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## CONTENTS

- 1 Student Perceptions of Collaborative and Blended Learning in Food Science and Technology  
VUSI VINCENT MSHAYISA
- 19 Comparisons between Flour Qualities Produced by Three Different Mills: Buhler, Quadrumat, and Industry Mills  
ABEER S. ALHENDI, TAMADHER H. AHMAD, WASAN S. ALBAYATI, BALSAM Q. ALMUKHTAR, ZAHRAA K. ALI AND NUHOODH K. AL-HAYANI
- 28 Development and Sensory Evaluation of a Cookie from Composite Sorghum and Cowpea Flour  
DJOULDE DARMAN ROGER, FADI GOYGOY AND DJOMDI
- 38 Influence of Extraction Methods on Phenolic Compounds from Pulp and Peel of Genipap (*Genipa Americana* L.) Fruit  
TENILA DOS SANTOS FARIA, MIRIA HESPANHOL MIRANDA REIS, VIVIAN CONSUELO REOLON SCHMIDT AND VICELMA LUIZ CARDOSO
- 51 Influence of Extraction Solvent on the Biological Properties of Maritime Pine Bark (*Pinus pinaster*)  
INÉS MÁRMOL, CATARINA VIEITO, VANESSA ANDREU, ANNABEL LEVERT, ANAÍS AMIOT, CÉDRIC BERTRAND, M<sup>a</sup> JESÚS RODRÍGUEZ-YOLDI, JOANA SANTOS AND MANUELA VAZ-VELHO
- 63 Storage Stability of Hot Smoked Spiced African Catfish (*Clarias gariepinus*)  
SOGO J. OLATUNDE, ANTHONIA F. AKINBISOYE, BEATRICE I. O. AND ADE-OMOWAYE
- 71 Chemical Composition, Nutritional, Functional and Pasting Properties of Yellow Root Cassava Grits and African Yam Bean Flour Blends  
VICTORIA FUNMILAYO ABIOYE, OLOLADE ABOSEDE OLODUDE AND BOLANLE AISHAT AKINWANDE
- 85 Practical Reflection and Benefits of Making a Food Garden at Home During Covid-19 Pandemic  
RENDY BAYU ADITYA AND AISYAH ZAKIAH
- 98 Physical and Chemical Characteristics of Beef Marinated by Cashew Apple Extract  
SITI SUSANTI, VALENTINUS PRIYO BINTORO, ANTONIUS HINTONO AND KHOIRUN NISA
- 106 A Systems Integral Approach in Exploring Creative Innovation in Culinary Research: The Example of Seaweed in the Context of the New Nordic Cuisine  
CHERYL M. CORDEIRO AND JAAP VAN HAL

# Student Perceptions of Collaborative and Blended Learning in Food Science and Technology

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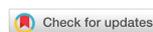
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## Abstract

Blended learning refers to the use of conventional face-to-face learning experiences in combination with online education resources and practices. An increase in enrolments and a more diverse student body has intensified the demand to develop first-year teaching and learning pedagogies. Food science and technology lecturers must facilitate constructive learning in order to develop student skills, including critical thinking, teamwork, and self-directed learning. The aim of this investigation was to evaluate student perceptions of collaborative and blended learning. Students were exposed to various technology-enhanced pedagogical tools and face-to-face teaching strategies such as online academic journal reflections, video screencasts, group assignments, food processing practicals, and group crossword puzzles. A mixed-method survey consisting of multiple-choice, a 5-point Likert scale, and open-ended qualitative questions was administered via Blackboard. A total of 133 students were registered for the module, and 72.1% (n = 96) completed the survey. In this study, respondents felt they were prepared to complete the online group assignments (82%), which illustrates that they could learn the course material through collaboration.

Moreover, 87% of the students agreed that they could keep up with the coursework in the blended format. Students recommended that there should be more lecture designed video screencasts, and they should be offered more opportunities to do oral presentations in this module. The respondents positively received collaborative and blended learning. The findings of this study, in general, affirm the merits of incorporating blended and collaborative learning in food science and technology curricula.

**Keywords:** Blended learning; Food Science and Technology; Collaborative learning; Flipped classroom; Constructivism; Blackboard

## 1 Introduction

Various studies and reports suggest a clear need for improved science education, not only in content but in the manner in which information is taught (Duffrin, 2006; Gezer-Templeton et al., 2017; Ma et al., 2018). Contrary to surface learning, deep learning is an essential strategy that enables students to extract meaning from

course material and experiences. In the context of higher education (HE), the transition from high school to university can be challenging for some students. The challenges of this transition are amplified by the fact that a large percentage of students come from low-income families with an under-resourced and sometimes dysfunctional educational system (Pillay & Gerrard, 2014). Given these challenges, educational in-

## Nomenclature

UoT	University of Technology	ogy
DFST	Department of Food Science and Technology	MCQ
		Multiple Choice Question
		LMS
		Learning Management System
CPUT	Cape Peninsula University of Technol-	FOT150S
		Food Technology 1

stitutions worldwide are increasingly embracing blended learning strategies to deliver course content to a diverse and dispersed cohort. Blended courses, which incorporate online and conventional instruction delivery, may be more conducive to classroom participation than purely online or face-to-face lessons (Bohlscheid & Davis, 2012), and they can also be used effectively in larger classes to improve learning (Meyer et al., 2014; Okaz, 2015; Poon, 2013). Understanding how students feel about blended learning can help inform future implementation of blended activities in food science and technology, tailoring educational activities to suit student preferences likely to increase student engagement. This paper describes a study conducted in an undergraduate food science and technology course to examine the students' perceptions of blended and collaborative learning.

### 1.1 Blended learning

From a pedagogical point of view, electronic education can shift the paradigm from passive, teacher-centred learning to active student-centred learning (Flores et al., 2016; Kavadella et al., 2012; Liceaga et al., 2011). This new paradigm positions students at the centre of the learning process, with models that stimulate curiosity, creativity, collaboration, and knowledge that is acquired outside the classroom. Compared to student-centred learning, the conventional didactic lecturing model with teacher-centred learning seems less interesting for students in terms of motivation and achievement (Marchalot et al., 2018). In the context of this

study, blended learning is defined as a thoughtful amalgamation of classroom face-to-face and online learning experiences with the view to enhance student learning experiences. Lecturers can combine online and face-to-face training in several ways.

Graham (2006) categorizes blends into three types: facilitating blends, which concentrate on simplicity and accessibility; enhancing blends, which supplement but do not radically alter the pedagogical style; and transforming blends, which shift the instructional delivery to an active learning model. According to Graham (2006), transforming blends enable students to actively build knowledge and participate in intellectual activity that would be difficult without technology.

One common form of blended learning that lecturers use allows students to complete activities online prior to face-to-face meetings to ensure that everyone is on the same page. The material can then be augmented and enriched with application-based and problem-solving exercises during class time. The flipped classroom is a term used to characterize this form of combination (Flores et al., 2016; Mason et al., 2013; Nouri, 2016). The face-to-face time can be used to learn the material at a deeper level and link the content to broader topics (Bates, 2015; Mason et al., 2013). Another type of blend involves teaching the course content during class time and allowing students to think critically and discuss their views about the material through online activities (Thai et al., 2017). Under the blended learning approach, students interact using different online and offline tools (Bliuc et al., 2007; Cabero et al., 2010). To support students'

needs, various forms of convergence between technology-based environments and traditional settings have been proposed, including virtual laboratories (Flint & Stewart, 2010; Hubackova & Semradova, 2016) and flipped classrooms (He et al., 2016; Marchalot et al., 2018; Nouri, 2016). With students' widespread use of laptops and the proliferation of low- and no-cost tools to facilitate online education, the supplemental blended learning model has more opportunities than ever before (Bailey & Smith, 2013). Vaughan (2010) offers some helpful hints for developing a blended learning atmosphere, including a thoughtful mix of asynchronous and synchronous dialogue, versatility and independence in online learning, and expert guidance in a purposeful face-to-face setting. Olmos et al. (2014) suggest replacing around one out of every three lessons with media-rich online experiences, while Korte et al. (2016) advocate for more student-centred learning and reshaping lecturer and student positions. Online learning involves providing students with access to learning resources, facilitating communication and collaborative working among and between students and lecturers (Smyth et al., 2012). The benefits of blended learning pedagogy include enhanced student learning outcomes, greater flexibility for students and lecturers, reduced student withdrawal rates and an ability to foster a professional learning environment, especially when a large number of students are to be taught (Güzer & Caner, 2014; López-Pérez et al., 2011). In food science and technology education, it is imperative to create instructional environments (classrooms) where students are actively involved and engaged in fostering student learning and critical thinking, conflict resolution, and collaboration skills among students to develop competencies in that regard, which will allow them to cope better in a working environment in the future (Ma et al., 2018).

## 1.2 Collaborative learning

Collaborative web-based applications have created new opportunities for students to interact with their peers, lecturers, and content. Although they are sometimes defined differently, collaborative, cooperative, and team-based

learning terms are usually considered to represent the same concept (Kirschner, 2001); in this paper, these concepts are considered comparable, and the term “collaboration” is used throughout the paper. Students engage in small-group activities to share their knowledge and expertise as part of collaborative learning. The lecturer typically works as a facilitator in these student-led events. (Kirschner, 2001; Scager et al., 2016). Frameworks identifying the basic skills for 21<sup>st</sup>-century learning emphasize the importance of collaboration for facing a constantly changing world (Ellis et al., 2016). Collaborative learning provides social skills such as oral and written communication, cultural intelligence, critical thinking, problem-solving, professionalism, and teamwork, which are essential for future professional work in the field of food science and technology (Hollis & Eren, 2016). Furthermore, collaborative learning is crucial when adapting and responding to new professional requirements of the radically changing workplace. Linton et al. (2014) found that students in group settings achieved significantly better conceptual understanding compared to students in courses with an individual setting. In a study conducted by Hassanien (2006), students perceived that group work fosters the development of a broader range of knowledge by encouraging discussion, clarifying ideas, and evaluating others' ideas. So and So and Brush (2008) and Biggs and Tang (2004) encourage interactive classrooms with learning facilitation, where students can have high-quality experiences with lecturers and receive real-time feedback. The benefits of blended learning activities for collaborative learning are captured in several studies (Ellis et al., 2016; Kirschner, 2001; Mshayisa, 2020; Osborne et al., 2018). Some studies suggest that the mere inclusion of blended learning activities will improve the engagement of students (Owston et al., 2013) and foster positive attitudes towards collaboration and satisfaction (So & Brush, 2008).

## 2 Research context

### 2.1 Theory of Change

Although blended learning has been reported in various disciplines such as nursing (Posey & Pintz, 2017), computer engineering (Yigit et al., 2014), language (Hassan Ja'ashan, 2015; Olivier, 2016), just to mention a few, no studies have been reported in food science and technology programs, especially from developing countries. Food science and technology is an interdisciplinary field in which the engineering, biological, and physical sciences are used to study the properties of foods, the principles underlying food processing, and the improvement of foods for the consuming public (Campbell-Platt, 2009). Therefore, this study aimed to investigate student perceptions of a collaborative and blended learning approach in a first-year food science and technology course at a large University of Technology (UoT). To achieve this, our primary research questions were:

1. What are student perceptions of blended learning?
2. What are student perceptions of collaborative learning?
3. What are student preferences on course format?

## 3 Learning context

The research was carried out in Cape Town, South Africa, at a larger public university of technology (UoT). A blended learning approach was introduced to first-year students (n= 133) enrolled in Food Technology 1 (FOT150S) in 2018. The 20-credit course contains both theoretical and practical elements.

There are no prerequisites for FOT150S, which is a foundation module in food science and technology curricula. In order to provide a basis for potential classes, the course takes a wide view of food production and its products. The majority of students who enrol for the course have no prior knowledge of food processing terminology. Gelatinization, viscosity, filtration, retrogradation,

aseptic processing, blanching, fermentation technology, and sensory evaluation are all terms that these students are unfamiliar with. The course content and student-centred learning practices implemented in this study are housed on the course website (MyClassroom, Blackboard Inc., Washington, D.C., USA), an institutional learning management system (LMS) that supports collaborative and blended learning. Students could interact with the lecturer and with each other over discussion forums e.g. WhatsApp group for the module. Students had the opportunity to meet each other and the lecturer during weekly contact sessions that lasted 3.75 hours. In the introductory session, students received training on using Blackboard and obtained all the information required to work online. Students were expected to log on to the course individually whenever convenient, read that week's course material, download resources, and follow instructions to complete tasks. Fig. 1 exhibits the teaching and learning activities implemented in FOT150S as part of blended learning pedagogy. A description of the course activities undertaken in this module is provided below.

### 3.1 Teaching and learning activities in an undergraduate food technology module

#### Face-to-face classes

Interaction among students is necessary for successful learning activities such as developing problem-solving skills, critical higher-order thinking, and knowledge application, which reflect the types of skills needed in practice (Bates, 2015; Rocca et al., 2014; Shu & Gu, 2018). Face-to-face lectures still provide a meaningful and effective mode of supporting student learning (Thai et al., 2017), and this conjecture was endorsed by regular lecture attendance of between 70 - 80% of students. The face-to-face activities were designed to incorporate student-centred active learning practices. For example, student groups solved crossword puzzles to improve their food science and technology vocabulary and participated in class discussions. The lectures were supported by copies of the lecture

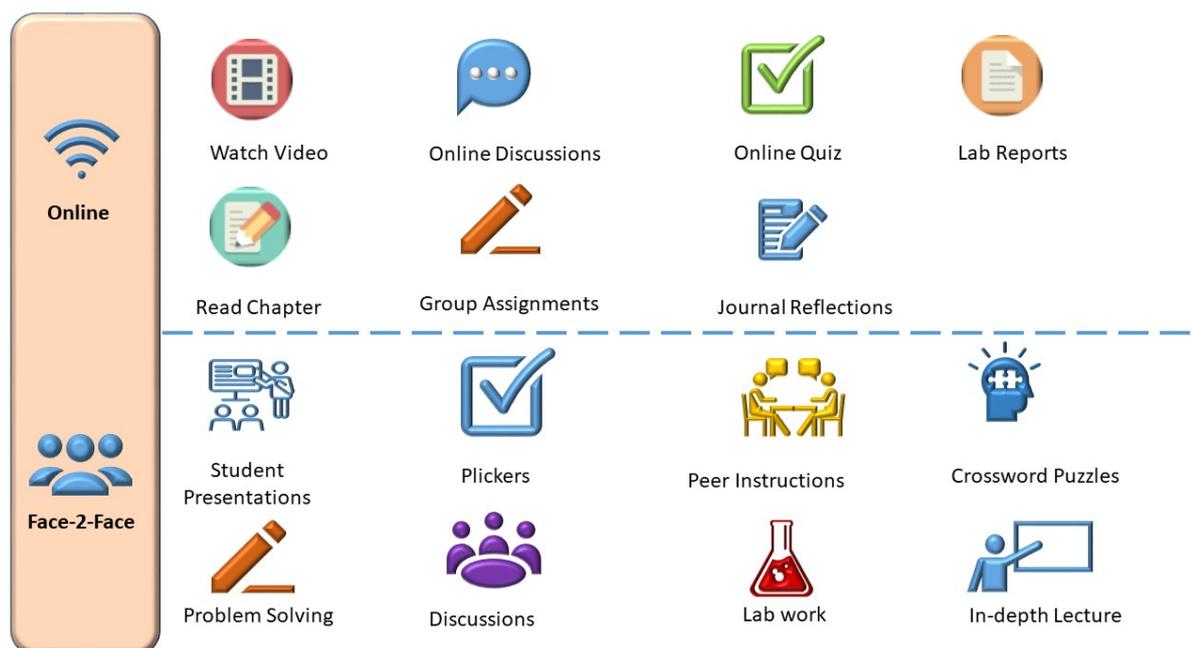


Figure 1: Online and face-to-face teaching and learning activities implemented in FOT150S

notes (as slides and handouts in PDF format) posted to the Blackboard site at least two weeks before the class time. The content was delivered using Microsoft PowerPoint presentations with infusions of multimedia and online tools such as Plickers or YouTube videos.

### Tutorial sessions

Tutorials played a major role in integrating the teaching and learning activities with the assessment activities. Tutorial sessions (1.5-hour sessions) served to reinforce the material presented in lectures, provide a forum for students to gain assistance with progressive assessment and revision for examinations, and provide formative feedback on the progress examination. Tutorials were optional and attended by 55 - 70% of enrolled students, depending on the topics covered in the tutorial session.

### Practical sessions

Food technology 1 (FOT150S) is a practical laboratory-focused discipline, and, as such, the practical component was an integral part of the module. During the first two practical sessions, the students were familiarised with all of the pilot plant equipment that they would later use during their practical sessions. Hygiene code of practice, laboratory conduct and pilot plant safety rules were also emphasised. These sessions include facilitators explaining the equipment, their functions, unit operations and use of the sensory evaluation facility. The equipment studied in detail includes those used to process bakery products, meat products, fermented foods, chocolate-based products and fruits and vegetables. During these sessions, the students were also exposed to the analytical equipment available to evaluate manufactured products. Instruments described and demonstrated include the refractometer, pH meter, colourimeter, viscometer, water activity meter and moisture analyser. Students were required to either watch a video (lecture prepared

screencast) or read a prepared note regarding the practical in addition to the practical manual. Screencasts were uploaded to YouTube as unlisted content and then shared via Blackboard by embedding the links. Students had a week before the practical session to familiarize themselves with the material in all of the materials, so the video links were triggered a week before the practical session. A pre-practical quiz on Blackboard was completed individually as a requirement for the practical to be conducted with the view to ensure adequate preparation and gauge understanding of the content. During practical sessions, students were placed in groups (6 students) to provide experience in building an effective team, sharing a workload, and dealing with team problems. The team organization facilitates overall learning, as many students learn by explaining concepts to their teammates and by having concepts explained to them by teammates. Following the Journal of Food Science manuscript submission format, a group practical report written as a scientific paper was then submitted on Blackboard, and formative feedback was provided using a rubric.

### Team project

A group (6 students) assignment was given to the students in the form of a brief to come up with a product idea. Each group gave an oral presentation using a PowerPoint during a face-to-face session about their product idea incorporating essential concepts covered in face-to-face sessions such as unit operations, packaging and product shelf life. The lecturer and peers provided constructive feedback and had the opportunity to ask questions and comment on the presentations' strengths and weaknesses.

## 4 Data collection and analysis

A survey was administered via Blackboard at the end of the second semester. Students were informed that their grade for the course would not be affected by their participation, or lack thereof, in the survey, which was completely voluntary and anonymous. The unique, 23-item mixed-method survey instruments were created to elicit

student responses. The first ten items identified the students' demographics and perceptions of the blended course. The next eight items identified the students' perception of collaborative learning, and the last two were multiple choice questions in the course format preferences. The surveys included two types of questions:

- quantitative questions including Likert scale ratings (5-point, "Strongly Agree", "Agree", "neither agree nor disagree", "Disagree", and "strongly disagree");
- respondents were provided with an opportunity to respond to open-ended questions about their study experiences, suggestions for improving learning enhancement activities, as well as their general comments related to the course. A total of 133 students were enrolled in the course, while 72.18% (n = 96) of the students completed the survey. All quantitative data were analysed using SPSS 25.0 (SPSS Inc., Chicago, Ill., U.S.A. 2017). The open-ended question responses were processed using Microsoft Excel<sup>®</sup> (2018) by selecting the frequently appearing responses and/or keywords in the responses to identify emerging themes. The internal reliability of the survey questions was measured by calculating Cronbach's Alpha ( $\alpha$ ) which was 0.84 and 0.92 for blended learning and collaborative learning questions, respectively. Since these Cronbach's alpha values suggest a high degree of internal consistency, the analysis presented in this paper can be considered accurate and relevant for obtaining student perceptions on blended and collaborative learning.

## 5 Results and discussion

### 5.1 Demographics

The online survey elicited students' responses to two demographic questions in order to provide context for the study findings. The breakdown of student profiles by demographics indicate that the participants were 24 and 74% male and female respectively (Fig. 2). The age of the respondents indicated that 34.4% were aged under

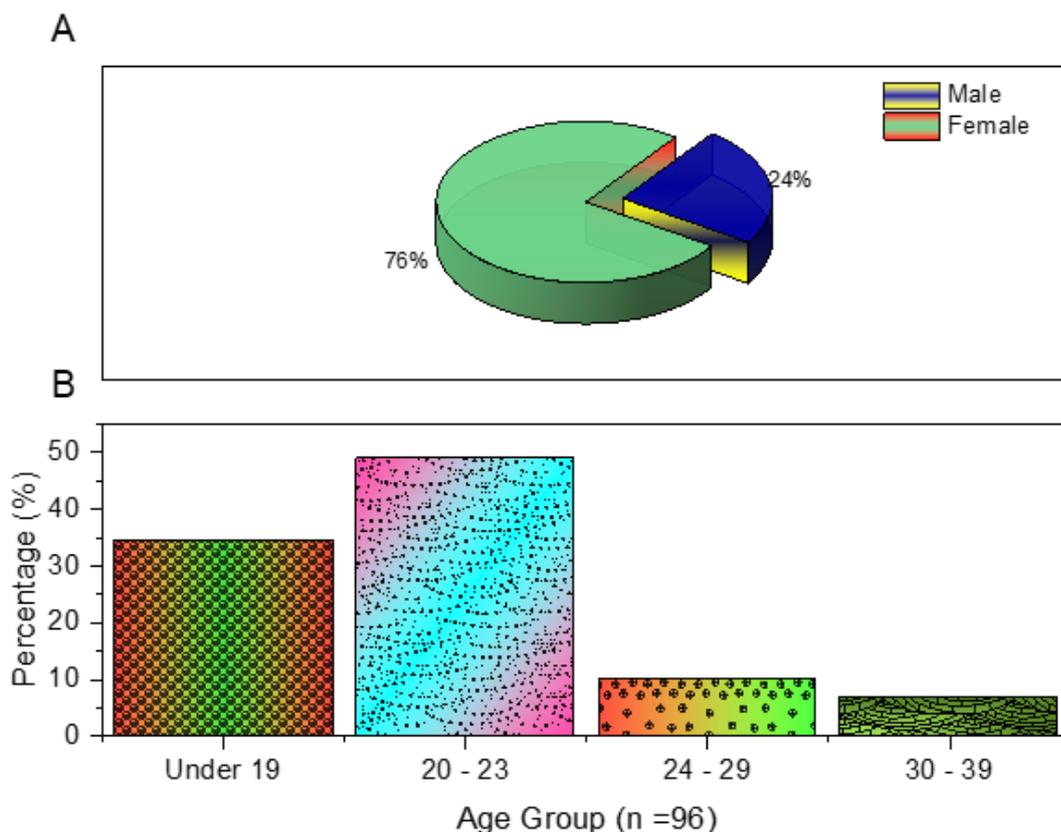


Figure 2: Student gender A) and age (B) demographics in FOT150S

19 years, 48.9% indicated 20 - 23 years, 10% indicated between 24 - 29 years while 6.7% indicated between 30 - 39 years old. Therefore, most of the respondents were aged between 20 - 23 years, exhibiting a young population regarded as more technologically savvy. All students participated in collaborative and blended learning activities implemented in FOT150S, and as a result, all of the respondents had the required experience to complete the questionnaire.

## 5.2 Students' perceptions of blended learning

The first research question aimed to elicit student responses to perceptions of the blended learn-

ing approach implemented in FOT150S. The student participants had no prior experience of blended learning at an institution of higher learning since these were primarily first-year students from high schools which are in predominantly underprivileged socio-economic environments characterized by the traditional chalk-and-talk approach. Learning is fostered under certain circumstances, including the motivational context and interaction with peers and lecturers. Evidence from the literature also suggests that it is imperative to be cognizant of the student's motivation to ensure student readiness and ability to cope with independent learning (Güzer & Caner, 2014). As shown in Fig. 3, a high percentage of the students felt that the course was inspir-

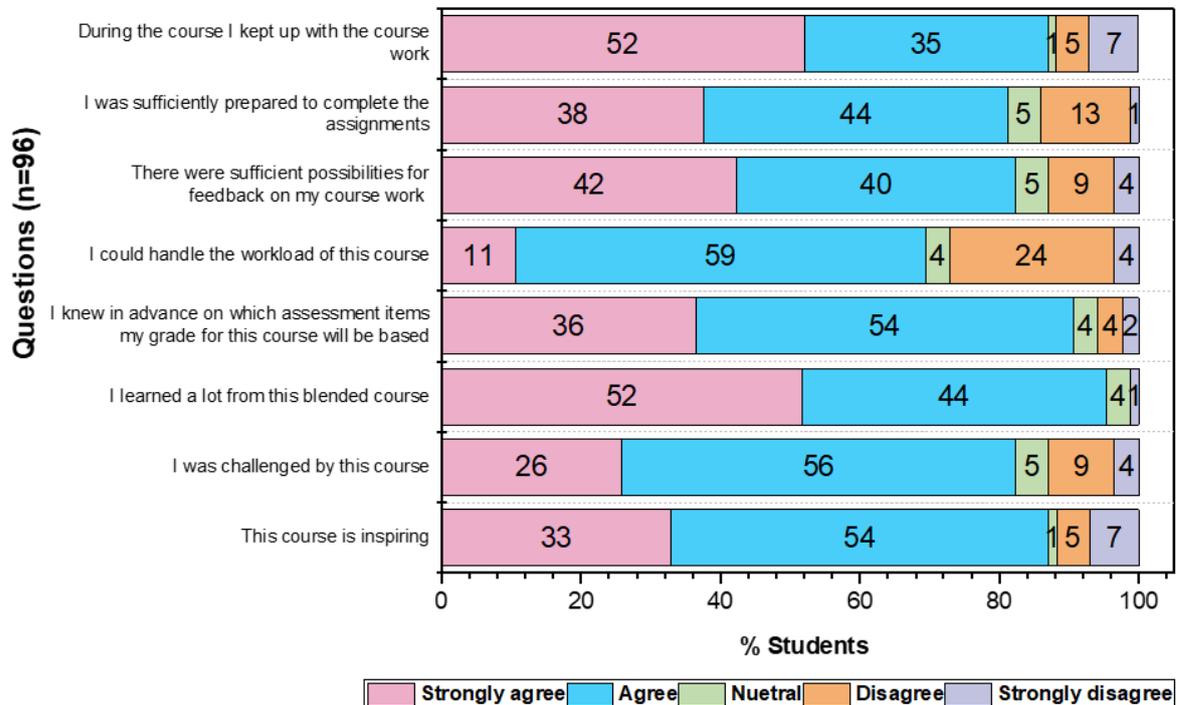


Figure 3: Students' perceptions of a blended food technology 1 (FOT150S) course

ing (Strongly Agree 38% and Agree 54%). For example, one student commented,

I liked the fact that the lecturer made the subject very interesting and inspiring, especially when reflecting on real-life problems it made me look forward to participating in class.

Moreover, 70% of the students felt that they could handle the course workload, and 87% agreed that they could keep up with the course work. One student commented that

There was a lot of work, but as time went by, I was able to manage my work through using the online course calendar and keeping up to date with required submissions.

It is essential to afford the students a reasonable workload to allow them to collaborate and independently learn outside the classroom. This

is consistent with the findings of Mason et al. (2013), who reported that a blended learning approach allowed students to cover more content and increased opportunities for active and collaborative learning without adding to the students' overall workload.

However, these students perceived the course as challenging (82%), perhaps due to the technical content and the fact that they were in their first year of study. One student commented that

FOT150S is becoming more challenging yet so educational, interactive and think on your feet to the extent that I am beginning to understand what food technology is all about.

This course required students to think critically and collaborate with others in a diverse setting to which they may not have been accustomed. Thus some may have found this learning approach challenging. Regarding preparedness to complete assignments, 82% of the students

agreed that they were prepared to complete the assignments, while 14% of the students disagreed with the same question (Fig. 3). One student noted,

What I liked most about FOT150S was that we were provided with the best resources for us to study, pre-practical materials for example videos, crossword puzzles, etc. All these resources enabled me to prepare and complete the assignments.

These findings were positive, suggesting that students could learn at their own speed using their available tools. A blended learning approach had no adverse effect on their learning. These results are consistent with research showing that blended learning offers students flexibility (Yigit et al., 2014) as they had access to the content at any time, anywhere with Internet access from university or home. The students agreed that they knew in advance which assessment items on which their grades would be based (Fig. 3). This is attributed to the use of Blackboard as an effective LMS where students have an overview of their assessment due dates and the fact that they could access their study guide, which had the assessment weights on the same LMS. Students also commented that

The way the LMS site is set out is helpful. You are given clear instructions on what is to be completed before each class and what is expected of you for practical sessions and assignments.

Perhaps the most welcome aspect of this module from the student perspective was the provision of varied, timely and relevant feedback, with frequent comments that it was the best feature of the FOT150S module. A large number (82%) of the participants agreed that they had sufficient possibilities for receiving feedback on their learning process (Fig. 3). For instance, one student discussed the benefits of receiving constant feedback from the instructor

As a first-year student, it is important for you to know how well you are doing and what areas you need to improve

on the lecturer feedback gave me confidence that I was following the correct study techniques to understand and apply the principles in food technology.

Students were given prompt feedback on their group assignments, group practical reports and individual learning journals via Blackboard. This technology-mediated approach ensured that the students always had access to the provided feedback at any time on the LMS to effect changes or improvements. According to Giacalone (2016), timely feedback can help students evaluate how they are performing. Participants commented favourably that they learned more through this blended format (26% Strongly Agree and 56% Agree).

I am obtaining more useful information through the videos, crossword puzzles, online quizzes, etc. They come in handy to my knowledge,

one student commented. These findings concur with studies that suggested that blended learning increased student knowledge, collaboration skills, performance and confidence (Gill, 2009; Kavadella et al., 2012; Kuhn et al., 2018). What makes blended learning particularly effective in food science and technology is its ability to facilitate a community of inquiry and collaboration. The student ceases to be a passive element and develops critical competencies such as selecting information, teamwork, critical thinking, and self-management of the learning process. Moreover, students must apply and use in practice what they have learned. This would support the notion of constructivism guided by Piaget and Vygotsky (Smyth et al., 2012). In constructivist learning, students build up their own body of knowledge centred on individual experiences and then apply this knowledge directly to the setting. This research provides empirical evidence that complements previous findings on blended learning in higher education (De la Flor López et al., 2016; López-Pérez et al., 2011; Trujillo Maza et al., 2016).

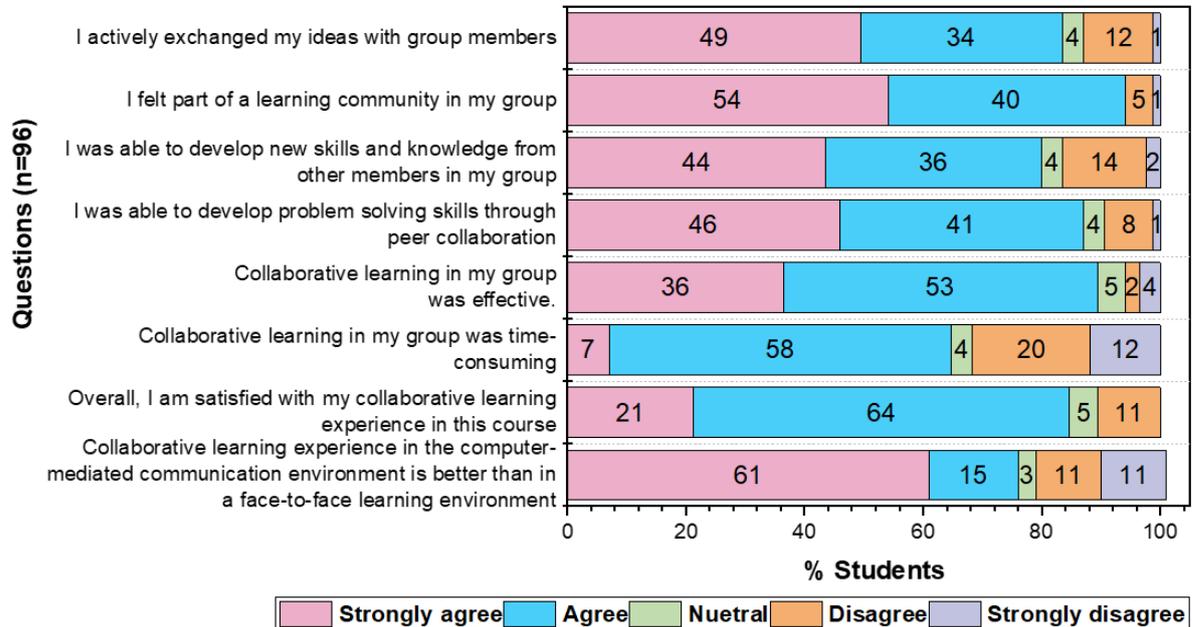


Figure 4: Students' perceptions of collaborative learning in Food Technology 1 (FOT150S) course

### 5.3 Collaborative learning

Active learning techniques support higher education goals and objectives specifically through familiarizing the students with diverse ways of knowing, fostering cross-disciplinary interactions and exposure to differing viewpoints. The large class size (> 80) provides an ideal setting to test the activities with a diverse student population, particularly given that the students in food science and technology will be required to work in diverse groups to produce a food product as part of an integrated module in the first year of study. The use of various teaching techniques in this cross-disciplinary course capitalizes on the cultural diversity of student experiences and ways of knowing. The course activities studied ranged from the common, in-class group discussions, group food production practicals and report writing, group assignments, and individual web-based reporting (quizzes), to the not-so-common in food science and technology, academic journal reflections.

Therefore, the second research question was to investigate the students' perceptions of collaborative learning in the course offered in a blended learning approach. The results obtained after administering the questionnaire were generally positive as many (84%) of the students felt that the collaborative learning experience in the blended learning environment is better than the traditional face-to-face learning environment (Fig. 4). This can be attributed to social media applications such as WhatsApp and the group tools in the Blackboard learning management system which the students utilised. In FOT150S, the students used WhatsApp extensively to facilitate communication, collaboration, scheduling meetings, and sharing and discussing food science and technology-related information. This is in line with the studies of Gachago et al. (2017) and (Owston et al., 2013) who observed that social media apps such as WhatsApp can be used to complement blended learning and facilitate student learning.

Moreover, collaborative learning activities led to

more interactions among students as more than 90% of the participants felt part of a learning community, actively exchanged ideas (83%), developed new skills (80%) and were able to develop problem-solving skills through peer collaboration (87%) (Fig. 4). One student commented,

What I enjoyed the most about this course was the group activities. It was challenging but yet fun, and yet it is meaningful to work with others. I always learn something new.

Combining online activities and learning resources with synchronous discussions may have encouraged participation and facilitated greater flexibility in learning than before, through opportunities for interaction with content and peers prior to, during, and after face-to-face classes. This approach may have been particularly valuable among students with different learning styles. In collaborative learning, the students are responsible for their own and the group's learning. The respondents were satisfied with the overall collaborative learning experience FOT150S offered using the blended learning approach. In this study, 94% of the students reported having felt as part of a learning community. One student noted,

I learned a lot from my group members, it was helpful to bounce ideas with them even if it is just to validate your ideas.

It is vital to communicate with others to build a culture of inquiry characterized by reflective written or spontaneous verbal discourse. This module also offered the students the opportunity to communicate with one another, and one student noted,

... module is the way we interacted with one another in class because of the groups that we had been allocated. The discussions we engaged in were also interesting and eye-opening, allowed us to polish our communication skills and most of all educating.

According to Garrison and Kanuka (2004), a sense of community is also necessary to sustain

the educational experience over time, so essential to moving students to higher levels of thinking. This is important as students with a stronger sense of community tend to possess greater perceived levels of cognitive learning.

The students' ability to systematically and methodically think and solve problems improved. For example, the first practical session was very chaotic because students were not prepared and had not worked in groups before. Many students had neglected to bring a copy of their practical manual, and they had trouble interacting with one another. The use of pre-practical online tests later eliminated this as students were compelled to read or watch online videos posted on Blackboard before coming to the sessions either as individuals or as groups. This study confirms the results obtained by Gregory and Di Trapani (2012). There is a lack of information about students' perceptions of blended and collaborative learning from a food science and technology point of view in the current literature. The present study addresses this gap by exploring a blended and collaborative approach to undergraduate perceptions.

#### 5.4 Student preferences on course format

To further probe student preferences on the blended learning pedagogy, two Multiple Choice Questions (MCQ) were asked pertaining to class attendance and course format, respectively. The first MCQ aimed to elicit student preferences on class attendance format (Fig. 5). In this study, 14% of the students preferred only accessing online downloadable videos of lectures, while 26% preferred attending face-to-face. Interestingly, 60% of the respondents preferred a combination of both - a blended learning approach. The blended learning approach allows students to access online components whenever and wherever they prefer while also having personal contact with peers and instructors (Poon, 2013). The second MCQ focused on the respondents preferred course format (Fig. 6). In this study, 10% of the students preferred to have the course offered entirely online, while 58% preferred the blended course format.

**If you had a choice between attending lectures face-to-face or accessing lectures online which would you choose?**

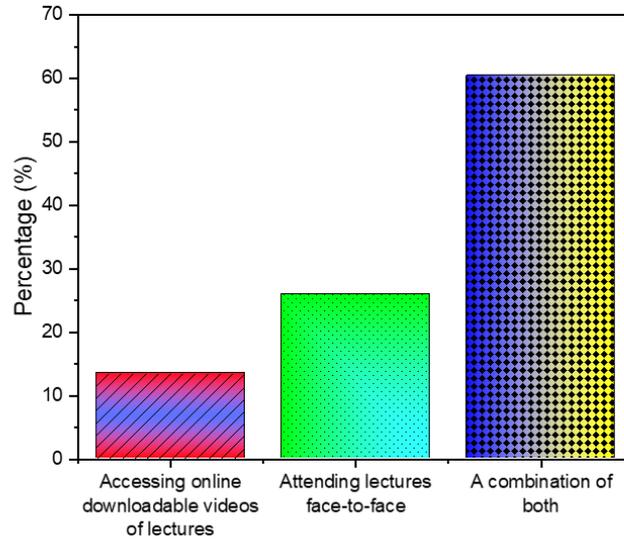


Figure 5: Student preferences on course attendance

**If the same course is being offered in different formats, which course format would you prefer?**

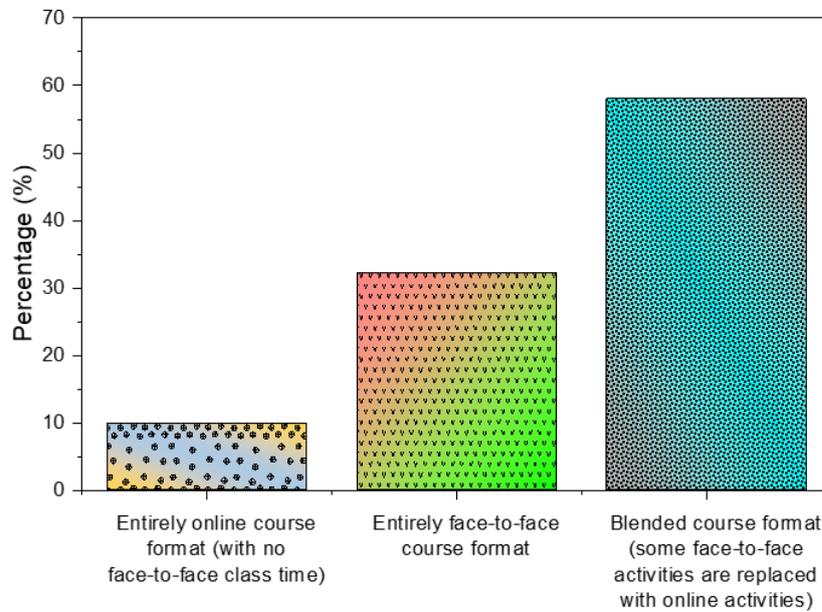


Figure 6: Student preferences on course format

Frequent meetings with peers and lecturers in class helped to build a powerful personal learning network,

one student said.

Students have expressed a preference for posing their questions directly to the instructor in class. These students found the mix of face-to-face and online mode is beneficial for their learning experiences. On the whole, students with a preference for the blended mode value the freedom of choice as regards the ways of learning. According to Waha and Davis (2014), the most common factors that cause students to prefer a blended learning mode are flexibility, convenience, interaction with peers and lecturers, interaction, independence, and balancing work commitments. The results of this study illustrate the importance of educating food science and technology students using an amalgamation of instructional modalities.

### 5.5 Themes emerging from student responses to open-ended questions

In addition to responding to Likert-scale questions, students also wrote in comments in response to the open-ended questions: ‘What did you like best or least about FOT150S?’ A representative selection of comprehensive responses showing different themes of students’ perspectives is depicted in Table 1. Peer-learning/instructions, communication, self-directed learning and self-monitoring of learning progress were the most predominant themes cited by the students in terms of the benefits of this pedagogical approach. These voluntary comments were generally very positive and showed that the students have an appetite for a blended and collaborative learning environment. The results of the student perception surveys highlighted the importance of educating food science and technology students using a combination of instructional modalities. According to the results, blended learning is appropriate and favoured by students in the field of food science and technology. Today’s students readily accept new technology and quickly learn how

to use, navigate, and handle it (Hubackova & Semradova, 2016). Eliciting student feedback or perceptions of the course is vital to improving course delivery. Student satisfaction is imperative and needs to be continuously assessed to assure students’ quality of learning experiences. Satisfied students are more motivated (Shantakumari & Sajith, 2014) and committed and hence better learners than their dissatisfied counterparts. In order to ensure that high-quality learning is achieved in a situation where their instructor and students are physically separated, research on student satisfaction with blended learning is required. As revealed in the open-ended questions, a blended approach which included: pre-practical online test, crossword puzzles and screencasts designed by the lecturer, was well received by the students.

## 6 Limitations of the study

There are a few limitations of this study to note. The absence of a comparator group is a limitation of the cross-sectional design, as there were no earlier or concurrent studies of students’ experiences or perceptions of the traditional teaching modalities of the course. It is also worth noting that all of the findings were related to better learning to improved learning, and the effectiveness of learning are based on students self-declared perceptions and not on independent measures.

## 7 Conclusion

This study illustrates students’ satisfaction with blended learning in food science and technology as it offers them more flexibility, which allows them to collaborate and independently learn outside the classroom. Moreover, this reflects the interaction between instructors, the content and peers and gives them enough time to do their tasks. So, students are encouraged to take responsibility for their own learning process and learners can decide when and how to use the resources provided. In the blended model, students’ preference for individual and independent learning was discovered to be an advantage. The findings of this study, in general, support the

Table 1: Themes that emerged from the open-ended questions on students perceptions of the blended FOT150s course

Themes	Illustrative quotes
<b>Benefits</b>	
Critical thinking	“The assessment in FOT150s challenges you to apply your knowledge through the use of cases...I liked the fact that I had to apply the concepts that I have been learning”.
Peer-learning	“Each team member brought a unique strength to the group that helped make our practical reports successful”.
Communication	“Communication through WhatsApp is very useful because if you don’t understand something, then you can ask for help, and you will be assisted very quickly.”
Self-directed learning	“I always look forward to doing the practicals in the pilot plant because of the pre-practical videos, which were interesting and made me research more about the practical”.
Self-monitoring of learning progress	“Using online learning Journals, we can easily monitor and track our learning progress. I can clearly see what I have learnt before and the way until now. I can see the progression of my learning”.
<b>Drawbacks/suggestions</b>	
Practical sessions	“The long hours of practicals and there are no breaks even in between the hours of the practical labour by the time you go home you are worn out.”
Learning styles	“I think there should be more videos because it is easier to understand something that you can see”.
Tutor	“I think it would be better for everyone if we got a new tutor or another one to assist the one that we already have, and we need more of those videos”.
Internet connectivity	“Since I don’t have the internet at home, it was very challenging to do some of the online stuff, especially if the IT centre was closed or full”.

benefits of integrating blended and collaborative learning into food science and technology curricula. Future research could use qualitative research methods, including interviews or focus groups, to better understand the complexities of students’ perspectives on blended learning. This would allow researchers to investigate factors that might improve their engagement.

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## Comparisons between Flour Qualities Produced by Three Different Mills: Buhler, Quadrumat, and Industry Mills

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### Abstract

Three types of mills (Buhler, Quadrumat, and industry mill) have been used to determine the effect of mill type on the quality of the produced flour. Quadrumat and Buhler mills are usually used to produce flour at a laboratory level. Flour quality has been determined physically, chemically, and rheologically. Results showed that the particle size of flour produced by Buhler mill (FPB) was finer (mostly less than  $132\mu\text{m}$ ) than other produced flour, while flour produced by Quadrumat mill (FPQ) had 8% particle size bigger than 50gg, which is more than Iraqi accepted limit (5%). The moisture content of FPQ exceeded the moisture content limit (14%). While, all flour produced by industry mills (FPI) was within the Iraqi standard in term of particle sizes and moisture content. Gluten content of FPB was higher than other produced flours; however, most increments were not significantly different. The results also showed that using different mills has no clear effect on the gluten index and alpha-amylase activity. Farinogram and extensogram results showed that FPQ was stronger than other produced flour followed by FPI. In conclusion, the quality of FPQ was closer to the quality of FPI, however, Quadrumat mill needs to be adjusted to produce flour with finer particle sizes and lower moisture content. The Buhler mill, on the other hand, needs to be adjusted to produce flour with bigger particle size. Both laboratory mills (Quadrumat and Buhler) need to be adjusted to produce flour that expresses FPI correctly.

**Keywords:** Flour quality; Mills; Rheological properties

## 1 Introduction

Wheat is a unique cereal, which contains gluten protein that gives viscoelastic properties to dough, which is necessary for bread production. Several instruments have been developed to study chemical and rheological properties of dough (Kaur et al., 2011). Quality and quantity of gluten proteins play a key role in determining baking quality because it is responsible for determining water absorption capacity, viscos-

ity, elasticity, and cohesively (Wieser, 2007). A Farinograph is an important device used to determine dough rheological properties such as development time, water absorption, stability, and degree of softening, which are useful parameters for the optimization of baking quality (Yazar et al., 2016). Measuring extensibility and resistance to deformation of dough can be determined with the use of an extensograph device (Di Cagno et al., 2002). Starch gelatinization, degradation of starch pastes by  $\alpha$ -amylase, of flour can be

determined by amylograph and falling number devices (Pertin, 1964).

Iraqi domestic wheat varieties were classified as weak wheats that need to be mixed with strong imported wheat to produce a suitable flour for Iraqi bread (Tanorry) (Alhendi et al., 2019). The Grain Board of Iraq (GBI) determines the percentage mix of domestic and imported wheat at laboratory level by using the Quadrumat mill. The State Company for Grain Processing (SCGP) monitors flour quality at industry level either by testing the flour produced by industry mills or by monitoring wheat mixture by using a laboratory Buhler mill. Therefore, the aim of this study is to define the flour quality produced by different mills, namely, the Quadrumat mill, Buhler mill, and several industry mills. The physical, chemical, and rheological properties of the produced flour will be determined. The aim of this study is to compare the flour quality produced by Quadrumat mill, which is used to regulate the wheat mixtures, and flour produced by Buhler mill, which is used to monitor produced flour express the flour quality produced at industry level.

## 2 Materials and Methods

Wheat samples used in this study were taken from two different silos in Baghdad, which were Khan Bani Saad silo and Altaji silo. A wheat mixture of Altaji silo (one truck) was about 60% Iraqi domestic wheat (different varieties) + 40% Australian wheat, while a wheat mixture of Khan Bani Saad silo (two trucks) was about 90% domestic wheat (different grades) + 10% Australian wheat. In Baghdad silos, all domestic wheat varieties are stored together, and all imported wheat varieties stored together depending on the origin country. Three different, 20 to 25 tonne loads, two from Khan Bani Saad silo and one from Altaji silo, were used in this study. Although similar wheat mixtures were used in the two trucks of Khan Bani Saad silo, some differences between wheat mixtures were expected because of some technical issues. From each truck, two identical samples were taken to produce flour by using the Quadrumat mill and the Buhler mill, while the whole truck was monitored

from a silo to an industry mill to produce flour (Fig. 1).

### 2.1 Produced Flour

A truck loaded with wheat was followed by the research team to an industry mill; the wheat was placed in an empty storage place (Fig. 1) and moisturized overnight ( $\approx 20$ -24h) before milling. The moisturization rate was based on an industry mill, which accounted for the long bath process, heat produced from machines, etc. to produce flour with moisture content about 14% wet basis (wb) or less. The impurities of wheat after cleaning were between 0.1 to 0.2%. The extraction rate of the produced flour was 80%, which is the percent of the produced flour in Iraq. Three different industry mills were used, which were named 1, 2, and 3 industry mills throughout the paper. The three mills were Buhler mills (Buhler mill, Buhler Group Company, Switzerland), and they were in Baghdad/ Iraq.

The first wheat samples (three samples from three different trucks), about 5 kg, were cleaned manually to ensure that all the impurities and stones were removed (approximately zero% impurities). The samples were moisturized to 16% (wb) overnight (about 20-24h) by determining wheat moisture content and calculating the required water amount to reach 16% wet weight (ww) by following The Pearson square of balancing rations. Milling was done by using the laboratory Quadrumat mill (Brabender<sup>®</sup> OHG, Brabender GmbH Co. KG, Duisburg, Germany) at Quality Control Department (QCD)/GBI. The extraction rate of the produced flour increased to 80% by addition of some of the sieved bran.

The second wheat samples (three samples from three different trucks), about 5kg, were cleaned using a mechanical sifter (Tripette & Renaud Chopin, Marcellin Berthelot, France) (approximately zero% impurities) and moisturized to 16% overnight (20-24h) (similar to the method mentioned above) before milling using laboratory Buhler mill (Buhler MCKA 202, Buhler Group Company, Uzwil, Switzerland) at QCD/SCGP. Moisturizing wheat to 16% (ww) is the percentage used to follow in both laboratories to give

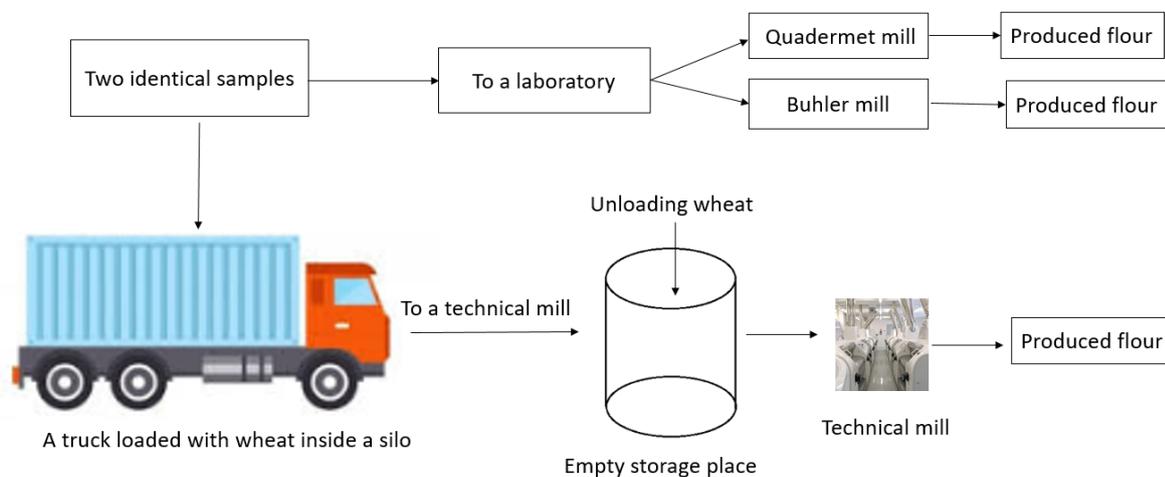


Figure 1: Diagram of wheat samples and mills used to produce flour.

flour within 14% (ww) moisture content flour. Sieves of Buhler mill consisted of three roll break (B) sieves with the following mesh sizes: B1 (710 and 150 $\mu\text{m}$ ), B2 (600 and 132 $\mu\text{m}$ ), B3 (530 and 132 $\mu\text{m}$ ) and three smooth roll reduction (C):C1 (530, 150, 150 $\mu\text{m}$ ), C2 (400, 132, and 132 $\mu\text{m}$ ), and C3 (132 and 132 $\mu\text{m}$ ). The extraction rate of the produced flour was raised to 80% through addition of some sieved bran produced by a bran finisher (Chopin BS, Tripette & Renaud Chopin, Marcellin Berthelot, France).

## 2.2 Physical, Chemical, and Rheological Analyses

Flour produced from different mills was analysed for particle size by using the Buhler Laboratory Siftermin 300 (Buhler Group Company, Uzwil, Switzerland) for five minutes. Also, they were analysed for moisture content (AACC 44-10), ash (AACC 08-01), wet gluten and gluten index (AACC 38-12), falling number AACC (56-81B), Farinograph (ICC 115/1), Amylograph (ICC 126/1), and Extensograph (AACC 54-10). All the analyses were made in the QCD/ SCGP.

## 2.3 Statistical Analysis

One-way analysis of variance (ANOVA) performed for statistical analysis of data. Least Significant Difference (LSD) of means implemented by using SAS version 9.0 (Cary, NC, USA). Significant differences considered at  $\alpha = 0.05$  level. All analysed data accomplished in duplicates.

## 3 Results and Discussion

### 3.1 Physical and Chemical Analyses

Table 1 shows some chemical and physical properties of produced flour from different mills. Moisture content values of FPQ were the highest (14.8 to 15.6%) compared to other produced flour, and this result was expected because of the way that flour was produced by Quadrumat mill, which is a one-step produced flour (one flour product and one bran product), short bath, and one sieve. In the laboratory Buhler mill, there were several flour products and several bran products, several sieves, and a relatively longer bath process; all these factors led to reduce the moisture content of the produced flour. For the industry mill, heat produced by big machines and

a long bath process were the main reasons to reduce moisture content, therefore, wheat usually moisturized to higher moisture (more than 16%) content to produce flour at 12-14% moisture. Moisture content values of FPB were between 11.6 and 12.9% and moisture content values of FPI were between 12.4 and 14.0%, which were within the Iraqi Standard  $14.0 \pm 0.1\%$  (Quality Standard of Iraq 37, 1988). The difference of moisture content of FPIs was probably due to the moisture level used in each mill. FPQ was the only one that exceeded the limit and should be moisturized to less than 16% mc to produce flour within the limits of industrial quality. Alhendi et al. (2019) mentioned that the moisture content of flour produced from four Iraqi wheat varieties was between 14.4 and 14.7% (ww), which agrees with the moisture content that was reported in this study. The two studies followed the same method for producing flour.

Ash or mineral content of the produced flour is shown in s in Table 1. The highest ash values were found in the FPI which were between 1.1 and 1.2% and the lowest was found in the FPB which were between 0.84 and 0.86%. There were significant differences between FPB and FPI for all produced flour. Although ash content of produced flour was mostly significantly different, ash content was less than the Iraqi limit (1.2% dw) (Quality Standard of Iraq 37, 1988). Kaur et al. (2011) mentioned that ash content was from 0.50% to 0.62%, which is lower than the ash content of flour in this study. Ash content of flour was higher because of the high extraction rate of the produced flour, which was 80% compared to common extraction rate, which was 71-73% (Hassan et al., 2015; Posner & Deyoe, 1986). Alhendi et al. (2019) stated that the ash content of flour produced from four Iraqi wheat varieties was between 0.81 to 0.93%, and the extraction rate of the flour was 80%, which is similar to the extraction rate of this study.

Gluten and gluten index values of the produced flour are shown in Table 1. Gluten content of FPB was higher than other produced flour; however, some increments were not significantly different. Wet gluten was significantly different between the three flours produced at the first mill, while there was no significant difference between the flour produced at the second mill.

Flour produced from the first mills had higher gluten index than others and that was due to the wheat mixture used in the first mills, which was 40% Australian wheat compared to other wheat mixtures, which were 10% Australian wheat. The quality and quantity of wheat gluten are known to be controlled by a wheat variety (Hadenadev et al., 2011).

The particle size distribution of the produced flour was presented in Table 1. The first and third industry mills produced very similar flour in terms of particle size, which was 2% larger than 50gg for both mills and 43% and 44% for particle size less than 10xx for both mills respectively. While the second industry mill produced a lower amount of 50gg (0.3%) and more amount of 10xx particle size (57%), which refers to a fine particle size compared to other FPI counterparts although the wheat mixture of second and third industry mills was same. The difference was probably due to adjustment of the industry mills. For FPB, the particle size was generally finer than other flour counterparts; the percentage of flour with particle size more than 50gg was between 0.2% and 0.7%, and the amount of flour with a particle size less than 10xx were between 86% and 94%. The particle size of FPQ was 8%g (above 50gg) and between 46% and 48% for particle size less than 10xx. All the produced flour had more than 40% (the minimum amount according to Quality Standard of Iraq 37 (1988) particle size less than 10xx. The similarity of particle size of FPQ and FPB individually was expected because of the use of the same mill and same adjustment in all mixtures. FPI and FPB were within the allowable limit of particle size bigger than 50gg, which is 5% of the produced flour (Data from SCGP), while all the FPQs were more than 5%. However, FPQ was close to FPI in the amount of particle size less than 10xx. Particle size influences the flour properties; reducing the particle size of the flour leads to increase starch damage and increase the surface area that causes more dough absorption and consequently more dough stickiness (Gaines, 1985). Further, Bressiani et al. (2017) reported that particle size affects the functionality of the gluten network and subsequently the bread volume. Therefore, FPQ and FPB should be controlled to be similar to FPI to express its quality appropriately.

Table 1: Chemical and physical properties of flour produced from three different industry mills, Buhler, and Quadrumat mill from three different wheat samples

Silo	Milling type	Moisture (%)	Ash (%) at dw*	Wet gluten% at 14%ww**	Gluten index (%)	Above 50gg sieve% (g/g)	Pass 10xx sieve% (g/g)
Altaji (truck 1)	Industry 1	12.9	1.09 ± 1.74a	29.2 ± 0.2b	66.0 ± 1.4b	2.0	43
	Buhler	12.9	0.87 ± 0.00b	30.0 ± 0.0a	72.9 ± 0.6a	0.2	94
	Quadrumat	15.6	1.03 ± 0.00ab	28.0 ± 0.0c	64.0 ± 1.4b	8.0	48
Khan Bani Saad (truck 2)	Industry 2	12.4	1.18 ± 0.00a	27.8 ± 0.7a	39.2 ± 0.9a	0.3	57
	Buhler	11.6	0.86 ± 0.00c	30.5 ± 0.7a	29.3 ± 3.8b	0.4	93
	Quadrumat	14.9	0.96 ± 0.00b	29.0 ± 0.5a	47.9 ± 0.6a	8.0	46
Khan Bani Saad (truck 3)	Industry 3	14.0	1.10 ± 0.00a	24.0 ± 1.4b	44.9 ± 0.3a	2.0	44
	Buhler	12.0	0.86 ± 0.01b	31.3 ± 0.3a	38.0 ± 0.7b	0.7	86
	Quadrumat	14.8	0.95 ± 0.04b	29.3 ± 0.4a	38.0 ± 0.7a	8.0	46

Values are expressed as a mean ± SD. Means with different letters within the same column and silo are significantly different at  $p < 0.05$ .

\*dw (Dry weight)

\*\*ww (Wet weight)

### 3.2 Farinograph Characteristics

Water absorption of FPB was higher than other produced flour, which was around 65% at 14% (ww) moisture content (Table 2). For FPI had water absorption 58.0% to 59.2%, which was the lowest compared to others, except for the first industry mill. Water absorption of FPQ had a wider value range, which was the lowest (58.1%) in the first sample and highest (63.2%) in the second sample (Table 2). Water absorption of FPI was closer to water absorption of FPQ than to FPB.

Abang Zaidel et al. (2009) mentioned that water absorption of flour between 61.4 and 65.4% is considered a strong flour, while between 57.5 and 61.5% is a weak flour. Hadnadev et al. (2011) reported that water absorption value is the greatest value of the farinograph parameters, which directly indicates the volume of the bakery products. However, Gaines (1985) stated that reducing particle sizes of flour were responsible for high water absorption due to increasing the starch damage, which is an acceptable explanation for increasing water absorption of FPB because the other farinograph parameters (Table 2) were not within the level of the strong flour. Diósi et al. (2015) reported that the grade A flour should have a minimum 60.0% water absorption and a minimum 10.0 min stability value, while

the grade B should have a minimum 55.0% water absorption and a minimum 6.0 min stability value.

The dough stability values of produced flour was presented in Table 2. The stability of FPB was the lowest values (from 3.2 to 4.0 min) compared to other produced flour, which is the opposite of the water absorption values. Dough stability of FPQ was the highest value. Depending on the above wheat flour classification (Diósi et al., 2015), all the produced flours were weak and had stability values less than quality B except for FPQ sample 3, which was 6.1 min. High dough stability refers to a high-quality dough that suitable for bread production (Wahyono et al., 2016). Dough development time of FPQ was longer than that of other produced flour except for the third sample, which was the same as the FPI. Dough-development time determines the optimum mixing time during dough formation. Some flour properties, such as gluten and protein content, gluten index, ash content, etc. influence dough development time (Abbasi et al., 2015). Wahyono et al. (2016) mentioned that increasing development time gives an indication to a high-quality dough that suitable for bread production.

The degree of softening (DOS), the lowering of the consistency line after 10 and 12 min of the development time point calculated in BU (Mohammed et al., 2012), of the produced flour was

Table 2: Farinograph characteristics of flour produced from three different industry mills, Buhler, and Quadrumat mill from three different wheat samples

Silo	Milling type	Water absorption (%)	Stability (min)	Development time (min)	DoS* (BU) 10 12min	Q number
Altaji (truck 1)	Industry 1	59.2	4.0	4.3	55, 87	63
	Buhler	64.6	3.2	4.2	93, 126	56
	Quadrumat	58.1	4.8	4.7	66, 96	65
Khan Bani Saad (truck 2)	Industry 2	59.2	4.8	4.8	47, 91	77
	Buhler	65.3	4.0	5.0	78, 117	67
	Quadrumat	63.2	5.9	6.0	36, 82	93
Khan Bani Saad (truck 3)	Industry 3	58.0	5.9	5.7	44, 94	86
	Buhler	65.3	3.5	4.4	65, 105	63
	Quadrumat	61.3	6.1	5.7	55, 91	78

presented in Table 2. The degree of softening values of FPB were the highest values compared to the other produced flours, while DOS for FPQ and FPI were closer to each other. A strong flour is characterized by a low degree of softening value ((Mohammed et al., 2012).

Farinograph quality number (FQN), is a time from the beginning of the mixing to a fall of 30 BU from the highest point of the curve (development time) (Weipert, 2006), of produced flour shown in Table 2. The lowest FQN was in FPB for all the three samples. There was no definite pattern between FPQ and FPI. High FQN indicates a strong flour (Mohammed et al., 2012), and it integrates the development time and stability, in addition to the degree of softening value (Weipert, 2006). Dencic et al. (2011) mentioned that the range of FQN of 140 cultivars was between 24.4 and 100.0 BU, and the FQN of the flour produced of this study was within the mentioned range.

### 3.3 Extensogram Characteristics

The extensogram characteristics of dough produced from different mills is shown in Table 3. The extensogram measures energy ( $\text{cm}^2$ ), extensibility (mm), resistance (BU) after 50 mm, maximum resistance, and maximum resistance to extension ratio (R/E) (BU). The energy or the dough strength of FPQ was the highest compared to other produced flour for all the tested times

(45, 90, and 135 min). However, the energy of FPQ was less than the reported energy (115, 116, and 106  $\text{cm}^2$ ) and (170, 145, and 135  $\text{cm}^2$ ) by the studies of Hassan et al. (2015) and Mohammed et al. (2012) respectively for the same resting times. Dough produced from FPB and FPI were closer to each other. The dough extensibility (mm) of FPB was higher than other produced flour for all the samples and for all the resting times (Table 3). The dough extensibility values of FPI and FPQ were close to each other (Table 3). The extensibility values of wheat flour mentioned by Mohammed et al. (2012) were 170, 145, and 135 mm for 45, 90, and 135 min respectively, which were within the range of this study. Increasing dough extensibility does not indicate a strong flour if the energy value and resistance are low. Resistance after 50 mm and maximum resistance of dough produced from FPQ were higher than other produced flour. The highest maximum resistance was in sample 2 of FPQ, which was 421, 574, and 557 BU for the three resting times, and they were less than the maximum resistance reported by Hassan et al. (2015), which were 510, 571, 538 BU. FPQ had higher maximum R/E ratio compare to other produced flour followed by FPI. From extensogram characteristics, FPQ was the strongest flour followed by FPI.

Table 3: Extensogram characteristics of flour produced from three different industry mills, Buhler, and Quadrumat mill from three different wheat samples

Silo	Milling type (min)	Energy (cm <sup>2</sup> )			Extensibility (mm)			Resistance <sub>50</sub> (BU)			Max Resistance (BU)			Ratio (max)		
		45	90	135	45	90	135	45	90	135	45	90	135	45	90	135
Altaji (truck 1)	Industry 1	64	53	62	176	154	148	212	226	255	238	239	272	1.4	1.5	1.8
	Buhler	50	54	49	195	188	166	175	175	193	195	199	212	1.0	1.1	1.3
	Quadrumat	85	70	89	165	149	151	284	313	365	358	364	422	2.2	2.4	2.8
Khan Bani Saad (truck 2)	Industry 2	35	35	27	129	116	107	197	207	165	205	227	191	1.6	2.0	1.8
	Buhler	43	31	32	180	161	162	150	128	134	167	134	142	0.9	0.8	0.9
	Quadrumat	70	73	70	120	95	93	409	573	555	421	574	557	3.5	6.1	6.0
Khan Bani Saad (truck 3)	Industry 3	40	34	26	119	97	94	251	238	190	254	258	218	2.1	2.7	2.3
	Buhler	29	24	19	180	168	162	100	92	80	108	94	80	0.6	0.6	0.5
	Quadrumat	56	60	56	131	119	109	286	358	371	307	362	371	2.3	3.1	3.4

Table 4: Amylogram curve characteristics and falling number of flours produced from three different industry mills, Buhler, and Quadrumat mill from three different wheat samples

Silo	Milling type	Amylogram characteristics			Falling number (s)
		Begin of Gelatinization (°C)	Gelatinization temperature (°C)	Gelatinization maximum (AU)	
Altaji (truck 1)	Industry 1	62.1	87.8	1402	548 ± 18a
	Buhler	60.4	85.8	1155	566 ± 16a
	Quadrumat	61.0	86.5	1270	586 ± 16a
Khan Bani Saad (truck 2)	Industry 2	62.3	88.5	1444	595 ± 28a
	Buhler	60.5	88.8	1414	606 ± 99a
	Quadrumat	61.3	89.9	1730	697 ± 44a
Khan Bani Saad (truck 3)	Industry 3	61.9	88.4	1650	510 ± 58b
	Buhler	60.5	89.6	1328	522 ± 5b
	Quadrumat	61.3	90.3	1629	693 ± 31a

### 3.4 Alpha Amylase Activity

Amylogram characteristics and falling number values are shown in Table 4. The maximum gelatinization (AU) and falling number (s) values of all the produced flours were high, which refers to low enzyme activity. This result agrees with the Alhendi et al. (2019) study, which mentioned that the falling number of four wheat varieties cultivated in Iraq were between 400 to 700s. The maximum gelatinization of flour A, B, and C were 545, 727, and 660 AU respectively (Kaur et al., 2011), which are less than all the maximum gelatinization of this study (Table 4). As well for falling number, the best falling number for bread production is between 250 and 300s (Polat & Yagdi, 2017), which is less than the values of the produced flour of this study (Table 4). In comparisons between samples, no definite pattern observed.

### 4 Conclusions

In conclusion, the quality of FPQ was the strongest of produced flour, while FPB was the weakest. Therefore adjustment of Buhler and Quadrumat mills should be considered to produce flour that its quality close to FPI. Generally, FPQ had closer properties to FPI than FPB. FPQ should be adjusted to express the wheat mixing in a suitable way or considering the differences when the wheat mixture percentage is determined.

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# Development and Sensory Evaluation of a Cookie from Composite Sorghum and Cowpea Flour

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## Abstract

There are many opportunities in the global food market for innovations, through the valorization of artisanal technologies based on the local raw material. In this context we were interested in the development of cookies based on a local variety of sorghum from northern Cameroon, the so-called S35 sorghum variety and a local cowpea from northern Cameroon the so called “sekem variety”. During the production of flours for cookies, the extraction yields were as follows: 46.67% for sorghum flour and 55.60% for cowpea flour. It was found that it was technically possible to produce these types of cookies. Several production trials were done and submitted to a panel for sensory analysis. The results showed that amongst different produced cookies, the one with 45% sorghum, 40% wheat, and 15% cowpea was the most appreciated by the members of the test panel. 70% of panelists considered them as “very good” against 30% who considered them as “good”. 60% of test panelists indicate that cookies with 50% sorghum, 40% wheat and 10% cowpea were “good”, against 40% who thought they were “not too bad”. Meanwhile 50% of the test panelists considered that the cookies made of 55% sorghum, 40% wheat and 5% cowpea were “good” against 50% who indicate this as “bad” and not too bad”. Proximate analysis of the cookies of trial 3 showed that it contained about 12.50% proteins, 84.10% carbohydrates, 27.34% lipids and 1.50% fiber.

**Keywords:** Cookies; Sorghum; Cowpea; Northern Cameroon; Sensory evaluation

## 1 Introduction

Cowpea and sorghum are among main food crops in the northern region of Cameroon (Boukar et al., 2019; Guei et al., 2011; Ingenbleek et al., 2019; Ishikawa et al., 2020). These species are the main staple food for people living in this part of the country (Silue et al., 2011; Sterns & Bernsten, 1994). The use of cowpea and sorghum for human food is very diverse in the area (Ngambeki et al., 1990). They are used as porridge, cake, bread, soft drink as well as form of alcoholic beverage (Carine et al., 2019; Singh,

2020; Visarada & Aruna, 2019). The Sudan-sahelian zone is generally known as a chronic food insecurity area. This is because of several internal factors that are linked to demography, climatic and soil conditions, farmers, agricultural inputs, post-harvest conservation technology, socio-cultural practices, famine and the invasion of local markets by imported products (Burfisher, 1984; Ngongang, 2019). Add to these natural events we have had in the past five years civil insurgents, such as the Boko haram (Abeh, 2003; Fungo et al., 2016). Increased pressures on food supply is also due to the massive move-

ment of people across the border with Nigeria and Chad (Kah, 2017). For this reason, we proposed to develop a new product based on cowpea flour and sorghum for peoples suffering from malnutrition, some immune deficiencies; in the humanitarian field, to reduce food insecurity and instability, and finally in order to promote our local products. The objective of this study is to diversify the industrial use of sorghum and cowpea in order to boost the use of these two locally produced speculations. More specifically, it will focus first in the production of sorghum and cowpea flours which will be used to make cookies; to identify problems related to the cookie processing based on sensory tests and then propose corrective actions to improve cookie manufacturing process.

## 2 Material and Methods

### 2.1 Material

The plant material used for this work consisted of cowpea grains as well as sorghum grains all supplied respectively by the cowpea section and the sorghum section of Institute of Agricultural Research for Development, Maroua research station in the far north of Cameroon. The plant material also consisted of wheat flour, ingredients such as: margarine, salt, water, baking powder, sugar, all bought in food at the central market of Maroua. The sorghum flour used for this work came from an improved variety of sorghum commonly known as S35. S35 is a sorghum variety of ivory yellow color, with a fairly short development cycle and whose characteristics in terms of health and nutrition are more important compared to local varieties (Kamuanga & Fobasso, 1994; Ndjomaha et al., 1998). The cowpea flour, was developed from a local variety, the so called “sekem variety” cowpea (Gonné et al., 2013).

### 2.2 Methods

#### Sorghum flour production

The S35 sorghum variety, was winnowed, sorted, washed and dried then crushed and sieved with a coarse mesh sieve (Fig 1). The coarse grains

were again crushed and sieved with this time a fine mesh sieve. The fine flour was kept in dry conditions while the grits was mixed again with unground grain to be crushed once more.

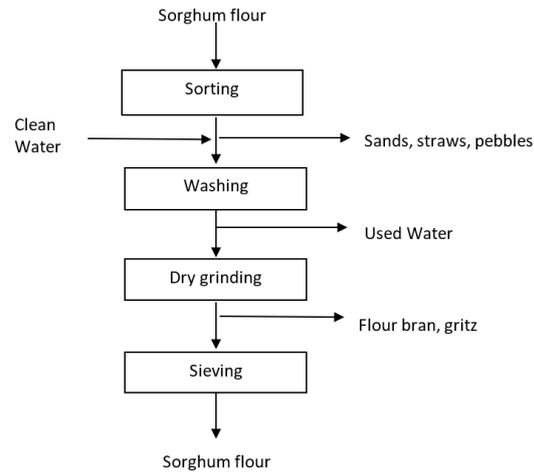


Figure 1: Sorghum flour processing

#### Cowpea flour production

Cowpea was sorted, washed, soaked, stripped of its film, and then dried. The clean dry grain was then crushed and sieved using a fine mesh sieve and the grits was again crushed to obtain as much flour as possible (Fig 2).

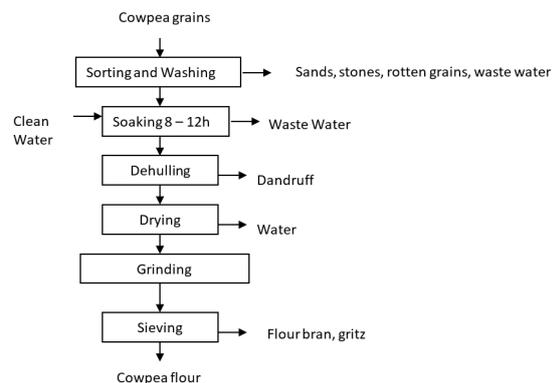


Figure 2: Process production of cowpea flour

## Processing of cookies

The classic processing method of cookies was used and consisted at weighting and mixing flour and ingredients, until a homogeneous paste was obtained and then allowed to let stand for about 15 minutes. Then the dough was cut according to the desired shape then finally baked in an oven at 150 °C for 1.5h. Three formulas of cookies with known different levels of ingredients were tested; there were not a specific experimental design for mixtures but the majors ingredients (Sorghum, wheat and cowpeas) were mixed to proportions with a step of 5 decrease or increase accordingly. Several cookies with fixed levels of ingredients were made as follows: Formula 1 was composed of 45% sorghum, 40% wheat, and 15% cowpea; formula 2 was composed of 50% sorghum, 40% wheat and 10% cowpea, the formula 3 was 55% sorghum, 40% wheat and 5% cowpea.

## Sensory evaluation

“Sorghum/Cowpea”, cookies and commercial bran cookie purchased from local market, were presented to a panel of experienced panelists who were regular cookies users and university students. The university students were regular cookies consumers but not experienced testers. The sensory analysis procedure suggested by Rivella (1987) was used. The tasting panel consists of 65 people of different genders with ages ranging from 25 to 50 with an average age of 30. They were selected based on the fact that they're generally practicing activities that require enough energy and especially physical effort, hence there's a need to consume items rich enough in energy in order to be able to compensate losses of energy. The ranking of different cookie formulas based on these flours varies according to the quality of the products obtained. Each panelist was given an evaluation form for each of the cookie samples. The form included five sensory attributes: overall appearance, texture, taste, aroma, color, and overall acceptability (harmony). Panelists were asked to assess the samples in terms of the listed attributes using a nine-point hedonic scale with 9 representing like extremely and 1 indicating dislike extremely. The tasting was carried out in a highly illumi-

nated tasting room. Panelists were provided with water to rinse their mouth after each round of tasting and were prevented from communicating with each other to avoid undue biases. Each panelist was served with 10 g of each “cookie” sample and commercial cookie in different coded form. All experiment was done in triplicate for each of the in four sessions.

## 3 Physicochemical analysis

### 3.1 Flour extraction rates

The determination of the yield of sorghum and cowpea flours was carried out by a conventional method, which is based on the measurement of the difference in mass of the samples of raw materials before and after dry milling. To do that, a small quantity of grain sorghum or cowpea were collected after they were removed from the bags. Then sorted until clean seeds are obtained. The clean sets of grains were weight ( $S_1$  for sorghum and  $C_1$  for cowpea). The samples were milled after tempering and drying in a Buhler mill to obtain bran and flour yields. The obtained grits were again weight ( $S_2$  for sorghum and  $C_2$  for cowpea). The flour yield calculation is given by the following relationship (Bhatty, 1997):

$$\text{Sorghum Yield Rate (RS)} = \frac{(S_1 - S_2)}{S_1} \times 100 \quad (1)$$

With respectively:

$S_1$  - sorghum mass before grinding;

$S_2$  - grits mass after grinding.

$$\text{Cowpea Yield Rate (RC)} = \frac{(C_1 - C_2)}{C_1} \times 100 \quad (2)$$

With respectively:

$C_1$  - Cowpea mass before grinding;

$C_2$  - Cowpea mass after grinding.

All results were the average of three determinations and are expressed as a percentage (%).

### 3.2 Determining the nutritional composition of cookies

The determination of nutritional composition of cookies was carried out as described by Bognár (2002). It is based on the addition of the

different nutritional compound content of each ingredient used to produce cookies.

**Protein** The protein content is given by the relationship:  $P = \frac{P_i}{M} \times 100$ , with P - protein content in the cookie, it expresses itself as a percentage (%);  $P_i$  - protein content of all ingredients and  $M$  = Mass of the cookies.

**Carbohydrate** Carbohydrate content is expressed by the following relationship:  $G = \frac{G_i}{M} \times 100$ , with: G - carbohydrate content in the cookie, it expresses as a percentage (%)  $G_i$  - carbohydrate content of all ingredients and  $M$  = Mass of the cookies.

**Lipids** The lipid content is given by the relationship:  $L = \frac{L_i}{M} \times 100$ , with L - lipid content in the cookie, it expresses percentage (%),  $L_i$  - fat content of different ingredients and  $M$  = Mass of the cookies.

**Fibre** The fiber content is given by the relationship:  $F = \frac{F_i}{M} \times 100$ , with: F - fibre content in the cookie, it expresses itself as a percentage (%),  $F_i$  - fibre content in each ingredient and  $M$  = Mass of the cookies.

**Energy** The energy value of the cookie is given by the following relationship:  $E = \frac{E_i}{M} \times 100$ , with E - the energy value of the cookie, it expresses itself in kilocalorie (Kcal),  $E_i$  - energy value of different ingredients and  $M$  = Mass of the cookies.

**Statistical Analysis** Data was subjected to analysis of variance and means were separated using Duncan's multiple range test at  $P < 0.05$  (Steel et al., 1980).

## 4 Results and discussion

### 4.1 Production of sorghum flour

750g of sorghum of the improved variety S35 was weighed for processing into flour as shown in Figure 1. According to this figure, sorghum was not peeled before being ground as indicated in the literature. This is in order to obtain a whole meal flour, that is to say to be quite rich in nutrients

such as fiber, carbohydrates and especially proteins, vitamins and mineral (Desikachar, 1981), the quantities of which decrease significantly during the shelling operation. In fact, slightly different milling processes were used for the various grains, but the process can generally be described as grinding, sifting, separation and re-grinding. The final nutrient content of a cereal after milling will depend on the extent to which the outer bran and aleurone layers are removed, as this is where the fiber, vitamins and minerals tend to be concentrated (McKevith, 2004). As the objective of this is the maximum possible preservation of nutrients hence the interest of eliminating the shelling operation while processing the S35 into flour.

### 4.2 Production of cowpea flour

500g cowpea grains were weighed for this flour and proceed as describe in fig 2. However, unlike the fact that it is generally say to avoid soaking during production of this flour (Coffigniez et al., 2019) we have given preference to the method of production of cowpea flour with an improvement in the soaking operation (Fig 2). Soaking time has been increased, meaning that cowpea grains were soaked from 8 to 12 hours. This was done to allow swelling of the grains, improvement of digestibility and, above all, to eliminates indigestible sugars (Ibrahim et al., 2002; Idun-Acquah et al., 2019).

### 4.3 Flour extraction yields

Analysis of sorghum flour extraction yields indicate a rate of 46.67%. The resulting yield is explained by the abrasive milling method used for flour production. The abrasive milling extraction method is said to causes more losses (Kebakile et al., 2007). Because during this transformation, the physical force exerted by the grinder is less intense compared to that of a mechanical roller grinder (Birania et al., 2020). Therefore, it is important to use a more suitable grinder for a better flour yield. The yield obtained, i.e. 46.67%, is also explained by the fact that the amount of crushed flour does not completely pass through the mesh of the sieves used as set by the CODEX-

STAN 173-1989 World Standard (1989). This is due to the coarse granularity of the flour via a not efficient enough grinding that can make the flour thinner.

#### 4.4 Analysis of cowpea flour yields

Analysis of cowpea flour extraction yields was found to be 55.60% meaning that from 500 g of cowpea grains weighed before sorting, soaking, drying and washing, we get 222 g of waste, and 278 g of cowpea fine flour. As previously notice for sorghum flour, this yield is explained by the abrasive milling method used for cowpea flour production used (Ningsanond & Oraikul, 1989). This method is said also to causes more losses. During this operation, when the cowpea grains are ground, the physical force exerted by this type of grinder is not intense. Therefore, it is important to use a more suitable grinder for a better cowpea flour yield. The yield obtained, i.e. the 55.60% cowpea flour, is also explained by the fact that the amount of crushed flour does not completely pass through the mesh of the sieves used as set by the CODEX-STAN 173-1989 World Standard (1989). This is due to the coarse granularity of the flour via a not efficient enough grinding that can make the flour thinner.

#### 4.5 Processing of sorghum and cowpea flours into cookies

The amount of mix sorghum/cowpea flour was 500 g at the laboratory level and each test was repeated three times. The percentages of each flours and other ingredients vary in order to get the right formula for making a cookie that consumers can found appreciable, whether in terms of texture, taste, smell and even color (Fig 3). No food additives or preservatives neither aromas, have been added as shown in Figure 4. This is because we wanted to produce a cookie purely based on natural products. Wheat flour was added to the preparation method of these cookies in order to make the dough bind as wheat flour contains gluten. Three formulas of cookies were used as follows:

The composite flours used for cookies varies as follow:

**Formula 1** 40% Wheat flour, 55% S35 sorghum flour, 5% cowpea flour

**Formula 2** 40% Wheat flour, 50% S35 sorghum flour, 10% cowpea flour

**Formula 3** 40% Wheat flour, 45% S35 sorghum flour and 15% cowpea flour.



Figure 3: Appearance of the cookies from the three trials (from right to left, trials 1, 2, 3)

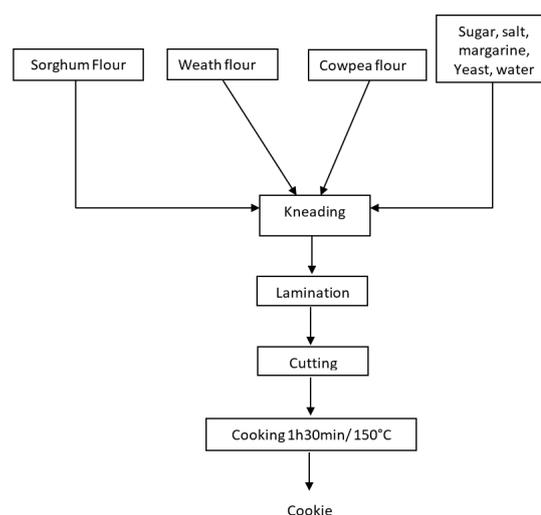


Figure 4: Process production of cookies

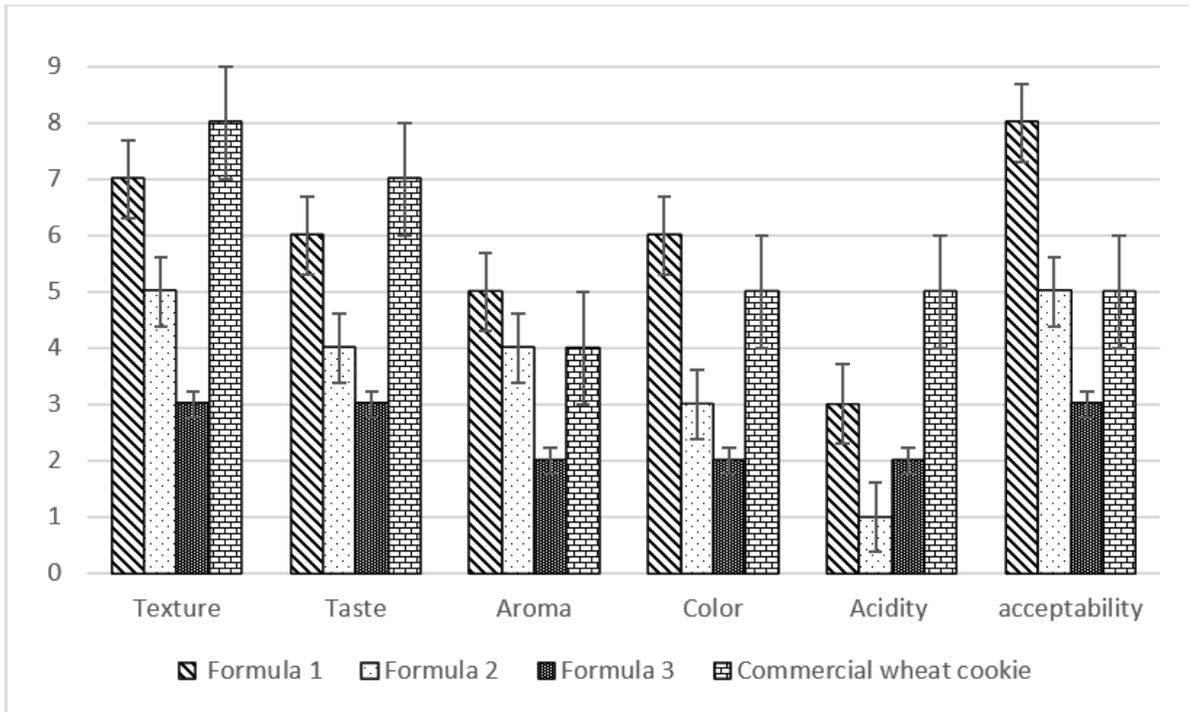


Figure 5: Sensorial attributes of different formulas of sorghum/cowpeas cookies

Table 1: Proximate composition of Sorghum/cowpeas cookies

	Formula 1	Formula 2	Formula 3
Proteins (g)	62.9 ± 21.2 <sup>a</sup>	90.75 ± 11.8 <sup>b</sup>	152 ± 31.6 <sup>c</sup>
Sugar (g)	420.7 ± 48.1 <sup>a</sup>	210.7 ± 21.0 <sup>b</sup>	130.6 ± 21.0 <sup>c</sup>
Lipids (g)	136.7 ± 16.8 <sup>a</sup>	167.55 ± 31.3 <sup>bc</sup>	190.41 ± 12.1 <sup>d</sup>
Fibers (g)	7.5 ± 2.1 <sup>a</sup>	4.1 ± 1.1 <sup>ba</sup>	2.5 ± 0.1 <sup>c</sup>
Energy (kcal)	1711.7 ± 13.8 <sup>a</sup>	1206.7 ± 10.8 <sup>bc</sup>	812.4 ± 12.8 <sup>c</sup>

In the same line, values with the same superscript letter are not significantly different (p>0.05)

#### 4.6 Sensorial attributes of the cookies

According to the panelists, sorghum and cowpea cookies are acceptable as cookies and they found that the composite flour cookies differ slightly more from cookies made from 100% wheat flour ( $P < 1$ ). The only differences were found at the levels of texture and color (fig 5). The mixed flour cookies is harder than 100% wheat cookies. This may be due to the absence of some ingredients such as milk and eggs in cookies (Gani et al., 2015) and especially by the fact that the granularity of sorghum flour and cowpea flour is denser than the one of wheat flour (Barak et al., 2014). In terms of color, cookies made from mixed flours are darker than the one done with 100% wheat flour. Cookies obtained are of acceptable quality. 70% of the panelists say mixed sorghum/cowpea flour cookies are acceptable and only 30% dislike. Formula 1 cookie was more appreciated considering color factor only, than the other formula including 100% wheat cookie. Regarding the aroma, the panelists find that the sorghum is much more striking than the other ingredients in these cookies, this may be due to the higher amount of sorghum flour during preparation. However, cookie from formula 1 was more appreciated than remaining formulas including wheat cookies.

As for texture, the cookies for formula 1 are harder than the two other mixed flours formulas samples, according to panelists. This is explained by the fact that there is enough sorghum flour whose grain size are denser than other flours which are finer (Ferreira et al., 2016).

Regarding the taste, the panelists found that the mixed flours formula tastes like rancid flour lacking in sugar. This is explained by the fact that it has not been dried in the oven sufficiently that is to say that the cooking time was insufficient. Regarding sugar, it was introduced during production in very small quantities. For Harmony, the formula 1 score was better than the other. The harmony is in relation with all other criteria, in particular the texture odor and taste that is to say that, according to the panelists, cookies from mixed flour have a better characteristics compared to other formulas.

#### 4.7 Nutritional attributes of cookies

From table 1, it can be notice that sorghum /cowpea cookies have a high energy value as well as protein. As amount of cowpea increase in the formula as the amount au protein increase in the final cookies. The protein content of formula 3 cookies seems better and can be explained by the fact that several ingredients with high nutrient content were added, in particular cowpea which is a good source of protein and energy (Giami, 1993; Vasconcelos et al., 2010). This can be interesting for infants as well as individuals who suffer malnutrition (Iqbal et al., 2006).

Concerning the fiber content of cookies, we can notice that it decreases or increases according to the amount of sorghum added or reduce (Table 1). The interesting amount of fiber can thus be link to sorghum flour, which during its processing into flour has not undergone the de-hulling stage in order to reduce as much as possible these fibers.

### 5 Conclusion

This work aims at valorizing local innovations, through the characterization of artisanal technologies based on the local raw material. In this context, we developed cookies based on a local variety of sorghum and a local cowpea variety. The process production was designed, and three formulas were tested. It was found that it was technically possible to produce these types of cookies and several productions trials were done and submitted to a panel for sensory analysis. The extraction yields of sorghum flour and cowpea flour were determined. Results showed that amongst the different cookies produced, the one composed of 45% sorghum, 40% wheat, and 15% cowpea was the most appreciated by the members of the test panel. Proximate analysis of the cookies of trial 3 showed that it contained about 12.5% proteins, 84.10% carbohydrates, 27.34% lipids and 1.50% fibers.

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# Influence of Extraction Methods on Phenolic Compounds from Pulp and Peel of Genipap (*Genipa americana* L.) Fruit

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## Abstract

Brazil has a great variety of fruits which are rich in bioactive compounds, such as the genipap fruit. Both the peel and the pulp of genipap have beneficial components for health, making the study of this fruit important for the proper use of its functionalities. The objective of this work was the extraction of bioactive compounds from the peel and pulp of genipap by different techniques. Extraction processes were carried out using different devices (orbital incubator shaker, ultrasonic bath, and ultrasonic probe) and at different temperatures (40, 60, 70, 80 and 90 °C). The best process for extracting phenolic compounds from the pulp of genipap fruit was with the ultrasonic probe at 40 °C, which indicated the efficiency of applying the sound waves directly to the sample. Regarding the peel, the best method for extracting phenolic compounds was using the orbital incubator shaker at 80 °C.

**Keywords:** Extraction methods; Genipap fruit; Phenolic compounds; Bioactive compounds; Antioxidant

## 1 Introduction

The Cerrado of Brazil holds a great biodiversity with diverse species of fruits, such as the genipap, which are little known or studied. In this biome, native and exotic fruits are found, which have great economic, nutritional and functional potential. Thus, the processing of non-conventional tropical fruits contributes to the local economy, as well as to the dissemination of fruits from the Brazilian biome (Luiz Cardoso Bailao et al., 2015).

Fruit processing produces residues with different characteristics that are commonly used for composting or production of animal feed. The po-

tential of these residues, which have high antioxidant activity, carbohydrate and fibre contents, biosorption properties, amongst others, are not being exploited (Evangelista Vasconcelos Schiassi et al., 2018; Gupta & Verma, 2015). Thus, it is necessary to make a better use of the residues from fruits, such as genipap. The genipap peel represents about 12.5% of the total fruit mass but there are few studies in relation to its use (Chagas Barros et al., 2017). The genipap peel has fibre, minerals, carbohydrates and bioactive compounds, such as iridoids (cyclopentane-[C]-pyran skeleton and carbocyclic iridoids) (Nathia-Neves et al., 2018). Some studies have reported the use

of unripe genipap fruit as source of iridoids and natural antioxidants. Genipin, genipinic acid, geniposide, sigmasterol,  $\beta$ -sitosterol, among others, were identified in genipap. Genipin has anti-inflammatory and anticarcinogenic actions, also acting in the control of cholesterol, while geniposide and geniposidic acid have a purgative activity (Belle et al., 2018; Nathia-Neves et al., 2017; Shanmugam et al., 2018). The literature shows that genipin and geniposide are in higher concentration in unripe genipap fruits (Belle et al., 2018). The degree of maturation of genipap influences the content of these bioactive compounds i.e., decreasing by about 90% with maturation (Belle et al., 2018). Bentes and Mercadante (2014) reported that the seeds presents greater concentration of genipin than the pulp of unripe genipap.

Several methods can be used to extract genipin and other bioactive compounds from genipap fruit. The selection of proper technologies depends on the compound of interest, the cost and the scale of production (Nathia-Neves et al., 2019). Some nonconventional extraction methods are reported in the literature that increase the extraction efficiency (Barba et al., 2016; Sagar et al., 2018). Nathia-Neves et al. (2019) carried out the extraction of genipin from pulp and peel of ripe genipap fruit by using pressurized liquid extraction at 50 °C, and reported the pulp and peel extracts contain genipin concentrations of  $20.7 \pm 0.9$  mg/g and  $6.18 \pm 0.07$  mg/g, respectively. Bentes and Mercadante (2014) reported a geniposidic acid concentration of  $2.54 \pm 0.05$  mg/g in the methanolic extract of unripe genipap pulp after a stirring extraction at 22 °C. Mayela Ramos-de-la-Pena et al. (2014) reported a genipin concentration of  $7.85 \pm 0.33$  mg/g after an ultrasound assisted extraction at 285 W and 24 kHz. Nathia-Neves et al. (2017) applied a pressurized extraction process with ethanol and reported that the endocarp gives the highest recovery of genipin ( $48.6 \pm 0.6$  mg/g raw material) and the extraction from the mesocarp allowed the greatest recovery of geniposide ( $59 \pm 1$  mg/g raw material). Enzyme-assisted extraction in liquid-liquid two-phase aqueous system was applied by Belle et al. (2018). Belle et al. (2018) tested different commercial enzymes for extract, and found that cellucast enzyme (10 %,

36 °C and pH 3.7) resulted in an extract with 196 mg/g of genipin. Madrona et al. (2019) reported that the optimal conditions for aqueous extraction of polyphenols from genipap fruit pulp were at 71 °C for 49 min by using a magnetic stirrer (400 rpm), fruit pulp:water ratio of 1:3 (w/w), which resulted in an extract containing 3.18 mg GAE/g.

The ultrasonic assisted extraction process has been investigated mainly to extract compounds of interest from a solid matrix, since the bubbles which are created by sound waves promote the breaking of the cell walls, resulting in better extraction (Gonzalez-Centeno et al., 2015). Basically, there are two types of ultrasound equipment, the ultrasonic bath and the ultrasonic probe. In the ultrasonic bath, the waves are emitted at the bottom of the tank reaching the raw material placed inside the equipment, whilst the ultrasonic probe is used directly in the sample and, thus, gives higher efficiency than the ultrasound bath (Dolatowski & Stasiak, 2012).

Therefore, the objective of this work was to evaluate the effects of different extraction methods, as well as the influence of temperature on the extraction of bioactive compounds from the pulp and peel of genipap fruit, by using incubator shaker, ultrasonic bath, and ultrasonic probe.

## 2 Materials and Methods

The unripe genipap fruits were purchased in a local market of Uberlândia city (Minas Gerais state, Brazil), peeled, cut in half, vacuum-packed (peels and pulps were individually packed) and frozen in the Food Engineering Laboratory of the Federal Universidad of Uberlândia, campus Patos de Minas (Minas Gerais state, Brazil), as shown in Figure 1.

Extractions of the bioactive compounds were performed for the pulp and peel of genipap by using different equipment: orbital incubator shaker at 120 rpm (SolabSL-223, Piracicaba, Brazil), ultrasonic bath at 40 kHz (Unique, USC-1400, Indaiatuba, Brazil) and ultrasonic probe at amplitude 70 %, 20 kHz, 500 W (COLE-PARMER, EW-04711-30 Ultrasonic homogeniser, Vernon Hills, USA), as well as at different temperatures (40, 60, 70, 80 and 90 °C). This temper-

ature range was chosen based on the values suggested in the literature for extraction of bioactive compounds from natural sources (Bindes et al., 2019). Water was used for the extraction at a ratio of 5:1 by mass of raw material, and for an extraction time of 45 min, as suggested by Madrona et al. (2019). Water was chosen in order to favour the extraction of phenolic compounds and in order to use an environmentally and health-friendly solvent.

A kinetic study was carried out to evaluate the concentration of phenolic compounds in relation to the extraction time. Extractions of genipap pulp were made using the incubator shaker at 60 °C for a total time of 180 min.

The moisture, ash, protein, lipid and dietary fibre contents were determined according to AOAC International (2010). The carbohydrate levels were calculated using the formula: 100 - (% ash + % lipids + % protein + % total dietary fibre).

Total phenolic analyses were performed using the Folin-Ciocalteu method proposed by Singleton and Rossi (1965). Hence, 0.125 mL of extract, 0.125 mL of Folin-Ciocalteu reagent (Sigma-Aldrich) and 2.25 mL of 2.8% sodium carbonate (Dinâmica, PA) solution were added into a test tube. The contents were mixed and held for 30 min at room temperature (25 °C), protected from light. Then, the total phenolic concentration was determined with a spectrophotometer (Ionlab, IL-226, Araucária, Brazil) at wavelength of 725 nm. Gallic acid (Sigma-Aldrich, 98.5%) was used as the standard acid, and the concentration was expressed in milligrams of gallic acid equivalents per gram of fresh genipap pulp (mg GAE/g).

For the analysis of the antioxidant activity of the peel and pulp extracts of genipap *in natura*, the procedure was performed according to the Avila et al. (2018) adapted method. Thus, 0.1 mL of sample and 2.46 mL of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich) at a concentration of 2.4 mg/100 mL were added to a test tube of 50% ethanol (Dinâmica, 99.5%). The tubes were shaken and stored in the dark at room temperature for 50 min. The absorbance was then read in a spectrophotometer Ionlab IL-226 at wavelength of 515 nm. The analysis was performed in triplicate, with the ability to se-

quester the radical expressed as the percentage of decrease of the absorbance in relation to the control (0.1 mL of water with 2.46 mL of DPPH). The percentage of reduced DPPH (% DPPH) was calculated using Equation 1.

$$\%DPPH = \left( \frac{ABS_C - ABS_A}{ABS_C} \right) \times 100 \quad (1)$$

where % DPPH is the percentage of reduced DPPH, ABSC is the absorbance of the control and ABSA is the absorbance of the sample.

Then a linear curve was drawn of the antioxidant capacity of the extract against its concentration. Linear regression of the data gave the regression equation that was used to calculate the EC<sub>50</sub> (Brand-williams et al., 1995). Analyses were performed in triplicate. All the analyses were compared with the control extract, which was obtained from the peel and pulp of the genipap without the use of heat, that is the extraction process was done at room temperature (25 °C). The free radical scavenging activity was expressed as the concentration required to inhibit 50% of free radicals (EC<sub>50</sub>). To obtain the EC<sub>50</sub> values (concentration of the extract necessary to reduce 50% of the DPPH radical) of the extracts, the antioxidant activity in different concentrations was calculated using Equation 2.

$$EC_{50} = \frac{\text{concentration of sample (mg/mL)} \times 50\%}{\% \text{ reduce of DPPH of the sample}} \quad (2)$$

For chromatographic analysis of some samples, a Shimadzu HPLC chromatograph (LC-20A Prominence, Barueri, Brazil) equipped with a Discovery HS C18 column at 280 and 320 nm at a temperature of 40 °C was used. Following the methodology of Ribeiro et al. (2015), the mobile phase was 2 % (v/v) acetic acid in water (eluent A) and 0.5 % acetic acid and water in acetonitrile (50:50 v/v, eluent B): gradient from 10 to 24 % B (20 min), from 24 to 30 % B (20 min), from 30 to 55 % B (20 min), from 55 to 100 % B (15 min), 100 % B isocratic (8 min), from 100 to 10 % B (2 min). Total run time was 90 min. The injection volume for all samples was 10 µL at flow rate of 0.7 mL/min. Genipin (Sigma-Aldrich), geniposidic acid (Sigma-Aldrich) and gallic acid (Sigma-Aldrich) were used as standards.

Extractions and analyses were carried out in triplicate. Statistical analyses were performed using

the Statcamp software, version 3.5.152.34 build 4 (Statcamp, Campinas, São Paulo, Brazil) at a 5% level of significance. The effects of the main factor on the content of phenolic compounds and antioxidant activity were determined by analysis of variance (ANOVA) and, if necessary, by Tukey's test, according to the following model (Equation 3):

$$Y_i = \beta_0 + \beta_1 x_i + \varepsilon_i \quad (3)$$

where,  $Y_i$  is the response variable in the  $i$ -th observation;  $x_i$  represents the value of the explanatory variable temperature;  $\varepsilon_i$  is a random variable that represents the experimental error;  $\beta_0$  and  $\beta_1$  are the parameters of the model, which were estimated, and which defined the regression line.

### 3 Results and Discussion

Table 1 presents the composition of peel and pulp of genipap fruit. These results were in agreement with that found in the literature (Bentes & Mercadante, 2014; Nathia-Neves et al., 2017; Nathia-Neves et al., 2020). The genipap is a juicy fruit, with a relatively high moisture percentage. Genipap fruits can be considered as a source of healthful carbohydrates. The genipap peel presented higher content of fibre and lipids than the genipap pulp, which could be used as a source of bioactive compounds instead of being discharged. Concentrations of total phenolic compounds in the extracts of genipap pulp and peel with all the proposed extraction methods and at temperatures varying from 40 to 90 °C are presented in Tables 2 and 3.

The increase in temperature had a negative influence on the content of phenolic compounds from genipap pulp using the shaker and ultrasonic probe (Table 2). These results may have been due to the agitation provided by the shaker, which in excess can promote oxidation due to the contact of the compounds with oxygen. In addition to the agitation, excessive temperatures can degrade sensitive compounds and the ultrasonic probe has a heater at its end which together with the temperature of the system may have negatively influenced the concentration of phenolic compounds (Das & Eun, 2018; Vinatoru et al.,

2017). At 40 °C, the extracts obtained with ultrasonic probe and shaker presented higher contents of phenolic compounds than the extract obtained without mixing at room temperature (control). This behaviour was likely due to the effect of mixing and heating extraction technique. Due to the cavitation effect provided by ultrasonic waves, there was a greater heat and mass transfer through disruption of plant cell walls. Thus, ultrasound is commonly used in the extraction of phenolic compounds, such as those present in genipap (Barba et al., 2016). The mechanical agitation provided greater contact of the solvent with the solid phase, contributing efficiency of the extraction (Bergman et al., 2017). This could explain the higher value obtained when using the incubator shaker at low temperature (40 °C) for the genipap peel extraction when compared to the control. In addition, the incubator shaker provided uniformly distributed heating due to its temperature-controlled chamber with fully agitated flask, in which diffusion of the solvent into the sample could be increased and thereby improved mass transfer in the extraction system, and consequently contributed to the outcome. At almost all temperatures, the use of the ultrasonic bath gave lower extraction of compounds from the pulp than the other equipment. This could be because of the relatively high frequency of the ultrasonic bath (40kHz) or due to loss in the energy distribution in the bath, since the frequency generator is mounted in the bottom of the tank, thus the size of the equipment and the position of the sample inside the bath could influence or diminish the extraction efficiency. On the other hand, the ultrasonic probe has the advantage of transmitting the energy directly to the sample which contributes to better extraction (Luque-Garcia & de Castro, 2003). Therefore, the best extraction of phenolic compound gave  $11.05 \pm 0.08$  mg GAE/g of genipap pulp when using the ultrasonic probe at 40 °C. This technique promoted an efficient extraction even at low temperatures due to the cavitation phenomenon, as reported by Barba et al. (2016). Genipap has about 12% of peel, which is often discarded. However, this residue contains components of technological and biological interest and nutritional and economic benefits (Singh et

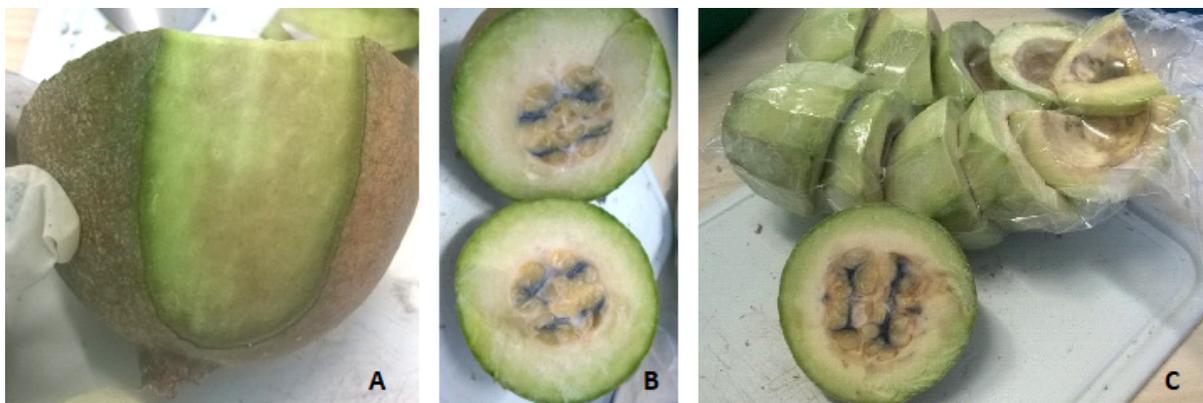


Figure 1: A) genipap fruit peel removed; B) fruit cut in half and C) genipap pulp vacuum packed and frozen

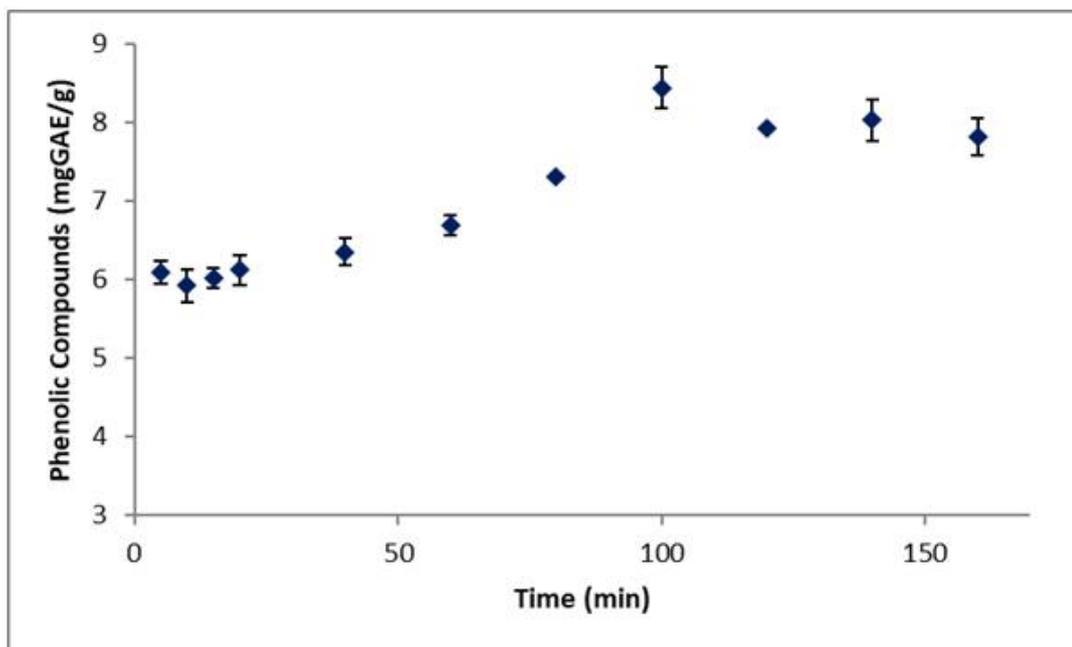


Figure 2: Kinetic extraction of the phenolic compounds from genipap pulp at 60 °C using the incubator shaker equipment

Table 1: Composition of the peel and pulp of genipap fruit

Physical and chemical characteristics	Peel	Pulp
Moisture (%)	69.60 ± 1.34 <sup>Y</sup>	76.60 ± 1.86 <sup>X</sup>
Ash (%) *	3.65 ± 0.02 <sup>Y</sup>	4.50 ± 0.21 <sup>X</sup>
Proteins (%) *	4.06 ± 0.09 <sup>X</sup>	4.05 ± 0.01 <sup>X</sup>
Carbohydrates (%) *	65.00 ± 1.20 <sup>Y</sup>	76.40 ± 0.62 <sup>X</sup>
Lipids (%) *	1.94 ± 0.04 <sup>X</sup>	1.50 ± 0.01 <sup>Y</sup>
Total fibre (%) *	25.35 ± 1.05 <sup>X</sup>	13.55 ± 0.39 <sup>Y</sup>

\* dry basis.

Treatment means followed by different superscript letters (X – Y) in the rows differ from each other at 5% level of significance, according to the Tukey test.

Table 2: Content of total phenolic compounds (mgGAE/g) in the extract of genipap pulp after extractions by orbital incubator shaker, ultrasound bath and ultrasound probe at temperatures ranging from 40 to 90°C

Extraction temperature (°C)	Extraction equipment		
	Orbital Incubator Shaker	Ultrasound Bath	Ultrasound Probe
40	9.37±0.08 <sup>a,B</sup>	4.34±0.23 <sup>b,C</sup>	11.05±0.08 <sup>a,A</sup>
60	7.90±0.20 <sup>b,B</sup>	4.34±0.58 <sup>b,C</sup>	8.31±0.08 <sup>c,A</sup>
70	7.50±0.02 <sup>c,A</sup>	5.12±0.76 <sup>b,B</sup>	9.31±0.02 <sup>b,A</sup>
80	6.31±0.03 <sup>d,C</sup>	8.73±0.05 <sup>a,B</sup>	9.51±0.01 <sup>b,A</sup>
90	6.05±0.07 <sup>d,C</sup>	7.50±0.16 <sup>a,B</sup>	8.81±0.06 <sup>c,A</sup>
Control <sup>1</sup>	8.08±0.07 <sup>b,A</sup>	8.08±0.07 <sup>a,A</sup>	8.08±0.07 <sup>c,A</sup>

\* Treatment means followed by different superscript uppercase letters (A – C) in the rows, and different superscript lowercase letters (a - d) in the columns differ from each other at 5% level of significance according to the Tukey test.

<sup>1</sup>Extract obtained from the sample without mixing and at room temperature (25 °C).

Table 3: Content of total phenolic compounds (mgGAE/g) in the extract of genipap peel after extractions by orbital incubator shaker, ultrasound bath and ultrasound probe at temperatures ranging from 40 to 90 °C

Extraction temperature (°C)	Orbital Incubator Shaker	Ultrasound Bath	Ultrasound Probe
40	4.87±0.21 <sup>e,C</sup>	6.99±0.04 <sup>a,B</sup>	8.03±0.04 <sup>a,A</sup>
60	7.27±0.26 <sup>c,A</sup>	6.28±0.27 <sup>b,A</sup>	6.60±0.27 <sup>c,A</sup>
70	7.44±0.04 <sup>c,A</sup>	5.63±0.17 <sup>cd,C</sup>	6.50±0.17 <sup>c,B</sup>
80	9.71±0.03 <sup>a,A</sup>	5.31±0.03 <sup>d,C</sup>	7.34±0.03 <sup>b,B</sup>
90	8.95±0.03 <sup>b,A</sup>	4.27±0.02 <sup>e,C</sup>	6.45±0.02 <sup>c,B</sup>
Control	5.90±0.07 <sup>d,A</sup>	5.90±0.07 <sup>bc,A</sup>	5.90±0.07 <sup>d,A</sup>

\* Treatment means followed by different superscript uppercase letters (A – C) in the rows, and different superscript lowercase letters (a – e) in the columns differ from each other at 5% level of significance according to the Tukey test.

al., 2018). Table 3 presents the phenolic compound contents extracted from the genipap peel. The increase in the temperature gave higher extraction of the compounds when using the incubator shaker. Since higher temperatures could decrease the viscosity of the solvent extract, thus it contributed to the transfer of mass and consequently the improvement of the extraction (Magalhaes et al., 2018). However, high temperatures can degrade the compounds in the plant matrix, justifying the decrease in the phenolic content at 90 °C for all three extraction methods. Therefore, the best extraction of phenolic compounds was  $9.71 \pm 0.03$  mg GAE/g of genipap peel when using the incubator shaker at 80 °C.

The values found in this study were similar to those found by Porto and Cardoso (2014) in which were  $8.57 \pm 0.05$  mg GAE/g using the dry whole genipap. Nathia-Neves et al. (2017) obtained  $7.4 \pm 0.2$  mg GAE/g using pressurized liquid extraction at 80 °C with ethanol for the genipap pulp *in natura* and obtained  $2.38 \pm 0.02$  mg GAE/g using pressurized liquid extraction at 80 °C for the genipap peel *in natura*. Madrona et al. (2019) reported  $3.18 \pm 0.12$  mg GAE/g from the aqueous extract of genipap fruit obtained by ultrasound assisted at 71 °C. Terra et al. (2019) reported 1.50 mg GAE/g by performing an aqueous extraction at 60 °C of genipap fruit.

Comparing the results using the ultrasonic bath, as the temperature increased the content of phe-

nolic compounds decreased in the extract of the peel (Table 3) of genipap, whereas the reverse occurred with the pulp (Table 2). The differences between the results for pulp and peel could be explained by differences in the structure of the matrix, since in the peel there is twice as much fibre,  $25.35 \pm 1.05$  % (Table 1), when compared to the pulp. In this way, the effect of agitation and/or temperature can be altered by the different structures in the peel and the pulp matrix, and therefore influencing the extraction efficiency of the process (Mayela Ramos-de-la-Pena et al., 2014).

In relation to the ultrasonic probe, the extracts obtained from the pulp presented a phenolic profile similar to the peel. Thus, as the temperature increased the content of the phenolic compounds decreased. This may have been due to the instability of the compounds at high temperatures, since the probe causes greater heating at its extremity, and so there may have been greater degradation of the phenolics (Arruda et al., 2017; Nathia-Neves et al., 2017).

Some compounds present in fruits have antioxidant properties that inhibit reactions that promote the oxidation of molecules or cellular structures. The main compounds that have this potential are vitamins C and E, carotenoids, minerals and phenolic compounds and their derivatives (Shahidi & Ambigaipalan, 2015). When fruits containing these components are consumed

they contribute beneficially to health, helping to prevent cardiovascular and degenerative diseases. Thus, it is important to study these components and verifying the best way to preserve their bioactive activity (Singh et al., 2018).

Among the evaluation methods of antioxidant activity, DPPH stands out. This technique has as principle the reaction of the antioxidant compounds with 2,2-diphenyl-1-picrylhydrazila (DPPH) by converting it to diphenylpicrylhydrazine and changing the colour of the solution, indicating the degree of activity (Aruda et al., 2017). The percentages of antioxidant activity (%DPPH) of each condition are found in Table 4 for the pulp and Table 5 for the peel.

For the shaker and the ultrasonic bath, the increase in temperature positively influenced the antioxidant activity. These results were observed in both pulp and peel. In general, the results were satisfactory, since almost all the conditions resulted in higher antioxidant percentages than the control extract, indicating the efficiency of the extraction process of each method. In addition, all values were above 50%, showing the potential of genipap in relation to beneficial health properties.

The variations of the values of antioxidant activity in relation to the content of phenolic compounds can be related to the fact that there are several compounds present in the matrix can exhibit antioxidant ability. The efficacy of iridoid-rich extracts from genipap fruits, as peroxy radical scavengers points to their potential to prevent and/or treat oxidative stress-related diseases. However, phenolic acids, tannins, and phytochemicals can be found in fruits, which also have an antioxidant activity. In addition, phenolic compounds may act in synergy with other active components, which are affected by the processing conditions of the fruit such as high temperatures. Therefore, heating may result in an increase in antioxidant activity depending on how the compound is present in a raw material (Neri-Numa et al., 2020).

Thus, the best values of antioxidant activity of the analysed extracts of the peel were given at 90 °C; however, using the ultrasonic bath gave the highest value, being  $87.54 \pm 0.51\%$ , with an  $EC_{50}$  equal to  $114.23 \pm 0.68$  mg/mL. The  $EC_{50}$  repre-

sents the equivalent concentration in mg required to reduce the DPPH reagent by 50%. Therefore, the lower this value the higher the antioxidant potential of the raw material. Regarding the pulp (Table 4), the highest percentage of activity was given using the ultrasonic probe at 70 °C, with a value of  $79.79 \pm 0.11\%$  ( $EC_{50} = 125.33 \pm 0.18$  mg/mL). In studies conducted by Silva and Jorge (2019)  $EC_{50}$  values of the extracts of some fruits were: lemon  $93.25 \pm 1.13$  mg/mL; orange  $40.58 \pm 0.13$  mg/mL; kinkan  $115.59 \pm 0.63$  mg/mL; and for passion fruit  $108 \pm 1.58$  mg/mL (Silva et al., 2015).

As reported by several authors, the degree of maturation, harvest season and condition, geographic origin, storage process and other factors may influence the content of the compounds present in fruits affecting their bioactivity (Bindes et al., 2019). There is a wide variety of phenolic compounds with antioxidant capacity, and flavonoids (flavones, flavanones, isoflavones, flavonols, flavanols and anthocyanins) which are known to capture and neutralize oxidizing species such as hydroxyl radical, superoxide anion ( $O_2^-$ ) or peroxide radical. These phenolic compounds and flavonoids can act synergistically with other antioxidants such as vitamins C and E (Cushnie & Lamb, 2011). We determined the extraction kinetic curve of the phenolic compounds at 60 °C using the incubator shaker (Figure 2). An increase of the compounds with time was observed reaching 8.44 mg GAE/g of genipap pulp. At 180 min, the content of phenolic compounds was reduced to 7.93 mg GAE/g. This was explained by the fact that the process reached the maximum point of extraction with thermal degradation of the compounds occurring after that time. Vega Arroyo et al. (2017) and Vinatoru et al. (2017) reported that long extraction times caused the degradation of phenolic compounds, since light, oxygen and high temperatures have a great influence on the process and may contribute negatively to the levels of these compounds.

The consumption of foods such as genipap, containing significant amounts of bioactive compounds contributes to health, and may have an effect against chronic and degenerative diseases. Thus, the identification and quantification of these compounds becomes important,

Table 4: Antioxidant activity (%DPPH) of genipap pulp extract

Extraction temperature (°C)	Orbital Incubator Shaker	Ultrasound Bath	Ultrasound Probe
40	35.14±0.22 <sup>e,C</sup>	40.09±0.22 <sup>e,B</sup>	73.56±0.11 <sup>d,A</sup>
60	68.92±0.09 <sup>b,C</sup>	73.07±0.26 <sup>a,B</sup>	76.99±0.01 <sup>bc,A</sup>
70	43.49±0.37 <sup>d,C</sup>	57.03±0.22 <sup>d,B</sup>	79.79±0.11 <sup>a,A</sup>
80	68.13±0.28 <sup>b,B</sup>	57.27±0.11 <sup>cd,C</sup>	77.48±0.22 <sup>b,A</sup>
90	76.12±0.11 <sup>a,A</sup>	62.62±0.22 <sup>b,B</sup>	75.88±0.22 <sup>c,A</sup>
<b>Control</b>	58.02±1.21 <sup>c,A</sup>	58.02±1.21 <sup>c,A</sup>	58.02±1.21 <sup>e,A</sup>

\* Treatment means followed by different superscript uppercase letters (A – C) in the rows, and different superscript lowercase letters (a – e) in the columns differ from each other at 5% level of significance according to the Tukey test.

Table 5: Antioxidant activity (%DPPH) of genipap peel extract

Extraction temperature (°C)	Orbital Incubator Shaker	Ultrasound Bath	Ultrasound Probe
40	50.79±0.46 <sup>f,C</sup>	65.89±0.11 <sup>c,B</sup>	80.03±0.22 <sup>c,A</sup>
60	67.67±0.37 <sup>c,B</sup>	85.53±0.26 <sup>b,A</sup>	84.58±0.11 <sup>a,A</sup>
70	64.94±0.33 <sup>d,B</sup>	63.42±0.22 <sup>d,C</sup>	83.30±0.11 <sup>b,A</sup>
80	71.56±0.22 <sup>b,B</sup>	62.86±0.33 <sup>d,C</sup>	83.08±0.22 <sup>b,A</sup>
90	81.53±0.28 <sup>a,C</sup>	87.54±0.51 <sup>a,A</sup>	85.30±0.00 <sup>a,B</sup>
<b>Control</b>	58.02 ± 1.21 <sup>e,A</sup>	58.02 ± 1.21 <sup>e,A</sup>	58.02 ± 1.21 <sup>d,A</sup>

\* Treatment means followed by different superscript uppercase letters (A – C) in the rows, and different superscript lowercase letters (a – f) in the columns differ from each other at 5% level of significance according to the Tukey test.

Table 6: Iridoid content present in unripe genipap

Sample	Genipin (mg/g)	Geniposidic Acid (mg/g)	Gallic Acid (mg/g)
Shaker Peel, 80 °C	nd*	22.752± 0.455 <sup>a</sup>	0.642± 0.012 <sup>a</sup>
Control Peel, 25 °C	nd*	2.796± 0.060 <sup>c</sup>	0.626± 0.013 <sup>a</sup>
Control Pulp, 25 °C	nd*	15.080± 0.302 <sup>b</sup>	0.660± 0.013 <sup>a</sup>

\* Treatment means followed by different superscript letters (a – c) in the columns differ from each other at 5% level of significance according to the Tukey test. nd = not detected

since it can show which bioactive components are present in the fruit under the conditions of analysis (Alves et al., 2017). Table 6 presents the contents of the iridoids, i.e., genipin, geniposidic acid and gallic acid found in unripe genipap under three different extraction conditions. These values were in accordance with those found in the literature. Nathia-Neves et al. (2017) found genipin concentration of  $20.7 \pm 0.9$  mg/g of ripe genipap pulp and  $6.18 \pm 0.07$  mg/g of ripe genipap peel using pressurized liquid extraction at 50 °C. However, Bentes and Mercadante (2014) did not find the presence of genipin in the unripe genipap pulp, but found geniposidic acid in the concentration of  $2.54 \pm 0.05$  mg/g of unripe genipap pulp when using stirring extraction with a methanolic solution at 22 °C.

In accordance with Nathia-Neves et al. (2018), genipin and geniposide were at higher concentration in unripe genipap fruits. Thus, the degree of maturation of genipap influences the content of bioactive compounds, since the geniposide is present in a larger quantity in unripe fruits, which decreases by about 90% with maturation. This is due to the increase in the levels of enzymes responsible for glycosylation of the iridoids with maturation (Bentes & Mercadante, 2014). As presented in Figure 1 the genipap fruits we used were in the unripe stage of maturation, with the characteristic blue colour in the pulp after air exposure.

Genipin is the compound present in genipap which reacts with primary amines of amino acids, peptides and proteins in the presence of oxygen to form blue pigments. In this way, genipin has an antimicrobial, anti-inflammatory and anticarcinogenic action. Gallic acid has antioxidant, antimicrobial and antimutagenic properties. However, genipap has been little studied with respect to its edible parts and degree of maturation (Nathia-Neves et al., 2018).

In addition, the solvent used for extraction may influence the content of compounds, as genipin is more polar it tends to be more soluble in polar solvents like water. However, the geniposidic acid is a precursor of the geniposide. Thus, these three compounds were found in greater quantity in green genipap. However, the geniposide was the most bioactive compound (Nathia-Neves et al., 2017).

## 4 Conclusion

The best method for extracting phenolic compounds from the genipap pulp *in natura* in this work was the ultrasonic probe at 40 °C due to its propensity to promote greater mass transfer, and having a direct contact with the sample, which provided greater efficiency. Regarding the peel, the best extraction method of phenolic compounds was using the incubator shaker at 80 °C. The highest percentage of antioxidant activity occurred the ultrasonic bath was used at 90 °C. Higher antioxidant activities were obtained at higher temperatures.

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## Influence of Extraction Solvent on the Biological Properties of Maritime Pine Bark (*Pinus pinaster*)

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### Abstract

Maritime pine bark (*Pinus pinaster* Aiton subsp. *atlantica*) is rich in polyphenols with known bioactive properties which are beneficial for human health. However, biological activities of bark extracts depend on the type of polyphenols extracted and the characteristics of these extractives depend on several factors such as the type of solvents used. The influence of the extraction solvent on the composition and consequently on the properties of the extracts has been poorly described. Thus, in this study the influence of the extraction solvent (water, ethanol and ethanol-water (50/50 v/v%)) on the antibacterial and anticancer properties of *P. pinaster* bark samples were evaluated. LC-DAD-MS profiling of the different extracts was also carried out to study their polyphenol composition. Results show that extraction solvent must be carefully chosen with respect to foreseeing use of bark extracts, since ethanolic and hydroethanolic extracts displayed the greatest antibacterial activity whereas water extracts showed increased anticancer properties.

**Keywords:** *Pinus pinaster*; Pine bark; Extraction solvent; LC-DAD-MS; Antibacterial; Anticancer

## 1 Introduction

Plants have been used worldwide for traditional medicine remedies due to their antioxidant, anti-inflammatory and antimicrobial properties (El-gazar et al., 2019; Ginovyan et al., 2017; Toiu et al., 2019). Nowadays, plant-derived metabolites are of great interest as alternatives to the current treatments of a wide range of diseases,

including cancer, metabolic syndrome and neurodegenerative disorders (Taghipour et al., 2019; Zhao et al., 2019). Among all plant metabolites, polyphenols are highlighted due to their health-promoting properties that include antioxidant, anti-inflammatory, immunomodulatory, chemoprotective and anti-diabetic activities (Ganesan & Xu, 2017; Gorzynik-Debicka et al., 2018; Sánchez-González et al., 2017). It remains un-

clear whether single molecules are responsible for the observed effects or it is the result of a synergy between two or more compounds, since evidence supporting both hypotheses have been obtained (Gascón et al., 2018; Kristek et al., 2019; Lewandowska et al., 2014).

Bioactive compounds are present in edible and non-edible parts of plants, but the use of edible parts for medicinal purposes raises an ethical issue due to a growing global hunger crisis. For this reason, non-edible parts such as bark or peel have been studied as new sources of molecules with promising biological activities (Amri et al., 2017; Mármol et al., 2019).

Pine bark is an abundant by-product of the pulp, paper and sawmill, and building industry, in which trees are felled with different ages/trunk diameters according to their use. Pine bark has been widely investigated due to its high content of polyphenols, thus repurposing a by-product from forest processing as a source of bioactive compounds. Of all the pine species, maritime pine (*Pinus pinaster* Aiton subsp. *atlantica*) has been one of the most investigated with respect to its health-promoting effects (Mármol et al., 2019). Three maritime pine bark extracts are commercially available as dietary supplements, all claiming that the pine originates from Les Landes de Gascogne forest in southwestern France: Pycnogenol<sup>®</sup>, (Horphag Research, Switzerland), Olygopin<sup>®</sup>, (DRT, France) and Flavangenol<sup>®</sup>, (Toyo Shinyaku Inc., Japan). Pycnogenol<sup>®</sup>, is by far the most studied extract of *P. pinaster* with proven health-promoting effects (Feragalli et al., 2018; Oliff & Blumenthal, 2019; Pourmasoumi et al., 2020).

Some authors have reported the influence of different extraction methods on the biological properties of plant-derived fractions. Final composition of the extracts might be heavily influenced by extraction procedure, which in turn modifies their biological activities as well as other properties such as taste (Mzid et al., 2017; Rodrigues et al., 2015; Xu et al., 2018). Since the loss of a bioactive compound and/or a group of them might invalidate the therapeutic potential of plant extracts, all extraction steps must be carefully planned according to the intended purpose.

There is a growing concern to use greener sol-

vents for extraction, such as water and ethanol, in order to reduce the environmental impact.

Herein, we analyse the composition and biological properties of samples from *Pinus pinaster* Aiton subsp. *atlantica* bark from the Minho region, Northwest of Portugal, extracted with three different solvents: ethanol, water, and ethanol-water (50/50 v/v%), in order to evaluate the influence of the extraction solvent on antimicrobial and anticancer activities.

## 2 Materials and Methods

### 2.1 Obtention of pine bark extracts

Maritime pine bark (*Pinus pinaster* Aiton subsp. *atlantica*) was collected in the Minho region, Northwest of Portugal, from trees aged 15 years. Whole bark was manually separated into an inner layer (phloem) and outer layer (rhytidome) based on morphological and colour differences between bark layers. Only the outer layer was used in this experiment. Bark samples were cut into smaller pieces, oven dried at 40 °C for 72 h, ground (Termomix TM31, Vorwerk, Germany) and sieved at the amplitude of 0.2 mm for 1 min to select the particles of 200 to 800 µm diameter. Bark samples were subjected to Soxhlet liquid-solid extraction using deionized water (PW), ethanol (PE) and water-ethanol (50/50 v/v%) (PWE) as solvents, as described in previous experiments (Vieito et al., 2018). 12.5 g of dry ground pine bark was put into an extraction thimble and placed inside the upper reservoir. Then, 220 mL of each solvent was added to the lower reservoir and the mixture boiled for 4 h under reflux. After cooling, the extract was filled up to 250 mL with the respective solvent (extract stock solution). Water and ethanol were selected as environmentally safe and food grade solvents. These liquid extracts were used in the subsequent analyses.

## 2.2 Analysis of pine bark extracts' composition by liquid chromatography-photodiode-array-mass spectrometry

Liquid chromatography-photodiode-array-mass spectrometry (LC-PDA-MS-MS) data was obtained with a Vanquish liquid chromatography system (Thermo Fisher Scientific®), coupled to Ultimate 3000 UV Detector (Dionex®), and Q Exactive Plus mass spectrometer (Thermo Fisher Scientific®). LC conditions were as follows: Luna C-18 column (150 x 3 mm i.d., 3 $\mu$ m, Phenomenex®, Torrance, USA); solvent system, (A) H<sub>2</sub>O containing 0.1% formic acid, (B) MeCN with 0.1% formic acid; gradient mode for aqueous pine bark extracts (PW): 5% of B for 1 min, 5 to 50% of B for 15 min, 50 to 100% of B for 5 min, and 100% of B for 3 min; gradient mode for ethanolic (PE) and hydroethanolic (PWE) extracts: 5% of B for 1 min, 5 to 100% of B for 19 min, and 100% of B for 3 min; flow rate at 500  $\mu$ L/min; injection volume was 10  $\mu$ L; sample concentration was 5 mg dry extract/mL H<sub>2</sub>O/MeOH. UV detection was performed at 254 and 280 nm. ESI-MS conditions were as follows: collision energy 35 eV; capillary temperature 320°C; electrospray negative ion mode (source voltage: 2500 V) in full scan and ms<sup>2</sup>, mass range: 100-1500.

## 2.3 Determination of antibacterial activity of pine bark extracts

15 mL of the extract stock solution were lyophilized for 48 h, under vacuum, in an Alpha 1-2 LDplus freeze-dryer (Christ, Germany) and reconstituted in 2 mL of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA). DMSO, used here as a "negative control", has a broad dissolving capacity both polar and non-polar compounds, and is relatively inert and widely used in biological assays. The final concentration of the applied extracts was 30 mg extract/mL DMSO for PW and 65 mg extract/mL DMSO for extraction using PWE and PE. They were reconstituted in DMSO equivalent to the extraction yield obtained in the Soxhlet extraction. The extraction yield (defined as the amount of solid

extract recovered in mass compared with the initial amount of dry bark) of water (PW) extracts (7.84  $\pm$  0.56%) was significantly lower compared to ethanolic (PE) and hydroethanolic (PWE) extracts respectively, 17.55  $\pm$  0.16 % and 17.08  $\pm$  0.23 % (Vieito et al., 2018). Hence, it was decided to use the same volume of extract that reflects the different mass corresponding to the yield of each respective solvent. The extracts with antibacterial properties will be applied directly, just after reconstitution in water, as well as a food flavouring onto a salami-like product.

A disk diffusion assay was used to determine the diameter of the inhibition zone of tested extracts and was performed following the method by CLSI 2012 CITA. Each disk (Oxoid, England) (6 mm in diameter) was impregnated with 10  $\mu$ L of extract or control (two disks per extract in a total of six disks per plate and two disks for the controls – DMSO and commercial solution of sodium hypochlorite coded as Lx (Neoblanc, Fater SpA, Italy)). Strains of *Bacillus cereus* NCTC 11143 and ATCC 11778, *Clostridium perfringens* ATCC 13124, *Escherichia coli* ATCC 25922 and ATCC 8739, *Listeria monocytogenes* ATCC 13932, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and ATCC 29213, and *Salmonella enterica* serovar Enteritidis ATCC 25928 were inoculated in Columbia Agar + 5% Sheep Blood (COS, Biomérieux, France). Active cultures (0.5 McFarland) were spread with a cotton swab onto Mueller-Hinton Agar (MHA, Oxoid, England). Plates were allowed to dry for 3 to 5 min. The disks were placed onto inoculated MHA plates, one disk in each of the eight equal parts. The plates stood for 15 min and then were inverted and incubated for 22 h  $\pm$  2 h at 37 °C  $\pm$  1 ° C. Zones of inhibition were measured in mm with the help of ImageJ software (Rasband, 1997-2018). The values presented correspond to the mean of the two inhibition halos. DMSO and bleach were used, respectively, as negative and positive controls.

Bacterial strains were selected as the most representative of the gram-positive and gram-negative foodborne pathogenic and food spoilage bacteria.

## 2.4 Cell lines

Three types of tumoral human cell lines were tested: colorectal adenocarcinoma Caco-2 cells, breast adenocarcinoma MCF-7 cells and hepatocellular carcinoma HepG2 cells. The cell lines were provided by different sources which are further acknowledged. All cell lines were maintained in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. Cells (passages 20-40) were grown in Dulbecco's Modified Eagles medium (DMEM) (Gibco Invitrogen, Paisley, UK) supplemented with 20% fetal bovine serum, 1% non-essential amino acids, 1% penicillin (1000 U/mL), 1% streptomycin (1000 µg/mL) and 1% amphotericin (250 U/mL). Culture medium was replaced every two days and cells were passaged enzymatically with 0.25% trypsin-1 mM EDTA and sub-cultured on 25 cm<sup>2</sup> flasks at a density of 2·10<sup>4</sup> cells/cm<sup>2</sup>.

Experiments in undifferentiated Caco-2 cells as well as on MCF-7 and HepG2 cells were performed 24 h post-seeding. For assays on differentiated Caco-2 cells, cells were cultured on 96-wells plates under standard culture conditions for 7 to 9 days, until reaching 80% confluence as confirmed by optic microscopy observation.

## 2.5 Cell viability assays

Cells were seeded in 96-well plates at a density of 4·10<sup>3</sup> cells/well. The culture medium was replaced with fresh medium (without foetal bovine serum) containing pine bark samples at concentrations varying from 0 to 1000 mg/L (for PE samples) or from 0 to 125 mg/mL (for PW and PWE samples), with an exposure time of 72 h. Thereafter, cell growth was analysed by the sulforhodamine B assay as previously described (Jiménez et al., 2016). Absorbance was measured with a scanning multiwell spectrophotometer (Biotex Sinergy ht Siafrtd, Vermont, USA) at wavelength between 540 and 620 nm. The effect on cell growth was expressed as a percentage of the control and calculated as % control. Experiments were conducted in quadruplicate wells and repeated at least two times. Results were expressed as mean ± SD.

## 3 Results and Discussion

### 3.1 Extraction solvent influences composition of pine bark extracts

Figures 1, 2 and 3 show, respectively, chromatographic data (LC-PDA-MS-MS) obtained for aqueous (PW), hydroethanolic (PWE) and ethanolic (PE) pine bark extracts. Major identified compounds in *Pinus pinaster* bark extracts using HPLC-DAD-MS analysis with electrospray negative ionization (ESI-) are numbered and listed in Tables 1 (aqueous extract) and 2 (hydroethanolic and ethanolic extracts, analysed under the same analytical conditions). The major polyphenolic compounds identified in the three types of *Pinus pinaster* bark extracts were catechin and taxifolin, two flavonoids of great interest due to their health-promoting properties (Li et al., 2020; Shafabakhsh et al., 2020; Sunil & Xu, 2019). Catechin, epicatechin and taxifolin represent “monomeric procyanidins” of which catechin was the most common (Oliff & Blumenthal, 2019). However, as it can be observed (Tables 1 and 2), PWE and PE extracts contained hydroxybenzoic acid, procyanidin, caffeic acid, abietic acid derivate and 15-hydroxydehydroabietic acid in their composition, whereas PW did not. On the other hand, the amount of taxifolin was greater in PW extracts than in PE and PWE extracts. Previous studies of Vieito et al. (2018) on pine bark extracts reported the higher antioxidant activity of hydroethanolic (PWE) extracts compared to water (PW) or ethanolic (PE) extracts. The dietary supplement Pycnogenol<sup>®</sup>, extracted from maritime pine bark using an ethanol-water solvent mixture (70/30 v/v%), showed significant antioxidant activity, based primarily on its procyanidin content (about 75%) (Oliff & Blumenthal, 2019).

### 3.2 PE and PWE extracts display greater antibacterial activity than PW extracts

The measurements of the inhibition halos of PW, PWE and PE extracts against the tested bac-

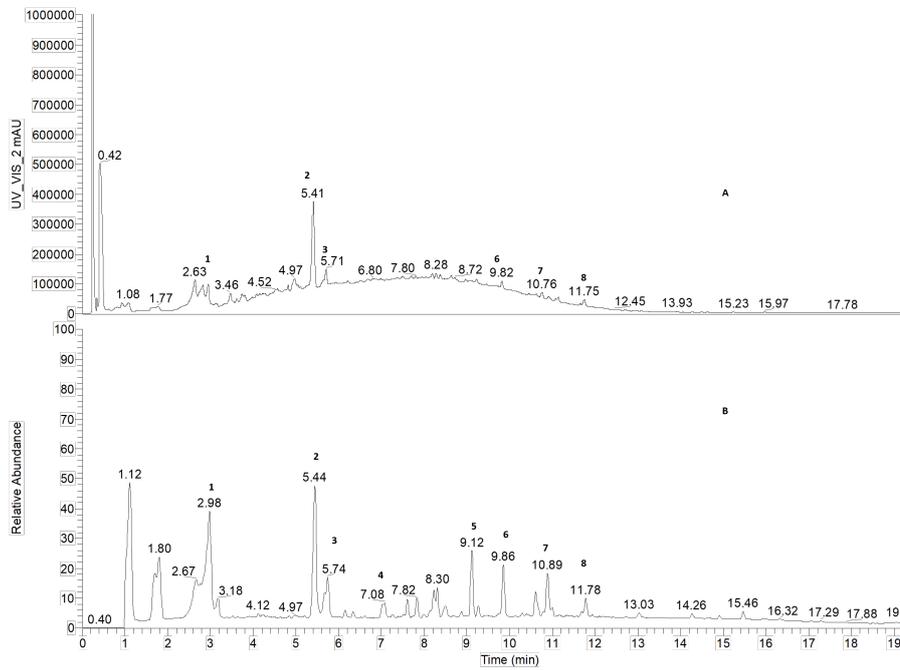


Figure 1: Chromatographic (LC-PDA-MS-MS) data obtained for aqueous *Pinus pinaster* bark extract (PW). A) HPLC profiles detected at 280 nm; B) ESI base peak ion chromatogram.

Table 1: Tentative structural elucidation of several chemical constituents contained in *Pinus pinaster* bark aqueous (PW) extract using HPLC-DAD-MS analysis with electrospray negative ionization (ESI-).

Peak <sup>a</sup>	Retention time (min)	$\lambda_{max}$ (nm) <sup>b</sup>	m/z ESI- (MS2)	M	Area	Tentative identification	Reference
1	2.9	280	289.071	290	243 487	Catechin	a,b
2	5.4	290	303.050	304	1 158 143	Taxifolin 1	a,b
3	5.7	290	303.051 (285.040)	304	247 348	Taxifolin 2	a,b
4	7.0	290	505.192	-	11 192	ND	-
5	9.1	290	291.160	-	17 662	ND	-
6	9.8	286	289.144 (245.154)	-	66 895	ND	-
7	10.8	286	349.201, 331.191	-	49 871	Dehydroxydehydro abietic acid derivate	-
8	11.7	298	331.191	332	60 964	7,15-dihydroxydehydro abietic acid	c,d

<sup>a</sup> Peak numbering are shown in Fig. 1

<sup>b</sup>  $\lambda_{max}$  in UV spectrum from the PDA detector

ND- Not determined

References: a= Yesil-Celiktas et al. (2009), b= Almeida et al. (2016); c= Lee et al. (2018); d= Mulholland et al. (2017)

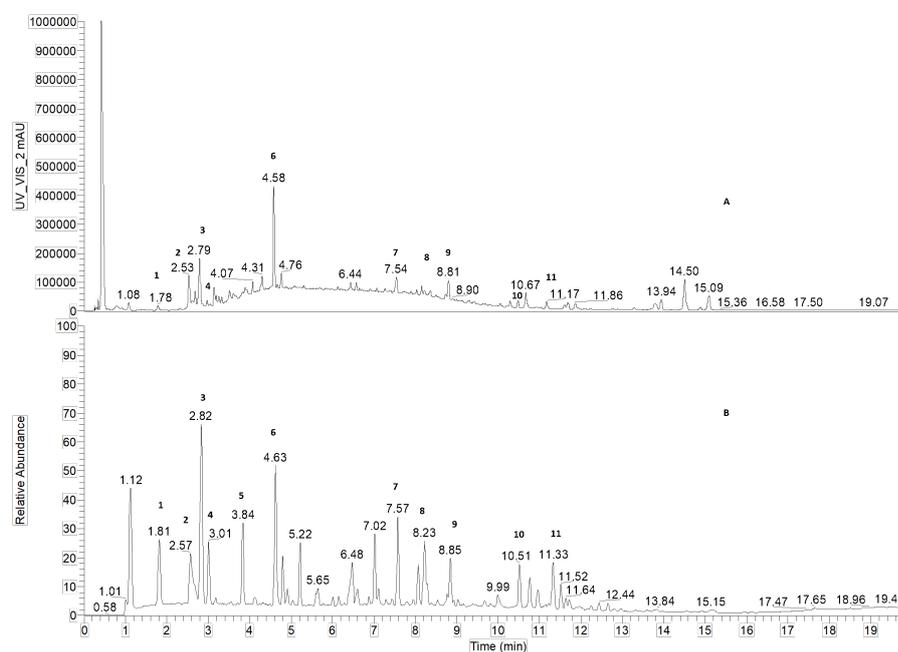


Figure 2: Chromatographic (LC-PDA-MS-MS) data obtained for hydroethanolic *Pinus pinaster* bark extract (PWE). A) HPLC profiles detected at 280 nm; B) ESI base peak ion chromatogram.

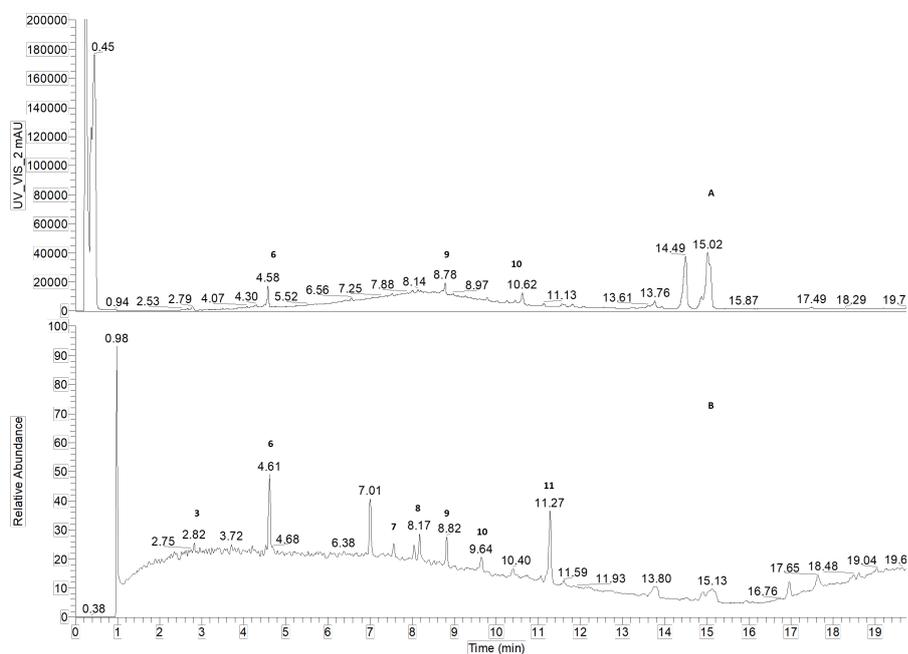


Figure 3: Chromatographic (LC-PDA-MS-MS) data obtained for ethanolic *Pinus pinaster* bark extract (PE). A) HPLC profiles detected at 280 nm; B) ESI base peak ion chromatogram.

Table 2: Tentative structural elucidation of several chemical constituents contained in *Pinus pinaster* bark hydroethanolic (PWE) and ethanolic (PE) extracts using HPLC-DAD-MS analysis with electro-spray negative ionization (ESI-).

Peak <sup>a</sup>	Retention time (min)	$\lambda_{max}$ (nm) <sup>b</sup>	m/z ESI- (MS2)	M	Tentative identification	Area		Reference
						PWE	PE	
1	1.8	229, 280, 308	137.023	138	Hydroxybenzoic acid	66 526	-	a
2	2.5	280	577.134	578	Procyanidin	256 425	7 402	b
3	2.8	279	289.071 (245.081)	290	Catechin	403 653	-	b, c, d
4	3.0	244, 323	179.034 (135.044)	180	Caffeic acid	38 250	-	c, d
5	3.7	263	186.112 (125.096)	-	ND	22 405	-	-
6	4.6	290	303.050	304	Taxifolin	715 433	44 845	c, d
7	7.5	263	289.144	-	ND	144 568	5 115	-
8	8.1	245, 303	275.165 (233.118)	-	ND	56 859	4 033	-
9	8.8	299	331.191	332	7,15-dihydroxydehydroabietic acid	148 233	17 046	e, f
10	10.5	284	333.207	-	Abietic acid derivate	68 784	27 323	-
11	11.2	276	315.196	316	15-hydroxydehydro-abietic acid	69 512	5 223	f

<sup>a</sup> Peak numbering are shown in Fig. 2 and 3

<sup>b</sup>  $\lambda_{max}$  in UV spectrum from the PDA detector

ND- Not determined

References: a= Touriño et al. (2005); b= Yesil-Celiktas et al. (2009); c= Almeida et al. (2016); d= Celhay (2013); e= Lee et al. (2018); f= Mulholland et al. (2017)

Table 3: Antibacterial activity of aqueous (PW), hydroethanolic (PWE) and ethanolic (PE) maritime pine bark extracts determined by the disk diffusion method in MHA (inhibition halos measurements).

Bacterial species	Strain reference	PW <sup>a</sup>	PWE <sup>a</sup>	PE <sup>a</sup>
<i>B. cereus</i>	NCTC 11143	8.4±0.0	11.2±0.2	10.7±0.3
	ATCC 11778	8.4±0.4	10.6±0.1	10.6±0.3
<i>C. perfringens</i>	ATCC 13124	11.9±0.7	15.6±0.3	14.4±0.3
	ATCC 8739	0.00±0.00	0.00±0.00	0.00±0.00
<i>E. coli</i>	ATCC 25922	0.00±0.00	0.00±0.00	0.00±0.00
	ATCC 13932	0.00±0.00	8.5±0.0	8.4±0.1
<i>L. monocytogenes</i>	ATCC 27853	0.00±0.00	0.00±0.00	0.00±0.00
<i>P. aeruginosa</i>	ATCC 25923	9.2±0.5	11.5±0.1	11.4±0.2
	ATCC 29213	7.7±0.0	8.8±0.1	8.3±0.1
<i>Salmonella</i> Enteritidis	ATCC 25928	0.00±0.00	0.00±0.00	0.00±0.00

<sup>a</sup> Inhibition halos measurements in mm

Table 4: IC50 values ( $\mu\text{g}/\text{mL}$ ) of *Pinus pinaster* bark ethanolic (PE), hydroethanolic (PWE) and aqueous (PW) extracts after 72 h of incubation on each cell line.

Pine bark extracts	Caco-2 cells IC50 values ( $\mu\text{g}/\text{mL}$ )	MCF-7 cells IC50 values ( $\mu\text{g}/\text{mL}$ )	HepG2 cells IC50 values ( $\mu\text{g}/\text{mL}$ )
PE	204.55 $\pm$ 22.89	587.77 $\pm$ 93.61	465.99 $\pm$ 86.21
PWE	40.62 $\pm$ 8.63	136.24 $\pm$ 23.32	8.54 $\pm$ 3.92
PW	15.06 $\pm$ 9.37	53.23 $\pm$ 9.14	2.36 $\pm$ 0.73

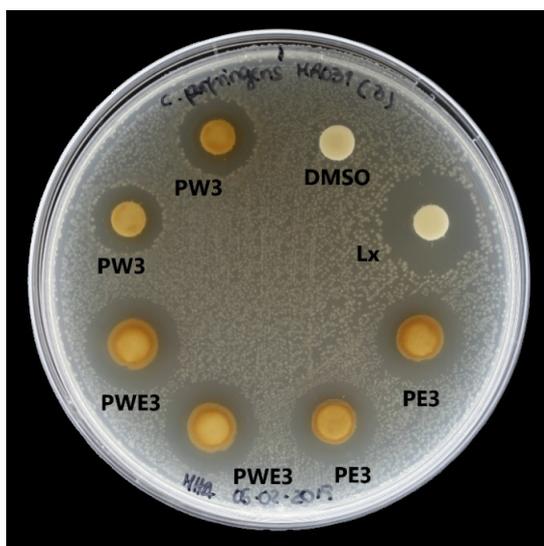


Figure 4: Antibacterial activity of maritime pine bark aqueous (PW), ethanolic (PE) and hydroethanolic (PWE) extracts against *Clostridium perfringens* ATCC 13124 by disk diffusion assay in MHA.

terial strains are presented in Table 3. All extracts presented bacterial activity against gram-positive bacteria, namely *B. cereus* NCTC 11143 and ATCC 11778, *L. monocytogenes* ATCC 13932, *S. aureus* ATCC 25923 and ATCC 29213, with the exception of PW which did not show antibacterial activity against *L. monocytogenes* ATCC 13932. The highest inhibition found was against *C. perfringens* ATCC 13124 whose inhibition halos can be observed in Figure 4. The extracts did not show antibacterial activity against the tested gram negative bacteria, namely *E. coli* ATCC 25922 and ATCC 8739, *P.*

*aeruginosa* ATCC 27853 and *Salmonella* Enteritidis ATCC 25928. These results are in agreement with other studies that reported gram-positive bacteria to be more susceptible to antimicrobials than gram negative bacteria (Kumar & Brooks, 2018; Ramos et al., 2016). Activity of antimicrobials may be limited against gram-negative bacteria due to the outer double-layer membrane, highly hydrophilic lipopolysaccharide molecules and a unique periplasmic space (Kumar & Brooks, 2018). It also seems that hydroethanolic (PWE) and ethanolic (PE) solvents are better at extracting antibacterial compounds. As pointed out before, PWE and PE extracts contained hydroxybenzoic acid, procyanidin, caffeic acid, abietic acid derivate and 15-hydroxydehydroabietic acid. These polyphenolic compounds, known to have antioxidant properties and not found in PW extracts, may also have antimicrobial properties, and thus may be responsible for the inhibitory action against *L. monocytogenes* ATCC 13932. However, the same polyphenol may be effective on one type of gram-positive (or gram-negative) strain and ineffective on another type so indicating a strain-dependent effect (Bouarab-Chibane et al., 2019). Nevertheless, the antibacterial activity of maritime pine bark extracts against gram-positive bacteria can be a useful tool for food preservation purposes and, furthermore, a potential complement to antibiotic therapies in the treatment of bacterial infections.

### 3.3 PW extracts display the highest antiproliferative effect

The antiproliferative activity of *P. pinaster* bark extracts was analysed using a panel of selected

tumour cell lines, namely Caco-2 (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma) and HepG2 (human hepatocellular carcinoma). The obtained IC<sub>50</sub> values are summarized in Table 4. Although all samples were able to significantly reduce cell viability, water (PW) extracts displayed the highest antiproliferative effect against all the analysed cell models, followed by water:ethanol (PWE) extracts and finally ethanol (PE) extracts, which could be due to the higher content of taxifolin in PW extracts. In terms of effectivity, HepG2 cells showed a better response to both PW and PWE samples followed by Caco-2 and finally MCF-7 cells, whereas Caco-2 cell line displayed a higher sensitivity to PE extracts than HepG2 and MCF-7.

In order to determine the selectivity of the extracts on healthy tissue, the cytotoxicity of all samples was evaluated on differentiated Caco-2 cells. This cell line undergoes spontaneous differentiation after reaching confluence, thus has been used as an intestinal barrier model to evaluate, for example, the absorption of nutrients and/or drugs. Differentiated Caco-2 cells displayed intracellular tight junctions, the characteristic brush border of healthy enterocytes and some of the most relevant enzymes of this cell type (Sambuy et al., 2005). In this line, all *P. pinaster* bark extracts were incubated 72 h on differentiated Caco-2 at different concentrations and IC<sub>50</sub> values higher than 100 µg/mL were obtained for each extract, which suggested that samples might display tumour-selectivity.

The differences between the three extracts tested, in terms of antiproliferative effect, might be a consequence of the variations in their chemical composition as a result of the selected extraction solvent. As shown in Table 1, PW extracts contain a greater amount of taxifolin than PWE and PE extracts (Tables 1 and 2), which is a molecule of great interest due to its antitumoral activity. As reported by Sunil and Xu (2019), taxifolin presents several promising pharmacological activities of which anticancer activity is more prominent than other activities evaluated in either *in vitro* or *in vivo* models.

## 4 Conclusions

The influence of three extraction solvents, water, ethanol and ethanol-water (50/50 v/v%), on the chemical composition and biological activities of maritime pine bark (*P. pinaster* Aiton subsp. *atlantica*) was analysed. It was observed that the extraction solvent strongly determines the antibacterial and anticancer effectivity of the samples which might be due to differences in phenolic composition. The choice of an extraction solvent is dependent on the desired biological effect and, consequently, determines its potential applications.

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# Storage Stability of Hot Smoked Spiced African Catfish (*Clarias gariepinus*)

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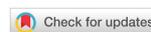
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## Abstract

Hot Smoked Spiced Catfish (HSSC) samples prepared under optimal conditions (garlic, 7.29 g/100 ml; ginger, 7.50 g/100 ml; turmeric, 2.5 g/100 ml; soaking temperature, 38.68 °C and soaking time, 7.51 min) were stored at ambient temperature (30±2 °C) for a period of 20 days to evaluate storage stability; un-spiced hot smoked catfish served as control. Moisture Content (MC), Thiobarbitric Acid (TBA), Peroxide Value (PV), Free Fatty Acid (FFA), Total Viable Counts (TVC) and Mould Counts (MoC) were monitored at two-day intervals as a measure of the storage stability indices. Sensory attributes of the Optimized Hot Smoked Spiced Catfish (OHSSC), control (unspiced) and commercial hot-smoked catfish were determined using a preference test. The storage stability indices values for HSSC were in the range of 6.64 - 7.01% (MC), 4.50 - 13.77 mg MDA/kg (TBA), 0.20 - 2.84 mEq/kg (PV), 0.72 - 9.64% (FFA), 9.50 - 57.00 cfu/g (TVC), 8.00 - 34.50 cfu/g (MoC). The control sample values were in the range of 6.97 - 7.30% (MC), 5.51 - 14.92 mg MDA/kg (TBA), 0.23 to 2.86 mEq/kg (PV), 2.24 - 11.88% (FFA), 13.50 - 113.00 cfu/g (TVC), and 10.00 - 49.00 cfu/g (MoC). The sensory evaluation indicated that OHSSC was most preferred in all the evaluated sensory parameters. This study established the synergistic effects of garlic and turmeric on the keeping quality and sensory attributes of hot-smoked catfish with the prospect of reducing post harvest losses.

**Keywords:** Ambient temperature; Shelf-life; Spices; Chemical parameters; Microbial load; Sensory attributes

## 1 Introduction

Fish, like all food products, is composed of biological raw materials which inherently spoil and deteriorate overtime (Ahmad et al., 2021). Consequently, preservation is required unless it is consumed soon after capture; because of its susceptibility to spoilage. Various food products have different spoilage times that are referred to as shelf-life (Humaid & Jamal, 2014). Catfish is a very important freshwater fish in Nigeria. It has enjoyed wide acceptability across the country, because of its unique taste, nutrients, flavour and

good texture (Adebowale et al., 2008). Catfish commonly sold in Nigeria comes in various sizes and weight ranging from small (average weight, 250- 330 g) to large (> 1.0 kg) that depend on several factors, such as feeds, age and others (Usman, 2017). In Nigeria, the shelf life of caught fish is estimated to be between 16 and 20 h in the southern part and between 20 and 36 h in the northern part at ambient temperature (Eyabi, 1996). The rate with which fish spoils depends on hygienic conditions, storage temperature, acidity and the structure of the muscular tissue (Clucas, 2006). It is estimated that post-harvest losses of

fish accounts for more than 50% of the total fish caught in Nigeria (Tyokumbur, 2014). Hence, to ensure the availability of fish throughout the year, especially during the lean season, it is essential to process the fish to preserve it in appreciable quantities in good condition until its use is required (FAO, 2001). There are different methods of prolonging the shelf-life of fish which commonly include use of chemical preservatives, chilling, freezing, canning, drying, salting and smoking.

Smoking has long been employed for preservation and is still widely used for extending the shelf life of fish among several communities in developing countries (Adeyeye, 2016; Ward, 1995). More than 50% of fish caught in Nigerian inland waters are preserved by smoke curing (Daramola et al., 2007). Smoking favours lengthy preservation by removing moisture, which contributes to bacteriological and enzymatic spoilage. Several factors such as raw material preparation, use of additives, type and time of smoking, relative humidity, air velocity, temperature, and others have been identified as factors that affect the quality and shelf life of the smoked product (Hilderbrand, 2001; Osibona & Ezekiel, 2014). Several authors have reported various levels of success with the use of spices (garlic, ginger, turmeric, and others) on shelf-life or organoleptic properties enhancement of fish (Tiamiyu et al., 2005). In a recent study the optimum conditions (garlic, 7.29 g/100 ml, ginger, 7.50 g/100 ml, turmeric, 2.5 g/100 ml, soaking temperature, 38.68 °C and soaking time, 7.51 min) for producing hot smoked spiced catfish with improved organoleptic and nutritional status were reported (Akinbisoye et al., 2019). This present study evaluated the storage stability of the catfish produced under these optimum conditions.

## 2 Materials and Methods

### 2.1 Materials

Twenty mature catfish with weight range of 250 to 350 g were procured live from Owena market in Ondo State, Nigeria. The fish were immediately transported to the laboratory for further processing. The fish were slaughtered, beheaded

and gutted, washed and kept in the freezer (-18 °C) overnight unsalted and without packaging material prior to the experiment. Smoked commercial catfish, ginger, garlic and turmeric were purchased from a local market in Ado-Ekiti, Ekiti State, Nigeria.

### 2.2 Methods

#### Preparation of the optimized hot smoked spiced catfish

The unsalted, cleaned and eviscerated whole fish were soaked in the optimized solution and optimized conditions (garlic;7.29 g/100 ml, ginger;7.50 g/100 ml, turmeric;2.5 g/100 ml, soaking temperature, 38.68 °C and soaking time, 7.51 min) as described and established in a previous publication (Akinbisoye et al., 2019). After draining of the spiced fish samples for about 5 min, both spiced and unspiced (control) fish samples were smoked in a smoking kiln. Smoking was performed at a temperature of 80 °C for 7 h (Abdel-Hamied et al., 2009). The spiced and unspiced (control) smoked fish samples were cooled before subjection to storage studies.

### 2.3 Storage stability studies

The unspiced (control) and optimized smoked spiced catfish were stored at ambient temperature (30 ± 2 °C) and monitored at two-day intervals for a period of 20 days to evaluate its stability. The monitored storage indices were moisture, thiobabaturic acid (TBA), peroxide value and free fatty acid (FFA) content of the fish samples using the standard method of AOAC (2005). Also, total viable and mould counts in the catfish samples were determined as described by Downes and Ito (2001) and Okechalu et al. (2011), and Montville and Matthews (2005) respectively.

### 2.4 Sensory evaluation

The sensory evaluation was carried out on Optimised Hot Smoked Spiced Catfish, unspiced (control) smoked catfish and smoked commercial catfish samples. Sensory attributes (taste,

texture, appearance, after-taste, aroma and general acceptability) were evaluated separately according to the method of Stone and Sidel (2004). A panel of 75 semi-trained people (50% male) comprised of staff and undergraduate students of the Department of Food Science and Technology, Federal Polytechnic Ado Ekiti, Nigeria, was constituted. The assessors fell within the age group of 25-55 years. The evaluation was carried-out in a well illuminated room with cross ventilation at ambient temperature. A 9-point hedonic scale, with 9 for extremely liked down to 1 for extremely disliked, was adopted. Consumer ethical conduct was followed by explaining the whole experimental process to the panellists before the organoleptic testing. Assessors were briefed about the type of fish species, which they would taste and the sources of the fish including the mode of processing e.g. the fish samples were smoked. Each panelist therefore participated in the study with full knowledge of the process. Approval from Ethics Committee is optional for common food sensory analysis hence, approval was not sought for this exercise being a commonly consumed food in the country. Nevertheless, these analyses were carried out following international tenets and informed consents were obtained from the panellists.

## 2.5 Statistical analysis

All the data obtained from the experiments were analysed with the statistical programme SPSS for Windows version 20 with analysis of variance (ANOVA) for the normalized data. Means that were statistically different were separated using Duncan's Multiple range test. The statistical significance for all the parameters was identified at 95% confidence level ( $p < 0.05$ ). In the case of two samples comparison, t- test: paired two samples for means was used.

## 3 Results and Discussion

### 3.1 Moisture content of the stored catfish samples

The moisture content (Table 1) of the Optimised Hot Smoked Spiced Catfish (OHSSC) decreased

as storage period progressed and was consistently lower than the control sample. The moisture content of OHSSC decreased from 7.01 to 6.65% while that of the control sample decreased from 7.30 to 6.98%. Significant differences ( $p < 0.05$ ) based on the t-test analysis existed in the moisture content of the OHSSC and control sample before and during storage. The storage period was also observed to have a significant effect on the moisture content in both the spiced and unspiced (control) fish samples. The difference in the initial moisture content of the OHSSC and the control sample might be attributed to the impact of the spices in the fish (Tagoe et al., 2011). The observed moisture content is close and falls within the range of moisture content (7-9%) for smoked *Clarias gariepinus* as reported by Usman (2017). However, the decreasing trend observed in both the OHSSC and control sample during storage might be attributed to loss of moisture into the atmosphere. The loss of moisture into the surrounding air may be dependent on the air's humidity. It was reported that the moisture content of fish subjected to different treatments decreased after smoking from 6.95 to 4.93% (Oyelese, 2006). The moisture content (which is of great importance in storage) of both the spiced and unspiced smoked *Clarias gariepinus* was between 6 and 7% which is within the moisture content reported for dried fish (6% to 8%) (Yanar, 2007). This observation indicates that the spiced smoked fish could be preserved successfully for 20 days and beyond without spoilage thus reducing postharvest losses of the fish.

### 3.2 Thiobabitoric acid content of the stored catfish samples

Thiobabitoric acid (TBA) content is a widely used indicator for the assessment of degree of secondary lipid oxidation. It evaluates the second stage of autoxidation, during which the peroxides are oxidised to aldehydes and ketones, which impart the disagreeable fishy or rancid odours and flavour (Romero et al., 2008). The TBA values (Table 1) increased consistently in both the spiced and unspiced samples as storage days increased, however the OHSSC had

Table 1: Effect of Storage Days on the Control and Optimised Hot Smoked Catfish

Storage Period (Days) S/N	MC (%)		PV (mEq/kg)		FFA (%)		TBA (mg MDA/kg)	
	Optimised	Control	Optimised	Control	Optimised	Control	Optimised	Control
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
0	7.010±0.000 <sup>j</sup>	7.300±0.00 <sup>j</sup>	0.209±0.001 <sup>a</sup>	0.230±0.003 <sup>a</sup>	0.724±0.000 <sup>a</sup>	2.248±0.397 <sup>a</sup>	4.503±0.022 <sup>b</sup>	5.516±0.028 <sup>a</sup>
2	6.937±0.002 <sup>i</sup>	7.267±0.02 <sup>i</sup>	0.471±0.004 <sup>b</sup>	0.489±0.004 <sup>b</sup>	2.976±0.01 <sup>b</sup>	5.220±0.012 <sup>b</sup>	4.685±0.017 <sup>a</sup>	5.682±0.017 <sup>b</sup>
4	6.905±0.002 <sup>h</sup>	7.235±0.02 <sup>h</sup>	0.735±0.004 <sup>c</sup>	0.753±0.004 <sup>c</sup>	3.716±0.012 <sup>c</sup>	5.961±0.012 <sup>c</sup>	5.534±0.017 <sup>c</sup>	6.712±0.017 <sup>c</sup>
6	6.905±0.002 <sup>h</sup>	7.203±0.00 <sup>g</sup>	0.999±0.004 <sup>d</sup>	1.017±0.004 <sup>d</sup>	4.457±0.01 <sup>d</sup>	6.701±0.012 <sup>d</sup>	6.564±0.017 <sup>d</sup>	7.742±0.017 <sup>d</sup>
8	6.873±0.002 <sup>g</sup>	7.171±0.002 <sup>f</sup>	1.263±0.004 <sup>e</sup>	1.281±0.004 <sup>e</sup>	5.198±0.012 <sup>e</sup>	7.442±0.012 <sup>e</sup>	7.593±0.017 <sup>e</sup>	8.771±0.017 <sup>e</sup>
10	6.841±0.002 <sup>f</sup>	7.139±0.002 <sup>e</sup>	1.527±0.004 <sup>f</sup>	1.545±0.004 <sup>f</sup>	5.938±0.012 <sup>f</sup>	8.182±0.012 <sup>f</sup>	8.623±0.017 <sup>f</sup>	9.801±0.017 <sup>f</sup>
12	6.709±0.002 <sup>c</sup>	7.107±0.002 <sup>d</sup>	1.791±0.004 <sup>g</sup>	1.809±0.004 <sup>g</sup>	6.679±0.01 <sup>g</sup>	8.923±0.012 <sup>g</sup>	9.653±0.017 <sup>g</sup>	10.830±0.01 <sup>g</sup>
14	6.747±0.002 <sup>d</sup>	7.075±0.002 <sup>c</sup>	2.055±0.004 <sup>h</sup>	2.073±0.004 <sup>h</sup>	7.419±0.01 <sup>h</sup>	9.663±0.012 <sup>h</sup>	10.682±0.01 <sup>h</sup>	11.860±0.01 <sup>h</sup>
16	6.704±0.002 <sup>c</sup>	7.035±0.002 <sup>c</sup>	2.483±0.004 <sup>h</sup>	2.501±0.004 <sup>h</sup>	7.419±0.01 <sup>h</sup>	9.663±0.012 <sup>h</sup>	10.702±0.01 <sup>h</sup>	11.960±0.01 <sup>h</sup>
18	6.681±0.002 <sup>b</sup>	7.011±0.002 <sup>b</sup>	2.583±0.004 <sup>i</sup>	2.601±0.004 <sup>i</sup>	8.909±0.012 <sup>i</sup>	11.144±0.01 <sup>i</sup>	12.741±0.017 <sup>i</sup>	13.919±0.017 <sup>i</sup>
20	6.649±0.002 <sup>a</sup>	6.979±0.002 <sup>a</sup>	2.847±0.004 <sup>j</sup>	2.865±0.004 <sup>j</sup>	9.641±0.012 <sup>j</sup>	11.885±0.01 <sup>j</sup>	13.771±0.017 <sup>j</sup>	14.949±0.017 <sup>j</sup>

The t-tests conducted for the optimised and control samples for each day along each row are all significantly different ( $p = 0.05$ ). Means with different letters along each column were significantly different at  $p < 0.05$ . (Mean  $\pm$  Std. deviation). MC: Moisture Content, PV: Peroxide Value, FFA: Free Fatty Acid, TBA: Thiobarbitric Acid

lower values of TBA compared to the control samples. The TBA values ranged from 5.51 to 14.92 mg MDA/kg in the control sample while for OHSSC, the values ranged from 4.50 to 13.77 mg MDA/kg. This observation showed that spiced and unspiced samples had the rancidity tendencies but the level was more in control samples than the spiced fish samples. The differences between the spiced and unspiced samples were significant throughout the storage period indicating the impact of the spices to minimise rancidity tendency in the catfish. These results are in line with TBA result documented for *Scomber japonicus* (Goulas & Kontominas, 2005) and *Argyrosomus regius* (Hernández et al., 2009). This observation also agrees with the findings of Famurewa et al. (2017) on storage of African catfish in freezer over a period of six weeks where increasing TBA was recorded with storage. The result indicates that spices are effective in retarding lipid oxidation. A previous report had also demonstrated that spices possess antioxidant and antimicrobial properties (Srinivasan, 2014).

### 3.3 Peroxide value of the stored catfish samples

The peroxide value as presented in Table 1 ranged from 0.23 to 2.87 mEq/kg for the control sample and 0.21 to 2.85 mEq/kg for the

OHSSC. Similar trends as recorded for TBA were also observed for the peroxide values in both the stored spiced and unspiced samples. The values increased as the storage period increased but the values for OHSSC were generally lower than that of the control sample. The differences ( $p \leq 0.05$ ) within the spiced sample or control sample, and between the spiced and unspiced samples were significant during the storage period. The spices and storage period had impact on the peroxide value as also noted in TBA. The peroxide value is an initial evidence of rancidity development in oils and fats, it is therefore inferential that the OHSSC would have reduced rate of primary peroxidation, compared to the control sample. This result is in agreement with report by Silva et al. (2008) which showed that ginger is effective in retarding rancidity in hot smoked catfish. It also agrees with the studies of Pakawatchai et al. (2009), that spices' activities as antioxidants are directly related to their concentration.

### 3.4 Free fatty acid of the stored catfish samples

The free fatty acid (FFA) values (Table 1) in Optimised Hot Smoked Spiced Catfish (OHSSC) ranged from 0.72 to 9.64% and from 2.25 to 11.88% in the control sample. The notable differences within each sample (OHSSC and control)

and between the control sample and OHSSC were significant during the storage period. The control sample had higher FFA values than OHSSC. This suggests that the addition of the local spices might have inhibited FFA production. Similar increasing trend in FFA was reported for smoked catfish stored in the freezer for six weeks (Famurewa et al., 2017). FFA content in a product can be an indicator of spoilage and sensory quality of the product (Clucas, 2006). It is used also to measure the rancidity of foods. The observed trend was similar to the other indices (TBA and PV) used to evaluate the rancidity tendencies in the stored catfish. The results further established the effectiveness of garlic, ginger and turmeric as antioxidants which are able to inhibit the synthesis of free fatty acid in the OHSSC stored for 20 days under ambient temperature.

### 3.5 Microbiological Characteristics

The microbial status of Optimised Hot Smoked Spiced Catfish (OHSSC) during storage is presented in Table 2. The TVC values ranged from 9.50 (after two days) to  $57.0 \times 10^2$  cfu/g (after twenty days) and from 13.50 (after two days) to  $113.00 \times 10^2$  cfu/g (after twenty days) for OHSSC and control, respectively. The relatively lower TVC values of OHSSC may be attributed to the antibacterial activities of the spices. Kumolu-Johnson and Ndimele (2011) had earlier reported lower microbial load of *Clarias gariepinus* treated with ginger paste. The microbial load is within the safe limit ( $<10^5$   $<10^7$  cfu/g) as reported by Frazier and Westhoff (1998) and FAO and WHO (2013). For mould count, the values were between 8.00 and  $10.00 \times 10^6$  cfu/g (day 2); and 35.00 and  $49.00 \times 10^6$  cfu/g (day 20) for OHSSC and control, respectively. There was an increasing trend in both TVC and MoC within the two samples and between the spiced and unspiced samples with increased storage period. However, the extent of increase of TVC between spiced and the control samples was more than 7-fold. For instance, between the 2<sup>nd</sup> and 4<sup>th</sup> day of storage there was about 95% increase in the OHSSC while the percentage increase in the control was about 737%. Gupta and Ravishankar (2005)

demonstrated the antibacterial activity of ginger, garlic and turmeric on *Escherichia coli* and the findings from this study also support this claim. Another study on the antibacterial activity of Allium plants including garlic has been documented (Benkeblia, 2004).

### 3.6 Sensory attributes of the OHSSC, Control and Commercial Smoked Catfish

The sensory attributes of the optimised smoked spiced catfish, control and commercial smoked catfish are presented in Table 3. Significant differences ( $p < 0.05$ ) existed among the three samples in almost all the parameters evaluated. From the table, it was clear that OHSSC product was the most preferred among the three samples based on their sensory scores. The mean sensory scores for aroma (7.44), texture (7.31), taste (7.51) and overall acceptability (7.47) of OHSSC were consistently higher than that of the control and commercial smoked catfish. The sensory evaluation showed over 80% preference for the optimised hot smoked spiced catfish. The higher preference for the spiced fish might be attributed to the inclusion of spices which have been demonstrated to enhance organoleptic quality of foods (Ade-Omowaye et al., 2015). This observation agrees with the findings of Kumolu-Johnson and Ndimele (2011) where panelists rated *Clarias gariepinus* treated with ginger paste better than untreated sample.

## 4 Conclusions

The anti-oxidative and antimicrobial properties of the garlic, ginger and turmeric spices were demonstrated on hot smoked spiced catfish with significant reduction in rancidity tendencies and total viable and mould growths during storage under ambient temperature for a period of 20 days. The low TBA and peroxide value, as well as reduced total viable and mould counts in the spiced catfish samples as compared to the unspiced sample are good indicators of the potentials of local spices in extending shelf life of catfish. Organoleptically, the general pattern of consumer preference of the products indicated that

Table 2: Microbial Load of the Control and Optimised Hot Spiced Smoked Catfish

Storage Period (Days)	TVC ( $\times 103$ )		MoC ( $\times 106$ )	
	Optimised (A)	Control (B)	Optimised (A)	Control (B)
0	-	-	-	-
2	0.950 $\pm$ 353.553 <sup>a</sup>	1.350 $\pm$ 212.132 <sup>a</sup>	8.000 <sup>ab</sup>	10.000 <sup>a</sup>
4	41.850 $\pm$ 70.711 <sup>b</sup>	11.300 $\pm$ 141.421 <sup>b</sup>	5.500 <sup>a</sup>	13.000 <sup>a</sup>
6	2.250 $\pm$ 212.132 <sup>bc</sup>	11.600 $\pm$ 144.214 <sup>bc</sup>	9.000 <sup>ab</sup>	15.500 <sup>a</sup>
8	2.550 $\pm$ 212.132 <sup>cd</sup>	12.950 $\pm$ 353.553 <sup>bcd</sup>	12.500 <sup>b</sup>	17.000 <sup>a</sup>
10	2.800 $\pm$ 282.843 <sup>d</sup>	13.200 $\pm$ 339.113 <sup>bcd</sup>	18.500 <sup>c</sup>	18.5.000 <sup>ab</sup>
12	2.950 $\pm$ 212.132 <sup>d</sup>	15.800 $\pm$ 565.685 <sup>cde</sup>	23.000 <sup>cd</sup>	19.5.000 <sup>ab</sup>
14	3.450 $\pm$ 353.553 <sup>e</sup>	16.850 $\pm$ 3323.402 <sup>def</sup>	25.000 <sup>d</sup>	28.000 <sup>bc</sup>
16	4.250 $\pm$ 70.711 <sup>f</sup>	18.250 $\pm$ 919.239 <sup>ef</sup>	31.000 <sup>e</sup>	35.000 <sup>cd</sup>
18	5.250 $\pm$ 353.553 <sup>g</sup>	19.250 $\pm$ 3464.823 <sup>ef</sup>	30.500 <sup>e</sup>	40.000 <sup>de</sup>
20	5.700 $\pm$ 141.421 <sup>g</sup>	20.350 $\pm$ 353.553 <sup>f</sup>	34.500 <sup>e</sup>	49.000 <sup>e</sup>

The t-tests conducted for the optimised and control samples for each day along each row are all significantly different ( $p = 0.05$ ). Means with different letters along each column were significantly different at  $p < 0.05$ . (Mean  $\pm$  Std. deviation). TVC: Total Viable Counts and MoC: Mould Counts

Table 3: Mean Sensory Attributes of the Control and Optimised Hot Smoked Spiced Catfish

Categories	Appearance	Aroma	Texture	Taste	After taste	Overall acceptability
OHSSC	7.48 $\pm$ 0.20 <sup>a</sup>	7.44 $\pm$ 0.18 <sup>a</sup>	7.31 $\pm$ 0.21 <sup>a</sup>	7.51 $\pm$ 0.13 <sup>a</sup>	7.53 $\pm$ 0.14 <sup>a</sup>	7.47 $\pm$ 0.30 <sup>a</sup>
CSC	7.52 $\pm$ 0.13 <sup>a</sup>	7.35 $\pm$ 0.11 <sup>b</sup>	6.61 $\pm$ 0.14 <sup>c</sup>	5.19 $\pm$ 0.11 <sup>c</sup>	6.67 $\pm$ 0.23 <sup>c</sup>	6.97 $\pm$ 0.23 <sup>c</sup>
CC	7.25 $\pm$ 0.24 <sup>b</sup>	6.12 $\pm$ 0.13 <sup>c</sup>	7.25 $\pm$ 0.07 <sup>b</sup>	7.25 $\pm$ 0.07 <sup>b</sup>	7.19 $\pm$ 0.11 <sup>b</sup>	7.31 $\pm$ 0.31 <sup>b</sup>

Means without a common superscript in a column are significantly different ( $p < 0.05$ ). (Mean  $\pm$  Std. deviation). (OHSSC) - Optimised Hot Smoked Spiced Catfish; (CSC) - Control smoked catfish; (CC) Commercial smoked catfish

the optimised spiced samples were most acceptable compared to the control and commercial catfish samples.

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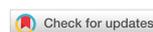
# Chemical Composition, Nutritional, Functional and Pasting Properties of Yellow Root Cassava Grits and African Yam Bean Flour Blends

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## Abstract

The effect of African Yam Bean (AYB) flour substitution on the nutritional, functional and pasting properties of yellow root cassava grits was investigated. Cassava grits were obtained by peeling, washing, cutting (5.5 cm thickness in cubes), soaking (72 h, 28±2 °C), dewatering, roasting (120 °C, 20 min), sieving and milling. Roasted AYB flour was obtained by cleaning, roasting (190 °C, 10 min), dehulling, milling and sieving (425 µm). Simple lattice design was used to generate different formulations, 100:0; 90:10; 80:20; 70:30; 60:40 and 0:100 of cassava grits and AYB flour, respectively. These were analyzed for chemical composition, amino acid profile, minerals, functional and pasting properties. Total ash, protein, β-carotene and hydrogen cyanide were in the ranges of 2.16-2.66%, 2.72-20.43%, 1.33 to 3.97 µg/g and 0.07-4.47 mg/kg, respectively. Total essential amino acids and total non-essential amino acids were in the ranges of 32.51-40.18% and 59.82-67.48%. Potassium, calcium, magnesium, iron, copper, zinc, manganese and sodium of the blends ranged from 338.00-646.75, 188.00-508.00, 358.00-532.50, 59.25-140.00, 0.12-0.19, 1.07-1.71, 7.25-38.25, 25.25-161.50 mg/100 g, respectively. Bulk density, water absorption capacity, swelling capacity and swelling index ranged from 0.67-0.81 g/ml, 151.05-503.29 g/ml, 1.67-5.68 g/g and 2.86-13.32%, respectively. The blends of yellow root cassava grits and African yam bean flour could provide nutritious food formulations and offer good potential for food security.

**Keywords:** Cassava grit; Yellow root cassava; African yam bean; Anti-nutrients; Malnutrition; Vitamin A

## 1 Introduction

Cassava (*Manihot esculenta* Crantz) is a starchy crop grown and consumed widely in tropical regions of Africa, Asia and Latin America (Esuma et al., 2019). Due to the high perishability of the fresh cassava tubers, they are usually processed and consumed as food products such as *gari*, *akpu (fufu)*, *chikwangue*, *lafun* (fermented

cassava flour), cassava cakes, tapioca and alcoholic drinks (Asonye, 2001). The various types of cassava meals provide about 65% of total calorie intake of the people in the lower economic ladder in Nigeria and sub-Saharan Africa (Asonye, 2001; Montagnac et al., 2009). However, consumption of cassava and its products has been implicated in malnutrition problems (Aykroyd et al., 1992; Falade & Akingbala, 2010; Osho, 2003).

African yam bean (*Sphenostylis stenocarpa*) is an underutilized leguminous plant crop that is relatively rich in protein and can supplement the protein requirements of many families throughout the year, especially low and medium income earners (Adebowale et al., 2009; Babarinde et al., 2019). AYB is a good source of protein, fibre, carbohydrate and minerals (Abioye et al., 2015; Baiyeri et al., 2018; Fasoyiro et al., 2006; Ojo et al., 2014). Research has revealed that one of the best ways to lessen nutritional, environmental and financial vulnerability in developing countries is by increasing the use of underutilized crops in the area where they are grown (Jaenicke & Pasiecznik, 2008). The contribution of these crops to food security has been reported to be of great significance (Naylor et al., 2004).

Sayre et al. (2011) reported that a typical cassava-based diet, provides less than 30% of the minimum daily requirement for protein and only 10%-20% of that for iron, zinc, and vitamin A. African yam bean is a highly nutritious legume, high in protein, mineral and fibre contents (Abioye et al., 2015). Also, Vitamin A-biofortified cassava varieties have been developed as a sustainable strategy to reduce the prevalence of vitamin A deficiency in areas where cassava is a staple food. However, information on its nutritional and health promoting potentials is limited (Oluba et al., 2017). Hence, the current trend in terms of acceptability and production of vitamin A-biofortified cassava is low when compared with the white cassava varieties in Nigeria. This study, therefore, investigated the effects of African yam bean supplementation on the nutritional, functional and pasting properties of yellow root cassava grits.

## 2 Materials and Methods

UMUCASS 37 variety of yellow root cassava used for this study is as shown in Figure 1. Fresh yellow cassava tubers were obtained from Ladoko Akintola University of Technology Teaching and Research farm, Ogbomoso, Oyo State Nigeria. African yam bean (AYB) seeds were obtained from a local market in Oyo State and identified at the LAUTECH teaching and research farm. African yam bean seeds are as shown in Figure

2.

### 2.1 Production of Cassava grits and African yam bean flour

Cassava grits were produced from yellow root cassava using the method of Sanni and Jaji (2003) with some modifications as shown in Figure 3a while African yam bean flour was produced as described by Aniedu and Aniedu (2014) as shown in Figure 3b.

### 2.2 Supplementation of yellow root cassava grits with African yam bean flour

Simplex lattice design was used to obtain different formulations from blends of yellow root cassava grit and AYB flour. The formulations were produced with (60-100%) cassava grit and (0-40%) AYB flour, while 100% AYB flour and 100% cassava grits were used as controls. The blends were thoroughly mixed for about 20 minutes using a Kenwood mixer (Model: Chef XL KVL4100S, made in China) to achieve uniform blending. The samples were then taken into the laboratory for further analyses.

### 2.3 Analyses

Protein and total ash content of the blends were determined as described by the standard methods of AOAC (1990). The method of Rodriguez-Amaya and Kimura (2004), using extraction with acetone, was used to determine the  $\beta$ -carotene content of the flour blends. Hydrogen cyanide was determined by the simple picrate method as described by Nwokoro et al. (2009). The method of AOAC (1990) was used for the mineral analysis of the samples, which were previously ashed in a furnace for 5 h at 600 °C, and then refluxed with 20% hydrochloric acid. The mixture was filtered into a 100 mL standard flask; the filtrate was then made up to the mark with deionized water. The resulting digest was filtered with Whatman No. 1 filter paper. Filtrate from each sample was analyzed for mineral (potassium, calcium, magnesium, iron,



Figure 1: Yellow root cassava



Figure 2: African yam bean seeds

copper, zinc, manganese and sodium) contents using an Atomic Absorption Spectrophotometer (AA Analyse Perkin Nerma) at standard wavelengths. The amino acid composition of the samples was measured on hydrolysates using an amino acid analyzer (Sykam-S7130) based on the

high performance liquid chromatography technique according to the method of Moore and Stein (1963). The bulk density of the samples was determined as described by Onwuka (2005). The water absorption capacities of the formulated samples were determined using the method described by Onwuka (2005). The methods described by Hirsch and Kokini (2002) were used to determine the swelling power and solubility index. Pasting properties of the blends were determined using a Rapid Visco Analyzer (Model RVA-4; Newport Scientific Pty. Ltd, Warriewood, Australia) as described in the Newport Scientific report (1998). All analyzes were performed in triplicate.

## 2.4 Statistical Analysis

All analyses were performed in triplicate and data obtained were subjected to Analysis of Variance (ANOVA). The means were separated using Duncan's multiple range test to detect significant difference ( $p < 0.05$ ) among the samples.

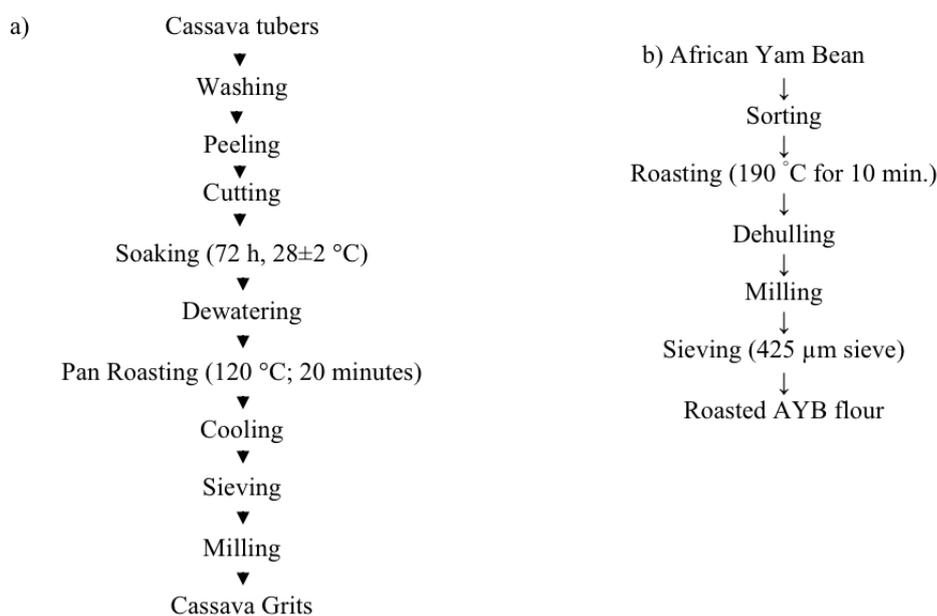


Figure 3: a) Flow chart for the preparation of yellow root cassava grits (Source: Sanni and Jaji (2003); and b) flow chart for the production of AYB flour (Source: Aniedu and Aniedu (2014)).

### 3 Results and Discussion

#### 3.1 Nutritional Properties of cassava grits and AYB flour blends

The amino acid profile of the samples is as shown in Table 1. Essential amino acids identified in AYB flour were valine, threonine, phenylalanine, histidine, isoleucine, leucine, lysine, methionine and tryptophan. The AYB flour is rich in leucine and glutamate. There were significant ( $p < 0.05$ ) differences in the leucine content among different blends of cassava grits and African yam bean flour except for the blends with 80:20 and 70:30, of cassava grits and African yam bean flour, respectively. All essential amino acids are significantly present in the blended samples. This is in line with the reports of other researchers that AYB flour contain all the essential amino acids (Atinuke, 2015; Esan & Fasasi, 2013; Oshodi et al., 1995). However, tryptophan is relatively low when compared to other essential amino acids

and significantly different ( $p < 0.05$ ) among the samples except with samples made from 100:0 and 80:20, of cassava grits and African yam bean flour, respectively. The amino acid content in the formulations increased with increase in level of AYB flour substitution. The percentage of essential amino acids increased from 32.51% in 100% cassava grits to 39.56% in 40% AYB flour substitution. The total essential amino acids of the flour blends ranged from 37.6% to 39.56%. This is higher than the 36% considered for an ideal protein (FAO, 2007; Iyenagbe et al., 2017) but slightly lower than the egg reference of 51.2%. The most abundant amino acid in all the samples evaluated was glutamic acid. Ade-Omowaye et al. (2015) also reported that glutamic acid was the most abundant amino acid in some legumes that were evaluated. The most concentrated essential amino acid in all the formulations was leucine, which increased as the level of substitution of AYB increased from 3.6 g/kg in 100% cassava grit to 7.01g/kg in the sample substituted with 40% AYB flour. Leucine is a nutritionally essential branched-chain amino acid in animal

Table 1: Amino acid profile of yellow root cassava grits and African yam bean flour blends (g/kg)

Amino Acid	Type	100% CG	100% AYB	90%CG 10%AYB	80%CG 20%AYB	70%CG 30%AYB	60%CG 40%AYB
Glycine	N	2.310±0.127 <sup>a</sup>	3.370±0.099 <sup>d</sup>	2.730±0.042 <sup>b</sup>	2.920±0.028 <sup>c</sup>	2.980±0.014 <sup>c</sup>	3.310±0.014 <sup>d</sup>
Alanine	N	3.630±0.042 <sup>a</sup>	4.750±0.070 <sup>d</sup>	4.060±0.085 <sup>b</sup>	4.520±0.028 <sup>c</sup>	4.460±0.085 <sup>c</sup>	4.190±0.127 <sup>b</sup>
Serine	N	1.640±0.057 <sup>a</sup>	3.560±0.014 <sup>d</sup>	2.820±0.028 <sup>b</sup>	3.250±0.071 <sup>c</sup>	3.320±0.028 <sup>c</sup>	2.830±0.042 <sup>b</sup>
Proline	N	2.070±0.099 <sup>a</sup>	4.060±0.085 <sup>d</sup>	3.200±0.283 <sup>b</sup>	3.420±0.283 <sup>bc</sup>	3.540±0.057 <sup>c</sup>	3.940±0.056 <sup>d</sup>
Valine	E	1.620±0.028 <sup>a</sup>	3.040±0.056 <sup>c</sup>	2.760±0.085 <sup>b</sup>	2.780±0.113 <sup>b</sup>	2.820±0.028 <sup>b</sup>	4.150±0.071 <sup>d</sup>
Threonine	E	2.730±0.042 <sup>a</sup>	2.620±0.028 <sup>ab</sup>	2.540±0.057 <sup>a</sup>	2.560±0.000 <sup>a</sup>	2.650±0.071 <sup>ab</sup>	2.620±0.028 <sup>ab</sup>
Isoleucine	E	1.550±0.071 <sup>a</sup>	2.620±0.028 <sup>ab</sup>	2.540±0.057 <sup>a</sup>	2.560±0.000 <sup>a</sup>	2.650±0.071 <sup>ab</sup>	2.620±0.028 <sup>ab</sup>
Leucine	E	3.620±0.028 <sup>a</sup>	8.760±0.028 <sup>d</sup>	7.060±0.085 <sup>b</sup>	7.770±0.099 <sup>c</sup>	7.700±0.000 <sup>c</sup>	7.040±0.056 <sup>b</sup>
Aspartate	N	8.140±0.056 <sup>d</sup>	8.760±0.028 <sup>e</sup>	7.940±0.057 <sup>c</sup>	6.860±0.085 <sup>b</sup>	6.920±0.028 <sup>b</sup>	5.820±0.028 <sup>a</sup>
Lysine	E	3.560±0.085 <sup>c</sup>	6.860±0.028 <sup>d</sup>	3.200±0.283 <sup>b</sup>	3.540±0.057 <sup>c</sup>	3.510±0.014 <sup>bc</sup>	2.860±0.085 <sup>a</sup>
Methionine	E	1.070±0.099 <sup>a</sup>	1.640±0.057 <sup>c</sup>	1.450±0.071 <sup>b</sup>	1.440±0.056 <sup>b</sup>	1.440±0.056 <sup>b</sup>	2.650±0.070 <sup>d</sup>
Glutamate	N	13.600±0.141 <sup>a</sup>	15.770±0.099 <sup>c</sup>	15.160±0.141 <sup>b</sup>	15.760±0.085 <sup>c</sup>	15.300±0.424 <sup>bc</sup>	17.390±0.014 <sup>d</sup>
Phenylalanine	E	3.470±0.099 <sup>a</sup>	4.740±0.056 <sup>c</sup>	3.730±0.042 <sup>b</sup>	4.620±0.282 <sup>c</sup>	4.060±0.085 <sup>b</sup>	5.030±0.042 <sup>d</sup>
Histidine	E	2.480±0.014 <sup>a</sup>	3.680±0.028 <sup>d</sup>	3.270±0.07 <sup>c</sup>	3.060±0.08 <sup>b</sup>	3.740±0.000 <sup>b</sup>	2.540±0.057 <sup>a</sup>
Arginine	N	8.898±0.007 <sup>f</sup>	5.850±0.071 <sup>b</sup>	7.500±0.000 <sup>e</sup>	6.150±0.071 <sup>c</sup>	6.510±0.14 <sup>d</sup>	5.100±0.141 <sup>a</sup>
Tyrosine	N	1.530±0.042 <sup>a</sup>	2.470±0.099 <sup>b</sup>	2.290±0.127 <sup>b</sup>	2.320±0.023 <sup>b</sup>	2.350±0.707 <sup>b</sup>	3.780±0.113 <sup>c</sup>
Tryptophan	E	0.150±0.071 <sup>a</sup>	0.360±0.084 <sup>b</sup>	0.300±0.000 <sup>ab</sup>	0.340±0.057 <sup>b</sup>	0.550±0.071 <sup>c</sup>	1.050±0.071 <sup>d</sup>
Cystine	N	0.850±0.071 <sup>c</sup>	0.800±0.000 <sup>b</sup>	0.770±0.099 <sup>a</sup>	0.740±0.566 <sup>a</sup>	0.770±0.099 <sup>a</sup>	7.900±0.042 <sup>ab</sup>
Total AA		63.33	79.35	74.35	75.11	75.64	80.50
Total EAA		20.59	31.88	27.94	29.15	29.52	31.84
Total NEAA		42.73	47.47	46.41	45.96	46.12	48.66

Mean values with different superscript along the rows are significantly different from each other ( $p < 0.05$ )  
 AA - Amino acid, EAA - Essential amino acid, NEAA - Non essential amino acid, CG - cassava grit, AYB flour - African yam bean flour.

nutrition and it is usually one of the most abundant amino acids in high-quality protein foods (Duan et al., 2016).

### 3.2 The mineral content of cassava grits and AYB flour blends

The mineral content of cassava grits and AYB flour blends is as shown in Table 2. Higher values were recorded for potassium, calcium, magnesium, sodium and iron while lower values were recorded for copper, zinc and manganese in AYB flour. The calcium content obtained for the samples ranged between 188.02 to 508.00 mg/100 g and the values were significantly ( $p < 0.05$ ) different from each other. The calcium content of all the samples increased as the level of substitution of AYB flour increased. This may be attributed to the high calcium content of AYB flour as reported by other researchers (Anya & Ozung,

2019; Atinuke, 2015; Ndidi et al., 2014). The current recommended nutrient intake (RNI) for calcium is 600 mg a day for children and 1000 mg a day for adults (FAO, 2002), indicating that these food products could help in meeting the daily recommended intake. The high calcium content in these food products could help bone and teeth development in infants and youths. The iron content of the blended samples ranged from 59.25 to 140.00 mg/100 g, which increased with increasing addition of AYB flour. The potassium content of the blended samples ranged from 338.00 to 646.75 mg/100 g, which is line with the value reported for African yam bean flour (Atinuke, 2015; Ene-Obong & Carnovale, 1992; Oshodi et al., 1995). This may be attributed to the high potassium content in AYB flour. The copper and the zinc contents of the blended samples ranged from 0.12-0.19 mg/100 g and 1.07-1.71 mg/100 g, respectively.

Table 2: Mineral content of yellow root cassava grits and African yam bean flour blends (mg/100 g)

Sample	K	Ca	Mg	Fe	Cu	Zn	Mn	Na
100%CG	338.00 <sup>a</sup>	188.0 <sup>a</sup>	358.00 <sup>a</sup>	59.25 <sup>a</sup>	0.14 <sup>c</sup>	1.26 <sup>c</sup>	7.25 <sup>a</sup>	25.25 <sup>a</sup>
100%AYB	646.75 <sup>f</sup>	508.00 <sup>f</sup>	532.50 <sup>e</sup>	140.00 <sup>e</sup>	0.19 <sup>f</sup>	1.71 <sup>f</sup>	38.25 <sup>f</sup>	161.50 <sup>f</sup>
90%CG10%AYB	402.50 <sup>b</sup>	353.25 <sup>b</sup>	477.20 <sup>b</sup>	60.45 <sup>a</sup>	0.12 <sup>a</sup>	1.07 <sup>a</sup>	10.50 <sup>b</sup>	45.00 <sup>b</sup>
80%CG20%AYB	440.50 <sup>c</sup>	389.00 <sup>c</sup>	490.20 <sup>c</sup>	80.75 <sup>b</sup>	0.13 <sup>b</sup>	1.18 <sup>b</sup>	13.00 <sup>c</sup>	61.25 <sup>c</sup>
70%CG30%AYB	510.00 <sup>d</sup>	422.50 <sup>d</sup>	511.00 <sup>d</sup>	96.00 <sup>c</sup>	0.15 <sup>d</sup>	1.38 <sup>d</sup>	16.65 <sup>d</sup>	74.25 <sup>d</sup>
60%CG40%AYB	585.00 <sup>e</sup>	492.75 <sup>e</sup>	533.70 <sup>e</sup>	103.25 <sup>d</sup>	0.16 <sup>e</sup>	1.55 <sup>e</sup>	18.90 <sup>e</sup>	93.95 <sup>e</sup>

Mean values with different superscript along the same column are significantly different from each other ( $p < 0.05$ ). CG - cassava grit, AYB flour - African yam bean flour

### 3.3 The chemical composition of cassava grits and AYB flour blends

The chemical composition of the flour blends is as shown in Figure 4. The total ash (mineral content) ranged from 2.16 to 2.66% with the highest value recorded in 100% AYB flour. There was a gradual increase in the mineral content of yellow cassava grits with increase in AYB substitution. This confirms the report of other researchers that AYB is an excellent source of minerals (Anya & Ozung, 2019; Ekpe et al., 2018; Mbah et al., 2015; Ndidi et al., 2014). The protein content of the samples ranged from 2.72 to 20.43% with the highest protein content recorded in 100% AYB flour and there was significant ( $p < 0.05$ ) difference in the blends with increase in AYB substitution. There was an increase of about 43% in protein content of yellow root cassava grits with 40% AYB flour substitution. Mbah et al. (2015) also reported an increase in yam pottage with increase in AYB flour substitution. This is a reflection of the protein content of AYB flour which is in line with the reports of other researchers (Ajibola & Olapade, 2021; Anya & Ozung, 2019). The values of beta-carotene are in the range of 1.33 to 3.97  $\mu\text{g/g}$ . The hundred percent yellow root cassava grit had the highest value of beta-carotene due to the variety of the cassava used which was enriched with beta-carotene. The value obtained is within the range reported for food products obtained from bio-fortified yellow root cassava (Odoemelam et al., 2020). As the substitution level of AYB flour increased, the

beta-carotene content of the blended samples decreased. Consumption of these blended samples could serve as a contributor to the daily intake of Vitamin A. Hydrogen cyanide levels ranged from 0.07 to 4.47 mg/kg. JECFA (1995) reported that a level of up to 10 mg hydrogen cyanide/kg is the Codex Standard for cassava flour and it's not associated with acute toxicity (Mburu et al., 2012). This indicates that the formulated samples are within the safe level and will pose no hazard to the consumers.

### 3.4 Functional properties of cassava grits and AYB flour blends

The functional properties of the flour blends are as shown in Table 3. The functional properties indicate how a food material will interact with other food components which directly or indirectly affect the processing, application, food quality, and ultimate acceptance of the food material (Awoyale et al., 2021). The bulk density of the flour blends ranged between 0.67-0.81 g/ml, and there was a gradual increase with increase in AYB flour substitution. Samples made with 100% cassava grits, 100% AYB and 90% cassava grits and 10%AYB were significantly ( $p < 0.05$ ) different while there was no significant differences among the samples substituted with 10-30% AYB. Bulk density measures the heaviness of solid samples, which is important in determination of packaging requirements, material handling and its application in the food industry (Falade & Okafor, 2015; Oladele & Aina, 2007).

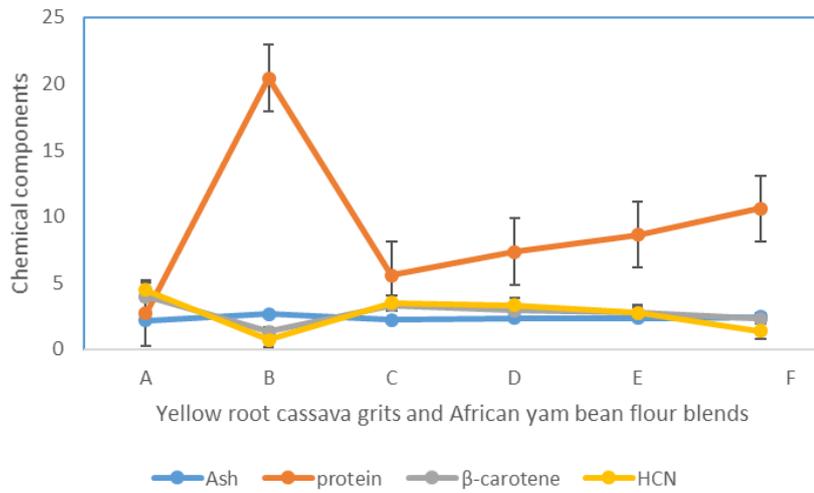


Figure 4: Chemical composition of yellow root cassava grits and AYB flour blends. X-axis Categories: A =100% Yellow root cassava grit, B = 100% AYB flour, C = 90% Yellow root cassava grit and 10% AYB, D = 80% Yellow root cassava grit and 20% AYB, E =70% Yellow root cassava grit and 30% AYB, F = 60% Yellow root cassava grit and 40% AYB.

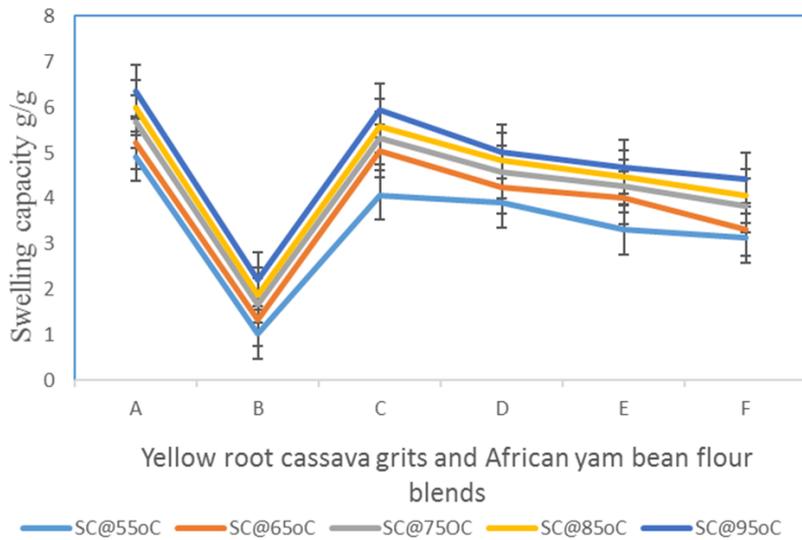


Figure 5: Swelling capacity of yellow root cassava grits and AYB flour blends at different temperatures. X-axis Categories: A =100% Yellow root cassava grit, B = 100% AYB flour, C = 90% Yellow root cassava grit and 10% AYB, D = 80% Yellow root cassava grit and 20% AYB, E =70% Yellow root cassava grit and 30% AYB, F = 60% Yellow root cassava grit and 40% AYB. Legend: SC@55 °C - Swelling capacity at 55 °C, SC@65 °C - Swelling capacity at 65 °C, SC@75 °C - Swelling capacity at 75 °C, SC@85 °C - Swelling capacity at 85 °C, SC@95 °C - Swelling capacity at 95 °C

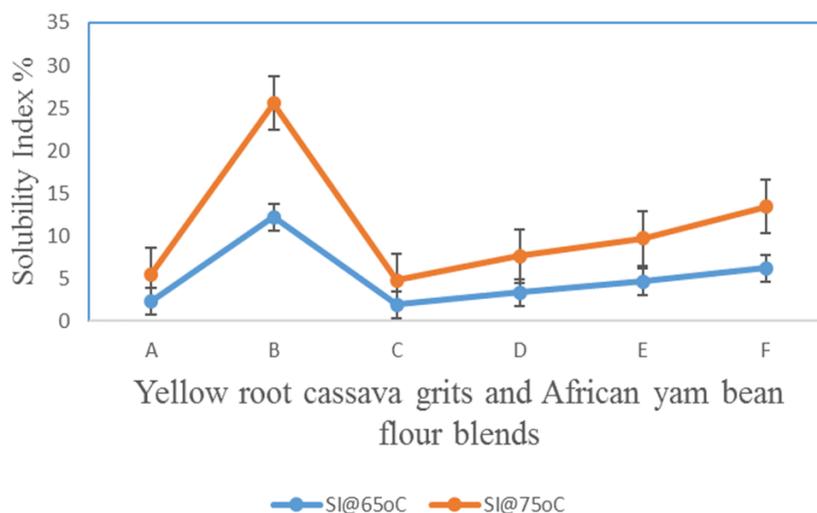


Figure 6: Solubility index of yellow root cassava grits and AYB flour blends at different temperatures. X-axis categories: A = 100% Yellow root cassava grit, B = 100% AYB flour, C = 90% Yellow root cassava grit and 10% AYB, D = 80% Yellow root cassava grit and 20% AYB, E = 70% Yellow root cassava grit and 30% AYB, F = 60% Yellow root cassava grit and 40% AYB. Samples: SI@65 °C - Swelling capacity at 65 °C, SI@75 °C - Swelling capacity at 75 °C

Table 3: Functional properties' of yellow root cassava grits and African yam bean flour blends

Sample	Bulk Density (g/ml)	WAC (g/ml)
100%CG	0.70±0.01 <sup>b</sup>	503.29±0.99 <sup>f</sup>
100%AYB	0.67±0.00 <sup>a</sup>	151.05±0.41 <sup>a</sup>
90%CG10%AYB	0.77±0.00 <sup>c</sup>	456.97±1.59 <sup>e</sup>
90%CG10%AYB	0.80±0.01 <sup>d</sup>	430.50±0.37 <sup>d</sup>
90%CG10%AYB	0.81±0.00 <sup>d</sup>	378.37±1.23 <sup>c</sup>
90%CG10%AYB	0.81±0.00 <sup>d</sup>	337.27±27.62 <sup>b</sup>

Mean values with different superscript along the same column are significantly different from each other ( $p < 0.05$ ). CG - cassava grit, AYB flour - African yam bean flour, WAC - Water Absorption Capacity

Table 4: Pasting properties of yellow root cassava grits and African yam bean flour blends

Sample	Peak viscosity (RVU)	Trough (RVU)	Break down (RVU)	Final viscosity (RVU)	Set back (RVU)	Peak time (min)	Pasting temp (°C)
A	3234.00 <sup>f</sup>	3000.00 <sup>f</sup>	266.50 <sup>d</sup>	3985.00 <sup>f</sup>	897.00 <sup>e</sup>	6.40 <sup>d</sup>	74.58 <sup>a</sup>
B	1060.00 <sup>a</sup>	1017.50 <sup>a</sup>	42.50 <sup>a</sup>	1276.50 <sup>a</sup>	259.00 <sup>a</sup>	7.00 <sup>d</sup>	84.80 <sup>c</sup>
C	2618.00 <sup>e</sup>	2380.00 <sup>e</sup>	238.50 <sup>b</sup>	3077.00 <sup>e</sup>	697.00 <sup>d</sup>	6.15 <sup>b</sup>	81.48 <sup>b</sup>
D	2454.00 <sup>d</sup>	2105.00 <sup>d</sup>	248.50 <sup>d</sup>	2676.00 <sup>d</sup>	570.50 <sup>d</sup>	5.85 <sup>a</sup>	81.83 <sup>b</sup>
E	2297.00 <sup>d</sup>	1949.50 <sup>d</sup>	247.50 <sup>d</sup>	2479.00 <sup>d</sup>	529.50 <sup>d</sup>	5.80 <sup>a</sup>	81.48 <sup>b</sup>
F	1942.00 <sup>b</sup>	1712.00 <sup>b</sup>	225.00 <sup>b</sup>	2087.50 <sup>b</sup>	370.50 <sup>b</sup>	5.85 <sup>a</sup>	81.95 <sup>b</sup>

Mean values with different superscript along the same column are significantly different from each other ( $p < 0.05$ ). Keys: A - 100% Cassava grit, B - 100% AYB flour, C - 90% Cassava grit and 10% AYB, D - 80% Cassava grit and 20% AYB, E - 70% Cassava grit and 30% AYB, F - 60% Cassava grit and 40% AYB

The change in bulk density is generally affected by the particle size and the density of the flour (Adeoye et al., 2020). Aniedu and Aniedu (2014) reported that when the bulk density is higher, the quality of fufu meals will be better when reconstituted in boiling water. This is because high density determines the ability of the flour to disperse easily when reconstituted in hot water. This indicates a better quality of the meals with AYB substitution. Water absorption capacity of the flour samples ranged from 151.05 to 503.29 g/ml and there were significant differences among all the samples. The lowest value was observed in AYB flour, while cassava grit had the highest value (503.05 g/ml), and there was a significant ( $p < 0.05$ ) difference among the blends. The values obtained are within the range (231-610%) reported for cassava grits (Eke et al., 2010; Sanni et al., 2004). Water absorption capacity is important in determining the capacity of flour to take up water and swell to improve uniformity in food. It is also advantageous in food processing for improving yield, uniformity, and giving shape to food products (Ngoma et al., 2019). The swelling capacity and the solubility index are as shown in Figure 5 and Figure 6, respectively. The swelling capacity ranged between 1.33-6.34 g/g and there was an increase with increase in temperature. The highest value was recorded for 100% cassava grits (6.43 g/g) at 95 °C while 100% AYB flour had the least value (1.33 g/g) at 95 °C. Inclusion of AYB flour in cassava grits reduced the swelling capacity of the blends. Starch swells on heating in water and the

extent of swelling depends on the type of starch. The swelling power of an aqueous suspension of starch is an indication of the strength of the hydrogen bonding between the granules (Eke et al., 2010; Safo-Kantanka & Acquistucci, 1995). This implies that the hydrophilic tendency of the samples decreased with increase in AYB supplementation level. This decrease might also be as a result of the presence of naturally occurring non-carbohydrates such as lipids, protein and others which could restrict swelling. This restriction occurs when amylase lipid complexes are formed (Odunlade et al., 2016), while on the other hand there was an increase in solubility in all the supplemented samples as temperature increased.

### 3.5 Pasting properties of cassava grits and AYB flour blends

The pasting properties of the flour blends are as shown in Table 4. The peak viscosity ranged between 1060-3234 Rapid Visco Units (RVU). The sample with 100% cassava grits recorded the highest value while the lowest value was recorded with AYB flour. There was a gradual decrease in the peak viscosity with increase in the level of AYB substitution. Higher peak viscosity was recorded with the higher proportion of cassava grits which could be attributed to the higher degree of swelling of cassava starch granules and the amylose content (Chisenga et al., 2019). Peak viscosity is an indication of the thickening power of the starch and the higher the peak viscos-

ity, the higher the thickening power (Chinma et al., 2013; Ironi et al., 2019; Offia-Olua, 2014). The value obtained for the trough ranged between 1017.50 and 3000 RVU, the highest value was observed in 100% cassava grit and the least value was observed in the AYB flour. There was a gradual decrease with increase in the level of AYB flour substitution. Breakdown viscosity ranged between 42.50 and 266.50 RVU with a gradual decrease with increase in the level of AYB substitution. The low breakdown viscosity exhibited by the blends is an indication of their ability to withstand breakdown during heating and shearing (Ocheme et al., 2018). The final viscosity ranged between 1276.50 and 3985.00 RVU and higher values were in blends with a higher proportion of cassava grits. The peak viscosity, trough, breakdown viscosity and the final viscosity all generally increased with a higher proportion of cassava grits in the blend which is in line with other reports (Ocheme et al., 2018). The setback value of the samples ranged between 259 and 897 RVU, with cassava grit having the highest (897 RVU) and AYB the lowest (259 RVU). There was a gradual decrease in the setback value with increase in the level of AYB inclusion. This indicates that the higher the level of inclusion of AYB flour, the lower the setback value and hence higher resistance to retrogradation. Pasting temperature of the flour blends ranged between 74.58 and 81.95 °C. One hundred percent Cassava grit had the lowest pasting temperature which is in line with other researchers (Bassey & Dosunmu, 2003; Onyeneke, 2019). Lower pasting temperature, with inclusion of AYB flour, will form pastes much easier.

#### 4 Conclusions

This study has established that inclusion of AYB flour has the potential to improve the nutritional, functional and pasting properties of yellow root cassava grits. The substitution increased the micronutrients such as calcium and potassium of the yellow root cassava grits. The protein quantity and quality of yellow root cassava also increased while the setback viscosity and retrogradation potentials of the blends reduced. The blends of yellow root cassava grits and African

yam bean flour could provide nutritious food formulations and offer good potential for food security.

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# Practical Reflection and Benefits of Making a Food Garden at Home During Covid-19 Pandemic

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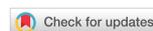
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## Abstract

The partial lockdown during the Covid-19 pandemic in Indonesia pushed people to work from and spend more time at home. During this unprecedented time, many people pursued new hobbies in gardening, which proved to enhance physical and mental health. With anxieties regarding food insecurity, food gardens became a new urban trend. With a relatively tiny space available, it is possible to make an urban food garden in the front yard of a house using various cultivation techniques to maximize space. However, the implementation of food gardens in urban houses is quite challenging due to limited space. Then, we reflect on the practical process and personal benefits gained from developing a tiny food garden at home. The tiny food garden could produce a variety of vegetables and herbs, such as the spinach family, lettuce, Asian greens, the tomato family, eggplants, the basil family, mint, rosemary, moringas, and butterfly-pea flowers. It may support a household with few amounts of fresh emergency food in the worst scenario during the Covid-19 pandemic. Besides, developing a tiny food garden at home may also provide co-benefits such as enhanced subjective well-being, increased appreciation of food and the environment, motivating others to start gardening at home, and great personal satisfactions of consuming home-grown food. With all these socio-ecological co-benefits, home food garden must be integrated as a strategy to achieve urban sustainability and increase household food resilience.

**Keywords:** Covid-19; Food garden; Kitchen garden; Urban farming; Urban gardening

## 1 Introduction

The partial lockdown during Covid-19 pandemic in Indonesia pushed people to work from and spend more time at home. The stay-at-home orders during Covid-19 have affected mental health concerns related to anxieties, negative thoughts, and insomnia (Jacobson et al., 2020). During this unprecedented time, many people pursued a new hobby in gardening. Regular gardening activities proved to enhance physical and mental health (Soga et al., 2017). Moreover, engaging with nature through gardening could

improve psychological and physical well-being through biophilic features and high-intensity activities (Scott et al., 2020). Gardening may also improve human cognitive functions, such as better memory performance (Park et al., 2019).

The pandemic is predicted to disturb the urban food systems (Reardon et al., 2020) and threaten the food supply in urban areas (Carey et al., 2020). Furthermore, concerns arise regarding the possibility of Covid-19 transmission through the food supply chain (Rizou et al., 2020). Anxieties of food insecurity and disrupted food supply in the market during pandemic Covid-19 raise a

concern and demand that families be more resilient in the food supply. Therefore, we took the initiative to convert the tiny urban house yard into a tiny food garden to grow a small amount of fresh emergency food and improve household food resilience. Literature has suggested that home gardening and urban farming are important strategies to a more resilient household food supply and further benefit the environment, economy, and ecosystem services (Galhena et al., 2013; Jacobs et al., 2016; Lal, 2020; Spilková & Vágner, 2018).

Some authors have discussed the importance and benefits of urban gardens (Altieri & Nicholls, 2020; Pulighe & Lupia, 2020) and home gardens (Lal, 2020; Montefrio, 2020) for enhancing food security in urban areas during pandemics. But there is a limited number of articles discussing the experiences of developing urban food gardens during the Covid-19 pandemic. As far as observed, only one article discusses the experiences of making a food garden during the Covid-19 pandemic in a Mediterranean country (Sofa & Sofa, 2020). Inspired by their work, this article aims to contribute new findings to the literature about the experiences of making an urban food garden and its benefits during a pandemic situation.

## 2 Method and Process

### 2.1 Identification of Available Space and Sunlight

The initial preparation for setting up a food garden is by observing the site. The site is situated in a tropical city setting called Yogyakarta, Indonesia. Yogyakarta is located at latitude -7.797068, and longitude 110.370529. The characteristic of the climate is hot and humid. The city has rainy seasons from October to April and dry seasons from April to October. The preparation of the food garden started at the end of May, and the planting intensively began in June. Living in a small urban house with a tiny front yard has its challenges, even more so when it comes to building a food garden. Various studies explain that limited space is one of the biggest challenges of urban gardening (Glatron & Gran-

champ, 2018; Lu & Grundy, 2017; Ranasinghe, 2009). The observation found that the house only provides 7 square meters of available space for gardening. The area is located in the front yard of the building. The front area of the house is facing North, thus a good amount of sunlight during the day is available (Figure 1). Sunlight is an essential aspect of food plant cultivation as it contributes to photosynthesis (Hemenway, 2001). Then, the available site is planned into several cultivation strategies and types of plants, based on sun exposure throughout the day and space availability.

### 2.2 Maximising Space and Cultivation Techniques

With limited space available, strategies to maximize space are needed (see Figure 2). Most techniques require containers in various shapes and sizes. Basic containers and vertical cultivation are an efficient technique for limited building space (Lu & Grundy, 2017). As containers are made of plastic, we reuse them as long as possible. Besides containers, other cultivation techniques are still feasible (Al-Mayahi et al., 2019) such as raised beds and in-ground beds (see Figure 3). The practical reflection in practicing various cultivation techniques for maximizing space is elaborated in Table 1.

An initial financial outlay is needed to build a kitchen garden. The cost estimation and materials are divided based on cultivation techniques (see Table 2). Some of the listed materials are free resource. It should be noted that the estimated prices here may differ from prices in other cities or countries. In addition, during unstable pandemic conditions, it is preferable to buy materials from online marketplaces or find the nurseries closest to the location of the garden and always wear a mask.

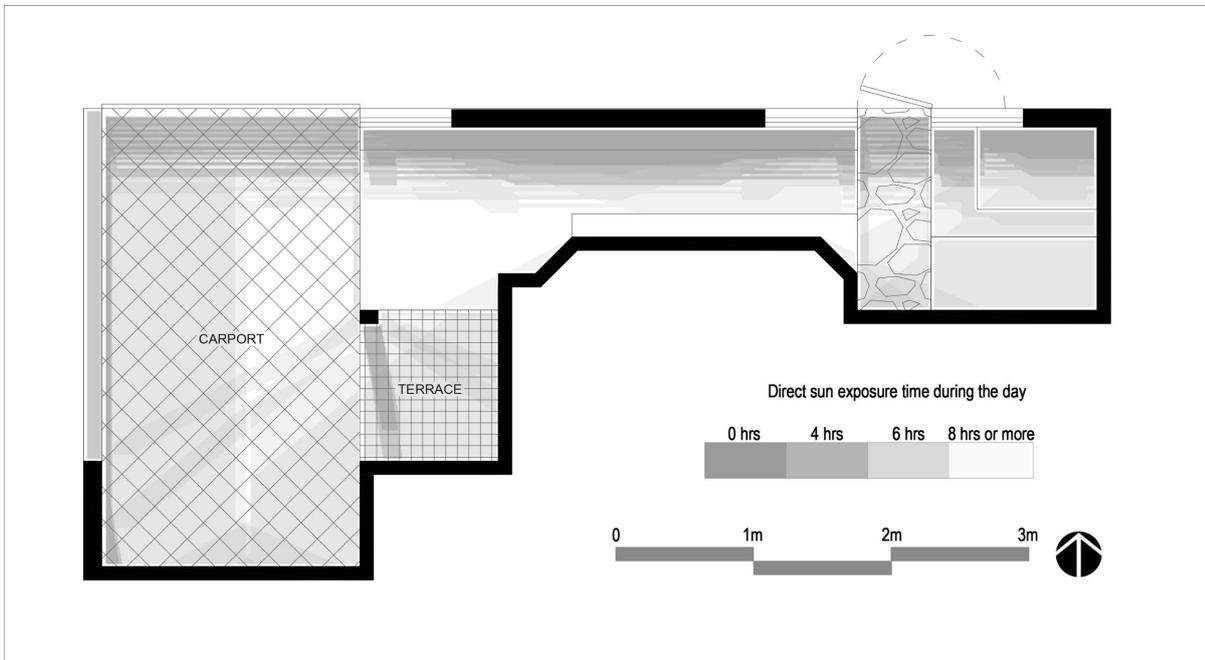


Figure 1: The area exposed to direct sunlight during June - August.

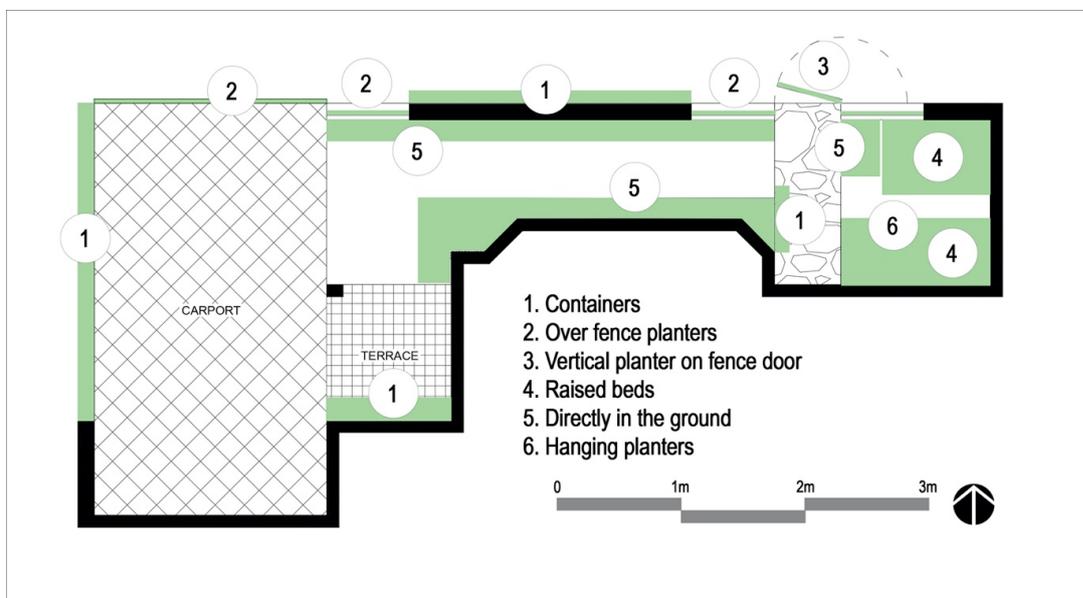


Figure 2: An Illustration of cultivation techniques for a small space.

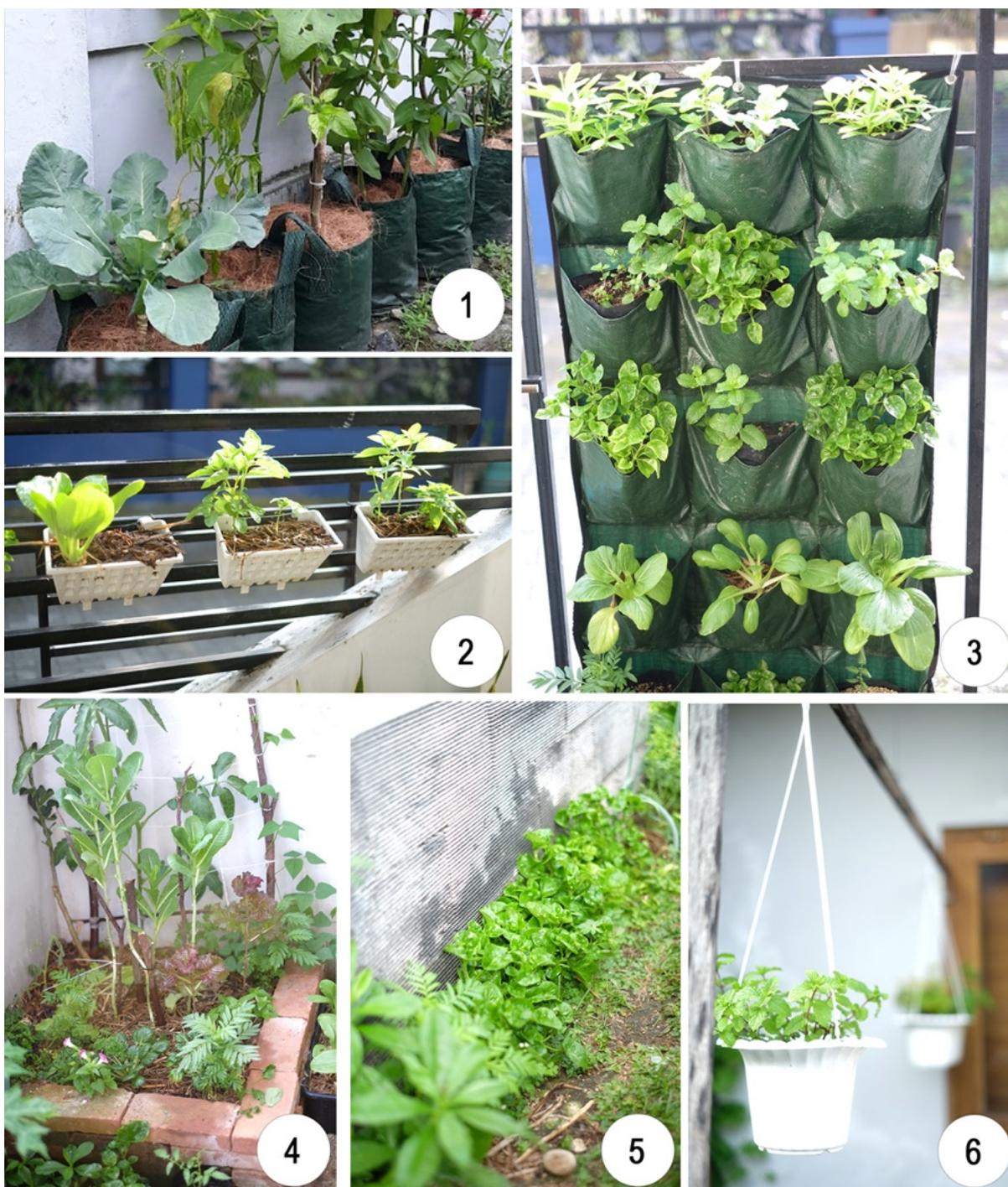


Figure 3: Cultivation techniques adopted for the kitchen garden; 1) containers, 2) over fence planters, 3) vertical planters on fence door, 4) raised bed, 5) directly in the ground, 6) hanging planters.

Table 1: Cultivation techniques for maximizing space for a food garden

No.	Cultivation Techniques	Practical reflection	Examples	Plants
1	Basic Containers	Containers are the most common and practical method to maximize the limited yard. They are available in various shapes, sizes, and colours to fit the colour tones and design space.	Pots; polybags; planter bags	Tomatoes, chili peppers, spinach, basil, rosemary, cilantro, kale
2	Over fence planters	Over fence planters can maximize the unused vertical fence space for vines and herbs.	Hooked pots	Basil, butterfly-pea flowers, betel leaf plants
3	Vertical planters	Vertical planters can maximize wall and fence space. Plants like herbs and small Asian greens are the most suitable for this method.	Pocket planters	Spinach, mint, Asian greens
4	Raised beds	Raised beds need certain ground space to build on. They also require a large number of materials for filling; however, we anticipate using the hügelkultur method (layered organic materials) to fill half of the raised beds. Raised beds are useful to anticipate pests like slugs and allow us to control the quality of growing medium or soil by adding fertile materials before we start planting. Another benefit of a raised bed is that it can be built over concrete or pavement.	Raised beds	Lettuce, tomatoes, parsley, spinach, basil, Asian greens, eggplants, dill, marigolds, cosmoses
5	In-ground beds	It is a traditional method of gardening. Planting in the ground requires space in the yard. We converted some tiny areas of grasses and shrubs for vegetables, edible companion flowers, and rhizomes.	Planting directly in the ground	Rhizomes, carrots, spinach, chili peppers, cosmoses, marigolds, moringas
6	Hanging planters	Hanging planters are 'space-less as long as we have scaffolding or structure to hang planters on.	Hanging pots	Mint, edible flowers

### 2.3 Growing Method

After site identification and selecting techniques to maximize space, the plants' selection is started. The selection of plant types is based on the family's food needs, including vegetables and herbs. Living in a tropical city is an advantage as we can propagate all year long. Various propagation methods have been practiced including sowing seeds, growing bought-seedlings, propagating from cuttings and side shots, even re-growing from kitchen scraps. Every propagation method has different advantages and challenges (Table 3).

### 2.4 Mulching and Watering

In a hot and humid climate like in Yogyakarta, evaporation happens more rapidly. More evaporation means more watering for plants. To avoid it, we apply organic mulching on the surface of soils for every plant. Mulching is beneficial to reduce evaporation which leads to efficient use of water (Krebs & Bach, 2018; Lal, 2020) since water is one of the limited yet precious resources for urban agriculture (Knorr et al., 2018; Lu & Grundy, 2017). There are a variety of organic mulches to use such as rice straw, hay, dry leaves, or wood chips. By applying mulch to minimize evaporation, gardeners can use water as efficiently as possible.

### 2.5 Composting and Fertilising

To fertilize the soil at a home food garden, it is important to create homemade natural liquid fertilizers and compost from kitchen scraps. In the urban farming context, domestic composting can be applied to improve soil nutrition while simultaneously upcycling domestic organic waste (Cleveland et al., 2017). Liquid fertilizer could be made once a week regularly. Ingredients include leftover rice, banana peels, rice wash, brown sugar, and additional bought bacteria starter. All ingredients are placed in one container and kept for two weeks for fermentation. The fermentation process is anaerobic which needs no oxygen, so the lid has to be closed tightly during the process. Liquid fertilizer can be used by di-

luting it with water using a 1:10 ratio. For compost, daily organic scraps such as fruits' peels and vegetable scraps are suitable. To minimize space, a composter bag can be used for domestic composting (see Figure 4).



Figure 4: Composter bags for domestic composting.

Table 2: Materials and Cost Estimation

Cultivation Techniques	Materials	Amount	Cost
Basic containers	Various size polybags	30 bags	\$ 5
	Various size pots	10 pots	\$ 10
Over fence planters	Various size hooked pots	8 pieces	\$ 8
	Potting mix	2 bags	\$ 4
Vertical planters	15 pocket vertical planter	1 planter	\$ 5
	Potting mix	2 bags	\$ 4
Log raised Beds	Logs	3 m	No Cost
	Compost	1 bag	\$ 2
	Potting soil	1 bag	\$ 2
Brick raised Beds	Bricks	50 pieces	\$ 5
	Branch and browns	Cover 10 cm layer thick	No cost
	Leaves (greens)	Cover 10 cm layer thick	No cost
	Compost	1 bag	\$ 2
	Potting Soil	1 bag	\$ 2
In-ground beds	Compost	1 bag	\$ 2
Hanging planters	Hanging pots	3 pieces	\$ 3
	Potting mix	1 bag	\$ 2
Additional	1 liter watering can	1	\$ 3
	8 liter watering can	1	\$ 10
	Compost	2 bags	\$ 4
	Potting mix	2 bags	\$ 4
	Seeds & seedlings	-	\$20
TOTAL COST			\$93

Table 3: Growing Method

Growing Method	Practical reflection
Seeds (bought)	Growing new plants from seeds need a longer time. We need to learn about seeds' characteristics as they are unique. It is suggested not to grow seeds too much, and to match the space and number of seeds carefully. After the seedlings have around 4 to 6 true leaves, they can be transplanted to new larger containers or in the ground. Additionally, some plants can be sowed directly and do not need to be transplanted, which is very convenient for novice gardeners.
Seedlings (bought)	Going to the local and closest nurseries and buying small plants are one of the most convenient methods of adding new plants to the garden. Usually, seedlings are planted in smaller containers by nurseries, which means that they need to be transplanted.
Cuttings	Some plants could grow from cuttings. Growing new plants from cuttings is the cheapest (and even at no cost) method of growing new plants.
Side shoots	This is also the cheapest method of growing new plants. Propagating new plants from side shoots or commonly known as sucker branches work well on tomatoes. Soil or water propagation techniques can be used.
Regrowing from kitchen scraps	Kitchen scraps can provide us free (no cost) seeds, cuttings, bulbs, rhizomes, even tubers to be regrown in the garden. Some plants that grow from this technique include chili peppers, lemon basil, spring onions, cilantro, and rhizomes.

## 2.6 Harvesting

'Cut and come again' harvesting method is applied, especially for leafy greens such as lettuce and spinach. The method allows home gardeners to harvest in small quantities for direct consumption. Also, it makes plants live longer and provide more yields (Johnson et al., 2016), so no sowing or transplanting is needed for a longer period. It is found that the 'cut and come' harvesting method is very convenient for amateur (non-full-time) home gardeners.

## 2.7 Access to Information

For novice gardeners with extremely limited gardening knowledge, access to information is essential. Interestingly, popular sources of information about urban gardening are available online at relatively diverse platforms such as blogs, microblogs, and video blogs. Novice gardeners

who would like to start gardening can select their sources of information according to their preferences and learn from them. Practical reflections on various sources of gardening information are elaborated in Table 4.

## 3 Results and Discussion

Within around 7 months of gardening, co-benefits of home food gardening are discussed as follow:

### 3.1 Fresh foods 'storage'

Initially, the first motivation of us developing the food garden is as an alternative to fresh emergency food sources if the worst thing happened during the Covid-19 pandemic. The food system is a sector that is predicted to be disrupted by partial (or full) quarantine activities caused by

Table 4: Information Sources of Gardening Tips

Source of Information	Practical Reflection
Microblogs (Instagram and Pinterest)	Microblogs are beneficial to find quick information about gardening. Many Instagram and Pinterest accounts provide simple and short gardening information. Additionally, Pinterest may also provide links to the original authors (blogs and websites) to learn more detailed information.
Gardening blogs/websites	Gardening blogs and websites provide more detailed information than microblogs. Interestingly, some blogs and websites even cite peer-reviewed references to support information. With so many available gardening-related blogs and websites, accessing blogs and websites that have similar garden specifications is recommended.
YouTube Channels	There is an enormous number of YouTube channels providing gardening information for different countries and climate regions. Watching gardening videos from the same climate region or country is the easiest way to learn.

the Covid-19 pandemic (Reardon et al., 2020). It is also recognized that the yield of urban domestic food gardens is not significant (Hallsworth & Wong, 2015), however, the food garden then may function as fresh vegetables and herbs ‘storage’ that can be harvested 2 - 4 times a week. Scholars have discussed the roles of domestic food gardens as alternative sources of food and nutrition (Galhena et al., 2013; Jacobs et al., 2016; Lal, 2020; Spilková & Vágner, 2018).

During the pandemic, many people pay more attention to the quality of their foods and practice healthy eating in order to maintain and boost the immune system (Sharma, 2020). A previous study has also suggested that health concern is one of the most motivating factors for people to eat a healthy diet (Gustavsen, 2020). The tiny food garden gives stocks of fresh greens and herbs which provide micronutrients (vitamins, minerals) and fibers. Micronutrients are a significant source for boosting the immune system (McAuliffe et al., 2020; Sharma, 2020) and for possibly reducing mortality among Covid-19 patients (Akhtar et al., 2021). Moreover, freshly harvested yields, salads, and other types of vegetables-based food were prepared for a balanced diet (see Figure 5). They are healthy, natural (no synthetics/chemicals), and have fewer environmental footprints.

### 3.2 Subjective well-being

Tiny food gardens can also function as a family stress reliever and mood fixer, as suggested by studies (Scott et al., 2020; Soga et al., 2017). Playing and interacting with natural elements in the garden while reducing the time spent indoor may result in increased cognitive functions, increased academic performance, lowered short-sightedness, as well as reduced childhood obesity and negative health effects for children (Gleeson, 2019). Based on the experience, it is highly recommended to allocate time and a small space at home to grow vegetables.

### 3.3 Appreciation of Food and the Environment

From a tiny food garden, so many things can be learned about vegetables and herbs, from soil and space preparation, production (growing), waste management, and harvesting. This experiential learning process builds appreciation and respect for food, its growing process, ecosystem, and all food growers (farmers). Previous studies also found that sustainable and ethical values predominantly underlie urban gardening activities (Al-Mayahi et al., 2019; Hirsch et al., 2016).



Figure 5: Homemade food with homegrown vegetables. Most vegetables, herbs, and garnishes come from our tiny food garden.

### 3.4 Motivating others

Sometimes we share our activities on social media (Instagram) in order to motivate others to grow vegetables at home. We also answered all questions from colleagues and responded to them based on our personal experiences growing food in our small garden. So far, we successfully influenced friends and family to grow vegetables and fruits at their urban houses with various techniques, as well as motivated our neighbourhood's community to convert a small area of grass in a little green open space into a community food garden.

### 3.5 Small economic benefits, great personal satisfaction

Our personal experience found that the direct economic benefits of the tiny food garden are relatively small. On contrary, many urban garden-

ers place economic benefits as one of their motivations and gain profit from the yields (Kirkpatrick & Davison, 2018). Our experience shows that we could frequently harvest vegetables and herbs (2 - 4 times a week) for 2 servings or about 100 - 350 grams. It may be worth only 2 to 5 dollars a week. However, despite the small economic benefit, we found that it is very rewarding to harvest home-grown vegetables and serving the food directly from the garden to the table.

### 3.6 Urban Planning Policy Implication

A previous study argues that urban gardens may not provide a sufficient amount of food for urban citizens (Hallsworth & Wong, 2015). Although the contribution of urban gardens to urban food sufficiency is questioned, many studies agree that home food gardens can still bring co-benefits in urban socio-ecological aspects. For

instance, Cleveland et al. (2017) analysed the potential of domestic food gardens in reducing greenhouse gas (GHG) emissions. They found that people who participate in urban gardening activities tend to use household resources in more sustainable manners, such as using recycled or harvested water more, reducing the carbon footprint of transported food, and reducing organic solid waste by composting and applying it to food plants. All of these activities potentially reduce GHG emissions and contribute to a sustainable lifestyle at the household level. To link these co-benefits with the Covid-19 pandemic, it is observed that people's awareness of the environment increases due to the pandemic (Rousseau & Deschacht, 2020). Therefore, starting urban food gardening at home during the pandemic is a good beginning for a more ecologically friendly family lifestyle. Additionally, from the nutritional security point of view, previous studies claim that urban gardens may improve the nutritional diversity and healthy diet of households (Jacobs et al., 2016; Lal, 2020; Rybak et al., 2018). It means that urban food gardens may provide people with alternative food and nutrition during the pandemic which may help to maintain health. With all these co-benefits, decision-makers, environmental activists, and people who are concerned about the environment could leverage the moment to promote and integrate home food gardening and its circularity as an urban sustainable initiative.

#### 4 Conclusions

The Covid-19 pandemic reminds us that the urban food system is vulnerable (Carey et al., 2020; Paganini et al., 2020; Reardon et al., 2020), as many experience anxieties of food insecurity. Thus, with relatively limited knowledge of urban gardening, we decided to convert our small urban house yard into a tiny food garden to enhance the family's food resilience. With a relatively small space, new gardeners in the urban residential area may harness various cultivation strategies, including containers and in-ground beds, depending on the available space. The tiny food garden could produce a variety of vegetables and herbs, such as the spinach family, lettuce, Asian

greens, the tomato family, eggplants, the basil family, mint, rosemary, moringas, and butterfly pea flowers. Such a garden may support a household with few amounts of fresh emergency food in case of the worst scenario during the Covid-19 pandemic. Besides, developing a tiny food garden at home may also provide co-benefits, such as enhanced subjective well-being, increased appreciation of food and the environment, others being motivated to start gardening at home, and great personal satisfaction of consuming home-grown food. Ultimately, with all these socio-ecological co-benefits, home food gardens must be integrated as a strategy to achieve urban sustainability and increase household food resilience.

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# Physical and Chemical Characteristics of Beef Marinated by Cashew Apple Extract

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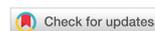
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## Abstract

Marination is one of the methods that are often used in beef processing in an attempt to obtain high quality of beef. Cashew apple extract marinade (CAM) improves the microbiological characteristics of meat by inhibiting the growth of meat bacteria. The effect of CAM on other aspects such as physical (microbiological, tenderness, cooking loss, water holding capacity and pH) and chemical (moisture, fat and protein content) characteristics of meat have not been evaluated, which would be beneficial for the utilisation of agroindustry waste in the meat industry. In this study, the effect of CAM on the physical and chemical characteristics of beef, including microbiological characteristics, tenderness, cooking loss and water holding capacity, was evaluated. CAM (0%, 10%, 20% and 30%) was used during beef processing for 4 h at refrigeration temperature. Significant effects of CAM were observed on the physical and chemical characteristics of beef. CAM increased beef tenderness and reduced total bacteria, cooking loss, moisture, fat, and protein content. The optimum concentration of CAM for which significant changes were observed in the physical and chemical characteristics of beef was 20%. Thus, cashew apple can be utilised as a promising marinade agent in beef processing with the criteria of food for specific health use. This approach will help reduce cashew apple waste and is an eco-friendly approach.

**Keywords:** Beef; Cashew apple extract; Marination; Marinade; Cashew nut

## 1 Introduction

Physical, chemical and microbiological characteristics of beef are one of the main appeals of this meat for consumers (Henchion et al., 2017). To obtain good quality beef, several methods are used during its processing and one of them is marination. Earlier, marination was used only for seasoning of beef (Smith & Acton, 2010). With the development of the processing

technique, marination evolved to function as a flavouring and tenderising agent, and to extend the shelf life of beef (Çetinkaya, 2017). The liquid used for marination is known as a marinade, which can be either acidic or enzymic or neutral in pH (Yusop et al., 2011). Recently, a juice obtained from some fruits was used as a marinade for improving the quality of meat (Guo et al., 2020). In a previous study, cashew apple extract marinade (CAM) was used to improve the micro-

biological characteristics of meat by inhibiting meat bacteria (Susanti et al., 2018). However, further studies about the effect of CAM on meat quality including physical and chemical properties are required.

Cashew apple is a waste product produced in the cashew nut industry; it is abundant in quantity reaching approximately 30 million tonnes per year globally (Oliveira et al., 2020). Although availability of cashew apple is high, only 10% of cashew apples are utilised commercially, whereas the rest (90%) remain un-utilised (Oliveira et al., 2020). This pseudo fruit is generally left on the ground in the shade to rotting after separation of the nuts. Cashew apple contains phytochemical compounds containing acidic groups, polyphenols and flavonoids (Rufino et al., 2010). This study aimed to evaluate the effect of CAM on the physical and chemical characteristics of beef. It showed the potential use of cashew apple as a marination agent in the meat processing industry.

## 2 Materials and Methods

### 2.1 Preparation of CAM

Cashew apples were collected from several farm areas along the Java Island. After washing and drying, the fruits were extracted using the percolation method as described earlier by Susanti et al. (2018). CAM was prepared by mixing the indicated concentration of cashew apple extract with mineral water to obtain final concentrations of 10%, 20% and 30%. Mineral water without any extract (0%) was used as the control.

### 2.2 Beef Marination Process

Beef samples were prepared by cutting tenderloin into  $2 \times 2 \times 2$  cm parts. CAM was prepared by diluting the indicated concentration of cashew apple extract in the required volume of mineral water. The samples were soaked in CAM for 4 h in 4 concentrations: 0% (control), 10%, 20% and 30% (Figure 1). The samples were stored in the refrigerator. After the marination time, the samples were flushed once with mineral water

for neutralisation and then physical and chemical characteristics were determined.

### 2.3 Evaluation of Physical Characteristics of Beef

#### Microbiological Test

Total bacteria present in beef was determined by counting the colonies under sterile conditions. The beef samples were soaked in 45 mL of distilled water for 10 min. Samples were diluted by serial dilution and 1 mL of each pipetted into petri dishes followed by 20 mL of nutrient agar medium. The medium was allowed to solidify at the room temperature. The petri dishes were then incubated at 37 °C for 24 h. The number of colonies was counted using a colony counter, and the dilution factor was used to calculate log CFU/mL.

#### Tenderness Test

Beef tenderness was measured using a texture analyser (Brookfield CT-03, USA). The samples were placed under a cylindrical probe of 10-mm diameter. The probe was moved downwards at  $2 \text{ mm.s}^{-1}$ . The probe continued moving downward until penetration of 75% of the sample thickness was attained, retracted to the initial point of contact with the sample, and stopped for 2 s of set time period before initiation of the second compression cycle. During the test, the force-time data of the sample was recorded and plotted on the force-time plot (de Huidobro et al., 2005). Tenderness ( $\text{mm/g}/10\text{s}$ ) was calculated using the standard procedure (Honikel, 1998; Railton & Aronstam, 1987).

$$\text{Tenderness} = \frac{\text{Mean of recorded data}}{10 \text{ seconds}} \quad (1)$$

#### Cooking Loss Test

Beef cooking loss was evaluated to determine the loss of beef mass during the cooking period. The samples were drained for 10 min at room temperature and then kept in trays after marination for 4 h. The samples were weighed, kept inside polypropylene plastic bags, and then heated in

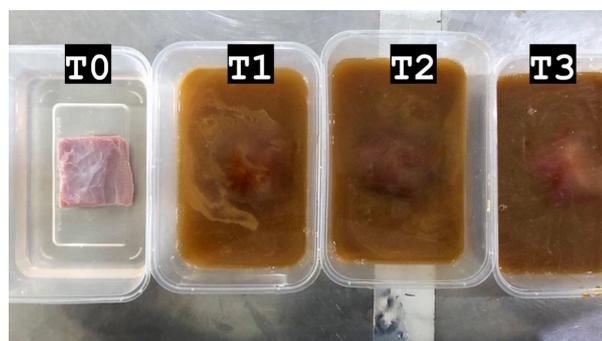


Figure 1: Cashew apple extract marinated (CAM) beef with the concentrations of 0% (T0), 10% (T1), 20% (T2) and 30% (T3) CAM.

water bath (Memmert, Germany) at a temperature of 60 °C for 60 min. Cooking loss was expressed as the percentage of difference between weights before and after cooking.

#### Water-holding capacity (WHC) Test

The WHC of beef was estimated as reported by Miller et al. (1980) by determining expressible juice using a modification of the filter paper press method. The sample (300 mg) was weighed and kept on an 11-cm diameter filter paper between Plexiglas plates and pressed at 200 psi for 1 min. The outline areas of the meat film and the expressible juice were traced, and both the areas were determined using a compensating planimeter. The percentage of expressible juice was calculated as described by Qiao et al. (2001). The increase in expressible juice percentage is related to the decrease in beef WHC.

$$\text{WHC}(\%) = \text{Moisture}(\%) - \text{Moisture}_{\text{wet area}}(\%) \quad (2)$$

$$\text{Moisture}_{\text{wet area}}(\%) = \frac{(\text{Area}_{\text{total surface}} - \text{Area}_{\text{meat film}})}{\text{Weight}_{\text{Sample}}} \times 100\% \quad (3)$$

#### pH Test

The pH of beef was measured by using a pH meter (pH1120x, Mettler Toledo, USA) according to the manufacturer's instructions. First, the pH meter was calibrated by using buffer solutions of pH 7 and 4. Beef was minced and then loaded

into a plastic tube filled with 10 mL of Aquadest. The tip of the pH meter was dipped into the sample and the pH recorded.

## 2.4 Evaluation of Chemical Characteristics of Beef

### Determination of Moisture

An empty porcelain cup was kept in an oven (100–105 °C) for 1 h, transferred into a desiccator, cooled for 30 min, and then weighed. Approximately 2–3 g of beef sample was taken into the cup, weighed, and then dried in an oven (100–105 °C) for 3 h. Drying and weighing were performed continuously. After a constant weight was obtained, the sample was transferred into a desiccator, cooled for 30 min, and then weighed. Moisture content was calculated by using the following formula.

$$\text{Moisture}(\%) = \frac{(\text{Weight}_{\text{initial sample}} - \text{Weight}_{\text{dried sample}})}{\text{Weight}_{\text{Sample}}} \times 100\% \quad (4)$$

### Determination of Protein Content

Protein content was measured using the Kjeldahl method. The sample (10 g) was put into a Kjeldahl flask and 2 g of  $\text{K}_2\text{SO}_4$  and 20 mL of  $\text{H}_2\text{SO}_4$  were added. The digestion was performed for 30 min until a clear, light-green solution was obtained. Digestion solution (10 mL) was added

to distilled water and shaken to mix thoroughly. NaOH (20 mL) and phenolphthalein indicator (3 drops) were added to the solution and then distilled using Erlenmeyer flasks.  $H_3BO_3$  (3%, 20 mL) and 2 drops of red/blue methyl, respectively, were used as the indicators. The yield of the distillation process was determined by titration with 0.1 N HCl until the solution turned light purple. Blank solution was prepared just before the determination of protein content as reported by Kolawole et al. (2020).

$$\text{Proteint Content(\%)} = \text{Total Nitrogen} \times 6.25 \quad (5)$$

$$\begin{aligned} \text{Total Nitrogen (\%)} = \\ \frac{(\text{Mol}_{NH_4Cl} \times \text{Equivalent Weight}_N)}{\text{Sample weight}} \times 100\% \end{aligned} \quad (6)$$

### Determination of Fat Content

Fat content was measured by the Soxhlet method. Approximately 1-2 g of beef sample was weighed into a paper thimble coated with cotton. The paper sleeve was plugged with cotton and dried in an oven at 105 °C for 1 h. The sleeve was inserted into the Soxhlet apparatus connected to the fat extraction flask containing dry boiling stones and having known weight. The samples were extracted using hexane for 6 h until the fat extract was obtained. The fat extract was cooled and weighed. Fat content was determined by using the following formula.

$$\begin{aligned} \text{Fat content (\%)} = \\ \frac{\text{Weight}_{\text{Flask with fat extract}} - \text{Weight}_{\text{Empty flask}}}{\text{Weight}_{\text{Sample}}} \times 100\% \end{aligned} \quad (7)$$

### 2.5 Statistical Analysis

One-way analysis of variance was performed to determine the significance of effects shown by CAM for the indicated parameters. Each parameter consisted of 4 treatments with 6 replicates per treatment. Significant differences among the treatment groups were confirmed by post hoc multiple comparison Duncan multiple range tests. Statistical analyses were performed using

SPSS for Windows. A *p-value* of <0.05 was considered statistically significant (Dawson & Trapp, 2001).

## 3 Results and Discussion

The quality of meat is the measure of characteristics that determine the suitability of fresh or stored meat for consumption for a reasonable period without deterioration (Elmasry et al., 2012). Usually, the quality of meat is assessed by consumers using sensory/organoleptic testing. To avoid subjectivity, quality assessment evolved as an assessment of these characteristics by physical and chemical analysis. In the present study, the quality of marinated meat as a highly nutritional food was evaluated. Ideally, marination is the soaking of beef in the marinade (solution or sauce), which facilitates the passive transport of nutrients from the marinade to the beef by osmosis (Çetinkaya, 2017).

The effect of CAM on the microbiological characteristic, tenderness, cooking loss and water holding capacity (WHC) of beef was evaluated in this study (Table 1). The effect on the microbiological characteristics was significant; concentrations of CAM between 10% to 30% showed a significant reduction in bacterial growth (Figure 2). Phenolic compounds present in the cashew apple extract might have inhibited the bacterial growth in beef. Phenol destroys the bacterial cell membrane and enzymes, thus resulting in bacterial death (Lima et al., 2019). Thus, CAM ameliorated the microbiological status of beef and also extended the shelf life of beef.

CAM beef was more tender than the control, and tenderisation increased with the increase in CAM concentration. The optimum level of tenderisation was found at 20% CAM; however, no significant difference was found between tenderisation at 20% and 30% CAM. Thus, CAM improved beef tenderness effectively. This might have been because of the acid content of the cashew apple. Acidic compounds hydrolyse and break the cross-linking of connective tissues in marinated beef. Akinwale (2000) reported the highest quantity of ascorbic acid in the cashew apple juice (203.5 mg 100 mL<sup>-1</sup>). This finding showed that the ascorbic acid content of cashew apple juice

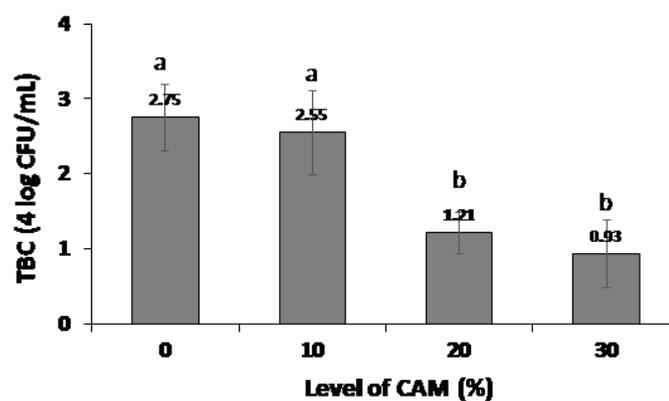


Figure 2: Effect of cashew apple extract marination (CAM) on the total bacterial count (TBC) of beef. TBC was expressed as a mean of 4 log CFU/mL unit  $\pm$  SD of 5 replicate analyses. Different superscripts letters show significant differences ( $p \leq 0.05$ ).



Figure 3: Beef appearance after the cooking process expressed as cooking loss. T0 is beef without cashew apple extract marination (CAM) and T1-3 are beef with CAM concentration of 10%, 20% and 30%, respectively.

Table 1: Physical characteristics of cashew apple extract (CAE) marinated beef

Parameters	CAE			
	0%	10%	20%	30%
Tenderness (g)	95.80 ± 9.38 <sup>a</sup>	82.50 ± 8.28 <sup>b</sup>	74.70 ± 6.69 <sup>b,c</sup>	68.60 ± 4.72 <sup>c</sup>
Cooking loss (%)	56.46 ± 6.56 <sup>a</sup>	47.14 ± 6.46 <sup>b</sup>	42.38 ± 8.41 <sup>c</sup>	36.40 ± 4.91 <sup>d</sup>
WHC (%)	26.69 ± 0.77 <sup>a</sup>	25.45 ± 0.55 <sup>b</sup>	23.63 ± 0.41 <sup>c</sup>	23.04 ± 0.23 <sup>c</sup>
pH	5.92 ± 0.13 <sup>a</sup>	5.56 ± 0.08 <sup>b</sup>	5.34 ± 0.05 <sup>c</sup>	5.24 ± 0.06 <sup>c</sup>

Data are expressed as mean ± standard deviation. Different superscripts letters on the same line show significant differences ( $p \leq 0.05$ ) between treatments. WHC, water-holding capacity

Table 2: Chemical characteristics of cashew apple extract (CAE) marinated beef

Parameters	CAE			
	0%	10%	20%	30%
Moisture (%)	77.18 ± 3.38 <sup>a</sup>	77.68 ± 4.99 <sup>a</sup>	74.67 ± 3.04 <sup>b</sup>	71.59 ± 3.56 <sup>c</sup>
Protein content (%)	20.89 ± 2.55 <sup>a</sup>	19.10 ± 2.52 <sup>b</sup>	15.10 ± 1.16 <sup>c</sup>	14.71 ± 3.33 <sup>c</sup>
Fat content (%)	8.54 ± 0.31 <sup>a</sup>	7.80 ± 0.15 <sup>b</sup>	7.23 ± 0.25 <sup>c</sup>	6.57 ± 0.36 <sup>d</sup>

Data are expressed as mean ± standard deviation. Different superscripts letters on the same line show significant differences ( $p \leq 0.05$ ) between treatments.

was almost four times higher than that of popular citrus fruits (54.7 mg 100 mL<sup>-1</sup>). Based on this finding, the tenderisation observed in our study was attributed to the high ascorbic acid content. Besides acid content, the sodium content of cashew apple (12 mg 100 g<sup>-1</sup>) may improve the beef texture by activating the binding of the water component to the proteins in the beef (Ahmad et al., 2020). Furthermore, sodium reduces fluid loss when beef is cooked at a high temperature under vacuum. Its manifestation was evident in the physical characteristics of beef, including cooking loss, which describe the degree of meat shrinkage during cooking (Figure 3). The percentage of cooking loss of CAM beef significantly decreased with the increasing concentration of cashew apple extract (Table 1). WHC describes the ability of meat to hold its native and added moisture during fabrication and processing. The WHC of beef progressively decreased ( $p \leq 0.05$ ) with the increasing concentration of CAM from 10% to 30%. WHC is the ability of proteins to prevent water release from the

three-dimensional structure of proteins. This is probably because of the proteolysis of myofibrillar proteins by ascorbic acid present in CAM and pH shifting towards the isoelectric point of myofibrillar proteins. The pH of CAM-treated beef decreased significantly with the increase in CAM concentration up to 20% (Table 1). Although the pH tended to decrease as an effect of treatments, the pH of CAM-treated beef was higher than its isoelectric point (5.0–5.1) and cooking loss was also low (Table 1).

Furthermore, CAM also influenced the moisture, protein and fat content of the beef. As described in Table 2, a decrease in moisture and protein content of the marinated beef was observed up to 20% CAM, whereas a decrease in fat content was observed up to 30% CAM.

The decrease in beef moisture and protein content in this study was thought to be correlated with lower WHC and increased concentration of cashew apple extract because of proteolysis caused by ascorbic acid. The fat content of beef decreased significantly with the increase in CAM

concentration because of the action of phenolic compounds in cashew apple extract. Beef fat, an ester of fatty acids, is hydrolysed into fatty acids during the marination process that involves water. Fatty acids are broken down into constituent esters by phenol through the esterification reaction (Figueroa-Espinoza & Villeneuve, 2005), and this could be the reason for the decrease in total fat content of the marinated beef.

Beef processing by the marination method in this study showed that cashew apple extract application as a meat marinade was able to reduce the fat and protein content of beef. The presence of low fat-protein beef can be useful for providing diet for humans with specific health conditions such as renal failure, coronary heart disease, diabetic mellitus, obesity and breast cancer (Rhee et al., 2018; Rubio-Patino et al., 2018; Shai et al., 2008). In addition, a low fat-protein diet can also be a clinically relevant lifestyle-intervention strategy for delaying the onset of cognitive impairment and dementia, especially in females (Buccarello et al., 2017).

## 4 Conclusions

CAM increased beef tenderness and reduced the bacterial growth, cooking loss, moisture and protein content of beef. The optimum level of CAM for significant changes in the physical and chemical characteristics of beef was 20%. Thus, cashew apple extract could be utilised as a promising marinade agent in beef processing.

## Acknowledgements

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# A Systems Integral Approach in Exploring Creative Innovation in Culinary Research: The Example of Seaweed in the Context of the New Nordic Cuisine

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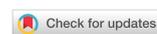
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## Abstract

Creativity and innovation in culinary research have gained steady academic interest over the last decade. The scholastic interest in creative innovation ranges from its artistic value to culinary creations, gastronomic experiences, and food science and technology. Creative innovation is important for food enterprises to succeed in a highly competitive market. In the context of the New Nordic Cuisine, entrepreneurs and chefs are constantly challenged to bring something new to the dining table. In this context, the processes of creative innovation remain under researched, particularly in the use of seaweed. As such, using the example of seaweed, a relatively new food in the New Nordic Cuisine, the objective of this corpus based study was to explore creative innovation from a systems integral approach, in order to uncover salient themes that contribute the processes of creative innovation in culinary research, and bringing new foods to market. For a corpus driven study, we built a small corpora of interviews with chefs, and food entrepreneurs. We enquired after what inspired and motivated them when faced with a challenge of bringing a relatively new food to market, or in creating new dishes with new available food technologies. The results suggested that food technology plays a critical role in creative innovation, and the resulting new dishes that can be presented to customers. They also suggested that seaweed in the New Nordic Cuisine is an emerging food concept, and that it is embedded in a social and cultural history and familiarity of the Nordic people.

**Keywords:** Culinary research; Creativity; Innovation; New Nordic Cuisine; Systems integral theory

## 1 Introduction

Creativity and innovation in culinary research have gained steady academic interest over the last decade. This interest ranges from the artistic value (Stierand & Lynch, 2008), the scientific aspects in cooking (Mouritsen et al., 2018; Mouritsen et al., 2012), how the processes are managed (Feuls, 2018), to their role in haute cuisine (Messeni Petruzzelli & Savino, 2014). Creative innovation is after all considered the very

ingredient needed for organization and enterprise success (Harrington & Ottenbacher, 2013; Tohidi & Jabbari, 2012). It can for instance also be applied when developing sustainable seaweed based materials for creations in fashion (Buet, 2020; Hurtado et al., 2020).

We use the term "creative innovation" following the example of scholars in the field of international business (Feldman, 2008). These creativity researchers, in light of the global market-

## Nomenclature

**NNC** the New Nordic Cuisine (NNC) is a food movement that began as a manifesto in Denmark in 2003. It was formulated by gourmet chefs and entrepreneurs who emphasized foods sourced from the Nordic region.

**HoReCa** in this study, HoReCa (hotels, restaurants, and cafés) refer to business enterprises, and their actors and stakeholders that made up part of the group of individuals interviewed. As business owners, they provided rich insights on their processes of innovation and creation of new dishes, new practices as well as their food and life philosophies.

**IMP** Integral methodological pluralism (IMP) refers to a theoretical approach

attributed to the works of Ken Wilber (Esbjörn-Hargens, 2010; Marquis, 2007; Wilber, 2007). The theoretical framework It combines notions from philosophy, psychology, and human cognitive development, that transcend disciplinary boundaries in a unified or holistic perspective that supports sustainable ecology.

**Kwic** Keyword-in-context (kwic) analysis, refers to a function of the concordance software AntConc. It allows users to retrieve search terms and keywords, as they are used by the respondents in the corpora. Kwic analysis is a means to locate, and provide this study with relevant respondent transcript examples, as part of the corpus data driven findings in this study.

place and increasing convergence of technology and science, tend to view creativity and innovativeness as essentially synonyms (Amabile & Pratt, 2016; Woodman et al., 1993). Similarly, creativity and innovation are co-constructing material processes, particularly in the field of culinary research, where advancements in technology enable new food applications and culinary creations (Feuls, 2018; Mele & Russo-Spena, 2016; Schumpeter, 1947). Creative innovation has also been studied from various theoretical perspectives, such as enterprise or firm ambidexterity in the field of international business studies (Chang et al., 2014; Kurniawan et al., 2020), relational sociology in economic theory (Feuls, 2018), actor network theory (Huggins & Thompson, 2015; Voeten et al., 2015), and entrepreneurship theory in small social businesses (de Bruin & Shaw, 2011; Messeni Petruzzelli & Savino, 2014). Using phycogastronomy (seaweed gastronomy) set in the context of the New Nordic Cuisine (NNC) as example, this empirically based study built upon

these studies to develop a holistic model of creative innovation in culinary research. We aimed to employ an empirical basis in the field of creative innovation to build a model using a systems integral theoretical approach. The novelty of our contribution was both practical and theoretical. The model contributes to business practice knowledge by using transcribed empirical data and turned it in into a small corpus database. As creative innovation is both an activity and a process that takes place in culinary related arenas, from drawing boards to kitchens, and in the contexts of HoReCa (hotels, restaurants, and cafés), we used text analysis to uncover common creative innovation elements across different contexts and individuals. We contributed to the theory by applying a systems integral approach towards a creative innovation model/tool. Holistic models of culinary innovation have been created in order to understand the phenomenological or lived experience of chefs (Stierand, 2009) and to understand personality, socio-

culture, time and the development of talent in the field of art (Csikszentmihalyi, 2015). We invited scholars/practitioners to pursue the challenging task of holistic, unified or system integral perspectives and theories, consolidating views from various disciplines in order to give more comprehensive overviews to objects / subjects of study. There were three research questions (RQ) addressed in this study.

**RQ1** What general elements can be identified in the creative innovation process?

**RQ2** How do these elements apply in the context of phycogastronomy in the New Nordic Cuisine context?

**RQ3** How does a system integral perspective and model of creative innovation compare to other holistic models in extant literature?

The first two pertained to the pragmatic aspects of (i) identifying creative innovation elements and (ii) how they are observed / applied in the field of phycogastronomy. The last RQ addressed theory.

The structure of this paper is as follows. The Introduction in section 1 states the research focus of this paper within the academic inquiry of the processes of creative innovation, using phycogastronomy in NNC as an example. Section 2 gives brief overviews of state-of-the-art literature of the three subjects at hand:

1. the New Nordic Cuisine and phycogastronomy,
2. creative innovation including a systems perspective to creative innovation and
3. systems integral theory.

Section 3 details the data collection and analysis methods. Section 4 discusses the findings of the data to address the RQs of this study. We propose how elements of the creative innovation process can be modelled using a system integral perspective. Section 5 gives a synopsis of the study, as well as the limitations of our model and suggestions for further research.

## 2 Literature review

### 2.1 New Nordic Cuisine and phycogastronomy

The NNC began as a manifesto in Denmark in 2003. It was formulated by gourmet chefs who emphasized foods sourced from the Nordic region (Byrkjeflot et al., 2013; Leer, 2016; Micheelsen et al., 2014). A long-standing notion in the field of gastronomy, particularly in wine and cheese is the concept of terroir. Terroir refers to the unique resource of soil, climate and processing methods that connect products with a geographical region, people, and food heritage. These qualities allow food producers to differentiate products (Charters, 2010; Li et al., 2017; Wilkin & Sinclair, 2007). The NNC expresses the following four principles: freshness, purity, simplicity and ethics (Mithril et al., 2012). It employs the concept of Nordic terroir, leveraging the region's cool climate, slow growth conditions and seasonal fluctuations. These properties of Nordic ingredients allow chefs to offer and be creative with them. The NNC also upholds the Nordic perspective of circular / regenerative farming, sustainable food sourcing and keeps high ethical standards of animal welfare (Bech-Larsen et al., 2016; Micheelsen et al., 2014). Nordic cuisine was put on the international culinary map with the support from actors and stakeholders from various sections of society. They include key players such as chefs, entrepreneurs, policy makers. It was further aided by participation from private and public organizations, institutions and communities of practice in the NNC (Bech-Larsen et al., 2016; Leer, 2016; Micheelsen et al., 2013). As part of the NNC, restaurants began offering menus consisting of culinary creations that were a remix of traditional Nordic staples, as well as foods that were up to that time rarely associated with human consumption in the Nordic countries. Seaweed, the subject of our paper, is such an example (Bech-Larsen et al., 2016; Haugan, 2015; Mouritsen et al., 2013). Seaweeds are found along coastal regions with suitable habitats around the globe. It is believed to have been part of the diet of coastal living humans from ancestral times. Newer studies

show that seaweed consumption contributes to the rapid development of the human brain (Cornish et al., 2017; Haskell-Ramsay et al., 2018). There is also a significant body of evidence suggesting that regular consumption of seaweed can contribute to the overall human health (Brown et al., 2014; Michalak & Chojnacka, 2018). Phycogastronomy has developed quite differently in Asia and Northern Europe / the Nordic region, as evidenced by the culinary history and heritage. During the Heian Period in Japan (794-1185), wild-gathered seaweeds were considered extraordinarily luxurious, consumed mostly by the noble classes. Nori became a popular ingredient in Japanese cuisine maki-zushi in the Edo period in Japan (1603-1867). Nori was specially reserved for Japanese nobility and could not be purchased in public markets. It was used in a traditional dish of boiled rice marinated with rice vinegar, sugar, salt and other ingredients such as fish, vegetables and seafood (Mouritsen et al., 2018). The consumption of seaweeds is similarly widespread in the neighbouring countries China and Korea. As such, Far Eastern countries such as Japan, Korea and China are considered phycophages and have kept the inclusion of seaweeds in their everyday foods from ancient times (Mouritsen et al., 2018).

In the European context from about 1400 years ago, seaweed harvested in coastal Scotland and Ireland was used by the poor to feed themselves in the form of Lhavan or Lhawvan (black butter). Lhavan was derived when seaweed was added to seafood and boiled for several hours, then spread on top of oat bread (Newton, 1951; Sexton, 1998). In the Nordic context, the use of seaweed was associated with poverty and thrift. Going back to the early Viking period, seaweeds washed on shore during storms along Nordic shores were picked, dried, and fed to animals, as well as used as soil fertilizers. It occasionally supplemented human diets during times of famine. This connotation of seaweed as "poor man's food" or "animal feed" remains until today in most general Nordic households, with the exception from the early 2000s. Phycogastronomy was heavily promoted by elite European and Nordic chefs (Efstathiou & Myskja, 2019; Indergaard, 2010; Mouritsen et al., 2012). Seaweed for human consumption is a relatively new concept. It is part of

the NNC diet that encourages a more vegetable based nutrition. This concept might prove challenging to introduce to Nordic consumers (Haugan, 2015; MacArtain et al., 2008; Mouritsen et al., 2012). It is this challenge of repositioning seaweed in the world of Nordic cuisine and gastronomy that makes for empirical study of the elements of creative innovation particularly interesting, as it is an evolving concept aiming to entice general consumption.

## 2.2 Creative innovation

Using normative definitions in language use, creativity refers to the act of conceiving something original or unusual. Innovation refers to the act of implementing something new. Creativity and innovation have mostly been studied as separate concepts. In the context of culinary research, the material process of creative innovation, which is the conception and implementation of new gastronomic delights in various culinary settings is key to success for the organization's (as well as industry) competitiveness. If one were to however, consider "creative innovation" in its own semantic context, the very nature of "creativity" flouts the rigidity of structure and labelling/naming. Its very naming pigeonholes and constrains its very conceptual development. For the study of "creative innovation" as a process, one could attempt to name its elements and characteristics in a structural form that is allowed to morph and be malleable according to its context. As early as the late 1800s (Galton, 1869) different schools of thought have approached the study of creativity from various perspectives. They include: the Freudian and Jungian psychoanalytic frameworks (Ferrell, 2015; Freud, 1971; Oremland, 1999), behaviourist theory from the works of Skinner and Watson (Skinner, 1984, 1985; Watson, 1926) and human cognition from the works of Maslow (Maslow, 1961; Maslow, 1962, 1964).

Moving beyond normative definitions however, processes of creative innovation seem to permeate human evolution. Humans have been solving problems and facilitating efficiency from the fashioning of tools in the Palaeolithic Age, through the development of organized agriculture and

the domestication of animals in the Neolithic Age, to modernity. Humans constantly strive to better organize ways of living in accordance to different contexts, from tribes to urban cities (Stearns et al., 2014). The multidisciplinary nature and omni-contextual presence of creative innovation has encouraged a systems approach in both the practitioner world and in scientific theory building. This is defined as a framework that is pluralistic, with elements that are constantly evolving and interacting, striving towards a way of living that is ecologically sustainable (Capra, 2005, 2009). A systems approach has been used to understand creative innovation processes from an organizational perspective of creativity at the work place (Puccio & Cabra, 2012). This has been achieved by delving into the understanding of individuals and using case studies as method as a means towards a deeper understanding of the human mind as it goes through various stages of cognitive development (Gruber, 1983, 1988); studying personality types and creativity (Krippner & Combs, 1998); by studying tangent outcomes of creativeness such wisdom in later life and lifelong learning (Rathunde, 1995) and growth of talent in adolescents (Rathunde & Csikszentmihalyi, 1993). A systems perspective characterises the context for experiencing productivity and flow, where "flow" is defined as optimal states of performance without seemingly much effort, as introduced by Csikszentmihalyi (Csikszentmihalyi, 1996; Csikszentmihalyi, 2015; Nakamura & Csikszentmihalyi, 2001). Figure 1 presents Csikszentmihalyi's systems model of creativity (Csikszentmihalyi, 2006). Csikszentmihalyi's body of work that began more than four decades ago has several illustrative models of creative processes derived from various contexts of study (Csikszentmihalyi, 2015). Figure 1 (Csikszentmihalyi, 2015) is a key example, if not the most comprehensive model of the creative processes developed by Csikszentmihalyi. The model is based on the model of Darwinian biological evolution where Csikszentmihalyi views creativity as part of the developmental force that drives cultural evolution. For Csikszentmihalyi, "Creativity" occurs at the interface of 3 subsystems. They include the Individual who is selected by the Field of gatekeepers (part of Society) into the Domain from where the novelty will

then be accessible to the next generation.

Csikszentmihalyi's systems model of creativity is used here as comparative foundation to the systems integral model/tool developed and presented in this paper.

### 2.3 Systems integral theory

In creating the systems model of creativity, Csikszentmihalyi (2014) drew analogies between biological and cultural evolution (Atran, 1998; Darwin, 1859; Dawkins, 2006). The interrelation between biology and human culture has been noted in diverse fields of study. The past two decades saw an increasing body of literature reflecting this relationship (Eisler, 2015; Garcia Coll, 2004; Goodman, 2013; Keller, 2016; Reynolds, 2007). In alignment with the evolutionary systems model of creativity presented by Csikszentmihalyi, the framework of systems integral theory in this study has its foundation in the works of Fritjof Capra (Capra, 1985, 2005; Capra & Luisi, 2014) and Ken Wilber (Esbjörn-Hargens, 2010; Wilber, 2001a, 2001b). The difference is that systems integral theory is situated in the field of unified science and theories. Capra's approach is based on evolutionary science which can be defined as a systems approach to systems theory. It combines notions that transcend disciplinary boundaries in a unified or holistic perspective that supports sustainable ecology. Wilber's integral methodological pluralism (IMP) is based transpersonal theory and integral epistemology. IMP is a methodological framework, transcending notions found in various theories of philosophy, psychology and human cognitive development (Marquis, 2007; Saiter, 2009; Wilber, 2007).

At the core of systems integral theory are synthesis and differentiation, depending on the context of application, researcher/practitioner perspectives adopted and the subject of study. Our framework is based on a four-quadrant model that reflects the perspective of the language pronoun system, I (singular subjective), We (plural intersubjective), It (singular objective), and Its (plural interobjective). Systems integral describes the relationship between the part and the whole, both in its structural nature as well as

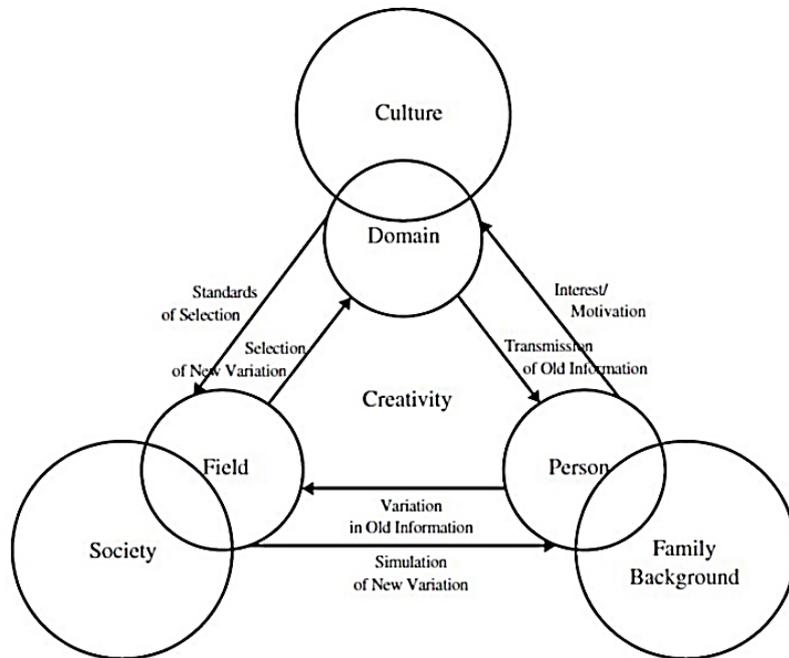


Figure 1: Systems model of creativity by Csikszentmihalyi (2014), p.166)

in its dynamic interactions (Capra, 1985). The idea is that in order to understand any complex system (creative innovation is one such phenomenon), you will need to break it into smaller pieces in order to explain it. And you keep on splitting and deciphering until you end with fundamental building blocks such as elements, substances, particles, sub-particles etc. From these fundamental building blocks or holons (constitute part-wholes), we study their fundamental laws of interaction in context. We are then able to reconstruct the larger whole and explain its dynamics in terms of its properties and parts (Capra, 1985; Koestler, 1970; Wilber, 2001b). Figure 2 shows our adapted version of Wilber’s IMP model.

The four quadrants reflect a language-based pronoun perspective (singular subjective, I, in the upper left (UL) quadrant; plural intersubjective, We, in the lower left (LL) quadrant; singular objective, It, in the upper right (UR) quadrant and plural interobjective, Its, in the lower right (LR)

quadrant). Consistent inquiries from these basic perspectives from an interior and exterior view will over a period of time, render specific types of knowledge based on this enabling methodology. Moving anti-clockwise starting with the UL, then LL, UR and LR, what can be noted is the transcending and inclusive aspect of the knowledges when integrated. All knowledge pertaining to the quadrants is related and relative in nature, where the perspectives are considered true but partial (Wilber, 2007; Wilber, 1982). Depending on context of study and perspective taken, disciplines can employ several types of paradigms and methodologies rendering different points of view, and knowledge findings. The eight methodologies and paradigms for gaining irreducible knowledge are: structuralism, phenomenology, autopoiesis, empiricism, hermeneutics, ethnomethodology, systems theory and social autopoiesis (McGregor, 2009; Saiter, 2009; Wilber, 2007). The systems integral model of creative innovation presented in this study will il-

illustrate how elements of creative innovation from other scholastic work such as Csikszentmihalyi's systems model of creativity can be understood in the four quadrant framework shown above.

### 3 Data collection and method

To develop the dataset, we set out to define the landscape at a seaweed conference held in September 2019 with 8 informal interviews with individuals who worked specifically with seaweed in Northern Europe and the Nordic region. This conference was chosen for the ease of access to individuals with expert knowledge and skillset pertaining to the use and cultivation of seaweed. The individuals represented various industry sectors that worked with seaweed. They were producers, product developers as well as chefs and HoReCa business owners, who used their interest and knowledge of seaweed in their menus and culinary presentations.

Data was then collected from online interviews / presentations. A total of 25 interviews and presentations were retrieved from online sources both in text as well as audio-visual formats. The texts were transcribed in accordance to the Gothenburg Transcription Standard (GTS) 6.4 (Nivre et al., 2004), using Modified Standard Orthography version 6 (MSO6) reflecting spoken language. The transcribed texts were compiled into a small, topic focused corpus that consisted of 82 427 word tokens. The text examples shown in this study occur in GTS 6.4 MSO6.

This study was corpus (data) driven – the visualisation followed a two-step data extraction process using

1. VOSviewer, a software tool for constructing and visualizing bibliometric networks (Cordeiro, 2019; van Eck & Waltman, 2007, 2014; Waltman et al., 2010) and
2. AntConc, a concordance software designed to facilitate text queries / text mining for regular phrases and expressions, performing kwic (keyword in context) analysis (Anthony, 2019).

The corpus was described as "small" as per Flowerdew (1996) with our data falling well in the

range for small corpora (20 000- 200 000 words). Our study took advantage of the design and use of small corpora by:

1. greater access and ease of use for collective concordance analysis, from keywords in context (kwic) analysis to word clusters and word collocates (words that co-occur frequently) that make up the foundation of the qualitative findings of this study;
2. facilitating researcher familiarity with the corpus for more efficient retrieval of findings of relevant text examples for illustrations by means of examples;
3. a systemic approach in corpus construction where the corpus was designed and constructed towards a specific topic query and
4. a focus on a specialised subject.

In this case the corpus reflected individuals with expertise in creative innovation as well as seaweed from various industry sectors, enabling focused thematic finds in qualitative text analysis and small corpora (Flowerdew, 1996). The main purpose of constructing such a corpus was to have a depth of understanding of a phenomenon, in this case, uncovering the elements of creative innovation that were then further investigated from a systems integral perspective.

VOSviewer and AntConc both use natural language processing techniques in text mining. They differ in the extent of purpose of construct and use. In VOSviewer, the idea of visualizing bibliometric networks is often referred to as "science mapping". In this study, it was applied to analyse the small corpus for co-occurrence relations between keywords (also known as nodes) or words most often referred to in the data (van Eck & Waltman, 2014). The result of this was thematic salience sorting through word clusters. VOSviewer created a weighted network that showed the strength of the co-occurrence of keywords through the thickness of lines indicated between nodes, the more salient the node, the larger the sphere in the visualization (see Figure 3). The concordance software AntConc gave a deeper analysis into the text, and into language in use in context. While VOSviewer rendered clusters of keywords that frequently co-occur,

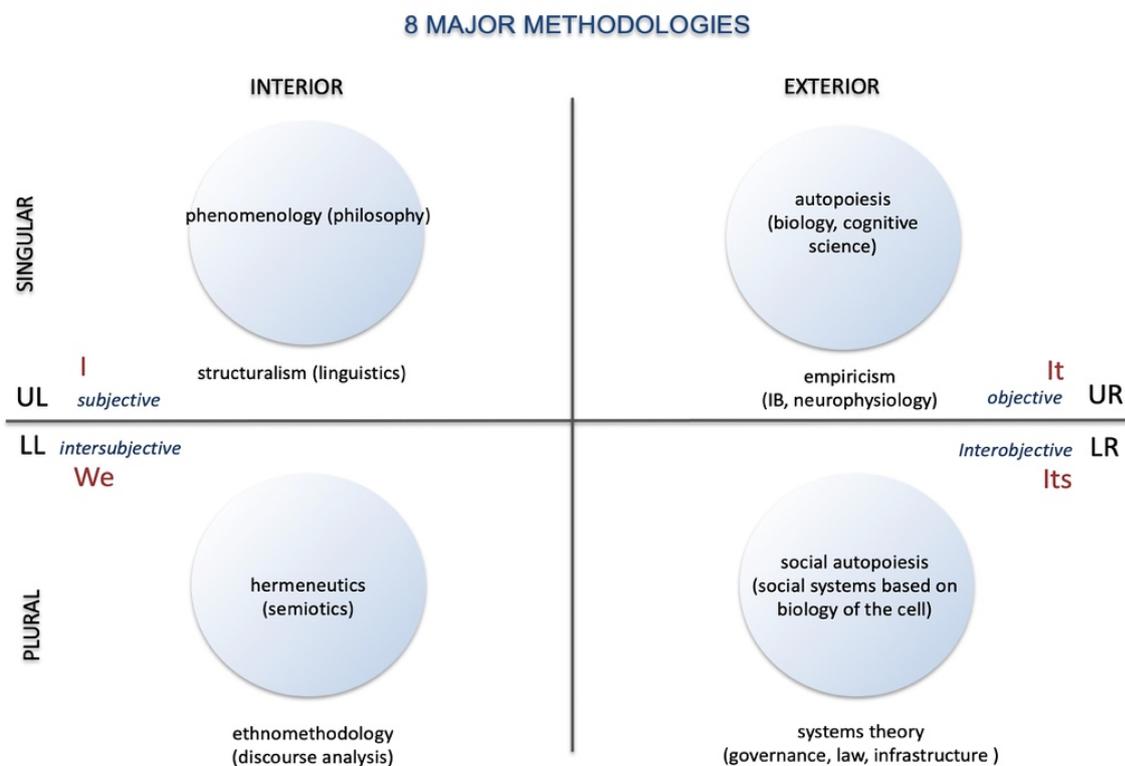


Figure 2: Integral methodological pluralism (IMP) model (Wilber, 2007): 36-37)

AntConc helped identify high frequency occurring words, word collocates, and word clusters in the corpus. It also helped identify in which context these words were used (kwc analysis) in order for it to be used in text examples. Kwc analysis also helped identify and sort accordingly, material actions, mental processes such as thoughts and behavioural intentions of the interlocutors. The purpose of the synergetic use of these two software was to create a triangulation of methods in which clusters of keywords were identified in a rigorous and systematic manner.

## 4 Findings and discussion

### 4.1 VOSviewer findings

Figure 3 shows the VOSviewer word co-occurrences network for the data. This data addressed RQ1 and RQ2 (vide supra) by means of identifying and clustering salient themes pertaining to the field of the culinary arts and science. The visual mapping was a result of binary counting with a minimum of 10 occurrences of a term. Of the 5903 terms identified, VOSviewer parameters identified 147 that met this threshold. For each of the 147 items, a relevance score was calculated. Based on this score, the most relevant terms were selected. Default choice in VOSviewer was to select 60% most relevant terms. A total of 77 terms were retrieved in this

manner in 4 semantic clusters (Figure 3).

Table 1 lists the semantic clustering of words that illustrate salient themes from our corpus. Themes can also be retrieved with axial coding, i.e. coding across the clusters. An example of an axially coded theme from this corpus is the reference to names of places, such as London, New York, Singapore, Spain etc. When focusing on creative innovation processes, we identified the first keywords of interest in this study by listing them as they occur in the VOSviewer retrieved clusters. Noticeable in all 4 semantic clusters was that the word "creativity" or its corresponding transitive verbal state "to create" was absent as a salient theme in the corpus. The word "innovate" occurred in Cluster 3 (with 13 items). The words that occurred in collocation with "innovate" were "child", "concept", "element", "field", "fun", "important thing", "ingredient", "inspiration", "molecular gastronomy", "science" and "study". The semantic cluster of words found in Cluster 3 seemed to occur in specifically addressing RQ1, with elements that bolstered or contributed to the innovation processes in the field of culinary science.

Cluster 1 had 33 items and addressed RQ2. It was the cluster where the word "seaweed" occurred. Seaweed occurred in cluster 1 despite it being marketed as a "sea vegetable" in the NNC context or in the example of one company as "sea pasta". Sea pasta is the trade name for *Himanthalia elongate* or thongweed. Seaweed is being marketed as part of the sustainable future foods scenario. We have been made aware of that phycogastronomy is currently outside mainstream in the context of the NNC and northern European diet (Transcript 15):

there are eight there are over six billion people on earth / and they're needing to eat / needing nutrition / and we all know that if we do that under the circumstances we have today we will be challenging ourselves / and seaweed might be one of the options so the future of food for us means finding new alternatives / and also new sources of nutrition / and it has to be sustainable / and bringing them to the middle of the plate as daily food and that's actu-

ally... what we challenge ourselves and each other in getting seaweed as an alternative to rice [and] pasta / which we now / we eat every day or possibly eat everyday / so it has to be sustainable and with lots of nutrition and of course those who eat it / it needs to be delicious / otherwise we don't eat it / and that's why we say eat yourself happy / and with that we try to bring a new product / a new category / or new because it [has] already [been] existing for quite a while / but we're not used to eating it and bringing it into the future of food

In Transcript 15 the speaker addressed the challenges of introducing a new product to an emerging market within the NNC diet: that seaweed is not a staple food, that even if people are becoming more familiar with seaweed as a food, seaweed in itself might not have a reputation of being too flavourful. The culinary heritage of eating rice and pasta in some cultures is difficult to sway and influence. The brief text example from Transcript 15 also carried connotations from other Cluster 1 collocations such as "first time", "challenge", "front", "future", "journey", "ocean", "sea" and "story". Contrasting connotations also occurred such as "meat", where in the NNC, a dietary pattern rich in fruits and vegetables including the use of rapeseed, flaxseed and olive oils is highly recommended. The NNC dietary guidelines do not necessarily discourage the eating of meat but note that highly processed meats in long term consumption may be detrimental to health. Semantic connotations in Cluster 2 with 26 items revolved around the individuals as chef or cook and the interests they have outside of their culinary profession, or what inspires them in their own work. Cluster 2 reflected words that could pertain to the person such as "chef", "book" (many chefs are involved in writing their own books), or disseminating their "techniques", "dream" as well as inciting the same "passion" in others. To some chefs, the "show" and "art" of culinary presentation is important. An axially coded theme between Clusters 1 to 3 showed the advancing technologies in the culinary field and "industry" that enable new

"techniques" and "innovations" to occur so that "molecular gastronomy" can be experienced and further refined in "kitchens".

Data bias is a common challenge in small corpora. One such potential bias is the place London, which occurred in cluster 4. This may have been caused by the experiences described in some of the transcripts. An AntConc concordance plot search for the place name "London" revealed that 6 (of 25) transcripts referred to London in relation to their kitchen experiences. A prominent feature of cluster 4 though was the value word "respect", falling into the same semantic cluster as "head chef", "cook" and "kitchen". A concordance plot analysis returned a total of 19 hits with 9 (of 25) transcripts speaking about respect in various contexts, from "respect for nature and the ingredients we use" and respect in relation to the culinary art and craft as found in Transcript 10:

respect your predecessors [because]  
without them you would not have a job  
there / you might be offered a job at  
a company that's 100 or 50 or 20 years  
old / treat it with care / you can change  
some things / life involves changes and  
you have to be cold about it / *respect is  
vital*

In Transcript 21, the word "respect" occurs in collocation with "head chef" in the context of being a chef, being in leadership position and creating good team rapport:

as the *head chef* you have to be a good  
leader, mentor and trainer / you have to  
adapt your kitchen to whatever circum-  
stances you are in / and make sure you  
have a *mutual respect with your team  
members* / this is not a popularity con-  
test / but it's important that as a *head  
chef you lead your team* in the right di-  
rection / and *treat people as individuals  
with respect*

## 4.2 AntConc keyword-in-context (kwic) findings

The VOSviewer clusters highlighted salient semantic webs that occur in the corpus. While this was helpful for identifying words associated with different contexts in being a chef and the creative innovation process, the notable absence of the word "creative" or "create" for example, indicated possible gaps in the key topic of inquiry for this study. In this section, we discuss how transitive verbs associated with the intangible aspects of creative innovation such as "to create" and "to know" are used in context. While the end product of creation is a tangible, material object, the processes of creation from idea and concept to actual prototype / object is mostly intangible. To fill this inquiry gap, we performed a wild card search on "creat\*" and "know\*". This retrieved kwic findings for variations of these two word groups.

### Findings for "creat\*"

AntConc results showed a total of 307 concordance hits with 21 (of 25) transcripts containing words with the base "create\*". There were 12 concordance hits with 4 (of 25) transcripts talking about "creative processes"! Some chefs view the creative process as being both structured and unstructured. The need for structure comes in when creative processes need to be managed in a systematic manner, for example, the study of ingredients for their properties, documenting results from different cooking techniques and designs for presentation and plating. As found in Transcript 13, the creative process for this chef is one that is structured into eight elements:

unique / pure / texture / memory / salt  
/ south / artisan / terroir // these are  
the eight most important words in <  
name of restaurant > / they are the  
backbone of everything that is created  
at < name of restaurant > / from a sin-  
gle dish to the restaurant's overall phi-  
losophy / [it is] our method of encourag-  
ing and managing the creative process  
/ and a principle to live by



Table 1: VOSviewer corpus salient themes reflected in semantic clusters

Cluster 1 (33 items)	Cluster 2 (26 items)	Cluster 3 (13 items)	Cluster 4 (5 items)
answer	anyone	child	cook
audience	art	concept	head chef
beginning	book	element	kitchen
challenge	chef	eye	london
event	cooking	field	respect
first time	cuisine	fun	
fish	dish	important thing	
front	dream	ingredient	
future	el bulli	innovation	
home	fact	inspiration	
industry	flavor	molecular gastronomy	
job	france	science	
journey	friend	study	
kid	god		
line	house		
meat	meal		
mother	middle		
ocean	music		
order	new york		
picture	passion		
piece	show		
salt	singapore		
sea	spain		
seamore	table		
seaweed	technique		
sense	wife		
sort			
story			
stuff			
thousand			
top			
vegetable			
water			

nian. That the key actor in Transcript 11 also said "I have a very pragmatic approach to creativity" further supported this idea of accessibility. "Anyone" (found in VOSviewer Cluster 2) can be creative and partake in creative innovation, and it is not only reserved for elite chefs experimenting with rarely used ingredients and innovative cooking techniques.

**Findings for "know\*"**

Close reading of the transcripts indicated that ideas were generally developed through a concerted effort of refining existing concepts and ex-

tending / testing new applications from an existing body of knowledge and practices. While chefs might come across as creative geniuses and one with great expert knowledge in kitchens, presenting dishes in a way unexpected by the consumer leading to that unforgettable dining experience, the findings in this section stemmed from looking among others at what chefs say they "don't know". Aside from building from an existing knowledge base, the aspect of knowing and "to know" was also tied to personal curiosity, learning and exploration. From Transcript 10:

I'm sick of know-it-alls / I know noth-

ing I'm here to learn / I'm just sharing my experience [of] the things that have happened to me

A similarly expressed thought about "knowing" was found in Transcript 13, where the speaker associated the unknown with the perception of what is unique:

[to be] unique / [it is] things that we know yet we don't know / ... it is about something that appears at the right environment [at the] right timing / nothing is really unique or not unique

And from Transcript 22:

I remember the first time I saw the [name of] restaurant website / I was astonished / it was my first glimpse of food that had been prepared and presented in a range of ways / which I had never seen before and could barely comprehend / it was all so simple and understated / yet even as a novice cook at the time / I could tell that the complexity of preparing such dishes must be immense / but how was it all done and more importantly how could I do it / this triggered a desire to learn more about this new / exotic form of cuisine / and my research into a field I came to know as molecular gastronomy / this journey for knowledge opened up my culinary eyes / and introduced me to a whole host of new names and faces I would otherwise have been ignorant of

Both transcript text examples illustrated how "knowing" includes knowing one's own limitations and boundaries, i.e., knowing what you don't know. To understand how "knowing" and "knowledge" contributed to the creative innovation process, a wildcard AntConc search term "know\*" used to retrieve variations of the keyword root "know". In this case, it retrieved keywords in contexts that included "know", "knows", "known", "knowledge" and "knowledgeable". Variations of the transitive verb "to know" did not appear in the VOSviewer cluster findings, but it had a concordance hit of 573

instances with 23 (of 25) transcripts containing references to "to know", further supporting the use of a kwic analysis.

An example of taking a familiar ingredient (such as salt) and working with existing consumer knowledge, expectation, and experience with the ingredient, one chef decided to deconstruct the facets of "saltiness" in this food presentation. From Transcript 13:

[salt is] the first seasoning that we know- so salt has been very important / for me / we are always thinking that can we have a flavour that everybody understands / whether it's lemongrass / or it's chili / or it's curry- / I don't know- a cheese // somehow it's trapped in a certain boundary / but can we have one dish that everybody understands / so I was thinking salt / it's not just the physical salt / but the depth of saltiness / what I mean is for example / soy sauce / fish sauce / ham / anchovy / and sea water or seaweed / they are all different depths of saltiness

However, personal exploration and deconstruction of the familiar is but one facet of knowing. When it comes to the field of phycogastronomy and trying to make seaweed consumption more mainstream in the Nordic countries, know-how of the acquisition of raw produce is vital. Knowledge and know-how for a sustainable business and the challenges faced in seaweed growing is voiced in Transcript 15. It explained in part the core reasons why phycogastronomy is challenging to foster / cultivate due to steady access to raw material:

it's a strict environment [much] like the wild west / by families who are doing their harvesting and fishing over years / and this being a new [seaweed] specimen / or it's not a new species of seaweed / but it's now being harvested / ... there's not a lot of [government] legislation around it / so you need to find a way looking to different types of seaweed / [and it's] hard to specify with quotas [what is being allowed] to harvest in sustainable ways / so this for

example we only harvest fifty percent [in] certain areas because we know it's proven that it grows back year after year / so you're still being sustainable / and that's also why we are harvesting from < name of country A > / ... and probably will we are still in < name of country B > next year / where the seaweed harvesting protocols are a little bit further ahead because we need to make sure that we can grow our business

### 4.3 Systems Integral Approach

This section addresses RQ3. Figure 4 shows our proposed systems integral model of creative innovation in culinary science, boiled down to the elements distilled from the small corpus. Elements from current creative innovation literature, and in particular Csikszentmihalyi's systems model, were reflected in the four quadrants following the 4 primordial perspectives and were also reflected in the language pronouns. Csikszentmihalyi's domains and its sub-domains, "social system - field", "cultural system - domain" and "genetic make-up - person" fell broadly under the UL and LL quadrants of our systems integral model. The interactions and feedback loops are illustrated in Figure 4 by the circling arrows between the various communities of practices and groups of individuals in the plural intersubjective "We" (LL quadrant) perspective. These feedback loops occurred in all four quadrants, but for reasons of clarity they are only shown in the LL quadrant in Figure 4.

The main difference between a systems model such as Csikszentmihalyi's and the systems integral model, is how the systems integral model can give multi-levelled, multi subjective / objective perspectives. Figure 4 reflects two types of perspectives, the first (labelled "A" in all quadrants) considers the chef (and the team) as singular subjective "Individual" or "I". Table 2 shows in broad outline, the human-centric perspective "A" and the product-centric perspective "B" as well as their elements that contribute to creative innovation into the four quadrants through applying language-based pronouns.

Starting with the human-centric perspective,

when chefs who speak of their own conviction, their food and life philosophy and their passion, these elements could be categorised to form the singular subjective perspective in the UL quadrant of "I". From Transcript 19, a chef narrates his journey to finding his professional identity in what he describes as a personal and individual calling:

my brother took me there and said / this is the perfect place for you / it's the place where you can express yourself through the food / through your passion / I said to < name > are you sure / he said / yes this is the perfect place / one week later / I was there / because the food world chose me / It's not that I chose that / that was the perfect timing / and in that moment / I realized I had to put all of myself into this world // and the simplest interest for food / step by step became a passion / and through the passion / I realized you can transfer emotions / that's what I think food is / and how I interpret food - just transfer emotions

The plural intersubjective "We" in the LL quadrant are the communities of practice, and different stakeholders in society that support a food movement such as the NNC. They may even support the emerging field of phycogastronomy, either by consumers buying the product, or HoReCa actors placing seaweed dishes on their menus. The consumers can then give feedback to the chefs through interaction either at restaurants or through online feedback forms. As the human being is at the radial centre of this perspective, the singular objective perspective in the UR refers to the enabling technologies of food prepared and presented in a different manner. An example from Transcript 11:

let's see a technique that is a very new technique / you know liquid nitrogen / well that may seem quite strange but it's a very common product / it comes in a gas form / in this case we use liquid nitrogen / and in this case it allows us to make things that are otherwise impossible / we're making pure alcohol

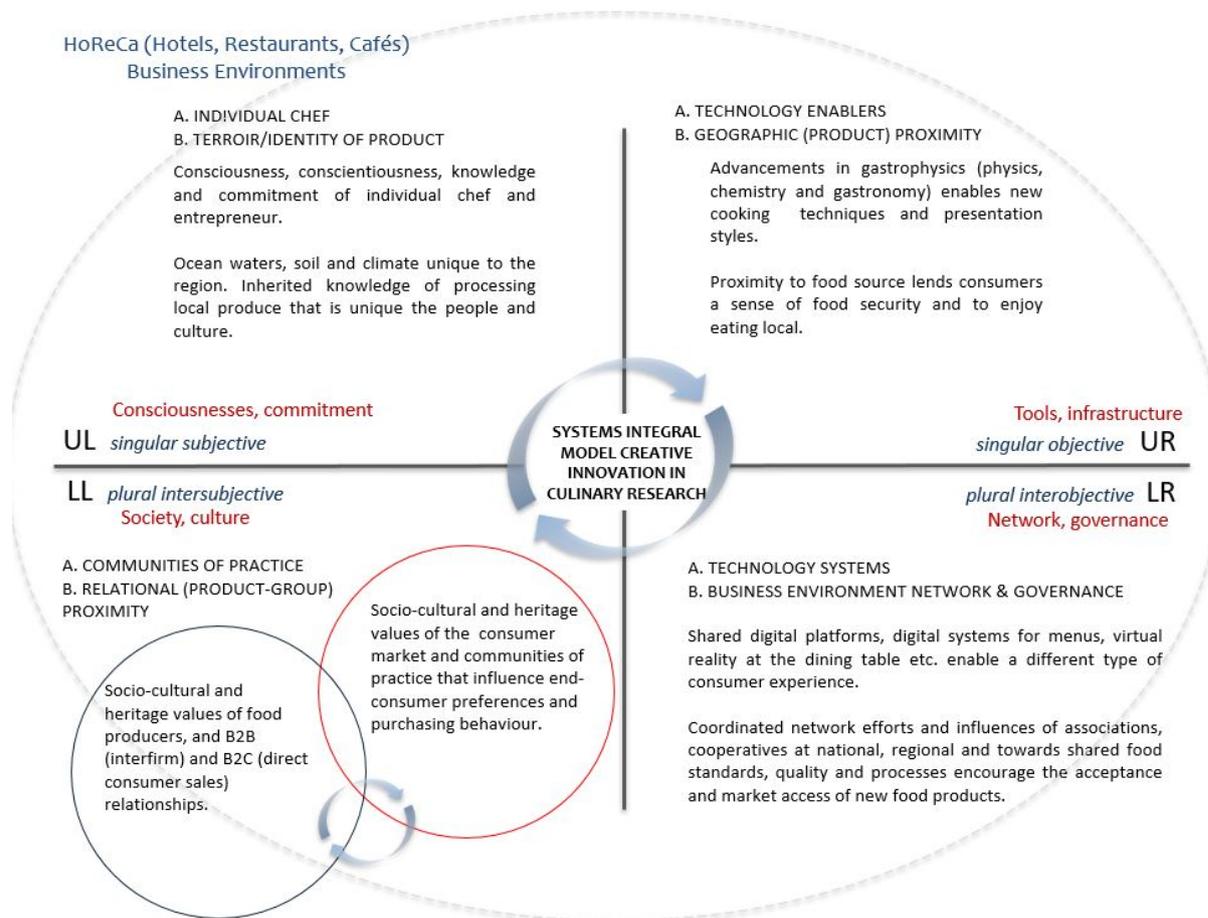


Figure 4: Systems Integral Model of Creative Innovation in Culinary Science

sorbet / if you put alcohol in the freezer you know that it won't freeze / this is -196 deg C

A continuing theme with regards to the challenges faced in phycogastronomy is technology enablers. This was an element in the UR quadrant, and discussed under Csikszentmihalyi's systems model of creativity under "domain" which refers to "knowledge, tools, values, practices". From Transcript 7, the issue of productivity in seaweed harvest depended on the available harvest technologies, and access to technology. The need for communities of practice and interaction between chefs, food producers and consumers (situated in the LL quadrant) was also high-

lighted in the following text example, as a means to creative innovations:

we hire inner-city kids to package / cook / process all of our food / they learn about 3D farming / they learn about sustainability / one of the kids at the school / it's called < name of the school > actually took the kelp and invented a 12 volt kelp powered biodegradable battery // so you know / on the blue green economy / I think we can think much bigger than this / why can't we take my farm and embed it in offshore wind farms / why don't we just harvest wind / let's harvest food / fuel / fertilizer / let's bring it back

Table 2: Systems integral model, human-centric ("A" perspective) and product-centric ("B" perspective)

Perspective	I (UL quadrant)	We (LL quadrant)	Its (UR quadrant)	Its (LR quadrant)
<b>A</b>	Consciousness	Social practices	Technology enablers in the kitchen for the Chef	Industry structure and network
	Commitment	Organizational support	Digitalisation / Internet for HoReCa	Business Environment Network
	Belief Passion	Culture Consumer awareness	Alternative food product Relatively new ingredients	Governance Logistics Infrastructure
	Inspiration	Intergroup communication and feedback		Trade agreements
	Aspiration	Values Heritage		
<b>B</b>	Terroir	Product-consumer proximity	Geographic proximity and access to raw produce	Industry structure and network
	Product Identity	Product branding through cultural practices, values, food heritage	Geographic proximity of product to consumers	Systems architecture and network
	Country of origin (COO)	New product support through purchase and consumption	Harvesting technologies	Logistics infrastructure
			Food processing technologies	Trade agreements

The second perspective (labelled "B" in all quadrants) considered the material product as singular subjective "I". In the field of food production and gastronomy, food products are branded, and given characteristics / qualities pertaining to terroir and place-of-origin. For example, in the NNC, products native to the Nordics are used and spoken about as being characteristic of / reflecting the Nordic region climate, soil, air and values (in how the food is processed) and marketed as "flavours of the North". The personification of raw products and products, through conscious and conscientious product branding, builds product identity. This product identity can also be studied from the perspective of the above mentioned four quadrants. Taking the example of seaweed pasta, from Transcript 15, product branding and identity is achieved via association with credible partners such as a bank as business partner, and new food wrapping technology companies that will help decrease the carbon footprint of each package of seaweed pasta sold. All branding and product identity building,

depending on the perspective of study, can be situated in the LL quadrant (for supporting business / social partners), to the UR quadrant (for technology enabling partners and product geographic proximity, from harvest to plate) and in the LR quadrant, which is looking towards future regional trade agreements:

besides the Netherlands / the seaweed pasta is also sold in Germany / Switzerland / Denmark / Great Britain and Australia / various other countries are expected to follow / after the crowdfunding the < name of bank > entered into a partnership with < company name > / the bank not only functions as its personal banker / but even introduces its products at trade fairs abroad // ...the bank is an important bank known for investing in the food and agriculture section // ...as to the transport of the harvest / that is being looked at too / transporters whose trucks would formerly

travel back empty after having delivered their flowers in < country name > / in the harvesting months now return to < name of country > with a load of seaweed / [so that] the company's ecological footprint will remain as small as possible

The broadest perspective that included and transcended all other perspectives in the four quadrant model was the plural interobjective perspective located in the LR quadrant. The LR quadrant pertains to the broadest perspective of network and governance. For the individual chef (the "A" perspective) or food producer, what could be identified as a subject of study located in this quadrant was how digitalisation is revolutionizing their workspaces and how they communicate with their own community of practice as well as their consumers. One example is how digitalisation and the use of the internet for consumers has made the flow of serving food more efficient. From Transcript 11, the chef and restaurant owners explained how productivity and dining experiences were enhanced for the consumers by use of the internet:

it's not [the most] logical thing that we'll find in the world / [but] you find long wine lists in restaurants / so if you get there maybe three hours before / [okay] / otherwise you won't have time to read it / in this case the internet can be used very efficiently and wonderfully / you can hang your wine list on internet / and you can look through it at ease / and you get an idea of the wines / and what things are a bit special / and at least you've got an idea when you're out at the restaurant

Because creative innovation is a delicate balance between structure and freedom from structure, project management and management of ideas is important. This can be improved by digitalisation, and software that help HoReCa manage the organization of documents better, whether it's coordinating their supply chain or table bookings, or managing staff shifts etc. From Transcript 17, structured innovation was referred to

and helped by placing projects in an organisation wide internal digital platform:

by actually bringing these projects to life in a digital platform / you increase your productivity significantly / and further / you also increase the quality of the output of the projects / because you've been efficient in producing something that actually adds value / and when the productivity is there the quality is there / the profitability also sneaks in on you because then you've been efficient in providing something that that creates value to your customers // ...it's also about joint work because it is more fun to go to work every day if you're part of the well-oiled machine / rather than spending most of your day discussing with your colleagues who should do what and when / and why don't I get the information I need when I need it etc

Systems of technological networks, available software that can be implemented at the organization or between organizations in a shared digital platform, will affect how creative innovation proceeds.

From the second "B" perspective of the product, the successful acceptance and adoption of new food products might require both an upward movement such as the NNC and the encouragement of eating more greens, that includes sea vegetables, as well as a top-down legislation effort, whether in the form of NNC dietary guidelines, or lowering trade barriers to favour access to certain raw foods. Pertaining to individual chefs and food producers as active stakeholders in ground up movements, the actions and commitment made by them will influence the relationship towards a great number of network actors including those from farm cooperatives to consumer associations, both governmental and non-governmental that in turn have the power to initiate/create the overarching legal frameworks within which their businesses operate.

Our four quadrant model enabled an unfolding of deeper elements as compared to the more traditional systems models for describing creativity

in current culinary research. One can use the systems integral model of creative innovation to elucidate how, depending on the researcher's purpose of study, the model, using a language-based pronoun system, can encompass multiple points of view. Various types of inquiries such as Individual, Group, Social, National, Environmental etc. are made possible with our model.

## 5 Conclusion

This study used phycogastronomy in the context of the NNC as example to study the processes of creative innovation. Based on 25 transcripts turned into a small but topic focused corpus, elements and characteristics of the creative innovation process were identified using a systems integral approach. A model of the findings from the small corpus was proposed and shown in Figure 4. The model showed that creative innovation can be studied from three perspectives, two of which are human-centric and the third one is product-centric. While our systems integral approach gave wider and deeper insights into creative innovation, the advantage of applying a systems integral approach was also its limitation. Depending on the researcher's expertise, interest and study perspective, the four quadrant model can be applied in different contexts, to study different themes. Some might find this model too broad and all-encompassing so that it becomes unhelpful to them. With growing bodies of knowledge, most academic fields are coming to realise the need for a unified / holistic approach of which systems integral theory is one such approach. In the case of studying creative innovation in gastronomy per se, our corpus driven findings suggested that a holistic approach to understanding its elements and management is more helpful compared to focusing solely on individual psychology and passion or cultural / community support.

Our data corpus revealed that phycogastronomy in the NNC context remains an emerging concept. Its challenge is reflected through the entire supply chain from harvest to consumer acceptance. Within our small corpus, several chefs and HoReCa stakeholders have voiced how technology affects their businesses, as well as given them

inspiration towards new dining experiences. Our data found a clear effect of technology enablers in gastronomic innovation. They included digital, technical, and innovative food preparation technologies. It has, according to those who implement it, accelerated, and structured the process of creative innovation, suggesting that there are clear advantages of a more widespread implementation of such technology enablers.

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