ISEKI Food Association

ISSN: 2182-1054

International Journal *of* Food Studies

Volume 7 | October, 2018

International Journal of Food Studies

The ***International Journal of Food Studies (IJFS)***, a journal of the ISEKI_Food Association, is an international peer-reviewed open-access journal featuring scientific articles on the world of Food in Education, Research and Industry. This journal is a forum created specifically to **improve the dissemination of Food Science and Technology knowledge between Education, Research and Industry** stakeholders. Core topics range from raw materials, through food processing, including its effect on the environment, to food safety, nutrition and consumer acceptance. To enrich this forum the journal is also open to other food-related topics such as food policy and food anthropology.

INTERNATIONAL JOURNAL OF FOOD STUDIES

Volume 7, nº 2 (2018)
(Published 18 October 2018)

CONTENTS

1 Are We Doing Our Homework? An Analysis of Food Engineering Education in Brazil
VIVIAN-LARA SILVA, FAUSTO MAKISHI, MARCUS MAGOSSI, IZABEL CRISTINA FREITAS MORAES, CARMEN SILVIA FÁVARO TRINDADE & PAULO JOSÉ DO AMARAL SOBRAL

17 Food Safety Implementation in the Perspective of Network Learning
ZAM-ZAM ABDIRAHMAN, LESLIE D. BOURQUIN, LOÏC SAUVÉE & DEEPA THIAGARAJAN

30 Antioxidant and Antibacterial Activities of Exopolysaccharides Produced by *Lactic Acid Bacteria* Isolated from Yogurt
ZOUAOUI BENATTOUCHE, DJILLALI BOUHADI & GHALEM BACHIR RAHO

38 Consumers' Willingness to Consume Cassava Leaves as a Leafy Vegetable in the Kumasi Metropolis, Ghana
FRED NIMOH, GODFRED O. ASARE, ISMAEL TWUMASI & RICHMOND ANAMAN

51 Production of Camel Milk Yoghurt: Physicochemical and Microbiological Quality and Consumer Acceptability
OBAKENG GALEBOE, EYASSU SEIFU & BONNO SEKWATI-MONANG

64 Antioxidant Activities of Aqueous Extracts from Nine Different Rose Cultivars
HAMADIA KHURSHID, SYED MUBASHAR SABIR, SHAHID IQBAL AWAN, SYED RIZWAN ABBAS & MUHAMMAD IRSHAD

76 Extraction Kinetics of Saponins from Quinoa Seed (*Chenopodium quinoa* Willd)
R. M. TORREZ IRIGOYEN & S. A. GINER

89 Physicochemical and Antioxidant Properties of Banana Varieties and Sensorial Evaluation of Jelly Prepared from those Varieties Available in Sylhet Region
ABDULLAH A. SAD, M. M. HOQUE & WAHIDU ZZAMAN

98 A Nutritional Evaluation of the Berry of a New Grape: 'Karaerik' (*Vitis vinifera* L.)
AYNUR KURT, NESRIN COLAK, AYDIN SÜKRÜ BENGU, ALI GUNDOĞDU, ERDAL AKPINAR & SEMA HAYIRLIOGLU-AYAZ & FAIK AHMET AYAZ

117 'Made-in-transit' Yoghurt Processing: A Review of Basic Concepts and Technological Implications
M. A. R. NOR-KHAIZURA, S. H. FLINT, O. J. MCCARTHY, J. S. PALMER, M. GOLDING & A. JAWORSKA

International Journal of Food Studies | IJFS October 2018 | Volume 7 | pages 1–16

Are We Doing Our Homework? An Analysis of Food Engineering Education in Brazil

Vivian-Lara Silva[a*], Fausto Makishi[b], Marcus Magossi[a], Izabel Cristina Freitas Moraes[a], Carmen Silvia Fávaro Trindade[a], and Paulo José do Amaral Sobral[a]

[a] Food Engineering Dept., University of São Paulo, 225 Duque de Caxias Norte Avenue, Zip code: 13635-900, Pirassununga (SP), Brazil
[b] Institute of Agricultural Sciences, Federal University of Minas Gerais, 1000 University Avenue, Zip code: 39.404-547, Montes Claros (MG), Brazil
[*]Corresponding author
vivianlara@usp.br
Tel Fax: (+55 19) 35 65 42 84

Received: 2 November 2016; Published online: 18 October 2018
Invited paper from the 4th International ISEKI Food Conference - ISEKI Food 2016 - Bridging Training and Research for Industry and the Wider Community - Responsible Research and Innovation in the Food Value

Abstract

What is the profile of Food Engineering education in Brazil? Are we following the contemporary professional renewal trend? Driven by these questions, the present study analyzed data regarding 21 academic courses, which represent approximately 22% of the total bachelor's degree in food engineering courses offered in the country. Samples were defined considering a Brazilian annual ranking of undergraduate programs: very good (four stars) and excellent (five stars). Next, information was recovered from both the Brazilian Ministry of Education and institutional homepages of each analyzed program. The results suggest that food engineering programs exhibit relative identity, naturally due to their history and the path of each program and their faculty, shaping particularities in how fields of knowledge are constituted, in addition to their representativeness in the total workload of the program. However, initial analysis is suggestive regarding understanding that Brazil is not properly doing its homework, based on global movement, concerning food engineering education. The need to rethink Brazilian technical education, without culminating in additional workload, is emphasized, not only regarding new materials and technologies for learning and teaching, but also in terms of bringing a human and market approach. The achievement of this complex goal seems to be provided by the encouragement of student associations, transversal learning processes, and learning experiences outside the classroom as a means of improving undergraduate programs and human resources.

Keywords: Food science and technology; Engineering education; Curriculum development

1 Introduction

Over the past decades, Brazil has consolidated itself not only as a global food producer but also as an important consumer market in terms of industrialized goods. This context follows the recent development of the Brazilian manufacturing industry, in which the food industry plays a unique role. Part of this contemporary phenomenon reflects the growing demand for human resources in general, and engineers in particular (Batalha et al., 2005). In response to this context, an increasing amount of food engineering programs has been instituted across the country in the last decades.

 10.7455/ijfs/7.2.2018.a1

Currently, according to the Brazilian Ministry of Education, there are 96 undergraduate bachelor degree programs in this field in the country, equivalent to 7,000 admissions per year (MEC, 2016). It is important to state that Brazilian food engineering programs in the EU are equivalent to integrated masters courses (BSc+MSc) in Europe, being completed in five years, on average.

Therefore, the main purpose of the present study was to make observations regarding food engineering education in Brazil, emphasizing a particular issue: are we aligned with the effervescent discussion that occurs at the international level concerning future challenges of the career?

In this regard, the American Society for Engineering Education (ASEE) introduced a series of discussions through the "Advancing the Scholarship of Engineering Education: A Year of Dialogue", in order to ensure greater consistency between engineering education and current demands from society (Melsa, Rajala, & Mohsen, 2009).

This initiative counted on the support of the North American industry, which was interested in promoting changes in the curricular and educational matrix, given the alignment of human resources with their particular needs. Similar movements were also observed in different parts of the world, such as Europe, Americas and Oceania (Alves, Restivo, & da Silva, 2015; Borrego & Bernhard, 2011).

It is interesting to note, however, that despite this, in the European Union, Flynn, Bejarano, Wahnstrom, Echim, and Quintas (2013) carried out a profile analysis of professionals in the field of Food Science and Technology in order to analyse why the sector exhibits low innovation rates when compared to other industries.

Flynn et al. (2013) suggested that the education of these professionals is on track. However, it has not yet reached sufficient amplitude or required depth in soft skills, which encompass abilities such as leadership, teamwork, and proactivity, as well as resilience and communication skills. Moreover, Saguy and Cohen (2016) highlight food engineering education should address entirely new topics and dimensions such as sustainability, economic environments, social responsibility, population growth, and aging. This is because a food engineering career is now confronted with unique challenges involving health, food security, and well-being (Silva, Sereno, & do Amaral Sobral, 2018); and must play a proactive role in the ecosystem of innovation. At least in part, this movement comes from the most challenging moment that food engineering faces in the two centuries of history of the food industry (Aguilera, 2006). Indeeed, food engineers face increasingly complex challenges, such as a growing concern for health and wellness, development of functional foods, adapting nutritional profiles and foods for the elderly, high performance products for athletes, foods with lower calorie density, socioenvironmental performance, ethical food trades and production and so on (Besterfield-Sacre, Cox, Borrego, Beddoes, & Zhu, 2014; Saguy & Cohen, 2016). As a result, a multi and interdisciplinary approach is required (Saguy & Cohen, 2016). Indeed, the need for scientific and technological development is intertwined with the social dynamics in which the food processing industry operates (Kasemodel, Makishi, Souza, & Silva, 2016). Culminating in new, distinct and interrelated knowledge domains that must be approached in an articulated manner by food engineering education, such as biology, medicine, molecular gastronomy, new materials, and nanotechnology, in addition to concepts of market, and business economics (Saguy, Singh, Johnson, Fryer, & Sastry, 2013).

An interesting highlight, however, is that while the topic of new routes of training in food engineering is increasingly discussed in different international forums around the world, in Brazil, the agenda remains unexplored and restricted to the specific context of undergraduate programs and their departments. With this in mind, the primary objective of the present study was to discuss if we are doing our homework in Brazil.

2 Materials and Methods

The curricula of 21 food engineering programs (approximately 22% of those offered in Brazil) were analyzed. The sample was defined by considering the 2016 Brazilian Student Guide ("Guia do Estudante 2016"), a traditional annual assess-

ment of undergraduate programs in the country. This annual report contains the evaluation results of programs classified in stars: good (three stars), very good (four stars), and excellent (five stars). The evaluation system consists of an annual collection of qualitative and quantitative data per program, in electronic format, of a commission formed by at least seven experts (referees), including course coordinators, department directors, and professors. The analyzed criteria gather information on faculty, didactic-pedagogical projects, scientific production, extension activities, internationalization, insertion of students in the job market, infrastructure, and supply of postgraduate courses. Each of the seven referees awarded grades according to criteria: excellent (5), very good (4), good (3), regular (2), poor (1) and "I'd rather omit myself"; in which case a new assessor is invited to participate. The top and bottom scores were disregarded to eliminate distortion. The mean was calculated from five assigned scores. The final grade was calculated from the weighted average of the grades obtained in the last three years. Programs receiving scores between 4.3161 and 5 were classified as five stars, and those receiving scores between 3.6322 and 4.3161 (Guia do Estudante, 2016).

For the 2016 Brazilian Student Guide, the analysis was performed using programs certified as five stars (a total of 5 programs) and four stars (a group of 16 programs). Once selected, undergraduate program information was recovered from the official databases of the Brazilian Ministry of Education (MEC, 2016), as well as from the institutional homepages of each program. The following data were collected: 1. year of establishment; 2. location; 3. annual number of admissions; 4. required and elective courses; 5. credit hours per course, and 6. total workload. Required and elective courses were divided into groups, representing their particular field of knowledge, totaling nine groups: 1. Basic Sciences; 2. Engineering Sciences; 3. Food Sciences; 4. Human Sciences; 5. Technologies; 6. Supervised Internship; 7. Monograph; 8. Elective Courses, and 9. Learning Experiences Outside the Classroom. The proposed division was based on the model and taxonomy utilized by the University of São Paulo (USP).

Table 1 summarizes the criteria applied for the proposed division. The data collection process covered the period from 15 to 25 December, 2015. Data were grouped into a spreadsheet in Excel format, which formed the basis for further analysis and discussion.

Additionally, in order to analyze the role of learning experiences outside the classroom (not included in the educational plan), an assessment of student associations linked to food engineering programs was carried out. These associations are organizations that are formed exclusively by undergraduate students who represent the students' interests, and which retain civic, cultural, educational, sporting, business, and social purposes.

The following student associations were considered: 1) AIESEC (originally a French acronym for *Association Internationale des Etudiants en Sciences Economiques et Commerciales*; dedicated to empowering young people for peace and fulfillment of mankind's potential); 2) Athletic Associations; 3) Enactus (whose name comes from the combination of three words: Entrepreneurial, Act, and Us; representing a community of student, academic, and business leaders committed to using the power of entrepreneurial action to transform lives and shape a better, and more sustainable world); 4) Junior Companies and 5) Program of Tutorial Education (PTE).

The selection of this set of five student associations was deliberate. Athletic Associations, Junior Companies, and PTE are a few of the leading traditional and disseminated student organizations in Brazil. In turn, AIESEC and Enactus emerge as distinctive international student associations around the world. These student associations have been identified as strategic organsiations for learning outside the classroom through interdisciplinary exposure to the real-world problems (Gair, 1997; Dillon et al., 2006; Paisley, Furman, Sibthorp, & Gookin, 2008).

Table 1: Characterization of areas of knowledge: Main content and course examples

KNOWLEDGE GROUPS	MAIN CONTENT	COURSES EXAMPLES
Basic Sciences	Related to basis of engineering knowledge and that are used as tools for other courses throughout the program. These courses are common to all engineering programs.	Calculus, Physics, and Statistics
Engineering Sciences	Linked with chemical and food processing.	Transport Phenomena, Unit Operations, and Biochemical Engineering
Food Sciences	Courses that study the reactions, composition, and analysis of food.	Food Biochemistry, Food Analysis, and Food Microbiology
Human Sciences	Related to humanities and social sciences.	Sociology, Economy, and Food Distribution
Technological Processes	Linked with the study of food processing, in terms of specific methods or technological packaging.	Processing of Meat and Derivatives, Milk Technology, and Packaging Technology
Monograph	Related to the concluding course assignment.	Final Project
Supervised Internship	Related to the supervised internship.	Training Program
Elective Courses	Free courses chosen by the student (having to attend a minimum amount of credits).	Excel, Autocad, Emerging Technologies for Food Processing, Eco-Design
Learning Experiences Outside the Classroom	Linked with culture and extension activities (with a maximum amount of hours to benefit).	Technical visits, Participation in Student Associations, Attendance in Workshops and Conferences.

Source: The authors, based on information available on the websites of the analyzed food engineering programs

3 Results and Discussions

3.1 Food engineering: professionals to relieve world hunger and more

Before presenting the results obtained in this study, we will present an overview of food engineering in the world and in the Brazil.

The search for a better-aligned preservation for safe and healthy foods drives the development of this sector and its profession. Although each country seems to have developed its own identitity in structuring food engineering as a profession and a career, Karel (1997) identified two primary branches at the origin of contemporary food engineering education: 1) food engineering, which originated as an agricultural engineering specialization; and 2) food science and technology, which is mainly associated with chemistry but also incorporates elements of microbiology and agronomy. The first is related to the European school, more specifically the French one, while the second refers to the American or Anglo-Saxon school (Kostaropoulos, 2012).

Indeed, in some European countries, such as France, food engineering is a derivation of agricultural engineering, *l'Ingénieur Agro-alimentaire*, and emphasis should be given to the formation of *l'École Nationale des Industries Agricoles* (ENSIA), which occurred in 1893 (Agroparistech, 2018). Starting from that and benefiting from the European industrial revolution that occurred in the early 1900s, food engineering was strongly associated with small-scale agroindustrial production (nearly one century before the emergence of food engineering in the USA), which began to supply the growing European working class (Abramovay, 1992). Preservation techniques, such as appertization and pasteurization, date back to the same period.

On the other hand, in the USA, food engineering developed differently and was initially introduced as Food Technology, consolidating itself as a career in the early 1920s and having matured recently in the academic field (Kostaropoulos,

2012; Saguy et al., 2013). The profession emphasizes the industrialization process and the competitiveness of the segment, particularly in gaining scale. In other words, the American Food Engineering School was developed amidst the green revolution, based on large-scale rural and industrial production, retaining a high degree of automation and increasing productivity. In this context, Loncin (2012) described food engineering as an adaptation of techniques from chemical engineering, focusing on the food industry.

Besides these two branches discussed by Karel (1997), Kostaropoulos (2012) described a third branch concerning the origin of contemporary food engineering, which originated from the combination of mechanical and chemical process engineering in Germany. In that view, Kupriano (Kostaropoulos, 2012) emphasized that the Technical University of Karlsruhe launched its first food engineering program, denominated Food Techniques, in 1948. In Germany, agricultural development was followed by strong industrial advancement in the mechanical and chemical fields. This path led to a direct unfolding in German food engineering programs, which are distinguished by knowledge production in the field of projects and industrial process dimensioning (Costa, Mozina, & Pittia, 2014). The program was primarily structured as a field of chemical engineering, particularly associated with food science, food chemistry, physical chemistry, and, on a smaller scale, biology and microbiology (Kostaropoulos, 2012).

In summary, the three previously described schools (European, American and Germany) contributed considerably to the contemporary understanding of food engineering. Evidently, the distinction among them is much less sensitive in the current global context in which similarities and differences between curricula occur and in which new and more complex challenges seem to be placed on this profession.

As observed by Barbosa-Canovas and Ibarz (2002), food process engineering aims to study the principals and laws that govern the physical, chemical, or biochemical stages of distinct processes, as well as the apparatus or equipment by which such stages are industrially carried out. It incorporates food engineering principles, such as essential elements of food processing, transformation, preservation, material sciences, food equipment, and plant design to deal with operations of whole food processing units, including storage and logistics, instrumentation and automatic control, and feasibility studies (Kostaropoulos, 2012).

But this seems to be only part of the training currently required of the food engineer. With the increasing demand for products that meet the specific needs of particular consumer groups, the food engineer's education is undergoing a time of review. The future seems to cover professional responsibility for operationalizing the efforts conducted so far in food science and technology, while respecting the constraints of economic viability, social impact, and environmental conservation (Karel, 1997; Saguy et al., 2013; Silva et al., 2018).

This discussion is globally underway in different food engineering programs and, of course, it is still necessary to adjust to the local context. Indeed, although food production and distribution occur at a global level, each country seems to have developed its own identitity in structuring food engineering as a profession and a career. In particular, the present study aims to assess how the topic is perceived in Brazil, as discussed in the following section.

3.2 Food engineering in Brazil

With a delay, relative to the development of the sector in Europe and the USA, the history of food engineering in Brazil only began to develop properly in the late 1960s, early 1970s.

This time marks a beginning to the development of the food industry in Latin America. The industrial sector as a whole was incipient in these countries, including Brazil, in which the exportation of commodities was the primary economic activity (Bulmer-Thomas, 2003; Skidmore, 2009). Particularly within the Brazilian context, the industrial process was encouraged and stimulated by the government, which predominantly focused on the steel and transportation industries. The Brazilian food industry in turn developed during this period, absorbing external technologies, such as UHT milk processing (a memorable example of the role of a food en-

gineering career and the offer of products with low cost, and extended shelf life, versus longer distribution routes). Moreover, the oil crisis and increase in prices led to a concern regarding food production. It was exactly in this period that the first engineering programs were founded in Brazil.

However, the awakening of Brazil to food engineering training would pass through another moment of numbness. This was due to another economic crisis that paralyzed Brazilian industrial development and impacted negatively on industrial food processing. More specifically, the 1980s were considered a lost decade, in which economic and political uncertainty prevailed in different Latin American countries, including Brazil.

In turn, in the 1990s, economic stabilization, market deregulation, and trade liberalization led to a new impulse for the Brazilian food industry. And so, from the second half of the 1990s, the country consolidated itself as a global food producer, becoming one of the leading agricultural product suppliers in the new millennium. The development of the Brazilian consumer market awakened the interest of many multinational food companies, significantly increasing the job supply in the sector.

Following this story, the origin of Brazilian food engineering was related to the creation of ITAL (Food Technology Institute) in 1962, resulting from the dismemberment of a section of the IAC (Agronomic Institute of Campinas, situated in the State of São Paulo). A few years later, in 1967, the first food engineering School was instituted by UNICAMP (State University of Campinas), followed by UFV (Federal University of Viçosa) in 1975, UFPB (Federal University of Paraíba) and UFC (Federal University of Ceará) in 1976, and UFSC (Federal University of Santa Catarina) and FURG (Federal University of Rio Grande) in 1979.

Afterward, as illustrated in Table 2, the program was introduced to UNIFEB (Educational Foundation of Barretos) in 1980, UNESP (São Paulo State University) in 1983, FENVA (Engineering College of Varginha) in 1984, Maua Engineering School in 1986, UNIMEP (Metodista de Piracicaba) in 1988, and PUC/PR (Pontifical Catholic University of Paraná) in 1989. São Paulo University (USP) inaugurated its course in 2001.

The UNICAMP program was structured by a multidisciplinary team of professors from the fields of mechanical, agronomic, agricultural, and chemical engineering, as well as mathematics and biology, having as its main reference the American school, as discussed in the previous section. More specifically, the influence came from the Food Science and Technology curriculum of the University of California. In turn, at UFV, the program evolved from the Food Technology specialization offered in the Agronomy program; while at UFPB, the food engineering program was associated with the Department of Chemical and Food Technology.

In addition to the mechanical, agronomic, agricultural and chemical engineering programs, other fields of knowledge are also verified in the genesis of other food engineering programs currently active in Brazil (MEC, 2016), such as production engineering, animal science, economy and business. From its establishment, food engineering programs have been implemented in various colleges and universities in several regions of the country. Figure 1 shows their distribution in Brazil.

But it is precisely from the end of 1990s that training in food engineering reached its boom in Brazil. Regarding the previous discussion, Figure 2 outlines the relatively recent dissemination of food engineering programs in Brazil, with respect to the number of students entering the programs, which has become more pronounced over the past 15 years, when approximately 70% of the existing courses were instituted. This period corresponds to the increasing stability of the Brazilian economy, inferring an attractive economic context to the growing mass of food engineers provided annually by schools since then.

As illustrated in Figure 1, there is a greater concentration of food engineering programs in the South and Southeast regions of Brazil, highlighting São Paulo and Minas Gerais states that together account for 36% of all programs offered. The mentioned states are known for retaining some of the largest centers of consumption in the country, and the highest concentration of large food processing industries.

However, another result should not be overshadowed. The progression of food engineering programs towards the countryside (Figure 1) sug-

Table 2: Top 21 Brazilian food engineering programs: Characterization by year of course establishment, location, and the number of students enrolled annually

Program	Year of course establishment	Location (City/State)	Number of students enrolled annually
UNICAMP – Universidade Estadual de Campinas	1967	Campinas-SP	115
UFV – Universidade Federal de Viçosa	1975	Viçosa-MG	60
UFC – Universidade Federal do Ceará	1976	Fortaleza-CE	100
UFPB – Universidade Federal da Paraíba	1976	João Pessoa-PB	30
FURG – Universidade Federal do Rio Grande	1979	Rio Grande-RS	50
UFSC – Universidade Federal de Santa Catarina	1979	Florianópolis-SC	50
UNIFEB – Fundação Educacional de Barretos	1980	Barretos-SP	40
UNESP – Universidade Estadual Paulista	1983	S. J. do Rio Preto-SP	30
FENVA – Faculdade de Engenharia de Varginha	1984	Varginha-MG	30
Instituto Mauá	1986	S. C. do Sul-SP	40
UNIMEP – Metodista de Piracicaba	1988	Piracicaba-SP	60
PUC/PR – Pontifícia Universidade Católica do Paraná	1989	Curitiba-PR	60
UFRRJ – Universidade Federal Rural do Rio de Janeiro	1991	Seropédica-RJ	60
UNISINOS – Universidade do Vale do Rio dos Sinos	1992	São Leopoldo-RS	90
UFRGS – Universidade Federal do Rio Grande do Sul	1995	Porto Alegre-RS	30
UPF – Universidade de Passo Fundo	1998	Passo Fundo-RS	NF
UFG – Universidade Federal de Goiás	1999	Goiânia-GO	60
UESB – Universidade Estadual do Sudoeste da Bahia	1999	Itapetinga-BA	40
UFPa – Universidade Federal do Pará	2000	Belém-PA	32
UEM – Universidade Estadual de Maringá	2000	Maringá-PR	40
USP – Universidade de São Paulo	2001	Pirassununga-SP	100
UCS – Universidade de Caxias do Sul	2001	Caxias do Sul-RS	50
UFS – Universidade Federal de Sergipe	2001	São Cristovão-SE	50
UFLA – Universidade Federal de Lavras	2003	Lavras-MG	100
UFRJ – Universidade Federal do Rio de Janeiro	2004	Rio de Janeiro-RJ	40
IFGoiano – Instituto Federal Goiano	2007	Rio Verde-GO	50

Note: NF (Information not found). Source: The authors, based on information obtained from the Brazilian Ministry of Education (MEC, 2016)

gests an open issue: the impact on the standard curricula regarding topics of regional or local interest, such as Amazon and Cerrado fruit processing, reductions in water use, and technologies to generate income in vulnerable populations.

3.3 Total Workload

The total workload of the 21 courses analyzed presents a standard deviation of 300 hours more or less, which suggests a quantitative difference between courses. Reviewed courses rated as five stars have a leaner workload compared to most of the courses rated four stars.
Figure 3 illustrates the total workload in hours of each of the 21 programs analyzed in the present study. The light gray bars represent programs certified as four stars by the 2016 Brazilian Student Guide (Guia do Estudante, 2016), while the dark gray bars characterize programs that were considered as five stars by the same ranking. In general, the programs rated as five stars exhibit reduced workload when compared to those rated as four stars.
In the international context, some authors suggested reduction of hours in the classroom could encourage students to pursue different activities such as voluntary work, practice in laboratories or in industry (following internship programs), as well as the development of research projects (introduction to science research) or even expe-

Figure 1: Distribution of the 96 food engineering programs in Brazil. Source: The authors, based on information obtained from the Brazilian Ministry of Education (MEC, 2016)

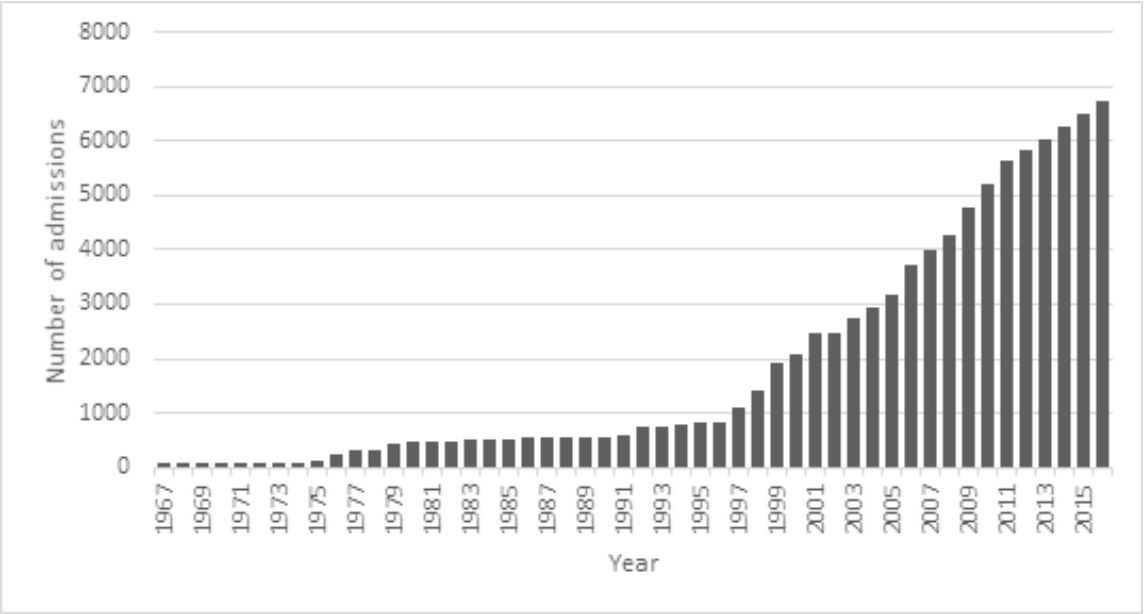

Figure 2: Number of admissions per year since the implementation of food engineering education in Brazil. Source: The authors, based on information available on the websites of the analyzed programs

rience abroad (in university exchange programs). This in turn allows the enhancement of new and complementary content (Flynn et al., 2013; Saguy et al., 2013; Saguy & Cohen, 2016). On the other hand, it is difficult to say if students willprefer more free time. Finding the incentive and monitoring mechanisms for complementary training in engineering courses are challenges. Any way, in Brazil, some of the courses analysed have incorporated hours dedicated to activities outside the classroom as mandatory requirements of their programs. This is the case of UFRRJ, FURG, UESB, UFRGS and Unisinos, which account for an average of 180 hours as experiences outside the classroom. In other courses, students' participation in these activities is voluntary.

Regardless of the quantitative aspect of the global workload of the programs in question, with respect to the qualitative analysis, Brazilian curricula display a particularly proportional pattern regarding 'fields of knowledge'. This statement was derived from a complementary assessment that refers to an in-depth qualitative analysis of the 21 programs analyzed in the present study. This is shown in detail in the next section.

3.4 Fields of Knowledge Analysis

A comparative analysis of the curriculum of very good (four stars) and excellent (five stars) food engineering programs in Brazil is shown in Figure 4. In spite of other classifications of possible courses, the present study proposed a set of knowledge groups, considering the Brazillian curriculum, as follows: 1. Basic Sciences; 2. Engineering Sciences; 3. Food Sciences; 4. Human Sciences; 5. Technological Processes; 6. Supervised Internship; 7. Monograph; 8. Elective Courses, and 9. Learning Experiences Outside the Classroom.

In general, Basic Sciences, Engineering Sciences, Food Sciences, Human Sciences, Supervised Internship, Monograph and Elective Courses have similar workloads considering the 21 studied curricula, disabling the visualization of significant differences between the programs classified as very good and excellent. A point of difference between the two groups concerns the workload attributed to Technology disciplines and Learning

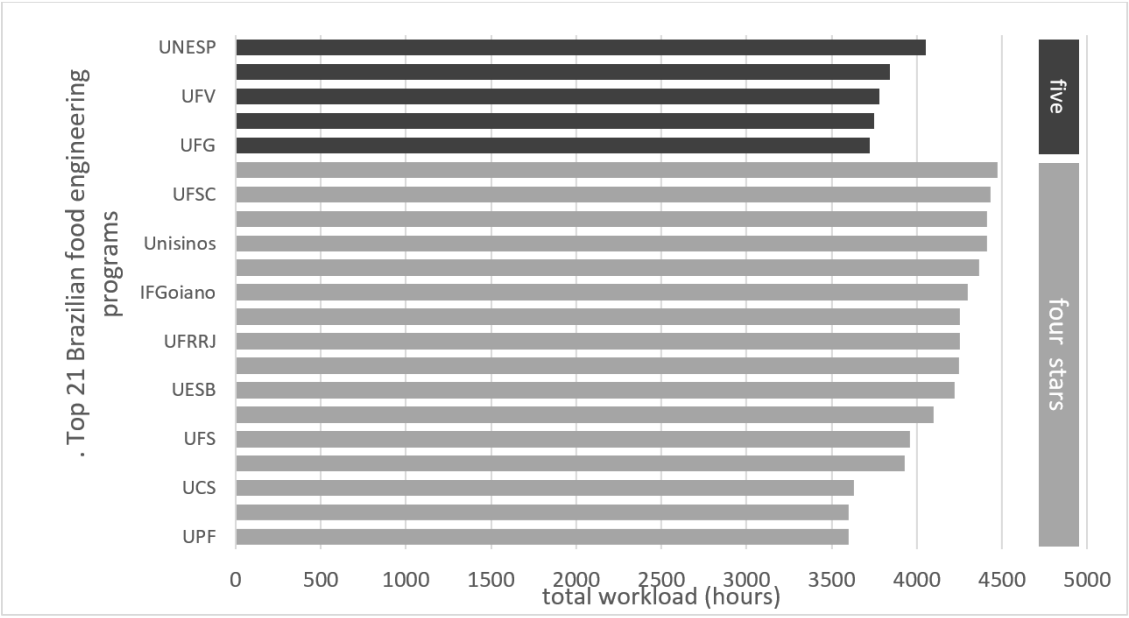

Figure 3: Top 21 Brazilian food engineering programs (four and five stars): characterization by total workload in hours. Source: The authors, based on both the Brazilian Ministry of Education ((MEC, 2016) and information available on the websites of the analyzed programs. Note: The Brazilian Government sets a minimum of 3,600 hours for food engineering education (distributed in 5 years)

Experiences Outside the Classroom. Programs classified as excellent have higher workloads in Food Technology disciplines and lower ones in Learning Experiences Outside the Classroom. It is noteworthy that the infrastructure, generally associated with the disciplines provided by the Technological processes group, such as laboratories and pilot processing units, are attributes evaluated by the Brazilian ranking. Including substantial financial investments, the incorporation of this type of discipline in the curriculum can be difficult for some programs, thus considered an obstacle in curriculum development.

The disaggregated analysis of the Top 5 programs (five stars), in the nine areas of knowledge, assists in understanding the heterogeneity of the Brazilian food engineering programs (Figure 5). The programs developed at the University of São Paulo (USP) and the Federal University of Goiás (UFG) proportionally exhibit more hours dedicated to core disciplines. The State University of Campinas (UNICAMP) and the Federal University of Viçosa (UFV) display smaller representations of these disciplines, with greater importance for technological processes such as meat, grain, fish and fruit processing and baking.

Basic Sciences represent 34.5% of the average workload of the 21 analyzed programs. Furthermore, it is noteworthy that the UFG and USP programs exhibit values well above average, accounting for 39.9% and 39.7%, respectively. This result indicates a considerable concern for the solid formation of basic or initial concepts, in addition to providing a foundation for an improved learning experience of supplementary fields encountered by the student. Part of the discussion takes place at international debates (Besterfield-Sacre et al., 2014; Roos et al., 2016; Saguy & Cohen, 2016), which have argued that the solution to increasingly complex problems is the deepening of basic sciences, such as mathematics, statistics,

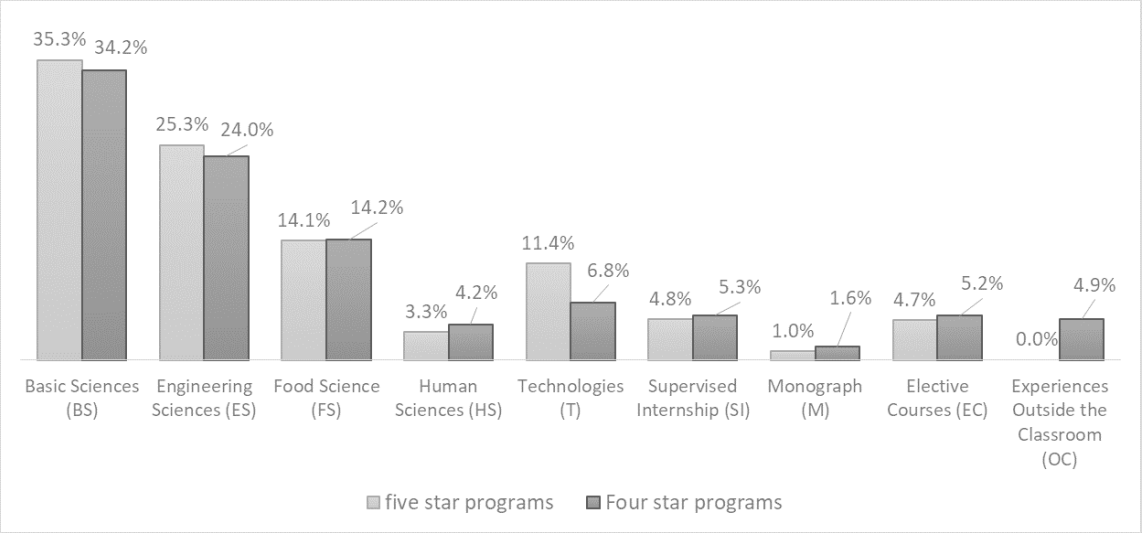

Figure 4: Comparative analysis of the Engineering program curriculum: very good (four stars) and excellent (five stars), considering nine fields of knowledge. Source: The authors, based on information available on the websites of the analyzed programs

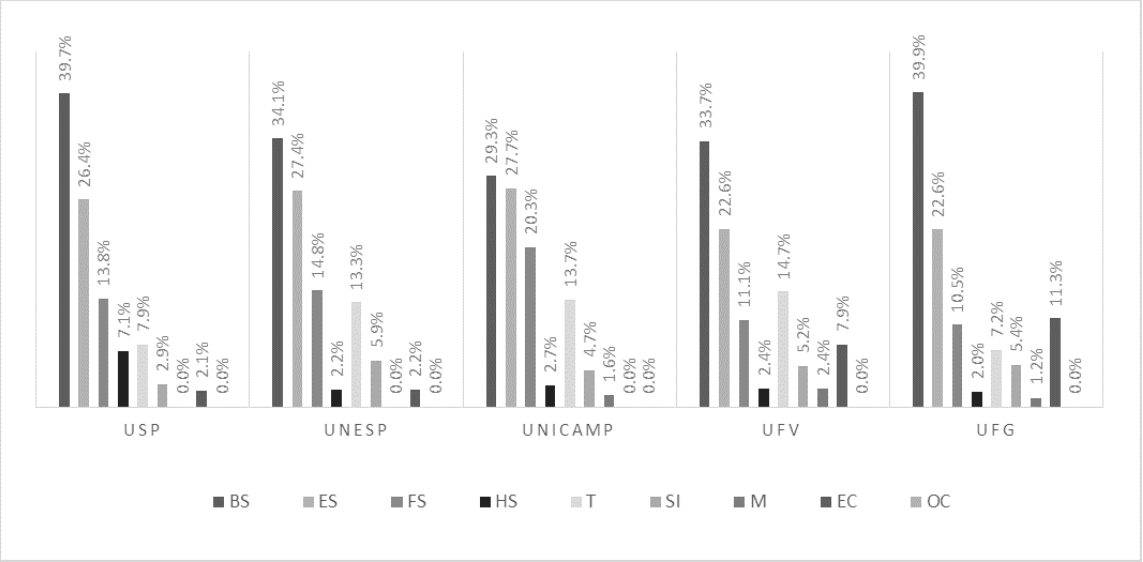

Figure 5: Individualized analysis of the top 5 engineering programs in Brazil according to area of knowledge: Basic Sciences (BS); Engineering Sciences (ES); Food Sciences (FS); Human Sciences (HS); Technological Processes (T); Supervised Internship (SI); Monograph (M); Elective Courses (EC), and Learning Experiences Outside the Classroom (OC). Source: The authors, based on information available on the websites of the analyzed programs

physics, chemistry, and biology.

Engineering Sciences represent 24.3% of the average workload of the 21 analyzed programs. Moreover, as shown in Figure 5, this field of knowledge displayed a higher percentage in programs rated as five stars than in the combined averages of programs rated as five and four stars. No significant discrepancy was verified between the values corresponding to each food engineering program. This result indicates certain homogeneity between the total workload regarding this knowledge group for all of the food engineering programs analyzed, indicating that this factor exhibits similar importance in all programs.

Food Sciences represent 14.2% of the average workload of the 21 programs analyzed. The combined averages of the programs reveal similar percentages. However, it is noteworthy that UNICAMP showed a value of 20.3%, which is superior to the mean of the 21 programs (14.2%), as well as the average of the programs rated as five stars (14.1%).

Human Sciences represent 4% of the average workload of the 21 analyzed programs. Programs rated as five stars exhibited a lower percentage when compared to the combined averages of the programs rated as four and five stars. In addition, emphasis should be given to the USP program, which displayed a 7.1% average regarding this knowledge group, superior to the mean of the 21 programs (4%).

Technological processes represent 7.9% of the average workload with respect to the 21 programs analyzed. This field of knowledge displayed a higher percentage in programs rated as five stars than in the combined averages of the programs rated as four and five stars. Moreover, it is relevant to note that UNESP, UNICAMP, and UFV exhibited superior values when compared to the mean of the programs rated as five stars, as well as the combined averages of the programs rated as four and five stars.

In turn, the consolidation of the concepts regarding classroom knowledge, as well as familiarizing students with operational and managerial routines, occurs mainly by way of a mandatory **Supervised Internship**. As observed by Roos et al. (2016) and Flynn et al. (2013), the approach of academia and industry proves to be a determining factor in the professional training of the engineer. In the present study, approximately 5% of the workload was devoted to compulsory internship activities, such as a training program, regarding the average workload of the 21 analyzed programs. Moreover, in most of the courses, students are encouraged to develop additional hours in this type of experience.

Monograph represents 1.4% of the average workload of the 21 analyzed programs. A relevant discrepancy was observed between each food engineering program and the average of the programs rated as five stars, as well as the combined averages of programs rated as four and five stars. This occurs because some food engineering programs do not require the integration of a final project in their curricula.

Elective Courses represent 4.9% of the average workload of the 21 analyzed programs. This percentage refers to the minimum quantity of credits that must be completed in each program. Additionally, a small discrepancy between the average of the programs rated as five starts and the combined averages of the programs rated as four and five stars was verified. However, a relevant variation was observed among the programs rated as five stars.

Lastly, it is relevant to note that **Experiences Outside the Classroom** are not recog-

nized as workloads in the programs rated as five stars (USP, UNESP, UNICAMP, UFV, and UFG). In contrast, programs rated as four stars offer courses regarding this field of knowledge. It is worth noting that the programs rated as five stars do not include experiences outside the classroom in their workloads since they are deemed voluntary.

After considering the workloads of the top 21 Brazilian food engineering programs, it would be interesting to discuss how the reduction in workload can contribute to promote and/or improve the out-of-class activities, contributing to complementary education, including certain soft skills. In this regard, several authors have suggested that the involvement in activities outside the classroom in real-world problems allow acquired knowledge to be added to other skills, forming essential skills for future professionals (Dillon et al., 2006; Paisley et al., 2008). While, in the European Union, professionals are required to gain soft skills (Flynn et al., 2013), in Brazil this is not different. However, these characteristics are often difficult to develop in the classic teaching-learning models.

3.5 Student Associations and Soft Skills

The Programme for International Student Assessment (PISA) defines as a key competency the ability to successfully meet complex demands in a particular context. Competent performance or effective action implies the mobilization of knowledge and cognitive and practical skills, as well as social and behavioral components, such as attitudes, emotions, and values and motivations (Rychen & Salganik, 2003).
However, if such skills are valued and furthermore necessary, how can they be developed in the academic environment? Additionally, how can such attributes be incorporated into academic education?
An initial insight was obtained from data shown in Table 3, which indicate the presence or absence of student associations in food engineering programs.

Many authors have suggested that the participation in student organizations is an essential method for the development of key competencies (Eccles & Barber, 1999; Knight, 2004; Berman & Ritchie, 2006; Lucena, Downey, Jesiek, & Elber, 2008).
Aligned to that approach, to integrate student associations provide a way of developing soft skills that are usually not fully developed throughout undergraduate programs. These initiatives allow students to implement competencies such as leadership, teamwork, proactivity, resilience, and communication skills, among others, which will be demanded of them when they enter the job market.
The foundation of these pedagogical tools is supported by a problem-based learning approach, which proposes the students' exposure to sufficient situations in order to enable them to seek knowledge for themselves when faced with a problem (Wood, 2003).
The learning experiences outside the classroom represent the main incentive that food engineering programs in Brazil provide in order to meet the market demand for complete professionals, whether in soft or hard skills, whohave the professional knowledge, tools, and techniques to be qualified for the career in question.
Nevertheless according to the evidence in Table 3, there is still a long path to follow in food engineering education, not only in implementing initiatives in all of the programs but also in disseminating them among the entire student population.

4 Conclusion

The present study developed a critical analysis of food engineering education in Brazil, and its results suggest that the Brazilian programs retain similar curricular structures, although variations were observed. In addition to the curricula, incentives provided by food engineering programs to student associations were described, culminating in a learning experience of abilities that are commonly referred to as soft skills. The results also suggest that these programs exhibit relative identity, naturally due to their history and the path of each program and their faculty, shaping

Table 3: Active student associations in each food engineering program

Program	Program of Tutorial Education	Enactus	Junior Company	AIESEC	Athletic Association
USP – Universidade de São Paulo	X	X	X	X	X
UNESP – Universidade Estadual Paulista	-	-	X	X	X
UNICAMP – Universidade Estadual de Campinas	-	X	X	X	X
UFV – Universidade Federal de Viçosa	-	X	X	X	X
UFG – Universidade Federal de Goiás	X	-	X	X	X
UFC – Universidade Federal do Ceará	-	-	X	X	X
IFGoiano – Instituto Federal Goiano	-	-	-	-	X
UFLA – Universidade Federal de Lavras	X	-	X	-	X
UFPa – Universidade Federal do Pará	-	-	-	X	X
UEM – Universidade Estadual de Maringá	-	X	X	X	X
UFRJ – Universidade Federal do Rio de Janeiro	-	X	X	X	X
UFRRJ – Universidade Federal Rural do Rio de Janeiro	-	-	X	-	X
UCS – Universidade de Caxias do Sul	-	-	X	-	X
UPF – Universidade de Passo Fundo	-	-	X	X	X
UFRGS – Universidade Federal do Rio Grande do Sul	-	X	-	X	X
FURG – Universidade Federal do Rio Grande	X	-	X	-	X
UFSC - Universidade Federal de Santa Catarina	-	-	X	X	X
UFS – Universidade Federal de Sergipe	-	-	-	-	X
Instituto Mauá	-	X	X	X	X
FENVA – Faculdade de Engenharia de Varginha	-	-	-	-	-
PUC/PR – Pontifícia Universidade Católica do Paraná	-	-	-	-	-
UESB – Universidade Estadual do Sudoeste da Bahia	-	-	-	-	-
UFPB – Universidade Federal da Paraíba	-	-	-	-	-
UNIFEB – Fundação Educacional de Barretos	-	-	-	-	-
UNIMEP – Metodista de Piracicaba	-	-	-	-	-
UNISINOS – Universidade do Vale do Rio dos Sinos	-	-	-	-	-

Note: The "X" indicates the existence, while "-" the absense of an association in the respective program. Source: The authors, based on information available on the websites of the analyzed programs

particularities in how fields of knowledge are constituted, in addition to their representativeness in the total program workload.

However, initial analysis is suggestive with respect to understanding that Brazil is not properly doing its homework based on global changes in food engineering education. The most important task will be the reduction of time spent inside classrooms. Some other aspects related to the economic, political, social, and environmental context stand out, giving a particular identity to the profile of the Brazilian school. Nevertheless, there is still a long way to go to integrate and standardize learning experiences for all students of distinct food engineering programs.

As homework, the initial analysis suggests an early opportunity to rethink certain issues, such as workload, transversal content, and teaching tools required to improve the alignment of Brazil with the vanguard movement facing food engineering education.

As well as improving the suggested methodology, a second stage of the study was structured, consisting of interviews with program coordinators, in order to search for a comparative analysis of the content; and even a comparison between the American, European, and Brazilian food engineering schools.

Acknowledgements

Our special thanks to the CORS (Center for Organizational Studies) and the GEPEC (Group of Studies and Research on Strategy and Vertical Coordination) for providing a rich environment

for the development of the present study. The authors are also grateful to Marcia G. Kasemodel, for her comments, suggestions, and technical assistance. Finally, the authors acknowledge the São Paulo Research Foundation (FAPESP) for the financial support (CEPID FoRC 13/07914-8), as well as the Brazilian National Council for Scientific and Technological Development (CNPq) for the Research fellowship of Paulo J.A. Sobral.

References

Abramovay, R. (1992). Paradigmas do capitalismo agrário em questão-hucitec. *Anpocs/Edunicamp-São Paulo-Campinas*.

Aguilera, J. M. (2006). Seligman lecture 2005 - food product engineering: building the right structures. *Journal of the Science of Food and Agriculture*, *86*(8), 1147–1155. doi:10.1002/jsfa.2468

Alves, G. R., Restivo, M. T., & da Silva, J. B. (2015). Special issue on engineering education: challenges for innovation. *European Journal of Engineering Education*, *40*(5, SI), 451–458. doi:10.1080/03043797.2015.1059409

Barbosa-Canovas, G. V. & Ibarz, A. (2002). *Unit operations in food engineering*. CRC press.

Batalha, M., Marchesini, M., Costa, M., Bergamaschi, M., Rinaldi, R., & Moura, T. (2005). *Recursos humanos e agronegócio: a evolução do perfil profissional* (J. Berman & L. Ritchie, Eds.). São Paulo: Novos Talentos.

Berman, J. & Ritchie, L. (2006). Competencies of undergraduate business students. *Journal of Education for Business*, *81*(4), 205–209. doi:10.3200/JOEB.81.4.205-209

Besterfield-Sacre, M., Cox, M. F., Borrego, M., Beddoes, K., & Zhu, J. (2014). Changing engineering education: views of us faculty, chairs, and deans. *Journal of Engineering Education*, *103*(2, SI), 193–219. doi:10.1002/jee.20043

Borrego, M. & Bernhard, J. (2011). The emergence of engineering education research as an internationally connected field of inquiry. *Journal of Engineering Education*, *100*(1), 14–47. doi:10.1002/j.2168-9830.2011.tb00003.x. eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/j.2168-9830.2011.tb00003.x

Bulmer-Thomas, V. (2003). *The economic history of latin america since independence*. Cambridge University Press.

Costa, R., Mozina, S. S., & Pittia, P. (2014). The regulation of food science and technology professions in europe. *International Journal of Food Studies*, *3*(1). doi:10.7455/ijfs/3.1.2014.a10

Dillon, J., Rickinson, M., Teamey, K., Morris, M., Choi, M. Y., Sanders, D., & Benefield, P. (2006). The value of outdoor learning: evidence from research in the uk and elsewhere. *School Science Review*, *87*(320), 107–111.

Eccles, J. S. & Barber, B. L. (1999). Student council, volunteering, basketball, or marching band: what kind of extracurricular involvement matters? *Journal of Adolescent Research*, *14*(1), 10–43. doi:10.1177/0743558499141003

Flynn, K., Bejarano, B. R., Wahnstrom, E., Echim, C., & Quintas, M. A. C. (2013). Profile of currently employed european food scientists and technologists: education, experience and skills. *International Journal of Food Studies*, *2*(2).

Gair, N. P. (1997). *Outdoor education: theory and practice*. Weidenfeld and Nicolson.

Guia do Estudante. (2016). Entenda como é feita a avaliação dos cursos do guia do estudante. Retrieved from http://guiadoestudante.abril.com.br/universidades/entenda-como-e-feita-a-avaliacao-de-cursos-do-guia-do-estudante/

Karel, M. (1997). The history and future of food engineering. *Food Engineering 2000*, 3–19.

Kasemodel, M.-G. C., Makishi, F., Souza, R. C., & Silva, V.-L. (2016). Following the trail of crumbs: a bibliometric study on consumer behavior in the food science and technology field. *International Journal of Food Studies*, *5*(1).

Knight, J. (2004). Internationalization remodeled: definition, approaches, and rationales. *Journal of Studies in International*

Education, *8*(1), 5–31. doi:10 . 1177 / 1028315303260832

Kostaropoulos, A. (2012). Food engineering within sciences of food. *International Journal of Food Studies*, *1*(2).

Loncin, M. (2012). *Food engineering: principals and selected applications*. Food science and technology. Elsevier Science. Retrieved from https://books.google.pt/books?id=YqH1d5FaC20C

Lucena, J., Downey, G., Jesiek, B., & Elber, S. (2008). Competencies beyond countries: the re-organization of engineering education in the united states, europe, and latin america. *Journal of Engineering Education*, *97*(4), 433–447. doi:10.1002/j.2168-9830.2008.tb00991.x

MEC. (2016). Cadastro e-MEC de instituições e cursos de educação superior: Sistema e-Mec. Ministério da Educação. Retrieved from http://emec.mec.gov.br

Melsa, J. L., Rajala, S. A., & Mohsen, J. P. (2009). Creating a culture for scholarly and systematic innovation in engineering education. *Journal of Engineering Education*, *98*(3), 209–209.

Paisley, K., Furman, N., Sibthorp, J., & Gookin, J. (2008). Student learning in outdoor education: a case study from the national outdoor leadership school. *Journal of Experiential Education*, *30*(3), 201–222. doi:10.1177/105382590703000302. eprint: https://doi.org/10.1177/105382590703000302

Roos, Y. H., Fryer, P. J., Knorr, D., Schuchmann, H. P., Schroen, K., Schutyser, M. A. I., ... Windhab, E. J. (2016). Food engineering at multiple scales: case studies, challenges and the future-a european perspective. *Food Engineering Reviews*, *8*(2), 91–115. doi:10.1007/s12393-015-9125-z

Rychen, D. S. & Salganik, L. H. (2003). *Key competencies for a successful life and well-functioning society*. Hogrefe Publishing.

Saguy, I. S. & Cohen, E. (2016). Food engineering: attitudes and future outlook. *Journal of Food Engineering*, *178*, 71–80. doi:10.1016/j.jfoodeng.2016.01.009

Saguy, I. S., Singh, R. P., Johnson, T., Fryer, P. J., & Sastry, S. K. (2013). Challenges facing food engineering. *Journal of Food Engineering*, *119*(2), 332–342. doi:10.1016/j.jfoodeng.2013.05.031

Silva, V. L., Sereno, A. M., & do Amaral Sobral, P. J. (2018). Food industry and processing technology: on time to harmonize technology and social drivers. *Food Engineering Reviews*, *10*(1), 1–13. doi:10.1007/s12393-017-9164-8

Skidmore, T. E. (2009). Brazil: five centuries of change. *New York, NY, USA: Oxford University Press*.

Wood, D. F. (2003). Problem based learning. *BMJ: British Medical Journal*, *326*(7384), 328–330. doi:10.1136/bmj.326.7384.328. eprint: https://www.bmj.com/content/326/7384/328.full.pdf

International Journal of Food Studies | IJFS October 2018 | Volume 7 | pages 17–29

Food Safety Implementation in the Perspective of Network Learning

Zam-Zam Abdirahman[a], Leslie D. Bourquin[b], Loïc Sauvée[a*], and Deepa Thiagarajan[b]

[a] INTERACT Research unit, UniLaSalle, 60000 Beauvais, France
[b] Department of Food Science and Human Nutrition, Michigan State University, Michigan, USA
[*]Corresponding author
loic.sauvee@unilasalle.fr

Received: 5 June 2017; Published online: 18 October 2018

Abstract

The food sector frequently faces difficulties in implementing food safety standards. Indeed, there are many barriers to appropriation of quality management standards which make effective implementation difficult for small and medium enterprises (SMEs), such as limited access to information, lack of financing and cognitive resources, food hazard perception, and insufficient access to adequately trained personnel. Consequently, one fundamental objective for practitioners such as managers, public bodies and development agencies is to help these food SMEs in improving their implementation capacity, which is usually done through the launch of different forms of collective initiatives such as associations, clubs, learning platforms, regional actions and other forms of collaboration. Globally speaking, the objective of these initiatives typically is to develop a step by step approach providing guidance on good practices associated with the implementation of these systems. The objective of the article is to explore and test the validity of this hypothesis, rooted in a general idea of "organizational network learning": the capacity of SMEs to adopt new food safety schemes is seen as a whole and necessitates mobilizing, at the same time, 1) formal innovation networks, which bring cognitive resources and institutional credibility, and 2) the practice by managers of informal network activities through interactive exchanges of information, benchmarking, knowledge transfer and translation, and experiential learning.

Keywords: Food safety; Implementation; Learning; Network; SME

1 Introduction

In modern agrifood systems, the development and effective implementation of food safety and quality management system standards (hereafter FSMS) such as ISO 22000, BRC (British Retail Consortium), IFS (International Food Standard) and other similar management standards is crucial (Scott & Chen, 2010). The food sector, mainly composed of SMEs, frequently faces difficulties in implementing these standards. Indeed, there are many barriers to appropriation of quality management standards which make effective implementation difficult for SMEs, such as limited access to information, lack of financing and cognitive resources, food hazard perception, and insufficient access to adequately trained personnel (Trienekens & Zuurbier, 2008).

Consequently, one fundamental objective for practitioners such as managers, public bodies and development agencies is to help these food SMEs in improving their implementation capacity, which is usually done through the launch of different forms of collective initiatives such as associations, clubs, learning platforms, regional actions and other forms of collaboration (Abdirah-

 10.7455/ijfs/7.2.2018.a2

man & Sauvée, 2012; Geith, Vignare, Bourquin, & Thiagarajan, 2010; Mensah & Julien, 2011; Trienekens & Zuurbier, 2008). Globally speaking, the objective of these initiatives typically is to develop a step by step approach to identify the benefits of engaging its members in food quality management programs and providing guidance on good practices associated with the implementation of these systems. More specifically, these initiatives aim to address the following tasks: the enhancement of the awareness in food quality management principles; the selection of adequate and competent partners such as consultants and coaches; the mobilization of the relevant services; the efficiency of the overall coordination over time; and the implementation of some global managerial recommendations. Nevertheless, the underlying hypothesis of these collective initiatives is rarely adressed, nor is it analyzed and compared in a systematic way. This hypothesis is rooted in a general idea of "network learning": the capacity of SMEs to adopt new food quality management schemes is seen as a whole and necessitates mobilizing at the same time, the following: a) formal innovation networks, which bring cognitive resources and institutional credibility, and b) the practice by managers of informal network activities through interactive exchanges of information, benchmarking, knowledge transfer and translation, and experiential learning.

In this context, the aim of this article is threefold. Firstly, it is to craft an original analytical framework in line with the literature on innovation networks, managerial innovation, network learning and related learning effects, specifically devoted to the study of quality management standards appropriation and implementation. This first part is mainly devoted to the identification of three categories of so called "network effects" that are provided by collective initiatives. The second objective of this article is to apply this framework to specific collective initiatives conducted in two countries (USA and France) in order to identify and compare the key relevant network effects induced at SME level by these collective initiatives which occur during the process of FSMS implementation by the involved SMEs. Thus, the research will identify strengths and weaknesses of these initiatives using a common grid based upon sound theoretical foundations. Indeed, a better understanding of learning processes at the individual as well as collective levels, both in informal (interpersonal) and formal (organizational) relationships, will providing insights into the major relevant learning principles and their possible adaptation to specific agrifood system sectors and to different national or regional contexts. Finally, we propose some concluding comments about the managerial implications derived from this analysis.

2 Materials and Methods

Yin (2013) case study methodology is followed. The case study is selected with an objective of an analytic generalization and comparison between cases. This approach of analytic generalization is relevant when "a previously developed theory is used as a template with which to compare the empirical result of the study". The research protocol in such an approach is based on interviews, which according to Eisenhardt and Graebner (2007) is a rich source of information and well adapted when the phenomenon is complex or unknown. Thus, several face-to-face interviews were conducted. In practice, the data was collected from a questionnaire and processed manually. Data collection is carried out among four actors: network coordinator, SMEs (adherents and beneficiaries of the network), public body and consultants (experts). Interviews with SMEs focused on a number of areas including membership motivations and network contributions. In total, seven semi-structured interviews were conducted: one with the network coordinator (CCI representative), one with the training organization, one with a consultant of quality and four with SMEs. The interviewees within the SMEs were the CEO (three interviews) and a quality referent (one interview). The consultant followed the company for a period of six months in order to realize a diagnosis, implement an action plan and monitor the implementation of the action plan. The training organization, meanwhile, carried out collective training for all companies of the collective.

This information was augmented by secondary data about the environment, the quality proce-

dures and the market characteristics relevant to the case study. The research protocol was conducted by the authors and based on extensive discussions with all members of the initiatives. More specifically, the two case studies followed a strict protocol, with iterative interviews of all the participants of the initiatives, and completed with interviews of the SMEs involved in the initiatives through contact with their CEOs and quality managers.

3 Results and Discussions

3.1 Network effects in Food Safety Management Standard (hereafter FSMS) implementation: emphasizing the interests of collective initiatives

Based upon a literature review, we considered three categories of network effects that are relevant to the topic. These effects are categorized as follows (Abdirahman & Sauvée, 2012):

1. The *structural effect* which finds its roots mainly in the structural analysis of networks;

2. The *interactive effect* which more specifically questions the idea of a networking activity that will support the implementation process;

3. The *cognitive effect* which focuses on the impact of the time dimension on any networking activity, leading to irreversibility, to path dependency and to the accumulation of new and specific knowledge useful for implementation of FSMS.

3.2 Exploring the structural dimensions of collective initiatives

For (Conway & Steward, 1998, 2009), the network perspective applied to innovation research has considerably renewed and extended our knowledge of innovation processes across different categories of innovation, including technological as well as marketing and organizational innovations. The starting point of the process of structural analysis is to consider any collective initiative, seen as a network, as a combination of actors and relationships (Burt, 2000; Borgatti & Li, 2009). In the structural analysis of networks, the actors are not independent but rather interdependent and influence each other. To take into account the unique situation of each member and the network structure as such, the structural approach combines two complementary perspectives: the global network, that is to say its density, the average distance between each of its members and the existence of subsets more or less structured; and the ego network, that is to say the situation of an actor (an individual, a SME) in its environment, its degree of inclusion and its mode of insertion into the global network (Borgatti & Li, 2009, 2009; Coulon, 2005). Another point to be considered is the evolution over time of the structural aspects of global and ego networks, which reinforces the importance of phases in FSMS implementation. Actors are considered as nodes, and relationships between them as ties. Thus, research on innovation, which mobilizes the structural analysis of networks (Coulon, 2005), produces a representation of innovation processes as maps (Conway, Jones, & Steward, 2001) or charts of nodes and relationships.
Consequently, within the FSMS context, two families of components must first be identified: actors and relationships. The identification of relationships that these actors have with one another is the second component. In line with social network theorists, these relationships can be of several types: continuous (similarities, relationships, interactions, such as common physical locations and cultural similarities) or discrete (financial flows, knowledge flows, such as permanent exchange of information), directed or not, measured by value or not, and formal or informal (Borgatti & Li, 2009). The systematic process of implementing management system standards such as FSMS typically involves two groups of major actors (Abdirahman, Kisempa Muyuala, & Sauvée, 2013; Hatanaka, Bain, & Busch, 2005; Hatanaka, Bain, & Busch, 2006): individuals

(managers and consultants) and organizations (SMEs, standardization bodies such as ISO, the International Organization for Standardization, consular agencies, auditors, governmental bodies and banks). Finally, the network reveals itself, by its structural properties, as facilitating (or hindering) the implementation.

3.3 Networking activity within collective initiatives

When implementing FSMS principles, knowledge transfer to the organization necessitates the mobilization of new cognitive resources and the activation of formal structures. An analytical approach applied to the implementation of FSMS is, therefore, assumed to provide a better understanding of the necessary learning processes. For Boris, Sandra, and Isabelle (2007), the mechanistic perspective is an essential step in that "the transfer of knowledge, considered as the dependent variable, proceeds from an optimal layout between the nature of network and the types of knowledge. The question is often that of a systematic identification of structural and relational properties of the network, as brakes or levers of the knowledge transfer." However, this structural determinism cannot alone explain the implementation process. Implementing FSMS implies a set of interdependencies and a permanent adjustment between the actors, their objectives and the context in question. Thus emerges a vision of co-constructed knowledge. In the end, a more complete representation of the relationship between network and organizational learning should show that the network is a "channel for learning but, recursively, that the network is transformed by the learning taking place. In other words, the network is at least partly constructed by the learning processes, dynamically, deliberately and in an emergent manner" (Boris et al., 2007).

The ambivalent dimension of the network in the phenomena of innovation is demonstrated by Owen-Smith and Powell (2004), Powell, Koput, and Smith-Doerr (1996), Powell, White, Koput, and Owen-Smith (2005) and Conway and Steward (2009). By distinguishing the network itself from the networking event, they show that the study of the innovation process involves taking into account both the structural dimension and interactivity. For Conway and Steward (2009), there is an interaction between the network as a structure and the networking event taking place in this network, with "on the one hand, the network may constrain or liberate the patterns of interaction and exchanges between network members; on the other, networking behavior may serve either to ossify (i.e. fix) the existing network membership and relationships, or create a dynamic in the membership and relationships within the network" (Conway & Steward, 2009). In the FSMS context, mobilizing transfers of knowledge, social networks and learning processes are involved. Thus, "the formal structure of network, but also the quality and relational characteristics that are played out, have a role on the nature of the learning that occurs there" (Boris et al., 2007). According to these authors, simultaneous consideration of structural and relational dimensions are necessary, in part, due to the fact that the individual is demanding of both resources and information but also demands a sense of belonging and social ties.

3.4 Collective initiatives as drivers of cognitive resources

The implementation of a FSMS goes through qualitatively distinct stages (Henson & Humphrey, 2009, 2010) with an evolutionary perimeter of actors involved in the process. These steps are mostly a reflection of the types of actors mobilized and of their changing status or role from one phase to another. Therefore, it is necessary to consider explicitly the time dimension and its corollary, namely its influence over the types of actors involved, and over the process of adopting the FSMS. This reflects the fact that the implementation is done in the long run and differentially mobilizes actors and resources. More precisely the time dimension in FSMS implementation impacts on the degree and number of involved SMEs and partners, with the idea of threshold effects: as soon as a threshold is reached, for instance in terms of number of consultancy firms involved in the initiative, a new stage of development is

possible.
The corollary of such a time dimension in the long run is the impact of knowledge creation and accumulation. Consequently, the implementation of a FSMS within a company, with its deep impacts on organizational structures and management procedures, requests an original view of the combination between the implementation process and learning phenomena. Change in organization related to learning is an important body of literature, stemming mainly from the seminal works of Argyris and Schön (1996) and Levitt and March (1988). According to Pawlowsky (2001) and his extensive survey of literature on learning, it is clear that "there are distinct perspectives on organizational learning that differ in respect to certain basic assumptions"; nevertheless, this author suggests that it is possible "to see outlines of a picture that visualizes basic building stones of an integrative model of organizational learning". His review suggests four different dimensions of learning: system-levels (from individual to network), learning modes (cognitive, cultural, action), learning types (single-loop, double-loop, deutero) and phases (Dierkes, Antal, Child, & Nonaka, 2003).
Following Podolny and Page (1998) and authors in social capital theory (Burt, 2000; Inkpen & Tsang, 2005; Nahapiet & Ghoshal, 1998), we will identify some characteristics of these cognitive effects that are paramount in the understanding of FSMS implementation. The basic idea for these effects is the fact that at a certain period of its development, learning processes lead to different forms of institutionalization within a formal network, which thus become a kind of "institution", producing its own rules, norms, values and culture, and aspects themselves embedded in idiosyncratic resources and skills. In the terms of Powell et al. (1996), the network becomes progressively the "locus of innovation".

3.5 Network effects in FSMS implementation: synthesis and managerial implications from a collective initiative point of view

The approach developed of FSMS implementation is the delineation of the structural characteristics of network, of the characteristics of the networking activity and of the network seen as a source of specific cognitive resources (Abdirahman & Sauvée, 2012). We have seen that this idea of three categories of network effects finds its source in the social capital theory (Burt, 2000; Nahapiet & Ghoshal, 1998; Inkpen & Tsang, 2005) and has already been developed in the context of innovation in general (Zheng, 2010) and managerial innovations in particular (Pitsis, 2013). Nahapiet and Ghoshal (1998) for instance define social capital as 'the sum of the actual and potential resources embedded within, available through, and derived from the network of relationships possessed by an individual of a social unit, it comprises both the network and the assets that may be mobilized through that network". As suggested by Pittaway, Robertson, Munir, Denyer, and Neely (2004) and Conway and Steward (2009), the connection has been made between the benefits of network and innovation. But the literature on the role and functions of networks on innovation can be approached through at least two interpretations (Conway & Steward, 2009). In the first one, the network is seen as a new way of organizing innovation activities, between market and hierarchy: it is thus the governance aspect that is emphasized. In the second one, the network is not considered per se as a specific mode of organizing activities benefiting (or not) to innovation. Instead, it is viewed as a new analytical lens which is interesting to focus on because it produces a wide range of effects, of externalities, that will influence the innovation processes. Doing so, the network is tracked via the effects it may produce, as a phenomenon affecting behavior of individuals and companies.
For instance, interaction effects between individuals probably will be more important at early stages of the implementation processes, while

structural dimensions are more predominant in well-established network relationships. Finally, cognitive effects will be mainly related to the institutionalization of a formal innovation network, especially when it becomes formalized into rules, routines and procedures which also tend to create path dependency, organizational memory and common resources. Through two examples in the USA and in France, we will show the nature of these effects and the necessary conditions under which these collective initiatives may be beneficial to SMEs.

4 Case study of collective initiatives for FSMS implementation in the USA and France

4.1 In the USA (with global implementation): the Food Safety Knowledge Network developed by Michigan State University (MSU)

Beginning in 2008 and in collaboration with several international partners, Michigan State University launched the Food Safety Knowledge Network (FSKN) initiative (Geith et al., 2010). The overall objectives of the FSKN initiative are to

1. develop internationally recognized competences in relation to food safety for individuals at all levels and in all sectors of the food supply chain, and

2. promote knowledge transfer within the food safety community.

The FSKN achieves these aims by harmonizing existing technical food safety training schemes through the development of the competencies of food safety professionals, recognized by international stakeholders, both from the public and the private sectors.
The FSKN is a collaborative platform that provides free access to high-quality, standardized learning resources in a highly scalable manner. To that end, all content (cognitive resources) is shared on the internet as Open Educational Resources (OER) under Creative Commons licensing via the FSKN web portals. The FSKN uses open source tools and openly-licensed materials encouraging development of derivative works that only require attribution to source and sharing under similar license as standardized FSKN content. This approach enables other users to customize, translate, and localize content for specific audiences or sectors of the food industry, and share these derivative works through either the MSU FSKN portals or their own web sites. Beyond content development, the FSKN initiative utilizes formalized training delivery mechanisms (e.g. face-to-face training, eLearning, blended learning) as well as coaching and mentoring of participants on effective strategies for implementing food safety management systems. The FSKN approach has been pilot-tested in a number of countries in collaboration with numerous partners from the food industry (individual companies and associations), development agencies, academic institutions and other service providers (Heyboer, Kim, Bourquin, & Thiagarajan, 2010). The specific approach has varied somewhat from country to country, but in general the target audience for capacity development has been small-and medium-scale suppliers (both primary producers and food processors) who are seeking to execute sales contracts with multi-national food retailers or other high-value markets within their country, or to engage in regional or distant trade of their products to more discriminating markets. Gaining access to these higher-value markets (both domestic and export) requires the suppliers to reach a much higher level of sophistication with respect to food safety and quality management systems, and ultimately the execution of sustainable contracts in these markets requires certification of the food safety management systems that are being implemented by these suppliers against recognized international standards.

Organizational level

The FSKN project engages a wide variety of organizations in accomplishing its mission. As the leader of the FSKN initiative, Michigan State University (MSU) and the faculty leading the effort are principally focused on the creation and transmission of knowledge to improve the competitiveness of primary producers (i.e. farmers) and SMEs in less-developed countries. Beyond improving food safety systems implemented by these suppliers, another long-range objective of these efforts is to improve the livelihoods of farm families and front-line workers in these less developed businesses.

Content development in the FSKN initiative is guided by international standards, with programs being delivered on international food safety guidelines adopted by the Codex Alimentarius Commission managed by the Food and Agricultural Organization of the United Nations (which are recognized as the de minimis food standards in member countries of the World Trade Organization) and other programs focused on helping suppliers meet the expectations of international private food safety standards such as those benchmarked by the Global Food Safety Initiative (GFSI). Individuals from several GFSI-member companies have participated in content development for the FSKN since its inception, and engagement with public sector food standards representatives (e.g. UN agency representatives or individual governments) has been encouraged where possible.

Content delivery in the FSKN project typically has been conducted by MSU researchers in partnership with academic institutions based in the countries where training occurs. The partner academic institutions are essential to the effective delivery of the content for local clientele because of the ability to deliver the training and mentoring in local language(s) and also because of their capabilities to localize the content with respect to local practices and cultural norms. It is preferable for MSU to work with local academic institutions in this manner as they share a similar culture of academic inquiry and knowledge dissemination. These collaborations also have a high likelihood of sustainability over the longer term.

The beneficiaries of the capacity development (e.g. farmers or processing establishments and their employees) may self-select for participation in these capacity development programs, but more commonly they are identified as potential or existing suppliers for multinational companies (either for the domestic market or export) who are in need of training and mentoring on the development and implementation of internationally-recognized food safety management systems. Many of the participating beneficiary farmers or manufacturers also are members of cooperatives or other food industry associations, which often work collectively to address key challenges such as compliance with food safety and quality standards. The multinational companies are motivated to identify suppliers for participation in these programs for a variety of reasons, but chiefly it is to help ensure the overall safety and quality of products sourced from these suppliers and, therefore, serves to protect the brand of these multinationals.

A variety of service providers also have engaged in the FSKN project since its inception. These have included third-party certification bodies who provide food safety certification, organizations offering food traceability support, equipment suppliers, sanitation services organizations, chemical suppliers, and providers of other ancillary services to farms or food processors.

Finally, several donor organizations, UN organizations and other NGOs have participated in or contributed to the FSKN initiative since its inception. Donor organizations such as the United States Agency for International Development (USAID) and the World Bank have provided financial support for FSKN development and delivery of programs. In addition, organizations such as the United Nations Industrial Development Organization (UNIDO) and the International Finance Corporation (IFC) of the World Bank Group have utilized FSKN-created materials in their own development projects that are focused on food safety capacity development in a number of countries. For the FSKN initiative, organizations such as UNIDO and IFC have been continuously engaged throughout the program. These collaborations have been critical to the successful implementation of FSKN and its dissemination to several economies outside the

US.
Clearly, the FSKN initiative has engaged with and benefitted from this large number and variety of international partners. Each has been critical to the successful implementation of FSKN and dissemination of its content to beneficiaries in several economies.

Individual level

At the individual level, there has been a tremendous amount of networking among key individuals working for FSKN partner organizations. Although some of this networking and collaboration has occurred through events organized explicitly for FSKN development and implementation, a considerable amount of networking has occurred through other fora such as the Global Food Safety Initiative of the Consumer Goods Forum, the Partnership Training Institute Network of the Asia Pacific Economic Cooperation Forum, and the World Bank-organized Global Food Safety Partnership. This networking has involved a relatively small, yet highly influential, group of individuals who collaborate on FSKN and similar globally-focused initiatives in the area of food safety standards and food safety management systems implementation. This core network interacts less directly with beneficiary groups such as farmers or SME food processors, who typically have been identified for participation in the programs by their buying companies (e.g. multinational food corporations) or donor organizations such as UNIDO or IFC. Content delivery in FSKN-related projects has been conducted by a select group of highly-qualified experts working in academia (e.g. MSU), the food industry, or as consultants. In many cases, the same experts have been enlisted to implement training and capacity development programs by multiple food industry companies, associations or donor agencies. This highlights the need for engagement of more experts in networks such as FSKN, but also speaks to a relative dearth of recognized international experts in this specific discipline.

4.2 In France: the ISO 22000 club launched by CCI Picardie

In spring 2007, the Picardie region Chamber of Commerce and Industry (CCI) decided to launch for ten regional food companies (including Paris Caramel) an informal 'ISO 22000 club', a regional program to support ISO 22000 standards. This ISO 22000 program has consisted of business leader coaching along with accompanying collective actions for all participating companies. The Paris Caramel's management decided to embark on the process of certification because of new customers' requirements and changes in the business environment. The certification was not an absolute necessity for this profitable company but appears as a possible supplementary marketing asset, in accordance with the policy of sustainable customer satisfaction, by ensuring the safety of products sold. It would also eliminate the different and heterogeneous customers' specifications and create differentiation towards competitors. Ultimately, Paris Caramel attained ISO 22000 certification in October 2008.
Founded in 1957, Paris Caramel is a food SME located in the Picardie region in Northern France and manufactures chocolate and confectionery products. The company manufactures three main types of products of the highest quality: caramel, fruit pulp and chocolate, for a turnover of 900 000 Euros per year. Their customers are pastry confectioners, delicatessens and shops selling local products. The company has forty employees, mainly makers of caramels, fruit jellies and chocolate candies. In 2000, the company decided to develop the certification of various stages of the production process, starting with Hazard Analysis and Critical Control Points (HACCP) certification. As a small family-owned company, with a mostly self-educated staff, Paris Caramel is very cost-efficient with a short decision process. Another important characteristic of the company is its human dimension: human capital is more important than financial returns, and the managers put more emphasis on training their employees and on maintaining employment than on profits.

Organizational level

Continuous ties such as spatial (the location in the Picardie region, the role of the Regional Council) and cultural ties have been acknowledged by the company as important features, as they provide trust and easy communication. The tacit knowledge dimension of the standard is also to be considered: for that type of knowledge, considered as soft information, organizational proximity is sufficient. For the responsible person in charge of ISO 22000 implementation at Paris Caramel, the institutional embeddedness of the initiative, promoted both by AFNOR (French branch of ISO) and by the Chamber of Commerce, has played a crucial function in providing seriousness and credibility. The congruence of goals between all the stakeholders of the initiative, creating a specific relationship and a sense of responsibility, provided an environment for mechanisms such as emulation and mimicry. Indeed, these effects can be considered as learning effects as well, in reinforcing/auto-promoting the exchange of skills and information.
The learning by doing effects have been identified mainly between the consultancy firm and Paris Caramel: the role at that organizational level is significant at the initiation stage (establishment of a first contact and of a formal tripartite contract between the CCI, the consultancy firm and the company), but the main interaction effects have occurred at the individual level.
Organizations involved in the process of standard adoption are: AFNOR, CCI, consultancy firms, and other SMEs.

- The CCI (Chamber of Commerce and Industry) had no role in the definition phase. Its action is crucial in the adoption phase: the organization has acted as a pivotal organization between AFNOR, consultancy firms and SMEs, through defining the program funding and the setup of the tripartite contracts.

- Consultancy firm: Protechnic, a consulting firm, had a central role in the adoption stricto sensu phase. It is difficult to separate its role as a company and as a person. Indeed, the manager of the company has been largely convinced by the consultant to adopt the standard, however, the company also has very good experience and a reputation in working with SMEs. The specific expertise is the basis of the successful interaction process.

- ISO 22000 club for SMEs: this club is the heir of another previous club devoted to HACCP. Its role has been to connect companies from different industries (thus not in competition) to exchange views and questions about the standard and its consequences. Its role is both formal (membership) and informal (interpersonal relations, cf. below).

- Third party certifier: the certification body, Bureau Veritas, has conducted the certification process and has been the main player, with Paris Caramel, during the conformity assessment phase.

Individual level

The inter-individual aspects of the network learning effects are more difficult to evaluate for confidentiality reasons. Nevertheless, there is a clear complementarity of the continuous ties between the two levels, leading to strong coupling effects. The managers from Paris Caramel (the CEO, the quality manager responsible) are part of a coherent community of leaders in the Picardie region and everybody knows each other quite well. This fact has played an important role in the decision to adopt. However, the individual level is also of tremendous importance for learning in terms of discrete ties and interactions (mainly with the consultant, but also with other food managers during the period of the CCI initiative as well as with some customers).
Informal contacts and exchanges may occur at any time and, for confidentiality and privacy reasons, interviewees are reluctant to answer. Nevertheless, interpersonal contacts seem to play an important role especially with one consultant and with all the managers from the ISO 22000

group.
According to the analytical framework, the learning effects are different from one phase to another. We will consider successively the five main phases, namely standard setting (antecedents), decision of adoption, implementation *stricto sensu*, conformity assesment (certification), and enforcement (post certification).
During the standard setting phase, only limited network learning phenomena occurred, at the individual level, in the form of previous personal experiences of the quality manager of Paris Caramel in similar fields. Indeed, no formal contacts between the company and AFNOR had existed, showing that during its definition, the ISO 22000 standard does not include all potential users such as food SMEs.
The adoption decision is reached thanks to contact between the company and CCI: the learning effect can be defined as the rise of awareness of the company leaders involved in the initiative in the development of the standard and soft information exchange for the establishment of the tripartite contract.
The implementation phase is obviously the period of time (almost 2 years) that has witnessed important learning phenomena. Of course, from a financial point of view, it is not certain that two years will be sufficient to amortize the investment, which is why support from organizations and public bodies is necessary. The most important learning phenomena has occurred at the interindividual level, in the form of a strong interaction between the quality manager and the consultant in charge of the program. The formal explicit knowledge included in the ISO standard specifications necessitates adaptations and translations in the real world of the Paris Caramel specificities. On the contrary, formal contacts between organizations are limited during this period of time. Another significant network learning effect is the permanent contact between the food managers involved in the initiative, in terms of comparisons, informal exchange and emulation.
The conformity assessment phase is more formal: this is the recognition of compliance with the specification by means of a certification audit. The process of learning is done through an exchange of explicit information (such as files and information control procedures) between the company and the certifying body.
The enforcement phase is the post certification period of continous improvement. Learning effects occur mainly in-house, with the practical involvement of employees. Nevertheless, the informal contacts established during the implementation phase with other managers remain active, in the form of informal meetings, cross auditing practices and informal exchanges.

5 Conclusion

The objective of the article is twofold: (i) to propose an original framework, using a network effect perspective, for the analysis of the implementation of internationally-recognized food safety management systems (e.g. ISO 22000, GFSI-benchmarked food safety schemes); and (ii) to apply this framework to two case studies. The major motivation is the fact that the implementation of a FSMS is a long and complex process strongly related to its organizational and individual context. Quality management standards are immaterial in nature and difficult to implement: food SMEs and their managers will inevitably rely heavily upon collective initiatives (Ropkins & Beck, 2000; Mensah & Julien, 2011). The focus of this article is mainly food SMEs and one must acknowledge that this could create a bias in the results, as network effects have more consistency and accuracy in this specific context of small firms.
From this analysis, it is possible to summarize a few key features. At the preliminary stages of the implementation process, the learning effects do not extend outside the organizations. These effects occur more clearly during the implementation phase. The dynamic approach shows an interesting phenomena: in the latter phases of the implementation process (i.e. conformity and assessment phases), new types of partners emerge and their roles are of tremendous importance for the success of ISO 22000 adoption. It suggests that the position/relationships of the individual companies vis à vis these partners during that period of time must be clearly emphasized as a key component of the success of the initiative.
For SMEs the interest of such initiatives is clearly

to overcome strong limitations, not only in resource access, but also in cognitive gaps, as we have seen the importance of specific capabilities that must be developed at collective levels.
Preliminary results, still to be confirmed and extended to other cases, could have interesting managerial implications for food SMEs. First of all, the collective (i.e. network) dimension of the process is shown. No food SMEs in these initiatives could have decided in isolation to set up FSMS. Instead, the food companies of the case studies are strongly embedded in a web of partners, defining a networking activity for innovation. Within this network, the process of learning is doubly collective: at the institutional level, where institutions, (e.g. Chamber of Commerce and Industry, academic institutions), consultancy firms and associations (e.g. AFNOR, GFSI) have worked together to promote the initiatives; at a micro-analytic level, with the SMEs building strong relationships with service providers (e.g. consultancy firms, certification bodies) and, at the same time with a broader community of food business leaders having its own dynamics, objectives and social interaction mechanisms. One must also acknowledge the fact that this process is not one-sided: consultancy firms themselves, as well as probably other partners, adapt to the situation and improve their own knowledge in such processes.
A second idea is that of resources. The critical success factor in FSMS implementation by SMEs in these case studies seems not to be financial resources, but rather access to cognitive resources, (i.e. the ability to connect and to be connected through a web of relationships to the relevant people and organizations). Learning phenomena appear to be complex, multifaceted and done through several mechanisms and mediation. Consequently, an important managerial implication of the research, to be validated by other situations and countries, would be to enhance these cognitive resources and mechanisms, to identify more precisely their nature, the partners involved and their roles for learning in relation with the requirements of the different phases of implementation.
For the Picardy Food Safety Club, the cognitive effects of this initiative have been limited: no real creation of common memory, no identification and formalization, inside the network, of codified knowledge related to ISO 22000 implementation. This knowledge has mainly remained at the consultant level, without significant socialization and embeddedness at an upper level. For this particular case it seems that the main benefits of the initiative have been in the mimicry phenomena and socialization around the interests of food safety standards such as ISO 22000 that has led to a strong involvement and motivation of the concerned companies.
In the case of the FSKN initiative in the USA and other countries, the focus on a standardized, competency-based approach to capacity development linked to a concrete framework (the GFSI Global Markets Programme) provided a consistent framework for SME development. The use of open education resources within the network encouraged partner organizations to adapt, localize and share derivative training content in multiple countries and in several languages.

Acknowledgements

This project was supported in part by Agriculture and Food Research Initiative Competitive Grant no. 2011-51110-31104 from the United States Department of Agriculture (USDA) National Institute of Food and Agriculture. The project has also received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 245301, as a part of the project "*Enhancing the innovativeness of food SME's through the management of strategic network behavior and network learning performance*" coordinated by Ghent University.

References

Abdirahman, Z.-Z., Kisempa Muyuala, G., & Sauvée, L. (2013). Mapping formal networks and identifying their role for innovation in EU food SMEs. In G. W. Schiefer & J. Deiters (Eds.), (Chap. France: Case studies 3D Network, ARI Picardie and BioBourgogne, pp. 67–88). Bonn: Centmapress.

Abdirahman, Z.-Z. & Sauvée, L. (2012). The implementation of a quality management standard in a food sme: a network learning perspective. *International Journal on Food System Dynamics*, *3*(3), 214–227. doi:10.18461/ijfsd.v3i3.333

Argyris, C. & Schön, A., Donald. (1996). *Organizational Learning II: Theory, Method, and Practice*. Reading: Addison-Wesley.

Borgatti, S. P. & Li, X. (2009). On social network analysis in a supply chain context. *Journal of Supply Chain Management*, *45*(2), 5–22. doi:10.1111/j.1745-493X.2009.03166.x

Boris, B., Sandra, C. P., & Isabelle, H. (2007). Réseaux sociaux et processus d'apprentissage, une relation complexe et ambivalente. In *Communication à la 16ème conférence de l'aims* (Vol. 6). Retrieved from https://basepub.dauphine.fr/handle/123456789/2261

Burt, R. S. (2000). The network structure of social capital. In *Research in organizational behavior, vol 22, 2000* (Vol. 22, pp. 345–423). Research in Organizational Behavior. doi:10.1016/S0191-3085(00)22009-1

Conway, S., Jones, O., & Steward, F. (2001). Social interaction and organizational change: aston perspectives on innovation networks', series on technology management. In F. Oswald Jones Steve Conway & Fred Steward (Ed.), *In social action and organisational change: aston perspectives on innovation networks, eds* (Chap. Realizing The Potential Of The Network Perspective, Vol. 6, *11*, pp. 349–366).

Conway, S. & Steward, F. (1998). Mapping innovation networks. *International Journal of Innovation Management*, *02*(02), 223–254. doi:10.1142/S1363919698000110. eprint: https://www.worldscientific.com/doi/pdf/10.1142/S1363919698000110

Conway, S. & Steward, F. (2009). *Managing and shaping innovation*. Oxford University Press.

Coulon, F. (2005). The use of social network analysis in innovation research: a literature review "lund university". Sweden. Retrieved from http://gent.uab.cat/diego_prior/sites/gent.uab.cat.diego_prior/files/Article_to_comment_10_bis.pdf

Dierkes, M., Antal, A. B., Child, J., & Nonaka, I. (Eds.). (2003). *Handbook of organizational learning and knowledge*. New York, NY, USA: Oxford University Press, Inc.

Eisenhardt, K. M. & Graebner, M. E. (2007). Theory building from cases: opportunities and challenges. *Academy of Management Journal*, *50*(1), 25–32. doi:10.5465/AMJ.2007.24160888

Geith, C., Vignare, K., Bourquin, L., & Thiagarajan, D. (2010). Designing corporate training in developing economies using open educational resources. *Online Learning*, *14*(3). doi:10.24059/olj.v14i3.142

Hatanaka, M., Bain, C., & Busch, L. (2005). Third-party certification in the global agrifood system. *Food Policy*, *30*(3), 354–369. doi:10.1016/j.foodpol.2005.05.006

Hatanaka, M., Bain, C., & Busch, L. (2006). Differentiated standardization, standardized differentiation: the complexity of the global agrifood system. In *Between the local and the global* (pp. 39–68). doi:10.1016/S1057-1922(06)12003-X

Henson, S. & Humphrey, J. (2009). The impacts of private food safety standards on the food chain and on public standard-setting processes. *Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Thirty-second Session, FAO Headquarters, Rome*, *29*. Retrieved from http://www.fao.org/tempref/codex/Meetings/CCEXEC/ccexec64/ex64_04e.pdf

Henson, S. & Humphrey, J. (2010). Understanding the complexities of private standards in global agri-food chains as they impact developing countries. *Journal of Development Studies*, *46*(9), 1628–1646. doi:10.1080/00220381003706494

Heyboer, G., Kim, S., Bourquin, L. D., & Thiagarajan, D. (2010). *Building capacity in developing countries: oer for food safety*. Universitat Oberta de Catalunya. Retrieved from https://oerknowledgecloud.org/sites/oerknowledgecloud.org/files/Heyboer.pdf

Inkpen, A. C. & Tsang, E. W. K. (2005). Social capital, networks, and knowledge transfer. *Academy of Management Review*, *30*(1), 146–165. doi:10.5465/AMR.2005.15281445

Levitt, B. & March, J. G. (1988). Organizational learning. *Annual Review of Sociology*, *14*, 319–340. doi:10.1146/annurev.so.14.080188.001535

Mensah, L. D. & Julien, D. (2011). Implementation of food safety management systems in the uk. *Food Control*, *22*(8), 1216–1225. doi:10.1016/j.foodcont.2011.01.021

Nahapiet, J. & Ghoshal, S. (1998). Social capital, intellectual capital, and the organizational advantage. *Academy of Management Review*, *23*(2), 242–266. doi:10.2307/259373

Owen-Smith, J. & Powell, W. W. (2004). Knowledge networks as channels and conduits: the effects of spillovers in the boston biotechnology community. *Organization Science*, *15*(1), 5–21. doi:10.1287/orsc.1030.0054

Pawlowsky, P. (2001). The treatment of organizational learning in management science. *Handbook of organizational learning and knowledge*, 61–88.

Pitsis, T. (2013). *Handbook of organizational and managerial innovation*. Elgar original reference. Edward Elgar Publishing, Incorporated. Retrieved from https://books.google.pt/books?id=TjTiTqP9zPsC

Pittaway, L., Robertson, M., Munir, K., Denyer, D., & Neely, A. (2004). Networking and innovation: a systematic review of the evidence. *International Journal of Management Reviews*, *5-6*(3-4), 137–168. doi:10.1111/j.1460-8545.2004.00101.x

Podolny, J. M. & Page, K. L. (1998). Network forms of organization. *Annual Review of Sociology*, *24*, 57–76. doi:10.1146/annurev.soc.24.1.57

Powell, W. W., White, D. R., Koput, K. W., & Owen-Smith, J. (2005). Network dynamics and field evolution: the growth of interorganizational collaboration in the life sciences. *American Journal of Sociology*, *110*(4), 1132–1205. doi:10.1086/421508

Powell, W. W., Koput, K. W., & Smith-Doerr, L. (1996). Interorganizational collaboration and the locus of innovation: networks of learning in biotechnology. *Administrative science quarterly*, 116–145.

Ropkins, K. & Beck, A. J. (2000). Evaluation of worldwide approaches to the use of haccp to control food safety. *Trends in Food Science & Technology*, *11*(1), 10–21. doi:10.1016/S0924-2244(00)00036-4

Scott, V. N. & Chen, Y. (2010). Pathogens and toxins in foods: challenges and interventions. In V. K. Juneja & J. Sofos (Eds.), (Chap. Food Safety Management Systems, pp. 477–492). Washington: ASM Press.

Trienekens, J. & Zuurbier, P. (2008). Quality and safety standards in the food industry, developments and challenges. *International Journal of Production Economics*, *113*(1), 107–122. doi:10.1016/j.ijpe.2007.02.050

Yin, R. K. (2013). Validity and generalization in future case study evaluations. *Evaluation*, *19*(3), 321–332.

Zheng, W. (2010). A social capital perspective of innovation from individuals to nations: where is empirical literature directing us? *International Journal of Management Reviews*, *12*(2), 151–183. doi:10.1111/j.1468-2370.2008.00247.x

International Journal of Food Studies | IJFS October 2018 | Volume 7 | pages 30–37

Antioxidant and Antibacterial Activities of Exopolysaccharides Produced by *Lactic Acid Bacteria* Isolated from Yogurt

Zouaoui Benattouche[a*], Djillali Bouhadi[b], and Ghalem Bachir Raho[c]

[a] Laboratory of Bioconversion, Microbiology Engineering and Alimentary Security, Department of Biology Mascara University - 22900 Algeria
[b] Laboratory of Bioconversion, Microbiology Engineering and Alimentary Security, Department of Biology Mascara University- 22900 Algeria
[c] Laboratoire de Microbiologie Moléculaire Proteomics et Santé, Department of Biology Sidi Belabbes University- 22300 Algeria
[*]Corresponding author
benattouche_22@yahoo.fr
Tel: +213557172691
Fax: +21349646312

Received: 19 July 2017; Published online: 18 October 2018

Abstract

The objectives of this study were to optimize the conditions for cell growth and exopolysaccharides (EPS) production by using pure and mixed microbial cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, and to evaluate the antioxidant and antibacterial activities of EPS *in vitro*. The mixed cultures of two strains showed a higher cell growth whereas the higher EPS production was detected in pure culture with *S. thermophilus*. The optimal medium were determined as follows (g/l): sucrose 50, yeast extract 10, KH_2PO_4 3 , $MgSO_4.7H_2O$ 0.05 and pH initial 6.5 at 30 °C. Under the optimized conditions, the values of dry cell weight (DCW) and EPS were 5.2 ± 0.8 g/l and 56.8 ± 0.62 mg/ml, respectively. The EPS demonstrated a positive antioxidant potential on DDPH radical scavenging. The agar diffusion assay showed that purified EPS exhibited antibacterial activities against tested pathogens such as *Escherichia coli* ATCC 250922 and *Staphyloccocus aureus* ATCC 250923 at (62-1000) μg/mL. In conclusion, EPS have an antioxidant activity and could have applications in the food industry.

Keywords: Xopolysaccharide; antioxidant and antibacterial activities; *Streptococcus thermophilus*; *Lactobacillus bulgaricus*

1 Introduction

Recently, lactic acid bacteria (LAB) have received attention for their exopolysaccharides (EPS) producing ability. A broad range of EPS from LAB with variable functionality can have a wide range of industrial application (Cerning & Marshall, 1999) as gelling agents, biosurfactants, emulsifiers, viscosifiers (Poli, Anzelmo, & Nicolaus, 2010) and biosorbents (de Oliveira Martins, de Almeida, & Ferreira Leite, 2008) In addition, EPS play important roles in human health owing to their antitumor, antioxidant, anti-ulcer and antibacterial activities (Kocharin, Rachathewee, Sanglier, & Prathumpai, 2010; Liu, Chu, Chou, & Yu, 2011) However, owing to the lack of expansive knowledge on EPS from food-associated LAB, EPS have remained largely underexploited. Fermentation is a very versatile process technology for producing value added products such as microbial biopolymers and since fermentation

 10.7455/ijfs/7.2.2018.a3

parameters have a high impact upon the viability and economics of the bioprocess, their optimization holds great importance for process development. Especially, microbial polysaccharide production is greatly influenced by fermentation conditions such as pH, temperature, oxygen concentration and agitation as well as by the composition of the culture medium (Bryan, Linhardt, & Daniels, 1986).

Yogurt is a fermented dairy product resulting from the symbiotic growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* during milk fermentation. A classical yogurt starter culture produces a smooth viscous gel, with a desirable fermented product aroma and flavor polysaccharides derived from *S. thermophilus* and *L. bulgaricus* show large variation in composition, charge, spatial arrangement, rigidity and ability to interact with proteins. Furthermore no defining correlation between EPS concentration and viscosity has been established in studies to date (Welman & Maddox, 2003). With the aim of increasing knowledge of the functional EPS from LAB in yogurt, we have isolated LAB from yogurt. Among the LAB, a crude EPS of *S. thermophilus* was obtained, optimized and examined for its antioxidant and antibacterial activities to find a functionally active EPS.

2 Materials and Methods

2.1 Microorganisms

Standard strains of *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used for determination of the antibacterial activity. Lactic acid bacteria were isolated from yogurt samples obtained within the Mascara regions of Algeria in 2017.

2.2 Isolation of Lactic Acid Bacterial Strains with High EPS Production

Three EPS producing strains were isolated by selective plating (from yogurt) on MRS Agar and anaerobic incubation at 37 °C for 48h. EPS producing colonies were evaluated by touching colonies with a sterile inoculating loop. The presence of ropy strands between the loop and the colony as the loop was slowly raised was considered EPS positive. The colonies were picked up and propagated in MRS broth for 24 h. The EPS producing isolates were verified for gram positive and catalase negative rods and cocci. The purity of cultures were verified by spreading cultures on MRS agar, and repeating several times until a pure culture was obtained.

The two EPS producing isolates were revived by transferring 0.5 ml of each frozen stock to 10 ml of MRS broth and incubating at 37 °C for 48h. The revived cultures were reactivated again by transferring 1.0 ml to 10 ml MRS broth and incubating at 37 °C for 48 h. The reactivated cultures were used as standard inoculums culture. In this experiment, the highest EPS producing isolates (*S. thermophi-lus* and *L. bulgaricus*), were used for EPS production in MRS broth. MRS was used as the EPS production media.

2.3 Growth and EPS Production

Lactic acid bacteria candidates (*S. thermophilus* and *L. bulgaricus*) were isolated from yogurt and used after selection and identification according to Bergey's Manual of determinative bacteriology, 9th edition (Holt, Krieg, Sneath, Staley, & Williams, 1994) and confirmation of identification by the anlaytical profile index (API) system. Pure and mixed cultures of the strains were tested for cell growth and production of exopolysaccharides. The pure and mixed microbial cultures were inoculated with a ratio of 1% (v/v) into 100 ml MRS broth in 500 ml conical flasks in triplicate. The pH was adjusted to 6.5. The flasks were incubated on a rotary shaker at room temperature for 72 hrs. The exopolysaccharides were precipitated by adding 3 volumes of cold absolute ethanol and stored overnight at 4°C. Finally, the recovered precipitates were redissolved with distilled water and dialyzed against the same solution for 24h at 4 °C (Garcia-Garibay & Marshall, 1991). The total amount of carbohydrates in the polysaccharides was determined by the phenoland sulfuric acid method described by (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The exopolysaccharides production was

expressed as mg/ ml and biomass dry weight (g/l) was monitored by measuring the D600nm of the culture in the final fermented samples.

2.4 Optimization of exopolysaccharide production

To study the effect of different parameters, 1% inoculum containing 3.10^8 UFC/ml were inoculated in 100 ml of production medium. EPS production was optimized under different environmental and nutritional conditions viz incubation period (1 to 3 days), carbon sources (glucose, sucrose), nitrogen sources (peptone, yeast extract) and different sucrose concentrations (1, 2, 3, 4, 5 and 6 %)

2.5 Measurement of growth

The optical density at 620 mm was used to monitor cell growth after appropriate dilution of samples (Adebayo-Tayo & A. Onilude, 2008). Growth was measured as the dry weight per volume by centrifugation (5000 g for 10 min) and then dried to a constant weight in an oven at 60 °C overnight to obtain cell dry weight (CDW).

2.6 Determination of total carbohydrate content

Total carbohydrate content was determined by the method of (Dubois et al., 1956). To the dried pellet, 1 mL of 5% phenol and 5 mL of 96% concentrated sulfuric acid was added and the mixture was kept in a boiling water bath for 20 min. The optical density of the sample was read spectrophotometrically at 490 nm and total carbohydrate content was calculated, using glucose as the standard.

2.7 Antibacterial activity of exopolysaccharides

The disk diffusion method was used to determine antibacterial activity by quantifying the clear zone of inhibition around the filter paper disk. In this method, the autoclaved filter paper discs were impregnated with 50 μl of the EPS and positioned on a nutrient agar plate, seeded with the test organisms, namely *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Penicillin, Gentamicin and Colistin were used as a positive control, while distilled water was used as a negative control. After firm placement of the discs, the plates were incubated at 37 °C in an inverted position for 1-2 days to allow different species of bacteria to grow. The zones of inhibition in discs were measured by a millimeter scale (Bauer, Kirby, Sherris, & Turck, 1966).

2.8 Free Radical Scavenging Activity

The antioxidant activity was determined by the DPPH scavenging assay (Khalaf, Shakya, Al-Othman, El-Agbar, & Farah, 2008). Various concentrations (62.5, 125, 250 ,500 and 1000 µg/mL) of EPS were prepared in separate tubes. Ascorbic acid was used as a reference compound (0.3125, 0.625, 1.25, 2.5 and 5.0 mg/mL). A freshly prepared solution of 0.002 % DPPH (2, 2, Diphenyl-2-Picryl hydrazyl) in methanol was added to each tube containing different concentrations of extracts (2.0 mL). The samples were incubated in the dark at 37 °C for 20 min and read at 515 nm. The data were expressed as the percent decrease in the absorbance compared to the control. The percentage inhibition of radical scavenging activity was calculated.

2.9 Statistical analysis

The experiments were carried out in triplicate and results are given as the mean ± standard deviation. The data in all the experiments were analysed (Microsoft Excel 2007) for statistical significance using the Student's Test, and differences were considered significant at $p < 0.05$.

3 Results and Discussion

3.1 Isolation of Lactic Acid Bacterial Strains with High EPS Production

In this study, fifty strains of LAB were isolated from yogurt for determination of EPS production. The primary characterization of bacterial isolates indicated that they were gram positive, rods and cocci, catalase negative and anaerobic. All isolates were screened for EPS production in MRS broth, under anaerobic conditions at 37 ˚C for 48 h as the EPS production period. According to their efficiency for EPS produc-tion, all but two isolates were in two categories namely high and weak, with respec-tive EPS concentrations from 1 to 10 g/l and 0.1 to 1 g/l. 30 and 18 of these isolates were in the respective weak and high categories. Two out of the bacterial isolates from two different yogurts EPS (from 10 to over 25 g/l). These two isolates were subjected to complete identification using the API 20 System. They were identified as lactic acid bacterial strains *L. bulgaricus* and *S. thermophilus*.

3.2 Growth and EPS Production Kinetics

Growth Intensity

Dry cell weight (DCW) of the pure bacterial isolates of L.. Bulgaricus, *S. thermophilus* and mixed cultures in MRS broth for 72 h are presented in Figure. 1. During the first 20 h of the incubation period growth intensity in MRS medium increased for all three tested cultures. It was observed that the highest intensity (DCW = 10.8 ± 0.3 g/l) was recorded after 48 h by mixed cultures of *L. bulgaricus* and *S. thermophilus* followed by pure cultures of *L. bulgaricus* (DCW = 5.4 ± 0.42 g/l) and pure cultures of *S. thermophilus* (DCW = 4.6 ± 0.8 g/l). After 48 to 72 h of incubation period the growth intensity in MRS medium decreased for all tested cultures. In addition, during 20 - 48 h of the incubation period there was a symmetric growth intensity for the strains grown in MRS broth.

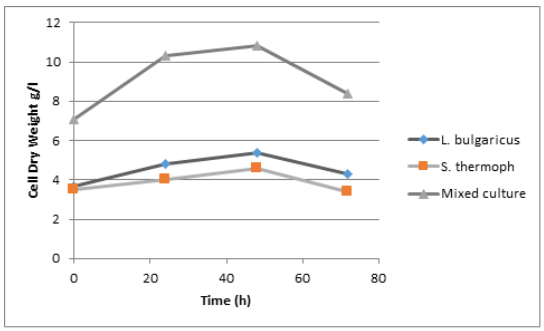

Figure 1: Cell dry weight of isolates of three cultures

EPS production

EPS production from the pure bacterial isolates of *L. bulgaricus*, *S. thermophilus* and mixed cultures grown in MRS broth at 37 ˚C for 72 h are presented in Figure 2 .The highest EPS production and EPS yield % were achieved at 48 hours of growth. The pure cultures of *S. thermophilus* gave the highest EPS concentrations (8.54 ± 0.22 g/l), followed by mixed cultures, and then the pure cultures of *L. bulgaricus*. The pure cultures of *S. thermophilus* were isolated, maitained on nutrient agar, and used throughout the study.

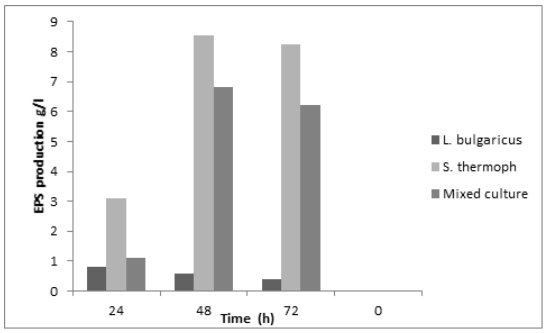

Figure 2: Exopolysaccharide content obtained from isolates of three cultures

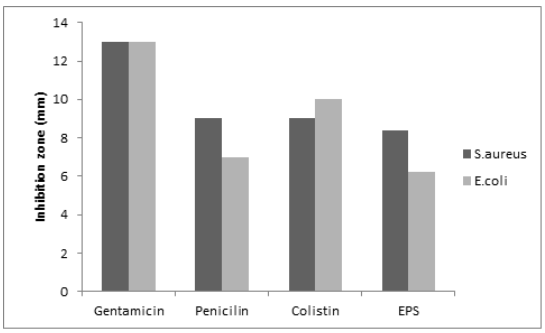

Figure 3: Agar-well diffusion test results of EPS and antibiotics

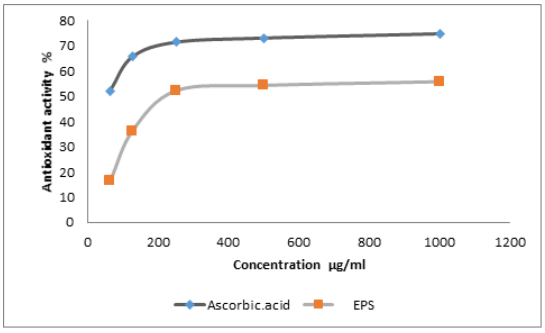

Figure 4: Antioxidant activity of different concentrations of EPS and ascorbic acid in DPPH radical scavenging method

3.3 Optimization of exopolysaccharides

The exopolysaccharide production of *S. thermophilis* was optimized under different environmental and nutritional conditions (Table 1). EPS production was determined during different periods of incubation (1, 2, and 3 days), and was highest after 2 days (8.54 ± 0.22 g EPS/L). Carbohydrates are the most abundant biomolecule and an important nutrient for cell growth and development (Zhang, Zhang, Li, Zhang, & Yang, 2011). The effects of different carbon sources on the production of EPS were studied. In this study, it was found that sucrose was a suitable carbon source (Table 1). However, Antonio Mata et al. (2006) reported that glucose was a suitable carbon source for EPS production. In this study, a 5% sucrose concentration in the medium gave higher EPS production (56.8 ± 0.62 g/l). Added peptone and yeast extract, as nitrogen sources, had negligible effect on EPS production (56.8 ± 0.62 g/l).

3.4 Antibacterial Activity

Figure 3 shows the antimicrobial activity of purified EPS as evaluated by the disc diffusion method. Purified EPS inhibited the growth of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. Antimicrobial activity was maximum when 1000 μg /ml of EPS were used and occurred after 72 h of incubation. The highest and lowest antimicrobial activities of purified *EPS* were 8.6 - 8.4 mm against *S. aureus* ATCC 25923, and 5.0 - 6.2 mm against *E. coli* ATCC 25922 respectively. Purified polysaccharides had a lower inhibitory effect on growth in comparison with antibiotics frequently used for the treatment of infectious bacteria, such as Gentamicin, Penicillin and Colistin.

The shown sensitivity of gram-positive bacteria to polysaccharides extracts agrees with previous studies (Venturini, Rivera, Gonzalez, & Blanco, 2008). This is due to the membrane composition of the bacterial stains (Holst & Müller-Loennies, 2007). It has been reported that EPS from microorganisms have a strong antimicrobial activity against several pathogens *in vitro*, and several possible antibacterial mechanisms of EPS have been proposed, such as impairment of cell division, disruption of the cell wall and cytoplasmic membrane, and decomposition of DNA (He, Yang, Yang, & Yu, 2010; Wu et al., 2010). According to these results, the antimi-crobial activity increased with increasing concentration of EPS.

3.5 Antioxidant activity of EPS

Antioxidant activities have been attributed to various reactions and mechanisms. In this study, the *in vitro* antioxidant activities of EPS from *S. thermophi-lus* were evaluated using the DPPH radical scavenging assay, and compared with ascorbic acid as a control. The antioxidant activ-

Table 1: Optimization of exopolysaccharide production by *S. thermophilus*

Parameter	Values	EPS g/l	Biomass gr/l
Incubation period (days)	1	3.10 ± 0.3	4.01 ± 0.64
	2	8.54 ± 0.22	4.6 ± 0.8
	3	8.26 ± 0.12	3.4 ± 0.21
Carbon source %			
Glucose	0.5	8.54 ± 0.22	4.6 ± 0.8
Sucrose	0.5	16.02 ± 0.12	4.2.± 0.24
Sucrose concentration %	1	17.4 ± 0.32	4.7 ± 0.62
	2	26.6 ± 0.24	4.8 ± 0.31
	3	33.4 ± 0.46	4.8 ± 0.6
	4	37.2 ± 0.17	4.9 ± 0.2
	5	56.8 ± 0.62	5.2 ± 0.8
	6	51.2 ± 0.6	5.2 ± 0.22
Nitrogen source %			
peptone	0.5	56.8 ± 0.62	5.2 ± 0.5
yeast extract	0.5	56.8 ± 0.62	5.6 ± 0.8

Each value is expressed as mean ±SD

ities of purified EPS at different con-centrations (62.5, 125, 250, 500 and 1000 μg /ml) are shown in Figure. 4. The highest antioxidant activity of 55.83 % was found for purified EPS at a concentration of 1000 μg /ml, followed by those at 500 μg /ml (54.34 %), 250 μg /ml (52.24 %), 125 μg /ml (36.12 %), and 62.5 μg /ml (16.34%). In the *in vitro* antioxidant assay, the purified EPS had a DPPH radical-scavenging activity, with an IC50 value of 225 μg/ml.which was much higher than that of the standard antioxidant ascorbic acid (48 μg /mL).

The bioactivities of polysaccharides can be affected by many factors including chemical components, molecular weight, structure, conformation, and even the extraction and purification methods. The molecular weight of polysaccharides could play an important role in the antioxidant activity (Chen, Zhang, Qu, & Xie, 2008).

4 Conclusions

In conclusion, the results of this study indicated that the extracellular polysaccharides (EPS) from lactic acid bacteria in yogurt have a significant antioxidant activity in the DPPH system. EPS could be used as antioxidative agents in the food industry.

Acknowledgements

The authors thank the Management of Mascara University for providing laboratory facilities and constant encouragement for this research work.

References

Adebayo-Tayo, B. & A. Onilude, A. (2008). Screening of lactic acid bacteria strains isolated from some nigerian fermented foods for eps production. *4*.

Antonio Mata, J., Bejar, V., Llamas, I., Arias, S., Bressollier, P., Tallon, R., ... Quesada, E. (2006). Exopolysaccharides produced by the recently described halophilic bacteria halomonas ventosae and halomonas anticariensis. *Research in Microbiology*, *157*(9), 827–835. doi:10.1016/j.resmic.2006.06.004

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, *45*(4), 493–496.

Bryan, B. A., Linhardt, R. J., & Daniels, L. (1986). Variation in composition and yield of exopolysaccharides produced by klebsiella sp strain-k32 and acinetobacter-calcoaceticus-bd4. *Applied and Environmental Microbiology*, *51*(6), 1304–1308.

Cerning, J. & Marshall, V. M. E. (1999). Exopolysaccharides produced by the dairy lactic acid bacteria. *Recent Results and Developments*, *3*, 195–209.

Chen, H., Zhang, M., Qu, Z., & Xie, B. (2008). Antioxidant activities of different fractions of polysaccharide conjugates from green tea (camellia sinensis). *Food Chemistry*, *106*(2), 559–563. doi:10.1016/j.foodchem.2007.06.040

de Oliveira Martins, P. S., de Almeida, N. F., & Ferreira Leite, S. G. (2008). Application of a bacterial extracellular polymeric substance in heavy metal adsorption in a co-contaminated aqueous system. *Brazilian Journal of Microbiology*, *39*(4), 780–786.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350–356. doi:10.1021/ac60111a017

Garcia-Garibay, M. & Marshall, V. M. E. (1991). Polymer production by lactobacillus delbrueckii ssp. bulgaricus. *Journal of Applied Microbiology*, *70*(4), 325–328.

He, F., Yang, Y., Yang, G., & Yu, L. (2010). Studies on antibacterial activity and antibacterial mechanism of a novel polysaccharide from streptomyces virginia h03. *Food Control*, *21*(9), 1257–1262. doi:10.1016/j.foodcont.2010.02.013

Holst, O. & Müller-Loennies, S. (2007). Microbial polysaccharide structures. In J. P. Kamerling (Ed.), (Chap. Comprehensive glycoscience. From chemistry to systems biology, Vol. 1.04, pp. 123–133). Elsevier.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994). *Bergey's manual of determinative bacteriology, 9th Ed. Baltimore* (Williams & Wilkins, Eds.).

Khalaf, N. A., Shakya, A. K., Al-Othman, A., El-Agbar, Z., & Farah, H. (2008). Antioxidant activity of some common plants. *Turkish Journal of Biology*, *32*(1), 51–55.

Kocharin, K., Rachathewee, P., Sanglier, J.-J., & Prathumpai, W. (2010). Exobiopolymer production of ophiocordyceps dipterigenabcc 2073: optimization, production in bioreactor and characterization. *BMC Biotechnology*, *10*(1), 51. doi:10.1186/1472-6750-10-51

Liu, C.-T., Chu, F.-J., Chou, C.-C., & Yu, R.-C. (2011). Antiproliferative and anticytotoxic effects of cell fractions and exopolysaccharides from lactobacillus casei 01. *Mutation Research-genetic Toxicology and Environmental Mutagenesis*, *721*(2), 157–162. doi:10.1016/j.mrgentox.2011.01.005

Poli, A., Anzelmo, G., & Nicolaus, B. (2010). Bacterial exopolysaccharides from extreme marine habitats: production, characterization and biological activities. *Marine Drugs*, *8*(6), 1779–1802. doi:10.3390/md8061779

Venturini, M. E., Rivera, C. S., Gonzalez, C., & Blanco, D. (2008). Antimicrobial activity of extracts of edible wild and cultivated mushrooms against foodborne bacterial strains. *Journal of Food Protection*, *71*(8), 1701–1706. doi:10.4315/0362-028X-71.8.1701

Welman, A. & Maddox, I. (2003). Exopolysaccharides from lactic acid bacteria: perspectives and challenges. *Trends in Biotechnology*, *21*(6), 269–274. doi:10.1016/S0167-7799(03)00107-0

Wu, M.-H., Pan, T.-M., Wu, Y.-J., Chang, S.-J., Chang, M.-S., & Hu, C.-Y. (2010). Exopolysaccharide activities from probiotic bifidobacterium: immunomodulatory effects (on j774a.1 macrophages) and antimicrobial properties. *International Jour-*

nal of Food Microbiology, *144*(1), 104–110. doi:10.1016/j.ijfoodmicro.2010.09.003

Zhang, T., Zhang, C., Li, S., Zhang, Y., & Yang, Z. (2011). Growth and exopolysaccharide production by streptococcus thermophilus st1 in skim milk. *Brazilian Journal of Microbiology*, *42*(4), 1470–1478. doi:10.1590/S1517-83822011000400033

International Journal of Food Studies | IJFS October 2018 | Volume 7 | pages 38–50

Consumers' Willingness to Consume Cassava Leaves as a Leafy Vegetable in the Kumasi Metropolis, Ghana

Fred Nimoh[a*], Godfred O. Asare[a], Ismael Twumasi[a], and Richmond Anaman[a]

[a] Department of Agricultural Economics, Agribusiness and Extension, KNUST, Kumasi, Ghana
[*]Corresponding author
frediemoh@yahoo.com

Received: 27 July 2017; Published online: 18 October 2018

Abstract

This study employs the logit model to assess the determinants of consumers' willingness to consume cassava leaves as a leafy vegetable in the Kumasi Metropolis of Ghana. A multistage sampling technique was used to select 180 respondents for the study. The study found that majority (76%) of the respondents had no knowledge of the nutritional value of cassava leaves, though they had consumed the product before. The empirical results showed that socioeconomic characteristics of respondents such as age, sex, household size and monthly income, as well as their perceptions on the attributes and use of cassava leaves as food have significant influence on willingness to consume cassava leaves as a leafy vegetable. There is the need to provide information on the nutritional benefits of cassava leaves to facilitate decision-making on its utilisation/consumption. Programmes aimed at promoting the consumption of cassava leaves should consider the significant variables that have influence on the consumption of the product.

Keywords: Cassava leaves; Willingness to consume; Perception; Logit regression; Kumasi-Ghana

1 Introduction

Cassava is one of the most important staple food crops widely cultivated in the lowland humid tropics. It plays a major role in alleviating African food crises because of its efficient production for energy year round, availability, tolerance to extreme conditions and suitability to the farming and food systems in Africa (Scott, Rosegrant, & Ringler, 2000). The world's total cassava utilization has been projected to 275 million tons by 2020 (Westby, 2002).

Cassava is grown nearly in every African country located between latitude 30°C north and south of the equator (Okigbo, 1980). The crop is Africa's second most important food staple in terms of per capita calories consumed (FAO, 2005). Africa contributes about half of the world's production of cassava; Nigeria leads with 19% of global market share and Ghana is the third largest producer of cassava in Africa after Nigeria and the Democratic Republic of Congo (FAO, 2009). Cassava production in Ghana has grown steadily from 8,107,000Mt in 2000 to 13,504,000Mt in 2010, and it is estimated to exceed 15,000,000Mt in 2015.

Since its introduction to Africa, cassava has become one of the most important crops in Africa. It is an important source of dietary energy for over 600 million people in developing countries within the tropics and sub-tropics (Scott et al., 2000). It is currently grown as a subsistence crop, cash crop, for animal feed and as an industrial raw material for starch extraction or alcohol production. The young shoots (stems, leaves and petioles) of cassava are edible and widely used as food in Africa (Lancaster & Brooks, 1983). The tuber has a number of uses in Ghana such as pro-

 10.7455/ijfs/7.2.2018.a4

cessing into *gari*, dough, tapioca, cassava flour and starch. In addition, the fresh tuber may be boiled and eaten as "*ampesi*" or pounded into a paste (*fufu*) and eaten with soup. Cassava leaves are also consumed to varying degrees in the cassava growing regions of Africa and constitute a major component of the diet in some countries (Bokanga, 1994).

Cassava leaves, as indigenous leaves, are classified as part of leafy vegetables. Cassava leaves are good source of proteins, vitamins and minerals (Gomez & Noma, 1986). Cassava leaves are either served as part of a sauce or as cooked green vegetables, but their role in the diet is very different from that of the roots (Bokanga, 1994). The cassava leaf meal has been included in schools in basic food packs distributed to families among low income population (Motta, Fukuda, & Costa, 1994). Moreover, cassava leaves have been found to have high nutrient value which can effectively boost the nutrition for animal production when preserved as hay, thereby assisting in formulating and processing of simple adoptable and low cost feed resource strategy during dry season when there is scarcity of forage (Wanapat, Puramongkon, & Siphuak, 2000). However the utilisation of cassava leaves for human consumption is fairly low to other vegetables (Keller, 2004).

Ghana has the potential to develop a more attractive and independent economy by taking interests in the kind of crops cultivated and consumed, especially those vegetables produced for local consumption and export for foreign income. Vegetables are important sources of vitamins and minerals for human diet. Approximately 1.7 million (2.8%) of deaths worldwide are attributable to low fruit and vegetable consumption (World Health Organization, 2003). FAO/WHO report on diet nutrition and prevention of diseases recommends a minimum daily intake of 200g of vegetables or about 73kg/year/person. Unfortunately, priority has been based mostly on few types of vegetables such as spinach, amaranths, okra, nightshade eggplant and cowpea leaves (Weinberger, 2004), which are sometimes scarce and relatively expensive compared to cassava leaves. Many consumers underestimate the benefits of cassava leaves; they consider them as waste or animal feed. Also, the forgone benefits to farmers as income in periods of scarcity of conventional leafy vegetables such as "*Kontomire*" have contributed to their economic implications. The negative perception about cassava leaves can affect the consumption of the leaves. A positive perception of any commodity implies an encouraging approach to consumption of such commodity (Padberg, Riston, & Albisu, 1997). Furthermore, the constraints associated with the consumption of any product determine the rate of intake of such commodity. Assessing consumers' perception and their willingness to consume cassava leaves as a leafy vegetable will help provide information and opportunity for food processors, farmers and other stakeholders along the commodity chain to boost the food industry and the economy at large.

2 Materials and Methods

2.1 Conceptual framework and empirical model specification of the study

The willingness of an individual to consume or not to consume a product can be explained as a distinct set of variables, regarding the choice of model. For this study, the dichotomous dependent variable, willingness to consume or not to consume, was used. According to Greene (2008), linear methods are inappropriate for dichotomous choices since they can lead to heteroscedastic variances. This problem is typically remedied by using maximum likelihood estimation. When heteroscedasticity is observed in likelihood estimation, such models require more general estimation (Wooldridge, 2002). However, such models are not often used, since logit and probit models with flexible functional forms in the independent variables tend to work well.

It is generally assumed that consumers maximize their utility subject to a budget constraint, and will therefore choose the option among a bundle of goods that gives them the highest utility. In considering the consumption of cassava leaves, consumers therefore expect their utility for consumption of cassava leaves as a leafy vegetable (assuming a monotonic relationship

between utility and benefits) to be higher than the other alternative leafy vegetables. According to Greene (2003), random utility models address these types of individual choice situations. A common specification is the linear random utility model.

Suppose an individual consumer's utility after consuming the new leafy vegetable for a given vector of socioeconomic characteristics, perception and product attributes (Z) is denoted by U_{re}(Z) and the utility without willingness to consume by UN_{re}(Z). The willingness to consume cassava leaves as a leafy vegetable or not can be defined as a linear relationship.

$$U_{WTC} = Z\beta_{WTC} + \varepsilon_{WTC} \quad (1)$$
$$Y_{NWTC} = Z\beta_{NWTC} + \varepsilon_{NWTC} \quad (2)$$

In this case β_{WTC}, β_{NWTC} and ε_{WTC}, ε_{NWTC} are response coefficient and random consumption associated with willingness to consume and non-willingness to consume respectively. Assuming that the qualitative variable Y_{NWTC} indexes the consumption, then it will take a value of one if the consumer is willing to consume cassava leaves as leafy vegetable and zero if otherwise. The probability that a consumer is willing to consume cassava leaves as a leafy vegetable could be expressed as a function of Z as follows:

$$p(y = 1) = p(U_{WTC} > U_{NWTC}) \quad (3)$$
$$p(Z\beta_{WTC} + \varepsilon_{WTC} > Z\beta_{NWTC} + \varepsilon_{NWTC}) \quad (4)$$
$$p[Z(\beta_{WTC} - \beta_{NWTC}) > \varepsilon_{NWTC} - \varepsilon_{WTC}] \quad (5)$$
$$p(Z\beta > \varepsilon) = F(Z\beta) \quad (6)$$

Where P is a probability function, $\varepsilon = \varepsilon_{NWTC}$ - ε_{WTC} is a random consumption term, $\beta = \beta_{WTC}$ - β_{NWTC}, a vector of unknown parameters which can be interpreted as net influence of the vector of independent variables on willingness to consume cassava leaves as leafy vegetable, and F(Z β) is the cumulative distribution function for ε evaluated at Z β. The exact distribution of F depends on the distribution of random term ε. The model arises from assuming a normal distribution, and a logit model arises from assuming a logistic distribution. Under the standard assumptions about the error term, there is no *a-priori* reason to prefer probit to logit estimation (Greene, 2003). Accordingly, in most applications, it seems not to make much difference. Considering all these aspects, a logit model was developed to study the factors affecting willingness to consume cassava leaves as a leafy vegetable in the Kumasi Metropolis.

According to the logit model, the probability of an individual's willingness to consume cassava leaves as a leafy vegetable *-(WTC)* given socioeconomic characteristics, perception variables and product attributes *(Z)* is, *P(WTC(Z))*and can be specified as:

$$P[WTC(Z)] = \frac{e^{Z\beta+\varepsilon}}{1 + e^{Z\beta+\varepsilon}} \quad (7)$$

Where a < Z β < a

The probability of not willing to consume cassava leaves as leafy vegetable is therefore:

$$P[NWTC] = 1 - P[WTC(Z)] \quad (8)$$
$$= 1 - \frac{e^{Z\beta+\varepsilon}}{1 + e^{Z\beta+\varepsilon}} \quad (9)$$
$$= \frac{1}{1 + e^{Z\beta+\varepsilon}} \quad (10)$$

The relative odds of willing to consume versus not willing to consume are given by:

$$\frac{P(WTC(Z))}{P(NWTC(Z))} = \frac{e^{Z\beta+\varepsilon}1 + e^{Z\beta+\varepsilon}}{1 + e^{Z\beta+\varepsilon}} \quad (11)$$
$$= e^{Z\beta+\varepsilon} \quad (12)$$

By taking the logarithms of both sides,

$$In[\frac{P(WTC/Z)}{P(NWTC/Z)}] = Z\beta + \varepsilon \quad (13)$$

The maximum likelihood approach can be used to estimate the above equation. The factors influencing the willingness to consume cassava leaves as a leafy vegetable in the Kumasi metropolis can be specified empirically as indi-

cated in (12) as;

$$WTC_{ij} = \beta_o + \beta_1 Sex_{ij} + \beta_3 HD_{size_{ij}} + \beta_4 No.\_yrs\_in\_sch_{ij} + \beta_5 M\_inc_{ij} + \beta 6 Sweet_{ij} + \beta_7 Bitter_{ij} + \beta_8 P\_essentialmin_{ij} + \beta_9 Animal\_feed_{ij} + \beta_1 0 eaten\_as\_food_{ij} + \beta_1 1 good\_substitute + \beta_1 2 poisonous\_comp_{ij} + \beta_1 3 Affect\_HHealth_{ij} + \beta_1 4 Used\_in\_household_{ij} + \beta_1 5 Affects\_tubber\_form_{ij} + \beta_1 6 Sold\_on\_market_{ij} + \beta_1 7 Saves\_cost_{ij} + \beta_1 8 For\_poor\_people_{ij} + \beta_1 9 Aroma_{ij} \beta_{ij} + beta_2 0 Texture_{ij} + \beta_2 1 Gen\_appearance_{ij} \quad (14)$$

Where WTC denotes willingness to consume cassava leaves as a leafy vegetable (WTC=1, if consumer is willing to consume cassava leaves as a leafy vegetable, $NWTC$=0, if otherwise). Sex, denotes gender of the consumer (1=*male* and 0=*female*). *Age*, represents the age of the consumer (years). Hd_size, denotes household size (number of people in the household). $No.\_yrs\_in\_sch$, denotes the number of years spent in school. M_inc denotes consumer's monthly income (GHC). *Sweet* represents if cassava leaves are sweet to taste. *Bitter* represents if cassava leaves are bitter to taste. *P_essential min.* denotes if cassava leaves provide essential nutrients when eaten as leafy vegetable. *Animal feed* represents if cassava leaves should be used as animal feed. *Eaten as food* represents if cassava leaves should be eaten as human food (leafy vegetable). *Good substitute* represents if cassava leaves are good substitute to other leafy vegetables. *Poisonous comp.* denotes if cassava leaves contain poisonous components like cyanide. *Affect HHealth* denotes if cassava leaves could affect human health when consumed as leafy vegetable. *Used in household* represents if cassava leaves should be used in households. *Affects_tuber_form* denotes if cassava leaves affects tuber formation when plucked and consumed. *Sold on market* represents if cassava leaves are considered as useful good and therefore should be sold on the market. *Saves cost* represents if cassava leaves could save cost if consumed as leafy vegetable. *For poor people* represents if cassava leaves are mostly for poor people. *Aroma* represents if the aroma of cassava leaves makes it unsuitable to be consumed as a leafy vegetable. *Texture* represents if the texture (chewiness) of cassava leaves makes it unsuitable to be consumed as a leafy vegetable. *Gen_appearance* denotes if the general appearance of cassava leaves makes it unsuitable to be consumed as a leafy vegetable and β_1, β_2, β_3, ..., $\beta_2 1$ represent the coefficients of the variables. $\varepsilon_i$denotes error term capturing all factors unknown to the researcher.

2.2 Statement of hypotheses

Hypothesis 1: Socio-economic variables such as age, number of years of formal education, household size, and monthly income have influence on willingness to consume cassava leaves as leafy vegetable in the Kumasi Metropolis.
Hypothesis 2: Perception on the product's attributes such as aroma, texture and general appearance consumption of cassava leaves have influence on willingness to consume cassava leaves as a leafy vegetable in the Kumasi Metropolis.

2.3 Data Collection and Sampling method

A multistage sampling technique was employed for this study. This was used to ensure fair representation within the Metropolis. The stratified random sampling technique was used to select communities from the metropolis since they were clustered into low, middle and high income groups (Table 1). The simple random sampling technique was also used to select communities within the residential income class of communities. In all, a total of 180 respondents from 12 randomly selected communities out of the 78 in the Kumasi Metropolis were considered in the survey for this study; 4 communities from low income category, 4 communities from middle income category and 4 communities from high income category. The systematic random sampling technique was used to select fifteen respondents each from the sampled communities (Table 2). The face-to-face interview technique was employed using a structured questionnaire. This was to provide the opportunity to explain ques-

tions which were difficult to answer, to obtain the exact information needed for the study, and also to afford the interviewer the opportunity to educate the respondents. The study population was targeted at all consumers of leafy vegetables and assessed based on income groups of the respondents. One reason for using income groups as a basis is that consumption is a function of income (Edgmand, 1987).

3 Results and Discussions

3.1 Socio-economic characteristics of respondents in the Kumasi Metropolis

Majority of the respondents (60%) interviewed were females. The average respondents' age interviewed was 37 years (Table 3). The average household size of the respondents was 4 persons. The average number of years of education among the respondents was 13.9 years representing secondary education. The average respondents' monthly income was GHC1,191.22.

3.2 Consumers' awareness of the nutritional status of cassava leaves

Amongst the total number of respondents interviewed, 43 representing 23.9% had knowledge of the nutritional status of cassava leaves whereas 137 representing 76.1 percent did not have any knowledge of the nutritional status of the product as depicted in the Figure 1.

3.3 Utilization of cassava leaves by Respondents

About 92% of the respondents interviewed had consumed cassava leaves before as food (either as vegetable salad, stew or for soup) in the past, 5% as food and as medicine, and about 3% had also consumed cassava leaves as food, as medicine, used as animal feed and as farm material (Figure 2).

3.4 Consumers' perception on consumption of cassava leaves as a leafy vegetable

The perception of a product determines the rate of consumption of a particular commodity as indicated by Padberg et al. (1997) that consumers' attitude towards a product depend heavily on their perception about the product. There is therefore a link between attitude and perception. Consumers' willingness to consume a product is influenced largely by their attitudes and determines their choice of decision making (Alvensleben & Meier, 1989). Consumers' opinions were sought on the nutritional, health and economic concepts on consumption of cassava leaves as leafy vegetable in the Kumasi Metropolis as presented in Table 4.
The study found an overall perception index as 1.98, implying a neutral idea about cassava leaves as a leafy vegetable and thus, indicates that majority of respondents did not know about the nutritional, health and economic benefits of cassava leaves as a leafy vegetable in the Kumasi Metropolis. The product attributes on assessment also provides a significant influence on whether respondents will consume cassava leaves as a leafy vegetable or not. This affirms the statements by Ragaert, Verbeke, Devlieghere, and Debevere (2004) that product attributes play a significant contribution on consumption of a particular commodity.

$$TCP = \frac{Np + Hp + Ep + Ap}{4} \tag{15}$$

$$= \sum\left(\frac{1.67 + 2.26 + 1.78 + 2.22}{4}\right) \tag{16}$$

$$= 1.98 \tag{17}$$

Where, TCP is the total perception index, Np is Nutritional perception; HP is Health perception; Ep is Economis perception and Ap is Attribute perception.

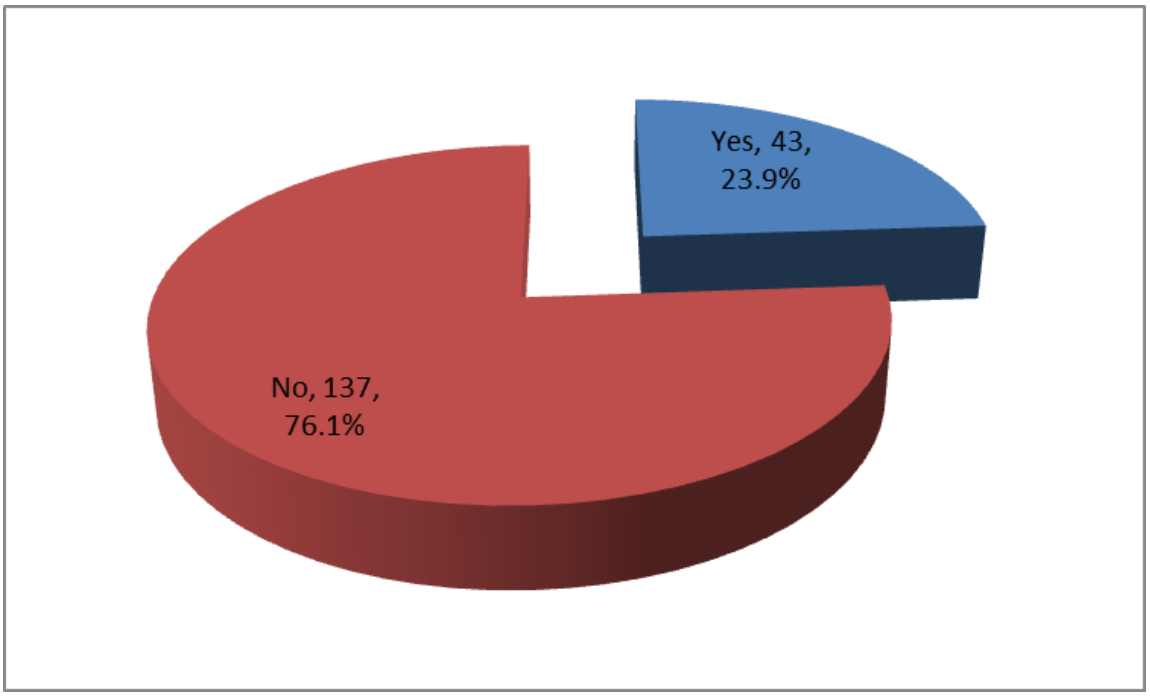

Figure 1: Consumers' awareness of the nutritional status of cassava leaves

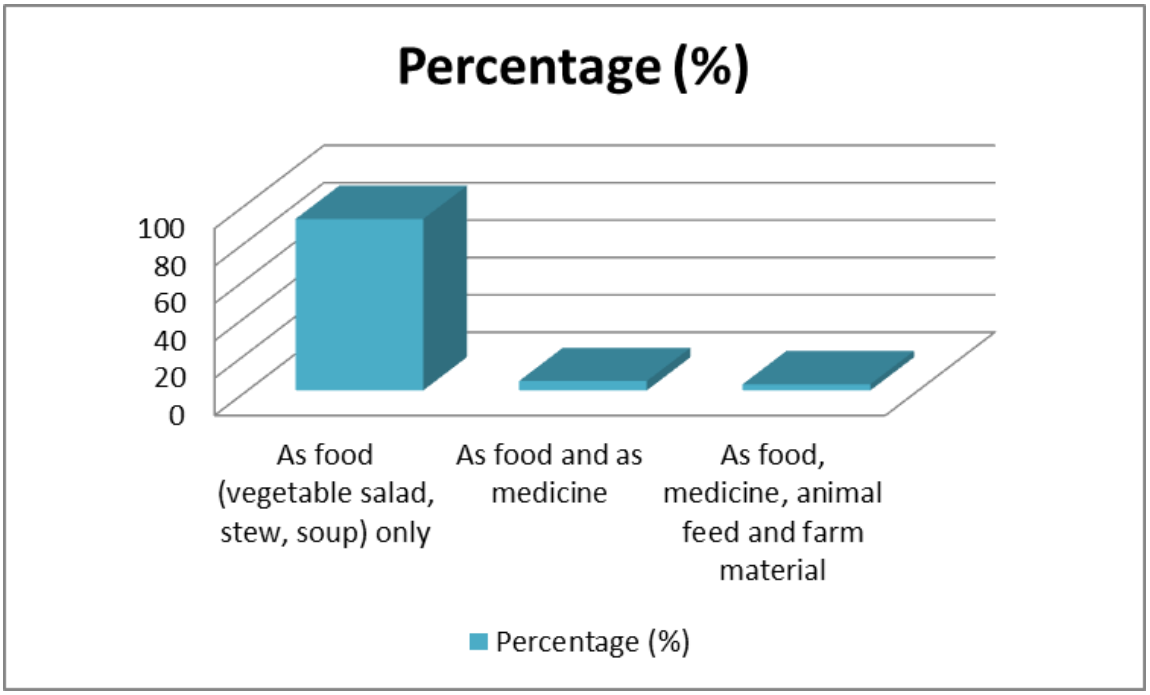

Figure 2: Utilization of cassava leaves by Respondents

Table 1: Residential Income Classes of Communities in the Kumasi Metropolis

High Income	Dadiesoaba, Asokwa, West Ayigya, Mbrom, Adiebeba, Adiembra, Ahodwo, Danyame, Odeneho Kwadaso, Aketego, Bomso, Bompe, Ridge, Nhyiaso, Extension, Parakuo Estate, Daban New Site, New Amakom Extension, Asokwa Residential Area
Middle Income	Asafo, Amakom, Airport, Bantama, Dichemso, Aprade, New Tafo, Asebi, Anyinam, Kuwait, Atonsu, New Atonsu, Gyenyase, New Agogo, Adoato, Kyirapatre Estate, Bohyen, Adumanu, Adumanu Extension, Asanti Newtown, Apiri, North Suntreso, Kotei, South Suntreso, Boadi West Patase, Ohwimase, Kwadaso Estate, Santase Odumase Extension, Patase, Kentinkrono
Low Income	Apatrapa, Dompoase, Aboabo, Moshie Zongo, Dichemso, Old Tafo, Ayigya Zongo, Dakwadwom, Sawaba, Yalwa, Daban, Kaase, Sokoban, Nsenee, Ahinsan, Anwomaso, Gyinyase, Adukrom, Asewase, Buobai, Nima, Pakuso, Abrepo, Sokoban, Amanfrom, Yenyawso, Buokrom, Ayeduase

Source: KMA, 2007

Table 2: Sampled communities within the Residential income class of communities

INCOME CATEGORY	RESIDENTIAL COMMUNITIES	NUMBER OF HOUSEHOLDS
HIGH INCOME	Bomso	15
	Ahodwo	15
	Ridge	15
	Asokwa residential area	15
MIDDLE INCOME	Asafo	15
	North Suntreso	15
	Boadi	15
	Amakom	15
LOW INCOME	Ayigya Zongo	15
	Aboabo	15
	Anwamaso	15
	Old Tafo	15

Source: Field survey, 2016

3.5 Estimation of factors affecting consumers' willingness to consume cassava leaves as a leafy vegetable in the Kumasi Metropolis

The study found a significant difference in the socioeconomic characteristics of the respondents and their perceptions with regards to the willingness to consume cassava leaves as leafy vegetable or not (Table 5). Based on the empirical results from the logit model (Table 6), age was found to be significant at 5% on consumers' willingness to consume cassava leaves as leafy vegetable. The direction of the variable was positive, meaning among the respondents interviewed those of older ages tend to consume cassava leaves as leafy vegetable. This can be explained that a unit change in the age of a consumer will increase willingness to consume cassava leaves by 0.39%. This is partly geared to the increase in knowledge of the benefits of fruits and vegetables (Elfhag, Tholin, & Rasmussen, 2008). In children and adolescents, consumption tends to decrease with age (Rasmussen et al., 2006). Sex of respondents was positive and significant at 10%. This finding agrees with Rasmussen et al. (2006). A unit change in male consumers will increase willingness to consume cassava leaves as leafy vegetable by 3.71%. Also respondents' monthly income was found to have a negative influence on willingness to consume cassava leaves as leafy vegetable at 10% significance level. This implies that, a unit change in monthly income of consumers in the Kumasi Metropolis will decrease willingness to consume cassava leaves as leafy vegetable by 6.2%.

The perception variable "cassava leaves are

Table 3: Socio-economic characteristics of respondents

Variable	**Category**		**Frequency (%)**	**Percentage**
Gender	Male		72	40
	Female		108	60
Marital status	Married		88	48.9
	Single		71	39.4
	Divorced/ Separated		21	11.7
Education level	Basic		41	22.8
	Secondary		58	32.2
	Tertiary		51	28.3
	Vocational		21	11.7
	Illiterate		9	5.0
Tribe	Akan		141	78.3
	Ga		8	4.4
	Ewe		10	5.6
	Northern		21	11.7
Continuous variables	**Minimum**	**Maximum**	**Mean**	**Std. deviation**
Age (years)	18	72	36.73	13.00
Household size (in persons)	1	9	4.36	1.55
Number of years in school	0	23	13.97	5.18
Monthly Income (GHC)	0.00	7000.00	1191.22	1203.20

Source: Field Survey, 2016

sweet" had a positive influence on consumption of cassava leaves and was significant at 1%. This result is consistent with the finding of Kamga, Kouame, Tchindjang, Chagomoka, and Drescher (2013). This means that, a unit change in the perception statement variable "cassava leaves are sweet" will increase willingness to consume cassava leaves as leafy vegetable by 10.55%. Also, the perception variable "Cassava leaves are bitter" was negative and was found to significantly affect consumption of cassava leaves at 1% level. This implies that, a unit change in the perception statement "Cassava leaves are bitter" will decrease willingness to consume cassava leaves as leafy vegetable by 12.2%. The perception variable "Cassava leaves should be used as animal feed" was negative and was found to significantly affect consumption of cassava leaves at 5% level. This indicates that, a unit change in the perception statement "Cassava leaves should be used as animal feed" will decrease willingness to consume cassava leaves as leafy vegetable by 4.58%. Moreover, the perception that "cassava leaves should be eaten as food" had a positive and a 1% significant influence on consumption of cassava as a leafy vegetable. A change in the unit of the perception variable "Cassava leaves should be eaten as food" will increase willingness to consume cassava leaves as leafy vegetable by 6.84%. "*Cassava leaves are good substitute*" was positive and significant at 5%. This connotes that a unit change in the perception variable "Cassava leaves are good substitute" will increase willingness to consume cassava leaves as leafy vegetable by 5.85%. The perception variable "*Cassava leaves are useful and therefore should be sold on the market*" was found to be positive and significant at 10%. This implies that a unit change in the perception variable "*Cassava leaves are useful and therefore should be sold on the market*" will increase willingness to consume cassava leaves as leafy vegetable by 2.66%. Also "*Aroma and General appearance*" were negative and have significant influence on consumption of cassava leaves at 1% and 5% respectively. This implies that a unit change in the cooked cassava leaves aroma will

Table 4: Consumers' perception on consumption of cassava leaves as a leafy vegetable

Nutritional perception statements on cassava leaves	**Agree (1)**	**Neutral (2)**	**Disagree (3)**	**Mean Score**
Cassava leaves are sweet	14	120	46	2.18
Cassava leaves are bitter	48	119	13	1.81
Cassava leaves provide some essential nutrients	57	123	0	1.68
Cassava leaves should be used as animal feed	156	1	23	1.26
Cassava leaves should be eaten as food	132	1	47	1.53
Cassava leaves are good substitute for other known leafy vegetables such as Spinach, lettuce	127	11	42	1.53
Nutritional Perception index				**1.67**
Health perception statement on cassava leaves	**Agree (1)**	**Neutral (2)**	**Disagree (3)**	**Mean Score**
Cassava leaves contains poisonous components like cyanide	52	64	64	2.07
Cassava leaves could affect human health when consumed	38	22	120	2.46
Health Perception index				**2.27**
Economic perception statements	**Agree (1)**	**Neutral (2)**	**Disagree (3)**	**Mean Score**
Cassava leaves should be used in households for cooking	130	2	48	1.54
Cassava leaves affect tuber formation if plucked and consumed	161	17	2	1.12
Cassava leaves are useful and therefore should be sold on the market	35	1	144	2.61
Cassava leaves save cost if consumed as leafy vegetable	126	19	35	1.49
Cassava leaves are mostly for poor people	43	66	71	2.16
Economic Perception index				**1.78**
Attribute statements on cassava leaves	**Agree (1)**	**Neutral (2)**	**Disagree (3)**	**Mean Score**
Aroma	6	116	58	2.29
Color (green)	0	57	123	2.68
Texture	42	113	25	1.91
General appearance	81	17	82	2.01
Attributes index				**2.22**

Source: Field Survey, 2016

decrease willingness to consume cassava leaves as leafy vegetable by 6.32% and 4.76%. This result is in line with Padberg et al. (1997), who stated that consumers' attitude towards a product depends heavily on their perception of the product. It was again found that, the product attribute "*Texture*" had positive influence on willingness to consume cassava leaves as leafy vegetable at 1% significance level. This indicates that a unit change in texture of cassava leaves will increase consumers' willingness to consume cassava leaves as leafy vegetable by 10.63%. This result is consistent with the finding of Kamga et al. (2013) that consumers' preferences of any particular commodity depend highly on the attributes of the product.

3.6 Consumers' constraints on utilization of cassava leaves as a leafy vegetable in the Kumasi Metropolis

Table 7 shows the constraints to consumption of cassava leaves in the study area. The study found "presence of other leafy vegetables" as the major limitation that hinders the respondents from consuming cassava leaves as leafy vegetable. Consumers in the Kumasi Metropolis who did not eat cassava leaves as a leafy vegetable attributed the "presence of other leafy vegetables" as the major constraint. This is in line with the statements of Weinberger (2004) that priority has been based mostly on few types of vegetables

Table 5: Descriptive statistics of respondents in the Kumasi Metropolis

Independent variable	NWTC	WTC	Mean difference	Significance
Sex	0.38	0.45	0.07	0.007***
Age	31.23	47.73	16.50	0.096*
Hd_size	3.98	5.13	1.15	0.110
No._yrs_in_sch	14.94	12.03	-2.91	0.000***
M_inc	1043.08	1487.50	444.42	0.002***
Sweet	0.01	0.22	0.21	0.000***
Bitter	0.03	0.73	0.70	0.000***
P_essential min.	0.27	0.42	0.15	0.001***
Animal feed	0.94	0.72	-0.23	0.000***
Eaten as food	0.63	0.95	0.33	0.000***
Good substitute	0.57	0.97	0.40	0.000***
Poisonous comp.	0.36	0.15	-0.21	0.000***
Affect HHealth	0.30	0.03	-2.27	0.000***
Used in household	0.61	0.95	0.34	0.000***
Affect tuber_form.	0.88	0.93	0.06	0.014**
Sold on market	0.15	0.28	0.13	0.000***
Saves cost	0.58	0.93	0.35	0.000***
For poor people	0.24	0.23	-0.01	0.805
Aroma	0.02	0.07	0.05	0.000***
Texture	0.02	0.67	0.65	0.000***
Gen_appearance	0.61	0.15	-0.46	0.000***

Source: Field Survey, 2016
***, **, * indicating significance at 1%, 5%, 10% respectively

such as spinach, amaranths, okra, nightshade eggplant and cowpea leaves which has caused some indigenous vegetables like cassava leaves to be extinct.

4 Conclusion

Based on the findings, it can be concluded that majority of consumers in the Kumasi Metropolis were not aware of the nutritional status of cassava leaves and this tended to inform their decision to accept and consume cassava leaves as a leafy vegetable. Respondents who were aware of the nutritional contents did not have a thorough knowledge of all the nutritional components and benefits. Most of the respondents in the Kumasi Metropolis agreed to consume cassava leaves as a leafy vegetable after being educated on its specific nutrients present. Therefore, awareness of the nutritional status informed consumers' willingness to consume cassava leaves as a leafy vegetable. Respondents did not have a positive perception on consumption of cassava leaves as a leafy vegetable. Due to inadequate knowledge of the nutritional status of cassava leaves, majority of the respondents did not agree or were neutral on the nutritional, health and economic benefits of cassava leaves.

Negative perception on consumption had influence consumers' willingness to consume cassava leaves as a leafy vegetable. Factors such as socio-demographic characteristics (age, sex, household, monthly income) and product attributes (aroma, texture and general appearance) were found to determine consumers' willingness to consume cassava leaves as leafy vegetable in the Kumasi Metropolis. Moreover, the presence of other leafy vegetables in the study area was identified as affecting the consumption of cassava leaves.

Based on key findings, the study recommends that research, educational and health institutions should promote the consumption of cassava

Table 6: Logit regression model on assessing consumers' willingness to consume cassava leaves as leafy vegetable in the Kumasi Metropolis

WTC	**Coefficient**	**Standard error**	**dy/dx**	**P-value**
Sex	2.2743*	1.3243	0.0371	0.0860
Age	0.2425**	0.1105	0.0039	0.0280
Household size	0.6782**	0.3268	0.0110	0.0380
Number of years in school	0.0673	0.0944	0.0010	0.4760
Monthly income	-0.0147*	0.0007	-0.0000	0.0620
Perception variables				
Cassava leaves are sweet	6.4674***	1.5455	0.1055	0.0000
Cassava leaves are bitter	-7.4814***	1.9811	-0.1220	0.0000
Cassava leaves provide some essential nutrients	-1.3464	1.1876	-0.0219	0.2570
Cassava leaves should be used as animal feed	-2.8124**	1.2005	-0.0458	0.0190
Cassava leaves should be eaten as food	-4.1952***	1.2848	-0.0684	0.0010
Cassava leaves has a good substitute	3.5895**	1.7513	0.0585	0.0400
Cassava leaves contains poisonous components	1.3895	1.4395	0.0226	0.3340
Cassava leaves could affect human health	0.8460	1.8167	0.0138	0.6410
Cassava leaves should be used in households for cooking	1.0881	1.3673	0.0177	0.4250
Cassava leaves affect tuber formation if plucked and consumed.	-0.3775	1.3637	-0.0061	0.7820
Cassava leaves are useful and therefore should be sold on the market	1.6329*	0.8852	0.0266	0.0650
Cassava leaves save cost if consumed as leafy vegetable	-2.4913	1.5899	-0.0406	0.1170
Cassava leaves are mostly for poor people	-2.3271	1.4643	-0.0379	0.1120
Aroma	-3.8735***	1.4041	-0.0632	0.0060
Texture	6.5161***	1.5851	0.1063	0.0000
General Appearance	-2.9215**	1.4071	-0.0476	0.0380
Obs., 180; Wald chi-square (21), 89.20; Prob. > chi-square, 0.0000; Pseudo R^2, 0.9088; Log pseudo likelihood, -10.44604				

***, **, * indicating significance at 1%, 5%, 10% respectively.
Source: Authors' calculations, 2016

Table 7: Consumers' constraints on utilization of cassava leaves as leafy vegetable in the Kumasi Metropolis

Constraints	Most serious (1)	More serious (2)	Moderate (3)	Slightly serious (4)	Least (5)	Mean score	Rank
Presence of other leafy vegetables	122	16	12	12	7	1.6154	$\mathbf{1}^{ST}$
Unaware if consumable	6	4	3	1	1	2.1333	$\mathbf{2}^{ND}$
Toxic components	9	7	3	5	2	2.3846	$\mathbf{3}^{RD}$
Product attributes	8	36	15	11	11	2.7654	$\mathbf{4}^{TH}$
Unaware of nutritional benefits	5	25	24	17	8	2.9747	$\mathbf{5}^{TH}$
No thorough education on consumption	7	24	31	26	13	3.1386	$\mathbf{6}^{TH}$
Time of cooking	5	7	13	11	6	3.1429	$\mathbf{7}^{TH}$
Not motivated	3	9	9	9	10	3.3571	$\mathbf{8}^{TH}$
Not preferred by household	2	10	10	13	13	3.5208	$\mathbf{9}^{TH}$
Unavailability at market	2	7	6	5	8	3.3571	$\mathbf{10}^{TH}$
Not consumed by parents	4	7	21	22	18	3.5972	$\mathbf{11}^{TH}$
Effect to tuber formation	4	5	11	10	15	3.6000	$\mathbf{12}^{TH}$
Stigmatization	2	10	10	12	24	3.7931	$\mathbf{13}^{TH}$
Dirty on sight	0	3	4	8	14	3.5972	$\mathbf{14}^{TH}$
Perceived as animal feed	0	1	2	5	7	4.2000	$\mathbf{15}^{TH}$
Pests infestation	0	1	4	9	16	4.3333	$\mathbf{16}^{TH}$

Source: Field survey, 2016

leaves as a leafy vegetable by providing thorough information which will make respondents aware of the nutritional benefits of cassava leaves. Educating consumers on the health benefits of cassava leaves would help re-orient their perception on consumption of cassava leaves as a leafy vegetable.

References

Alvensleben, R. v. & Meier, T. (1989). The influence of origin and variety on consumer perception. In *Workshop on measuring consumer perception of internal product quality 259* (pp. 151–162).

Bokanga, M. (1994). Processing of cassava leaves for human consumption. In *International workshop on cassava safety 375* (pp. 203–208).

Edgmand, M. R. (1987). *Macroeconomics theory and policy. third edition* (N. J. .-.-. U. Prentice-Hall Englewood Cliff, Ed.). Retrieved from https://www.amazon.com/Macroeconomics-Theory-Michael-R-Edgmand/dp/0135429528

Elfhag, K., Tholin, S., & Rasmussen, F. (2008). Consumption of fruit, vegetables, sweets and soft drinks are associated with psychological dimensions of eating behaviour in parents and their 12-year-old children. *Public Health Nutrition*, *11*(9), 914–923. doi:10.1017/S1368980008002371

Gomez, G. & Noma, A. T. (1986). The amino-acid-composition of cassava leaves, foliage, root tissues and whole-root chips. *Nutrition Reports International*, *33*(4), 595–601.

Greene, W. H. (2003). *Econometric analysis, 5th edition* (P. Hall, Ed.). Retrieved from http://www.worldcat.org/title/econometric-analysis/oclc/50252029

Greene, W. H. (2008). *Econometric analysis, 6th edition* (P. Hall, Ed.). Retrieved from http://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1883856

Kamga, A., Kouame, C., Tchindjang, M., Chagomoka, T., & Drescher, A. W. (2013). Environmental impacts from overuse of chemical fertilizers and pesticides amongst market gardening in bamenda, cameroon. *1*, 6–19.

Keller, G. (2004). African nightshade, eggplant, spiderflower et al.–production and consumption of traditional vegetables in tanzania from the farmers point of view. *Masterarbeit im wissenschaftlichen Studiengang Agrarwissenschaften an der Georg-August Universität Göttingen, Fakultät für Agrawissenschaft*.

Lancaster, P. A. & Brooks, J. E. (1983). Cassava leaves as human food. *Economic Botany*, *37*(3), 331–348. doi:10.1007/BF02858890

Motta, J. S., Fukuda, W. G., & Costa, Z. M. (1994). Farinha da folha de mandioca: uma alternativa como complemento alimentar. *Mandioca em foco*, *4*(1), 1–2.

Okigbo, B. N. (1980). Nutritional implications of projects giving high priority to the production of staples of low nutritive quality: the case for cassava (manihot esculenta, crantz) in the humid tropics of west africa. *Food and Nutrition Bulletin*, *2*(4), 1–10.

Padberg, D. I., Riston, C., & Albisu, L. M. (1997). Agro-food marketing cab international. (pp. 209–224). CABI Publishing, Oxford, UK.

Ragaert, P., Verbeke, W., Devlieghere, F., & Debevere, J. (2004). Consumer perception and choice of minimally processed vegetables and packaged fruits. *Food Quality and Preference*, *15*(3), 259–270. doi:10.1016/S0950-3293(03)00066-1

Rasmussen, M., Krolner, R., Klepp, K.-I., Lytle, L., Brug, J., Bere, E., & Due, P. (2006). Determinants of fruit and vegetable consumption among children and adolescents: a review of the literature. part i: quantitative studies. *International Journal of Behavioral Nutrition and Physical Activity*, *3*. doi:10.1186/1479-5868-3-22

Scott, G. J., Rosegrant, M. W., & Ringler, C. (2000). *Roots and tubers for the 21st century: trends, projections, and policy options*. International Food Policy Research Institute (IFPRI) and International Potato Centre (CIP).

Wanapat, M., Puramongkon, T., & Siphuak, W. (2000). Feeding of cassava hay for lactating

dairy cows. *Asian-australasian Journal of Animal Sciences*, *13*(4), 478–482. doi:10.5713/ajas.2000.478

Weinberger, K. (2004). *Indigenous vegetables in tanzania: significance and prospects*. AVRDC-WorldVegetableCenter.

Westby, A. (2002). Cassava utilization, storage and small-scale processing. *Cassava: Biology, Production and Utilization*, 281–300.

Wooldridge, J. M. (2002). *Econometric analysis of cross section and panel data*. Econometric Analysis of Cross Section and Panel Data. MIT Press. Retrieved from https://books.google.pt/books?id=cdBPOJUP4VsC

World Health Organization. (2003). Effectiveness of interventions and programmes promoting fruit and vegetable intake. Geneva, Switzerland.

International Journal of Food Studies | IJFS October 2018 | Volume 7 | pages 51–63

Production of Camel Milk Yoghurt: Physicochemical and Microbiological Quality and Consumer Acceptability

Obakeng Galeboe[a], Eyassu Seifu[a*], and Bonno Sekwati-Monang[a]

[a] Department of Food Science and Technology, Botswana University of Agriculture and Natural Resources, Private Bag 0027, Gaborone, Botswana
[*]Corresponding author
eseifu@buan.ac.bw

Received: 23 August 2017; Published online: 18 October 2018

Abstract

The objectives of this study were to make yoghurt from camel milk and determine its physicochemical, microbiological and sensory qualities. The quality of camel milk yoghurt was compared with cow milk yoghurt and all parameters were analyzed following standard procedures. Yoghurt of acceptable consistency was made from camel milk using 1.2% gelatin, 5% bovine skim milk powder, 1.5 ml/L of calcium chloride, 40 ml/L of maple strawberry syrup and 6% yoghurt culture (YF-L811) and by incubating the milk at 42 ^{o}C for 18 h. The average values for moisture, ash, syneresis, pH, titratable acidity and total solids of camel milk yoghurt were 83.4%, 1.13%, 58%, 4.37, 1.255% lactic acid and 16.7%, respectively. The corresponding values for cow milk yoghurt were 80.6%, 0.71%, 56%, 4.67, 0.865% lactic acid and 19.5%, respectively. The titratable acidity of camel milk yoghurt was significantly higher ($P<0.05$) than cow milk yoghurt; however, no significant difference was observed between the two yoghurt types for the other parameters. Coliforms were not detected in both yoghurt types. The sensory analysis showed that cow milk yoghurt was more preferred by the panellists than camel milk yoghurt. Production of yoghurt from camel milk using the same procedure as for cow milk yoghurt proved to be difficult. Further research is called for to improve the acceptability of camel milk yoghurt using locally available and acceptable flavouring agents. Research needs to be conducted to optimize the operating parameters and standardize the production procedures of camel milk yoghurt in the future.

Keywords: Botswana; Camel milk yoghurt; Physicochemical properties; Microbial quality; Consumer acceptability

1 Introduction

Camels are very reliable milk producers during dry seasons and drought years when milk from cattle, sheep, and goats is scarce. In drought-stricken areas of the world, where continuous drought decimates cattle, sheep and goat populations, only the camel survives and continues to produce milk. Camel milk has an important role in food security and rural economy of arid zones of north and east Africa, the Middle East, central Asia, and the Indian subcontinent. In some regions, such as the horn of Africa, 10% of the milk is derived from dromedary camels (Faye & Bonnet, 2012).

Components of camel milk differ considerably from milk of ruminant animals (cows, sheep and goats). Camel milk has high concentrations of niacin and vitamin C, and high water content especially during the hot summer months. Camels produce diluted milk in hot weather when water is scarce. Camel milk has low fat content and it

 10.7455/ijfs/7.2.2018.a5

creams less rapidly and less completely than cow milk (Farah, 2011). Camel milk has higher unsaturated fatty acids, lower saturated and short chain fatty acids and lower content of carotene than bovine milk (Claeys et al., 2014). The high vitamin C content of camel milk is of significant importance especially in arid and desert environments where green vegetables and fruits are not readily available. β-Lactoglobulin, the main whey protein of bovine milk, is not found in camel milk (Farah, 2011) and thus milk allergy which is usually associated with cow milk is not common with camel milk. Camel milk contains high proportions of antibacterial substances (Al-Otaibi & El-Demerdash, 2013) and it keeps for a longer period of time as compared with cow milk (Farah, 2011).

In recent years, interest in camel milk has grown among specific consumer groups in North America and Europe due to its potential medical benefits (Al Haj & Al Kanhal, 2010; Mullaicharam, 2014; Sharma & Singh, 2014). Camel milk has medicinal properties and contains protective proteins, which may have a possible role for enhancing the immune defence mechanism (Al-Otaibi & El-Demerdash, 2013). Camel milk has been used to treat tuberculosis, dropsy, jaundice, and anaemia (Yagil, 1982; Hashim, Khalil, & Habib, 2009). It has high insulin content (Agrawal, Beniwal, Sharma, et al., 2005; Shori, 2015) and it has traditionally been used to treat diabetes (Al-Numair, Chandramohan, & Alsaif, 2011). Agrawal, Beniwal, Kochar, et al. (2005) reported that camel milk improved long-term glycaemic control and reduced insulin dose in patients with type-1 diabetes.

In traditional pastoral communities, camel milk is consumed predominantly fresh or after it turns sour. Camel milk does not coagulate easily and as a result it is difficult to make fermented dairy products such as cheese, yoghurt and butter from camel milk (Breulmann et al., 2017). The manufacturing of products like butter, ghee, yoghurt and cheese from camel milk is still not well developed and standardized (Farah, 1996). The main reason for the difficulty of product making from camel milk is due to the unique structural and functional properties of the milk components. Camel milk contains low amounts of kappa casein resulting in a weak casein network that is destroyed during cutting and the loss of dry matter of cheese to the whey (Ramet, 2001). Processing of set-type yoghurt by use of gelatin or alginate plus calcium with acceptable sensory quality was reported for camel milk (Hashim et al., 2009). Reports also indicate the possibility of production of cheese from camel milk (Mehaia, 1993; Khan, Athar, & Aslam, 2004). However, these reports indicate the need for more research to improve the quality, efficiency and the yield of dairy products from camel milk.

Production of yoghurt from cow milk is well established. The procedure for yoghurt production from cow milk involves pasteurization of the milk at 85 ^{o}C for 30 min, cooling the heat treated milk to 42 ^{o}C, addition of thermophilic yoghurt culture, addition of stabilizers and sweeteners depending on the type of yoghurt produced, and incubating the milk at 42 ^{o}C for 4 h (Tamime & Robinson, 2000). However, production of yoghurt from camel milk using the same technology and procedure as for cow milk proved to be difficult due to the inherent characteristics of camel milk. In recent years, attempts have been made by different researchers to make yoghurt from camel milk (Hashim et al., 2009; Al-Zoreky & Al-Otaibi, 2015; Ibrahem & El Zubeir, 2016). However, the results reported so far are different from one another and sometimes conflicting. This suggests that the procedure for yoghurt production from camel milk is not yet established and more research needs to be done in order to standardize the manufacturing procedure.

In Botswana, camels are kept in Tsabong which is a semi-arid region in Kgalagadi District. They are kept in an enclosed park known as Tsabong Ecotourism Camel Park. The camels are under the care of the local community and the Botswana Tourism Organization oversees the overall management of the park. Despite the potential of camels in Botswana, no research has been carried out on camels or their products to date. Camels kept in Tsabong are mainly used for tourism (riding) purposes. The milk produced is consumed raw and is not processed into value-added products. To date no attempt has been made to make products from camel milk in Botswana.

Yoghurt production from camel milk would diversify dairy products in the market and in-

crease income of camel keepers and improve their livelihoods. This study was conducted to develop yoghurt from camel milk and determine the physicochemical and microbiological properties and consumer acceptability of camel milk yoghurt.

2 Materials and Methods

2.1 Description of the study area

Tsabong is the administrative centre of the Kgalagadi District located in south-western Botswana. The human population of the area was 8939 according to the 2011 census. The geographical coordinates of Tsabong are 26^o 3' 0" South, 22^o 27' 0" East. The study area, Tsabong Ecotourism Camel Park, is found in this region and is located at a distance of 520 km from the capital city Gaborone and 10 km north of Tsabong town and comprises a fenced area of 3200 hectares. The area is characterized by poor and unreliable rainfall with an annual precipitation of less than 250 mm and with an average ambient temperature of above 35 ^{o}C during summer and less than 2 ^{o}C in winter (Kgaudi, 2014). The area has sparsely distributed vegetation dominated by *Acacia* and *Grewia* species and some species of grass.

2.2 Sampling and milk sample collection

Milk samples were collected from camels kept in Tsabong Ecotourism Camel Park. Milk from different lactating camels that were at their sixth month of lactation was pooled together and a total of seven litres of milk was sampled and placed into a sterile container. Prior to hand milking the camels, the milkers washed their hands in order to prevent contamination of the milk.
The milk samples were then kept in a cool box containing packs of ice blocks and transported to Botswana University of Agriculture and Natural Resources Laboratory immediately after collection and kept at 4 ^{o}C in a refrigerator pending laboratory analysis. In the laboratory, the milk sample was divided into five lots of one litre each. Cow milk samples were used for production of yoghurt as controls.

2.3 Yoghurt preparation from camel milk

After a number of preliminary trials, the following formulation was used to make yoghurt samples for physicochemical, microbiological and sensory analyses. Prior to yoghurt making, all equipment used was thoroughly washed and sterilized by boiling in hot water for 1 hour in order to kill vegetative cells of microorganisms on the surface of the equipment. Before pasteurization, 12 grams of gelatin and 50 grams of bovine skim milk powder were weighed separately and added into the milk (1 L) and mixed. The milk was then pasteurised at 85 ^{o}C for 30 min in a thermostatically controlled water bath. The milk was then cooled to 42 ^{o}C, followed by addition of 1.5 ml /L (40% w/v) of food-grade calcium chloride, 40 ml/L of maple strawberry maple syrup (Tongaat Hullets Sugar, South Africa) and 6% commercial yoghurt culture (YF-L811 thermophilic yoghurt culture, Chr. Hansen, Denmark). The inoculated milk sample (1 L) was then divided into three lots of 333 ml each, incubated at 42 ^{o}C for 18 hours and stored at 4 ^{o}C overnight before testing. The same procedure was followed for the production of cow milk yoghurt except that 25g of skimmed milk powder was used instead of 50 grams and it was incubated for 4 hours at 43 ^{o}C. Figure 1 shows the flow diagram of the procedure used for production of the yoghurt samples.

2.4 Physicochemical properties

Moisture content

The moisture content of the yoghurt samples was determined according to the method specified by Association of Official Analytical Chemists (Association of Official Analytical Chemists, 1995). Ten grams of yoghurt sample were placed in a dried and weighed moisture dish which was then placed in air oven for 1 hour at 105 ^{o}C. The moisture dish was then removed and placed in a desiccator to cool and then weighed. The loss in weight was regarded as moisture content which

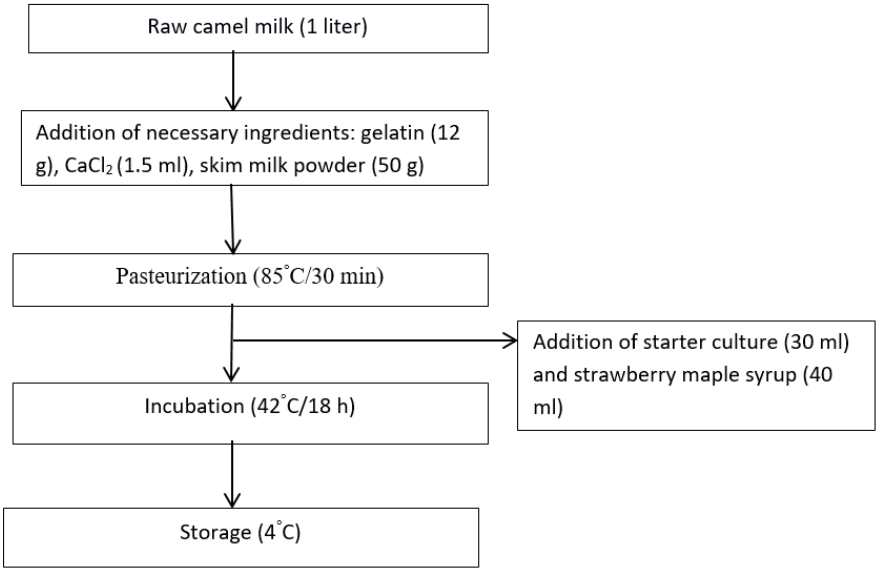

Figure 1: Production steps of camel milk yoghurt

was calculated using the following formula:
Moisture content (%) =

$$\frac{weight\ of\ fresh\ sample - weight\ of\ dry\ sample}{weight\ of\ fresh\ sample} \times 100$$

Total solids

The weight of the residue obtained from moisture content analysis was used to compute the total solids using the formula below (Association of Official Analytical Chemists, 1995):

$$\text{Total solids (\%)} = \frac{(dry\ yoghurt)}{weight\ of\ the\ sample} \times 100$$

Titratable acidity

The titratable acidity of yoghurt was determined using the method described by Richardson (1985). Yoghurt sample (9 g) was weighed in 100 ml Erlenmeyer flask and 20 ml of fresh distilled water was added to it and then titrated against 0.1 N NaOH after adding 3-5 drops of 1% phenolphthalein solution until persistent (30 sec.), faint pink colour was observed. The titratable acidity was then expressed as percentage of lactic acid using the following formula:

$$\text{Lacticacid(\%)} = \left[\frac{(ml\frac{N}{10}alkali \times 0.009)}{ml\ of\ sample\ used}\right] \times 100$$

pH

For determination of pH of yoghurt samples, a digital electronic pH meter (Orion Star A111 Benchtop pH Meter, Thermo Scientific USA) was used after calibrating it using standard buffer solutions of pH 4 and 7. The pH was then measured by inserting the glass electrode into the sample in a beaker and the reading was taken when the displayed value was steady.

Ash content

The ash content of yoghurt samples was determined according to the Association of Official Analytical Chemists (1995) method No.945.46. Using the dried yoghurt samples from the determination of total solids content, a sample weighing approximately 3 g was measured and put in a crucible, placed into a muffle furnace and ignited at $\leq$ 550 °C until ash was carbon free. Then, it was placed in a desiccator for

cooling and re-weighed. The initial and final weights of the sample were recorded. The ash weight was divided by the original sample weight and expressed in percent. The ash content was calculated using the following formula:

$$\text{Ash } (\%) = \frac{weight\ of\ residue}{weight\ of\ sample} \times 100$$

Syneresis

Syneresis (whey separation) was determined according to Celik and Bakirci (2003). Five ml of yoghurt was placed into a test tube and centrifuged at 5000 rpm for 20 min at 4 ^{o}C. After centrifugation, the test tube was then kept for 1 min before measuring the volume of separated whey. The rate of syneresis (%) was expressed as volume of separated whey per 100 ml of yoghurt.

2.5 Microbiological analysis

The microbiological quality tests conducted included coliform count (CC) and yeast and mould count (YMC). Violet Red Bile Agar (VRBA) (Oxoid, England) was used for CC and chloramphenicol agar (Merck, South Africa) was used for YMC. Yoghurt samples for microbiological analysis were prepared according to the method described by Richardson (1985). From a thoroughly mixed sample, 11 g of yoghurt was weighed into a 100 ml Schott bottle and mixed with 90 ml of peptone water (40 ^{o}C). The content was then mixed (10 min) until a homogenous dispersion was obtained, and then this 1:10 dilution was used for YMC and CC.

Coliform count

Coliform count was determined according to the method described by Richardson (1985) and Haddad, Al-Qudah, Abu-Romman, Obeidat, and El-Qudah (2017) using Violet Red Bile Agar (VRBA). From the 1:10 dilution of yoghurt, 1 ml sample was pour plated on duplicate Petri dishes and then VRBA was added (15-20 ml) onto each of them. Plates were then incubated at 30 ^{o}C for 24 hours. Typical dark red colonies ($>$ 0.5 mm in diameter) were considered as coliforms (Richardson, 1985).

Yeast and mould count

Yeast and mould count was determined as reported by Richardson (1985) using chloramphenicol agar. From the 1:10 dilution, 0.1 ml sample was placed on the surface of the chloramphenicol agar and evenly distributed using the streak plate technique. The plates were then incubated at 25 ^{o}C for 5 days. Yeast and mould count per gram of yoghurt was reported (Richardson, 1985).

2.6 Sensory evaluation

Sensory evaluation of yoghurt samples was carried out according to the method described by Barnes, Harper, Bodyfelt, and Mcdaniel (1991) using 25 consumer panellists consisting of 15 males and 10 females. Testing was conducted in the Food Processing Laboratory of Botswana University of Agriculture and Natural Resources (BUAN). Consumers were asked to write their preference (liking) of the yoghurt samples for the sensory attributes colour, aroma, sweetness, sourness, mouth feel and overall acceptability. Consumer panellists (BUAN students and staff) were selected based on their experience of yoghurt consumption. Yoghurt samples were served at temperature of less than 7 ^{o}C (Tamime, 2006) and a sample size of at least 40 ml of yoghurt was served in clear shot glasses. The yoghurt samples were presented in a randomised order. Panellists were asked to evaluate the sensory attributes of yoghurt, using the 9 point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Spring water was provided in disposable cups for cleansing their palates between samples. Prior to sensory evaluation, panellists read an explanation about the purpose of the study and gave their informed consent to participate in the sensory evaluation.

2.7 Statistical analysis

Comparison was made between yoghurt samples made from camel milk and cow milk for the parameters considered. The data generated was analysed using Student's t-test. The microbial

count data were $\log_{10}$ transformed before statistical analysis.

3 Results and Discussions

3.1 Yoghurt making

A number of preliminary trials were conducted in order to come up with a formulation that resulted in production of yoghurt of acceptable consistency from camel milk. In the initial trial, 0.6% gelatin and 2.5% milk solids-not-fat (MSNF) were added into camel milk and the mixture was pasteurized at 85 ^{o}C for 30 min and then cooled to 43 ^{o}C. This was followed by addition of 2% yoghurt culture (YF-L811 thermophilic yoghurt culture) and the milk was incubated at 43 ^{o}C for 4 h as recommended by Hashim et al. (2009). This did not result in the coagulation of the milk. In a second trial, 0.5% xanthan gum was added as a stabilizer (as suggested by Al-Zoreky and Al-Otaibi (2015)) in addition to 0.6% gelatin and yoghurt was made following the same procedure as above. The xanthan gum did not dissolve completely and formed lumps in the milk and the milk had thin and watery consistency. In a third trial, 2% gelatin and 7.5% MSNF were added into camel milk and the mixture was pasteurized at 85 ^{o}C for 30 min and then cooled to 43 ^{o}C. The cooled milk sample was inoculated with 6% yoghurt culture, 50 ml/L maple strawberry syrup and 1.5 ml/L food-grade calcium chloride and the milk was incubated at 43 ^{o}C for 22 h. This resulted in a very thick gel that was difficult to sample and analyze for parameters such as syneresis and was not appealing for sensory analysis.

Finally, yoghurt of acceptable appearance and consistency was obtained by adding 1.2% gelatin and 5% skim milk powder into the camel milk and pasteurizing the mixture at 85 ^{o}C for 30 min. The pasteurized milk was cooled to 42 ^{o}C and 1.5 ml/L of food-grade calcium chloride, 40 ml/L of maple strawberry syrup and 6% commercial yoghurt culture were added and the milk was then incubated at 42 ^{o}C for 18 h. The resulting yoghurt had a fairly thick consistency although it was less viscous and not as firm as cow milk yoghurt. The product was more like drinking yoghurt. This observation was in agreement with the findings of Ibrahem and El Zubeir (2016) who reported that yoghurt made from camel milk was less firm (had fluid-like texture) and was suitable for use as a drinking yoghurt. In the present study, 1.2% gelatin was used as a stabilizer and this is agreeed with Ibrahim and Khalifa (2015) who recommended that 1.5% gelatin can be added to camel milk to stabilize the texture of camel milk yoghurt without affecting the overall acceptability of the yoghurt.

Hashim et al. (2009) reported that acceptable camel milk yoghurt can be produced by dissolving milk solids-not-fat (2.5%) and stabilizer (0.6% gelatin or 0.75% sodium alginate (ALG)) and 0.075% calcium chloride in camel milk and pasteurizing the mixture at 85 °C for 30 min and incubating it at 43 ^{o}C for 4 h after inculcating the cooled milk with commercial yoghurt culture. They indicated that addition of 1% gelatin or 0.75% ALG + 0.075% Ca resulted in camel milk yoghurt with the highest intensities for firmness and body. They also reported that addition of 0.75% sodium alginate + 0.075% calcium chloride produced camel milk yoghurt with acceptable sensory attributes similar to cow milk yoghurt.

Al-Zoreky and Al-Otaibi (2015) made camel milk yoghurt using 6% stabilizers (carboxymethyl cellulose, pectin, gum acacia or alginate), which were added to pasteurized (85 ^{o}C/20 min) camel milk and then heated for 10 min at 85 ^{o}C after thorough mixing. The pasteurized camel milk formulations were cooled to 45 ^{o}C, inoculated with 2% (v/v) commercial yoghurt culture and incubated at 42±1 ^{o}C for 6 h. They reported that stabilizers did not improve the consistency and coagulum of camel milk yoghurt compared with cow milk yoghurt. They also indicated that camel milk yoghurt containing 0.6% alginate + 0.06% $CaCl_2$ showed higher rate (33.5%) of syneresis (whey separation) as compared with cow milk yoghurt (23.8%) indicating a weaker water-holding capacity.

Camel milk took longer time to ferment as compared to cow milk. The present observation contradicts the findings of Hashim et al. (2009) who reported that use of 1% gelatin and 0.075% calcium chloride results in camel milk yoghurt with firm body when incubated at 43 ^{o}C for 4 h. In

the current study, camel milk did not form a coagulum/gel after 4 hours of incubation at 43 ^{o}C irrespective of the level of ingredients used. However, this observation was in line with the findings of Ibrahem and El Zubeir (2016) who reported that camel milk takes longer time (17 h) to coagulate as compared with sheep milk. Similarly, Attia, Kherouatou, and Dhouib (2001) concluded that dromedary milk appears less favorable for lactic fermentation, because the activity of the inoculated lactic starter was lower in camel milk than in bovine milk. Generally, more research needs to be conducted to optimize the operating parameters and standardize the production procedures of camel milk yoghurt in the future.

3.2 Physicochemical properties

pH and acidity

Physical properties of yoghurt play an important role in determining its quality. Table 1 depicts the physical properties of yoghurt made from camel milk and cow milk. The sourness and refreshing taste of yoghurt are mainly attributed to its acidity. The average titratable acidity of camel milk yoghurt observed in the present study was higher than the value (0.78%) reported by Bhagiel, Musatafa, Tabidi, and Ahmed (2015) for camel milk. Al-Zoreky and Al-Otaibi (2015) reported a pH value of 4.59-4.63 and a titratable acidity of 0.71-0.87% lactic acid for camel milk yoghurt produced in Saudi Arabia. On the other hand, Hashim et al. (2009) reported a pH value ranging from 4.3 to 4.5 and a titratable acidity ranging from 0.98 to 1.16% for camel milk yoghurt produced with added gelatin, alginates and calcium chloride. In the present study, the titratable acidity of camel milk yoghurt was significantly higher ($P<0.05$) than that of cow milk yoghurt (Table 1). Camel milk yoghurt had a lower pH (4.37) which contributed to its higher acidity compared to cow milk yoghurt which had a pH of 4.67. Ibrahim and Khalifa (2015) reported that addition of stabilizer (gelatin and mono & diglycerides of fatty acids) caused highest acidity and lowest pH of camel milk yoghurt compared with those which did not have stabilizer. This was also observed by Kavas (2016) who found a significant increase in acidity when xanthum gum was used as a stabilizer in camel milk yoghurt.

Moisture

The average moisture contents of camel and cow milk yoghurt are indicated in Table 1. The moisture content of camel milk yoghurt observed in the present study was lower than that reported by Bhagiel et al. (2015) and Eissa, Mohamed, Yagoub, and Babiker (2010) for camel milk yoghurt which was 88.17% and 87.71%, respectively. It was also lower than the value of 87.71% reported by Eissa, Yagoub, Babiker, and Ahmed (2011) for the moisture content of camel milk yoghurt produced in Sudan. These differences in moisture can be attributed to seasonal variations in milk composition and availability of drinking water for camels (Bhagiel et al., 2015). The moisture content of the two yoghurt samples in the present study was not significantly ($P>0.05$) different.

Syneresis

Syneresis is an important defect in yoghurt. It is defined as the separation of whey (serum) from the coagulum in yoghurt and is related to shrinkage of the gel (Sahan, Yasar, & Hayaloglu, 2008). This quality defect occurs in yoghurt due to low total solids, over acidification, mechanical shaking of the gel network, insufficient denaturation of whey proteins, incompatibility of dairy and non-dairy ingredients (inappropriate amount and/or type of stabilizer), too high incubation temperature or too low acidification ($pH>4.6$) (Chandan & A., 2013). Syneresis can limit the shelf life and acceptability of yoghurt because of the undesirable appearances it causes. If yoghurt is subjected to a high degree of syneresis, its shelf life could be reduced as the gel formed could easily expel whey from the gel matrix leading to a suspension of yoghurt materials in whey within short period of time (Habtegebriel & Admassu, 2016). Yoghurt with high degree of syneresis is not liked by consumers. No significant difference ($P>0.05$) in syneresis was observed between camel milk and cow milk yoghurt (Table 1). The syneresis value observed

Table 1: Physicochemical properties of yoghurt made from camel and cow milk

Variable	Camel milk yoghurt	Cow milk yoghurt
Moisture (%)	83.40±0.59	80.60±2.32
Total solids (%)	16.65±0.06	19.45±2.32
Ash (%)	1.13±0.23	0.71±0.09
Syneresis (%)	58.00±2.83	56.00± 1.41
pH	4.37 ±0.01	4.67±0.01
Titratable acidity (%lactic acid)	1.255 ± 0.021^{a}	0.865 ± 0.007^{b}

Means with different superscript letters in a row are significantly different (P<0.05); Values in the table are means plus standard deviations of three samples

for camel milk yoghurt in the present study was higher than the value (33.5%) reported by Al-Zoreky and Al-Otaibi (2015). The difference in syneresis observed between the current study and that reported by Al-Zoreky and Al-Otaibi (2015) could be attributed to the difference in the type of stabilizer used which was gelatin in the former and sodium alginate in the latter study. The type and level of stabilizer used influence the degree of synersis in yoghurt Ibrahim and Khalifa (2015). Syneresis decreases with increase in stabilizer addition (Kiros, Seifu, Bultosa, & Solomon, 2016). Similarly, Ibrahim and Khalifa (2015) reported that addition of stabilizers significantly decreased syneresis, and increased viscosity and water holding capacity of camel milk yoghurt ($P \leq 0.05$).

Ash

The average ash contents of camel milk yoghurt and cow milk yoghurt are indicated in Table 1. The ash content of camel milk yoghurt observed in the present study was higher than the value of 0.84% reported by Bhagiel et al. (2015) and 0.71% reported by Eissa et al. (2011) for camel milk yoghurt produced in Sudan. It was also higher than the value (0.99%) reported by Ibrahem and El Zubeir (2016) for ash content of camel milk yoghurt. The ash content is an index of the mineral content of milk or yoghurt, which is needed for bone development, teeth formation and body functions (Bibiana & Joseph, 2014). The ash content of camel milk yoghurt was higher than cow milk yoghurt (Table 1). The results indicate that camel milk yoghurt is a good source of minerals.

Total solids

The average total solids (TS) content of camel milk yoghurt observed in the present study was higher than that reported by Bhagiel et al. (2015) and Bashir (2009), which were 11.83% and 11.3%, respectively. It was also higher than the values (12.2% and 9.24%) reported by Eissa et al. (2011) and Ibrahem and El Zubeir (2016), respectively, for TS of camel milk yoghurt. The TS content of yoghurt is influenced by the TS of the raw material (milk) from which it is produced. The total solids content of camel milk varies with season and it tends to be lower in the hot season as the water content of the camel milk increases during this season for the nourishment of young calves Bhagiel et al. (2015). The high amount of total solids observed in this study could be attributed to the addition of skim milk powder during the preparation of the yoghurt. For yoghurt manufacture, the solids content of the milk is usually increased to 16%. Increasing the solids content improves the nutritional value of yoghurt, makes it easier to produce firmer yoghurt and improves stability.

3.3 Microbiological quality

Presence of coliforms in yoghurt suggests unsanitary conditions during processing (Eissa et al., 2010). In the present study, coliforms were not detected in both yoghurt types (Table 2). This agreed with the findings of Eissa et al. (2011) who reported absence of coliforms in camel milk

yoghurt. The absence of coliforms in the present study could be attributed to the high hygienic conditions followed in the laboratory that prevented post-processing contamination.
Yeasts and moulds are major causes of spoilage of yoghurt and other fermented dairy products in which the low pH provides a selective environment for their growth. yoghurt produced under good manufacturing practices should contain no more than 10 yeast cells/g and should have a shelf life of 3–4 weeks at 5 °C (Ledenbach & Marshall, 2009). They also stated that yoghurts having initial counts of >100 cfu/g tend to spoil quickly. Yeasty and fermented off-flavours and gassy appearance are often detected in yoghurt when yeasts grow to 10^5–10^6 cfu/g (Ledenbach & Marshall, 2009). Total yeast and mould count recommended in yoghurt is <10 cfu/g (Mostert & Jooste, 2002). The yeast and mould count (YMC) of camel milk yoghurt observed in the present study (Table 2) was in line with the findings of Eissa et al. (2011) who reported YMC of 6.5 x 10^4 cfu/g for camel milk yoghurt produced in Sudan.

3.4 Sensory analysis

Results of the sensory analysis of the yoghurt samples are indicated in Table 3. Camel milk yoghurt had the lowest rating compared with cow milk yoghurt for all sensory attributes except for sourness. A significant difference ($P<0.05$) was observed between camel milk and cow milk yoghurts for colour, aroma, sweetness, mouth feel and overall acceptability. Cow milk yoghurt had higher scores for all these parameters. The sourness score for camel milk yoghurt was numerically higher than that of cow milk yoghurt (Table 3) and this corresponded with the higher acidity (lower pH) observed in camel milk yoghurt (Table 1). Plain yoghurt made from camel milk in the preliminary trials had a pungent smell and salty taste and this was the reason for inclusion of maple strawberry syrup in the production of the experimental yoghurt samples. This observation agreed with the findings of Eissa et al. (2011) who reported that camel milk yoghurt had lower consumer acceptability compared with cow milk yoghurt. They attributed the low acceptability of camel milk yoghurt to the high concentration of salt in camel milk. In the present study, inclusion of maple strawberry syrup improved the aroma and flavour of the camel milk yoghurt; however, even then the consumer acceptability scores for sensory attributes of the camel milk yoghurt were significantly lower than that of cow milk yoghurt. This suggested the need for further research aimed at improving the sensory quality of camel milk yoghurt using various indigenous fruits and other ingredients that are well accepted by the community.
Most of the panellists were able to notice a salty taste in the camel milk yoghurt which is not common in cow milk yoghurt. Also the panelists noticed that the yoghurt made from camel milk lacked firm texture, which was in agreement with the findings of Ibrahem and El Zubeir (2016) and Hassan, El Zubeir, and Babiker (2007). Attia et al. (2001) reported that fermented dromedary milk did not produce a curd structure but a few dispersed, small casein fragments at the surface and a film or firm gel at the bottom of the vessel. The low overall acceptability of camel milk yoghurt observed in the present study was in line with earlier reports. Ibrahem and El Zubeir (2016) reported low overall acceptability of camel milk yoghurt compared with yoghurt made from sheep milk or a mixture of sheep and camel milk. Similarly, Hashim et al. (2009) reported that yoghurt made from camel milk had the lowest ratings for all the sensory attributes and addition of gelatin and sodium alginate significantly improved acceptability of camel milk yoghurt. Also Al-Saleh, Metwalli, and Ismail (2011) reported that flavour scores of frozen yoghurt made from camel milk constituents were significantly lower ($P < 0.05$) than those made from cow milk.

4 Conclusion

Yoghurt of acceptable consistency was made from camel milk using 1.2% gelatin, 5% skim milk powder, 1.5 ml/L of calcium chloride, 40 ml/L of maple strawberry syrup as a flavouring agent and 6% yoghurt culture and by incubating the milk at 42 °C for 18 h. The results showed that camel milk yoghurt and cow milk yoghurt had comparable physicochemical and microbio-

Table 2: Microbial counts (cfu/g) of yoghurt made from camel and cow milk

Count	Camel milk yoghurt	Cow milk yoghurt
Yeast and mould	1.4 x 10^4	2.8 x 10^4
Coliforms	ND	ND

No significant difference (P>0.05) was observed in yeast and mould counts between the two yoghurt types; ND = not detected; Values in the table are means plus standard deviations of three samples

Table 3: Sensory quality of yoghurt made from camel and cow milk (n = 25)

Sensory attribute	Camel milk yoghurt	Cow milk yoghurt
Colour	5.80 ± 1.23^a	7.00 ± 0.91^b
Aroma	5.50 ± 1.30^a	7.60 ± 0.75^b
Sweetness	5.60 ± 1.20^a	6.30 ± 0.85^b
Sourness	6.00 ± 1.26	5.70 ± 1.32
Mouth feel	3.50 ± 1.17^a	6.30 ± 1.20^b
Overall acceptability	4.10 ± 1.03^a	6.70 ± 1.59^b

Means with different superscript letters in a row are significantly different (P<0.05); n = total number of panellists. Values are means and standard deviations

logical properties. However, cow milk yoghurt was more preferred than camel milk yoghurt. Production of yoghurt from camel milk using the same procedure as for cow milk yoghurt is difficult. Thus, more research needs to be conducted to optimize the operating parameters and standardize the production procedures of camel milk yoghurt in the future. The low consumer acceptability of camel milk yoghurt calls for further research to improve the acceptability of camel milk yoghurt using locally available and acceptable flavouring agents such as indigenous fruits.

Acknowledgements

The authors would like to thank Tsabong Ecotourism Camel Park workers for their assistance during the camel milk sample collection. We would also like to extend our appreciation to Botswana Tourism Organization for giving us permission to collect camel milk samples from the park. This study was funded by the Department of Tertiary Education of Botswana.

References

Agrawal, R. P., Beniwal, R., Kochar, D. K., Tuteja, F. C., Ghorui, S. K., Sahani, M. S., & Sharma, S. (2005). Camel milk as an adjunct to insulin therapy improves long-term glycemic control and reduction in doses of insulin in patients with type-1 diabetes. a 1 year randomized controlled trial. *Diabetes Research And Clinical Practice*, *68*(2), 176–177. doi:10.1016/j.diabres.2004.12.007

Agrawal, R. P., Beniwal, R., Sharma, S., Kochar, D. K., Tuteja, F. C., Ghorui, S. K., & Sahani, M. S. (2005). Effect of raw camel milk in type 1 diabetic patients: 1 year randomised study. *Journal Of Camel Practice And Research*, *12*(1), 27–31.

Al Haj, O. A. & Al Kanhal, H. A. (2010). Compositional, technological and nutritional aspects of dromedary camel milk. *International Dairy Journal*, *20*(12), 811–821. doi:10.1016/j.idairyj.2010.04.003

Association of Official Analytical Chemists. (1995). Official methods of analysis (16th ed.). Washington DC.: Association of Official Analytical Chemists.

Attia, H., Kherouatou, N., & Dhouib, A. (2001). Dromedary milk lactic acid fermentation: microbiological and rheological characteristics. *Journal Of Industrial Microbiology and Biotechnology*, *26*(5), 263–270. doi:10.1038/sj.jim.7000111

Barnes, D. L., Harper, S. J., Bodyfelt, F. W., & Mcdaniel, M. R. (1991). Prediction of consumer acceptability of yogurt by sensory and analytical measures of sweetness and sourness. *Journal Of Dairy Science*, *74*(11), 3746–3754. doi:10.3168/jds.S0022-0302(91)78566-4

Bashir, A. Y. M. (2009). *Effect of gum arabic on enzymatic coagulation of camel milk* (Doctoral dissertation, Sudan University of Science & Technology).

Bhagiel, I., Musatafa, E. A., Tabidi, M. M., & Ahmed, M. E. M. (2015). Comparison between the physiochemical attributes of yogurt processed from camel milk and that processed from cow milk and the effect of storage. *World Journal of Pharmacy and Pharmaceutical Sciences*, *4*(8), 1530–1540.

Bibiana, I. & Joseph, A., S. an Julius. (2014). Physicochemical, microbiological and sensory evaluation of yoghurt sold in makurdi metropolis. *African Journal of Food Science and Technology*, *5*(6), 129–135. doi:10.14303/ajfst.2014.052

Breulmann, M., Böer, B., Wernery, U., Wernery, R., El Shaer, H., Alhadrami, G., … Norton, J. (2017). *The camel from tradition to modern times. a proposal towards combating desertification via the establishment of camel farms based on fodder production from indigenous plants and halophytes*. United Arab Emirates: UNESCO Doha Office.

Celik, S. & Bakirci, I. (2003). Some properties of yoghurt produced by adding mulberry pekmez (concentrated juice). *International Journal Of Dairy Technology*, *56*(1), 26–29. doi:10.1046/j.1471-0307.2003.00070.x

Chandan, R. C. & A., K. (2013). *Manufacturing yogurt and fermented milks. (2nd ed.)* USA: Wiley-Blackwell. Retrieved from https://www.wiley.com/en-bw/Manufacturing+Yogurt+and+Fermented+Milks,+2nd+Edition-p-9781119967088

Claeys, W. L., Verraes, C., Cardoen, S., De Block, J., Huyghebaert, A., Raes, K., … Herman, L. (2014). Consumption of raw or heated milk from different species: an evaluation of the nutritional and potential health benefits. *Food Control*, *42*, 188–201. doi:10.1016/j.foodcont.2014.01.045

Eissa, E. A., Mohamed, A. I. A., Yagoub, A. E. A., & Babiker, E. E. (2010). Physicochemical, microbiological and sensory characteristics of yoghurt produced from goat milk. *Livestock Research for Rural Development*, *22*. Retrieved from http://www.lrrd.org/lrrd22/8/eiss22137.htm

Eissa, E. A., Yagoub, A. E. A., Babiker, E. E., & Ahmed, I. A. M. (2011). Physicochemical, microbiological and sensory characteristics of yoghurt produced from camel milk during storage. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *10*(6), 2305–2313.

Farah, Z. (1996). *Camel milk: properties and products*. St Gallen: Swiss Centre for Development Cooperation in Technology and Management.

Farah, Z. (2011). Encyclopedia of dairy sciences (2nd ed.) In J.W. Fuquay, P.F. Fox & P.L.H. McSweeney (Ed.), (Chap. Camel milk, pp. 512–517). Amsterdam: Elsevier Ltd.

Faye, B. & Bonnet, P. (2012). Camel sciences and economy in the world: current situation and perspectives. In *3rd Conference of International Society of Camelid Research and Development, held at Muscat, Sultanate of Oman, January-1 February 2012 (pp. 2-15. 29). Muscat: Sultan Qaboos University*.

Gaddour, A., Najari, S., & Abdennebi, M. (2013). Yoghurt produced from goat milk. *Journal of Animal and Veterinary Advances*, *12*(24), 1700–1703.

Habtegebriel, H. & Admassu, S. (2016). Optimization of the processing conditions of stirred yoghurt from camel milk using linear programming technique. *Food Science and Quality Management*, *53*, 33–38. Re-

trieved from http://iiste.org/Journals/index.php/FSQM/article/view/31687/0

Haddad, M. A., Al-Qudah, M. M., Abu-Romman, S. M., Obeidat, M., & El-Qudah, J. (2017). Development of traditional jordanian low sodium dairy products. *Journal of Agricultural Science*, *9*(1), 223–230. Retrieved from http://iiste.org/Journals/index.php/FSQM/article/view/31687/0

Hashim, I. B., Khalil, A. H., & Habib, H. (2009). Quality and acceptability of a set-type yogurt made from camel milk. *Journal Of Dairy Science*, *92*(3), 857–862. doi:10.3168/jds.2008-1408

Hassan, R. A., El Zubeir, I. E. M., & Babiker, S. A. (2007). Effect of pasteurization of raw milk and storage temperature on the chemical composition of fermented camel milk. *International Journal of Dairy Science*, *2*(2), 166–171. doi:10.3923/ijds.2007.166.171

Ibrahem, S. A. & El Zubeir, I. E. M. (2016). Processing, composition and sensory characteristic of yoghurt made from camel milk and camel-sheep milk mixtures. *Small Ruminant Research*, *136*, 109–112. doi:10.1016/j.smallrumres.2016.01.014

Ibrahim, A. H. & Khalifa, S. A. (2015). The effects of various stabilizers on physiochemical properties of camel's milk yoghurt. *Journal of American Science*, *11*(1), 15–24. Retrieved from http://www.jofamericanscience.org/journals/am-sci/am110115/003_27636am110115_15_24.pdf

Kavas, N. (2016). Yoghurt production from camel (camelus dramedarius) milk fortified with samphire molasses and different colloids. *Mljekarstvo*, *66*(1), 34–47.

Kgaudi, K. (2014). *Milk production potential and major browse species consumed by dromedary camels (camelus dromedarius) in tsabong area, southern kgalagadi district* (Doctoral dissertation, Botswana College of Agriculture, Gaborone, Botswana).

Khan, H., Athar, I. H., & Aslam, M. (2004). Evaluation of cheese prepared by processing camel milk. *Pakistan Journal of Zoology*, *36*(4), 323–326. Retrieved from https://www.yumpu.com/en/document/view/30039151/evaluation-of-cheese-prepared-by-processing-camel-zspcompk

Kiros, E., Seifu, E., Bultosa, G., & Solomon, W. K. (2016). Effect of carrot juice and stabilizer on the physicochemical and microbiological properties of yoghurt. *LWT-Food Science And Technology*, *69*, 191–196. doi:10.1016/j.lwt.2016.01.026

Ledenbach, L. H. & Marshall, R. T. (2009). Compendium of the microbiological spoilage of foods and beverages. In W. Sperber and M. Doyle (Ed.), (Chap. Microbiological spoilage of dairy products, pp. 41–46). New York: Springer Science + Business Media, LLC.

Mehaia, M. A. (1993). Fresh soft white cheese (domiati-type) from camel milk - composition, yield, and sensory evaluation. *Journal Of Dairy Science*, *76*(10), 2845–2855. doi:10.3168/jds.S0022-0302(93)77623-7

Mostert, J. & Jooste, P. (2002). Dairy microbiology handbook (3rd ed.) In R.K. Robinson (Ed.), (Chap. Quality control in the dairy industry, pp. 655–736). New York: John Wiley and Sons.

Mullaicharam, A. R. (2014). A review on medicinal properties of camel milk. *World Journal of Pharmaceutical Sciences*, *2*(3), 237–242. Retrieved from http://www.academia.edu/6245890/A_review_on_medicinal_properties_of_Camel_milk

Al-Numair, K. S., Chandramohan, G., & Alsaif, M. A. (2011). Influence of camel milk on glycoprotein components in streptozotocin-diabetic rats. *Journal Of Camel Practice And Research*, *18*(1), 15–20.

Al-Otaibi, M. & El-Demerdash, H. (2013). Nutritive value and characterization properties of fermented camel milk fortified with some date palm products chemical, bacteriological and sensory properties. *International Journal of Nutrition and Food Science*, *2*(4), 174–180. doi:10.11648/j.ijnfs.20130204.13

Ramet, J. P. (2001). The technology of making cheese from camel milk (camelus dromedarius). *Animal Production and Health Paper, No.113. Rome: Food and Agriculture Organization of the United Nations.*

Richardson, H. G. (1985). *Standard methods for the examination of dairy products (6th ed.)* Washington DC.: American Public Health Association.

Sahan, N., Yasar, K., & Hayaloglu, A. A. (2008). Physical, chemical and flavour quality of non-fat yogurt as affected by a beta-glucan hydrocolloidal composite during storage. *Food Hydrocolloids*, *22*(7), 1291–1297. doi:10.1016/j.foodhyd.2007.06.010

Al-Saleh, A. A., Metwalli, A. A. M., & Ismail, E. A. (2011). Physicochemical properties of probiotic frozen yoghurt made from camel milk. *International Journal Of Dairy Technology*, *64*(4), 557–562. doi:10.1111/j.1471-0307.2011.00699.x

Sharma, C. & Singh, C. (2014). Therapeutic value of camel milk – a review. *Advanced Journal of Pharmacie and Life Science Research*, *2*(3), 7–13. Retrieved from http : / / ajplronline . org / UploadFile / 635464294846827709.pdf

Shori, A. B. (2015). Camel milk as a potential therapy for controlling diabetes and its complications: a review of in vivo studies. *Journal Of Food And Drug Analysis*, *23*(4), 609–618. doi:10.1016/j.jfda.2015.02.007

Tamime, A. Y. (2006). *Fermented milks*. Oxford, UK: Blackwell Science Ltd.

Tamime, A. Y. & Robinson, R. K. (2000). *Yoghurt science and technology (2nd ed.)* Boca Raton, USA: Woodhead Publishing Ltd and CRC Press LLC.

Yagil, R. (1982). Camels and camel milk. *FAO Animal Production and Health Paper 26. Rome, Italy: Food and Agriculture Organization of the United Nations.*

Al-Zoreky, N. S. & Al-Otaibi, M. M. (2015). Suitability of camel milk for making yogurt. *Food Science And Biotechnology*, *24*(2), 601–606. doi:10.1007/s10068-015-0078-z

International Journal of Food Studies | IJFS October 2018 | Volume 7 | pages 64–75

Antioxidant Activities of Aqueous Extracts from Nine Different Rose Cultivars

Hamadia Khurshid[a], Syed Mubashar Sabir[a*], Shahid Iqbal Awan[b], Syed Rizwan Abbas[c], and Muhammad Irshad[d]

[a] Department of Chemistry, University of Poonch, Rawalakot, Azad Kashmir, Pakistan
[b] Department of Plant Breeding and Molecular Genetics, University of Poonch, Rawalakot, Azad Kashmir, Pakistan
[c] Department of Biological Sciences, Karakoram International University, Galgit Baltistan, Pakistan
[d] University of Kotli, Azad Jammu and Kashmir, Kotli, Pakistan
[*]Corresponding author
mubashersabir@yahoo.com
Tel: +923455171876
Fax: +9205824960007

Received: 3 October 2017; Published online: 18 October 2018

Abstract

Rose petals have been applied as food additives in teas, cakes and flavor extracts. The aim of this research study was to explore and reveal the antioxidant potential of aqueous extracts of rose petals belonging to nine genotypes of rose (wild as well as hybrid). The *in vitro* antioxidant activities of roses were studied by lipid peroxidation assay, DPPH radical scavenging assay, iron chelation assay, phosphomolybdenum reduction assay and total phenolic and flavonoid contents. The aqueous extract showed inhibition against lipid peroxidation (TBARS), induced by prooxidants (10 μM $FeSO_4$) in mice liver homogenate. The free radical scavenging activities of the extracts were determined by scavenging of the DPPH radical. Extracts also showed metal chelating activities and high antioxidant activity in the phosphomolybdenum assay. The high content of phenolics and flavonoids detected in aqueous extracts may be responsible for the antioxidant activity. Amongst the different rose genotypes, screened, *Rosa moschata* (musk rose) was found to carry slightly higher antioxidant potential, owing to its higher phytochemical content.

Keywords: Rose; Phenolics; Radical Scavenging Activity; Iron Chelation; Lipid Peroxidation

1 Introduction

Reactive oxygen species (ROS) are spontaneously generated in cells during metabolism and are implicated in the aeitology of different diseases, such as heart diseases, stroke, rheumatoid arthritis, diabetes and cancer (Halliwell, Gutteridge, & Cross, 1992). Oxidative stress is due to the decrease in natural cell antioxidant activity or due to an increased quantity of ROS in the organisms. It is well established that free radicals cause cell degeneration, especially in the liver (Shulman, Rothman, Behar, & Hyder, 2004). Normally, intracellular molecules including mitochondrial antioxidants prevent cellular damage produced by endogenous ROS. Previously it was proposed that the progression of cancer is strongly related to oxidative stress. Thus, the validation of antioxidant effect of tested plant material is nowadays routinely supplemented with the analysis of anticancer activities (Loizzo et al., 2014; da Costa

 10.7455/ijfs/7.2.2018.a6

et al., 2015). Antioxidants are the compounds that, when added to food products, act as radical scavengers, prevent the radical chain reactions of oxidation, delay or inhibit the oxidation process and increase shelf life by retarding the process of lipid peroxidation (Young & Woodside, 2001). Consumers are becoming more conscious of the nutritional value and safety of their food and ingredients. The preference for natural foods and food ingredients that are believed to be safer, healthier and less hazardous is increasing compared to their synthetic counterparts (Farag, Badei, Hewedi, & Elbaroty, 1989). A number of studies have shown that the use of phenolic compounds found in tea, fruits and vegetables is associated with the low risk of these diseases (Hertog, Hollman, & Vandeputte, 1993). Consequently, there is a growing interest in edible plants that contain antioxidants and phytochemicals as potential therapeutic agents. Foods are often contaminated with transition metal ions that may be introduced during processing. Bivalent transition metal ions catalyze the oxidative processes, resulting in the formation of hydroxyl radicals, in addition to hydroperoxide decomposition reactions, via the Fenton reaction (Wang & Fordham, 2007). These processes can be delayed by iron chelation and deactivation.

Rose is a woody perennial of the genus Rosa and belongs to the family Rosacea. There are over a hundred species, which are widely distributed in Europe, Asia, Middle East and North America. Rose flowers vary in size, shape and colors (Raj & Gupta, 2005). There are a number of studies from several decades ago on the chemical composition and antioxidant properties of rose in different countries of the world such as India, Chile, Iran, Turkey and Tunisia, since long (Yoshida, Wei-Sheng, & Okuda, 1993). Rose plants are used in perfumery and in food industry. Roses are known to be a rich source of polyphenolic compounds (Yoshida et al., 1993). Rose flowers, roots and leaves have been used in Chinese medicine to treat burns, injuries and rheumatic arthritis (Fenglin, Ruili, bao, & Liang, 2004). Rose flowers, petals and leaves have shown antioxidant activity. Some rose species also show antibacterial activities. Herbal teas of *R. moschata* studied in Chile have shown antioxidant properties (Speisky, Rocco, Carrasco, Lissi, & López-Alarcón, 2006). Rose hip (*Rosa cannina* L.) is the pseudo fruit of the rose plant which is a rich source of polyphenols and vitamin C (Fan, Pacier, & Martirosyan, 2014). Taif rose, Ward Taifi (*Rosa damascena trigintipetala* Dieck), is a type of Damask rose which is considered one of the most important economic products of Taif governorate, Saudi Arabia (Abdel-Hameed, Bazaid, & Salman, 2013). The genotype has a significant effect on the activity of bioactive compounds (Anttonen & Karjalainen, 2005). *Rosa damascena Mill* is rich in oil and is used for ornamental purposes (Rusanov et al., 2005). Along with *R. damascena*, other rose species, such as *Rosa centrifolia, Rosa gallica, Rosa alba* and *Rosa rugosa* show a similar chemical composition and are important therapeutically (Ranganna, 1986).

Therefore, it has been found that apart from the ornamental use of rose species, rose plants (petals, leaves and rose hip) have also been used in many countries of the world for their medicinal properties, both antioxidant and antimicrobial. The literature does not quote any studies carried out related to the properties of rose plants from Pakistan. Therefore, the present study is aimed to uncover the antioxidant properties of local rose species for their possible use in food and pharmaceuticals.

2 Materials and Methods

2.1 Chemicals

Thiobarbituric acid (TBA), malonaldehyde-bis-dimethyl acetal (MDA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, rutin, gallic acid, and phenanthroline were purchased from Sigma-Aldrich (St. Louis, MO, USA). Iron (II) sulfate was obtained from Lahore, Pakistan.

2.2 Preparation of plant extract

The petals of roses were collected from different areas of the district of Rawalakot Azad Kashmir during April-June, 2015 and identified by a taxonomist at the University of Poonch Rawalakot. The extracts were prepared following the method of Sabir et al. (2012). The petals of the plant

(25 g) were ground and soaked in boiling water (500 mL) for 15 minutes, allowed to cool and filtered using Whatman No. 1 filter paper. The resulting residue was further extracted twice and finally the whole extract was concentrated in a rotary evaporator (50 ^{o}C). Serial dilutions were prepared to obtain the desired concentration of plant for the experiments.

2.3 Test Animals

All animal procedures were done with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Poonch, Ethical Council (UPR 101). BALB/C mice (20–25g) were purchased from the National Institute of Health, Islamabad. The animals were housed in separate cages with access to water and food *ad libitum*, in a room with controlled temperature (22 $\pm$ 3 ^{o}C) and a 12h light/dark cycle.

2.4 Production of TBARS from animal tissues

Production of TBARS was determined using a modified method (Ohkawa, Ohishi, & Yagi, 1979). Chloroform was used to anesthetize the animals and sacrifice was by decapitation. The livers were immediately removed and placed on ice. Tissues (1:10, w/v) were homogenized in cold 100 mM Tris buffer pH 7.4 (1:10 w/v) and centrifuged at 1,000 x g for 10 minutes. The resulting homogenates (100 μL) were incubated with or without 50 μL of freshly prepared oxidant (iron) and different concentrations of the extracts together with the proper volume of deionized water to give a total volume of 300 μL at 37 ^{o}C for 1 h. The color reaction was done by adding 200, 500 and 500 μL each of the 8.1% Sodium dodecyl sulphate (SDS), acetic acid (pH 3.4) and 0.6% TBA, respectively. The reaction mixtures, including those of serial dilutions of 0.03 mM standard MDA (1.5-9 nM), were incubated at 97 ^{o}C for 1 h. The absorbance of tubes was read after cooling at a wavelength of 532 nm in a spectrophotometer.

2.5 DPPH radical scavenging activity

Scavenging of the stable DPPH radical (ethanolic solution of 0.25 mM) was assayed *in vitro* by the method of Hatano et al., (1988). Briefly, a 0.25 mM solution of the DPPH radical (0.5 mL) was added to extract sample solution in ethanol (1 mL) at concentrations from 25-200 μg/mL. The mixture was shaken vigorously and left to stand for 30 min in the dark, after which the absorbance was measured (Spectronic D-20; Thermo Scientific) at 517 nm. The capacity to scavenge the DPPH radical was calculated as:
DPPH radical scavenging (%) = [(Ao-A1)/Ao)] $\times$ 100

where Ao is the absorbance of the control reaction and A1 is the absorbance of the sample reaction. All determinations were carried out in triplicate.

2.6 Antioxidant potential assay

The total antioxidant potential of the extracts was estimated using the phosphomolybdenum reduction assay of Prieto, Pineda, and Aguilar (1999). The assay is based on the reduction of molybdenum, Mo (VI)-Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. The extracts (25-200 μg/mL) were mixed with 3 mL of the reagent solution (0.6 M H2SO4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 ^{o}C for 90 mins. The mixture was cooled to room temperature and the absorbance of the solution was measured at 695 nm.

2.7 Metal chelating activity

The iron chelating ability of the extract was determined using a modified method of Puntel et al., (2005). Briefly, 150 μL of freshly prepared 2 mM FeSO4·7H2O was added to a reaction mixture containing 168 μL of 0.1 M Tris-HCl (pH 7.4), 218 μL of saline, and plant extracts at concentrations of 25-200 μg/mL. The reaction mixture was incubated for 5 min before addition of 13 μL of 0.25% 1,10-phenanthroline (w/v). The

absorbance was subsequently measured at 510 nm using a spectrophotometer (Spectronic D-20; Thermo Scientific).

2.8 Determination of Phenolic content

The total phenolic content was determined by the method of Singleton, Orthofer, and Lamuela-Raventós (1999). Extracts (0.5 mL) were added to 2.5 mL of 10% Folin-Ciocalteu's reagent (v/v) and 2 mL of 7.5% sodium carbonate. The reaction mixture was incubated at 45 ^{o}C for 40 minutes and the absorbance was measured at 765 nm using a spectrophotometer (Spectronic D-20; Thermo Scientific). Gallic acid was used as a standard phenol. The total phenolic content was expressed as mg of gallic acid equivalents/g of extract.

2.9 Determination of total flavonoids

The total flavonoid content as quercetin equivalents/g extract was based on the method of Kosalec, Pepeljnjak, Bakmaz, and Vladimir-Knežević (2004). Quercetin was used for preparation of a calibration curve (0.04, 0.02, 0.0025, and 0.00125mg/mL in 80% (v/v) ethanol). Standard solutions or extracts (0.5 mL) were mixed with 1.5 mL of 95% (v/v) ethanol, 0.1mL of 10% (w/v) aluminum chloride, 0.1 mL of 1 mol/L sodium acetate, and 2.8 mL of water. The same volume of distilled water was substituted for 10% aluminum chloride in a blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer (Spectronic, D-20; Thermo Scientific). The total flavonoid content was expressed as mg of quercetin equivalents/g of extract

2.10 Data analysis

The results were expressed as means ± SD. The data were analyzed by one-way ANOVA and different group means were compared by applying Duncan's multiple range test (DMRT); $p<0.05$ was considered significant in all cases. The software package, Statistica was used for statistical analysis.

3 Results and Discussions

3.1 Antilipid peroxidative properties of roses

The present study was designed to investigate the antioxidant activity of different genotypes of roses. Lipid peroxidation in mice liver was induced with iron (10 μM) and the antioxidant effect of rose extracts was determined. There was a statistically significant increase ($P<0.05$) in the formation of TBARS in ferrous sulphate (81%) liver homogenate compared to the basal or normal (Fig. 1a). However, treatment with roses caused a concentration dependent inhibition ($P<0.05$) of TBARS production and brought the values close to the basal level (Fig. 1a and 1b). Fig. 1(a) shows that all the genotypes were effective in decreasing the level of lipid peroxidation (TBARS). However, certain genotypes like *R. moschata, R. hybrida* (tea pink), *R. hybrida* (white) showed a higher percentage of TBARS reduction compared to the control. In Fig. 1(b) different genotypes of roses such as *R. demascena, R. hybrida* (orange) and *R. hybrida* (pink yellow) showed a higher percentage decrease of lipid peroxidation.

Oxidative stress is now found to be associated with more than 200 diseases which include the normal aging process (Ghazanfari et al., 2006). There is a strong correlation between thiobarbituric acid-reactive substances (TBARS) as a marker of lipid peroxidation and products that reflect oxidative damage to DNA (Chen, Wu, & Huang, 2005). It is well established that metal-catalysed generation of ROS results in an attack to DNA and proteins, but also on important cellular components which involve polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Shacter, 2000). Iron overload is a less frequent condition, but high contents of iron in tissues is responsible for different pathological conditions, including liver, heart diseases, cancer and neurodegenerative disorders (Milman et al., 2001). The protection of-

fered by the aqueous extract of roses in mice liver homogenates confirms the antioxidant activity of the extract and indicates its use in accidental toxicities resulting from the overload of iron

3.2 DPPH radical scavenging activity of roses

The radical scavenging activity of the extract was tested against important *in vitro* models of free radicals namely DPPH (Fig. 2). The role of an antioxidant is to remove free radicals. Among the genotypes *R. moschata, R. hybrida* (tea pink yellow), *R. hybrida* (tea pink), *R. hybrida* (white), *R. hybrida* (yellow) and *R. demascena* showed higher potential in reducing the DPPH radical (Fig. 2). Antioxidants neutralize free radicals and their negative effects. They act at different stages (prevention, interception and repair) and by different mechanisms: reducing agents by donating hydrogen, quenching singlet oxygen, acting as chelators and trapping free radicals (Devasagayam et al., 2004). $DPPH^{\bullet}$ is a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is started by lipid autoxidation. Antioxidants react with $DPPH^{\bullet}$ and reduce the number of DPPH free radicals to the number of their available hydroxyl groups. Therefore, absorption at 517 nm is proportional to the amount of residual $DPPH^{\bullet}$. It is visually noticeable as a discoloration of $DPPH^{\bullet}$ from purple to yellow. The rose extracts also showed high radical scavenging activity which justifies their use in diseases arising from free radical attack.

3.3 Iron chelating ability of roses

The effect of rose extracts on iron chelation is shown in Fig. 3. Extracts exhibited strong chelating abilities in a dose-dependent manner (Fig. 3). Foods are often contaminated with transition metal ions during processing (Morgan, 1999). Bivalent metal ions speed up the oxidative processes, which results in the formation of hydroxyl radicals and hydroperoxide decomposition, via the Fenton reaction (Wang & Fordham, 2007). These processes can be delayed by iron chelation and deactivation by the extract. The ability of the extracts to chelate iron was measured as a percentage of iron chelating. Chelating agent disrupts the formation of complexes with 1,10-phenathroline and iron which leads to a decrease in color intensity. For normal health physiology, metals are necessary. On the other hand, metals can cause serious health complications. Transition metals such as iron, zinc and copper make complexes in biological systems. During complex formation there is a generation of ROS in the cells leading to metal toxicity. Metal toxicity can be treated by the chelation therapy. In this process the metal ions are chelated, and toxic and excess metal ions are removed from the system and reduce the effect. Oxidative stress caused by ferrous ions leads to many diseases such as Alzheimer's syndrome which is a neurological disorder (Ebrahimzadeh, Pourmorad, & Bekhradnia, 2008). Many types of metal chelators are available for toxic metal chelation. But selection of ideal chelators is very difficult. Metal chelators should be specific and properly administrated (Flora & Pachauri, 2010). Plants naturally contain phytochemicals such as phenols and flavonoids, which are responsible for the chelation of the metals and also prevent lipid peroxidation (Khan et al., 2014).

3.4 Total antioxidant activities of roses

The total antioxidant activity of the extract (equivalent to ascorbic acid) was found to be 50.26 ± 2.5 μg/mL at a maximal concentration (200 μg/mL) and increased with increasing concentrations of extract (Fig. 4). *R. hybrida* (yellow) showed the highest antioxidant activity while, *R. hybrida* (white) showed the least antioxidant activity in the phosphomolydenum reduction assay. In this assay, which measures total antioxidant capacity, the extract demonstrated electron-donating capacity showing its ability to act as a chain terminator, transforming reactive free radical species into more stable non-reactive products (Dorman, Kosar, Kahlos, Holm, & Hiltunen, 2003).

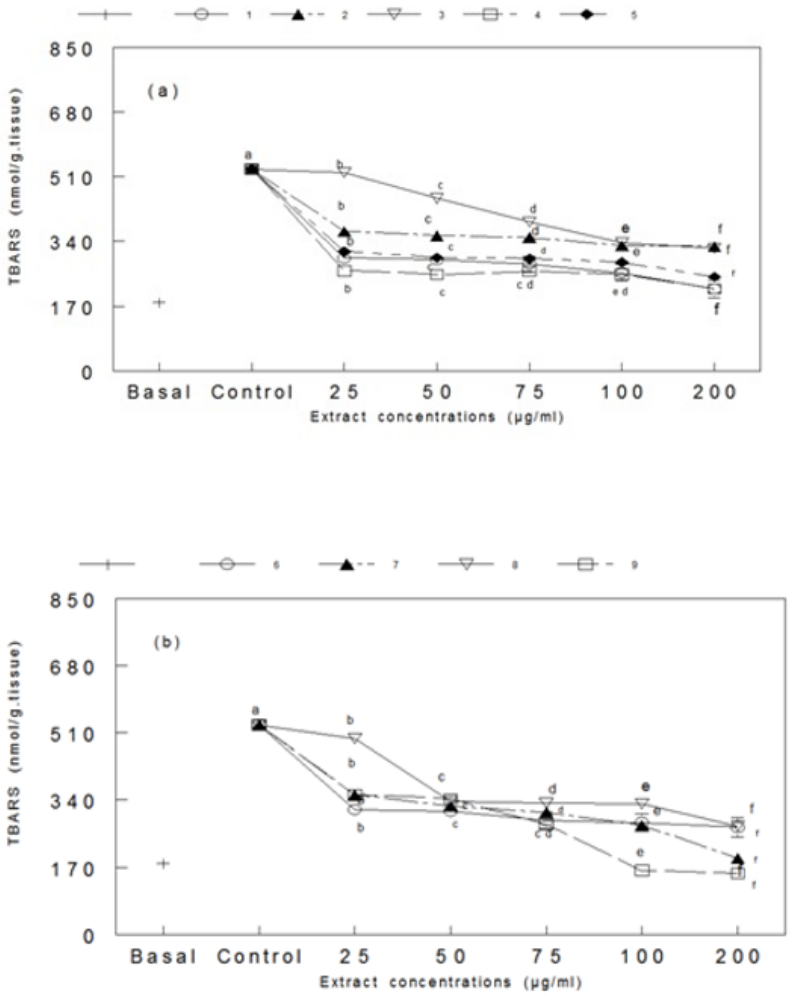

Figure 1: Inhibitory effect of Rose genotypes on lipid peroxidation induced by 10 μM Fe(II) in mice liver homogenate. (a) inhibitory effect of Rose genotypes on ferrous sulphate (Fe) induced lipid peroxidation in mice liver. 1= *R. moschata*, 2= *R. hybrida* (red), 3= *R. hybrida* (tea pink-yellow), 4= *R. hybrida* (tea pink), 5= *R. hybrida* (white) (b). inhibitory effect of Rose genotypes on ferrous sulphate (Fe) induced lipid peroxidation in mice liver. 6= *R. hybrida*(yellow), 7= *R. demascena*, 8= *R. hybrida* (orange), 9= *R. hybrida* (pink yellow). Values represent the means of three separate experiments in duplicate ± SD. $p<0.05$ is significantly different from control by DMRT. Values with different letters are significantly ($p<0.05$) different from each other by DMRT.

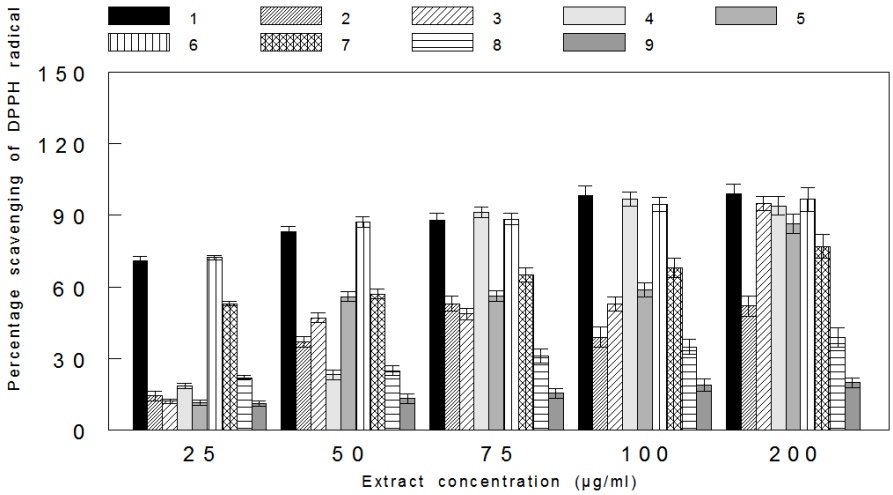

Figure 2: DPPH radical scavenging activities of Rose genotypes. 1= *R. moschata*, 2= *R. hybrida* (red), 3= *R. hybrida* (tea pink-yellow), 4= *R. hybrida* (tea pink), 5= *R. hybrida* (white), 6= *R. hybrida* (yellow), 7= *R. demascena*, 8= *R. hybrida* (orange), 9= *R. hybrida* (pink yellow). Values are means±SD (n=3).

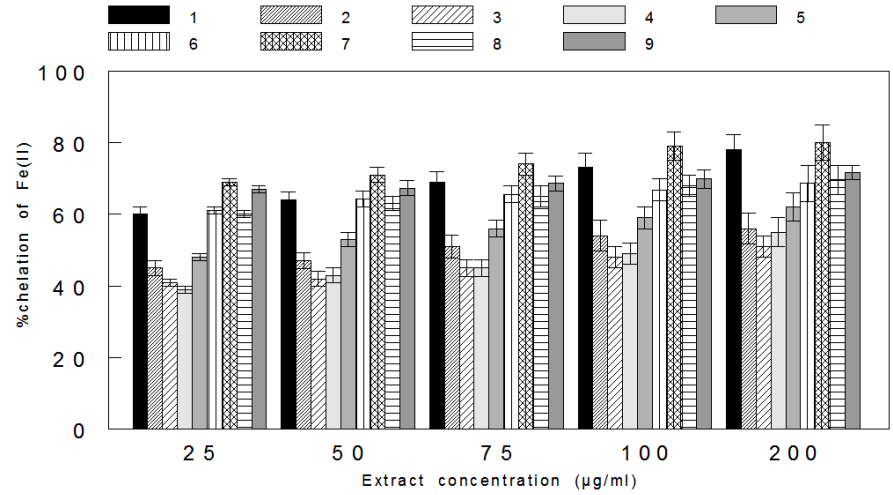

Figure 3: Iron chelating abilities of Rose genotypes. 1= *R. moschata*, 2= *R. hybrida* (red), 3= *R. hybrida* (tea pink-yellow), 4= *R. hybrida* (tea pink), 5= *R. hybrida* (white), 6= *R. hybrida* (yellow), 7= *R. damascena*, 8= *R. hybrida* (orange), 9= *R. hybrida* (pink yellow). Values are means±SD (n=3).

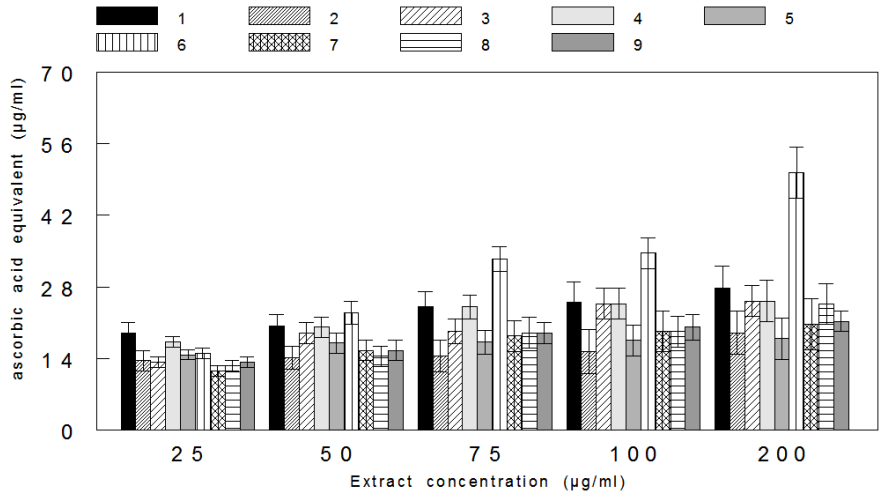

Figure 4: Total antioxidant activity of Rose genotypes as assessed by the phosphomolybdenum assay. 1= *R. moschata*, 2= *R. hybrida* (red), 3= *R. hybrida* (tea pink-yellow), 4= *R. hybrida* (tea pink), 5= *R. hybrida* (white), 6= *R. hybrida* (yellow), 7= *R. damascena*, 8= *R. hybrida* (orange), 9= *R. hybrida* (pink yellow). Values are means±SD (n=3).

Table 1: Total phenolic (*GAEA mg/g) and flavonoid contents (*Quer^B mg/g) among different genotypes of rose

Genotype of rose	Phenolic content (mg/g extract)	Flavonoid content (mg/g extract)
R. moschata	91.195±1.2^a	8.04±0.1^a
R. hybrida (red)	82.22±1.1^b	7.56±0.12^b
R. hybrida (tea pink-yellow)	77.51±0.89^c	7.20±0.2^c
R. hybrida (tea pink)	69±1^d	6.9v0.23^d
R. hybrida (white)	67.39±1.4^e	6.73±0.1^e
R. hybrida (yellow)	67.275±0.56^f	6.72±0.3^{ef}
R. damascena	49.45±0.34^g	4.95±0.1^g
R. hybrida (orange)	42.09±1.1^h	4.20±0.04^h
R. hybrida (pink yellow)	39.1±0.82^i	3.91±0.02^i

*GAE^A is gallic acid equivalent, *Quer^B is quercetin equivalent, Values with different letters are significantly (P<0.05) different from each other by DMRT.

3.5 Total phenolic and flavonoid contents

Table 1 shows the total phenolic and flavonoid content of roses. The phenolic content ranged between 39.1±0.82 to 91.195±1.2 mg/g gallic acid equivalents. *R. moschata* contained the highest amount of phenolics whereas, *R. hybrida* (pink yellow) contained the least. The total flavonoid content ranged between 3.91±0.02 to 8.04±0.1 mg/g quercetin equivalent. *R. moschata* contained the highest amount of flavonoid whereas, *R. hybrida* (pink yellow) contained the least. Plant-derived polyphenolic flavonoids are well known for exhibiting antioxidant activity through a variety of mechanisms including scavenging of ROS, inhibiting lipid peroxidation and chelating metal ions (Shahidi, 1997). The high content of phenolics and flavonoids in the extracts of plants contributes to the antioxidant activity. Joo, Kim, and Lee (2010) studied the secondary metabolites of white rose flower extract. Biological activities (antimicrobial and antioxidant properties) were determined in the extracts. Low molecular weight secondary metabolites of the white rose flower extracts were found effective in reproductive processes and showed resistance against environmental stresses and pathogens. The extracts of *Rosa rugosa* (white rose) flowers contained many volatile and phenolic compounds. These compounds were isolated, and their medicinal value was evaluated, in order to apply them for pharmaceutical purposes. The white rose flower extracts scavenged free radicals depending upon their concentration. Uggla, Gao, and Werlemark (2003) reported 90.5 mg/g phenolic content in rose species while Nowak and Gawlik-Dziki (2006) reported 83.4 mg/g phenolic content in rose petals which is in agreement with our studies. The total flavonoid content in methanolic rose extracts was reported to vary from 3.6-23.7 mg/g of extract (Li et al., 2014).

4 Conclusion

In conclusion, the aqueous extract of roses possesses anti-lipid peroxidative and free radical scavenging activities which may be associated with its high medicinal use as a functional food. The DPPH radical scavenging activities as well as the protective activities against lipid peroxidation, lead us to propose rose petal as a promising natural source of antioxidants suitable for application in the food and pharmaceutical fields and in the prevention of free radical-mediated diseases.

Acknowledgements

We are thankful to the Department of Chemistry, University of Poonch, Rawalakot, A.K. Pakistan for providing the necessary funds to carry out this research.

References

Abdel-Hameed, E.-S. S., Bazaid, S. A., & Salman, M. S. (2013). Characterization of the phytochemical constituents of taif rose and its antioxidant and anticancer activities. *Biomed Research International*. doi:10.1155/2013/345465

Anttonen, M. J. & Karjalainen, R. O. (2005). Environmental and genetic variation of phenolic compounds in red raspberry. *Journal of Food Composition and Analysis*, *18*(8), 759–769. doi:10.1016/j.jfca.2004.11.003

Chen, H. J. C., Wu, C. F., & Huang, J. L. (2005). Measurement of urinary excretion of 5-hydroxymethyluracil in human by gc/nici/ms: correlation with cigarette smoking, urinary tbars and etheno dna adduct. *Toxicology Letters*, *155*(3), 403–410. doi:10.1016/j.toxlet.2004.11.009

da Costa, L. S., Andreazza, N. L., Cerrea, W. R., da Silva Cunha, I. B., Tasca Gois Ruiz, A. L., de Carvalho, J. E., ... Salvador, M. J. (2015). Antiproliferative activity, antioxidant capacity and chemical composition of extracts from the leaves and stem of chresta sphaerocephala. *Revista Brasileira De Farmacognosia-brazilian Journal of Pharmacognosy*, *25*(4), 369–374. doi:10 . 1016/j.bjp.2015.04-005

Devasagayam, T. P. A., Tilak, J. C., Boloor, K. K., Sane, K. S., Ghaskadbi, S. S., & Lele, R. D. (2004). Free radicals and antioxidants in human health: current status and future prospects. *Journal of the Association of Physicians of India*, *52*(11), 794–804. Retrieved from http://www.japi.org/october2004/R-794.pdf

Dorman, H. J. D., Kosar, M., Kahlos, K., Holm, Y., & Hiltunen, R. (2003). Antioxidant properties and composition of aqueous extracts from mentha species, hybrids, varieties, and cultivars. *Journal of Agricultural and Food Chemistry*, *51*(16), 4563–4569. doi:10.1021/jf034108k

Ebrahimzadeh, M. A., Pourmorad, F., & Bekhradnia, A. R. (2008). Iron chelating activity, phenol and flavonoid content of some medicinal plants from iran. *African Journal of Biotechnology*, *7*(18), 3188–3192.

Fan, C., Pacier, C., & Martirosyan, D. M. (2014). Rose hip (rosa canina l): a functional food perspective. *Functional Foods in Health and Disease*, *4*(12), 493–509.

Farag, R. S., Badei, A., Hewedi, F. M., & Elbaroty, G. S. A. (1989). Antioxidant activity of some spice essential oils on linoleic-acid oxidation in aqueous-media. *Journal of the American Oil Chemists Society*, *66*(6), 792–799. doi:10.1007/BF02653670

Fenglin, H., Ruili, L., bao, H., & Liang, M. (2004). Free radical scavenging activity of extracts prepared from fresh leaves of selected chinese medicinal plants. *Fitoterapia*, *75*(1), 14–23. doi:10.1016/j.fitote.2003.07.003

Flora, S. J. S. & Pachauri, V. (2010). Chelation in metal intoxication. *International Journal of Environmental Research and Public Health*, *7*(7), 2745–2788. doi:10.3390/ijerph7072745

Ghazanfari, G., Minaie, B., Yasa, N., Nakhai, L. A., Mohammadirad, A., Nikfar, S., … Abdollahi, M. (2006). Biochemical and histopathological evidences for beneficial effects of satureja khuzestanica jamzad essential oil on the mouse model of inflammatory bowel diseases. *Toxicology Mechanisms and Methods*, *16*(7), 365–372. doi:10.1080/15376520600620125

Halliwell, B., Gutteridge, J. M. C., & Cross, C. E. (1992). Free-radicals, antioxidants, and human-disease - where are we now. *Journal of Laboratory and Clinical Medicine*, *119*(6), 598–620.

Hertog, M. G. L., Hollman, P. C. H., & Vandeputte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *Journal of Agricultural and Food Chemistry*, *41*(8), 1242–1246. doi:10.1021/jf00032a015

Joo, S. S., Kim, Y.-B., & Lee, D. I. (2010). Antimicrobial and antioxidant properties of secondary metabolites from white rose flower. *Plant Pathology Journal*, *26*(1), 57–62. doi:10.5423/PPJ.2010.26.1.057

Khan, A., Sabir, S. M., Nazar, H., Hamid, A., Usmanghani, K., & Ali, H. (2014). Antioxidant activities and inhibitory effects of dietary plants against sodium nitroprusside induced lipid peroxidation in the mouse brain and liver. *Food Science and Biotechnology*, *23*(4), 1305–1311. doi:10.1007/s10068-014-0179-0

Kosalec, I., Pepeljnjak, S., Bakmaz, M., & Vladimir-Knežević, S. (2004). Quantitative analysis of the flavonoids in raw propolis from northern croatia. *Acta Pharmaceutica*, *54*(1), 65–72. Retrieved from http://widgets.ebscohost.com/prod/customerspecific/ns000290/authentication/index.php?url=https%3a%2f%2fsearch.ebscohost.com%2flogin.aspx%3fdirect%3dtrue%26AuthType%3dip%2ccookie%2cshib%2cuid%26db%3dedselc%26AN%3dedselc.2-52.0-1942520353%26lang%3dpt-br%26site%3deds-live%26scope%3dsite

Li, L., Ham, H., Sung, J., Kim, I., Jeongand, H., & Lee, J. (2014). Antioxidant activities of methanolic extracts from four different rose cultivars. *Journal of Food and Nutrition Research*, *2*(2), 69–73. doi:10.12691/jfnr-2-2-2

Loizzo, M. R., Rashed, K., Said, A., Bonesi, M., Menichini, F., & Tundis, R. (2014). Antiproliferative and antioxidant properties of alhagi maurorum boiss (leguminosae)

aerial parts. *Industrial Crops and Products*, *53*, 289–295. doi:10.1016/j.indcrop.2013.12.049

Milman, N., Pedersen, P., Steig, T. A., Byg, K. E., Graudal, N., & Fenger, K. (2001). Clinically overt hereditary hemochromatosis in denmark 1948-1985: epidemiology, factors of significance for long-term survival, and causes of death in 179 patients. *Annals of Hematology*, *80*(12), 737–744. doi:10.1007/s002770100371

Morgan, J. N. (1999). Effects of processing on heavy metal content of foods. In L. S. Jackson, M. G. Knize, & J. N. Morgan (Eds.), *Impact of processing on food safety* (pp. 195–211). Boston, MA: Springer US. doi:10.1007/978-1-4615-4853-9_13

Nowak, R. & Gawlik-Dziki, U. (2006). Polyphenols of rosa l. leaves extracts and their radical scavenging activity. *Zeitschrift für Naturforschung C*, *62*(1-2), 32–38. doi:10.1515/znc-2007-1-206

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal-tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351–358. doi:10.1016/0003-2697(79)90738-3

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin e. *Analytical Biochemistry*, *269*(2), 337–341. Retrieved from http://widgets.ebscohost.com/prod/customerspecific/ns000290/authentication/index.php?url=https%3a%2f%2fsearch.ebscohost.com%2flogin.aspx%3fdirect%3dtrue%26AuthType%3dip%2ccookie%2cshib%2cuid%26db%3dedselc%26AN%3dedselc.2-52.0-0344348886%26lang%3dpt-br%26site%3deds-live%26scope%3dsite

Raj, D. & Gupta, P. K. (2005). Standardizing dehydration technology for ornamental herbaceous plants from outer himalayas. *Journal of Ornamental Horticulture*, *8*(1), 53–55.

Ranganna, S. (1986). *Handbook of analysis and quality control for fruit and vegetable products*. Tata McGraw-Hill Education.

Rusanov, K., Kovacheva, N., Vosman, B., Zhang, L., Rajapakse, S., & Atanassov, I., A .and Atanassov. (2005). Microsatellite analysis of rosa damascena mill. accessions reveals genetic similarity between genotypes used for rose oil production and old damask rose varieties. *Theoretical and Applied Genetics*, *111*(4), 804–809. doi:10.1007/s00122-005-2066-9

Sabir, S. M., Ahmad, S. D., Hamid, A., Khan, M. Q., Athayde, M. L., Santos, D. B., ... Rocha, J. B. T. (2012). Antioxidant and hepatoprotective activity of ethanolic extract of leaves of solidago microglossa containing polyphenolic compounds. *Food Chemistry*, *131*(3), 741–747. doi:10.1016/j.foodchem.2011.09.026

Shacter, E. (2000). Protein oxidative damage. In *Singlet oxygen, uv-a, and ozone* (Vol. 319, pp. 428–436). Methods in Enzymology.

Shahidi, F. (1997). Natural antioxidants, chemistry, health effects and applications. In F. Shahidi (Ed.), (Chap. Natural antioxidants: An overview, pp. 1–11). The American Oil Chemists Society.

Shulman, R. G., Rothman, D. L., Behar, K. L., & Hyder, F. (2004). Energetic basis of brain activity: implications for neuroimaging. *Trends in Neurosciences*, *27*(8), 489–495. doi:10.1016/j.tins.2004.06.005

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Oxidants and antioxidants part a* (Vol. 299, pp. 152–178). Methods in Enzymology. Academic Press. doi:10.1016/S0076-6879(99)99017-1

Speisky, H., Rocco, C., Carrasco, C., Lissi, E. A., & López-Alarcón, C. (2006). Antioxidant screening of medicinal herbal teas. *Phytotherapy Research*, *20*(6), 462. Retrieved from http://widgets.ebscohost.com/prod/customerspecific/ns000290/authentication/index.php?url=https%3a%2f%2fsearch.ebscohost.com%2flogin.aspx%3fdirect%3dtrue%26AuthType%3dip%2ccookie%2cshib%2cuid%26db%3dedb%26AN%3d64211910%26lang%3dpt-br%26site%3deds-live%26scope%3dsite

Uggla, M., Gao, X., & Werlemark, G. (2003). Variation among and within dogrose taxa (rosa sect. caninae) in fruit weight, percentages of fruit flesh and dry matter, and vitamin c content. *Acta Agriculturae Scandinavica, Section B-Plant Soil Science*, *53*(3), 147–155.

Wang, S. Y. & Fordham, I. M. (2007). Differences in chemical composition and antioxidant capacity among different genotypes of autumn olive (elaeagnus umbellate thunb.) *Food Technology and Biotechnology*, *45*(4), 402–409.

Yoshida, T., Wei-Sheng, F., & Okuda, T. (1993). Two polyphenol glycosides and tannins from rosa cymosa. *Phytochemistry*, *32*(4), 1033–1036. Retrieved from http://widgets.ebscohost.com/prod/customerspecific/ns000290/authentication/index.php?url=https%3a%2f%2fsearch.ebscohost.com%2flogin.aspx%3fdirect%3dtrue%26AuthType%3dip%2ccookie%2cshib%2cuid%26db%3dedselc%26AN%3dedselc.2-52.0-0008479119%26lang%3dpt-br%26site%3deds-live%26scope%3dsite

Young, I. S. & Woodside, J. V. (2001). Antioxidants in health and disease. *Journal of Clinical Pathology*, *54*(3), 176–186. doi:10.1136/jcp.54.3.176

International Journal of Food Studies | IJFS October 2018 | Volume 7 | pages 76–88

Extraction Kinetics of Saponins from Quinoa Seed (*Chenopodium quinoa* Willd)

R. M. Torrez Irigoyen[a,b] and S. A. Giner[a,b,c*]

[a] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA). Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 47 y 116 (1900)- La Plata, Provincia de Buenos Aires, Argentina
[b] Facultad de Ingeniería, Universidad Nacional de La Plata
[c] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina
[*]Corresponding author
saginer@ing.unlp.edu.ar

Received: 12 December 2017; Published online: 18 October 2018

Abstract

Quinoa has higher protein content (11-16% m/m) and better amino acid profile than cereals and represents a valuable resource for healthy nutrition. The aim of this work was to study the saponins extraction kinetics during washing of soaked quinoa. The experimental curves of saponins content as a function of time was measured at water temperatures of 20, 40, 60, and 70^{o}C. A spectrophotometric method was proposed to determine total saponins content, while an unsteady state diffusional model was applied to this extraction problem, assuming strict internal control to the mass transfer rate. As a first analysis, the complete analytical solution for constant diffusion coefficient (D_{eff}) using the initial radius (R_0) provided an accurate predicted curve at each temperature. The diffusion coefficients (around 10^{-10} m^{2}s^{-1}), were correlated with temperature using an Arrhenius-type relationship to obtain an activation energy E_a of 16.9 kJ mol^{-1}. The preliminary values of E_a and preexponential factor (D_0) thus obtained were used as initial values of a second, more robust fitting where the whole dataset of saponins concentrations as a function of time for all temperatures. The Arrhenius equation was directly inserted into the diffusional solution. The following parameters were obtained: E_a= 17.2 kJ mol^{-1} and, D_0= 3.232×10^{7}m^2 s^{-1}, respectively with an overall r^2=0.985. Saponins content agreed well with experimental values. As the equation is capable of predicting saponin extraction times for various operating conditions, it can be used within equipment design schemes.

Keywords: Quinoa; Saponins; Diffusive model; Spectrophotometric method

1 Introduction

The quinoa (*Chenopodium quinoa* Willd) is an ancient crop of the Andean region of South America. It is considered a pseudo-cereal with high levels of protein and a good balance of essential amino acids (Food and Agriculture Organization, 2011; Escuredo, Martin, Moncada, Fischer, & Hierro, 2014; Navruz-Varli & Sanlier, 2016). In addition, the lipid fraction is rich in essential fatty acids, such as linoleic and α-linolenic acids (Navruz-Varli & Sanlier, 2016; Peiretti, Gai, & Tassone, 2013) and, besides, quinoa has a high minerals content particularly calcium, magnesium, potassium, phosphorus and manganese, with high amounts of iron as well (Vega-Galvez et al., 2010). Having no gluten, quinoa is a food of choice for celiacs (Ridout, Price, Dupont, Parker, & Fenwick, 1991). On these grounds, in recent years, quinoa seeds have become important to an extent that FAO declared 2013 as the International Year of Quinoa (Food and Agriculture

 10.7455/ijfs/7.2.2018.a7

Nomenclature

D_{eff}	Effective diffusive coefficient, $m^2\ s^{-1}$
D_0	Pre-exponential factor, $m^2\ s^{-1}$
E_a	Activation energy, kJ mol^{-1}
n	Number of data points
r	radial position, m
R_g	Universal gas constant, 8.314 x 10^{-3} kJ $mol^{-1}K^{-1}$
R_0	Initial particle radius, m
S	saponins content, kg 100 kg dry $matter^{-1}$
t	Washing process time, s
T_w	Water temperature, in ^oC
T_k	Water absolute temperature, K
Greek symbols	
π	Dimensionless number
Subscripts	
0	Initial
e	Equilibrium
w	Water
m	average
d	Dimensionless
exp	Experimental
pred	Predicted

Organization, 2013).
However, these seeds have saponins, triterpenoid glycosides concentrated in the seed coat, that affect the taste of quinoa and protein digestibility, so they must be removed before consumption (Kozioł, 1992; Francis, Kerem, Makkar, & Becker, 2002). Traditionally, the saponins are removed by a solid-liquid extraction usually carried out domestically washing the seeds under running water with which saponins develop foam; as foam formation vanishes, washing is considered complete (Ridout et al., 1991). However, the saponins content that a human can consume in quinoa is still a topic of discussion in terms of its bitterness and negative biological effects (Chauhan, Eskin, & Tkachuk, 1992; Quispe-Fuentes et al., 2013). Recently, a standard was issued by the Codex alimentarius which establish a value of 0.12% m/m as a maximum limit to be considered convenient for consumption in quinoa with a moisture content of 13.5% w.b. which represents a 0.14% (m/m) in dry basis units (Codex Alimentarius, 2017).
Regarding the methodology for measuring the amount of saponins, Koziol (1991) developed a method based on measuring the foam height formed by adding quinoa seeds to water in a tube, which was agitated for a period of time. However, this method has some drawbacks because the production and stability of foam are dependent on the chemical structure and surfactant capacity of the saponins, presence of salts, pH and agitation method. Other authors, as San Martin and Briones (2000) and Quispe-Fuentes et al. (2013) utilized successfully the reversed-phase high performance liquid chromatography (RP-HPLC) to determine saponins. This is a well-established method in the pharmaceutical industry due to its high accuracy and precision. However, suitable standards of saponins are necessary for correct identification of the components and their quantitative determination (Ruales & Nair, 1993). On the other hand, Nickel, Spanier, Botelho, Gularte, and Helbig (2016) studied the effect of different types of processing (i.e.: washing, cooking and toasting) on the saponins content of quinoa and applied a spectrophotometric method to determine the total saponins content with good results. The basic principle of this method is the reaction of oxidized triterpene saponins with vanillin. Sulfuric acid is used as oxidant and the distinctive colour of the reaction is purple (Hostettmann & Marston, 1995). This method is simple, fast and inexpensive to

operate. However, some factors as the selection of standards and the optimum wavelength should be considered before applying this technique (Cheok, Salman, & Sulaiman, 2014).
Authors such as Ridout et al. (1991), Koziol (1991) and Nickel et al. (2016) investigated the effect of different treatments on the saponins content from quinoa using one of methods referred previously; nevertheless the information was limited only to the initial and final values for this compound. With respect to the saponin extraction kinetics few works were found in literature. Quispe-Fuentes et al. (2013) developed a diffusive model, considering spherical geometry, for the kinetics of saponins extraction in quinoa seeds during washing, obtaining accurate predictions. These authors utilized a chromatographic method for saponins quantification. It is possible that products experience swelling and thus structural modifications during washing (or soaking). From the technological point of view (for instance to develop equations of use in automatic control algorithms) simplified models as the analytical solutions of the unsteady state diffusion equation can be tested to represent the phenomenon (Torrez Irigoyen & Giner, 2014). Although information on the nutritional potential of quinoa has been published (Kozioł, 1992; Vega-Galvez et al., 2010; Navruz-Varli & Sanlier, 2016), only limited information is available on the kinetics of saponins extraction.
Therefore, the objective of this work is to study saponins extraction kinetics applying analytical solutions of the diffusion equation. Saponins were experimentally determined by a spectrophotometric technique. Knowledge of the kinetic behaviour may contribute to improve the understanding of the extraction mechanism and is useful for design purposes, since the diffusional model can be employed for estimating the extraction time to reach a safe saponins content for human consumption.

2 Materials and Methods

2.1 Material

Quinoa grains of the CICA variety, provided by the INTA EEA Famaillá, Provincia de Tucumán Argentina (Famaillá Experimental Station of the National Institute of Agricultural Technology, Province of Tucumán) were utilized. Moisture content at reception was 0.111 kg water kg dry matter^{-1}. The experimental work carried out can be described by the flow sheet shown in Fig. 1.

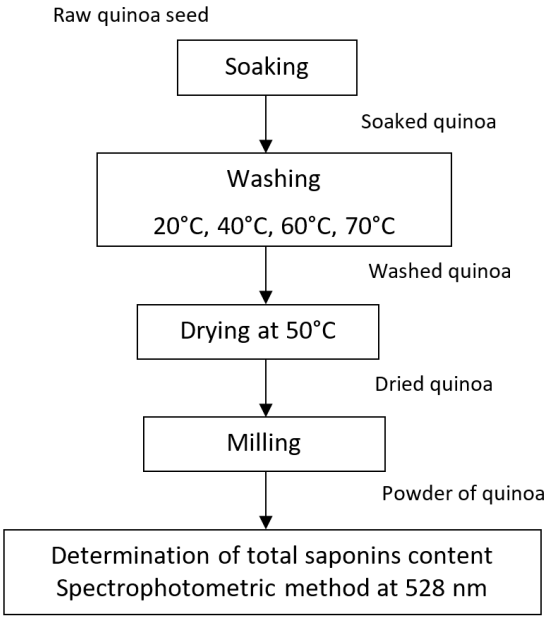

Figure 1: Flowsheet representing the experimental plan followed in this work

2.2 Soaking

Seeds were visually inspected to remove foreign materials and then immersed in distilled water, using a water-to-quinoa mass ratio of 5:1, and allowed to soak for 120 min. Figure 2 shows the characteristics dimensions for soaked quinoa seed measured by a digital caliber.

2.3 Washing

Experimental curves of quinoa moisture and total saponins content as a function of time were measured in a shaking water bath at 20, 40, 60 and 70°C. The grains were loaded in a steel bas-

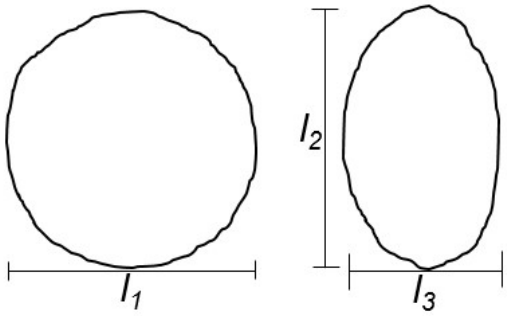

Figure 2: Drawing of front view and plan view of the quinoa seed and their axis measured utilizing a digital caliber. $l1$: 2.301 ± 0.113 mm, $l2$: 2.198 ± 0.077 mm and $l3$: 1.371 ± 0.408 mm

ket (initial mass of seeds: 60 ± 0.4 g) and immersed in the water bath. Samples were removed at various times between 5 and 70 min for moisture content and total saponins determination.

2.4 Drying

In order to obtain powder suitable for saponins content determination a thin layer of soaked and washed quinoa was dried by placing the seeds in a tray inside a mechanical convection oven set at 50^{o}C until reaching a final moisture content of 0.117 ± 0.001 kg water kg dry matter^{-1}. Moderate temperatures were considered for the drying step in order to avoid undesirable reactions such as Maillard (Brožková et al., 2018).

2.5 Determination of moisture content

Moisture content was determined in triplicate by the AOCS Ac 2-41 whole grain method (130^{o}C, atmospheric pressure, 3 h) in a mechanical convection oven (air velocity, 0.25 m s^{-1}) Sanjor Model SL30SDB, Argentina.

2.6 Milling

The dried samples were milled for three minutes in a grinder (Peabody, Pe-mc9100, China) and reduced to a powder sieved with a mesh size of 80 which correspond to a particle size of 0.177 mm.

2.7 Determination of saponins content by spectrophotometric analysis

For extraction of saponins, 2.5 g of sample was added to 25 mL of 50%(v/v) ethanol and left for 30 min at room temperature. Subsequently, the extracts were filtered through qualitative filter paper (grammage 80 g m^{-2}) into 25 ml glass volumetric flask and topped up to volume with 50% Ethanol. The analysis was carried out by adding 1 mL of the diluted extract (1:20 dilution) to 3.5 mL of the Lieberman-Buchard reagent (16.7% of acetic anhydride in concentrated sulfuric acid). The solution was vortexed and left to stand in the dark for 30 min at room temperature, before being placed in a spectrophotometer set at 528 nm. Quantification was performed with a standard of saponins provided by Biopack Chemical products. The calibration curve was performed at 50 – 350 μg mL^{-1} and the results were expressed as kg saponins per 100 kg dry matter (Gianna, Manuel Montes, Luis Calandri, & Alberto Guzman, 2012; Nickel et al., 2016). All measurements were carried out three times.

3 Mathematical modeling of saponins extraction

3.1 Microscopic mass balance with diffusional transport of mass

In general, taking the grain volume as a system and assuming mass transport by molecular diffusion, the microscopic mass balance can be expressed in the following way for constant volume of seed (Crank, 1975)

$$\frac{\partial S_I}{\partial t} = \nabla(D_{eff}\nabla S_I) \tag{1}$$

where D_{eff} is the effective diffusion coefficient of saponins relative to the dry matter. For radial water flux in spherical geometry, consider-

ing diffusion coefficient independent of saponins content, Eq. 1 can be developed to give (Pabis, Jayas, & Cenkowski, 1998).

$$\frac{\partial S_I}{\partial t} = D_{eff}\left(\frac{\partial^2 S_I}{\partial r^2} + \frac{2}{r}\frac{\partial S_I}{\partial r}\right) \quad (2)$$

This equation holds true for each internal point of the solid, and gives the local value of the diffusing component S_l kg saponins 100 kg dry matter^{-1} s^{-1} as a function of time t in seconds and the radial coordinate r in m, whose axis is always normal to the surface and whose origin is placed at the center of symmetry.

Initial and boundary conditions in mass transfer

The initial and boundary conditions to solve the partial differential equation Eq. 2, are the following: *Initial condition*:

$$t = 0 \ ; \ S_L = S_0 \ ; \ 0 \leq r \leq R_0 \quad (3)$$

where S_0 is the initial saponins content and R_0 is the initial seed radius (average value of 214 1.652 $\times$ 10^{-3} m). *Boundary condition in the particle centre*: The water flux is zero by symmetry

$$r = 0 \ ; \ \frac{\partial S_L}{\partial r} = 0 \ ; \ t > 0 \quad (4)$$

Boundary condition at the surface The surface boundary condition can be represented as follows:

$$r = R_0 \ ; \ S_s = S_e \ ; \ t > 0 \quad (5)$$

where S_s is the particular value of S_l at the surface. This represents that during washing the external resistance may be considered negligible compared to the internal, so a prescribed boundary condition could be proposed which means a strict internal control for mass transfer (Quispe-Fuentes et al., 2013).

Analytical solution of the diffusion equation

The unsteady state diffusion equation for spheres (Eq. 2), with the initial condition given by Eq. 3 and boundary conditions provided by equations 4 and 5, can be analytically solved after integration in the sphere volume to give the average saponins content S_m as a function of time (Quispe-Fuentes et al., 2013).

$$S_d = \frac{S_m - S_e}{S_0 - S_e} = \frac{6}{\pi^2}\sum_{n=1}^{n=\infty}\frac{1}{n^2}e^{-n^2\pi^2\frac{D_{eff}t}{R_0^2}} \quad (6)$$

where S_d is the dimensionless saponins content, S_e equilibrium saponins content in kg saponins 100 kg dry matter^{-1} and n number of data points. To implement the analysis, Eq. 6 was solved for S_m

$$S_m = S_e + (S_0 - S_e)\frac{6}{\pi^2}\sum_{n=1}^{n=\infty}\frac{1}{n^2}e^{-n^2\pi^2\frac{D_{eff}t}{R_0^2}} \quad (7)$$

With the purpose to determine the effective diffusion coefficient ten terms of the series was applied to fit the experimental data.

Dependence of the diffusion coefficient with temperature

With the aim of estimating the effect of temperature on D_{eff} the natural logarithms of the experimental diffusion coefficients were plotted as a function of the reciprocal of the water bath absolute temperature by means of an Arrhenius-type equation, as proposed in (Eq. 8)

$$LnD_{eff} = LnD_0 - \frac{E_a}{R_g T_k} \quad (8)$$

Where T_k stands for the absolute water temperature in K. Symbol D_0 represents the pre exponential factor in m^2 s^{-1}, while E_a is the activation energy in kJ mol^{-1}, being R_g the gas constant, 8.314 x10^{-3} kJ mol^{-1} K^{-1}.

3.2 Statistical analysis

Triplicate experiments were carried out for each determination, measuring saponins content. Different conditions were compared by the Tukey's test (Montgomery, 1991), at a confidence level of 95%. The goodness of fit was evaluated by two indicators, to have prediction errors expressed in the same units as the fitted variable, the root

mean square error ($RMSE$).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(Y_{pred,i} - Y_{exp,i})^2}{N}} \quad (9)$$

Where $y_{exp,i}$ stands for an experimental value and $y_{pred,i}$ represents the corresponding predicted number. The other statistical indicator is the coefficient of determination r^2, which was computed by using the following equation:

$$r^2 = 1 - \frac{\sum_{i=1}^{N}(Y_{exp,i} - Y_{pred,i})^2}{\sum_{i=1}^{N}(Y_{exp,i} - Y_m)^2} \quad (10)$$

Where y_m is the average value of the experimental data.

4 Results and discussion

4.1 Experimental saponins extraction curves

The Tukey's test of that three determination of the experimental saponins content did not present significant differences at a confidence level of 95% in all cases. Experimental extraction curves showing the average seed saponins content (S_m) as a function of time, in kg Saponins 100 kg dry matter^{-1} are presented in Fig. 2. The initial saponins content determined by the spectrophotometric method was 0.179 % (m/m) which correspond with the bitter variety of this seed, saponins content > 0.11 % m/m (Nickel et al., 2016). However, after the soaking process a value of 0.156 % (m/m) was measured. For sake of clarity, henceforth the values will be expressed in dry basis units, in this manner the 0.156% (m/m) becomes to 0.311% d.b. In this work this last value was considered as initial saponins content.

As observed in Fig. 3, saponins content falls with time in all cases with decreasing slope. This suggests an internal control for mass transfer as well as a gradual approach towards equilibrium values. As is shown in Fig. 3 the saponins content decrease faster during the first 5 min of process. From this time and until 40 min is observed a gradual decrease in the content reaching an equilibrium saponins content above this last process time. Besides, this decrease is faster at higher temperatures. Quispe-Fuentes et al. (2013) have reported a similar behavior during saponins extraction from quinoa seeds in a comparable temperature range. At the final process time the saponins content values are lower than the 0.140 % expressed in dry matter units proposed by the Codex Alimentarius (2017).

However, the scarce literature found agrees in that the effectiveness of saponins elimination can be expressed as a percentage of total extraction (Erramouspe, Armada, & Molina, 2013; Cheok et al., 2014). In this work, the final value for saponins content represents, in all experiments, an extraction percentage higher than 80% against the initial content ($S_{finalcontent}$/ $S_{initialvalue}$ *100). Nickel et al. (2016) used the spectrophotometric method and found a percentage of 17 % (p/p) for quinoa seeds washed during 15 min. On the other hand, authors as Ridout et al. (1991) and Ruales and Nair (1993) who analyzed the saponins content after different treatments have reported similar extraction percentages. However, these authors used the gas chromatography and HPLC methods, respectively for saponins quantification. It is difficult to compare the saponins content between different works because no official method exists so far (Codex Alimentarius, 2017).

The amount of elimination of saponins content obtained by different methods can be comparable. However, the absence of an official method to quantify these compounds in products intended for human consumption is not yet available, except for the recent standard established by the Codex Alimentarius.

4.2 Analytical series solution of diffusion

Table 1 lists the parameters resulting from the fitting of ten terms of the analytical series solution model (Eq. 7) to the data of Fig. 3 by a nonlinear least squares quasi newton method (Systat version 12, 2007). The table also includes statistical indices of goodness of fit. Values of r^2 and $RMSE$ are highly satisfactory.

Parameters of goodness of fit, r^2 and RMSE in Table reftable:1 indicate that the model (Eq. 7)

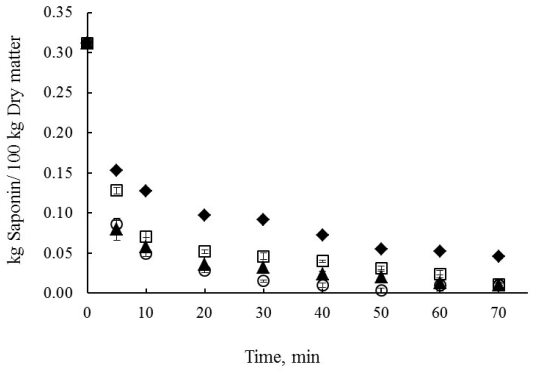

Figure 3: Experimental curves of quinoa saponins content as a function of time, at different water temperatures: (⧫) 20˚C, (□) 40˚C, (▲) 60˚C and (○) 70˚C

provides an adequate description of the experimental behavior (Eq. 7). The values for Effective diffusivity coefficient are in agreement with those presented by Abdelkader (1992) who studied the loss of glucose from potatoes and Varzakas, Leach, Israilides, and Arapoglou (2005) who developed a research on the theoretical and experimental approaches for the determination of solute effective diffusivities in foods. Figure 4 shows the predictions of Eq. 7 with the parameters of Table reftable:1 together with the experimental data.

The Fig. 4 shows a reasonably good prediction for all cases. The agreement between the experimental data and predicted values were obtained in spite of the assumptions made of constant diffusion coefficient and negligible shrinkage which are required to obtain the analytical solution (Eq. 7). The rapid elimination of saponins from quinoa seeds was favored by the previous stage of soaking, which is recommended by several authors with the purpose of facilitating the saponins extraction. Authors as Chauhan et al. (1992) and Nickel et al. (2016) attribute this effect to the hydration of particles, which allows water to penetrate in the particle, thus facilitating the release of saponins by diffusion. Secondly, the presence of saponins on the seed coat may also contribute to an easier removal of these compounds (Koziol, 1991; Vega-Galvez et al., 2010).

As the temperature increased, extraction of saponins was faster. However, for 60 and 70^{o}C, at the end of the process, the appearance of seeds became altered losing the seed coat and germ. Even when proteins and carbohydrates experience a high degree of hydration during washing. However, the total content of protein in quinoa seed varied between 10 and 13 % d.b. while the starch content in this product is five times as large, in the range of 58-66% d.b. (Kozioł, 1992; Food and Agriculture Organization, 2011; Srichuwong et al., 2017). From this point of view, it is possible to ascribe the loss of structure more to starch gelatinization than to protein denaturation. Authors as Kashaninejad, Maghsoudlou, Rafiee, and Khomeiri (2007) who investigated the hydration kinetics of a starchy grain as rice at different temperatures, suggested that soaking must be conducted at temperatures below that of starch gelatinization (65 -70^{o}C) to preserve kernel structure. For this reason, it is considered that a temperature of 50^{o}C should not be exceeded during the process (Jan, Panesar, Rana, & Singh, 2017; Li & Zhu, 2017). Besides, authors as Mota et al. (2016) who studied the loss of soluble solids (some proteins, minerals, some vitamins) during soaking in water at several temperatures for different seeds suggested that long periods of time in hot water may contribute to a higher reduction of water-soluble nutritional compounds in quinoa seeds.

4.3 Dependence of the diffusion coefficient with temperature

An Arrhenius-type Equation was fitted to data of the natural logarithm of the experimental diffusion coefficients (Ln D_{eff}) as a function of the reciprocal of the water absolute temperature of $1/T_k$, that is, (1/ (Tw+273.15)), by means of an Arrhenius-type equation (Eq. 7). The symbol T_w stands for the water temperature in ^{o}C. The E_a is a measure of the effect of temperature on the diffusion coefficient.

Predictions shown in Fig. 5 are in good agreement with the experimental coefficients. Results

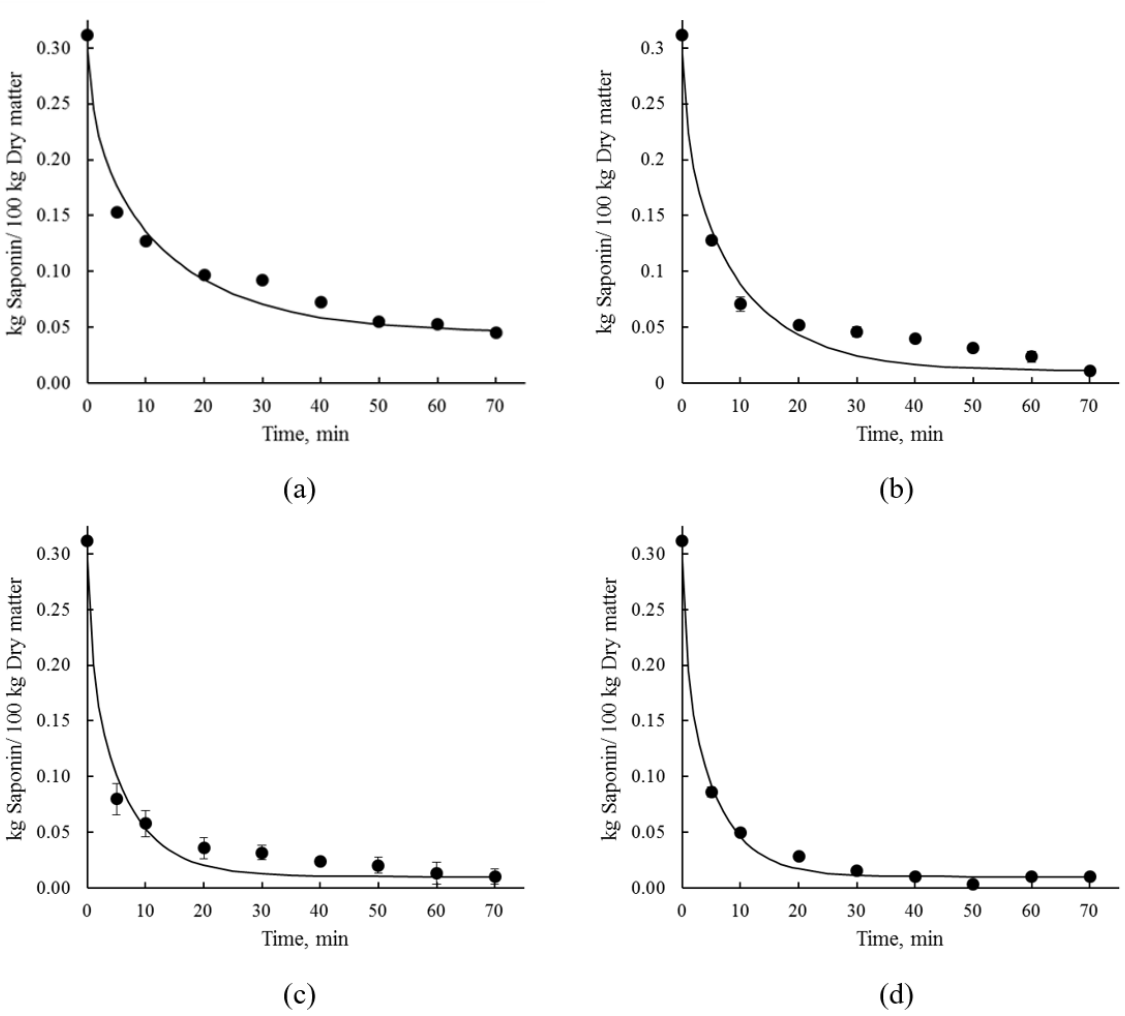

Figure 4: Saponins content curves from soaked quinoa during washing treatment. Experimental data (symbols), and predicted values (-) by f the analytical solution (Eq.6), for water temperatures of: (a) 20^{o}C, (b) 40^{o}C, (c) 60^{o}C and (d) 70^{o}C

Table 1: Effective diffusion coefficients of saponins in quinoa determined by fitting the Eq. 6 to experimental data and their Asymptotic Standard Estimation (S)

$T\ ^oC$	$D_{eff}\ \times\ 10^{-10}\ m^2/s$	$S(D_{eff})\ \times\ 10^{-11}$	r^2	$RMSE$
20	2.848	1.962	0.983	0.013
40	4.012	3.115	0.978	0.016
60	6.665	5.837	0.979	0.014
70	7.538	4.088	0.995	0.008

from the fitting of Eq.7 were: $E_a = 16.9$ kJ mol^{-1} and D_0 =2.875 × 10^{-7} m^2 s^{-1}, with a high coefficient of determination, r^2=0.987. The activation energy for saponins extraction was comparable to that obtained in a similar temperature range by Quispe-Fuentes et al. (2013) during saponins extraction and Chi et al. (2006) who studied the leaching of flavonoids from vegetables.

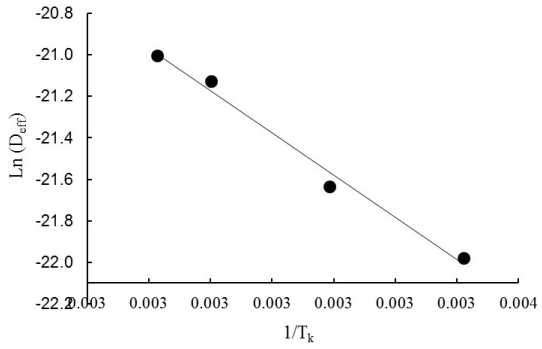

Figure 5: Dependency of the experimental diffusion coefficient with water temperature (symbols) together with predictions by an Arrhenius-type equation fitted to them (solid line)

4.4 A more accurate method to calculate the activation energy for saponins extraction process

In this section, a second method of fitting the analytical solution to whole dataset was tested to obtain an activation energy (E_a) that can be determined with more degrees of freedom. With this purposes the Eq.7 was rewritten in the following way:

$$S_m = S_e + (S_0 - S_e)\frac{6}{\pi^2}\sum_{n=1}^{n=\infty}\frac{1}{n^2}\cdot e^{-n^2\pi^2\frac{(D_0 e^{\frac{E_a}{8.314}\frac{1}{(T_w+273.15)}}t)}{R_0^2}} \quad (11)$$

The effective diffusion coefficient was replaced directly by the Arrhenius equation, Eq. 8 to find an overall activation energy (E_a)) and a pre-exponential factor (D_0). This equation was solved by a nonlinear least squares quasi newton method for whole dataset, using as initial values for E_a and D_0, those obtained previously in section 4.3.

An overall activation energy (E_a) of 17.2 (2.6) kJ mol^{-1} and a pre-exponential factor equal to 3.232×10^{-7} (3.234×10^{-9}) m^2 s^{-1} were estimated with a global coefficient of determination of 0.985 (Systat 12, 2007), which is higher than the average coefficient of determination of the fittings shown in Table reftable:1. The values presented between brackets correspond to the asymptotic standard error of the parameters.

The consideration of more degrees of freedom in a single fitting stage allows a more accurate determination of the activation energy and then a more reliable influence of temperature on for the process. Some authors as van Boekel (2008) who works about the estimation of kinetics parameters on some quality food process recommend the use of all data available in order to estimate the activation parameters with more precision.

The predictions obtained by this second fitting procedure were satisfactory and the figures obtained, between predicted and experimental data, were almost coincident to those shown in Figure (4). Therefore, to avoid repetition, it was considered that it was more than sufficient to inform the statistical parameters of the second fitting procedure, the overall coefficient of determination (r^2=0.985) and the asymptotic standard error (between brackets) for each parameter E_a=17.2 (2.6) kJ mol^{-1} and D_0= 3.232×10^{-7} (3.234×10^{-9}) m^2 s^{-1}.

In order to study the rate of the extraction process, Eq.12 was derived with respect to time, and the following equation was obtained:

$$\frac{dS_m}{dt} = -(S_0 - S_e)\frac{6}{R_0^2}D_0 e^{-\frac{E_a}{R_g T_k}}\cdot \sum_{n=1}^{n=\infty} e^{-\frac{1}{n^2}\pi^2 D_0 e^{-\frac{E_a}{R_g T_k}}\frac{t}{R_0^2}} \quad (12)$$

Figure 6 show the saponins extraction rates estimated from experimental saponins contents, and the predicted values a fair agreement is achieved.

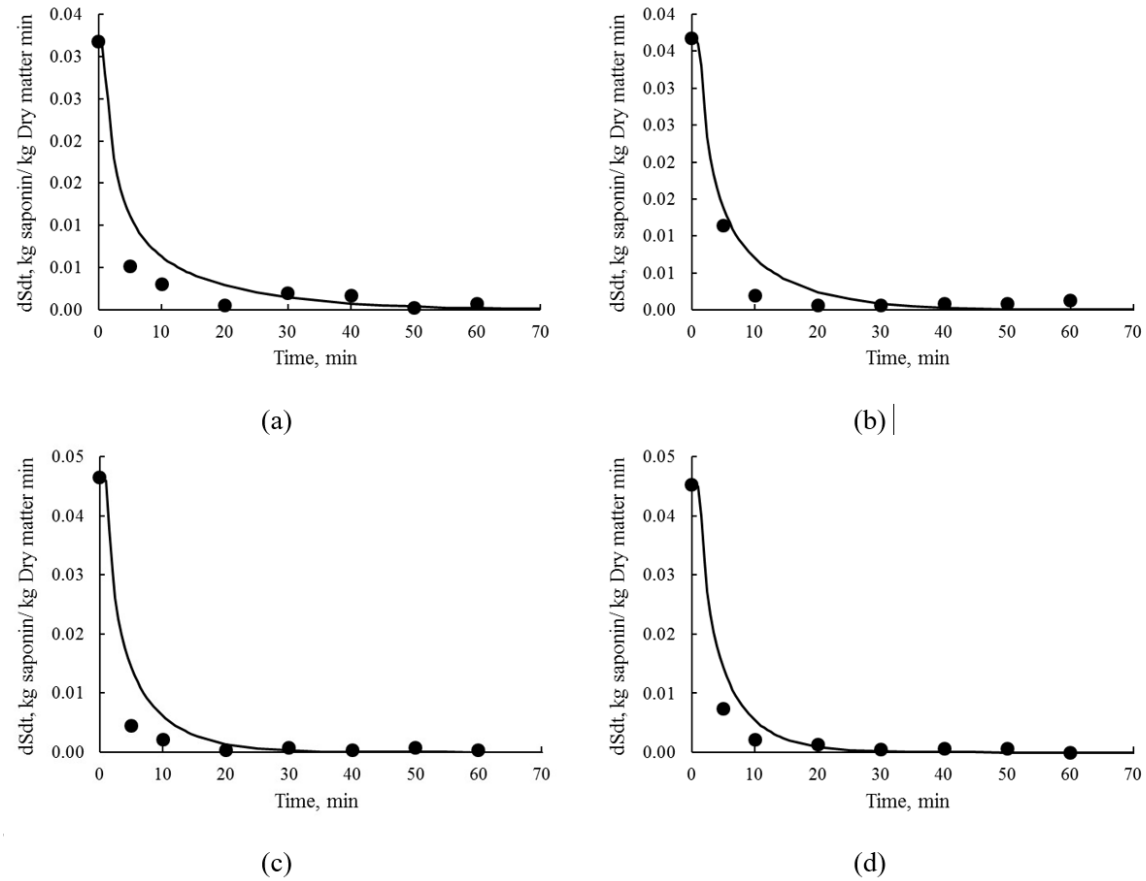

Figure 6: Saponins extraction rate ($-dS_m/dt$), kg saponins / kg dry matter min, obtained from (symbols) values obtained from experimental measurement and (solid line) predicted by the derivative of the diffusive model with respect to time for various water temperatures

The deviation observed at the beginning may be due to the error involved in calculating the experimental derivatives, which are approximated by two point forward finite differences.
Figure 6 shows that the saponins extraction rate is faster between in the first part of the process, which is then followed by a phase of much slower transfer rate. Lucas, Le Ray, and Marietteette (2007) and Machado, Oliveira, and Cunha (1999) who studied the water absorption and solute leaching during soaking of different breakfast cereals reports a similar behavior.

5 Conclusions

In this work, the experimental extraction kinetics of saponins from quinoa seeds was studied at various water temperatures to improve the understanding of this process.
The spectrophotometric method was adequate to determine the total saponins content. From this study, the treatment carried out at 40^{o}C for 6 min, can be considered optimum to reach a safe level of saponins for human consumption without visible damage to the seed.
An unsteady state diffusional model was proposed with strict internal control to the mass transfer rate, assuming constant diffusion coef-

ficient and using the initial particle size during the process. The complete analytical solution of the diffusion equation provided reasonably accurate predictions of the experimental curves.

The experimental diffusion coefficients determined in this work were correlated as a function of water temperature by means of an Arrhenius-type equation. Both the values of the diffusion coefficient (around 10^{-10}m^2 s^{-1}) and the activation energy (about 16.9 kJ mol^{-1}) are within the ranges expected for similar processes.

A second, more general method for fitting the diffusional model to the entire set of data involved the inclusion of the Arrhenius equation inside the diffusional model to directly fit an overall activation energy (E_a) of 17.2 kJ mol^{-1} and a pre-exponential factor equal to 3.232×107 m^2 s^{-1}. A slightly more accurate prediction was obtained compared with the first fitting method.

In future work, a model similar to that developed here will be combined with another model for water diffusion into the grain. In addition, leaching of minerals will be measured to know the extent of this possible phenomenon. Such a model, combined with mass balances that include the water increase content, will be useful for equipment design, by predicting saponins extraction times for different operating conditions.

References

Abdelkader, Z. M. (1992). Effect of blanching on the diffusion of glucose from potatoes. *Nahrung-Food*, *36*(1), 15–20.

Brožková, I., Zapletal, T., Kroutilová, L., Hájek, T., Moťková, P., & Červenka, L. (2018). The effect of soaking regime and moderate drying temperature on the quality of buckwheat-based product. *Journal of Cereal Science*, *81*, 15–21. doi:10.1016/j.jcs.2018.01.015

Chauhan, G. S., Eskin, N. A. M., & Tkachuk, R. (1992). Nutrients and antinutrients in quinoa seed. *Cereal Chemistry*, *69*(1), 85–88.

Cheok, C. Y., Salman, H. A. K., & Sulaiman, R. (2014). Extraction and quantification of saponins: a review. *Food Research International*, *59*, 16–40. doi:10.1016/j.foodres.2014.01.057

Chi, R. A., Tian, J., Gao, H., Zhou, F., Liu, M., Wang, C. W., & Wu, Y. X. (2006). Kinetics of leaching flavonoids from pueraria lobata with ethanol. *Chinese Journal of Chemical Engineering*, *14*(3), 402–406. doi:10.1016/S1004-9541(06)60091-8

Codex Alimentarius. (2017). Norma en estudio Nº CL 2017/44-CPL. Retrieved from http://www.fao.org/faowho-codexalimentarius/sh-proxy/en/?lnk=1%5C&url=https%5C%253A%5C%252F%5C%252Fworkspace.fao.org%5C%252Fsites%5C%252Fcodex%5C%2052%2052FCircular%5C%252520Letters%5C%252FCL%5C%2525202017-81%5C%252Fcl17_81e.pdf

Crank, J. (1975). *The mathematics of diffusion*. Oxford University Press.

Erramouspe, P. L. J., Armada, M., & Molina, S. E. G. (2013). Propiedades físico-químicas, estructurales y de calidad en semillas de quinua (chenopodium quinoa) variedad cica, con evaluación de la eficiencia de un proceso artesanal de escarificación en seco. *Revista Ciencia y Tecnología de los Cultivos Industriales*, *3*(5), 71–79. Retrieved from https://inta.gob.ar/sites/default/files/script-tmp-inta-revista-ciencia-y-tecnologa-de-los-cultivos-indu_4.pdf

Escuredo, O., Martin, M. I. G., Moncada, G. W., Fischer, S., & Hierro, J. M. H. (2014). Amino acid profile of the quinoa (chenopodium quinoa willd.) using near infrared spectroscopy and chemometric techniques. *Journal of Cereal Science*, *60*(1), 67–74.

Food and Agriculture Organization. (2011). Quinoa: an ancient crop to contribute to world food security. technical report. regional office for latin america and the caribbean. Retrieved from http://www.fao.org/docrep/017/aq287e/aq287e.pdf

Food and Agriculture Organization. (2013). Food and agriculture organization of the united nations regional office for latin america and the caribbean. International

Year of Quinoa Secretariat. Retrieved from http://www.fao.org/quinoa-2013/en/

Francis, G., Kerem, Z., Makkar, H. P. S., & Becker, K. (2002). The biological action of saponins in animal systems: a review. *British Journal of Nutrition*, *88*(6), 587–605. doi:10.1079/BJN2002725

Gianna, V., Manuel Montes, J., Luis Calandri, E., & Alberto Guzman, C. (2012). Impact of several variables on the microwave extraction of chenopodium quinoa willd saponins. *International Journal of Food Science and Technology*, *47*(8), 1593–1597. doi:10.1111/j.1365-2621.2012.03008.x

Hostettmann, K. & Marston, A. (1995). *Chemistry and pharmacology of natural products: saponins*. Cambridge University Press.

Jan, K. N., Panesar, P. S., Rana, J. C., & Singh, S. (2017). Structural, thermal and rheological properties of starches isolated from indian quinoa varieties. *International Journal of Biological Macromolecules*, *102*, 315–322. doi:10.1016/j.ijbiomac.2017.04.027

Kashaninejad, M., Maghsoudlou, Y., Rafiee, S., & Khomeiri, M. (2007). Study of hydration kinetics and density changes of rice (tarom mahali) during hydrothermal processing. *Journal of Food Engineering*, *79*(4), 1383–1390. doi:10.1016/j.jfoodeng.2006.04.019

Koziol, M. J. (1991). Afrosimetric estimation of threshold saponin concentration for bitterness in quinoa (chenopodium-quinoa willd). *Journal of the Science of Food and Agriculture*, *54*(2), 211–219. doi:10.1002/jsfa.2740540206

Kozioł, M. J. (1992). Chemical composition and nutritional evaluation of quinoa (chenopodium quinoa willd.) *Journal of Food Composition and Analysis*, *5*(1), 35–68. doi:10.1016/0889-1575(92)90006-6

Li, G. & Zhu, F. (2017). Physicochemical properties of quinoa flour as affected by starch interactions. *Food Chemistry*, *221*, 1560–1568. doi:10.1016/j.foodchem.2016.10.137

Lucas, T., Le Ray, D., & Mariette, F. (2007). Kinetics of water absorption and solute leaching during soaking of breakfast cereals. *Journal of Food Engineering*, *80*(2), 377–384. doi:10.1016/j.jfoodeng.2005.11.006

Machado, M. F., Oliveira, F. A. R., & Cunha, L. M. (1999). Effect of milk fat and total solids concentration on the kinetics of moisture uptake by ready-to-eat breakfast cereal. *International Journal of Food Science and Technology*, *34*(1), 47–57. doi:10.1046/j.1365-2621.1999.00238.x

Montgomery, D. C. (1991). *Design and analysis of experiments* (N. Y. J. W. bibinitperiod Sons, Ed.).

Mota, C., Nascimento, A. C., Santos, M., Delgado, I., Coelho, I., Rego, A., ... Castanheira, I. (2016). The effect of cooking methods on the mineral content of quinoa (chenopodium quinoa), amaranth (amaranthus sp.) and buckwheat (fagopyrum esculentum). *Journal of Food Composition and Analysis*, *49*, 57–64. doi:10.1016/j.jfca.2016.02.006

Navruz-Varli, S. & Sanlier, N. (2016). Nutritional and health benefits of quinoa (chenopodium quinoa willd.) *Journal of Cereal Science*, *69*, 371–376. doi:10.1016/j.jcs.2016.05.004

Nickel, J., Spanier, L. P., Botelho, F. T., Gularte, M. A., & Helbig, E. (2016). Effect of different types of processing on the total phenolic compound content, antioxidant capacity, and saponin content of chenopodium quinoa willd grains. *Food Chemistry*, *209*, 139–143. doi:10.1016/j.foodchem.2016.04.031

Pabis, S., Jayas, D. S., & Cenkowski, S. (1998). *Grain drying: theory and practice*. John Wiley and Sons.

Peiretti, P. G., Gai, F., & Tassone, S. (2013). Fatty acid profile and nutritive value of quinoa (chenopodium quinoa willd.) seeds and plants at different growth stages. *Animal Feed Science and Technology*, *183*(1-2), 56–61. doi:10.1016/j.anifeedsci.2013.04.012

Quispe-Fuentes, I., Vega-Galvez, A., Miranda, M., Lemus-Mondaca, R., Lozano, M., & Ah-Hen, K. (2013). A kinetic approach to saponin extraction during washing of quinoa (chenopodium quinoa willd.) seeds. *Journal of Food Process Engineering*, *36*(2), 202–210. doi:10.1111/j.1745-4530.2012.00673.x

Ridout, C. L., Price, K. R., Dupont, M. S., Parker, M. L., & Fenwick, G. R. (1991). Quinoa saponins-analysis and preliminary investigations into the effects of reduction by processing. *Journal of the Science of Food and Agriculture*, *54*(2), 165–176.

Ruales, J. & Nair, B. M. (1993). Saponins, phytic acid, tannins and protease inhibitors in quinoa (chenopodium-quinoa, willd) seeds. *Food Chemistry*, *48*(2), 137–143. doi:10.1016/0308-8146(93)90048-K

San Martin, R. & Briones, R. (2000). Quality control of commercial quillaja (quillaja saponaria molina) extracts by reverse phase HPLC. *Journal of the Science of Food and Agriculture*, *80*(14), 2063–2068.

Srichuwong, S., Curti, D., Austin, S., King, R., Lamothe, L., & Gloria-Hernandez, H. (2017). Physicochemical properties and starch digestibility of whole grain sorghums, millet, quinoa and amaranth flours, as affected by starch and non-starch constituents. *Food Chemistry*, *233*, 1–10. doi:10.1016/j.foodchem.2017.04.019

Torrez Irigoyen, R. M. & Giner, S. A. (2014). Drying-toasting kinetics of presoaked soybean in fluidised bed. experimental study and mathematical modelling with analytical solutions. *Journal of Food Engineering*, *128*, 31–39. doi:10.1016/j.jfoodeng.2013.12.009

van Boekel, M. A. J. S. (2008). Kinetic modeling of food quality: a critical review. *Comprehensive Reviews in Food Science and Food Safety*, *7*(1), 144–158. 12 World Congress of Food Science and Technology, Chicago, IL, JUL 16-20, 2003. doi:10.1111/j.1541-4337.2007.00036.x

Varzakas, T. H., Leach, G. C., Israilides, C. J., & Arapoglou, D. (2005). Theoretical and experimental approaches towards the determination of solute effective diffusivities in foods. *Enzyme and Microbial Technology*, *37*(1), 29–41. doi:10.1016/j.enzmictec.2004.06.015

Vega-Galvez, A., Miranda, M., Vergara, J., Uribe, E., Puente, L., & Martinez, E. A. (2010). Nutrition facts and functional potential of quinoa (chenopodium quinoa willd.), an ancient andean grain: a review. *Journal of the Science of Food and Agriculture*, *90*(15), 2541–2547. doi:10.1002/jsfa.4158

International Journal of Food Studies | IJFS October 2018 | Volume 7 | pages 89–97

Physicochemical and Antioxidant Properties of Banana Varieties and Sensorial Evaluation of Jelly Prepared from those Varieties Available in Sylhet Region

Abdullah A. Sad[a], M. M. Hoque[a], and Wahidu Zzaman[a*]

[a] Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh
[*] Corresponding author
wahid-ttc@sust.edu

Received: 12 January 2018; Published online: 18 October 2018

Abstract

The present research aimed to evaluate and compare the antioxidant activity in both pulp and peel and the physicochemical contents in the pulp of four local varieties of banana, i.e. *Musa acuminate* species named Sagor, Champa, Shail and Bichi, available in Sylhet region of Bangladesh. The antioxidant activity from a methanolic extract of banana peels and pulps was evaluated by determination of total phenolic content and DPPH scavenging activity. The physicochemical contents of banana pulp such as protein, fat, moisture, ash and carbohydrate were also determined. The results showed that the overall antioxidant activity of banana peel was higher than the pulp of banana. The pulp of the Shail variety had the highest antioxidant activity (TPC = 80.14 mg GAE/100 g, DPPH radical scavenging activity =91.37%) among the four varieties. Fat (1.38%) and moisture (76.23%) contents were higher in the pulp of Shail, while protein (1.82%) and carbohydrate (22.71%) contents were higher in the pulp of Bichi. The Champa variety contained higher ash content (1.05%). Among the jellies prepared from those banana varieties, the jelly of the Shail variety showed the highest score for overall acceptance (6.8) whilst the jelly prepared from the Bichi variety showed the highest score for taste (7.75). This may be due to higher carbohydrate content. The study suggests that banana peel could be used in the food industry as a raw material to produce bakery products and in cosmetics and pharmaceutical industries as an antioxidant supplement.

Keywords: Banana; Antioxidant; Physicochemical; Sensory; Sylhet

1 Introduction

Bananas are the creamy flesh and sweet fruit, with firmness, which are packaged in their yellow jackets. Banana is one of the most consumed fruits in the world (Alkarkhi, Saifullah, Yong, & Azhar, 2010).
Nutritionally, banana is a good source of minerals, vitamins, flavonoids, carbohydrate and phenolic compounds (Aurore, Parfait, & Fahrasmane, 2009). People of all classes of society can easily get banana. Banana can be utilized in the treatment of pains, intestinal lesions of colitis, inflammation and snakebite (Coe & Anderson, 1999). Diabetes mellitus is treated by the methanolic extract from banana.
In our body, free radicals may be produced continuously which cause serious diseases, like heart disease, cancer, arthritis, inflammation and aging. Antioxidants are those agents which can scavenge the free radicals and inhibit the damage caused by them. *Musa spp.* (banana) is the world's leading fruit and in terms of economic value, it is the number five agricultural crop in

 10.7455/ijfs/7.2.2018.a8

world trade. Banana contains antioxidants and phytochemicals such as Vitamin-C, Vitamin-E, flavonoids and β-carotene (Macheix, Fleuriet, & Billot, 1990) which have free radical scavenging activity. Some enzymes of banana flesh increase the antioxidant capacity (Someya, Yoshiki, & Okubo, 2002). That is why, banana consumption is so important to reduce the risk of those diseases. Since banana is a tropical plant, it is able to prevent the oxidative stress that is caused by sunlight of high intensity and the elevated temperature by means of increasing the antioxidant ability (Kanazawa & Sakakibara, 2000). Banana's antioxidant ability depends on cultivar, maturity and the stage of ripening. The ripening process of banana influences the changes in the antioxidant capacity (Sisler, Alwan, Goren, Serek, & Apelbaum, 2003).

The peel of banana is one of the main agricultural wastes that is being used in soap making, medicine, animal feed, fillers in rubber and blackening of leather (Olalekan & Ayodeji, 2010). It is about 40% of the total weight of the banana (Anhwange, Ugye, & Nyiaatagher, 2009). There is an increasing quantity of banana peel, much of which goes unused and is disposed of as waste at a large expense. That is why, it is important to find applications for this huge amount of banana peels to minimize environmental problems (Zhang, Whistler, BeMiller, & Hamaker, 2005).

The banana peel is a good source of phenolic compounds which are considered to act as antioxidants against heart disease and cancer (Someya et al., 2002). The banana peel contains different antioxidant compounds, including gallocatechin (Someya et al., 2002) and dopamine (Umamaheswari & Chatterjee, 2008). In banana peel, the total phenolic compounds range from 0.90 to 3.0 g/100 g dry weight (Someya et al., 2002; Nguyen, Ketsa, & van Doorn, 2003). Gallocatechin exists at a concentration of 160 mg/100 g dry weight (Someya et al., 2002). Many other phytochemicals like delphinidin, anthocyanin, cyanidin and catecholamines (Kanazawa & Sakakibara, 2000) are found in ripe banana pulp and peel. According to Someya et al. (2002), total phenolics are higher in banana peel (907 mg/ 100 g dry wt.) than in the pulp of banana (232 mg/100 g dry wt.).

The present study aimed to compare the physicochemical and antioxidant properties of both peel and pulp of banana varieties found in the Sylhet region of Bangladesh and to evaluate the sensorial properties of jelly prepared from those varieties.

2 Materials and Methods

2.1 Sample collection

The banana samples (Sagor, Champa, Shail, and Bichi varieties) were collected from the local market of Bondor, Sylhet in Bangladesh.

Sample extraction and preparation

Two types of samples were prepared from banana. One was from banana peel and the other was from banana pulp. At first the four types of bananas were peeled. Then, the peels of the banana varieties were dried in a freeze dryer for 12 h. The dried samples were powdered using a blender. Then 1 g from each dried powder was taken in a falcon tube and dissolved into 30 ml methanol for each sample. The samples were then centrifuged at 3000 rpm for 5 minutes. Then, the banana peel samples were filtered.

The samples from banana pulps were prepared by taking 1 g of banana pulp from each variety and dissolved into 15 ml methanol for each sample in a falcon tube. Then, the samples were centrifuged at 3000 rpm for 5 minutes. After centrifuging, the banana pulp samples were filtered.

2.2 Antioxidant activity determination by UV-vis spectrophotometer

The antioxidant activity of banana peels and pulps were evaluated by determination of: (1)total phenolic content and (2) DPPH scavenging activity.

Total phenolic content determination by spectrophotometer

The total phenolic content was determined by using the Folin-Ciocalteu phenol reagent as reported by Amorim et al. (2008). 0.5 ml of the

sample extract of four banana varieties, including both banana peel and pulp, was added to 8.5 ml of distilled water separately. 0.5 ml of the Folin-Ciocalteu phenol reagent was then added to each sample and kept at room temperature for 5 minutes. After that, 1 ml of 35% sodium carbonate solution was added to each sample. Then the mixtures were shaken well, and kept at room temperature for 20 minutes. The absorbance of the mixtures was then measured at 765 nm. A blank was prepared using distilled water. Then, a set of Gallic acid standard solutions was read against a blank. The results of phenolics were expressed in terms of Gallic acid in mg/g of dry extract. Total phenolic contents of banana varieties including both banana peel and pulp were determined as mg of Gallic acid equivalent per gram using the equation obtained from a standard Gallic acid calibration curve (R^2 =0.999).

DPPH radical scavenging activity determination by spectrophotometer

The DPPH radical scavenging activity of banana varieties was measured using the modified method described by Chang et al. (2001). 2 ml of 0.2 mM methanol DPPH solution was added to 2 ml of each solution which were extracted from both banana peel and pulp. Then the mixtures were vortexed vigorously for 1 minute and left to stand at room temperature for 10 minutes in the dark for the reaction to occur. After 10 minutes, absorbance was measured against a blank at 517 nm. The ability to scavenge the DPPH radical was calculated using the following formula:

$$DPPH(\%) = 1 - \frac{Sample\_absorbance}{Control\_absorbance} \times 100 \quad (1)$$

2.3 Physicochemical properties

For proximate analysis, the protein of pulp of the banana varieties was determined by the Micro-Kjeldahl method as described by Association of the Official Analytical Chemists (2004). A protein conversion factor of 5.2 was used to calculate the percent protein from the Nitrogen determination. The fat content of pulp of the banana varieties was determined according to the method of Ravichandran and Parthiban (2000). In this method, Chloroform/Methanol (2:1) solution was added toeach sample to separate the fat content from the banana pulp. The moisture content of pulp of the banana varieties was determined according to the official method 44-01 of American Association of Cereal Chemists (2000). The total ash of banana varieties was measured by direct incineration of each sample in a crucible, according to American Association of Cereal Chemists (2000) method 08-01. The carbohydrate content of the banana varieties was calculated by subtracting the protein, fat, moisture and ash contents from 100 (Lim, Lim, & Tee, 2007).

2.4 Sensory analysis

Jellies were made from the banana pulp of those varieties available in Sylhet region. Sensory evaluation was conducted using a 7 point hedonic scale (1=dislike very much to 7= like very much) in order to determine the acceptance of jelly by consumers. The sensory evaluation panel comprised thirty professors and students from Shahjalal University of Science and Technology, Sylhet, Bangladesh. Among the panel members, twenty were men and ten were women, and their age ranged from 18-45. Panel members met the following criteria: have affinity for the consumption of banana, able to feel the basic tastes, possess the ability to recognize aroma and smell, non-smoking, interested in this study and willing to reveal their decisions (Plemmons & Resurreccion, 1998). The attributes for the sensory evaluation were color, aroma, bitterness, taste and overall acceptance.

2.5 Statistical analysis

The experiments were performed in triplicate and data attained from experiments were collated and analyzed using the Statistical Package for the Social Sciences (Version 21; IBM Corporation). The mean scores were calculated for each attribute and one-way analysis of variance, using the Duncan test, was carried out (Maisuthisakul, Suttajit, & Pongsawat-

manit, 2007). Significant differences were determined at $p < 0.05$.

3 Results and Discussions

3.1 Antioxidant activity

Total phenolic content

Phenolic compounds are essential fruit constituents since they inactivate lipid free radicals or prohibit decomposition of hydroperoxides to free radicals (Maisuthisakul et al., 2007). Table 1 shows that the total phenolic content (TPC) of tested banana varieties varied significantly ($p<0.05$) from 7.47 to 80.14 mg GAE/100 g for banana pulp and from 34.43 to 119.47 mg GAE/100 g for banana peel. In the present study, methanol was used, which resulted in higher extraction yields of phenolic compounds due to its high polarity. The banana peel has a higher total phenolic content than the pulp of banana. The TPC of banana pulp recorded in the literature were 56.1 mg GAE/100 g (Sun, Chu, Wu, & Liu, 2002), 51 to 54 mg GAE/100 g in Pisang Mas banana (Lim et al., 2007; Alothman, Bhat, & Karim, 2009) and 90.4 mg GAE/100 g in Pisang Awak banana (Choo & Aziz, 2010). The TPC of pulp from the Sagor variety (7.47 mg GAE/100 g) was not in agreement with previous studies. The value may differ since it is a different variety. According to Fatemeh, Saifullah, Abbas, and Azhar (2012), the TPC was generally higher in the peel than in the pulp for a particular banana component which supports the present study.

DPPH radical scavenging activity

DPPH radicals react with reducing agents when the electrons pair off and the color of solution is lost stoichiometrically, which depends on the number of electrons taken up (Subhasree, Baskar, Keerthana, Susan, & Rajasekaran, 2009). The ability of banana peel extracts to scavenge DPPH radicals had been demonstrated (Okonogi, Duangrat, Anuchpreeda, Tachakittirungrod, & Chowwanapoonpohn, 2007). Therefore, the DPPH scavenging activity of each sample was reported as the percentage of DPPH inhibition, where a higher value is associated with a stronger antioxidant activity (Fatemeh et al., 2012). As the condensation of phenolic compounds increased, the DPPH radical scavenging activity also increased (Alothman et al., 2009; Gonzalez-Montelongo, Gloria Lobo, & Gonzalez, 2010) which is typical for plant materials. The DPPH radical scavenging activity of various banana pulp extracts are shown in Table 1. The DPPH radical scavenging activity was highest in the pulp of Shail (91.37±0.05%) and the peel of Shail (94.04±0.73%) although the peel of all varieties contained similar values. According to Baskar et al. (2011), the DPPH radical scavenging activity of banana was 98.19% at 10 mg ml^{-1}. It is evident that the extract obtained from the peel has higher antioxidant activity than that obtained from the pulp (Fatemeh et al., 2012).

3.2 Physicochemical properties

Proximate analysis provides information on the basic chemical composition of agricultural waste (Adeolu & Enesi, 2013). The carbohydrate, protein, fat and energy contents of banana were reported as higher than those of apple (Auta & Kumurya, 2015). The protein content of banana varieties ranged from 1.28% to 1.82% as shown in Table 2. The maximum protein content (1.82%) was in the Bichi variety and the minimum was in the Shail variety. Champa and Sagor varieties contained almost equal amounts of protein. The percentage of crude protein for banana was 7.30 according to Auta and Kumurya (2015). The protein content of banana reported in the previous study is higher than the present study due to regional differences. The fat content in banana varieties is also shown in Table 2. This attribute may be used as the basis to confirmiprocessing temperatures and autooxidation that may lead to rancidity which affects the flavour of food (Adeolu & Enesi, 2013). The fat content of banana varieties ranged from 0.98% to 1.38%. The Shail variety contained the highest amount of fat (1.38%) among the four banana varieties while the Sagor variety contained the lowest fat content (0.98%). According to the

Table 1: Total phenolic content and DPPH radical scavenging activity of different banana peel and pulp

Antioxidant Activity	Sagor	Champa	Shail	Bichi
Total phenolic content of banana peel (mg GAE/100 g)	**119.47±4.77**^{d}	70.03±2.7^{c}	35.88±3.37b	34.43±1.43^{a}
Total phenolic content of banana pulp (mg GAE/100 g)	7.47±0.94^{a}	38.92±0.58^{c}	**80.14±4.11**^{d}	9.8±0.38^{b}
DPPH radical scavenging activity of banana peel (%)	93.26±0.12^{b}	93.54±0.09^{b}	**94.04±0.73**^{b}	92.27±0.05^{a}
DPPH radical scavenging activity of banana pulp (%)	64.49±0.25^{b}	90.83±0.44^{c}	**91.37±0.05**^{c}	62.14±3.23^{a}

Values are mean ± standard deviation. Within column values followed by different superscript letter(s) are significantly different ($p<0.05$).

Table 2: Proximate analysis of various banana pulps

Composition	Sagor	Champa	Shail	Bichi
Protein (%)	1.45±0.05^{a}	1.46±0.03^{a}	1.28±0.02^{a}	**1.82±0.06**^{a}
Fat (%)	0.98±0.06^{a}	1.17±0.09^{b}	**1.38±0.08**^{c}	1.29±0.04b
Moisture (%)	75.47±0.05^{b}	76.13±0.34^{c}	**76.23±0.17**^{c}	73.49±0.49^{a}
Ash (%)	0.96±0.005^{ns}	**1.05±0.350**^{ns}	0.75±0.007^{ns}	0.72±0.008^{ns}
Carbohydrate (%)	21.13±0.13^{b}	20.17±0.76^{a}	20.37±2.11^{a}	**22.71±0.04**^{c}

Values are mean ± standard deviation. Within column values followed by different superscript letter(s) are significantly different ($p<0.05$).

Table 3: Sensory evaluation of jelly

Sensory Attribute	Sagor	Champa	Shail	Bichi
Color	5.2±0.42^{b}	**6.33±0.52**^{c}	5.67±0.52^{b}	4.5±0.53^{a}
Aroma	5.75±0.46^{b}	4.8±0.79^{a}	5.8±0.79^{b}	**6.4±0.52**^{c}
Bitterness	3.6±0.49^{ns}	4.2±0.42^{ns}	**4.5±0.53**^{ns}	3.75±0.46^{ns}
Taste	6.25±0.46^{b}	4.6±0.51^{a}	6.5±0.5^{b}	**6.75±0.46**^{c}
Overall acceptance	6.20±0.78^{c}	5.25±0.88^{a}	**6.8±0.42**^{c}	5.4±0.84^{b}

Values are mean ± standard deviation. Within column values followed by different superscript letter(s) are significantly different ($p<0.05$).

USDA National Nutrient data base, the fat percentage of banana pulp was 1% which is similar to the present value. Auta and Kumurya (2015) reported that the crude fat of banana was 1.05% which also supports the present value. The moisture content inthe pulp of banana varieties is also shown in Table 2. High moisture content food items are more susceptible to microbial attack and a shorter shelf life (Adepoju, 2012). The Shail variety contained the highest amount of moisture (76.23%) whereas the Bichi variety contained less moisture (73.49%). The approximate moisture content of Embul, Seeni and Kolikuttu varieties of banana at the harvest maturity were 77.86%, 72.46% and 75.97% respectively, according to Wasala, Dharmasena, Dissanayake, and Thilakarathne (2012) which supports the present study. Similar values were reported by Kachru, Kotwaliwale, and Balasubramanian (1995) for some Indian banana varieties at maturity. Auta and Kumurya (2015) found the moisture content of banana to be 73.63%. According to Ikegwu and Ekwu (2009), the moisture content of fruits has an impact on a number of engineering properties. Fraser, Verma, and Muir (1978) indicate that the moisture content of agricultural produce dominances their bulk density. The ash content of banana varieties is also shown in Table 2. A high percentage of ash is indicative of a high mineral content, particularly the macro-minerals (Adeolu & Enesi, 2013). Ash content was highest in the Champa variety at 1.05% while the Bichi variety contained the lowest amount of ash (0.72%). The percentage of ash content of banana should be 4.93% according to Auta and Kumurya (2015) which was higher than the present study. The carbohydrate content of the banana varieties is also shown in Table 2. Carbohydrate could be a good source of energy for animals (Adeolu & Enesi, 2013). The Bichi variety contained the highest carbohydrate (22.71%) content, while the Champa variety contained 20.17% carbohydrate. According to the USDA National Nutrient data base, the carbohydrate percentage of banana pulp is 18%. The total percentage carbohydrate content of banana found by Auta and Kumurya (2015) was 22.01%.

3.3 Sensory evaluation of jelly

Sensory evaluation results for jellies which were made from the pulp of the four banana varieties are shown in Table 3. Reducing sugars and amino acids are responsible for the Maillard reaction that provides food flavor and a desirable brown color. Sucrose was the main ingredient for the production of the jellies to provide a sweet taste and contribute to jelly formation (Silva, Lacerda, Santos, & Martins, 2008). A previous study found that fat content influences the taste (Bus & Worsley, 2003). The color of jelly prepared from the Champa variety was the best while the color of jelly of the Bichi variety was not accepted by the panelists. The panelists described the jelly from the Champa variety as having a fascinating color that was more attractive to the eye compared with the darker color of the jelly from the Bichi variety. As color is of primary importance in consumers' acceptability of a product, the color of the jelly of the Bichi variety should be improved. The aroma score was highest (6.4±0.52) in the jelly prepared from the Bichi variety which may be due to the higher carbohydrate content. The bitterness scores of the jelly from all banana varieties were almost average. The taste of the jelly prepared from the Bichi variety (6.25±0.46) was the best but not much better than the taste of the jelly prepared from the Shail variety. The jelly prepared from the Shail variety showed the highest overall acceptance (6.8±0.42).

4 Conclusion

The study showed that the Shail, Bichi, Champa and Sagor banana varieties of the Sylhet region in Bangladesh contained appreciable levels of nutrients. The Shail variety had the highest antioxidant activity as determined by the total phenolic content and DPPH scavenging activity, and contained the highest carbohydrate content and a protein content which was higher than in the Bichi variety. Among the jellies prepared from the banana varieties, the jelly of Shail variety showed the highest overall acceptance among the panelists. The peel of the bananas well as the pulp is a good source of antioxidants which may

help reduce the risk of a variety of chronic diseases. Banana is widely available in Bangladesh and can be found throughout the year. Both the pulp and peel of banana offer new product development opportunities in the food, cosmetics and pharmaceutical industries.

References

Adeolu, A. T. & Enesi, D. O. (2013). Assessment of proximate, mineral, vitamin and phytochemical compositions of plantain (*Musa paradisiaca*) bract-an agricultural waste. *International Research Journal of Plant Science*, *4*(7), 192–197.

Adepoju, O. T. (2012). Nutrient composition, antinutritional factors and contribution of enriched dry guinea corn (*Sorghum* sp) leaf extract to nutrient intake of nigeria consumers. In *Proc. intern. conf environ, biomed & biotech ipcbee aug* (pp. 4–5).

Alkarkhi, A. F. M., Saifullah, R., Yong, Y., & Azhar, M. E. (2010). Physicochemical properties of banana peel flour as influenced by variety and stage of ripeness: multivariate statistical analysis. *Asian Journal of Food and Agro-Industry*, *3*(3), 349–362.

Alothman, M., Bhat, R., & Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from malaysia, extracted with different solvents. *Food Chemistry*, *115*(3), 785–788. doi:10.1016/j.foodchem.2008.12.005

American Association of Cereal Chemists. (2000). Approved methods of the american association of cereal chemists. AACC. Retrieved from https://books.google.pt/books?id=xJwQAQAAMAAJ

Amorim, E. L. C., Nascimento, J. E., Monteiro, J. M., Peixoto Sobrinho, T. J. S., Araújo, T. A. S., & Albuquerque, U. P. (2008). A simple and accurate procedure for the determination of tannin and flavonoid levels and some applications in ethnobotany and ethnopharmacology. *Functional Ecosystems and Communities*, *2*(1), 88–94.

Anhwange, B. A., Ugye, T. J., & Nyiaatagher, T. D. (2009). Chemical composition of *Musa sapientum* (banana) peels. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *8*(6), 437–442.

Association of the Official Analytical Chemists. (2004). Official methods of analysis of AOAC International. 20th ed., Washington, USA: AOAC.

Aurore, G., Parfait, B., & Fahrasmane, L. (2009). Bananas, raw materials for making processed food products. *Trends in Food Science & Technology*, *20*(2), 78–91. doi:10.1016/j.tifs.2008.10.003

Auta, S. A. & Kumurya, A. S. (2015). Comparative proximate, mineral elements and anti-nutrients composition between musa sapientum (banana) and musa paradisiaca (plantain) pulp flour. *Sky Journal of Biochemistry Research*, *4*(4), 025–030.

Baskar, R., Shrisakthi, S., Sathyapriya, B., Shyampriya, R., Nithya, R., & Poongodi, P. (2011). Antioxidant potential of peel extracts of banana varieties (*Musa sapientum*). *Food and Nutrition Sciences*, *2*(10), 1128–1133. doi:10.4236/fns.2011.210151

Bus, A. E. M. & Worsley, A. (2003). Consumers' sensory and nutritional perceptions of three types of milk. *Public Health Nutrition*, *6*(2), 201–208. doi:10.1079/PHN2002417

Chang, S. T., Wu, J. H., Wang, S. Y., Kang, P. L., Yang, N. S., & Shyur, L. F. (2001). Antioxidant activity of extracts from acacia confusa bark and heartwood. *Journal of Agricultural and Food Chemistry*, *49*(7), 3420–3424. doi:10.1021/jf0100907

Choo, C. L. & Aziz, N. A. A. (2010). Effects of banana flour and beta-glucan on the nutritional and sensory evaluation of noodles. *Food Chemistry*, *119*(1), 34–40. doi:10.1016/j.foodchem.2009.05.004

Coe, F. G. & Anderson, G. J. (1999). Ethnobotany of the sumu (*Ulwa*) of southeastern nicaragua and comparisons with miskitu plant lore. *Economic Botany*, *53*(4), 363–386. doi:10.1007/BF02866715

Fatemeh, S. R., Saifullah, R., Abbas, F. M. A., & Azhar, M. E. (2012). Total phenolics, flavonoids and antioxidant activity of banana pulp and peel flours: influence of variety and stage of ripeness. *International Food Research Journal*, *19*(3), 1041.

Fraser, B. M., Verma, S. S., & Muir, W. E. (1978). Some physical-properties of fababeans. *Journal of Agricultural Engineering Research*, *23*(1), 53–57. doi:10.1016/0021-8634(78)90079-3

Gonzalez-Montelongo, R., Gloria Lobo, M., & Gonzalez, M. (2010). The effect of extraction temperature, time and number of steps on the antioxidant capacity of methanolic banana peel extracts. *Separation and Purification Technology*, *71*(3), 347–355. doi:10.1016/j.seppur.2009.12.022

Ikegwu, O. J. & Ekwu, F. C. (2009). Thermal and physical properties of some tropical fruits and their juices in nigeria. *Journal of Food Technology*, *7*(2), 38–42.

Kachru, R. P., Kotwaliwale, N., & Balasubramanian, D. (1995). Physical and mechanical-properties of green banana (*musa-paradisiaca*) fruit. *Journal of Food Engineering*, *26*(3), 369–378. doi:10.1016/0260-8774(94)00054-D

Kanazawa, K. & Sakakibara, H. (2000). High content of dopamine, a strong antioxidant, in cavendish banana. *Journal of Agricultural and Food Chemistry*, *48*(3), 844–848. doi:10.1021/jf9909860

Lim, Y. Y., Lim, T. T., & Tee, J. J. (2007). Antioxidant properties of several tropical fruits: a comparative study. *Food Chemistry*, *103*(3), 1003–1008. doi:10.1016/j.foodchem.2006.08.038

Macheix, J. J., Fleuriet, A., & Billot, J. (1990). *Fruit phenolics*. Boca Raton, FL, CRC Press.

Maisuthisakul, P., Suttajit, M., & Pongsawatmanit, R. (2007). Assessment of phenolic content and free radical-scavenging capacity of some thai indigenous plants. *Food Chemistry*, *100*(4), 1409–1418. doi:10.1016/j.foodchem.2005.11.032

Nguyen, T., Ketsa, S., & van Doorn, W. (2003). Relationship between browning and the activities of polyphenol oxidase and phenylalanine ammonia lyase in banana peel during low temperature storage. *Postharvest Biology and Technology*, *30*(2), 187–193. doi:10.1016/S0925-5214(03)00103-0

Okonogi, S., Duangrat, C., Anuchpreeda, S., Tachakittirungrod, S., & Chowwanapoonpohn, S. (2007). Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. *Food Chemistry*, *103*(3), 839–846. doi:10.1016/j.foodchem.2006.09.034

Olalekan, A. J. & Ayodeji, K. E. (2010). Antioxidative potentials of banana and plantain peel extracts on crude palm oil. *Ethnobotanical Leaflets*, (5), 1.

Plemmons, L. E. & Resurreccion, A. V. A. (1998). A warm-up sample improves reliability of responses in descriptive analysis. *Journal of Sensory Studies*, *13*(4), 359–376. doi:10.1111/j.1745-459X.1998.tb00095.x. eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1745-459X.1998.tb00095.x

Ravichandran, R. & Parthiban, R. (2000). Lipid occurrence, distribution and degradation to flavour volatiles during tea processing. *Food Chemistry*, *68*(1), 7–13. doi:10.1016/S0308-8146(99)00143-0

Silva, M. R., Lacerda, D. B. C. L., Santos, G. G., & Martins, D. M. d. O. (2008). Chemical characterization of native fruits of the cerrado. *Rural Science*, *38*, 1790–1793. doi:10.1590/S0103-84782008000600051

Sisler, E. C., Alwan, T., Goren, R., Serek, M., & Apelbaum, A. (2003). 1-substituted cyclopropenes: effective blocking agents for ethylene action in plants. *Plant Growth Regulation*, *40*(3), 223–228. doi:10.1023/A:1025080420990

Someya, S., Yoshiki, Y., & Okubo, K. (2002). Antioxidant compounds from bananas (*Musa Cavendish*). *Food Chemistry*, *79*(3), 351–354. doi:10.1016/S0308-8146(02)00186-3

Subhasree, B., Baskar, R., Keerthana, R. L., Susan, R. L., & Rajasekaran, P. (2009). Evaluation of antioxidant potential in selected green leafy vegetables. *Food Chemistry*, *115*(4), 1213–1220. doi:10.1016/j.foodchem.2009.01.029

Sun, J., Chu, Y.-F., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry*, *50*(25), 7449–7454. PMID: 12452674. doi:10.1021/jf0207530. eprint: https://doi.org/10.1021/jf0207530

Umamaheswari, M. & Chatterjee, T. K. (2008). In vitro antioxidant activities of the frac-

tions of coccinia grandis l. leaf extract. *African Journal of Traditional Complementary and Alternative Medicines*, *5*(1), 61–73.

Wasala, W. M. C. B., Dharmasena, D. A. N., Dissanayake, T. M. R., & Thilakarathne, B. M. K. S. (2012). Physical and mechanical properties of three commercially grown banana (*Musa acuminata Colla*) cultivars in sri lanka. *Tropical Agricultural Research*, *24*(1), 42–53.

Zhang, P., Whistler, R. L., BeMiller, J. N., & Hamaker, B. R. (2005). Banana starch: production, physicochemical properties, and digestibility-a review. *Carbohydrate Polymers*, *59*(4), 443–458. doi:10.1016/j.carbpol.2004.10.014

International Journal of Food Studies | IJFS October 2018 | Volume 7 | pages 98–116

A Nutritional Evaluation of the Berry of a New Grape: 'Karaerik' (*Vitis vinifera* L.)

Aynur Kurt[a], Nesrin Colak[a], Aydin Sükrü Bengu[b], Ali Gundoğdu[c], Erdal Akpinar[d], Sema Hayirlioglu-Ayaz[a], and Faik Ahmet Ayaz[a*]

[a] Department of Biology, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey
[b] Department of Medical Services and Techniques, Vocational School of Health Services Medical Laboratory Techniques Program Bingöl University, 12000 Bingöl, Turkey
[c] Department of Food Engineering, Faculty of Engineering & Natural Sciences, Gümüşhane University, 29100 Gümüşhane, Turkey
[d] Social Sciences Training Department, Faculty of Education, Erzincan Binali Yıldırım University, 24030, Erzincan, Turkey
[*]Corresponding author
faa@ktu.edu.tr
Tel and Fax: +90-462-377-37 12

Received: 29 January 2018; Published online: 18 October 2018

Abstract

Grape berries are a good source of nutrients and nutraceuticals and have many benefits for human health. Growing interest in the export potential and consumption of a new grape (cv. Karaerik), cultivated as a table grape in Turkey, encouraged us to profile its major nutrient contents from six different locations. Due to its popularity, the nutritional value of this grape berry needs to be investigated to ascertain its potential economic and health benefits. The most abundant sugars in the grape berry were fructose and glucose (peel/whole fruit; averages 236.57 and 127.87, and 183.36 and 108.60 g kg^{-1} fresh weight, respectively), while the major organic acids were tartaric and malic acids (7.17 and 2.81, and 2.61 and 1.76 g kg^{-1} fresh weight, respectively). Linoleic acid (peel/whole fruit/seed; 37.14, 33.12 and 57.83%, respectively) was the predominant fatty acid, while potassium (peel/whole fruit/seed; 9331.5, 10226.33 and 5354 μg/g dry weight, respectively) was the predominant mineral, followed by phosphorus (1592.8, 2672 and 3072.67) in the berry. Our results demonstrate that the nutrient components and physicochemical parameters varied significantly among the sampling locations. The grape berry contains considerable quantities of potentially beneficial healthy nutrients worthy of further evaluation.

Keywords: *Vitis vinifera* ; Karaerik, sugar; organic acid; fatty acid; mineral

1 Introduction

The consumption of fruits and vegetables is a key component of a healthy diet in humans and for protection against various degenerative diseases, such as cancer, cardiovascular diseases, diabetes, pulmonary disorders, and Alzheimer's (Ferretti, Turco, & Bacchetti, 2014; Hyson, 2011) by reducing the risk of their development (Slavin & Lloyd, 2012). The benefits obtained from foods are associated with their nutrients (vitamins, minerals, organic acids, and mono- and polyunsaturated fatty acids), dietary fibers and polyphenolic antioxidants (Kurt et al., 2017; Liu, 2013). Information regarding the quality and quantity of nutrients or neutraceuticals in fruits or vegetables during the pre- and post-harvest periods is particularly important when assess-

 10.7455/ijfs/7.2.2018.a9

ing the contribution of the consumption of these foods to protection against the diseases cited above (Kurt et al., 2017).

Grapes (*Vitis* spp.) are one of the most important and widely cultivated fruit crops around the world. Of the various *Vitis* species (*V. labrusca* , *V. rotundifolia* and *V. vinifera*), *V. vinifera* L. is the most prevalent and widely cultivated worldwide (Pavlousek & Kumsta, 2011). During the 2014-2015 production period, 21.7 million tons of grapes were produced. Turkey is one of the world's three largest producers, at 9 million tons (9.2%) (Xia, Deng, Guo, & Li, 2010).

Grapes can be consumed fresh (table grapes) or used in the production of wine, grape juice and raisins (Zhou & Raffoul, 2012). The beneficial effects of grapes and grape-derivative food products are associated with their nutritional and polyphenolic compositions (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012; Santos et al., 2011) and many studies have been published concerning their nutritional value (Kurt et al., 2017). The organoleptic quality, flavor and stability of grape berries depend to a large extent on the relative and total amounts of sugars and organic acids that maintain grape berry quality and determine its nutritive value. The nature and concentration of these constituents affect the market value because they contribute to grapes' sweetness and acidity, properties that vary among species, cvs. or varieties. For instance, grape seeds are a rich source of linoleic acid and a-linolenic acid, polyunsaturated fatty acids which prevent cardiovascular diseases (Kurt et al., 2017).

The 'Karaerik' grape, so named because of its black color and large-grained berries, is cultivated as a table grape, mainly around Üzümlü and the surrounding areas in Erzincan, Turkey (Figure 1). The taste lies on a fine point between mildly sour and sweet, with a specific aroma not found in other grapes (Akpınar & Yiğit, 2011; Güner & Aslan, 2012). It is generally regarded as a table grape. In addition, the syrup of the Karaerik grape is traditionally processed in different forms such as vinegar, molasses and dried pulp by local residents (Akpınar & Yiğit, 2011). Growing interest has focused on the phenolics and antioxidant capacity of the new grape berry. In this regard, the presence of a feruloyl derivative of anthocyanins (malvidin-3-feruloylglucoside) that has very recently been identified and quantified in the grape constitutes the first evidence for the *V. vinifera* grape (Ayaz et al., 2017).

The grape's increasing export potential and growing consumption as a table grape, together with its unique anthocyanin composition among all *V. vinifera* grapes (Ayaz et al., 2017), encouraged us to profile the nutritional value of the new grape cultivar, 'Karaerik', in detail. Apart from a very few phenological and ampelographic studies, the present research constitutes the first record of the nutrient composition of the grape berry. The aim of the present study was to characterize and compare various physicochemical parameters and nutritional composition changes (soluble sugars, organic acids, fatty acids, and minerals) in berries of the new grape (Karaerik) that is particularly widely grown in the region.

2 Materials and Methods

2.1 Chemicals and Reagents

All solvents were of HPLC quality and analytical grade. Natrium hydroxide (NaOH), KH_2PO_4, methanol and acetonitrile used for HPLC were purchased from MERCK (Darmstadt, Germany), and deionized water was prepared using a Simplicity 185 deionizer (Millipore, Bedford, MA, USA). Water for LC-MS analysis was of Milli-Q quality.

Analytical grade reference compounds used for sugar [(>99%, glucose (CAS 50-99-7), fructose (CAS 57-48-7), sucrose (CAS 57-50-1), maltose (CAS 6363-53-7) and lactose (CAS 64044-51-5)] and organic acid [(>99%, malic acid (CAS 6915-15-7), ascorbic acid (CAS 50-81-7) and citric acid (CAS 77-92-9)] calibration and quantification were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). The fatty acid methyl esters (FAME) mixture (Supelco 37 FAME mix, 10 mg/mL FAMEs in methylen chloride 47885-U, Supelco, USA) and HCSM (hexane/chloroform/sodium methoxide Sigma 403067) solution were also GC-MS or LC-MS grade (>99%).

Calibration and internal standards for ICP-MS

measurements were supplied by Agilent Technologies (Santa Clara, CA, USA) and Inorganic Ventures (Virginia, United States). These standards are traceable to the National Institute of Standards and Technology (NIST).

2.2 Plant material

Black berries of the 'Karaerik' (*V. vinifera* L.) grape at commercial maturity were sampled from six locations where they are widely grown in the district of Üzümlü and the surrounding area (Figure 1). Bunches of grape berries were randomly handpicked on sunny days from four 15-year-old grape plants in vineyards in the late morning at altitudes of 1250 and 1600 m above sea level (a.s.l.) on the hills of Üzümlü, Bayırbağ, Karakaya, Pişkidağ, Göllerköyü and Çağlayan in Erzincan (eastern Anatolia, Turkey). The distance between trees in each location was greater than 100 m. Grape bunches of moderate size, weighing 2-3 kg were collected in triplicate from six lots for each of the six sampling locations. These bunches were then combined for each location. From these bulk grape samples (2-3 kg), approximately 100 berries were separately prepared for analysis.

Sampled grape berries were immediately washed free of any residues (dead flower debris or decayed, abnormal or immature berries not at the correct maturity) using distilled water. They were kept cold below ± 4 ^{o}C and transported to the laboratory within approximately 2.5 - 3 h. The berry samples were treated with liquid nitrogen (-195.79 ^{o}C) and stored at -80 ^{o}C until further analysis. Part of each sample was set aside for physicochemical analysis. After lyophilization, the hard, dried grape berries were crushed with a steel hammer and then ground to a fine powder using a stainless steel mill for further analysis as described below.

2.3 Determination of physicochemical parameters

An Association of Official Agricultural Chemists (2003) with slight modification was used to determine pH values and titratable acidity (TA) contents as recently described elsewhere by Kurt et al. (2017). The TA was analyzed from a prepared 30 ml fresh juice sample by titration with standardized 0.1 N NaOH to pH 8.2 according to Kurt et al. (2017) and content was expressed as citric acid equivalents (CAE) g kg^{-1} of fw berry. The whole grape berry from each location in triplicate was analyzed in terms of both moisture content (MC) and dry matter content (DM) according to an official Association of Official Agricultural Chemists (2011) with slight changes as recently described elsewhere by Kurt et al. (2017). Total soluble solids (TSS, %) content was measured in juice pressed from the whole grape berry from each location using a digital refractometer (RE 5 Mettler-Toledo, Tokyo, Japan) at 21 ^{o}C. Firmness (g mm^{-1}) was measured with a penetrometer (FT-327) with an 11-mm diameter probe from three different areas (top, middle and bottom) of the whole grape berry. The fruit size (FS) of the whole grape berry (40 berries from six separate stalks) from each location was measured using a digital caliper with a sensitivity of 0.01 mm.

2.4 Extraction and determination of soluble sugars and organic acids

The extraction protocol described by Kurt et al. (2017) with slight changes was followed for the separation and quantification of sugars and organic acids in the berry of the 'Karaerik' grape. The fresh weighed peel (avg., 25 g fw) and deseeded whole grape berry (avg., 100 g fw) samples were first treated with liquid nitrogen (-195.79 ^{o}C) and homogenized at maximum speed in a blender using aqueous ethanol (80%, v/v, 20 ml x 3) for approximately 10 min, depending on tissue softness. The homogenates of both grape samples were centrifuged, and the supernatants were then separated. The residues were washed three times (20 ml x 2) with the same extraction solvent, and then centrifuged. All supernatants were combined, centrifuged and evaporated under vacuum using a rotary evaporator below 35 ^{o}C (Heidolph, Germany). The slurry was lyophilized, and the dry sample was dissolved in 5 ml deionized water and centrifuged under the same conditions and fractioned by solid-phase ex-

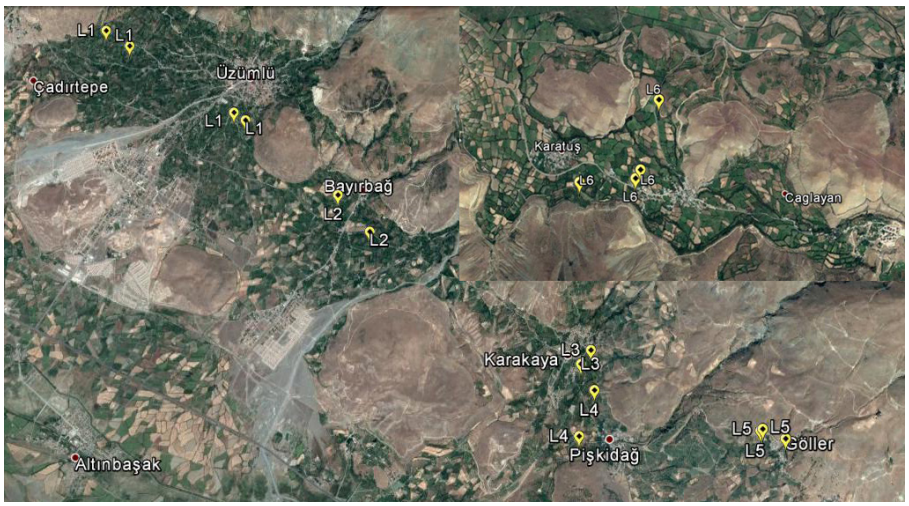

Figure 1: Locations of the 'Karaerik' black grape (*V. vinifera* L.) from east Anatolia (Erzincan, Turkey)

L1; Üzümlü (39°42'52.41"N, 39°40'38.17"E, 1361; 39°42'45.55"N, 39°40'52.80"E,1365; 39°42'13.09"N, 39°42'03.06"E, 1367; 39°42'16.26"N, 39°41'56.25"E, 1377), L2; Bayırbağ (39°41'41.32"N, 39°42'58.06"E, 1340; 39°41'25.64"N; 39°43'17.17"E, 1324), L3; Karakaya (39°40'31.66"N, 39°45'17.22"E,1365; 39°40'37.59"N, 39°45'22.39"E, 1395), L4; Pişkidağ (39°40'20.89"N, 39°45'24.99"E, 1352; 39°40'01.55"N, 39°45'17.41"E, 1306), L5; Göller Köyü (39°40'06.28"N, 39°46'53.38"E,1576; 39°40'07.03"N, 39°46'54.46"E, 1582; 39°40'03.41"N, 39°47'06.40"E, 1611), L6; Çağlayan-Yamaçlı (39°36'01.97"N, 39°41'36.21"E, 1239; 39°45'29.61"N, 39°41'24.96"E, 1243; 39°35'27.46"N, 39°40'55.44"E, 1261; 39°35'33.17"N, 39°41'27.44"E, 1240)

traction (SPE). Solid-phase extraction columns (Grace Pure C-18, max 500 mg packed bed, 3 mL, Deerfield, IL, USA) were rinsed with 100% and 80% methanol (5 mL), and then activated with deionized water (2 x 5 mL). The aqueous combined extract was then passed through the columns. Hydrophobic compounds were absorbed onto the columns, while sugars and other polar compounds were eluted with deionized water (aqueous fraction). The aqueous fraction obtained from the SPE fractionation was used for soluble sugar and organic acid analysis.

An Agilent 1100 equipped with a quarternary HPLC pump, microvacuum degasser (MVD), thermostated column compartment (TCC), refractive index detector (RID), multivariable wavelength detector (MWD) and diode array detector (DAD) (Palo Alto, CA, USA) was used for sugar and organic acid analysis. Sugars were detected using a HP 1100 series RI detector and elutions were performed on a Fortis C18 Nucleosil C18 carbohydrate analytical column (250 x 4.6 mm i.d., 5 μ particle size, Fortis Technologies Ltd., Neston, Cheshire, UK) with a column temperature of 25 ^{o}C. The mobile phase used was acetonitrile:water (79:21, v/v) for isocratic elution at flow rate of 2 mL min^{-1}. Calibration curves for the standard solutions at ranged between 10 – 0.5 mg mL^{-1} and were based on a five-point calibration calculated for each sugar (glucose, fructose and sucrose). These were later used for assessing the concentrations corresponding to the different peaks in the chromatograms. Quantification was performed by comparing the peak areas with those of the respective external standards using HP ChemStation (Hewlett-Packard, Palo Alto, CA, USA) software. Linearity, and limit of detection (LOD) calculated from three times the noise level of the response, limit of quantification (LOQ) calculated from 10 times the noise level of the response, and the average recovery (%RSD) of this method under the present chromatographic conditions were as follows:

- glucose, R^2 = 0.9999, y = 37802.74678x – 6536.136, LOD; 0.699, LOQ; 2.330, %RSD;

0.024

- fructose, R^2 = 0.9997, y = 78177.37605x + 9892.674, LOD; 0.237, LOQ; 0.791, %RSD; 0.016

- and sucrose, R^2 = 0.9999, y = 80451.7799x + 1092.3479, LOD; 0.139, LOQ; 0.460, %RSD; 0.009

Based on sugar concentration data, the sweetness index (SI) and total sweetness index (TSI) were calculated using the formulae SI = (1.00 [glucose]) + (2.30 [fructose]) + (1.35 [sucrose]) and TSI = (1.00 x [sucrose]) + (0.76 x [glucose]) + (1.50 x [fructose]), as previously described by Magwaza and Opara (2015).
Organic acids were detected using the same HPLC system. Elution of the organic acid standard solutions and samples was performed on a Fortis C18 (250 x 4.6 mm i.d., 5 μ particle size, Fortis Technologies Ltd., Neston, Cheshire, UK) column. The mobile phase was a 0.02 M potassium phosphate solution (KH_2PO_4, pH 3.01) at a rate of 0.9 mL min^{-1} and the injection volume was 10 μL. Temperature of the column was held constant at 25 ^{o}C. The automatic injection system used was a 10 mL sample loop and organic acids were detected using a HP 1100 series DAD set at 214 nm. Standard solutions and extracts were filtered through a prefilter and finally a 0.22 μm milipore membrane before they were injected onto the column. To prevent the loss of ascorbic acid, standard solutions and extracted samples were protected from light using amber flasks. Quantification was performed by comparing the peak areas with those of the respective external standards using HP ChemStation (Hewlett-Packard, Palo Alto, CA, USA) software. The calibration curves were plotted by the peak area versus concentration of each organic acid based on a five-point calibration in a concentration range of 0.05 - 0.5 mg mL^{-1}. Linearity, and limit of detection (LOD) calculated from three times the noise level of the response, limit of quantification (LOQ) calculated from 10 times the noise level of the response, and the average recovery (%RSD) of this method under the present chromatographic conditions were as follows:

- malic acid, R^2 = 0.9998, y= 897.44394x + 2.65149, LOD; 0.010, LOQ; 0.034, %RSD; 1.347

- tartaric acid, R^2 = 0.9998, y= 1772.18677x + 5.49515, LOD; 0.008, LOQ; 0.029, %RSD; 1.181

- citric acid, R^2 = 0.9999, y= 616.1977x +0.37935, LOD; 0.015, LOQ; 0.517, %RSD; 2.026

- and ascorbic acid; R^2= 0.9999, y= 7453.28005x + 23.56019, LOD; 0.021, LOQ; 0.071, %RSD; 2.616

Based on the above validation statistics, both methods of analysis possess good sensitivity, precision, and repeatability. Linearity was confirmed over a wide calibration range with regression coefficients higher than 0.999, suitable for detecting sugars and organic acids. These two methods can therefore be recommended for routine compositional analyses.

2.5 Lipid extraction and analysis of fatty acid methyl esters (FAME)

The conventional method of total lipid extraction described by Folch, Lees, and Stanley (1957) was used in triplicate for the skin, pulp, and seed of the grape berry. Derivatization of the fatty acids to methyl esters (FAME) was performed by adding 500 μl of HCSM (hexane/chloroform/sodium methoxide, 75/20/5, v/v/v) solution to the sample vials.
The FAME peaks were identified by comparison with FAME standards and the software library in GC-MS. An Agilent 7890 GC /5970 MS Series gas chromatograph (Agilent, Santa Clara, CA, USA) with an FID and MS and a fused (88% - cyanopropyl) aryl-polysiloxane and high polarity capillary column (HP-88, 100 m x 0.25 mm, 0.20 μm film (Part no: 112-88A7, Agilent, Santa Clara, CA, USA) was used. The oven temperature was initially set at 120 ^{o}C for 2 min, and then raised to 250 ^{o}C in increments of 5 ^{o}C min^{-1}. The total analysis time was 45 min. Other conditions were a split ratio of 1/10, solvent delay time 12 min, and injection volume

Table 1: Values of physiochemical parameters, and soluble sugar and organic acid concentrations in the whole berry and peel of 'Karaerik' (*V. vinifera*) grape collected from six locations. Means in rows followed by different letters at superscript are significant at $P < 0.05$

	Locations						
	Üzümlü (avg., 1368 m, a.s.l.)	**Bayırbağ (avg., 1332 m, a.s.l.)**	**Karakaya (avg., 1380 m, a.s.l.)**	**Pişkidağ (avg., 1329 m, a.s.l.)**	**Göllerköyü (avg., 1580 m, a.s.l.)**	**Çağlayan (avg., 1246 m, a.s.l.)**	*mean*
				Whole berry			
pH	3.56 ± 0.04 c	3.66 ± 0.05 d	3.56 ± 0.01 c	3.59 ± 0.02 c	3.39 ± 0.01 b	3.33± 0.01 a	3.51
TA (g CiA kg^{-1} fw)	6.27 ± 0.31 c	5.72 ± 0.1 ab	5.83 ± 0.31 b	5.63 ± 0.15 ab	5.47 ± 0.29 ab	5.23 ± 0.25 a	5.89
DM (%)	20.50 ± 0.78 b	20.17 ± 0.63 b	20.09 ± 0.71 b	20.48 ± 0.83 b	20.23 ± 0.68 b	19.34 ± 0.21 a	20.13
MC (%)	83.31 ± 0.66 b	81.98 ± 0.72 b	81.68 ± 0.73 b	81.25 ± 0.65 b	81.50 ± 0.73 b	78.62 ± 0.14 a	81.39
TSS (%)	18.57 ± 0.04 b	18.56 ± 0.05 b	18.50 ± 0.16 ab	18.55 ± 0.09 b	18.57 ± 0.04 b	18.33 ± 0.14 a	18.51
TSS:TA	2.96	3.24	3.17	3.29	3.39	3.50	3.25
FS (mm)	22.70 ±1.79 c	21.80 ± 1.09 bc	21.40 ± 1.95 bc	20.30 ± 0.76 b	18.00 ± 8.74 bc	16.80 ± 0.84 a	20.17
FF (g mm^{-1})	187.80 ± 2.55 a	188.77 ± 1.26 a	188.48 ± 2.32 a	187.55 ± 2.43 a	187.12 ± 3.57 a	190.81 ± 1.89 a	188.42
Sugar (g kg^{-1} fw)							
Fructose	143.78 ± 3.18 e	136.59 ± 1.21 d	129.68 ± 1.60 c	126.78 ± 0.99 c	120.61 ± 1.72 b	109.77 ± 1.21 a	127.87
Glucose	115.03 ± 0.23 c	112.75 ± 1.93 bc	111.25 ± 1.70 b	111.96 ± 1.52 b	112.86 ± 2.35bc	87.74 ± 0.72 a	108.60
Sucrose	0.72 ± 0.05 b	0.73 ± 0.04 b	0.77 ± 0.03 b	0.76 ± 0.04 b	0.75 ± 0.07 b	0.40 ± 0.03 a	0.69
TS	259.54 ± 2.92 e	250.07 ± 3.09 d	241.7 ± 2.47 c	239.50 ± 1.69 c	234.22 ± 1.91 b	197.91 ± 0.74 a	237.16
SI	446.69	427.89	410.55	404.58	391.27	340.75	403.62
TSI	303.81	291.30	279.84	276.02	267.43	231.73	275.02
Organic acid (g kg^{-1} fw)							
Tartaric acid	3.95 ±0.03 f	2.29 ± 0.02 b	2.52 ± 0.06 c	3.39 ± 0.03 e	3.03 ± 0.03 d	1.69 ± 0.06 a	2.81
Malic acid	2.18 ± 0.11 d	2.12 ± 0.01 d	1.94 ± 0.03 c	1.67 ± 0.01 b	1.34 ± 0.03 a	1.34 ± 0.03 a	1.76
Citric acid	0.28 ± 0.05 c	0.25 ± 0.02 c	0.23 ± 0.04 bc	0.26 ± 0.01 c	0.19 ± 0.02 ab	0.16 ± 0.02 a	0.23
TOA	6.41 ± 0.09 d	4.60 ± 0.01 b	4.70 ± 0.03 b	5.33 ± 0.06 c	4.62 ± 0.03 b	3.19 ± 0.08 a	4.81
				Peel			
Sugar (g kg^{-1} fw)							
Fructose	248.25 ± 2.74 b	244.82 ± 6.78 b	247.63 ± 5.64 b	247.81 ± 1.73 b	239.49 ± 2.23 b	191.45 ± 7.02 a	236.57
Glucose	192.60 ± 0.90 b	189.21 ± 5.84 b	185.74 ± 7.35 b	190.16 ± 8.01 b	189.86 ± 2.06 b	152.59 ± 4.48 a	183.36
Sucrose	0.19 ± 0.02 b	0.18 ± 0.03 b	0.17 ± 0.04 b	0.15 ± 0.02 ab	0.17 ± 0.03 b	0.11 ± 0.01 a	0.16
TS	441.03 ± 2.44 b	434.21 ± 12.49 b	433.54 ± 6.05 b	438.12 ± 7.55 b	429.52 ± 4.07 b	344.15 ± 4.44 a	420.09
SI	763.83	752.54	749.52	760.32	740.92	593.07	726.70
TSI	518.94	511.21	512.77	516.38	503.69	403.25	494.37
Organic acid (g kg^{-1} fw)							
Tartaric acid	8.72 ± 0.05e	7.63 ± 0.05 bc	7.85 ± 0.01 d	7.75 ± 0.02 cd	7.42 ± 0.05 b	3.67 ± 0.28 a	7.17
Malic acid	3.12 ± 0.05 d	2.95 ± 0.01 c	2.32 ± 0.10 b	2.94 ± 0.03 c	2.31 ± 0.06 b	2.05 ± 0.02 a	2.61
Citric acid	0.51 ± 0.01 d	0.48 ± 0.03 cd	0.44 ±0.03 bc	0.49 ± 0.02 cd	0.43 ± 0.01 b	0.32 ± 0.04 a	0.44
TOA	12.36 ± 0.02 e	11.01 ± 0.07 d	10.61 ± 0.11 c	11.17 ± 0.01 d	10.21 ±0.10 b	6.03 ±0.34 a	10.23

Abbreviations: TA; titratable acidity (g CiA kg^{-1}), DM; dry matter, MC; moisture content, TSS; total soluble solids, FS; Fruit size, FF; Fruit firmness (g mm^{-1}), TS (total sugar) and TOA (total organic acid) contents are the sum of individual component identified, a.s.l.; above sea level.

Table 2: Concentrations of fatty acids (%) and minerals (μg g^{-1} dw) in the peel, seed and the whole grape berry of 'Karaerik' (*V. vinifera*) collected from six locations (m, a. s. l). Analysis of variance (one-way ANOVA) was used for comparisons. Means in rows followed by different letters at superscript are significant at $P < 0.05$

	Üzümlü (avg., 1368 m, a.s.l.)	**Bayırbağ (avg., 1332 m, a.s.l.)**	**Karakaya (avg., 1380 m, a.s.l.)**	**Pişkidağ (avg., 1329 m, a.s.l.)**	**Göllerköyü (avg., 1580 m, a.s.l.)**	**Çağlayan (avg.,1246 m, a.s.l.)**	***mean***
Fatty acid (%)				Whole grape			
C 18:0	17.76 ± 0.54 a	14.75 ± 7.08 a	15.53 ± 3.9 a	12.99 ± 1.27 a	17.05 ± 3.76 a	12.40 ± 3.32 a	15.08
C 18:1	1.66 ± 0.24 a	7.11 ± 0.11 e	2.98 ± 0.29 b	4.50 ± 0.64 d	3.65 ± 0.17 c	4.01 ± 0.21 cd	3.98
C 18:2	33.66 ± 3.98b	20.78 ± 0.06a	38.95 ± 5.99b	35.95 ± 3.50b	33.27 ± 4.28b	36.10 ± 0.69b	33.12
C 18:3	5.65 ± 0.06 a	8.94 ± 0.36 d	5.99 ± 0.11 b	7.78 ± 0.11 c	17.82 ± 0.10 e	9.63 ± 0.99 d	9.30
∑SFA	59.02	62.77	52.08	51.76	45.26	50.17	53.51
∑UFA	40.97	36.83	47.92	48.23	54.74	49.84	46.42
∑MUFA	1.66	7.11	2.98	4.50	3.65	4.11	4.00
∑PUFA	39.31	29.72	44.94	43.73	51.09	45.73	42.42
∑Other Acids$^{\mho}$	15.38	14.09	8.84	9.86	3.62	11.10	10.48
Range (avg.)	0 – 12.53 (2.56)	0 – 7.67 (2.35)	0 – 7.05 (1.47)	0 – 7.78 (1.64)	0 – 2.02 (0.60)	0.1 – 5.03 (1.85)	
				Peel			
C 16:0	25.30 ± 1.14 ab	27.15 ± 1.39 b	23.45 ± 2.59 a	23.50 ± 2.40 a	22.97 ± 0.92 a	25.45 ± 1.00 ab	24.64
C 18:0	13.11 ± 0.13 ab	16.27 ± 0.78 b	11.29 ± 0.84 a	13.32 ± 3.93 ab	14.05 ± 0.90 ab	11.09 ± 0.22 a	13.19
C 18:1	6.59 ± 0.28 cd	7.37 ± 0.72 d	2.11 ± 0.02 a	4.43 ± 0.07 b	6.38 ± 0.89 c	6.38 ± 0.10 c	5.54
C 18:2	40.27 ± 7.28 a	37.87 ± 2.31 a	40.08 ± 5.43 a	34.66 ± 2.82 a	33.45 ± 2.16 a	36.54 ± 0.31 a	37.14
C 18:3	7.13 ± 0.08 b	3.28 ± 0.41 a	11.64 ± 1.32 d	10.40 ± 0.56 cd	7.98 ± 0.22 bc	11.35 ± 3.23 d	8.63
∑SFA	45.97	51.49	46.18	50.50	52.19	45.74	48.68
∑UFA	53.99	48.52	53.83	49.49	47.81	54.27	51.32
∑MUFA	6.59	7.37	2.11	4.43	6.38	6.38	5.54
∑PUFA	47.40	41.15	51.72	45.06	41.43	47.89	45.77
∑Other Acids$^{\mho}$	7.56	8.07	11.44	13.68	15.17	9.20	10.85
Range (avg.)	0.18 – 4.78 (1.51)	0 – 4.92 (1.61)	0 – 4.87 (2.29)	0.28 – 9.45 (2.74)	0.17 – 9.76 (3.03)	0.25 – 4.26 (1.84)	
				Seed			
C 16:0	11.35 ± 0.21ab	11.0 ± 0.18 a	11.15 ± 0.37 ab	11.67 ± 0.56 b	11.42 ± 009 ab	11.35 ± 0.34 ab	11.32
C 18:0	6.35 ± 0.00 d	5.51 ± 0.18 b	6.25 ± 0.14 cd	6.10 ± 0.10 bc	6.03 ± .05 c	5.41 ± 0.01 a	5.94
C 18:1	23.57 ± 0.05cd	23.50 ± 0.42 cd	20.15 ± 1.53 a	24.22 ± 0.38 d	22.49 ± 0.13 bc	22.12 ± 0.04 b	22.67
C 18:2	56.2 ± 0.31ab	57.63 ± 1.3 b	60.55 ± 1.17 c	56.08 ± 0.56 a	57.29 ± 0.36 ab	59.17 ± 0.28 c	57.83
C 18:3	1.13 ± 0.38 ab	1.17 ± 0.06 ab	1.19 ± 0.33 ab	1.06 ± 0.20 a	1.76 ± 0.57 b	1.28 ± 0.27 ab	1.26
∑SFA	18.72	17.38	17.90	18.26	18.12	17.11	17.91
∑UFA	81.29	82.63	82.10	81.74	81.88	82.89	82.09
∑MUFA	23.91	23.83	20.36	24.60	22.83	22.44	22.99
∑PUFA	57.38	58.80	61.74	57.14	59.05	60.45	59.09
∑Other Acids$^{\mho}$	1.36	1.20	0.71	0.87	1.01	0.67	0.97
Range (avg.)	0 – 0.34 (0.27)	0 – 0.63 (0.24)	0.04 – 0.28 (0.14)	0 – 0.38 (0.17)	0 – 0.42 (0.20)	0 – 0.32 (0.13)	
Mineral (μg g^{-1} dw)$^{\clubsuit}$				Whole grape			
Na	1079 ± 36 f	998 ± 41 e	557 ± 13 b	803 ± 18 d	614 ± 12 c	498 ± 15 a	758.11
Mg	512 ± 4 e	460 ± 10 d	368 ± 3 c	345 ± 4 b	300 ± 6 a	307 ± 4 a	381.90
P	3460 ± 53 e	2932 ± 12 d	2887 ± 59 cd	2842 ± 35 c	2457 ± 37 b	1456 ± 35 a	2672.33
K	17864 ± 62 a	12425 ± 23 e	12032 ± 257 e	10207 ± 334 d	9749 ± 55 c	9085 ± 255 b	10226.8
Ca	447.9 ± 7.2 f	180.3 ± 2.3 e	161.2 ± 3.3 d	66.2 ± 2.3 b	78.5 ± 0.7 c	52.2 ± 1.3 a	164.39
Mn	3.59 ± 0.09 e	2.55 ± 0.05 d	2.09 ± 0.05 c	2.12 ± 0.06 c	1.86 ± 0.05 b	1.74 ± 0.03 a	2.32
Fe	31.8 ± 0.85 e	22.7 ± 0.33 d	18.2 ± 0.21 c	16.7 ± 0.22 b	17.0 ± 0.34 b	15.2 ± 0.33 a	20.27
Zn	22.0 ± 0.9 a	18.6 ± 0.8 e	16.9 ± 0.5 c	18.2 ± 0.4 f	13.1 ± 0.4 d	9.0 ± 0.3 b	16.29
				Peel			
Na	682 ± 13 f	562 ± 12 e	504 ± 12 d	391 ± 12 c	360 ± 10 b	267 ± 7 a	460.89
Mg	530 ± 5 e	480 ± 5 d	477 ± 5 c	393 ± 7 b	336 ± 5 a	329 ± 4 a	424.21
P	2312 ± 33 d	1907 ± 26 c	1500 ± 29 b	1303 ± 46 a	1295 ± 40 a	1241 ± 27 a	1592.83
K	13498 ± 353 e	11252 ± 354 d	8487 ± 392 bc	8805 ± 416 c	7932 ± 141 b	6015 ± 111 a	9331.5
Ca	717.3 ± 5.6 f	238.4 ± 4.4 e	222.3 ± 3.4 d	206.2 ± 2.5 c	180.3 ± 2.1 b	90.3 ± 1.7 a	275.8
Mn	6.9 ± 0.20 d	3.2 ± 0.09 ab	3.4 ± 0.12 b	3.0 ± 0.15 a	3.9 ± 0.12 c	3.0 ± 0.11 a	3.88
Fe	32.2 ± 0.9 d	30.5 ± 0.9 c	23.6 ± 0.7 b	24.4 ± 0.8 b	24.0 ± 0.4 b	16.9 ± 0.4 a	25.26
Zn	20.5 ± 1.5 c	16.3 ± 0.6 b	16.0 ± 0.8 d	13.3 ± 0.1 a	14.7 ± 0.7 b	8.8 ± 0.5 b	14.94
				Seed			
Na	216 ± 8 f	184 ± 7 e	177 ± 6 d	145 ± 6 b	164 ± 9 c	131 ± 4 a	169.44
Mg	189 ± 4 f	152 ± 4 d	154 ± 3 e	140 ± 4 c	119 ± 2 b	104 ± 5 a	143.12
P	3879 ± 50 e	3451 ± 56 d	3211 ± 57 c	2961 ± 52 b	3240 ± 70 c	1693 ± 38 a	3072.67
K	5851 ± 203 c	5675 ± 121 c	5728 ± 233 c	5247 ± 118 c	5064 ± 135 b	4563 ± 58 a	5354.67
Ca	735.9 ± 5.9 e	686.1 ± 10.3 d	530.9 ± 4.7 c	511.7 ± 6.5 b	504.4 ± 11.5 b	455.9 ± 4.5 a	570.82
Mn	21.5 ± 0.15 f	15.4 ± 0.21 e	14.0 ± 0.17 d	10.2 ± 0.10 b	12.0 ± 0.21 c	9.7 ± 0.11 a	13.79
Fe	32.9 ± 0.7 e	30.6 ± 0.8 d	29.3 ± 0.4 c	28.6 ± 0.9 c	25.5 ± 0.6 a	27.4 ± 0.7 b	29.04
Zn	15.4 ± 0.5 a	20.3 ± 0.6 d	18.0 ± 0.6 c	20.2 ± 1.0 e	28.2 ± 0.8 b	26.6 ± 1.1 f	21.44

Abbreviations: C16:0; palmitic acid, C16:1; palmitoleic acid, C18:0; stearic acid, C18:1; oleic acid, C18:2; linoleic acid, C18:3; α-linolenic acid, SFA; saturated fatty acids, UFA; unsaturated fatty acids, MUFA; monounsaturated fatty acids, PUFA; polyunsaturated fatty acids, Na; sodium, Mg; magnesium, P; phosphorous, K; potassium, Ca; calcium, Mn; manganese, Fe; iron, Zn; zinc. , a.s.l; above sea level.
$\mho$: The other acids category is the sum of C14:0, C15:0, C16:1, C17:0, C22:0, C24:0.
$\clubsuit$: Analytical performance; LOD, mg kg^{-1} (Na; 0.12, Mg; 0.13, P; 0.17, K; 0.13, Ca; 0.15, Mn; 0.10, Fe; 0.05, Cu; 0.08, Zn; 0.06,), LOQ, mg kg^{-1} (Na; 0.41, Mg; 0.45, P; 0.57, K; 4.3, Ca; 4.9, Mn; 0.34, Fe; 0.16, Zn; 0.19,), RSD, % Na; 2.7, Mg; 2.7, P; 3.1, K; 3.1, Ca; 3.6, Mn; 2.5, Fe; 0.9, Zn; 1.9).

1μL. An injection system with auto sampler was used.

2.6 Element analysis

Briefly, 0.5 g dried finely powdered grape sample (deseeded whole grape, skin and seed) at 0.1 mg sensitivity was weighed into the Teflon vessels of a microwave digestion system (A Milestone START D, Sorisole, Italy). Next, 6 mL of concentrated HNO_3 and 2 mL of H_2O_2 were added. The content of the vessels was digested under microwave irradiation at 45 bar pressure, as described elsewhere (Bulut et al., 2008; Duran et al., 2007). After digestion, the limpid solutions were made up to 50 mL with ultrapure water, and finally the solutions were analyzed by ICP-MS to determine the element content of the samples. The limpid solutions were analyzed with an Agilent 7700 x ICP-MS device (Santa Clara, California, USA) equipped with a third-generation Octopole Reaction System (ORS^3) using helium gas under conditions recommended by the manufacturer.
In order to corroborate the accuracy of the ICP-MS method combined with the microwave digestion, spiked/recovery tests and analysis of a certified standard reference material, CRM NIES No. 7 Tea Leaves, were performed using the same method. Satisfactory results were achieved at the end of the accuracy test. The analytical characteristics of the method were also determined with the parameters LOD (limit of detection), LOQ (limit of quantification) and RSD (relative standard deviation). In order to determine the LOD and LOQ of each element, the standard deviations of the results obtained by measuring 20 blank solutions with ICP-MS method were calculated. Values three- and 10–fold greater than the standard deviation were adopted as LOD and LOQ, respectively. In order to determine the RSD value of each element, which represents the precision, the method was repeated 10 times for analyzing solutions containing a fixed amount of each element. The RSD values were calculated by dividing the standard deviation of each element by the mean value (Bulut et al., 2008). The results are shown in Table 2.

2.7 Statistical Analysis

All extractions and analyses were performed in triplicate (n = 3, mean) from harmonized triplicate samples, and the data are presented as mean ± pooled standard deviation. The data given in Table 1 and 2 were compared using one-way analysis of variance (ANOVA) and Duncan's Multiple Range test (IBM SPSS Statistics V22.0) at significance level of $P < 0.05$. The mean data were also subjected to Pearson correlation (r) using the same software at significance levels of $P < 0.01$ or 0.05. A statistical software package (XLSTAT version 2014.6) using ADDINSOFT (Damrémont, Paris, France) was employed to perform the Principal Component Analysis (PCA) on Microsoft Office Excel 2010.

3 Results and Discussions

3.1 Physicochemical parameters of the 'Karaerik' grape berry

The values for physicochemical parameters were strongly positively or negatively correlated with the concentrations of sugars and organic acids in the whole grape berry and the peel sampled from grape berries in the six locations in the district of Üzümlü and the surrounding area (Fig. 1, Table 1) ($P < 0.01$, 0.05, Table 3). The results show that the grape berries sampled from Çağlayan differed significantly ($P < 0.01$ or 0.05) from those from the other five locations, exhibiting lower physicochemical parameter values and concentrations of sugars and acids. The pH value in the berry of the 'Karaerik' grape agrees rather well with the average values for 101 grape berries previously reported (Akpınar & Yiğit, 2011; Eyduran, Akin, Ercisli, Eyduran, & Maghradze, 2015; Karasu et al., 2016; Rolle, Giacosa, Gerbi, Bertolino, & Novello, 2013; Xu et al., 2017; Yamamoto et al., 2015). The TA in the present grape berry was also in good agreement with ranges reported for 33 grape berries (avg. 4.3, range 0.3 -11.6 CAE g kg^{-1} fw) in various studies (Ejsmentewicz et al., 2015; Ochmian et al., 2013; Pavlousek & Kumsta, 2011; Rolle et al., 2013; Yamamoto et al., 2015). However, data re-

ported for dry matter (avg. 26.1%, range 22.2 - 28.8) and moisture (avg. 92.3%, range 85.6 - 98.6) contents in eight grape berry cvs are not in agreement with those reported by several authors (Karasu et al., 2016; Kurt et al., 2017; Ochmian et al., 2013; Ozcan & Al Juhaimi, 2017). Notably, Lijavetzky et al. (2012) also reported a low DM content (avg. 15.9%) in 'Muscat Hamburg' grape cultivar. In addition to these values, the TSS, FS and FF values of the 'Karaerik' grape berry in the present study exhibited good agreement with the findings for 104 grape cvs (avg. 18.2, range 10.9 – 25.7, avg. 16.4, range 12.1 – 26.6, avg. 276.3, range 69.4 – 605 g mm^{-1}, respectively) described in other studies (Conner, 2013; Ochmian et al., 2013; Ejsmentewicz et al., 2015; Yamamoto et al., 2015; Eyduran et al., 2015; Xu et al., 2017).

3.2 Soluble sugar composition of the 'Karaerik' grape berry

Fructose was the dominant soluble sugar, with a mean value of 236.57 g kg^{-1} fw (range 191.45 to 248.25), in the peel berry and of 127.87 g kg^{-1} fw (range 109.77 to 143.78) in the whole grape, followed by glucose (avg. 183.36; range 152.9 - 192.60 and 108.60; range 87.74 - 115.03) and the minor soluble sugar sucrose (avg. 0.16 and 0.69 g kg^{-1} fw). Among the six locations in this study, only the grape berries sampled from Çağlayan differed significantly ($P < 0.01$ or 0.05) with their low concentrations of soluble sugars, while the remaining five locations exhibited similar concentrations at high levels, the difference between them being statistically insignificant (Table 1). Fructose and glucose have been identified as the major soluble sugars in grape berries, as in the 'Karaerik' grape berry, while sucrose and other sugars are rarely found in *V. vinifera* and its hybrids with *V. labrusca* or others, as reviewed by Kurt et al. (2017). Estimated concentrations of fructose (avg. 83.4 g kg^{-1} fw; range 47.4 – 155.5) and glucose (avg. 88.2 g kg^{-1} fw; range 64 – 164.7) in a large number of grape berries have indicated wide variations in content (Eyduran et al., 2015; Karasu et al., 2016; Kurt et al., 2017; Liu, Wu, Fan, Li, & Li, 2006; Liang et al., 2011; Pavlousek & Kumsta, 2011; Sousa et al., 2014; Topalovic & Mikulic-Petkovsek, 2010). A similar pattern was observed in sugar profiles in berries of the 'Karaerik' grape to those reported for the above citations. A low level of sucrose in grape berries (*V. vinifera* x *V. labrusca*) (Kurt et al., 2017) has also very recently been confirmed in the grape berry in the present study (Karaerik).

3.3 Organic acid composition of the 'Karaerik' grape berry

The major organic acid in the grape berry was tartaric acid, the levels of which varied between 3.67 and 8.72 g kg^{-1} fw (avg. 7.17) in the peel and 1.69 and 3.95 g kg^{-1} fw (avg. 2.81) in the whole grape berry over the six sampling locations. This was followed by malic acid (avg. 2.61; range 2.05 – 3.12 and 1.76; 1.34 – 2.18, respectively). Similar to the sugar concentrations, berries sampled from Çağlayan had significantly ($P < 0.01$ or 0.05) lower organic acid concentrations than berries from the other five locations (Table 1). The minor acid was citric acid, as reported earlier elsewhere (Kurt et al., 2017) for grape berries, the concentration of which averaged 0.44 and 0.23 g kg^{-1} fw in the peel and the whole grape berry, respectively. Most grape berries are reported to contain tartaric acid as the major organic acid (Mpelasoka, Schachtman, Treeby, & Thomas, 2003). A compilation of data for 46 grape berry cvs or varieties revealed an average of 4.41 g kg^{-1} fw (range 1.40 – 12.71) of tartaric acid and 2.21 g kg^{-1} fw (range 0.97 – 5.19) of malic acid (Eyduran et al., 2015; Liu et al., 2006; Pavlousek & Kumsta, 2011; Rolle et al., 2013; Topalovic & Mikulic-Petkovsek, 2010). Our findings for the present grape (Karaerik) were also in agreement with the ranges previously reported in the literature. Some authors have reported quite low citric acid concentrations in grape berries (29 cvs) (avg. 0.26 g kg^{-1} fw; range 0.04 -0.96), (Kurt et al., 2017; Pavlousek & Kumsta, 2011; Rolle et al., 2013). Kurt et al. (2017), Pavlousek and Kumsta (2011), Rolle et al. (2013) and others have reported complete absence of citric acid (Eyduran et al., 2015; Liu et al., 2006). We determined approximately the same concentration of citric acid in the present grape berry (avg. 0.23 g kg^{-1} fw, range 0.16 –

Table 3: Pearson correlation (r) of sugars and organic acids compared with some physical parameters of the grape berry (Karaerik)

	TA	DM	MC	TSS	TSS/TA	FS	FF	fru^{w}	glc^{w}	*uc^{w}	TS^{w}	SI^{w}	TSI^{w}	fru^{p}	glc^{p}	suc^{p}	TS^{p}	SI^{p}	TSI^{p}	TaA^{w}	MaA^{w}	CiA^{w}	TOA^{w}	TaA^{p}	MaA^{p}	CiAp	TOAp
pH	0.648NS	0.672 ^{NS}	0.690 ^{NS}	0.645 ^{NS}	-0.666 ^{NS}	**0.894***	-0.382 ^{NS}	**0.822***	0.702 ^{NS}	0.687 ^{NS}	0.810 ^{NS}	**0.825***	**0.823***	0.779 ^{NS}	0.699 ^{NS}	0.659 ^{NS}	0.750	0.762 ^{NS}	0.761 ^{NS}	0.361 ^{NS}	**0.854***	**0.881***	0.597 ^{NS}	0.735 ^{NS}	0.804 ^{NS}	**0.834***	0.777 ^{NS}
TA	-	0.711 ^{NS}	**0.897***	0.620 ^{NS}	**-0.999****	**0.912***	-0.475 ^{NS}	**0.937****	0.699 ^{NS}	0.581 ^{NS}	**0.873***	**0.911***	**0.905***	0.712 ^{NS}	0.678 ^{NS}	0.810 ^{NS}	0.701	0.704 ^{NS}	0.706 ^{NS}	0.725 ^{NS}	**0.866***	**0.862***	**0.896***	0.809 ^{NS}	0.733 ^{NS}	0.799 ^{NS}	**0.830***
DM	-	-	**0.882***	**0.943****	-0.695 ^{NS}	0.704 ^{NS}	**-0.911***	0.765 ^{NS}	**0.941****	**0.892***	**0.895***	**0.850***	**0.859***	**0.932****	**0.958****	0.801 ^{NS}	**0.947****	**0.950****	**0.942****	**0.886***	0.531 ^{NS}	**0.845***	**0.911***	**0.947****	0.794 ^{NS}	**0.958****	**0.963****
MC	-	-	-	0.892*	-0.885*	0.859*	-0.751 ^{NS}	**0.933****	**0.926****	**0.826***	**0.982****	**0.973****	**0.975****	**0.898***	**0.912***	**0.971****	**0.908***	**0.907***	**0.905***	0.776 ^{NS}	0.765 ^{NS}	**0.841***	**0.903***	**0.960****	0.757 ^{NS}	**0.918****	**0.963****
TSS	-	-	-	-	-0.599 ^{NS}	0.651 ^{NS}	**-0.915***	0.743 ^{NS}	**0.978****	**0.925****	**0.901***	**0.844***	**0.855***	**0.933****	**0.986****	**0.888***	**0.959****	**0.958****	**0.950****	0.746 ^{NS}	0.504 ^{NS}	0.728 ^{NS}	0.787 ^{NS}	**0.937****	0.710 ^{NS}	**0.911***	**0.936****
TSS/TA	-	-	-	-	-	**-0.924****	0.450 ^{NS}	**-0.936****	-0.688 ^{NS}	-0.578 ^{NS}	**-0.867***	**-0.906***	**-0.901***	-0.709 ^{NS}	-0.664 ^{NS}	-0.799 ^{NS}	-0.695	-0.698 ^{NS}	-0.701 ^{NS}	-0.698 ^{NS}	**-0.882***	**-0.864***	**-0.880***	-0.802 ^{NS}	-0.723 ^{NS}	-0.790 ^{NS}	**-0.821***
FS	-	-	-	-	-	-	-0.413 ^{NS}	**0.966****	0.738 ^{NS}	0.666 ^{NS}	**0.909***	**0.944****	**0.939****	0.790 ^{NS}	0.718 ^{NS}	0.807 ^{NS}	0.765 ^{NS}	0.772 ^{NS}	0.775 ^{NS}	0.539 ^{NS}	**0.970****	**0.928****	0.782 ^{NS}	**0.822***	0.799 ^{NS}	**0.856***	**0.851***
FF	-	-	-	-	-	-	-	-0.503 ^{NS}	**-0.895***	**-0.887***	-0.725 ^{NS}	-0.639 ^{NS}	-0.654 ^{NS}	**-0.850***	**-0.912***	-0.717 ^{NS}	**-0.880***	**-0.874***	**-0.869***	**-0.819***	-0.204 ^{NS}	-0.551 ^{NS}	-0.743 ^{NS}	**-0.852***	-0.500 ^{NS}	-0.771 ^{NS}	**-0.825***
fru^{w}	-	-	-	-	-	-	-	-	0.790 ^{NS}	0.671 ^{NS}	**0.953****	**0.984****	**0.980****	0.795 ^{NS}	0.774 ^{NS}	**0.886***	0.790 ^{NS}	0.796 ^{NS}	0.793 ^{NS}	0.642 ^{NS}	**0.935****	**0.921****	**0.851***	**0.857***	**0.854***	**0.895***	**0.894***
glc^{w}	-	-	-	-	-	-	-	-	-	**0.969****	**0.939****	**0.887***	**0.896***	**0.982****	**0.997****	**0.921****	**0.993****	**0.992****	**0.990****	0.734 ^{NS}	0.589 ^{NS}	0.769 ^{NS}	0.809 ^{NS}	**0.982****	0.680 ^{NS}	**0.922****	**0.967****
suc^{w}	-	-	-	-	-	-	-	-	-	-	**0.856***	0.787 ^{NS}	0.800 ^{NS}	**0.981****	**0.967****	**0.831***	**0.980****	**0.978****	**0.981****	0.638 ^{NS}	0.495 ^{NS}	0.690 ^{NS}	0.701 ^{NS}	**0.942****	0.552 ^{NS}	**0.851***	**0.906***
TS^{w}	-	-	-	-	-	-	-	-	-	-	-	**0.992****	**0.994****	**0.932****	**0.928****	**0.953****	**0.936****	**0.938****	**0.935****	0.723 ^{NS}	**0.818***	**0.898***	**0.878***	**0.968****	**0.816***	**0.959****	**0.980****
SI^{w}	-	-	-	-	-	-	-	-	-	-	-	-	**1.000****	**0.885***	**0.874***	**0.936****	**0.885***	**0.888***	**0.886***	0.697 ^{NS}	**0.877***	**0.918****	**0.877***	**0.932****	**0.842***	**0.943****	**0.955****
TSI^{w}	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.894***	**0.884***	**0.940****	**0.894***	**0.897***	**0.895***	0.702 ^{NS}	**0.868***	**0.916***	**0.878***	**0.939****	**0.839***	**0.946****	**0.960****
fru^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.979****	**0.876***	**0.996****	**0.997****	**0.999****	0.700 ^{NS}	0.636 ^{NS}	**0.815***	0.800 ^{NS}	**0.982****	0.683 ^{NS}	**0.929****	**0.966****
glc^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.897***	**0.992****	**0.991****	**0.988****	0.754 ^{NS}	0.562 ^{NS}	0.775 ^{NS}	**0.816***	**0.976****	0.701 ^{NS}	**0.931****	**0.966****
suc^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.890***	**0.886***	**0.886***	0.632 ^{NS}	0.735 ^{NS}	0.728 ^{NS}	0.775 ^{NS}	**0.924****	0.648 ^{NS}	**0.845***	**0.910***
TS^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1.000****	**1.000****	0.726 ^{NS}	0.609 ^{NS}	0.803 ^{NS}	0.810 ^{NS}	**0.985****	0.694 ^{NS}	**0.935****	**0.971****
^{p}SI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.999****	0.727 ^{NS}	0.617 ^{NS}	**0.813***	**0.814***	**0.984****	0.707 ^{NS}	**0.941****	**0.973****
TSI^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.717 ^{NS}	0.620 ^{NS}	0.808 ^{NS}	0.807 ^{NS}	**0.985****	0.690 ^{NS}	**0.933****	**0.970****
TaA^{w}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.372 ^{NS}	0.726 ^{NS}	**0.945****	0.784 ^{NS}	0.694 ^{NS}	0.794 ^{NS}	0.808 ^{NS}
MaA^{w}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.846***	0.655 ^{NS}	0.679 ^{NS}	0.747 ^{NS}	0.734 ^{NS}	0.718 ^{NS}
CiA^{w}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.889***	**0.843***	**0.935****	**0.945****	**0.899***
TOA^{w}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.881***	**0.824***	**0.906***	**0.913***
TaA^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.723 ^{NS}	**0.946****	**0.990****
MaA^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.902***	**0.813***
CiA^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.981****

Abbreviations (plus superscripts): TA; titratable acidity, DM; dry matter, MC; moisture content, TSS; total soluble solid, TSS/TA; total soluble solid/titratable acidity, FS; fruit size, FF; fruit firmness, w; whole grape berry, p; peel, fru; fructose, glc; glucose, suc; sucrose, TS; total sugar, SI; sweetness index, TSI; total sweetness index, TaA; tartaric acid, MaA; malic acid, CiA; citric acid, TOA; total organic acid. Asterisks indicate significance at * P <0.05 and ** P <0.01, NS; non-significant.

0.28).
Sugars and organic acids have been described as an important key factor in evaluating organoleptic properties in grape berries. In the present study, sugars and organic acids were closely and significantly correlated (either positively or negatively) with physicochemical parameters and sampling locations (range, r = 0.813 – 1.000, P < 0.01 or 0.05, see Table 3).

3.4 Fatty acid composition of the 'Karaerik' grape berry

Linoleic acid, with average concentrations of 33.12% in the whole grape berry, 37.14% in the peel and 57.83% in the seed, was the most abundant fatty acid in the 'Karaerik' grape. Concentrations of "other acids", as the minor acids, (representing the sum of myristic acid, pentadecanoic acid, palmitoleic acid margaric acid, behenic acid, and lignoceric acid), averaged 10.48% in the whole grape berry, 10.85% in the peel and 0.97% in the seed. The highest C18:2 had been 40.27% in the peel of sampled berries from Üzümlü and 60.55% in the seed of sampled berries from Karakaya (Table 2). Table 4 clearly indicates that the fatty acids were largely insignificantly correlated with physicochemical parameters and sampling locations, although there were possible strong positive or negative correlations within the physicochemical parameters and the sum of fatty acids (range r = -0.817 – 1.000, P < 0.01 or 0.05). In general, fatty acid composition in grape berries, except for seeds, has rarely been described. More recently, fatty acid changes during berry maturation and ripening of the 'Isabel' grape have been well studied (Kurt et al., 2017). A notable large variation in concentrations of major saturated (C16:0; avg. 9.6%, range 7.1 – 8.24, C18:0; avg. 4.5, range 2.4 - 6.5) and unsaturated (C18:1; avg. 20.6%, range 13.4 - 32.3, C18:2; avg. 63.9%, range 47.3 - 70.7) fatty acids has been observed among 44 grape berry cvs or varieties (i.e. wine or table grapes), in agreement with the findings for 'Karaerik' in the present study, by some authors (Akin & Altindisli, 2011; Al Juhaimi, Gecgel, Gulcu, Hamurcu, & Ozcan, 2017; Kurt et al., 2017; Shiozaki & Murakami, 2016).

3.5 Mineral composition of the 'Karaerik' grape berry

Concentrations of eight elements (Table 2) in the present grape berry varied significantly (P < 0.05) and were strongly positively or negatively correlated (range, r = 0.813 – 0.999, P < 0.01 or 0.05, Table 5) with the physicochemical parameters or sampling locations. In general, mineral concentrations were higher in berries sampled from Üzümlü than in those from Çağlayan (the lowest concentration). Potassium was the most abundant mineral in the whole grape berry (avg. 10,226.8 μg g^{-1} dw), the peel (avg. 9331.5 μg g^{-1} dw) and the seed (avg. 5354.67 μg g^{-1} dw), followed by phosphorus (P, avg. 2672.33 μg g^{-1} dw), sodium (Na, avg. 758.11 μg g^{-1} dw) and magnesium (Mg, avg. 381.90 μg g^{-1} dw). 'Karaerik' grapes contained considerable amounts of calcium (avg. 164.39 μg g^{-1} dw) in the whole berry, in the peel (avg. 275 μg g^{-1} dw) and in the seed (avg. 570.82 μg g^{-1} dw). Concentrations of iron and zinc in the present grape berry were also high and comparable (avg. 20.27 and 16.29; 25.26 and 14.94; 29.04 and 21.44, μg g^{-1} dw, respectively, Table 4). K (potassium) was also the major element in the 'Shiraz' grape cultivar, with concentrations of 4380, 3660 and 3360 g^{-1} dw in the skin, seed and peel, respectively, (Rogiers, Greer, Hatfield, Orchard, & Keller, 2006). Earlier reported concentrations (avg. g kg^{-1} fw) of the first three major elements in grapes were 958.98 (range 10.80 to 3870) for potassium, 56.2 (range 095 to 92.3) for calcium and 39 (range 0.87 – 190) for phosphorus (Kurt et al., 2017; Rogiers et al., 2006; Sousa et al., 2014). Our findings are generally in agreement with the given citations regarding the composition of minerals in grape berries. In terms of tissue, most of the phosphorus accumulated in the seeds of grape berries, as concluded previously by Rogiers et al. (2006) and Kurt et al. (2017), and this was also confirmed in the seeds of the grape berry in the present study. It was determined with the present study that sampling locations having different soil characteristics affected the mineral composition of the grape berry. Güneş, Köse, and Turan (2015) have studied the effect of different boron concentrations on nutrient uptake

Table 4: Pearson correlation (r) of fatty acids compared with some physicochemical parameters of the grape berry (Karaerik)

	TA	DM	MC	TSS	TSS/TA	FS	FF	C16:0^{w}	C18:0^{w}	C18:1^{w}	C18:2^{w}	C18:3^{w}	SFA^{w}	UFA^{w}	MUFA^{w}	PUFA^{w}	C16:0^{p}	C18:0^{p}	C18:1^{p}	C18:2^{p}	C18:3^{p}	SFA^{p}	UFA^{p}	MUFA^{p}	PUFA^{p}	C16:0^{s}	C18:0^{s}	C18:1^{s}	C18:2^{s}	C18:3^{s}	SFA^{s}	UFA^{s}	MUFA^{s}	PUFA^{s}
pH	0.648NS	0.672 ^{NS}	0.690 ^{NS}	0.645 ^{NS}	-0.666 ^{NS}	**0.894***	-0.382 ^{NS}	0.708 ^{NS}	0.137 ^{NS}	0.328 ^{NS}	-0.460 ^{NS}	-0.567 ^{NS}	0.758 ^{NS}	-0.758 ^{NS}	0.312 ^{NS}	-0.750 ^{NS}	0.308 ^{NS}	0.534 ^{NS}	-0.150 ^{NS}	0.431 ^{NS}	-0.492 ^{NS}	0.216 ^{NS}	-0.218 ^{NS}	-0.150 ^{NS}	-0.090 ^{NS}	-0.304 ^{NS}	0.278 ^{NS}	0.312 ^{NS}	-0.263 ^{NS}	-0.643 ^{NS}	0.286 ^{NS}	-0.281 ^{NS}	0.300 ^{NS}	-0.349 ^{NS}
TA	-	0.711 ^{NS}	**0.897***	0.620 ^{NS}	**-0.999****	**0.912***	-0.475 ^{NS}	0.029 ^{NS}	0.671 ^{NS}	-0.426 ^{NS}	-0.072 ^{NS}	-0.576 ^{NS}	0.603 ^{NS}	-0.594 ^{NS}	-0.440 ^{NS}	-0.416 ^{NS}	0.125 ^{NS}	0.163 ^{NS}	-0.094 ^{NS}	0.708 ^{NS}	-0.325 ^{NS}	-0.256 ^{NS}	0.252 ^{NS}	-0.094 ^{NS}	0.231 ^{NS}	-0.179 ^{NS}	0.701 ^{NS}	0.154 ^{NS}	-0.313 ^{NS}	-0.438 ^{NS}	0.724 ^{NS}	-0.719 ^{NS}	0.144 ^{NS}	-0.302 ^{NS}
DM	-	-	**0.882***	**0.943****	-0.695 ^{NS}	0.704 ^{NS}	**-0.911***	0.060 ^{NS}	0.561 ^{NS}	-0.147 ^{NS}	-0.139 ^{NS}	-0.124 ^{NS}	0.274 ^{NS}	-0.271 ^{NS}	-0.168 ^{NS}	-0.198 ^{NS}	-0.239 ^{NS}	0.484 ^{NS}	-0.090 ^{NS}	0.052 ^{NS}	-0.368 ^{NS}	0.393 ^{NS}	-0.398 ^{NS}	-0.090 ^{NS}	-0.252 ^{NS}	0.233 ^{NS}	0.732 ^{NS}	0.463 ^{NS}	-0.661 ^{NS}	-0.158 ^{NS}	**0.843***	**-0.841***	0.457 ^{NS}	-0.670 ^{NS}
MC	-	-	-	**0.892***	**-0.885***	**0.859***	-0.751 ^{NS}	0.089 ^{NS}	0.790 ^{NS}	-0.221 ^{NS}	-0.269 ^{NS}	-0.232 ^{NS}	0.497 ^{NS}	-0.494 ^{NS}	-0.241 ^{NS}	-0.377 ^{NS}	0.011 ^{NS}	0.477 ^{NS}	0.001 ^{NS}	0.414 ^{NS}	-0.531 ^{NS}	0.172 ^{NS}	-0.176 ^{NS}	0.001 ^{NS}	-0.131 ^{NS}	-0.170 ^{NS}	0.692 ^{NS}	0.248 ^{NS}	-0.446 ^{NS}	-0.152 ^{NS}	0.755 ^{NS}	-0.751 ^{NS}	0.240 ^{NS}	-0.454 ^{NS}
TSS	-	-	-	-	-0.599 ^{NS}	0.651 ^{NS}	**-0.915***	0.148 ^{NS}	0.656 ^{NS}	0.027 ^{NS}	-0.354 ^{NS}	0.000 ^{NS}	0.285 ^{NS}	-0.287 ^{NS}	0.005 ^{NS}	-0.255 ^{NS}	-0.153 ^{NS}	0.675 ^{NS}	0.048 ^{NS}	-0.014 ^{NS}	-0.567 ^{NS}	0.576 ^{NS}	-0.580 ^{NS}	0.044 ^{NS}	-0.452 ^{NS}	0.019 ^{NS}	0.586 ^{NS}	0.398 ^{NS}	-0.586 ^{NS}	0.057 ^{NS}	0.698 ^{NS}	-0.695 ^{NS}	0.393 ^{NS}	-0.566 ^{NS}
TSS/TA	-	-	-	-	-	**-0.924****	0.450 ^{NS}	-0.055 ^{NS}	-0.645 ^{NS}	0.414 ^{NS}	0.064 ^{NS}	0.611 ^{NS}	-0.616 ^{NS}	0.607 ^{NS}	0.429 ^{NS}	0.430 ^{NS}	-0.132 ^{NS}	-0.143 ^{NS}	0.130 ^{NS}	-0.735 ^{NS}	0.305 ^{NS}	0.279 ^{NS}	-0.275 ^{NS}	0.130 ^{NS}	-0.265 ^{NS}	0.202 ^{NS}	-0.694 ^{NS}	-0.124 ^{NS}	0.275 ^{NS}	0.470 ^{NS}	-0.700 ^{NS}	0.696 ^{NS}	-0.114 ^{NS}	0.329 ^{NS}
FS	-	-	-	-	-	-	-0.413 ^{NS}	0.413 ^{NS}	0.456 ^{NS}	-0.063 ^{NS}	-0.293 ^{NS}	-0.654 ^{NS}	0.769 ^{NS}	-0.764 ^{NS}	-0.079 ^{NS}	-0.657 ^{NS}	0.272 ^{NS}	0.349 ^{NS}	-0.151 ^{NS}	0.695 ^{NS}	-0.444 ^{NS}	-0.081 ^{NS}	0.078 ^{NS}	-0.151 ^{NS}	0.129 ^{NS}	-0.349 ^{NS}	0.516 ^{NS}	0.177 ^{NS}	-0.232 ^{NS}	-0.596 ^{NS}	0.507 ^{NS}	-0.502 ^{NS}	0.165 ^{NS}	-0.308 ^{NS}
FF	-	-	-	-	-	-	-	0.189 ^{NS}	-0.631 ^{NS}	0.211 ^{NS}	0.004 ^{NS}	-0.239 ^{NS}	0.096 ^{NS}	-0.097 ^{NS}	0.230 ^{NS}	-0.143 ^{NS}	0.520 ^{NS}	-0.416 ^{NS}	0.130 ^{NS}	0.218 ^{NS}	0.240 ^{NS}	-0.535 ^{NS}	0.539 ^{NS}	0.130 ^{NS}	0.337 ^{NS}	-0.332 ^{NS}	-0.742 ^{NS}	-0.297 ^{NS}	0.568 ^{NS}	-0.230 ^{NS}	**-0.817***	**0.818***	-0.296 ^{NS}	0.525 ^{NS}
C16:0^{w}	-	-	-	-	-	-	-	-	-0.373 ^{NS}	**0.849***	-0.742 ^{NS}	-0.332 ^{NS}	0.716 ^{NS}	-0.725 ^{NS}	0.845*	**-0.854***	0.665 ^{NS}	0.599 ^{NS}	0.148 ^{NS}	0.173 ^{NS}	-0.554 ^{NS}	0.332 ^{NS}	-0.330 ^{NS}	0.148 ^{NS}	-0.315 ^{NS}	-0.522 ^{NS}	-0.471 ^{NS}	0.270 ^{NS}	0.003 ^{NS}	-0.518 ^{NS}	-0.441 ^{NS}	0.446 ^{NS}	0.262 ^{NS}	-0.075 ^{NS}
C18:0^{w}	-	-	-	-	-	-	-	-	-	-0.488 ^{NS}	-0.080 ^{NS}	0.166 ^{NS}	0.110 ^{NS}	-0.106 ^{NS}	-0.502 ^{NS}	0.032 ^{NS}	-0.155 ^{NS}	0.243 ^{NS}	0.115 ^{NS}	0.300 ^{NS}	-0.386 ^{NS}	0.052 ^{NS}	-0.055 ^{NS}	0.115 ^{NS}	-0.096 ^{NS}	-0.205 ^{NS}	0.632 ^{NS}	-0.072 ^{NS}	-0.218 ^{NS}	0.369 ^{NS}	0.659 ^{NS}	-0.656 ^{NS}	-0.074 ^{NS}	-0.155 ^{NS}
C18:1^{w}	-	-	-	-	-	-	-	-	-	-	-0.761 ^{NS}	0.174 ^{NS}	0.336 ^{NS}	-0.350 ^{NS}	**1.000****	-0.560 ^{NS}	0.513 ^{NS}	0.658 ^{NS}	0.333 ^{NS}	-0.315 ^{NS}	-0.519 ^{NS}	0.628 ^{NS}	-0.625 ^{NS}	0.333 ^{NS}	-0.621 ^{NS}	-0.358 ^{NS}	-0.723 ^{NS}	0.293 ^{NS}	-0.016 ^{NS}	-0.078 ^{NS}	-0.640 ^{NS}	0.643 ^{NS}	0.291 ^{NS}	-0.034 ^{NS}
C18:2^{w}	-	-	-	-	-	-	-	-	-	-	-	-0.133 ^{NS}	-0.672 ^{NS}	0.684 ^{NS}	-0.756 ^{NS}	0.795 ^{NS}	-0.754 ^{NS}	**-0.900***	-0.686 ^{NS}	0.005 ^{NS}	**0.941****	-0.557 ^{NS}	0.557 ^{NS}	-0.686 ^{NS}	0.739 ^{NS}	0.554 ^{NS}	0.499 ^{NS}	-0.468 ^{NS}	0.295 ^{NS}	0.025 ^{NS}	0.295 ^{NS}	-0.302 ^{NS}	-0.463 ^{NS}	0.294 ^{NS}
C18:3^{w}	-	-	-	-	-	-	-	-	-	-	-	-	-0.619 ^{NS}	0.609 ^{NS}	0.175 ^{NS}	0.496 ^{NS}	-0.324 ^{NS}	0.279 ^{NS}	0.369 ^{NS}	-0.791 ^{NS}	-0.139 ^{NS}	0.642 ^{NS}	-0.641 ^{NS}	0.369 ^{NS}	-0.651 ^{NS}	0.175 ^{NS}	-0.232 ^{NS}	0.021 ^{NS}	-0.115 ^{NS}	0.941**	-0.132 ^{NS}	0.129 ^{NS}	0.031 ^{NS}	0.016 ^{NS}
SFA^{w}	-	-	-	-	-	-	-	-	-	-	-	-	-	**-1.000****	0.330 ^{NS}	**-0.968****	**0.819***	0.504 ^{NS}	0.323 ^{NS}	0.639 ^{NS}	-0.683 ^{NS}	-0.080 ^{NS}	0.078 ^{NS}	0.323 ^{NS}	-0.096 ^{NS}	-0.561 ^{NS}	-0.115 ^{NS}	0.366 ^{NS}	-0.211 ^{NS}	-0.661 ^{NS}	-0.006 ^{NS}	0.013 ^{NS}	0.355 ^{NS}	-0.298 ^{NS}
UFA^{w}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.345 ^{NS}	**0.972****	**-0.823***	-0.515 ^{NS}	-0.328 ^{NS}	-0.631 ^{NS}	0.691 ^{NS}	0.067 ^{NS}	-0.065 ^{NS}	-0.328 ^{NS}	0.108 ^{NS}	0.567 ^{NS}	0.125 ^{NS}	-0.367 ^{NS}	0.210 ^{NS}	0.653 ^{NS}	0.016 ^{NS}	-0.024 ^{NS}	-0.356 ^{NS}	0.295 ^{NS}
MUFA^{w}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.555 ^{NS}	0.518 ^{NS}	0.646 ^{NS}	0.338 ^{NS}	-0.317 ^{NS}	0.739 ^{NS}	0.617 ^{NS}	-0.614 ^{NS}	0.333 ^{NS}	-0.615 ^{NS}	-0.357 ^{NS}	-0.737 ^{NS}	0.289 ^{NS}	-0.008 ^{NS}	-0.077 ^{NS}	-0.655 ^{NS}	0.657 ^{NS}	0.287 ^{NS}	-0.036 ^{NS}
PUFA^{w}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-0.859***	-0.618 ^{NS}	-0.375 ^{NS}	-0.480 ^{NS}	-0.509 ^{NS}	-0.095 ^{NS}	0.096 ^{NS}	-0.375 ^{NS}	0.249 ^{NS}	0.592 ^{NS}	0.295 ^{NS}	-0.397 ^{NS}	0.188 ^{NS}	0.598 ^{NS}	0.178 ^{NS}	-0.185 ^{NS}	-0.387 ^{NS}	0.264 ^{NS}
C16:0^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.433 ^{NS}	0.636 ^{NS}	0.396 ^{NS}	-0.653 ^{NS}	-0.086 ^{NS}	0.086 ^{NS}	0.636 ^{NS}	-0.239 ^{NS}	-0.607 ^{NS}	-0.618 ^{NS}	0.322 ^{NS}	-0.059 ^{NS}	-0.403 ^{NS}	-0.460 ^{NS}	0.467 ^{NS}	0.316 ^{NS}	-0.114 ^{NS}
C18:0^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.586 ^{NS}	-0.218 ^{NS}	**-0.922****	0.797 ^{NS}	-0.709 ^{NS}	0.586 ^{NS}	**-0.870***	-0.271 ^{NS}	-0.197 ^{NS}	0.609 ^{NS}	-0.549 ^{NS}	0.094 ^{NS}	0.040 ^{NS}	-0.035 ^{NS}	0.606 ^{NS}	-0.528 ^{NS}
C18:1^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.232 ^{NS}	-0.707 ^{NS}	0.318 ^{NS}	-0.319 ^{NS}	**1.000****	-0.712 ^{NS}	-0.112 ^{NS}	-0.518 ^{NS}	0.621 ^{NS}	-0.519 ^{NS}	0.243 ^{NS}	-0.169 ^{NS}	0.174 ^{NS}	0.624 ^{NS}	-0.473 ^{NS}
C18:2^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.076 ^{NS}	-0.697 ^{NS}	0.696 ^{NS}	-0.252 ^{NS}	0.626 ^{NS}	-0.588 ^{NS}	0.269 ^{NS}	-0.336 ^{NS}	0.339 ^{NS}	-0.576 ^{NS}	0.116 ^{NS}	-0.111 ^{NS}	-0.347 ^{NS}	0.258 ^{NS}
C18:3^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.529 ^{NS}	0.531 ^{NS}	-0.707 ^{NS}	0.730 ^{NS}	0.487 ^{NS}	0.225 ^{NS}	-0.517 ^{NS}	0.452 ^{NS}	-0.039 ^{NS}	-0.011 ^{NS}	0.004 ^{NS}	-0.512 ^{NS}	0.438 ^{NS}
SFA^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-1.000****	0.318 ^{NS}	**-0.892***	0.099 ^{NS}	-0.157 ^{NS}	0.454 ^{NS}	-0.453 ^{NS}	0.420 ^{NS}	0.007 ^{NS}	-0.007 ^{NS}	0.457 ^{NS}	-0.392 ^{NS}
UFA^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.319 ^{NS}	**0.892***	-0.102 ^{NS}	0.152 ^{NS}	-0.458 ^{NS}	0.458 ^{NS}	-0.419 ^{NS}	-0.013 ^{NS}	0.013 ^{NS}	-0.461 ^{NS}	0.397 ^{NS}
MUFA^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.712 ^{NS}	-0.112 ^{NS}	-0.518 ^{NS}	0.621 ^{NS}	-0.519 ^{NS}	0.243 ^{NS}	-0.169 ^{NS}	0.174 ^{NS}	0.624 ^{NS}	-0.473 ^{NS}
PUFA^{p}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.022 ^{NS}	0.360 ^{NS}	-0.635 ^{NS}	0.586 ^{NS}	-0.426 ^{NS}	0.071 ^{NS}	-0.073 ^{NS}	-0.638 ^{NS}	0.519 ^{NS}
C16:0^{s}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.325 ^{NS}	0.416 ^{NS}	-0.534 ^{NS}	0.059 ^{NS}	0.463 ^{NS}	-0.468 ^{NS}	0.424 ^{NS}	-0.517 ^{NS}
C18:0^{s}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.086 ^{NS}	-0.228 ^{NS}	-0.069 ^{NS}	**0.915***	**-0.917***	-0.090 ^{NS}	-0.229 ^{NS}
C18:1^{s}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.932**	-0.232 ^{NS}	0.293 ^{NS}	-0.289 ^{NS}	**1.000****	**-0.948****
C18:2^{s}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.066 ^{NS}	-0.589 ^{NS}	0.587 ^{NS}	**-0.933****	**0.990****
C18:3^{s}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.051 ^{NS}	0.048 ^{NS}	-0.222 ^{NS}	0.205 ^{NS}
SFA^{s}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-1.000****	0.291 ^{NS}	-0.581 ^{NS}
UFA^{s}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.287 ^{NS}	0.578 ^{NS}
MUFA^{s}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-0.948****

Abbreviations (plus superscripts): TA; titratable acidity, DM; dry matter, MC; moisture content TSS; total soluble solid, TSS/TA; total soluble solid/titratable acidity, FS; fruit size, FF; fruit firmness, w; whole grape berry, p; peel, s; seed, C16:0; palmitic acid, C18:0; stearic acid, C18:1; oleic acid, C18:2; linoleic acid, C18:3; α-linolenic acid, SFA; saturated fatty acids, UFA; unsaturated fatty acids, MUFA; monounsaturated fatty acids, PUFA; polyunsaturated fatty acids, Asterisks indicate significance at * P <0.05 and ** P <0.01, NS; non-significant.

of the same grapevine (Karaerik) in Üzümlü location and reported more or less the same findings, agreeing with those reported in the present study.

We have assumed that the higher nutrient content in the present grape berry, 'Karaerik', results from the soil characteristics and the unique microclimate in the district of Üzümlü. The district is located in the Esence Mountains in the upper Euphrates in the west of the Eastern Anatolian region, which covers a large part of the north of the Erzincan plain, with an area of 410 km^2 and a generally mountainous and rugged relief structure. Alluvium, hydromorphic alluvium and coluvial soils are common in the valleys and surrounding areas, while litosols are dominant in mountainous areas. Due to its lithological structure, land extruded from ophiolitic rocks, which are very prone to erosion and are subjected to intense tectonic movements, has acquired a lithozolic characteristic as a result of extreme soil erosion. The climate is terrestrial with significant summer-winter temperature differences, involving cold winters and short but quite hot summers due to factors such as altitude, distance from the sea and especially the Siberian High Pressure Center. The severity of continentality is evident, and clearly manifests itself in the region's temperature, pressure and precipitation regimens, in the snow cover inhabitation period, and the upper limit of permanent snow and forest. The region generally receives rainfall of over 500 mm, except for in depressions, including the Erzincan plains. However, on the relatively low slopes of the mountains and on brown ground on the high plateau plains, 'Karaerik' breeding is carried out in an uncommon microclimate (Akpınar & Yiğit, 2011; Güner & Aslan, 2012).

3.6 Principal component analysis (PCA) and Pearson correlation (r)

Analysis of the sugars and organic acids (Fig. 2A) and minerals (Fig. 2C) compared between sampling locations and the measured physicochemical parameters revealed that two principal components (PCs) accounted for 92.75% (PC1: 83.96% and PC2: 8.79%, Fig. 2A) of sugars and organic acids and 90.05% (PC1: 80.48% and PC2: 9.57%, Fig. 2D) of minerals. Data exhibited strong positive loadings at the right upper and lower quadrants on PC1 of each PC, distinguishing the Karakaya, Bayırbağ, Üzümlü and Pişkidağ locations for the sugar and organic acid concentrations, and the Üzümlü, Bayırbağ and Karakaya locations for the mineral concentrations. They were also closely associated and positively or negatively strongly correlated ($P < 0.01$ or 0.05) with most of the physicochemical parameters measured in the grape berry. The remaining three locations, Çağlayan, Göllerköyü and Pişkidağ, exhibited strong negative loadings on their PCs, 2 at the lower and upper left quadrants, associating less and correlating only with FF value, the ratio of TSS:TA and the zinc concentration. When compared, the berries sampled from Çağlayan (1246 m, a.s.l.) exhibited a noticeable altitude effect and contained the lowest concentration of sugars, organic acids and minerals, and this site was thus easily distinguished from the other five altitudes/locations (Fig. 2A,C).

Figure 2B shows PCs of the data for fatty acids in the berry. The PCA of the fatty acids explained a low total variation (61.82%), where PC1 accounted for 34.15% of the variance and PC2 for 27.67% (Fig. 2B). The association or correlation seen in the case of sugars and organic acids or minerals was not clearly observed for the fatty acids. The fatty acids could not be distinguished on basis of the PCs, and were not associated or correlated with the measured physicochemical parameters or sampling locations; to be more precise, they were cluttered.

3.7 Agglomerative hierarchical clustering (AHC) of the nutrients in the black grape berry

The dendrogram (Fig. 2D) shows that the sugars and organic acids of the berry sampled from six locations were homogeneous and that the Çağlayan location (cluster I), which had low nutrient concentrations, exhibited major differences from the other five locations (Üzümlü, Göllerköyü, Pişkidağ, Bayırbağ and Karakaya)

Table 5: Pearson correlation (r) of minerals compared with physicochemical parameters in the grape berry (Karaerik).

	TA	DM	MC	TSS	TSS/TA	FS	FF	Na^w	Mg^w	P^w	K^w	Ca^w	Mn^w	Fe^w	Zn^w	Na^p	Mg^p	P^p	K^p	Ca^p	Mn^p	Fe^p	Zn^p	Na^s	Mg^s	P^s	K^s	Ca^s	Mn^s	Fe^s	Zn^s
pH	0.648NS	0.672 ^{NS}	0.690 ^{NS}	0.645 ^{NS}	-0.666 ^{NS}	0.894*	-0.382 ^{NS}	0.716 ^{NS}	0.695 ^{NS}	0.809 ^{NS}	0.498 ^{NS}	0.414 ^{NS}	0.533 ^{NS}	0.472 ^{NS}	**0.862***	0.729 ^{NS}	0.783 ^{NS}	0.567 ^{NS}	0.701 ^{NS}	0.376 ^{NS}	0.115 ^{NS}	0.757 ^{NS}	0.629 ^{NS}	0.549 ^{NS}	0.734 ^{NS}	0.686 ^{NS}	**0.833***	0.665 ^{NS}	0.471 ^{NS}	0.692 ^{NS}	-0.788 ^{NS}
TA	-	0.711 ^{NS}	**0.897***	0.620 ^{NS}	**-0.999****	**0.912***	-0.475 ^{NS}	0.738 ^{NS}	**0.863***	**0.916***	**0.951****	**0.921****	**0.912***	**0.891***	**0.916***	**0.952****	**0.921****	**0.871***	**0.906***	**0.913***	0.797 ^{NS}	**0.840***	**0.941****	**0.920****	**0.990****	**0.843***	**0.905***	**0.831***	**0.917****	**0.860***	**-0.892***
DM	-	-	**0.882***	**0.943****	-0.695 ^{NS}	0.704 ^{NS}	**-0.911***	0.671 ^{NS}	0.494 ^{NS}	**0.902***	0.537 ^{NS}	0.481 ^{NS}	0.577 ^{NS}	0.524 ^{NS}	**0.853***	0.635 ^{NS}	0.535 ^{NS}	0.472 ^{NS}	0.705 ^{NS}	0.587 ^{NS}	0.469 ^{NS}	0.776 ^{NS}	0.774 ^{NS}	0.608 ^{NS}	0.703 ^{NS}	0.875*	0.710 ^{NS}	0.549 ^{NS}	0.510 ^{NS}	0.418 ^{NS}	-0.568 ^{NS}
MC	-	-	-	**0.892***	**-0.885***	**0.859***	-0.751 ^{NS}	0.753 ^{NS}	0.750 ^{NS}	**0.971****	0.796 ^{NS}	0.760 ^{NS}	0.783 ^{NS}	0.779 ^{NS}	**0.909***	**0.891***	0.793 ^{NS}	0.764 ^{NS}	**0.885***	0.773 ^{NS}	0.675 ^{NS}	**0.926****	**0.978****	**0.904***	**0.886***	**0.993****	**0.905***	0.801 ^{NS}	**0.819***	0.641 ^{NS}	-0.697 ^{NS}
TSS	-	-	-	-	-0.599 ^{NS}	0.651 ^{NS}	**-0.915***	0.649 ^{NS}	0.471 ^{NS}	**0.856***	0.463 ^{NS}	0.417 ^{NS}	0.500 ^{NS}	0.485 ^{NS}	0.768 ^{NS}	0.623 ^{NS}	0.492 ^{NS}	0.467 ^{NS}	0.684 ^{NS}	0.481 ^{NS}	0.392 ^{NS}	**0.814***	0.786 ^{NS}	0.643 ^{NS}	0.615 ^{NS}	**0.921****	0.707 ^{NS}	0.578 ^{NS}	0.504 ^{NS}	0.311 ^{NS}	-0.412 ^{NS}
TSS/TA	-	-	-	-	-	**-0.924****	0.450 ^{NS}	-0.723 ^{NS}	-0.862*	**-0.913***	**-0.944****	-0.911*	**-0.900***	**-0.877***	**-0.916***	**-0.953****	**-0.935****	**-0.863***	**-0.894***	**-0.896***	-0.771 ^{NS}	**-0.827***	**-0.930****	**-0.911***	**-0.993****	**-0.830***	**-0.915***	**-0.822***	**-0.907***	**-0.871***	**0.912***
FS	-	-	-	-	-	-	-0.413 ^{NS}	0.772 ^{NS}	**0.872***	**0.931****	**0.813***	0.753 ^{NS}	0.795 ^{NS}	0.759 ^{NS}	**0.957****	**0.943****	**0.966****	0.810 ^{NS}	**0.877***	0.706 ^{NS}	0.503 ^{NS}	**0.866***	**0.867***	**0.831***	**0.954****	**0.828***	**0.972****	**0.832***	0.787 ^{NS}	**0.872***	**-0.934****
FF	-	-	-	-	-	-	-	-0.378 ^{NS}	-0.167 ^{NS}	-0.709 ^{NS}	-0.283 ^{NS}	-0.247 ^{NS}	-0.299 ^{NS}	-0.274 ^{NS}	-0.584 ^{NS}	-0.371 ^{NS}	-0.234 ^{NS}	-0.192 ^{NS}	-0.437 ^{NS}	-0.375 ^{NS}	-0.353 ^{NS}	-0.558 ^{NS}	-0.624 ^{NS}	-0.438 ^{NS}	-0.429 ^{NS}	-0.773 ^{NS}	-0.492 ^{NS}	-0.277 ^{NS}	-0.297 ^{NS}	-0.045 ^{NS}	0.237 ^{NS}
Na^w	-	-	-	-	-	-	-	-	**0.896***	0.768 ^{NS}	0.771 ^{NS}	0.740 ^{NS}	**0.875***	**0.846***	**0.850***	**0.815***	0.722 ^{NS}	**0.861***	**0.942****	0.753 ^{NS}	0.599 ^{NS}	**0.926****	0.742 ^{NS}	0.731 ^{NS}	0.785 ^{NS}	0.727 ^{NS}	0.680 ^{NS}	**0.924****	0.757 ^{NS}	0.801 ^{NS}	-0.649 ^{NS}
Mg^w	-	-	-	-	-	-	-	-	-	0.765 ^{NS}	**0.919****	**0.897***	**0.942****	**0.938****	**0.838***	**0.949****	**0.917***	**0.981****	**0.956****	**0.832***	0.685 ^{NS}	**0.889***	**0.818***	**0.874***	**0.903***	0.710 ^{NS}	0.811 ^{NS}	**0.978****	**0.915***	0.954**	-0.806 ^{NS}
P^w	-	-	-	-	-	-	-	-	-	-	0.781 ^{NS}	0.727 ^{NS}	0.775 ^{NS}	0.741 ^{NS}	**0.973****	**0.893***	**0.844***	0.738 ^{NS}	0.877*	0.745 ^{NS}	0.591 ^{NS}	0.903*	**0.936****	**0.842***	**0.925****	**0.950****	**0.938****	0.777 ^{NS}	0.766 ^{NS}	0.714 ^{NS}	**-0.817***
K^w	-	-	-	-	-	-	-	-	-	-	-	**0.995****	**0.977****	**0.978****	0.810 ^{NS}	**0.936****	**0.880***	**0.955****	**0.920****	**0.969****	**0.891***	**0.815***	**0.884***	**0.928****	**0.939****	0.735 ^{NS}	0.788 ^{NS}	**0.892***	**0.979****	**0.898***	-0.805 ^{NS}
Ca^w	-	-	-	-	-	-	-	-	-	-	-	-	**0.969****	**0.981****	0.751 ^{NS}	**0.906***	**0.839***	**0.950****	**0.895***	**0.972****	**0.921****	0.782 ^{NS}	**0.861***	**0.920****	**0.900***	0.699 ^{NS}	0.736 ^{NS}	**0.876***	**0.982****	**0.867***	-0.747 ^{NS}
Mn^w	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.989****	**0.831***	**0.912***	**0.835***	**0.965****	**0.957****	**0.966****	**0.872***	**0.859***	**0.850***	**0.884***	**0.913***	0.724 ^{NS}	0.737 ^{NS}	**0.928****	**0.948****	**0.904***	-0.770 ^{NS}
Fe^w	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.778 ^{NS}	**0.913***	**0.822***	**0.980****	**0.948****	**0.958****	**0.892***	**0.857***	**0.861***	**0.917***	**0.885***	0.729 ^{NS}	0.726 ^{NS}	**0.940****	**0.976****	**0.871***	-0.708 ^{NS}
Zn^w	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.903***	**0.877***	0.786 ^{NS}	**0.912***	0.769 ^{NS}	0.580 ^{NS}	**0.906***	**0.881***	0.803 ^{NS}	**0.946****	**0.873***	**0.912***	**0.822***	0.766 ^{NS}	**0.820***	**-0.884***
Na^p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.966****	**0.944****	**0.953****	**0.847***	0.712 ^{NS}	**0.919****	**0.943****	**0.960****	**0.970****	**0.859***	**0.940****	**0.938****	**0.944****	**0.895***	**-0.853***
Mg^p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.878***	**0.868***	0.754 ^{NS}	0.581 ^{NS}	**0.817***	**0.855***	**0.877***	**0.955****	0.754 ^{NS}	**0.946****	**0.860***	**0.866***	**0.926****	**-0.931****
P^p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.954****	**0.889***	0.792 ^{NS}	**0.879***	**0.850***	**0.921****	**0.887***	0.724 ^{NS}	0.776 ^{NS}	**0.978****	**0.967****	**0.910***	-0.736 ^{NS}
K^p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.883***	0.754 ^{NS}	**0.969****	**0.911***	**0.912***	**0.926****	**0.852***	**0.838***	**0.973****	**0.920****	**0.870***	-0.766 ^{NS}
Ca^p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.958****	0.768 ^{NS}	**0.845***	**0.864***	**0.880***	0.705 ^{NS}	0.674 ^{NS}	**0.819***	**0.931****	0.809 ^{NS}	-0.716 ^{NS}
Mn^p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.635 ^{NS}	0.764 ^{NS}	0.803 ^{NS}	0.732 ^{NS}	0.612 ^{NS}	0.509 ^{NS}	0.69* ^{NS}	**0.877***	0.633 ^{NS}	-0.511 ^{NS}
Fe^p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.917***	**0.890***	**0.866***	**0.921****	**0.857***	**0.943****	**0.846***	0.755 ^{NS}	-0.686 ^{NS}
Zn^p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.971****	**0.924****	**0.961****	**0.917***	**0.854***	**0.913***	0.719 ^{NS}	-0.731 ^{NS}
Na^s	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.906***	**0.884***	**0.880***	**0.902***	**0.973****	0.771 ^{NS}	-0.706 ^{NS}
Mg^s	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.834***	**0.929****	**0.864***	**0.902***	**0.905***	**-0.928****
P^s	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.891***	0.779 ^{NS}	0.779 ^{NS}	0.574 ^{NS}	-0.630 ^{NS}
K^s	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.79* ^{NS}	0.799 ^{NS}	0.775 ^{NS}	**-0.864***
Ca^s	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.919****	**0.872***	-0.701 ^{NS}
Mn^s	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.841***	-0.719 ^{NS}
Fe^s	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-0.909***

Abbreviations (plus superscripts): TA; titratable acidity, DM; dry matter, MC; moisture content TSS; total soluble solid, TSS/TA; total soluble solid/titratable acidity, FS; fruit size, FF; fruit firmness, w; whole grape berry, p; peel, s; seed, Na; sodium, Mg; magnesium, P; phosphorous, K; potassium, Ca; calcium, Mn; manganese, Fe; iron, Zn; zinc. Asterisks indicate significance at $*P < 0.05$ and $**P < 0.01$, NS; non-significant.

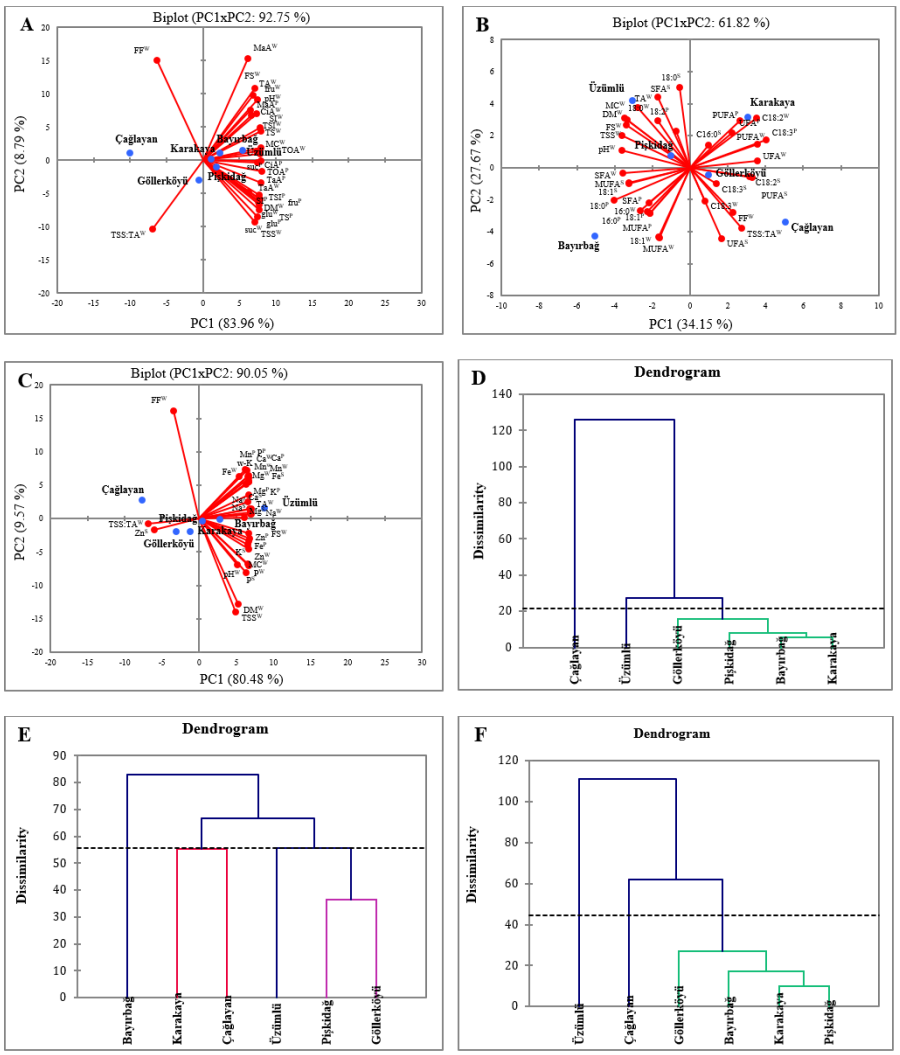

Figure 2: Bi-plot PCA of nutrients in the berry of 'Karaerik' grapes sampled from six locations in the district of Üzümlü and its surroundings

(A) sugars and organic acids, (B) fatty acids, (C) minerals in the peel, whole grape berry and seed. Agglomerative hierarchical clustering (AHC) for sugar and organic acids (D), fatty acids (E) and minerals (F) in the peel, whole grape berry and seed. Abbreviations: w; whole grape berry, p; peel, MC; moisture content, DM; dry matter, FS; fruit size, fru; fructose, glu; glucose, suc; sucrose, TS; total sugar, TSS; total soluble solids, TA; titratable acidity, TaA; tartaric acid, MaA; malic acid, CiA; citric acid, TOA; total acid (whole berry + pulp), SI; sweetness index, TSI; total sweetness index

(cluster II), with their high nutrient concentrations. The grapes sampled from Bayırbağ and Karakaya (cluster III) were capable of being clustered within a short hierarchical distance as a third cluster (III), indicating that they had similar nutrient profiles.

As shown in Fig. 2E, the fatty acids were irregularly clustered, as in the case of the PCs (Fig. 2B). The Bayırbağ location (cluster I) differed significantly from the other five locations. It may be suggested that the profiles of fatty acids in the grape berry sampled from Bayırbağ are significantly different from those in grape samples from the remaining five locations. The grape berry samples from Karakaya and Çağlayan (cluster II) exhibited a similar fatty acid fingerprint. The Üzümlü location (cluster III) was capable of being clustered within a long hierarchical distance at a similar concentration to the Karakaya and Çağlayan sites (cluster II). Pişkidağ and Göllerköyü (cluster IV), with a short hierarchical distance, exhibited similar concentrations of fatty acids, but rather lower than those of Üzümlü.

Three main groups and several subgroups within each group were considered in terms of the mineral/elements among the concentrations and the physical parameters measured. Cluster I included Üzümlü, interestingly clustered within a long hierarchical distance and differing from the grape berries sampled from the remaining five locations. Çağlayan (cluster II), with its low mineral concentrations, was capable of being clustered within a moderate hierarchical distance, exhibiting greater differences from the locations Göllerköyü, Bayırbağ, Karakaya and Pişkidağ (cluster III). The third cluster (cluster III) was capable of division into four subgroups (Fig. 2F). In their review, Mpelasoka et al. (2003) and Lasik (2013) emphasized that potassium is the major cation in grape berry or juice, and that high potassium juice reduces free acids and increases overall pH. They also reported that tartaric acid is a significantly stronger acid than malic acid, and at similar values of total acidity, a lower tartrate:malate (TA:MA) ratio may therefore result in a less acidic pH. It has also been reported that high malate enhances malolactic fermentation (Mpelasoka et al., 2003; Abrahamse & Bartowsky, 2012; Lasik, 2013). This secondary fermentation is carried out by many lactic acid bacteria and may have either positive or negative impacts on the organoleptic quality of wines. This necessitates the use of commercial lactic acid bacteria starter-cultures to control malolactic fermentation (Abrahamse & Bartowsky, 2012; Lasik, 2013). Tartrate also bestows a crisp and fresh acidic taste to wine and is therefore preferred to malate. During winemaking, high potassium increases the precipitation of tartrate in salt and hence reduces free tartrate. High potassium can therefore lead to a reduced TA:MA ratio, which is undesirable for high quality wines, as earlier reviewed by Mpelasoka et al. (2003). In the present study, the TA:MA ratio was 1.59 and the highest pH was 3.7 (avg., 3.5, over the six locations) in berries of the 'Karaerik' grape. However, berries of the 'Isabel' grape had a 0.77 TA:MA ratio and 3.1 pH in ripe and overly ripe berries (Kurt et al., 2017).

4 Conclusion

In conclusion, this study provides the first data in the literature concerning the nutrient profile in berries of the new grape cultivar 'Karaerik' among the various *V. vinifera* grapes. Sugars, organic acids and minerals quantified separately in the whole berry and the peel exhibited strong positive and negative correlations with the measured physicochemical parameters and sampling locations, although the fatty acids were not so correlated. Berries sampled from Üzümlü, the best potential growing location for the grape, contained higher concentrations of nutrients than berries from Çağlayan. Our findings represent important data for viticulturists and for the food science and technology industries. The berry of the grape investigated in this study has a unique taste and flavor that make it very popular with consumers. These properties distinguish the grape from *V. vinifera* fruits. We therefore conclude that the 'Karaerik' grape should be given "protected" status. More comprehensive nutritional studies are now needed to obtain deeper insights into this and other quality parameters and to open the possibility of establishing new vineyards to improve the grape in breeding pro-

grams without destroying the present form in terms of biological diversity conservation. Further studies are also needed to investigate the antioxidant properties and phenolic profile of the grape berry. An ongoing project investigating the detailed phenolic profiles of the berry supplemented with antioxidant properties is currently being performed in our laboratory.

Acknowledgements

Financial support for this study was provided by the Scientific and Technological Research Council of Turkey (TUBITAK- Project number: 115Z365).

References

Abrahamse, C. E. & Bartowsky, E. J. (2012). Timing of malolactic fermentation inoculation in shiraz grape must and wine: influence on chemical composition. *World Journal of Microbiology & Biotechnology*, *28*(1), 255–265. doi:10.1007/s11274-011-0814-3

Akin, A. & Altindisli, A. (2011). Determination of fatty acid composition and lipid content of some grape cultivar seeds in Turkey. *Biyoloji Bilimleri Araştırma Dergisi*, *4*(2), 13–15.

Akpınar, E. & Yiğit, D. (2011). Ekolojik faktörlerin karaerik üzüm çeşidi yetiştiriciliğine etkileri. *Doğu Coğrafya Dergisi*, *11*(16).

Al Juhaimi, F., Gecgel, U., Gulcu, M., Hamurcu, M., & Ozcan, M. M. (2017). Bioactive properties, fatty acid composition and mineral contents of grape seed and oils. *South African Journal of Enology and Viticulture*, *38*(1), 103–108.

Association of Official Agricultural Chemists. (2003). Official Methods of Analysis of AOAC International. 17th ed. 2nd revision. Gaithersburg, USA.

Association of Official Agricultural Chemists. (2011). Official Methods of Analysis of AOAC International. 18th ed. Gaithersburg, USA.

Ayaz, F., Colak, N., Kurt, A., Akpinar, E., Gomez-Alonso, S., & Hermosin-Gutierrez, I. (2017). First evidence of the occurrence of feruloyl derivatives of anthocyanins in *Vitis vinifera* black grape cultivar: the Turkish 'Karaerik' grape. Paper presented at 10th Symposium: In Vino Analytica Scientia (IVAS), Analytical Chemistry for Wine, Brandy and Spirits, Universidad de Castilla la Mancha, Spain.

Bulut, V. N., Duran, C., Gundogdu, A., Soylak, M., Yildirim, N., & Elci, L. (2008). A new approach to separation and preconcentration of some trace metals with co-precipitation method using a triazole. *Talanta*, *76*(2), 469–474. doi:10.1016/j.talanta.2008.03.040

Conner, P. J. (2013). Instrumental textural analysis of muscadine grape germplasm. *Hortscience*, *48*(9), 1130–1134.

Duran, C., Senturk, H. B., Gundogdu, A., Bulut, V. N., Elci, L., Soylak, M., ... Uygur, Y. (2007). Determination of some trace metals in environmental samples by flame aas following solid phase extraction with amberlite xad-2000 resin after complexing with 8-hydroxyquinoline. *Chinese Journal of Chemistry*, *25*(2), 196–202. doi:10.1002/cjoc.200790040

Ejsmentewicz, T., Balic, I., Sanhueza, D., Barria, R., Meneses, C., Orellana, A., ... Campos-Vargas, R. (2015). Comparative study of two table grape varieties with contrasting texture during cold storage. *Molecules*, *20*(3), 3667–3680. doi:10.3390/molecules20033667

Eyduran, S. P., Akin, M., Ercisli, S., Eyduran, E., & Maghradze, D. (2015). Sugars, organic acids, and phenolic compounds of ancient grape cultivars (*Vitis vinifera* L.) from Igdir province of Eastern Turkey. *Biological Research*, *48*. doi:10.1186/0717-6287-48-2

Ferretti, G., Turco, I., & Bacchetti, T. (2014). Apple as a source of dietary phytonutrients: bioavailability and evidence of protective effects against human cardiovascular disease. *Food and Nutrition Sciences*, *05*, 1234–1246.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Güner, A. & Aslan, S. (2012). *Türkiye bitkileri listesi:(damarlı bitkiler)*. Nezahat Gökyigit Botanik Bahçesi Yayınları.

Güneş, A., Köse, C., & Turan, M. (2015). Yield and mineral composition of grapevine (*Vitis vinifera* L. cv. Karaerik) as affected by boron management. *Turkish Journal of Agriculture and Forestry*, *39*(5), 742–752. doi:10.3906/tar-1412-13

Hyson, D. A. (2011). A comprehensive review of apples and apple components and their relationship to human health. *Advances in Nutrition*, *2*(5), 408–420. doi:10.3945/an.111.000513

Karasu, S., Baslar, M., Karaman, S., Kilicli, M., Us, A. A., Yaman, H., & Sagdic, O. (2016). Characterization of some bioactive compounds and physicochemical properties of grape varieties grown in Turkey: thermal degradation kinetics of anthocyanin. *Turkish Journal of Agriculture and Forestry*, *40*(2), 177–185. doi:10.3906/tar-1502-38

Kurt, A., Torun, H., Colak, N., Seiler, G., Hayirlioglu-Ayaz, S., & Ayaz, F. A. (2017). Nutrient profiles of the hybrid grape cultivar 'Isabel' during berry maturation and ripening. *Journal of the Science of Food and Agriculture*, *97*(8), 2468–2479. doi:10.1002/jsfa.8061

Lasik, M. (2013). The application of malolactic fermentation process to create good-quality grape wine produced in cool-climate countries: a review. *European Food Research and Technology*, *237*(6), 843–850. doi:10.1007/s00217-013-2083-x

Liang, Z., Sang, M., Fan, P., Wu, B., Wang, L., Duan, W., & Li, S. (2011). Changes of polyphenols, sugars, and organic acid in 5 vitis genotypes during berry ripening. *Journal of Food Science*, *76*(9), C1231–C1238. doi:10.1111/j.1750-3841.2011.02408.x

Lijavetzky, D., Carbonell-Bejerano, P., Grimplet, J., Bravo, G., Flores, P., Fenoll, J., ... Martinez-Zapater, J. M. (2012). Berry flesh and skin ripening features in *Vitis vinifera* as assessed by transcriptional profiling. *Plos One*, *7*(6). doi:10.1371/journal.pone.0039547

Liu, H.-F., Wu, B.-H., Fan, P.-G., Li, S.-H., & Li, L.-S. (2006). Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. *Journal of the Science of Food and Agriculture*, *86*(10), 1526–1536. doi:10.1002/jsfa.2541

Liu, R. H. (2013). Dietary bioactive compounds and their health implications. *Journal of Food Science*, *78*(1), A18–A25. doi:10.1111/1750-3841.12101

Magwaza, L. S. & Opara, U. L. (2015). Analytical methods for determination of sugars and sweetness of horticultural products-a review. *Scientia Horticulturae*, *184*, 179–192. doi:10.1016/j.scienta.2015.01.001

Mikulic-Petkovsek, M., Schmitzer, V., Slatnar, A., Stampar, F., & Veberic, R. (2012). Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. *Journal of Food Science*, *77*(10), C1064–C1070. doi:10.1111/j.1750-3841.2012.02896.x

Mpelasoka, B. S., Schachtman, D. R., Treeby, M. T., & Thomas, M. R. (2003). A review of potassium nutrition in grapevines with special emphasis on berry accumulation. *Australian Journal of Grape and Wine Research*, *9*(3), 154–168. doi:10.1111/j.1755-0238.2003.tb00265.x

Ochmian, I., Angelov, L., Chełpiński, P., Stalev, B., Rozwarski, R., & Dobrowolska, A. (2013). The characteristics of fruits morphology, chemical composition and colour changes in must during maceration of three grapevine cultivars. *Journal of Horticultural Research*, *21*(1), 71–78. doi:10.2478/johr-2013-0010

Ozcan, M. M. & Al Juhaimi, F. Y. (2017). Effect of microwave roasting on yield and fatty acid composition of grape seed oil. *Chemistry of Natural Compounds*, *53*(1), 132–134. doi:10.1007/s10600-017-1926-2

Pavlousek, P. & Kumsta, M. (2011). Profiling of primary metabolites in grapes of interspecific grapevine varieties: sugars and or-

ganic acids. *Czech Journal of Food Sciences*, *29*(4), 361–372.

Rogiers, S. Y., Greer, D. H., Hatfield, J. M., Orchard, B. A., & Keller, M. (2006). Mineral sinks within ripening grape berries (*Vitis vinifera* L.) *Vitis*, *45*(3), 115–123.

Rolle, L., Giacosa, S., Gerbi, V., Bertolino, M., & Novello, V. (2013). Varietal comparison of the chemical, physical, and mechanical properties of five colored table grapes. *International Journal of Food Properties*, *16*(3), 598–612. doi:10.1080/10942912.2011.558231

Santos, L. P., Morais, D. R., Souza, N. E., Cottica, S. M., Boroski, M., & Visentainer, J. V. (2011). Phenolic compounds and fatty acids in different parts of *Vitis labrusca* and *V. vinifera* grapes. *Food Research International*, *44*(5), 1414–1418. doi:10.1016/j.foodres.2011.02.022

Shiozaki, S. & Murakami, K. (2016). Lipids in the seeds of wild grapes native to japan: *Vitis coignetiae* and *Vitis ficifolia* var. ganebu. *Scientia Horticulturae*, *201*, 124–129. doi:10.1016/j.scienta.2016.01.038

Slavin, J. L. & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition*, *3*(4), 506–516. doi:10.3945/an.112.002154

Sousa, E. C., Uchoa-Thomaz, A. M. A., Beserra Carioca, J. O., De Morais, S. M., De Lima, A., Martins, C. G., ... Rodrigues, L. L. (2014). Chemical composition and bioactive compounds of grape pomace (*Vitis vinifera* L.), benitaka variety, grown in the semiarid region of Northeast Brazil. *Food Science and Technology*, *34*(1), 135–142. doi:10.1590/S0101-20612014000100020

Topalovic, A. & Mikulic-Petkovsek, M. (2010). Changes in sugars, organic acids and phenolics of grape berries of cultivar cardinal during ripening. *Journal of Food Agriculture & Environment*, *8*(3-4, 1), 223–227.

Xia, E.-Q., Deng, G.-F., Guo, Y.-J., & Li, H.-B. (2010). Biological activities of polyphenols from grapes. *International Journal of Molecular Sciences*, *11*(2), 622–646. doi:10.3390/ijms11020622

Xu, C., Yagiz, Y., Zhao, L., Simonne, A., Lu, J., & Marshall, M. R. (2017). Fruit quality, nutraceutical and antimicrobial properties of 58 muscadine grape varieties (*Vitis rotundifolia* michx.) grown in united states. *Food Chemistry*, *215*, 149–156. doi:10.1016/j.foodchem.2016.07.163

Yamamoto, L. Y., de Assis, A. M., Roberto, S. R., Bovolenta, Y. R., Nixdorf, S. L., Garcia-Romero, E., ... Hermosin-Gutierrez, I. (2015). Application of abscisic acid (S-ABA) to cv. Isabel grapes (*Vitis vinifera* x *Vitis labrusca*) for color improvement: Effects on color, phenolic composition and antioxidant capacity of their grape juice. *Food Research International*, *77*(3), 572–583. doi:10.1016/j.foodres.2015.10.019

Zhou, K. & Raffoul, J. (2012). Potential anticancer properties of grape antioxidants. *Journal of Oncology*, *1*(8). doi:10.1155/2012/803294

International Journal of Food Studies | IJFS October 2018 | Volume 7 | pages 117–135

'Made-in-transit' Yoghurt Processing: A Review of Basic Concepts and Technological Implications

M. A. R. Nor-Khaizura[a, b*], S. H. Flint[a*], O. J. Mccarthy[a], J. S. Palmer[a], M. Golding[a], and A. Jaworska[c]

[a] Institute Food, Nutrition and Human Health to Massey Institute of Food Science and Technology, Private Bag 11 222, Palmerston North 4442, New Zealand
[b] Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia
[c] Designer, 1011 HW Amsterdam, the Netherlands
[*]Corresponding author
norkhaizura@upm.edu.my
Tel: +603 89468533
Fax: +603 89423552

Received: 10 November 2017; Published online: 18 October 2018

Abstract

The manufacture of food during distribution, a concept known as "made-in-transit" (MIT) manufacture, has the potential to expand the distribution range, extend shelf-life, and provide the customer with the freshest possible product. Benefits for the manufacturer include maximising throughput while minimising manufacturing space and inventory. This concept is new, with mushrooms being the only MIT food developed so far. The feasibility of developing an MIT product from a fermented food was reviewed using yoghurt as a model system. Through the alteration of some of the yoghurt manufacturing parameters (e.g. milk base formulation, heat treatment, starter culture composition and fermentation temperature) it is possible to develop this form of yoghurt production. A predictive microbiology approach is suitable for predicting the effects of both time and temperature on designing and predicting the fermentation process. This review demonstrates the potential of the MIT concept for a fermented food.

Keywords: Made-in-transit (MIT); Fermentation; Yoghurt; Predictive microbiology

1 Fundamental features of MIT

Made-in-transit (MIT) is a supply chain concept in which the production or manufacture of a perishable food occurs partially or completely during transportation (Jaworska, 2007a). The MIT concept transforms manufacture where manufacture is merged with distribution. This has the potential to change the role of transportation from simply relocating material to include manufacture (Jaworska, 2007a). Jaworska (2007b). In such processes transportation has been described as a productive creator of value by taking a total chain perspective from the outset and skipping, merging or reversing the order of events. The MIT concept is an example of convergent technology where two or more activities are combined into one. Traditionally, product shelf-life is reduced by the time taken for transportation. While, the MIT concept avoids this loss of shelf-life and provides an opportunity for the consumer to harvest the fresh end product themselves.

 10.7455/ijfs/7.2.2018.a10

2 Application and advantages of MIT

One application of this concept is the growth of mushrooms which can occur in packs within 5 to 7 days during transportation. If packages arrive prematurely at the retailer's facility, the last part of growth could occur there (Jaworska, 2008). Consumers may wish to purchase before the "ready by" date and pick product units that suit their need, based on their planned time of consumption. The MIT concept has the potential to make use of a "ready by" date in preference to a "use by" date. This ensures that the consumer receives a fresh product ideal for consumption. There are several other benefits of MIT (Jaworska, 2007a, 2007b);

1. Reduced factory manufacturing and inventory space
2. Growth-enabling technology replacing post-harvest technology for plant produce
3. Expanding the distribution range of the product by making use of the manufacturing time for transportation
4. Extending the shelf-life as the product arrives at the retailer's facilities in a fresher state than would be possible if manufacture and distribution occurred in the normal sequence.
5. Preventing overproduction and consequent waste, when applied to on-demand supply chains, and
6. Providing higher quality, freshness and nutrient-rich products to the consumer.

3 Challenges of MIT

Jaworska (2007b) mentioned that experts have raised a concern about the ability of the product to stand the vibration of transport. In the case of mushrooms, one concern was that the bodies of the mushrooms during growth may be too brittle and this may result in damage during the transportation. It was hypothesized by another researcher that any damage may depend on the stage of development. The diverse species of mushroom mean the some may be more tolerant than others to vibration during transportation (Jaworska, 2007b).

Other challenges of producing MIT product are; producing a consistent product, applying special packaging to ensure ideal conditions for manufacture, controlling the conditions surrounding the package (i.e. environment) and changing the standard system of production and distribution. Many of the systems are in place but not being used in the right way for MIT. For example, containers are available with the capacity to control the environment (humidity, temperature, carbon dioxide levels) to preserve freshness of product rather than being set to enable manufacture (Jaworska, 2008).

As MIT is considered a new concept, research on potential applications is limited. Yet, some of the issues facing manufacturers for product distribution could possibly be resolved through applying the MIT concept. For instance, New Zealand is a long way from world markets and this is a challenge for NZ manufacturers to market their product outside New Zealand. The MIT concept would allow food manufacturers to make use of the time a product is in transit to distant markets. As much food is discarded as it exceeds the 'best before' date in the market (Kleijnen & Van der Vorst, 2005) and home (Parfitt, Barthel, & Macnaughton, 2010), product manufactured using the MIT concept may avoid some wastage of food.

4 Potential of MIT in food system

To the authors' knowledge, the concept of MIT is currently only applied to mushrooms. There is potential to apply the MIT concept to many foods. Such foods need to be capable of transformation or maturity during transport. Potential foods most suited to MIT are fermented products like cheese, salami, fermented drinks and yoghurt (Table 1). Among all fermented products, yoghurt and cultured dairy products are the fastest growing dairy categories worldwide (Oneil, Kleyn, & Hare, 1979). In one article the Fonterra Brands Managing Director (Anonymous, 2005) mentioned that yoghurt is not only

well received locally but has a potential to be applied in markets outside New Zealand. Hence, yoghurt could be a suitable model of fermented product to be manufactured using the MIT concept, extending the distribution of yoghurt from New Zealand. Yoghurt has a worldwide market as a fermented product yet has a short shelf–life. As yoghurt has a rapid fermentation time, it was selected as a suitable food for this feasibility study as results could be obtained in a short time frame (Nor-Khaizura, 2013).
To apply the MIT concept to yoghurt, fermentation needs to be carried out during distribution. Generally, the fermentation lasts for 6 to 12 h in the processing plant, which, for the MIT concept, would only allow only a short distribution of the product. Manipulation of crucial factors including milk base composition, heat treatment, starter culture composition and inoculum size (Tamime & Robinson, 2007) could extend the fermentation to give a yoghurt that could be used to test the feasibility of the MIT concept (Nor-Khaizura et al., 2012).

4.1 Yoghurt

Yoghurt is one of the best known and most popular cultured milk products internationally. Data provided by the USDA reveals that yoghurt consumption in the US gradually increased from 1954 to 2005 (Figure 1). Various factors influence the consumption of fermented milk, particularly yoghurt. These include the availability of milk, food habits, level of income, advertising, range of fermented milk products available in the market, distribution system and general acceptability of other dairy products (Kurmann, 1984).
Yoghurt is produced by the growth of *Streptococcus thermophilus and Lactobacillus delbrueckii* subsp. *bulgaricus* in heated milk (Kosikowski & Mistry, 1997) incubated at the optimum conditions of 40 to 45^{o}C for 2 to 3 hours (Tamime & Robinson, 2007). During this time, the starter culture metabolizes lactose in the milk, producing the lactic acid which reduces the pH of milk to pH within 4.6 to 4.2 (Tamime & Robinson, 2007). This is the major determinant in producing the characteristics of yoghurt terms of the flavour and texture. Damin, Alcantara, Nunes, and Oliveira (2009) described the texture of yoghurt resulting from the curdling of milk that occurs when casein becomes unstable and coagulates to form a firm gel. This gel is composed of strands of casein micelles and whey entrapped within this matrix. This matrix consists of, 1) the disulphide bonding between k-casein and denatured whey protein and 2) casein aggregation when the pH decreases to the isoelectric point of casein. Lactic acid also plays a major role in the preservation of the product by creating a pH that limits the growth of many microorganisms, including pathogens (Walstra, 1999). The shelf-life of yoghurt is about 20 days under refrigeration. (Oneil et al., 1979).
Yoghurt can be categorized due to its physical, chemical or flavour properties. Physically, yoghurt may be a set yoghurt with firm gel, a stirred yoghurt with smooth gel in which the gel has been broken or as drinking yoghurt with a viscous liquid (Spreer, 2017; Tamime & Robinson, 2007). Chemically, yoghurt may be a full, low or non-fat product. Flavour may be described as plain or natural or with fruit and other flavourings (Tamime & Robinson, 2007). Commercially, yoghurt processing involves the standardisation of milk, homogenization, heat treatment, inoculation of starter culture, fermentation, cooling and packaging. To adapt the MIT system to yoghurt processing, yoghurt fermentation could be carried out during distribution. Since the current fermentation time is very short, less than 12 h, an extended fermentation would be required in order to expand the yoghurt distribution and shelf-life. There are a number of challenges in preparing an MIT yoghurt, in particular controlling the growth of contaminants and ensuring the final product is acceptable in terms of the physical and flavour characteristics. The steps in yoghurt manufacture, including milk standardization, heat treatment, starter culture composition and inoculum level, and fermentation temperature could be altered to extend the fermentation time. These factors will affect the acidification and gelation processes (Peng, Serra, Horne, & Lucey, 2009).

Table 1: Examples of Fermented foods: substrate, cultured microorganism(s) and country

Product	Substrate	Cultured Microorganism(s)	Main Market
Yoghurt	Milk	*Streptococcs thermophilus, Lactobacillus delbrueckii subsp. Bulgaricus*	Worldwide
Acidophilus milk	Milk	*Lactobacillus acidophilus*	Several countries
Cheese	Milk	Lactic acid bacteria (L. lactis, S. thermophilus, L. shermanii, Propionibacterium) sometimes moulds (Penicillium spp.)	Worldwide
Fermented sausages	Meat	Lactic acid bacteria (lactobacilli, pediococci) Catalase positive cocci (S. carnosus, S. xylosus, M. varians) sometimes yeasts and/or moulds	Europe and United State
Soy sauce	Soybeans and wheat	*Aspergillus oryzae or A. soyae*, Lactobacillus, *Zygosaccharomyces rouxii*	The Orient (Japan, China, Philippines)
Bread	Wheat, rye, other grains	Saccharomyces cerevisiae, other yeasts, lactic acid bacteria	Worldwide
Sauerkraut	Cabbage	*Lactic acid bacteria Ln. mesenteroides, L. brevis, L. plantarum, L. curvatus, L. sake*	Worldwide
Kimchi	Cabbage, vegetables, sometimes seafood, nuts	*Lactic acid bacteria*	Korea

Source: Adapted from Doyle, Beuchat, and Montville (1997) and Jay (2000)

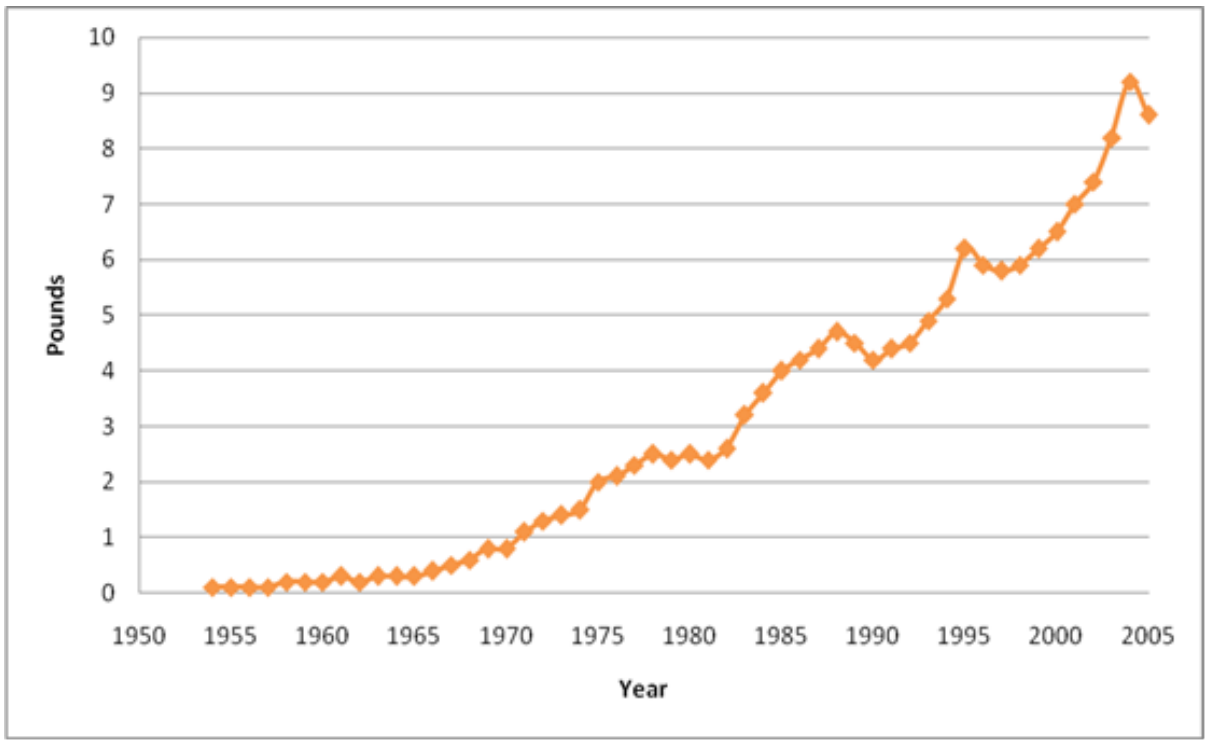

Figure 1: Per capita yoghurt consumption in United State from year 1954 to 2005 (Source: Economic Research Service, USDA

4.2 Factors affecting yoghurt fermentation

Milk standardization

The main and most crucial ingredient in yoghurt processing is milk. Milk composition is described in terms of milk fat and milk solids not fat (MSNF) which consists of protein, lactose and minerals. Standardization of fat and MSNF content in milk is essential in yoghurt manufacture as this influences the quality and consistency of the end product. The fat content of yoghurt varies, from as low as 0.1 to 10 g per 100 g depending on the type of yoghurt; full, medium or low-fat yoghurt (Tamime & Robinson, 2007). The percentage of MSNF (mainly lactose, protein and mineral matter) in milk for yoghurt manufacture depends on the legal standards of the country in which the product will be sold or the physical or flavour of the end product. The major component in milk is water (84.5 to 87.7%) (Swaisgood, 1996). Next is lactose (4.9 to 5.0%), the major carbohydrate of milk. Lactose is essential in yoghurt production by providing the nutrition or energy source for the yoghurt starter bacteria. Fat 3.4 to 5.1%), imparts richness or smoothness to dairy products and directly provides an excellent mouthfeel. Protein (3.3 to 3.9%) plays an important role in the formation of the coagulum, influencing the consistency or viscosity of yoghurt (Tamime & Robinson, 2007). The level of protein is proportional to the viscosity of yoghurt. The major proteins of milk are caseins and whey proteins. Caseins are insolubilized protein and begin to precipitate when the pH of milk is reduced to pH 4.6. The soluble portion at pH 4.6 is known as whey proteins consisting of albumins and globulins (Chandan & O'Rell, 2006).The total solids content of the milk base influences the yoghurt firmness (Penna, Converti, & De Oliveira, 2006; Tamime & Deeth, 1980; Nor-Khaizura et al., 2012). The milk base protein content (Tamime, Kalab, & Davies, 1984; Trachoo & Mistry, 1998) and protein type (Cho, Lucey, & Singh, 1999; Penna et al., 2006; Sodini, Remeuf, Haddad, & Georges, 2004; Tamime et al., 1984) are important factors in determining yoghurt texture.

The use of a reconstituted yoghurt milk base prepared from dried dairy ingredients is an alternative to standardised fresh milk. Skim milk powder (SMP) is widely used to prepare a yoghurt milk base (Isleten & Karagul-Yuceer, 2006). These authors also mentioned that the sensory properties of reconstituted milk ideally should be similar to fresh skim milk. The use of SMP is preferable to whole milk for the manufacture of fermented milks due to problems with oxidized flavours (McKenna, 1997; McKenna & Anema, 1993). The milk powder should be free from any inhibitory agents and have good microbiological and physical quality. Some specific requirements for SMP include a whey protein nitrogen index of 4.5-5.9; cysteine number, 38-48; thiol number, 7.5-9.4 and heat number, 80-83 (Wilcek, 1990). In the preparation of reconstituted skim milk from SMP, the hydration time is crucial in order to achieve the proper re-equilibration of the minerals, which requires around 3 h (Anema & Li, 2003). The normal practice is to rehydrate the powder to about 12 g per 100 g solid non-fats (SNF) (Tamime & Robinson, 2007). The Codex Standards (FAO/WHO, 2003) state that fermented milk products including yoghurt, must contain a minimum of 2.7% milk protein (% m/m) and less than 15% milk fat (% m/m).

To ensure the characteristics in yoghurt, stabilizers are often added into the yoghurt milk base. Stabilizers can improve the body and texture, viscosity or consistency, appearance and mouthfeel. Yoghurt coagulum is often subject to mechanical treatment during manufacture, for example stirring the coagulum in the fermentation tank for stirred yoghurt production, mixing to incorporate the fruit or flavours into the coagulum and subsequent post-fermentation treatment of the coagulum (e.g. pasteurization, UHT) (Tamime & Robinson, 2007). Stabilizers can avoid defects during stirring. Other functions of stabilizers incorporated into the yoghurt mix listed by Chandan and O'Rell (2006) are as follows: minimise whey separation and bind free water, maintain gel structure after pumping, mixing and cooling, and increase shelf-life of the product. Ingredients that are usually added as yoghurt stabilizers are starch, gelatin, guar gum, locust bean gum, carrageenan, pectin and xanthan gum. The addition of stabilizers is not suitable for plain yoghurt as they may affect the

product aroma and flavour (Tamime & Robinson, 2007) and may affect the consumer perception of yoghurt (Amatayakul, Sherkat, & Shah, 2006).

Yoghurt texture can be improved by increasing the milk total solids by three methods 1) concentrating the milk base through evaporation, 2) reverse osmosis (RO) and 3) fortification with dried dairy ingredient such as skim milk powder (SMP), skim milk concentrate (SMC) or buttermilk powder (BMP) (Sodini et al., 2004).

Many studies have been carried out to enhance the texture of yoghurt by fortification with dried dairy protein such as skim milk powder (Damin et al., 2009; Guzmán-González, Morais, Ramos, & Amigo, 1999), buttermilk powder (Trachoo & Mistry, 1998), whey protein concentrates (Damin et al., 2009; Guzmán-González et al., 1999; Lucey, Munro, & Singh, 1999; Patocka, Cervenkova, Narine, & Jelen, 2006; Remeuf, Mohammed, Sodini, & Tissier, 2003), whey protein isolates (Isleten & Karagul-Yuceer, 2006; Patocka et al., 2006), milk protein concentrate (Guzmán-González et al., 1999), sodium caseinate (Damin et al., 2009; Isleten & Karagul-Yuceer, 2006) and other milk-protein based ingredients (Lankes, Ozer, & Robinson, 1998; Rohm & Schmid, 1993) These ingredients have gained acceptance as a feasible way to increase total solids in low-fat and non-fat yoghurt (Tamime & Robinson, 2007). Sodini et al. (2004) also mentioned that the effect of milk base protein enrichment could be influenced by the heat treatment of the milk base.

Fortification with dried dairy ingredient

Increasing the total solids content in low-fat and non-fat yoghurt will prevent poor firmness and surface whey separation (Lucey, 2002). Skim milk powder (SMP) is dried non-fat milk and is the most commonly used fortification ingredient to increase the total solid content of the yoghurt milk base. Yoghurt fortified with SMP was observed to have a dense matrix, composed of short micellar chains and small micellar clusters (Tamime et al., 1984). Buttermilk powder (BMP) is the by-product of sweet cream butter manufacture. BMP can act as an emulsifier due to the high content of phospholipids (Tamime & Robinson, 2007). Yoghurt manufactured from a milk base fortified with BMP has been reported as acceptable (Trachoo & Mistry, 1998).

Whey protein concentrates (WPC) or isolates (WPI) are the by-products from cheese manufacture and often added to a yoghurt milk base (Penna, Baruffaldi, & Oliveira, 1997). The addition of WPC to the yoghurt milk base can reduce syneresis, increase yoghurt viscosity (Kailasapathy & Supriadi, 1996) and water holding capacity (Remeuf et al., 2003), yet, the undesirable flavour of WPC can limit its application in food (Damodaran, 1996).

Milk protein concentrate (MPC) is a concentrated milk product containing 40-90% of milk protein and sodium caseinate (NaCN) consisting mainly of casein. Both are produced by initially separating of whole milk into cream and skim milk. For the MPC, the skim milk is concentrated using ultrafiltration then the product is spray dried. NaCN is produced from casein that has been precipitated from milk using rennet enzyme. This casein is washed and the purified casein protein is treated with sodium hydroxide to produce a soluble casein compound, NaCN. The addition of MPC (Soukoulis, Panagiotidis, Koureli, & Tzia, 2007)and NaCN (Isleten & Karagul-Yuceer, 2006) to the milk base an improve yoghurt texture and reduce syneresis in set yoghurt. Sodium caseinate has a high protein content with emulsification and water binding properties that contribute to the texture of yoghurt (Isleten & Karagul-Yuceer, 2006).

Yoghurt fermentation time, is influenced by the protein components of yoghurt milk base (Puvanenthiran, Williams, & Augustin, 2002). The addition of WPC and NaCN does reduce the fermentation time (Damin et al., 2009; Lucey, Teo, Munro, & Singh, 1997), yet the opposite effect has been observed for SMP (Damin et al., 2009). The latter is similar to the finding obtained of Isleten and Karagul-Yuceer (2006), where the addition of dried dairy ingredients including whey isolate, SMP and NaCN did not affect the fermentation time, although these components greatly influenced the yoghurt texture. This may be explained the buffering effect of the increased solids content in yoghurt milk, meaning more acid development by the starter cul-

tures was necessary to achieve the casein isoelectric point (Lee & Lucey, 2010).

The influence of fortification material on the fermentation time may also depend on the starter cultures used (Isleten & Karagul-Yuceer, 2006). Using a probiotic as single starter culture (*L. acidophilus* LA5 or *L. rhamnosus* LR35) fortification of milk bases with SMP, MPC and casein hydrolysate increased the fermentation rate and increased the texture of the yoghurt. This was less pronounced in yoghurt prepared with mixed culture starters (probiotic with *S. thermophiles*) (Sodini, Lucas, Oliveira, Remeuf, & Corrieu, 2002). For the single culture, the addition of dried dairy ingredient really influenced the fermentation time with the shortest time produced with milk fortified with casein hydrolysate (Oliveira, Sodini, Remeuf, & Corrieu, 2001; Sodini et al., 2002).

For yoghurt texture, yoghurt fortified with NaCN is reported to have a stronger gel than the unfortified control and WPI-fortified yoghurts (Isleten & Karagul-Yuceer, 2006). Yoghurt enriched with NaCN produced a coarse texture when assessed visually using a spoon (Tamime et al., 1984). This was possibly due to large casein particles and a robust micellar chain. They found the yoghurt firmness made from a milk base fortified with NaCN was 30% higher than that from a milk base fortified with SMP, although the former had lower total solid content, 12.8% rather than 16%. The yoghurt rheology was also influence by the fortification of the milk base with dried dairy ingredients in yoghurt prepared using probiotic cultures (Sodini et al., 2002). They found the highest texture in yoghurt manufactured with added MPC and lowest in yoghurt with added casein hydrolysate. Peng et al. (2009) mentioned that the yoghurt texture based on physicochemical properties related to the nature and type of protein interactions are not well understood. Possible interactions in the yoghurt are hydrophobic and electrostatic interactions, hydrogen bonding, steric repulsion and dissolution of colloidal calcium phosphate (CCP), which collectively influence the yoghurt physical and rheological properties (Peng et al., 2009). Dissolution or solubilisation of CCP could weakened casein-casein interaction and may contribute to soft gel (Peng et al., 2009). Generally, the casein-based powders are more effective than whey protein products in producing a firmer yoghurt (Bhullar, Uddin, & Shah, 2002; Dave & Shah, 1998).

The milk base used may stimulate probiotic growth, providing some advantage in manufacturing yoghurts containing probiotics. For example, casein hydrolysate stimulates the growth of *L. acidophilus* LA5 (Sodini et al., 2002). Different casein hydrolysates may also have different influences on the growth of starter or probiotic cultures, and this is believed to be due to variations in the amino acid and peptide composition. Two casein hydrolysates (CH1 contain 73.2 of total nitrogen and CH2, 74.6) used to fortify a yoghurt milk base produced different result, with CH2 producing higher growth of starter culture and shorter fermentation time (Sodini et al., 2002). This could be due to slightly higher total nitrogen content in CH2. The opposite finding was obtained by several researchers (Isleten & Karagul-Yuceer, 2006; Soukoulis et al., 2007), with no major effect of milk base fortification on the starter culture growth when using the yoghurt cultures *S. thermophiles* and *L. delbrueckii* subsp. *bulgaricus*. In general, the starter and milk base interactions appear to have a major influence on yoghurt manufacture.

Heat treatment

Heat treatment is one of the crucial stages in yoghurt manufacture. The major purpose of heating is to eliminate all the pathogenic and spoilage microorganisms. In addition, the destruction of competitive microorganisms provides a favourable condition for yoghurt bacteria to grow (Chandan & O'Rell, 2006). In the industry, the yoghurt mix is usually heated at 90^{o}C with a minimum holding time of 30 min (N. Kusumaningrum, personal communication, May 10, 2009). For a high temperature-short time (HTST) pasteurization, the equivalent temperature and time combination is 73^{o}C for 15 s, while ultra-high temperature (UHT) treatment uses temperatures more than 90^{o}C and as high as 148^{o}C for 2 s (Chandan & Shahani, 1993). Treatment at 90-95^{o}C with a holding time of 5-10 min has also been found to be satisfactory (Labropoulos, Palmer, & Lopez, 1981; Mottar, Bassier, Joniau, & Baert, 1989; Parnell-Clunies, Kakuda,

& Deman, 1986; Schmidt, Vargas, Smith, & Jezeski, 1985). In yoghurt manufacture it is important that 70-95% of the whey protein is denatured to enhance water absorption. This ensures yoghurt with a smooth consistency and high viscosity (Chandan & O'Rell, 2006). The heat treatment of the yoghurt mix is normally achieved using industrial heat exchangers.

Heating milk also needed for changes in the physicochemical properties of the milk constituents which are relevant in yoghurt making (Tamime & Robinson, 2007). β-lactoglobulin, is the main whey protein that is denatured during heating (Lee & Lucey, 2010). This shifts the yoghurt gelation point towards higher pH values (Lucey, Tamehana, Singh, & Munro, 1998), producing a higher isoelectric point at pH 5.3. Denaturation of β-lactoglobulin up to 60% influences the yoghurt texture. Further denaturation, between 60 to 90% of β-lactoglobulin, has less effect on the yoghurt texture. Therefore, the heat treatment of milk base contributes to the fermentation time (Labropoulos et al., 1981; Parnell-Clunies et al., 1986; Shaker, Jumah, & Abu-Jdayil, 2000) and firmness of yoghurt (Augustin, Cheng, & Clarke, 1999; Dannenberg & Kessler, 1988).

The use of UHT as a heat treatment for the yoghurt milk base is not common. Yet, the sterilization effect of UHT is vital to prevent the growth of contaminating bacteria during the longer fermentation necessary for the production of MIT yoghurt. UHT can destroy all microorganisms including spores, inactivate some enzymes and affect the chemical changes, colour and flavour of milks (Fox, Mcsweeney, & Paul, 1998), producing an astringency flavour (Harwalkar, Boutinmuma, Cholette, Mckellar, & Emmons, 1989). UHT of milk is a continuous heating process at 135 to 150^{o}C for 2-8 sec (Krasaekoopt, Bhandari, & Deeth, 2003) and can be direct or indirect. Most studies use indirect UHT processes. This is due to a better texture and viscosity of yoghurt produced using the indirect compared with the direct method (Mottar et al., 1989).

There are several advantages in using UHT for yoghurt manufacture (1) better process control and sanitation, (2) energy and time savings, (3) high microbial quality, (4) longer shelf-life for the product (Labropoulos et al., 1981; Schmidt et al., 1985) and (5) stimulation of the growth and activity of yoghurt cultures (Smith, Schmidt, & Adams, 1982). The quality of yoghurt made from UHT milk compared with conventionally heated milk has been extensively reviewed by Krasaekoopt et al. (2003) (Table 2).

Briefly, yoghurt made from UHT milk has (a) lower viscosity and gel strength, (b) less syneresis, (c) a similar flavour to product manufactured from a pasteurized milk base, (d) minor differences in the microstructure (e) different texture that might be due to different denaturation effects of UHT heating and conventional heating on the whey protein, (f) improved texture when fortified with SMP, (e) enhanced the pH reduction (De Brabandere & De Baerdemaeker, 1999).

Starter culture composition

The commercial process of yoghurt manufacture uses a defined mixture of lactic acid bacteria. The combination of *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (STLB) is normally used as the starter culture. These starter cultures are thermophilic bacteria with an optimum growth at a temperature of 37 to 45^{o}C, homofermentative and some strains can produce exopolysaccharide (EPS) (Tamime & Robinson, 2007). The rationale for selecting the combination of starter cultures is to achieve the desired flavour and texture characteristics. The culture is added to the milk base either by direct inoculation using concentrated, frozen or freeze-dried cultures or indirect inoculation using a pre-cultured inoculum at levels from 1 to 5% (Sodini et al., 2004).

The Codex standard defines yoghurt as a milk product obtained by the fermentation of milk, or products obtained from milk, by the action of suitable microorganisms resulting in a reduction of pH, with or without coagulation (isoelectric precipitation) (FAO/WHO, 2003). The suitable microorganisms for yoghurt, according to Codex, are as follows; symbiotic cultures of *S. thermophiles* and *L. delbrueckii* subsp. *bulgaricus*. Alternative yoghurt cultures mentioned in the Codex standard include a mixture of *S. thermophiles* and any *Lactobacillus* species. More-

Table 2: Comparison on the quality of yoghurt produced by UHT and pasteurized milk

Evaluation	Yoghurt produced from UHT milk Vs pasteurized milk (conventional)	References
Texture (Firmness and/or Apparent viscosity)	Yoghurt produced using UHT milk has a weaker gel, lower viscosity and less shear time compared with conventional processes. Yoghurt made from UHT milk fortified with 16, 18 and 20% of low heat skim milk powder has delayed gelation with lower viscosity. 20% of the total solids fortified in UHT milk have a similar viscosity to 16% total solids in conventional processes.	(Labropoulos, Palmer, and Lopez (1981); Mottar, Bassier, Joniau, and Baert (1989); (Parnell-Clunies, Kakuda, and Deman (1986); (Krasaekoopt, Kew, Bhandari, and Deeth (2002); Krasaekoopt, Bhandari, and Deeth (2004))
Microstructure	The microstructure studied by SEM and TEM shows a minor difference between yoghurts produced by UHT milk and conventional processing. In conventional yoghurt, micelles tend to fuse and form a dense network that may result in firm gel texture and high viscosity. Compared with UHT yoghurt, the low gel strength and viscosity and loose microstructure could be due to the filamentous appendages that disrupt the fusion of casein particles by forming floccules by particle to particle attachment in UHT yoghurt.	(Parnell-Clunies, Kakuda, Deman, and Cazzola (1988); Parnell-Clunies, Kakuda, and Smith (1987)) (Krasaekoopt, Bhandari, and Deeth (2003)
Syneresis	UHT yoghurt was observed to have less syneresis compared with conventionally processed yogurt. This could be due to the increase in water holding capacity (WHC) by denaturation, whereas increased exposure of charged groups and increased surface area enhances protein-water interactions.	(Savello and Dargan (1997); Schmidt, Vargas, Smith, and Jezeski (1985)) Kinsella (1984); Parnell-Clunies, Kakuda, and Deman (1986))
Denaturation of whey protein	UHT processing was reported to produce less denature whey protein compared to conventional process. UHT and conventionally heated milk are observed to have similar levels of denaturation of whey protein	(Labropoulos, Palmer, and Lopez (1981); Krasaekoopt, Bhandari, and Deeth (2004)) (Dargan and Savello (1990); Mottar, Bassier, Joniau, and Baert (1989)

over, other microorganisms than those constituting the specific starter culture(s) specified above may be added (FAO/WHO, 2003).

Many types of lactobacilli and bifidobacteria have been used. These bacteria may be added as a probiotic or adjunct culture with the standard bacteria for yoghurt manufacture. The selection of the starter cultures can also affect the growth of probiotics, depending on protocooperation, inhibition or competition (Dave & Shah, 1997; Saxelin et al., 1999). Probiotic bacteria (*Lactobacillus* spp. e.g. *L. acidophilus, L. casei, Bifidobacterium* spp. and *Enterococcus* spp.) are usually added for producing a health promoting yoghurt. Probiotic bacteria have a beneficial effect on intestinal function and promote good health (Sanders, 1999). Some probiotic bacteria are claimed to aid lactose digestion (Vesa et al., 1996), prevent travellers' diarrhoea (Oksanen et al., 1990) and enhance the immune activity (Meydani & Ha, 2000). Certain levels of probiotic bacteria are required for these functions. For instance, the occurrence of travellers' diarrhoea can be reduced with 10^9 cfu day^{-1} of strain *L. acidophilus* GG (Oksanen et al., 1990). Therefore, it is important to maintain a high number of probiotic bacteria in yoghurt after manufacture in order for them to function as probiotics.

For yoghurt fermentation time, the yoghurt starter culture of *S. thermophiles* and *L. delbrueckii subspp. Bulgaricus* produce a very short fermentation time, 2 to 3 h at 40 to 45^{o}C (Tamime & Robinson, 2007). Yet, most probiotic bacteria grow slowly in milk and the rate of acid production is usually too slow to support adequate fermentation in yoghurt (Shah, 2000). Several other researchers have also reported that probiotic bacteria produce poor acidification in milk when compared to yoghurt starter cultures (Almeida, Tamime, & Oliveira, 2008; Marshall & Tamime, 1997; Oliveira et al., 2001; Saxelin et al., 1999; Sodini et al., 2002). This could be due to a lack of proteolytic activity in probiotic bacteria (Klaver, Kingma, & Weerkamp, 1993; Lucas, Sodini, Monnet, Jolivet, & Corrieu, 2004).

Starter culture composition has a great effect on fermentation time. When the mixed starter culture (*S. thermophiles* (ST) with probiotic, *L. acidophilus* (STLA) or *L. rhamnosus* (STLR)) were used, the fermentation time decreased two to three times compared to using a single culture of probiotic bacteria, *L. acidophilus* (LA) or *L. rhamnosus* (LR) (Dave & Shah, 1997; Oliveira et al., 2001; Sodini et al., 2002). Sodini et al. (2002) observed that a mixed culture of STLA produced a fermentation time of approximately 4 to 8 h compared with LA by itself which had a fermentation time of 8 to 13 h. In another study, Damin, Minowa, Alcantara, and Oliveira (2008) found the shortest fermentation time to reach pH 4.5 was obtained with milk fermented by *S. thermophiles* with *L. bulgaricus* (5.4 h),

and that the fermentation time was longer time when *S. thermophiles* was co-cultured with *Bifidobacterium lactis* (8.3 h) and *L. acidophilus* (9.3 h); the differences between them were significant ($p < 0.05$). The combination of STLA was found to extend the yoghurt fermentation to 168 h when incubated at 25^{o}C compared to STLB (Nor-Khaizura et al., 2012).

Probiotics tend to grow slowly in yoghurt milk base, do not compete well with many starter strains and the probiotics show poor stability during storage. This may be due to competition between lactobacilli, slowing the growth of probiotic lactobacilli (Sodini et al., 2002). Mixed cultures of *S. thermophilus* and a probiotic such as *L. acidophilus* showed that the former predominates under all culture conditions used (Dave & Shah, 1997; Oliveira et al., 2001; Vinderola, Gueimonde, Delgado, Reinheimer, & los Reyes-Gavilán, 2000). Probiotic growth was better when a single culture was used instead of a mixed culture (Sodini et al., 2002). However, Dave and Shah (1997) observed no difference in single or mixed culture for *L. acidophilus* growth.

The starter culture can influence yoghurt firmness (Hassan, Frank, Schmidt, & Shalab, 1996; Hess, Roberts, & Ziegler, 1997; Rohm & Kovac, 1994) depending whether or not the culture strain is an exopolysaccharide (EPS) producer. This is due to the EPS which has a large molecular mass, interacting with casein or physically preventing casein micelles from coming into close contact, therefore, restricting the increase of yoghurt firmness (Sodini et al., 2004). Some probiotic cultures also influence the rheological parameters, with higher values found in yoghurt with the single culture more than with mixed culture (Sodini et al., 2002). This contradicts Oliveira et al. (2001), where they observed the culture composition did not affected the yoghurt texture.

Inoculum level

The inoculum level of starter culture may influence the acidification process, and consequently the fermentation time (Nor-Khaizura et al., 2012) and yoghurt gelation (Lee & Lucey, 2004b; Peng et al., 2009). Lowering the inoculum level decreases the acidification rate (Kristo, Biliaderis, & Tzanetakis, 2003; Sebastiani, Gelsomino, & Walser, 1998). This also affects the rheology of yoghurt, which decreases under longer fermentation (Kristo et al., 2003). Higher inoculum levels increase the rheology of yoghurt (Lee & Lucey, 2004b). The permeability, pore size and whey separation of the yoghurt gel is increased with a long fermentation time due to a lower inoculum (Lee & Lucey, 2004b). However, according to Sodini et al. (2004), the inoculum level has a small effect. Ronnegard and Dejmek (1993) found not much effect on the yoghurt viscosity when the inoculum level varied between 1 to 5%.

Fermentation temperature

The fermentation temperature affects the yoghurt fermentation time and texture. Fermentation temperatures higher (43.5 and 45^{o}C) than the optimal (42^{o}C) for standard commercial yoghurt cultures, were reported not to affect pH development compared with lowering the temperature (40.5 and 39^{o}C) where the pH drop slowed (De Brabandere & De Baerdemaeker, 1999). Lowering the fermentation temperature causes a systematic decrease in the time required to reach the final pH of 4.5, which can be explained by a decrease in the metabolic activity of the bacteria (Haque, Richardson, & Morris, 2001). Mortazavian et al. (2006) observed that fermentation at 37^{o}C required approximately 6.17 h, compared with 40 and 44^{o}C requiring 5.26 and 4.39 h, respectively. At even lower fermentation temperatures (e.g. ∼30^{o}C), the fermentation time can be extended up to 12 h and good quality of yoghurt is produced (Lucey et al., 1998). Using probiotic bacteria, the fermentation temperature has a similar influence on the pH reduction in milk. Ostlie, Treimo, and Narvhus (2005) reported after 48 h of fermentation, depending on probiotic strains (*L. aciophilus* LA5, *L. acidophilus* 1748, *L. reuteri* SD2112, *L. johnsonii* LA1 and *Bifidobacterium animalis* BB12), pH decreased from 6.7 to 4.1-5.1 at a fermentation temperature of 30˚C, to 3.8-4.7 at 37^{o}C and 3.8-4.5 at 45^{o}C. Further lowering the fermentation temperature to 25^{o}C using the combination of STLA extended the fermentation time to 168 h, but the yoghurt texture was defective (Nor-Khaizura et al., 2012). At the typical fermentation temperature for yo-

ghurt, 42^{o}C or higher, yoghurt has a fast gelation time. This causes the yoghurt gel network to be more prone to rearrangements and these changes may lead to greater whey separation (Lucey, 2001; Mellema, Walstra, van Opheusden, & van Vliet, 2002). The yoghurt microstructure shows that gels fermented at 42^{o}C have less branches, coarser, thinner strands and larger pores compared to gels fermented at 30^{o}C (Lucey et al., 1998). Yoghurt incubated at a lower temperature (e.g. <40^{o}C) has a slightly longer gelation time, and the product is normally firmer, more viscous, less prone to syneresis and with less lumpy or grainy defects on stirring the coagulum during cooling (Lee & Lucey, 2004a; Lucey, 2002). A few studies observed, that stirred yoghurt viscosity was higher at lower incubation temperatures (<40^{o}C) compared with higher temperatures (>40^{o}C) (Lee & Lucey, 2006; Martin, Skokanova, Latrille, Beal, & Corrieu, 1999; Sodini et al., 2004). The micrograph structure of yoghurt fermented at lower temperatures showed a highly cross-linked and branch protein network and small pores (Lee & Lucey, 2003; Lee & Lucey, 2004b). However, when yoghurt is fermented at lower temperatures (e.g. 25^{o}C) using the probiotic bacteria as co-culture (STLA), the texture was defective compared to yoghurt fermented with the standard co-culture (STLB) (Nor-Khaizura et al., 2012).

4.3 Possible mechanism of yoghurt gelation during the long fermentation

During long fermentation, the acidification rate becomes slower. This condition directly increases the yoghurt gelation or coagulation. Lucey et al. (1998) describe the increased coagulation as a two-step phenomenon. Aggregation of heated milk base is expected to begin at higher pH, at about pH 5.3 (isoelectric pH of β-lactoglobulin) and continue to pH 4.6 (isoelectric pH of casein). During the long fermentation time, the elapsed time between these two pH levels is also long. At the first step of coagulation, the number of bonds created is higher due to the time needed to reach to pH 4.6. Therefore, at the second step of coagulation, rearrangement (further aggregation of strands and clusters) occur in gel network, causing whey separation and the formation of larger pores (Peng et al., 2009). This was exhibited in the microstructure of yoghurt made with a long fermentation time, where large strands and fewer apparent interconnections in the strands were seen compared to the fine structure and more branches in yoghurt prepared over a short fermentation time (Peng et al., 2009).
Based on Nor-Khaizura (2013), two possible formulations for MIT set yoghurt and standard set yoghurt were tested for the sensory using trained panelists (descriptive test) and untrained panelists (acceptance test). The finding showed no significant differences ($p > 0.05$) between the two MIT set yoghurts on descriptive test yet they were significantly different ($p < 0.05$) to the standard set yoghurt. The yoghurt attribute that assessed were appearance, aroma, texture and taste. For the acceptance test, MIT set yoghurts scored better than standard set yoghurt for overall acceptance.

5 Tool to assist in preparing an MIT product

Predictive microbiology or modelling can be used to assist in monitoring and predicting the fermentation as well as designing the best conditions for fermentation to fit with the requirements of an MIT food. Predictive microbiology describes microbial responses to different environmental conditions, which enable an objective evaluation of the effect of processing, distribution and storage operations on the microbiological safety and quality of foods (McMeekin, Olley, Ratkowsky, & Ross, 2002). Predictive microbiology is cost effective compared to the traditional microbiological testing to determine shelf-life and safety. Whiting (1993) classified predictive food microbiology according to three levels - primary, secondary and tertiary models. Primary models describe the change in the bacterial number with time under particular environmental and cultural conditions. The response can be measured directly by total viable count (TVC), toxin formation, substrate level or metabolic products and indirectly by absorbance, optical density or impedance. This generates information on the

generation time, lag phase duration, exponential growth rate and maximum population density (Whiting, 1995; Whiting, 1993; Whiting & Buchanan, 1994). Secondary models describe the response of one or more parameters of the primary model (e.g. generation time) in accordance with one or more changes in cultural or environmental conditions (e.g. pH, water activity, relative humidity, temperature). Tertiary models are the application of one or more primary and secondary models, incorporated into a user-friendly computer package.

The important aspects of practical model development are the range of characteristics investigated (growth, death, survival, toxin formation etc.). Variables consist of temperature, water activity, pH, nitrate concentration, gaseous atmosphere, organic acid or other preservative concentrations (Ross, Dalgaard, & Tienungoon, 2000). Reproducible responses are important for developing predictive microbiology in order to be able to predict future behaviour (McMeekin et al., 2002).

There are several examples of predictive microbiology research conducted in dairy manufacture. Roupas (2008) reported that statistical modelling accurately predicted curd pH and moisture during cheese making. The author added that mathematical models that can predict the cell growth and lactic acid production would be very useful in determining the quality of cheese. The modelling provided improved control in gelation during the cooling of rennet casein gels, and the structure and quality of dairy products such as processed cheese (Zhong & Daubert, 2004).

In yoghurt processing, Soukoulis et al. (2007) and De Brabandere and De Baerdemaeker (1999) proposed the use of predictive modelling as a monitoring system during yoghurt fermentation. Due to the complexity of the fermentation and the many factors involved in yoghurt coagulation, the mechanisms involved remain poorly understood (Peng et al., 2009). Prediction of fermentation is difficult, so it is a common practice to control it empirically (Soukoulis et al., 2007). In industry, pH measurement is used to control yoghurt manufacture, as acidification is the parameter for monitoring fermentation (De Brabandere & De Baerdemaeker, 1999).

The fermentation based on pH reduction could be illustrated with a three phase process 1) lag phase (slow pH reduction), 2) logarithm phase (rapid pH reduction) and 3) slowdown of acidification rate (Soukoulis et al., 2007). This three phase process forms a sigmoidal fermentation curve. The curve is dependent on many parameters (De Brabandere & De Baerdemaeker, 1999) such as the yoghurt milk base, fortification ingredients, heat treatment, starter culture composition and fermentation temperature (Soukoulis et al., 2007). The modified Gompertz model was shown to be excellent to describe the pH reduction (Soukoulis et al., 2007; De Brabandere & De Baerdemaeker, 1999) and viscosity development (Soukoulis et al., 2007) during yoghurt fermentation. This supports continuously monitoring pH during yoghurt fermentation as a useful tool for checking product quality and for predictive or corrective purposes (Soukoulis et al., 2007). Other than modelling, other monitoring systems suggested include a combination of near infrared (NIR) and the electronic nose (Cimander, Carlsson, & Mandenius, 2002; Navratil, Cimander, & Mandenius, 2004).

6 Potential and challenges for yoghurt as an MIT product

Based on the information on the manufacture of yoghurt presented in this review, should be possible to prepare MIT yoghurt. Yoghurt is an ideal product to investigate the development of MIT fermented foods as the fermentation period and shelf-life for the standard product are relatively short. This means that results from experiments are produced in a relatively short time frame, compared for the ripening of cheese, for example. In addition, using the MIT concept, it is possible to extend the shelf-life and distribution of this relatively short shelf-life product. In order to prepare an MIT product, the fermentation needs to be extended. This may present challenges in terms of the product texture, flavour and possible contamination. Possible ways to overcome these problems are fortification and UHT treatment of the yoghurt base. The MIT process will be best for products that undergo the transformation in the final package. Therefore, it could minimize the hygiene issue during processing in

the transit. To design and predict the fermentation of yoghurt under different conditions, predictive microbiology or modelling is an appropriate tool.

References

Almeida, K. E., Tamime, A. Y., & Oliveira, M. N. (2008). Acidification rates of probiotic bacteria in minas frescal cheese whey. *LWT-Food Science and Technology*, *41*(2), 311–316. doi:10.1016/j.lwt.2007.02.021

Amatayakul, T., Sherkat, F., & Shah, N. P. (2006). Physical characteristics of set yoghurt made with altered casein to whey protein ratios and eps-producing starter cultures at 9 and 14% total solids. *Food Hydrocolloids*, *20*(2-3), 314–324. 7th International Hydrocolloids Conference, Melbourne, AUSTRALIA, AUG 29-SEP 01, 2004. doi:10.1016/j.foodhyd.2005.02.015

Anema, S. G. & Li, Y. M. (2003). Effect of ph on the association of denatured whey proteins with casein micelles in heated reconstituted skim milk. *Journal of Agricultural and Food Chemistry*, *51*(6), 1640–1646. doi:10.1021/jf025673a

Anonymous. (2005). Fonterra Gains Anchor, Fresh n Fruity Brands in NZ. Retrieved from http://www.scoop.co.nz/stories/BU0508/S00134.htm

Augustin, M. A., Cheng, L. J., & Clarke, P. T. (1999). Effects of preheat treatment of milk powder on the properties of reconstituted set skim yogurts. *International Dairy Journal*, *9*(3-6), 415–416. 1997 Hannah Symposium on Caseins and Caseinates -Structures, Interactions, Networks, HANNAH RES INST, AYR, SCOTLAND, MAY 21-23, 1997. doi:10.1016/S0958-6946(99)00113-2

Bhullar, Y. S., Uddin, M. A., & Shah, N. P. (2002). Effects of ingredients supplementation on textural characteristics and microstructure of yoghurt. *Milchwissenschaft-Milk Science International*, *57*(6), 328–332.

Chandan, R. C. & O'Rell, K. R. (2006). Principles of yogurt processing. In A. K. R. C. Chandan C. H. White & Y. H. Hui (Eds.), (Chap. Manufacturing Yogurt and Fermented Milks, pp. 195–209). Oxford, England: Blackwell Publishing.

Chandan, R. & Shahani, K. M. (1993). Dairy science and technology handbook. In Y. H. Hui (Ed.), (Chap. Yogurt, pp. 1–56). VCH Publishers Inc., New York.

Cho, Y. H., Lucey, J. A., & Singh, H. (1999). Rheological properties of acid milk gels as affected by the nature of the fat globule surface material and heat treatment of milk. *International Dairy Journal*, *9*(8), 537–545. doi:10.1016/S0958-6946(99)00123-5

Cimander, C., Carlsson, M., & Mandenius, C. F. (2002). Sensor fusion for on-line monitoring of yoghurt fermentation. *Journal of Biotechnology*, *99*(3), 237–248. 10th European Congress of Biotechnology (ECB10), MADRID, SPAIN, JUL, 2001. doi:10.1016/S0168-1656(02)00213-4

Damin, M. R., Alcantara, M. R., Nunes, A. P., & Oliveira, M. N. (2009). Effects of milk supplementation with skim milk powder, whey protein concentrate and sodium caseinate on acidification kinetics, rheological properties and structure of nonfat stirred yogurt. *LWT-Food Science and Technology*, *42*(10), 1744–1750. doi:10.1016/j.lwt.2009.03.019

Damin, M. R., Minowa, E., Alcantara, M. R., & Oliveira, M. N. (2008). Effect of cold storage on culture viability and some rheological properties of fermented milk prepared with yogurt and probiotic bacteria. *Journal of Texture Studies*, *39*(1), 40–55. doi:10.1111/j.1745-4603.2007.00129.x

Damodaran, S. (1996). Food chemistry. In O. R. Fennema (Ed.), (Chap. Amino Acids, Peptides and Proteins, pp. 245–369). Marcel Dekker Inc., New York. Retrieved from http://drasalehi.iauq.ac.ir/assets/subdomains/drasalehi/file/Marcel%20Dekker,_Food%20Chemistry,%203rd%20Edition_%5B1997_ISBN0824793463%5D.pdf

Dannenberg, F. & Kessler, H. G. (1988). Effect of denaturation of beta-lactoglobulin on texture properties of set-style nonfat yogurt .2. firmness and flow proper-

ties. *Milchwissenschaft-milk Science International*, *43*(11), 700–704.

Dargan, R. A. & Savello, P. A. (1990). Ultra-high temperature treatment of skim milk and low fat milk for yogurt. *Journal of Dairy Science*, *73*(Supplement 1), 111.

Dave, R. I. & Shah, N. P. (1997). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal*, *7*(1), 31–41. doi:10.1016/S0958-6946(96)00046-5

Dave, R. I. & Shah, N. P. (1998). Ingredient supplementation effects on viability of probiotic bacteria in yogurt. *Journal of Dairy Science*, *81*(11), 2804–2816. doi:10.3168/jds.S0022-0302(98)75839-4

De Brabandere, A. G. & De Baerdemaeker, J. G. (1999). Effects of process conditions on the ph development during yogurt fermentation. *Journal of Food Engineering*, *41*(3-4), 221–227. doi:10.1016/S0260-8774(99)00096-5

Doyle, M. P., Beuchat, L. R., & Montville, T. J. (1997). *Food microbiology : fundamentals and frontiers*. Washington, DC : ASM Press. Retrieved from https://trove.nla.gov.au/work/15886054

FAO/WHO. (2003). Codex Standard for Fermented Milks CODEX STAN 243-2003.

Fox, P. F., Mcsweeney, P. L. H., & Paul, L. H. (1998). *Dairy chemistry and biochemistry*. Springer.

Guzmán-González, M., Morais, F., Ramos, M., & Amigo, L. (1999). Influence of skimmed milk concentrate replacement by dry dairy products in a low fat set-type yoghurt model system. i: use of whey protein concentrates, milk protein concentrates and skimmed milk powder. *Journal of the Science of Food and Agriculture*, *79*(8), 1117–1122. doi:10.1002/(SICI)1097-0010(199906)79:8⟨1117::AID-JSFA335⟩3.0.CO;2-F. eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/%28SICI%291097-0010%28199906%2979%3A8%3C1117%3A%3AAID-JSFA335%3E3.0.CO%3B2-F

Haque, A., Richardson, R. K., & Morris, E. R. (2001). Effect of fermentation temperature on the rheology of set and stirred yogurt. *Food Hydrocolloids*, *15*(4-6), 593–602. 5th International Hydrocolloids Conference, RALEIGH, NC, SEP 10-15, 2000. doi:10.1016/S0268-005X(01)00090-X

Harwalkar, V. R., Boutinmuma, B., Cholette, H., Mckellar, R. C., & Emmons, D. B. (1989). Isolation and partial-purification of astringent compounds from ultrahigh temperature sterilized milk. *Journal of Dairy Research*, *56*(3), 367–373. doi:10.1017/S002202990002882X

Hassan, A. N., Frank, J. F., Schmidt, K. A., & Shalab, S. I. (1996). Rheological properties of yogurt made with encapsulated nonropy lactic cultures. *Journal of Dairy Science*, *79*(12), 2091–2097. doi:10.3168/jds.S0022-0302(96)76582-7

Hess, S. J., Roberts, R. F., & Ziegler, G. R. (1997). Rheological properties of nonfat yogurt stabilized using lactobacillus delbrueckii ssp bulgaricus producing exopolysaccharide or using commercial stabilizer systems. *Journal of Dairy Science*, *80*(2), 252–263. doi:10.3168/jds.S0022-0302(97)75933-2

Isleten, M. & Karagul-Yuceer, Y. (2006). Effects of dried dairy ingredients on physical and sensory properties of nonfat yogurt. *Journal of Dairy Science*, *89*(8), 2865–2872. doi:10.3168/jds.S0022-0302(06)72559-0

Jaworska, A. (2007a). *Made in transit: a supply chain concept for on the way growth* (Doctoral dissertation, Master Thesis).

Jaworska, A. (2007b). *Made in transit: the end of factory?* Retrieved from http://www.foodproductiondaily.com/Supply-Chain/Made-in-Transit-The-end-of-the-factory

Jaworska, A. (2008). *The future of fungal freshness?* Retrieved from http://blog.mycology.cornell.edu/?p=418

Jay, J. M. (2000). *Modern food microbiology* (Sixth). Maryland: Aspen Publishers.

Kailasapathy, K. & Supriadi, D. (1996). Effect of whey protein concentrate on the survival of lactobacillus acidophilus in lactose hydrolysed yoghurt during refrigerated storage. *Milchwissenschaft-milk Science International*, *51*(10), 565–569.

Kinsella, J. E. (1984). Milk-proteins - physicochemical and functional-properties.

CRC Critical Reviews in Food Science and Nutrition, *21*(3), 197–262. doi:10.1080/10408398409527401

Klaver, F. A. M., Kingma, F., & Weerkamp, A. H. (1993). Growth and survival of bifidobacteria in milk. *Netherlands Milk and Dairy Journal*, *47*(3-4), 151–164.

Kleijnen, J. P. C. & Van der Vorst, J. G. A. J. (2005). Designing robust and sustainable fresh-food supply chains: improved simulation methodology for reducing waste. *Department of Information Systems & Management/Center for Economic Research, Tilburg University*.

Kosikowski, F. & Mistry, V. (1997). *Cheese and fermented milk foods: origins and principles*. Cheese and Fermented Milk Foods. F.V. Kosikowski. Retrieved from https://books.google.pt/books?id=EVydtQEACAAJ

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2003). Yogurt from uht milk: a review. *Australian Journal of Dairy Technology*, *58*(1), 26–29.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2004). Comparison of textures of yogurt made from conventionally treated milk and uht milk fortified with low-heat skim milk powder. *Journal of Food Science*, *69*(6), E276–E280.

Krasaekoopt, W., Kew, S. Y., Bhandari, B., & Deeth, H. C. (2002). Characterization of texture development of yogurt from uht milk during fermentation. In *International conference on innovations in food processing tech & eng* (pp. 355–363). AIT, Bangkok.

Kristo, E., Biliaderis, C. G., & Tzanetakis, N. (2003). Modelling of the acidification process and rheological properties of milk fermented with a yogurt starter culture using response surface methodology. *Food Chemistry*, *83*(3), 437–446. doi:10.1016/S0308-8146(03)00126-2

Kurmann, J. A. (1984). Aspects of the production of fermented milks. *International Dairy Federation*.

Labropoulos, A. E., Palmer, J. K., & Lopez, A. (1981). Whey-protein denaturation of uht processed milk and its effect on rheology of yogurt. *Journal of Texture Studies*, *12*(3), 365–374. doi:10.1111/j.1745-4603.1981.tb00545.x

Lankes, H., Ozer, H. B., & Robinson, R. K. (1998). The effect of elevated milk solids and incubation temperature on the physical properties of natural yoghurt. *53*, 510–513.

Lee, W. J. & Lucey, J. A. (2004a). Rheological properties, whey separation, and microstructure in set-style yogurt: effects of heating temperature and incubation temperature. *Journal of Texture Studies*, *34*(5-6), 515–536. doi:10.1111/j.1745-4603.2003.tb01079.x

Lee, W. J. & Lucey, J. A. (2004b). Structure and physical properties of yogurt gels: effect of inoculation rate and incubation temperature. *Journal of Dairy Science*, *87*(10), 3153–3164. doi:10.3168/jds.S0022-0302(04)73450-5

Lee, W. J. & Lucey, J. A. (2006). Impact of gelation conditions and structural breakdown on the physical and sensory properties of stirred yogurts. *Journal of Dairy Science*, *89*(7), 2374–2385. doi:10.3168/jds.S0022-0302(06)72310-4

Lee, W. J. & Lucey, J. A. (2010). Formation and physical properties of yogurt. *Asian-Australasian Journal of Animal Sciences*, *23*(9), 1127–1136. doi:10.5713/ajas.2010.r.05

Lee, W.-J. & Lucey, J. A. (2003). Rheological properties, whey separation, and microstructure in set-style yogurt: effects of heating temperature and incubation temperature. *Journal of Texture Studies*, *34*(5-6), 515–536.

Lucas, A., Sodini, I., Monnet, C., Jolivet, P., & Corrieu, G. (2004). Probiotic cell counts and acidification in fermented milks supplemented with milk protein hydrolysates. *International Dairy Journal*, *14*(1), 47–53. doi:10.1016/S0958-6946(03)00147-X

Lucey, J. A. (2001). The relationship between rheological parameters and whey separation in milk gels. *Food Hydrocolloids*, *15*(4-6), 603–608. 5th International Hydrocolloids Conference, RALEIGH, NORTH

CAROLINA, SEP 10-15, 2000. doi:10.1016/S0268-005X(01)00043-1

Lucey, J. A. (2002). Formation and physical properties of milk protein gels. *Journal of Dairy Science*, *85*(2), 281–294. doi:10.3168/jds.S0022-0302(02)74078-2

Lucey, J. A., Munro, P. A., & Singh, H. (1999). Effects of heat treatment and whey protein addition on the rheological properties and structure of acid skim milk gels. *International Dairy Journal*, *9*(3-6), 275–279. 1997 Hannah Symposium on Caseins and Caseinates -Structures, Interactions, Networks, HANNAH RES INST, AYR, SCOTLAND, MAY 21-23, 1997. doi:10.1016/S0958-6946(99)00074-6

Lucey, J. A., Tamehana, M., Singh, H., & Munro, P. A. (1998). Effect of interactions between denatured whey proteins and casein micelles on the formation and rheological properties of acid skim milk gels. *Journal of Dairy Research*, *65*(4), 555–567. doi:10.1017/S0022029998003057

Lucey, J. A., Teo, C. T., Munro, P. A., & Singh, H. (1997). Rheological properties at small (dynamic) and large (yield) deformations of acid gels made from heated milk. *Journal of Dairy Research*, *64*(4), 591–600. doi:10.1017/S0022029997002380

Marshall, V. M. & Tamime, A. Y. (1997). Starter cultures employed in the manufacture of biofermented milks. *International Journal of Dairy Technology*, *50*(1), 35–41. doi:10.1111/j.1471-0307.1997.tb01733.x

Martin, N. C., Skokanova, J., Latrille, E., Beal, C., & Corrieu, G. (1999). Influence of fermentation and storage conditions on the sensory properties of plain low fat stirred yogurts. *Journal of Sensory Studies*, *14*(2), 139–160. doi:10.1111/j.1745-459X.1999.tb00109.x

McKenna, A. B. (1997). Examination of whole milk powder by confocal laser scanning microscopy. *Journal of Dairy Research*, *64*(3), 423–432. doi:10.1017/S0022029997002331

McKenna, A. B. & Anema, S. G. (1993). The effect of thermal processing during whole milk powder manufacture and after its reconstitution on set-yoghurt properties. In *Protein and fat globule modifications by heat treatment, homogenization and other technological means for high quality dairy products. idf seminar, munich (germany)*. FIL-IDF.

McMeekin, T. A., Olley, J., Ratkowsky, D. A., & Ross, T. (2002). Predictive microbiology: towards the interface and beyond. *International Journal of Food Microbiology*, *73*(2-3), 395–407. 3rd International Conference on Predictive Modelling in Foods, LEUVEN, BELGIUM, SEP 12-15, 2000. doi:10.1016/S0168-1605(01)00663-8

Mellema, M., Walstra, P., van Opheusden, J. H. J., & van Vliet, T. (2002). Effects of structural rearrangements on the rheology of rennet-induced casein particle gels. *Advances in Colloid and Interface Science*, *98*(1), 25–50. doi:10.1016/S0001-8686(01)00089-6

Meydani, S. N. & Ha, W. K. (2000). Immunologic effects of yogurt. *American Journal of Clinical Nutrition*, *71*(4), 861–872.

Mortazavian, A. M., Ehsani, M. R., Mousavi, S. M., Reinheimer, J. A., Emamdjomeh, Z., Sohrabvandi, S., & Rezaei, K. (2006). Preliminary investigation of the combined effect of heat treatment and incubation temperature on the viability of the probiotic micro-organisms in freshly made yogurt. *International Journal of Dairy Technology*, *59*(1), 8–11. doi:10.1111/j.1471-0307.2006.00216.x

Mottar, J., Bassier, A., Joniau, M., & Baert, J. (1989). Effect of heat-induced association of whey proteins and casein micelles on yogurt texture. *Journal of Dairy Science*, *72*(9), 2247–2256. doi:10.3168/jds.S0022-0302(89)79355-3

Navratil, M., Cimander, C., & Mandenius, C. F. (2004). On-line multisensor monitoring of yogurt and filmjolk fermentations on production scale. *Journal of Agricultural and Food Chemistry*, *52*(3), 415–420. doi:10.1021/jf0304876

Nor-Khaizura, M.-A.-R. (2013). *Development and predictive modelling of set yoghurt as a made-in-transit (MIT) product: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Food Technology at Massey Uni-*

versity, Palmerston North, New Zealand (Doctoral dissertation, Massey University). Retrieved from http://hdl.handle.net/10179/4678

Nor-Khaizura, M.-A.-R., Flint, S. H., McCarthy, O. J., Palmer, J. S., Golding, M., & Jaworska, A. (2012). Development of made-in-transit set culture yoghurt: effect of increasing the concentration of reconstituted skim milk as the milk base. *International Journal of Food Science and Technology*, *47*(3), 579–584. doi:10.1111/j.1365-2621.2011.02880.x

Oksanen, P. J., Salminen, S., Saxelin, M., Hamalainen, P., Ihantola-Vormisto, A., Muurasniemi-Isoviita, L., ... Salminen, E. (1990). Prevention of travellers diarrhoea by lactobacillus GG. *Annals of Medicine*, *22*(1), 53–56.

Oliveira, M. N., Sodini, I., Remeuf, F., & Corrieu, G. (2001). Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria. *International Dairy Journal*, *11*(11-12), 935–942. doi:10.1016/S0958-6946(01)00142-X

Oneil, J. M., Kleyn, D. H., & Hare, L. B. (1979). Consistency and compositional characteristics of commercial yogurts. *Journal of Dairy Science*, *62*(6), 1032–1036. doi:10.3168/jds.S0022-0302(79)83368-8

Ostlie, H. M., Treimo, J., & Narvhus, J. A. (2005). Effect of temperature on growth and metabolism of probiotic bacteria in milk. *International Dairy Journal*, *15*(10), 989–997. doi:10.1016/j.idairyj.2004.08.015

Parfitt, J., Barthel, M., & Macnaughton, S. (2010). Food waste within food supply chains: quantification and potential for change to 2050. *Philosophical Transactions of the Royal Society B-biological Sciences*, *365*(1554), 3065–3081. doi:10.1098/rstb.2010.0126

Parnell-Clunies, E. M., Kakuda, Y., & Deman, J. M. (1986). Influence of heat treatment of milk on the flow properties of yoghurt. *Journal of Food Science*, *51*(6), 1459–1462. doi:10.1111/j.1365-2621.1986.tb13834.x

Parnell-Clunies, E. M., Kakuda, Y., Deman, J. M., & Cazzola, F. (1988). Gelation profiles of yogurt as affected by heat-treatment of milk. *Journal of Dairy Science*, *71*(3), 582–588. doi:10.3168/jds.S0022-0302(88)79594-6

Parnell-Clunies, E. M., Kakuda, Y., & Smith, A. K. (1987). Microstructure of yogurt as affected by heat-treatment of milk. *Milchwissenschaft-milk Science International*, *42*(7), 413–417.

Patocka, G., Cervenkova, R., Narine, S., & Jelen, P. (2006). Rheological behaviour of dairy products as affected by soluble whey protein isolate. *International Dairy Journal*, *16*(5), 399–405. doi:10.1016/j.idairyj.2005.05.010

Peng, Y., Serra, M., Horne, D. S., & Lucey, J. A. (2009). Effect of fortification with various types of milk proteins on the rheological properties and permeability of nonfat set yogurt. *Journal of Food Science*, *74*(9), C666–C673. doi:10.1111/j.1750-3841.2009.01350.x

Penna, A. L. B., Baruffaldi, R., & Oliveira, M. N. (1997). Optimization of yogurt production using demineralized whey. *Journal of Food Science*, *62*(4), 846–850. doi:10.1111/j.1365-2621.1997.tb15469.x. eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2621.1997.tb15469.x

Penna, A. L. B., Converti, A., & De Oliveira, M. N. (2006). Simultaneous effects of total solids content, milk base, heat treatment temperature and sample temperature on the rheological properties of plain stirred yogurt. *Food Technology and Biotechnology*, *44*(4), 515.

Puvanenthiran, A., Williams, R., & Augustin, M. A. (2002, December). Structure and visco-elastic properties of set yoghurt with altered casein to whey protein ratios. *12*, 383–391.

Remeuf, F., Mohammed, S., Sodini, I., & Tissier, J. P. (2003, December). Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yogurt. *13*, 773–782. doi:10.1016/S0958-6946(03)00092-X

Rohm, H. & Kovac, A. (1994). Effects of starter cultures on linear viscoelastic and physical properties of yogurt gels. *Journal of Texture Studies*, *25*(3), 311–329. doi:10.1111/j.1745-4603.1994.tb00763.x

Rohm, H. & Schmid, W. (1993, January). Influence of dry matter fortification on flow properties of yogurt. 1. evaluation of flow curves. *48*, 556–560.

Ronnegard, E. & Dejmek, P. (1993). Development and breakdown of structure in yoghurt studied by oscillatory rheological measurements. *Le Lait*, *73*(4), 371–379.

Ross, T., Dalgaard, P., & Tienungoon, S. (2000). Predictive modelling of the growth and survival of listeria in fishery products. *International Journal of Food Microbiology*, *62*(3), 231–245.

Roupas, P. (2008). Predictive modelling of dairy manufacturing processes. *International Dairy Journal*, *18*(7), 741–753. Milestone achievements in dairy science research and their current and future industrial applications. doi:10.1016/j.idairyj.2008.03.009

Sanders, M. E. (1999). Probiotics. food technology. *53*, 67–77.

Savello, P. A. & Dargan, R. A. (1997). Reduced yogurt syneresis using ultrafiltration and very-high temperature heating. *Milchwissenschaft*, *52*(10), 573–577.

Saxelin, M., Grenov, B., Svensson, U., Fondén, R., Reniero, R., & MattilSvensson, T. (1999). The technology of probiotics. *Trends in Food Science & Technology*, *10*(12), 387–392. doi:10.1016/S0924-2244(00)00027-3

Schmidt, R. H., Vargas, M. M., Smith, K. L., & Jezeski, J. J. (1985). The effect of ultra-high temperature milk processing on yogurt texture1. *Journal of Food Processing and Preservation*, *9*(4), 235–240. doi:10.1111/j.1745-4549.1985.tb00723.x

Sebastiani, H., Gelsomino, R., & Walser, H. (1998). Cultures for the improvement of texture in quarg. In *Texture of fermented milk products and dairy desserts-idf symposium, vicenza (italy), 5-6 may 1997*. IDF.

Shah, N. P. (2000). Probiotic bacteria: selective enumeration and survival in dairy foods. *Journal of Dairy Science*, *83*(4), 894–907. doi:10.3168/jds.S0022-0302(00)74953-8

Shaker, R. R., Jumah, R. Y., & Abu-Jdayil, B. (2000). Rheological properties of plain yogurt during coagulation process: impact of fat content and preheat treatment of milk. *Journal of Food Engineering*, *44*(3), 175–180.

Smith, K. L., Schmidt, R. H., & Adams, J. P. (1982). Growth of yogurt cultures in uht treated milk. *Journal of Dairy Science*, *65*(Suppl 1), 221.

Sodini, I., Lucas, A., Oliveira, M. N., Remeuf, F., & Corrieu, G. (2002). Effect of milk base and starter culture on acidification, texture, and probiotic cell counts in fermented milk processing. *Journal of Dairy Science*, *85*(10), 2479–2488.

Sodini, I., Remeuf, F., Haddad, S., & Georges, C. (2004). The relative effect of milk base, starter and process on yogurt texture: a review. *Critical Reviews in Food Science and Nutrition*, *44*(2), 113–137. doi:10.1080/10408690490424793

Soukoulis, C., Panagiotidis, P., Koureli, R., & Tzia, C. (2007). Industrial yogurt manufacture: monitoring of fermentation process and improvement of final product quality. *Journal of Dairy Science*, *90*(6), 2641–2654. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17517704

Spreer, E. (2017). *Milk and dairy product technology*. Routledge.

Swaisgood, H. E. (1996). *Characteristics of milk* (O. R. F. F. Chemistry, Ed.). Marcel Dekker.

Tamime, A. Y. & Deeth, H. C. (1980). Yogurt: technology and biochemistry. *Journal of Food Protection*, *43*(12), 939–977.

Tamime, A. Y., Kalab, M., & Davies, G. (1984). Microstructure of set-style yoghurt manufactured from cow's milk fortified by various methods. *Food Structure*, *3*(1), 11.

Tamime, A. Y. & Robinson, R. K. (2007). *Tamime and robinson's yoghurt: science and technology*. Elsevier.

Trachoo, N. & Mistry, V. V. (1998). Application of ultrafiltered sweet buttermilk and sweet buttermilk powder in the manufac-

ture of nonfat and low fat yogurts1. *Journal of Dairy Science*, *81*(12), 3163–3171.

Vesa, T. H., Marteau, P. H., Zidi, S., Briet, F., Pochart, P. H., & Rambaud, J. C. (1996). Digestion and tolerance of lactose from yoghurt and different semi-solid fermented dairy products containing lactobacillus acidophilus and bifidobacteria in lactose maldigesters–is bacterial lactase important? *European Journal of Clinical Nutrition*, *50*(11), 730–733.

Vinderola, C., Gueimonde, M., Delgado, T., Reinheimer, J., & los Reyes-Gavilán, C. (2000). Characteristics of carbonated fermented milk and survival of probiotic bacteria. *International Dairy Journal*, *10*(3), 213–220. doi:10.1016/S0958-6946(00)00031-5

Walstra, P. (1999). *Dairy technology: principles of milk properties and processes*. CRC Press.

Whiting, R. C. (1993). A classification of models in predictive microbiology-a reply to kr davey. *Food Microbiology*, *10*(2), 175–177.

Whiting, R. C. & Buchanan, R. L. (1994). Microbial modeling. *Food Technology*, *48*(6), 113–120.

Whiting, R. C. (1995). Microbial modeling in foods. *Critical Reviews in Food Science & Nutrition*, *35*(6), 467–494.

Wilcek, A. (1990). Methods for classification of skim-milk powder. (pp. 135–140). Brussels, Belgium: International Dairy Federation.

Zhong, Q. & Daubert, C. (2004). Kinetics of rennet casein gelation at different cooling rates. *Journal of Colloid and Interface Science*, *279*(1), 88–94. doi:10.1016/j.jcis.2004.06.059