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Food Oral Processing in Humans: Links Between Physiological Parameters, Release of Flavour Stimuli and Flavour Perception of Food

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Abstract

In humans, food oral processing (FOP) is the first step in the digestive process. It prepares the food for swallowing and to undergo the process of digestion. During chewing, the food is comminuted by the combined action of chewing and saliva to form a bolus. The particle size of the bolus is reduced due to the action of the tongue and the teeth, and the saliva is continuously produced by the salivary glands to humidify and impregnate the food. Saliva lubricates the bolus and enables the cohesion of particles to prepare for swallowing. During food oral processing, the compounds responsible for food flavour and taste are released, leading to the perception of food organoleptic properties and significantly contributing to the consumer's acceptability of the product. Understanding this process of food breakdown and bolus formation thus appears to be a way to revisit food functional properties. However, this process is extremely complex, and as such, its description necessitates a combination of many quantities from different disciplines, i.e., physics, chemistry, physiology, psychology, behavioural science and food science. It depends, on one hand, on food properties and on the other hand, it depends on oral physiology. However, large inter-individual variability is commonly observed, which has important consequences on flavour release and perception. The challenge for the food industry is to be able to develop food considering this large variability, and sensory and nutritional constraints. This challenge is particularly relevant when specific populations (i.e., elderly, infants or obese subjects) are considered.

Keywords: Mastication; Saliva; Food Bolus; Aroma; Taste

1 Food breakdown and bolus formation in the mouth

1.1 Role of mastication

In humans, the mastication process involves the coordinated activity of masticatory muscles (masseter temporalis, lateral and medial pterygoids), jaw, lips, tongue and saliva. Food breakdown begins by the rhythmic movements of opening and closing the mandible while the jaws and tongue reposition the food between the teeth. The tongue gathers the particles to form a bolus, and then transports the bolus to the posterior part of the oral cavity to initiate swallowing. Salivation, which is mechanically stimulated

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by chewing and is physico-chemically stimulated by the food components, enables lubrication of the bolus, aggregation of particles and swallowing (Salles et al., 2011).
Masticatory ability is closely related to the number of teeth, and a decrease in the ability to chew occurs when less than 20 teeth are present. Functional chewing difficulties particularly occur in the elderly, with a decline in general health that leads to tooth loss and hyposalivation, but ageing has little effect on chewing in older people with proper oral health status. In the case of infants, physiological changes allow them to move progressively from sucking to chewing and thus from liquid to solid foods. Infant masticatory efficiency is stimulated by exposure to more or less complex food textures (Nicklaus, Demonteil, & Tournier, 2015).
Chewing is mainly influenced by the composition of the food, its texture (Kohyama & Mioche, 2004) and the chewing parameters (Yven, Culioli, & Mioche, 2005). Indeed, the food's texture has a large influence on the chewing process. In particular, the number of chewing cycles preceding the first swallowing depends on the texture of the food. A dry, hard food requires many chewing cycles to be fragmented into particles and impregnated by saliva before being swallowed (Prinz & Lucas, 1995). In fact, to be swallowed, the food bolus must reach adequate levels of comminution (particle size) and of lubrication or moistening (Hutchings & Lillford, 1988). For hard, brittle foods, such as peanuts, almonds, carrots, it appears that the particle sizes are similar among individuals, but the duration of chewing sequences and the number of cycles vary (Mishellany, Woda, Labas, & Peyron, 2006). In most studies, the breakdown rate is determined by successive sieving bowls of particles (van der Bilt & Fontijn-Tekamp, 2004). However, for non-brittle foods, other bolus properties triggering swallowing have been evidenced. In particular, food bolus consistency has been highlighted as an important property to trigger swallowing during cheese consumption (Yven et al., 2012). In this case, it is likely that the continuous phase of the food bolus, resulting from a mix between saliva and food, is as important as particle size.

1.2 Role of salivation

Saliva is a biological fluid that bathes and moisturizes the oropharyngeal cavity. It is produced by three pairs of major (parotid, sublingual and submandibular) and minor (buccal, labial, lingual, and palate) glands. A distinction is made between saliva at rest, which corresponds to the liquid produced without any stimulation (mechanical, chemical or trigeminal), and stimulated saliva that results from a stimulation, more often during the consumption of liquid or solid food (Schipper, Silletti, & Vingerhoeds, 2007).
During the chewing process, stimulated saliva contributes to the formation of a food bolus that can be swallowed (Yven, Guessasma, Chaunier, Della Valle, & Salles, 2010) and to the release of active substances. Due to its moisturizing, lubrication and hydrolytic capacities, saliva changes the structure and mechanical and physical properties of the food bolus and thus the sensory perception of texture and flavour (Yven et al., 2005). Moreover, large inter-individual variability was observed (Quintana et al., 2009; Feron et al., 2014). Indeed, studies conducted on the incorporation of saliva during chewing showed a level of moistening of up to 80%, depending on the matrices and subjects considered (Guichard et al., 2008; Guinard, ZoumasMorse, Walchak, & Simpson, 1997).

1.3 Particle size reduction and swallowing event

At the end of the chewing sequence, a double threshold - comminution and lubrication - needed to trigger aspiration was proposed (Hutchings & Lillford, 1988). These two parameters can be connected to one dimension, represented by the cohesion of the food bolus (Prinz & Lucas, 1995). During chewing and thus the production of smaller and smaller particles, saliva gradually reduces the space between particles and thus increases the cohesive forces between them. For instance, an analysis of fragments of different kinds of cereal flakes (breakfast type) showed the importance of the breaking behaviour of the product on human mastication. The fragmentation phase is quickly followed, in less than ten cy-

cles of mastication, by a particle agglomeration phase. The two phenomena are related to the amplitude of the masticatory force and its change during the chewing process (Yven et al., 2010). The gradual disintegration of the food matrix in the mouth during chewing leads to variations in texture perception, which although they are specific to each type of food, they can change over time, reflecting particle size reduction and the increased cohesion of lubricated particles (Lenfant, Loret, Pineau, Hartmann, & Martin, 2009). The harder food is to swallow, the longer the time that is needed for Food Oral Processing (FOP) (Chen & Lolivret, 2011).
In conclusion, this part of in-mouth food breakdown and bolus formation can be likened to a food process that leads a product far from what is originally placed in the mouth. It will thus impact the dynamics of the release of active substances and their functionalities (organoleptic, nutritional, etc.) (Salles et al., 2011).

2 FOP, flavour release and perception

2.1 Aroma

In the mouth, aroma release and perception are strongly linked to oral parameters (chewing, salivation, breathing, swallowing) (Rolls & Rolls, 1997). Furthermore, individual differences in the profiles of chewing and swallowing explain inter-individual differences in the retronasal release of aroma compounds (Ruijschop, Burgering, Jacobs, & Boelrijk, 2009).
In vivo, aromatic perception depends on the concentration of flavour in the nasopharynx, which itself is affected by the rate of release of the food aroma compounds in the oral cavity (van Ruth & Roozen, 2000). The release of aroma compounds in the mouth is affected by the composition of the food matrix and the entire oral process (Figure 1). All phenomena that occur between food intake and swallowing increase the surface area available for exchange, dilution and partial dissolution in saliva, which may affect the release of aroma compounds (Bakker et al., 1996).
For instance, in cheese and for one subject, the time corresponding to the maximum release of aroma compounds, such as hexane-2-one, hexane-2-ol and ethylhexanoate, are very close, but large inter-individual differences regarding volatile release kinetic parameters have been reported (Pionnier et al., 2004). These differences are attributed to different oral physiology characteristics and oral processing behaviours. In a study conducted on 43 subjects consuming cheese products, a maximum concentration and total amount of aroma released in the mouth have been linked to the number of chewing bursts, high masticatory activity, low salivary flow and a low level of salivary α-amylase (due to a retention effect by this protein in this case) (Feron et al., 2014). These relationships are explained by a higher breakdown of the food, followed by a higher spreadability of the food bolus and a higher coating at the surface of the oral cavity. The consequence is a higher transfer of volatile compounds from the oral cavity to the nasal cavity through the retronasal way. Similar observations were reported for candies (Blissett, Hort, & Taylor, 2006). On perception, the release rate of aroma compounds, rather than the maximum concentration released in the mouth, has shown an important determinant for the perception of aroma (Mestres, Moran, Jordan, & Buettner, 2005; Boland, Delahunty, & van Ruth, 2006). However, aroma perception was explained by masticatory behaviour and saliva properties and was less extensively related to aroma release profiles (Feron & Guichard, 2014).
Inter-individual differences in swallowing have also been reported (Guichard et al., 2008). These differences have a significant effect on the release profile of flavour compounds measured in nasal emanations (Buettner, 2002b). Thus, some subjects will continuously release aroma compounds as they chew food, other subjects will release these compounds during chewing, with interruptions during swallowing, while some will release these compounds only after swallowing. This phenomenon is explained by differences in soft palate positioning, which allows opening and closing the mouth and the retronasal control of gas transfer (Mishellany-Dutour et al., 2012).
When moistening the bolus, various factors related to the composition of saliva will directly impact both the quality and quantity of aroma release. One can cite the work of Guinard and

co-workers (Guinard, ZoumasMorse, & Walchak, 1997), Van Ruth and co-workers (van Ruth & Roozen, 2000), Buettner (Buettner, 2002a; Buettner, Beer, Hannig, Settles, & Schieberle, 2002) and Pagès and co-workers (Pagès-Hélary, Andriot, Guichard, & Canon, 2014) as examples, as these authors have described the role of salivary flow and composition on in-mouth retention and the degradation of certain volatile compounds. Some of the salivary compounds that seem to be the most involved are mucins, certain enzymes and small molecules, especially salts, and the pH. For instance, the retention of aroma molecules (ketones and esters) by mucin increases as a function of the aliphatic chain length, suggesting the involvement of hydrophobic effects for these molecules (Pagès-Hélary et al., 2014). Salivary esterases and dehydrogenases can lead to the release of alcohols from esters and to the oxidation of alcohols to aldehydes, respectively (Ployon, Morzel, & Canon, 2017). Ultimately, a high salivary amount of salts increases aroma release in the oral cavity due to the "salting-out" effect, which principally depends on the hydrophobic properties of the volatile molecule (Salles et al., 2011).

Furthermore, the action of salivary α-amylase may induce a change in the viscosity of a solution containing starch, thus having an impact on the release of aroma compounds (Ferry, Hort, Mitchell, Lagarrigue, & Pamies, 2004). Authors have shown that action of the enzyme on two solutions of the same viscosity but different levels of starch resulted in a greater decrease in the viscosity of the solution richer in starch, which explains its more intense release of aroma compounds. In both cases, increasing the amount of α-amylase leads to a more rapid decrease of viscosity. The effect of saliva on the release of aroma compounds from bread was also shown in *in vitro* systems mimicking chewing conditions (Poette et al., 2010).

Finally, chewing behaviour and aroma release varies depending on food properties. For instance, interactions between the composition of cheese matrices and chewing behaviour of individuals affects the release of aroma compounds in the mouth (Figure 2). In particular, a reduction in fat content increases the release of aroma compounds (Feron et al., 2014). This is due to a greater masticatory work due to the increased hardness of the matrices with less fat content, which thus leads to a higher release rate from the matrix. Moreover, the use of fat to decrease the matrix melting point and a lower mixing speed of ingredients during cheese preparation leads to a less hard cheese. In this case, the expected increase in the release of aroma compounds due to the greater mobility of the compounds was partially compensated by lower chewing activity. Indeed, velum opening depends on chewing behaviour (frequency and amplitude). Thus, in this case, the transfer of aroma compounds from the oral cavity to the nasal cavity due to the velum opening was higher for the hardest cheeses.

2.2 Taste

As for the volatile compounds, the release of taste compounds in the mouth during consumption of a food depends on its composition and texture and on the oral physiology parameters of individuals (Figure 1).

The temporal release profile of tasting compounds is highly dependent on the nature of the food matrix. For instance, the citric acid of an orange will be released much more quickly in the mouth than in a gelatin gel, but it will also decrease more rapidly after reaching its maximum (Davidson, Linforth, Hollowood, & Taylor, 2000, 1998).

Similar observations have been reported for cheeses of different textures. Although soft cheese contains more salt than hard cheese, there is a much greater maximum saltiness intensity for salted hard cheese (Davidson et al., 2000, 1998). This significant difference is due to the matrix properties and chemical composition of cheeses (Figure 2). Soft cheese requires low chewing effort and thus breaks easily in the mouth, unlike the hard cheese, in which chewing requires more mechanical energy, and therefore the cheese undergoes a partial breakdown before swallowing. This means that some of the salts are not released into the oral cavity for soft cheese, thus explaining the much lower intensity than that observed for hard cheese. The distribution of the stimulus into the food matrix also plays a role in the release kinetics. When the salt is localized

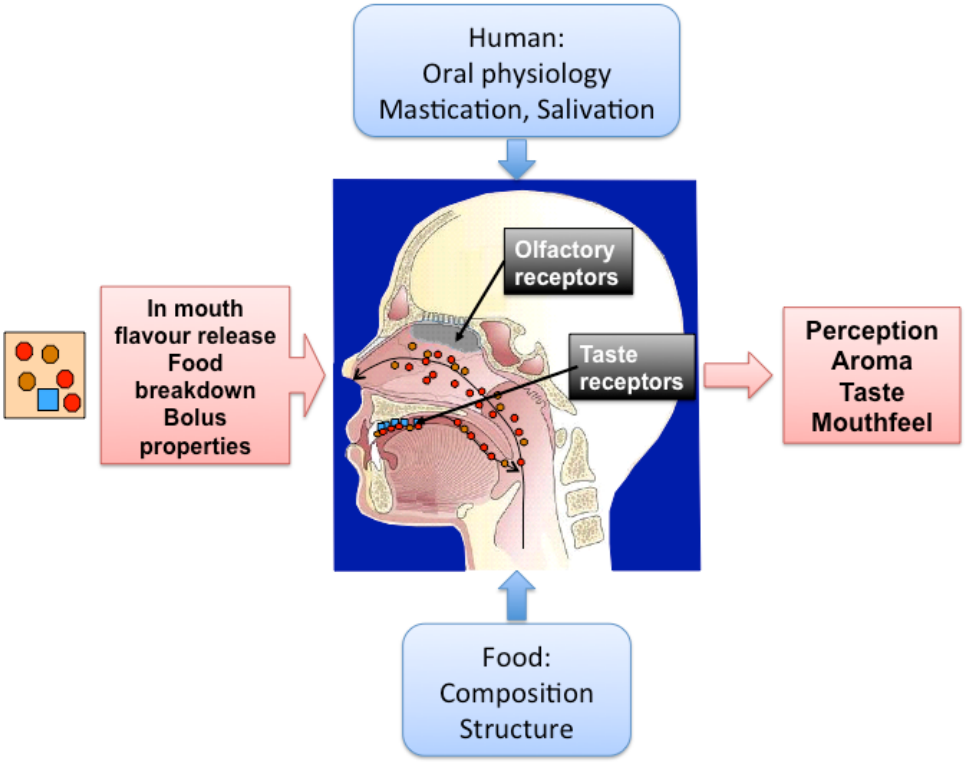

Figure 1: Schematic representation of oral food breakdown, leading to the release and perception of taste and aroma. During food oral processing (FOP), the food product is broken down in the mouth to form a food bolus. The properties of the food bolus and its dynamics of evolution in the mouth depends on the subject's physiology and on the food's properties. During FOP, aroma and taste compounds are released in the oral and nasal cavity, where they reach the receptors and are perceived. All these events contribute to acceptance or rejection of the food product by the consumer (adapted from Feron, Salles, and Guichard (2013).

only on the surface of the food, it is detected more quickly than if it is included in the matrix. Furthermore, the nature of the matrix is also important. For dry products, for example, a phase of hydration of the matrix by saliva is observed, in which saliva plays a solvent role and allows the compounds to come into contact with the receptors.

For the same food matrix in one individual, the release kinetics of a tasting compound are similar and differ only by the concentration of the compound in the matrix (Pionnier et al., 2004). Moreover, food matrix composition also plays a role in the release of tasting compounds in the mouth, either directly through interactions with the compounds of the matrix or indirectly by changes in the textural properties of the products, which induce a change in physiological behaviours during mastication of the product (Phan et al., 2008). In contrast, inter-individual differences are very important and essentially depend on the physiological characteristics of subjects. These characteristics are critical to the release kinetics of the stimuli. In the case of mastication of solid food, the release of sodium in the mouth is positively correlated to the total chewing duration, but it is negatively correlated with salivary flow, chewing frequency, rate of swallowing and masticatory efficiency. Flow and masticatory efficiency play a role in the speed of per-

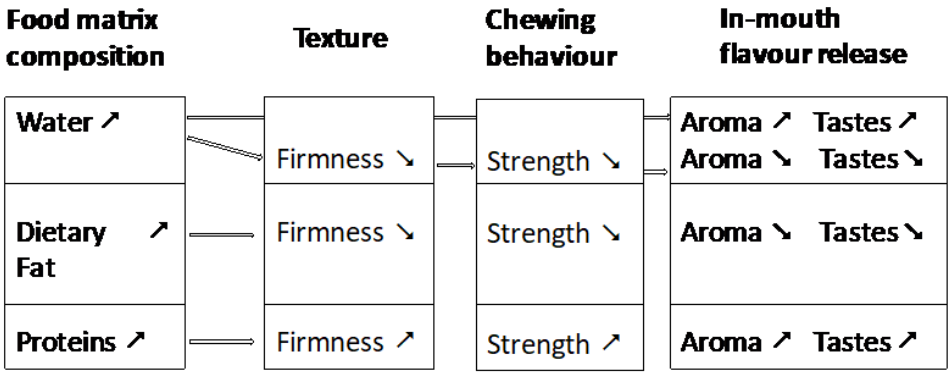

Figure 2: Influence of cheese matrix composition on texture, chewing behaviour and in-mouth release of flavour compounds (adapted from Feron, Salles, and Guichard (2013). Increasing the matrix water amount leads to two contradictory effects: (i) higher aroma and taste release, and (ii) decreased firmness, lower chewing activity and thus lower aroma and taste release. This second effect is also observed when the matrix fat content is increased. In contrast, increasing the matrix protein amount leads to higher firmness, higher chewing activity and thus higher aroma and taste release during consumption.

ception and not the intensity of saltiness (Phan et al., 2008).

If one focuses more on saliva and its composition, saliva may, due to its moisturizing properties and hydrolytic capacities, promote disintegration of the matrix and thus the release of palatable active substances. This is the case with the salivary α-amylase that hydrolyses the starch contained in food to release maltose, which has a sweetening power of 0.33 (compared to sucrose). This effect has also been particularly illustrated by the work of Ferry and co-workers, (Ferry et al., 2006) which showed a direct relationship between the level of α-amylase activity in saliva and the saltiness of starchy matrices. In this case, amylase has the effect of enhancing the disintegration of the product, which releases sodium in the saliva medium, and therefore enhances its perception.

Furthermore, saliva itself contains tasting compounds at a more or less high concentration. This is the case for certain amino acids and certain peptides with specific organoleptic properties (bitter, sweet and umami), certain salts (sodium, potassium and calcium), sugars (glucose, fructose) and fatty acids. These compounds constantly stimulate the taste receptors on the tongue. Interestingly, the salivary concentrations of some of these compounds largely cover the detection limits observed in humans. This is the case of sodium, calcium, and certain amino acids (histidine and glutamate). It is therefore likely that the sensory perception of these tasting substances, when they are added in the food, are dependent on their salivary concentrations, which are subject to high inter-individual variability. For instance, salivary calcium and sodium levels differed, respectively, from 22.6 to 1800 μg/ml and 140 to 275 μg/ml in saliva, depending on the subject. These differences may explain variability in the perception and thus the acceptability of some food products. For instance, it has been shown that an individual with a high salivary sodium concentration is less sensitive to saltiness (Bartoshuk, 1978). It is likely that this individual will tend to favour higher sodium levels in food that an individual with a very low salivary sodium concentration. This is of course a hypothesis that should be tested on a large number of salivary compounds, particularly those related to sweetness and fattiness.

2.3 Other food components

Beyond taste and aroma, other compounds may directly impact the perception of the food without having an "a priori" flavour. This the case

of CO_2 perception in carbonated beverages. It was long considered that the CO_2 perception was mainly due to physical phenomena linked to bubbles bursting in the mouth. However, studies have shown that this feeling was under the control of an enzyme found in saliva, carbonic anhydrase VI (CAH VI) or gustin. This enzyme catalyses the conversion of CO_2 into carbonic acid. With a turnover of 10^{-5} Mole per second, this enzyme has one of the highest velocities ever known, which makes it compatible with food in the mouth for a very short time. This role in CO_2 trigeminal perception has been demonstrated by Dessirier and co-workers (Dessirier, Simons, Carstens, O'Mahony, & Carstens, 2000) during psychophysical studies in humans using an inhibitor of the enzyme.
Mouth perception of phenolic compounds, such as tannins, is another known example of the possible role of saliva in perception through the sensation of astringency provided by these compounds. Astringency is often described as a drying sensation in the mouth. It is generally accepted that this sensation results from the interaction of certain proline-rich salivary proteins (PRP) and phenolic compounds in food (de Wijk & Prinz, 2005; Prinz & Lucas, 2000). These interactions result in a decrease in the lubricating properties of saliva, leading to this drying sensation.

3 Prospect

3.1 Linking dynamic of food bolus formation and sensory perception

To date, most of the published works that have attempted to relay food bolus properties, aroma and taste release and food sensory perception have been focused on the bolus just before swallowing. However, in-mouth food breakdown is a dynamic process. Therefore, it is of a high interest to evaluate how the food bolus changes during FOP in a dynamic way and the consequences on aroma and taste release and then perception. Some methodologies exist to continuously measure aroma release and flavour perception during food consumption. Sensors can be used to directly monitor taste release in the mouth (Emorine et al., 2012). Aroma release can also be measured continuously with spectroscopic measurements, such as APCI (Atmospheric Pressure Chemical Ionization) (Le Quéré, Gierczynski, Langlois, & Sémon, 2006) or PTR-MS (Proton Transfer Reaction – Mass Spectrometry) (Le Quéré & Guichard, 2011). For sensory analysis, TI (Time Intensity) or TDS (Temporal Dominance Sensations) are relevant methodologies (Labbe, Schlich, Pineau, Gilbert, & Martin, 2009). The most challenging aspect concerns the food bolus. As far as we know, it is not possible to follow food bolus property changes in situ. Moreover, evaluating food bolus rheological characteristics requires methodological developments with particular constraints (small samples, large heterogeneity, large between subject variability, etc.). These constraints lead to the fact that (i) the bolus must be collected at different times during the chewing sequence, (ii) we cannot use a single method for characterization of the bolus collected at different stages, and (iii) we need to find a methodology that can cover the large variability observed within the population. However, some very recent works showed clear relationships between bolus property changes, chewing behaviours and sensory perception during the consumption of sausages (de Lavergne, Derks, Ketel, de Wijk, & Stieger, 2015). This interesting study highlights an interest to conduct research in the field.

3.2 Specific populations

Most of the studies described in the previous chapters have been principally conducted on young, healthy populations (typically from 18 years old to 60 years old). However, it is a big challenge to characterize more specific populations, such as elderly people, infants or obese individuals.
To date, these populations have been poorly investigated, as they represent strong demographic challenges. However, some oral characteristics have been described in the literature for these populations.
For instance, elderly saliva is characterized by low flow and a high level of proteins, α-amylase

and ions (Vandenberghe-Descamps et al., 2016; Nagler & Hershkovich, 2005; Hershkovich & Nagler, 2004). In terms of dentition, elderly show a low amount of functional units compared to a younger population (Vandenberghe-Descamps et al., 2016). These differences significantly impact the capacity to chew and form a swallowable food bolus (Mioche, Bourdiol, Monier, Martin, & Cormier, 2004). In infants, the first year of infancy corresponds to food oral exposure and experiences. In parallel, infants develop masticatory and digestion capacities with the development of teeth and changes in saliva properties (Nicklaus et al., 2015; Morzel et al., 2012). However, in this population, a food bolus is difficult to collect, though some recent investigation methodologies have been proposed (Tournier, Rodrigues, Canon, Salles, & Feron, 2015). Thus, its rheological properties are difficult to evaluate.

Regarding in-mouth aroma and taste release and their link with FOP, the literature is quite scarce for infants and the elderly. However, it is likely that the phenomena in these populations will be different compared to other individuals. One of the difficulties is to develop investigation methodologies that are specific to these particular populations, especially infants. This issue represents a real challenge for the scientific community working in the field.

Another population that should be of high interest to investigate is overweight and obese individuals. This population represents a true demographic challenge in terms of health issues. Surprisingly, this population has been poorly investigated in relation to FOP, while some scientific results led to the hypothesis that this population has a particular FOP profile. For instance, it has been shown that obese subjects have lower salivary flow and altered oral health (Modeer, Blomberg, Wondimu, Julihn, & Marcus, 2010), particular salivary compositions (Vors et al., 2015) and different chewing behaviours in comparison to a normal weight population (Godlewski et al., 2011). Moreover, *in vitro* experiments conducted on obese subjects' saliva showed a lower aroma release pattern compared to saliva from normal-weight subjects (Piombino et al., 2014). Therefore, it is likely that the *in vivo* processes of aroma and taste release in the mouth and the nasal cavity are different in this population.

4 Conclusion

This review aimed to show the important role of chewing and salivation in in-mouth food breakdown and the release of flavour from the food bolus, with particular attention to organoleptic components. This review also tried to provide an interesting perspective on the role and contribution of FOP in specific populations.

An important point in all studies on chewing and salivation that have been conducted on a significant number of human subjects is the extreme difference between inter-subject variability and intra-subject variability. The links between this large physiological variability and that of the different sensory phenotypes observed in the human population are yet to be established. Moreover, this point clearly raises the question of considering this variability in reverse engineering approaches aimed at rationally designing food for the delivery of particular functionalities along the gut. Only integrated and systemic scientific approaches involving many different disciplines, starting from the physico-chemistry of food to physiology and genetics, will help prioritize different oral events and their effects and will then establish the best strategies for improving the food supply.

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Examination of Optimum Test Conditions For a 3-Point Bending and Cutting Test To Evaluate Sound Emission of Wafer During Deformation

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Abstract

The purpose of this study was to investigate optimum test conditions of acoustical-mechanical measurement of wafer analysed by Acoustic Envelope Detector attached to the Texture Analyser. Force-displacement and acoustic signals were simultaneously recorded applying two different methods (3-point bending and cutting test).
In order to study acoustical-mechanical behaviour of wafers, the parameters "maximum sound pressure", "total count peaks" and "mean sound value" were used and optimal test conditions of microphone position and test speed were examined. With a microphone position of 45° angle and 1 cm distance and at a low test speed of 0.5 mm/s wafers of different quality could be distinguished best. The angle of microphone did not have significant effect on acoustic results and the number of peaks of the force and acoustic signal decreased with increasing distance and test speed.

Keywords: Acoustic; Crispness; Texture; Wafer

1 Introduction

Quality of wafer products is generally examined and categorised according to textural property which is mainly called crispness considered as a primary textural attribute measured during bending and snapping of crispy foods at the first bite (Vickers, 1983; Duizer, 2004; Mallikarjunan, 2004; Martinez-Navarrete, Moraga, Talens, & Chiralt, 2004). In general knowledge, as higher is the crispness value, higher is quality of wafer however determination of crispness and its perception by consumers greatly varies from individual to individual and from country to country (Bourne, 2002). This brings food researchers huge difficulty to identify parameter that is scientifically meaningful and easy to measure. In order to identify crispness, many sensory test panels have been still performed and large variations of results have been estimated. Although, sensory test methods are fundamental methods to determine crispness (Christensen & Vickers, 1981; Vickers, 1984), due to the difficulties such as time consuming, not convenient for routine tests, requiring more statistical works and most of all providing participants who have

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good knowledge in texture attributes, other test techniques; mechanical-acoustical tests and their parameters are applied and evaluated by using some crispy foods which are mechanically brittle and emit sound during consumption (Duizer, 2004; Gregersen et al., 2015; Roudaut, Dacremont, Pamies, Colas, & Le Meste, 2002; Zdunek, Cybulska, Konopacka, & Rutkowski, 2011).
Sounds of crispy products during mechanical tests can be detected and evaluated by applying acoustical methods (Edmister & Vickers, 1985; Seymour & Hamann, 1988; Tesch, Normand, & Peleg, 1996; Duizer, 2004). Acoustic detection devices connected to texture analyser can provide to obtain information about crispness from the acoustic and force/displacement curves acquired during mechanical measurements of wafer and parameters on these curves can be calculated and correlated by sensory test results.
Recently, related studies were carried out by some researches and good correlation values between sensory and acoustical-mechanical tests were estimated in different types of crispy foods (Dematte et al., 2014; Piazza & Giovenzana, 2015; Wiktor et al., 2016; Zdunek et al., 2011; Saeleaw, Duerrschmid, & Schleining, 2012; Chanvrier, Jakubczyk, Gondek, & Gumy, 2014; Blonska, Marzec, & Blaszczyk, 2014; Giacosa et al., 2016; Jakubczyk, Gondek, & Tryzno, 2017). Moreover, parameters and deformation techniques were improved and test conditions such as test speed and microphone location were noticed as critical factors affecting the results of acoustical methods in some studies at the beginning of this type of researches (Chen, Karlsson, & Povey, 2005; Varela, Chen, Fiszman, & Povey, 2006; Varela, Salvador, & Fiszman, 2008).
In this study, in order to distinguish quality differences of wafer samples in terms of crispness, acoustical-mechanical tests were carried out by using acoustic envelop detector and microphone attached to texture analyser. Two different fracturing methods (3-point bending and cutting test methods) and acoustical parameters were used and optimum test conditions of microphone positions and test speeds were examined.

2 Materials and Methods

2.1 Materials

Nine different brands of wafers; Bella, Manner, Sweet Gold, Napoli, Biscoteria, Jadro, Fin Carre (Normal), Fin Carre Strawberry and Fin Carre Limon with the dimension of 16 mm thickness, 51 mm length and 18 mm width were purchased from local markets. Samples were kept in its original package in a dark place at about 24°C. For each experiment, in order to avoid humidity absorption, a new package was opened and in case samples were not of the same size, a sharp knife was used for trimming. All samples were measured within maximum 20 minutes.

2.2 Texture measurement

All measurements were carried out with a texture analyzer TAXT.plus (Stable Micro Systems, Surrey, U.K) connected with the standard microphone (Brüel Kjaer, Type 2671 Naerum, Denmark). The reaction force was determined by a 5 kg load cell and the microphone was calibrated with the acoustic calibrator type 4231 (Brüel Kjaer) at 94 and 114 dB sound pressure level (SPL). The amplifier was set to level 4.
Chen et al. (2005) explained that the intensity of the vibration of original source, the travel distance and the available sound paths affect the strength of the sound. Therefore, the position of the detecting microphone is important to measure acoustic signals. On this huge random of possibilities, nine positions were selected for microphone. Tests were made in 3 different angles (0°, 45°, 90°) and 3 different distances (1 cm, 5 cm and 10 cm) for each angle totally nine different location. Product Bella were used to investigate best microphone position by applying 3-point bending at 1.0 mm/s test speed.
Two deformation methods were used to investigate crispness of wafer samples; 3-point bending (Alvarez, Saunders, Vincent, & Jeronimidis, 2000; Baltsavias, 1996; Castro-Prada, Luyten, Lichtendonk, Hamer, & Van Vliet, 2007; Chen et al., 2005) and cutting test methods (Castro-Prada et al., 2007). For the 3-point bending, the sample lays on two supports and a compressing

bar moves down between these supports, bending the food until it snaps (Table 1). For the cutting test the samples lays on a flat metal platform, and a trapezium-shaped stainless steel blade (60 mm length, 19 mm height and 0.65 mm width) cuts the sample to for a distance of 9 mm (Table 2).
Four different test speeds of 0.1, 0.5, 1.0 and 1.5 mm/s were applied to examine the optimum test speed for the 3-point bending and cutting test (see table 1 and 2 for test settings). Measurements were repeated ten times for the product Bella.
Parameters of maximum sound pressure (MSP), total count peaks (TCP) and mean sound value (MV) were calculated from the acoustic-displacement curve within a range from 0 to 10 resp. 20 mm using the Exponent software (Stable Micro System Surrey, U.K) (see Figure 1 and 2).

2.3 Statistical analysis

Results were studied and evaluated by SPSS software with Analysis of Variance (ANOVA) and multiple range tests.

3 Results and Discussions

3.1 Selection of the microphone position

In order to find the best position for the microphone, nine different positions; 0˚-1 cm, 45˚-1 cm, 90˚-1 cm, 0˚-5 cm, 45˚-5 cm, 90˚-5 cm, 0˚-10 cm, 45˚-10 cm, 90˚-10 cm and test speed of 1.0 mm/s was applied for the product “Bella” with the 3-point bending method, which has been used for acoustic tests by several researchers (Castro-Prada et al., 2007; Chen et al., 2005).
The maximum sound pressure (MSP) and the total count peaks (TCP) were examined to see in which position these parameters have the smallest variability (see Figure 3 and 4). Table 3 and Table 4 show standard deviations (SD) and average values of MSP and TCP at the nine different microphone positions.
Table 3 demonstrates that generally the average values of the MSP and TCP decrease and the standard deviation of MSP increase with the microphone distance. For TCP there was no significant effect on the standard deviation.
Figure 3 and Figure 4 show that the angle had no significant effect on the MSP and TCP. This was also observed by Chen et al. (2005). Considering Table 3, optimum microphone angle and distance can be selected as 45˚ angle-1 cm distance since at this position lower standard deviations and high average values of MSP were measured compared to the other positions.
Because of low variability and high values at small distance for both parameters, the choice of the best microphone position according to the MSP and TCP was 1 cm distance 45˚.

3.2 Selection of the test speed

The microphone position of 45˚ angle and 1 cm distance was used to select best test speed. Parameters of MSP, TCP and mean sound value (MV) were evaluated from the obtained curves (Figure 1 and 2) and results are illustrated in Table 4 shows that how variances of MSP, TCP and MV change as a function of test speed in 10 replications (Bella brand of sample tested for each speed within ten replications).
Table 4 shows that for both test methods in general the average values for values MSP and MV increase with the test speed, whereas the values of TCP decrease for both tests. This could be explained that with higher speeds some acoustic events were lost. Statistically, the increase of MSP was not significant.
The values of MSP and MV of the 3-Point bending method were higher than for the cutting test, whereas the TCP values were lower except at 0.1 mm/s test speed. The higher number of peaks for the cutting test is obviously due to the longer measuring time.
For the 3-point bending test the standard deviations decrease with increasing test speed for TCP but are more or less the same for MSP and opposite for MV. For the cutting test the standard deviations generally decrease with increasing test speed for TCP and MV.
Chen et al. (2005) explained that the reliability of acoustic emission detector is higher in distin-

Table 1: Settings of 3-point bending test method

Load cell	5 kg
Test Type	Return to start
Pre-test speed	0.8 mm/s, 0.4 mm/s, 0.8 mm/s and 1.4 mm/s
Test speeds	0.1 mm/s, 0.5 mm/s, 1.0 mm/s and 1.5 mm/s
Back-test speed	10 mm/s
Distance	16 mm
Test mode	Compression
Microphone position	0 degree (parallel to sample) and 1 cm distance to sample
Data acquisition rate	500 pps
Envelope Corner Frequency	3.125 KHz

Table 2: Settings of cutting test method

Load cell	5 kg
Test Type	Return to start
Pre-test speed	0.8 mm/s, 0.4 mm/s, 0.8 mm/s and 1.4 mm/s
Test speeds	0.1 mm/s, 0.5 mm/s, 1.0 mm/s and 1.5 mm/s
Back-test speed	10 mm/s
Distance	16 mm
Test mode	Compression
Microphone position	0 degree (parallel to sample) and 1 cm distance to sample
Data acquisition rate	500 pps
Envelope Corner Frequency	3.125 KHz

Table 3: Maximum sound pressure (MSP) and total count peak (TCP) for the 3-point bending test at 1.0 mm/s test speed at different microphone positions, carried out with wafer Bella. Values are expressed as average ± standard deviation (n=10). Different indices indicate significantly different values per column based on the Tukey test, $p < 0.05$

Positions		Acoustic Parameters	
		MSP	TCP
0°-1 cm		79.81 ± 2.86^{cd}	232.80 ± 45.07^{abc}
0°-5 cm		75.82 ± 3.57^{bc}	265.70 ± 37.73^{c}
0°-10 cm		70.82 ± 3.50^{a}	204.90 ± 34.41^{ab}
45°-1 cm		80.39 ± 2.32^{d}	227.40 ± 51.97^{abc}
45°-5 cm		75.73 ± 2.59^{bc}	223.90 ± 51.27^{abc}
45°-10 cm		70.86 ± 4.58^{a}	193.80 ± 25.89^{a}
90°-1 cm		81.95 ± 3.21^{d}	253.30 ± 21.70^{bc}
90°-5 cm		75.83 ± 2.95^{bc}	249.30 ± 32.56^{bc}
90°-10 cm		72.12 ± 2.61^{ab}	193.90 ± 27.07^{a}
ANOVA	F-value	16.813	4.722
	P value	0.000	0.000

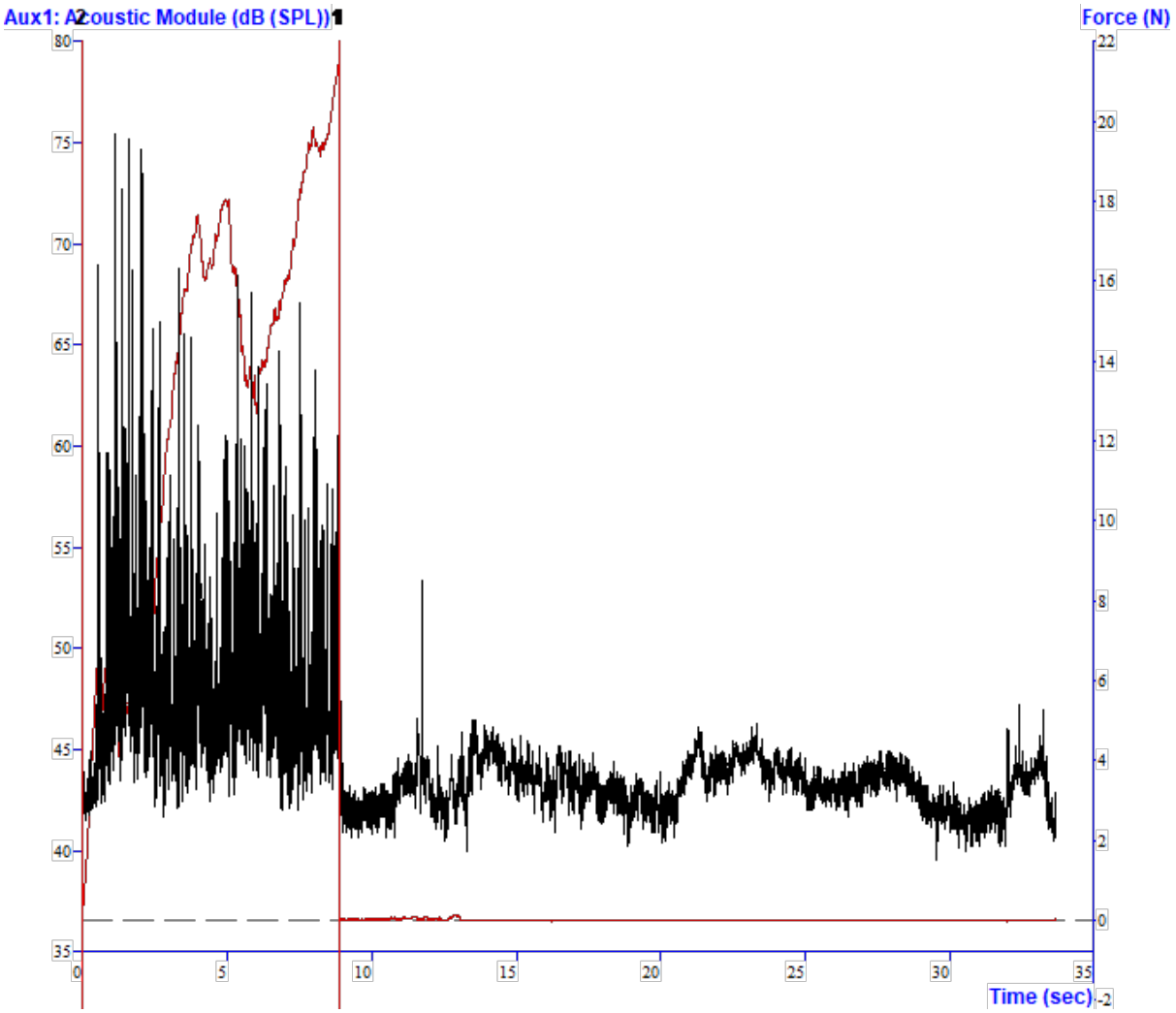

Figure 1: Results (acoustic and force) of the 3-point bending method (Red line corresponds force-displacement curve and black line corresponds acoustic-displacement curve)

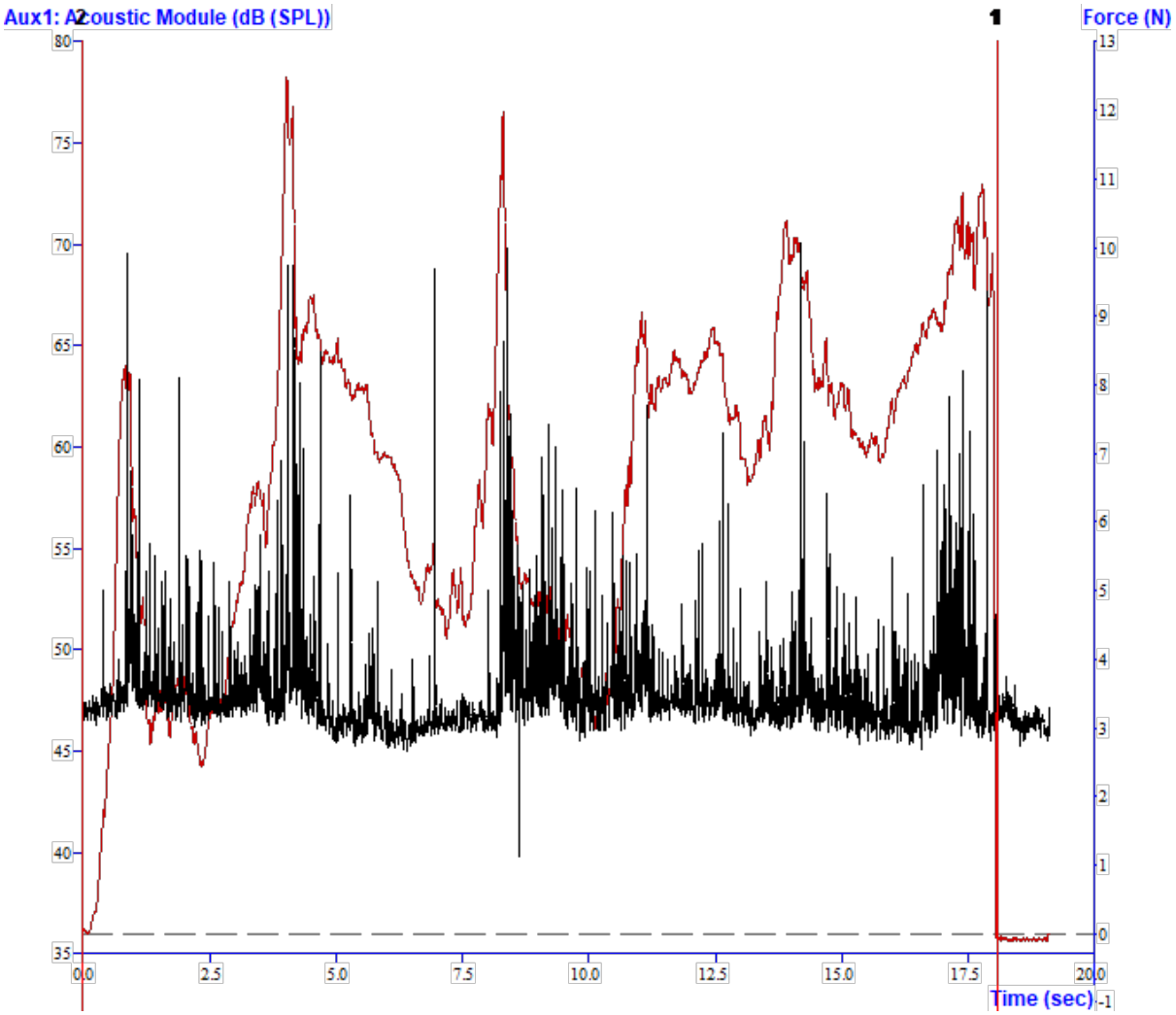

Figure 2: Results (acoustic and force) of the Cutting test method (Red line corresponds force-displacement curve and black line corresponds acoustic-displacement curve)

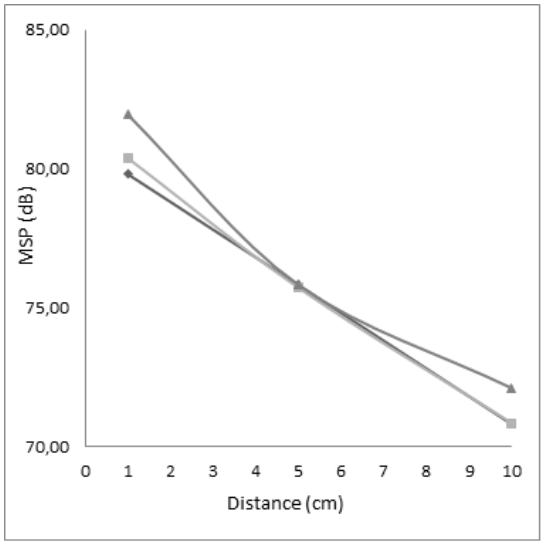

Figure 3: Maximum sound pressure (MSP) at various distances and angles in 3-point bending method (■-45˚, ◊-0˚, ▲-90˚)

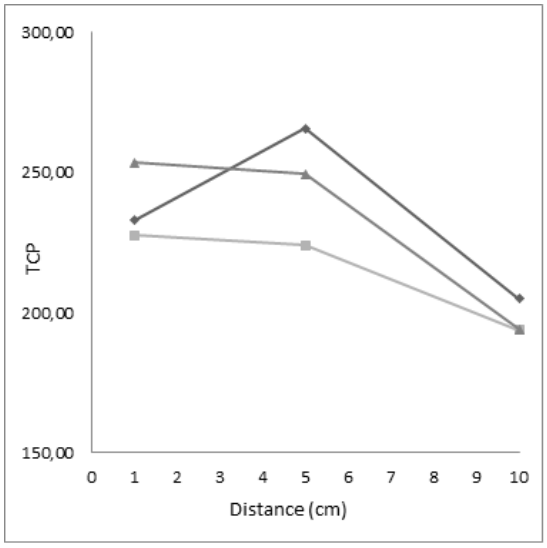

Figure 4: Total count peak (TCP) at various distances and angles in 3-point bending method (■-45˚, ◊-0˚, ▲-90˚)

guishing acoustic signals during breakup of biscuits at low speeds than fast speeds. It was noticed that the device probably fails to pick up all individual acoustic events at high testing test speed for some products. This is due to a too large integration time of the acoustic emission detector that makes the device unable to distinguish two sequential acoustic events. The current default integration time works well for the majority of crispy foods at a reasonably low test speed, but a shorter integration time should be considered for acoustic-rich products. Especially variations of other acoustic parameters, such as TCP, showed an obviously loss of this information at high speeds.
Although the variability was lower at high test speeds and especially high at 0.1 mm/s 0.5 mm/s was selected in order not to lose acoustical events at high test speeds.

3.3 Comparison of quality differences of wafers by means of crispness

A test speed of 0.5 mm/s and microphone position of 45˚ angle with-1 cm distance from the sample were used to distinguish quality differences of nine brands of wafers by applying a 3-point bending test.
Table 5 illustrates the average values and standard deviations of MSP, TCP and MV of the brands of wafers. There are significant differences ($p<0.05$) between MSP, TCP and MV values of nine brands of wafers.
The products Jadro, Fin Carre, Fin Carre Lemon, Fin Carre Strawberry and Napoli were placed in same group regarding MSP values more than 80 dB (see Fig. 5 and Table 5). However, highest TCP values more than 440 were examined for the products Napoli, Sweet Gold, Fin Carre Lemon and Fin Carre Strawberry. In addition, highest MV values more than 49.50 dB were also obtained for products Fin Carre, Fin Carre Strawberry and Sweet Gold.
According to these results, generally products Fin Carre group (Fin Carre, Fin Carre Lemon and Fin Carre Strawberry) and Sweet Gold displayed highest values of acoustic parameters. Crispness is evaluated with sound events dur-

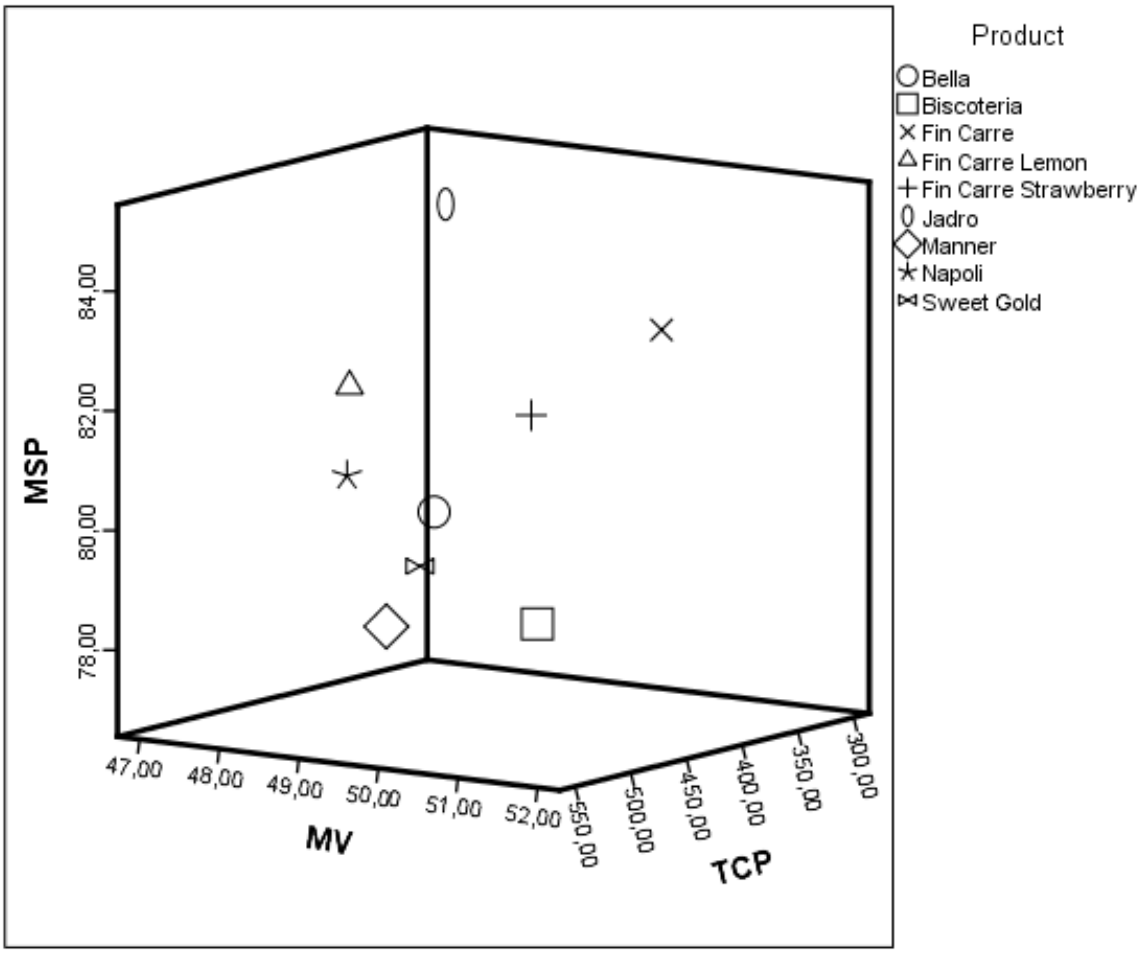

Figure 5: Distribution of brands of wafers as functions of Maximum sound pressure, Total count peak and Mean sound value

ing mechanical deformations and as higher number of sound events, the higher is the crispness. Based on this, it could be said that products Fin Carre group and Sweet Gold are more crispy than the other 5 brands of wafers.

4 Conclusion

Comparing the 2 methods, it could be concluded that the 3-point bending test is more convenient and reliable than the cutting test since there were difficulties to distinguish acoustical events whether caused by friction of the knife or by breaking of the wafer.

The best microphone position was selected as 45° and 1 cm distance from the breaking point of the wafer due to lower variability. The smaller the distance the higher are the values of the evaluated parameters and in general the lower the variability. The angle of microphone did not significant effect. The results of the test speed measurements displayed that especially number of recorded peaks at high test speeds (1.0 and 1.5 mm/s) were less than at slower test speeds, because there is loss of acoustic events at high speeds. As the evaluated parameters showed a much higher variability at the lowed test speed of 0.1 mm/s, 0.5 mm/s was chosen.

Table 4: Maximum sound pressure (MSP), total count peak (TCP) and mean sound value (MV) for the 3-point bending and cutting test at different test speeds, carried out with Product Bella. Values are expressed as average ± standard deviation (n=10). Different indices indicate significantly different values per column based on the Tukey test, $p < 0.05$.

Test Speeds		3-Point Bending test		
		MSP	TCP	MV
	0.1 mm/s	78.31 ± 3.31^{a}	1123.0 ± 120.96^{c}	42.97 ± 0.60^{a}
	0.5 mm/s	79.65 ± 2.47^{a}	374.7 ± 70.94^{b}	48.04 ± 0.79^{b}
	1.0 mm/s	79.81 ± 2.86^{a}	232.8 ± 45.07^{a}	51.65 ± 1.17^{c}
	1.5 mm/s	80.48 ± 2.61^{a}	163.8 ± 25.60^{a}	52.02 ± 0.84^{c}
ANOVA	F-value	1.030	349.263	230.482
	P-value	0.390	0.000	0.000
		Cutting Test		
		MSP	TCP	MV
	0.1 mm/s	75.06 ± 4.05^{b}	989.2 ± 127.57^{d}	$46.12 \pm 0{,}36^{a}$
	0.5 mm/s	70.89 ± 2.60^{a}	448.7 ± 67.06^{c}	47.93 ± 0.34^{b}
	1.0 mm/s	74.49 ± 3.35^{ab}	349.0 ± 23.43^{b}	49.67 ± 0.30^{c}
	1.5 mm/s	73.96 ± 3.28^{ab}	256.4 ± 19.16^{a}	50.61 ± 0.32^{d}
ANOVA	F-value	3.071	198.972	354.031
	P-value	0.040	0.000	0.000

Table 5: Maximum sound pressure (MSP), total count peak (TCP) and mean sound value (MV) for the 3-point bending at optimum test conditions (at 0.5 mm/s test speed and 45˚-1 cm microphone position), carried out with 9 different brand of wafers. Values are expressed as average ± standard deviation (n=10). Different indices indicate significantly different values per column based on the Tukey test, $p < 0.05$.

Brand of Wafers		MSP	TCP	MV
Manner		77.89 ± 3.38^{a}	411.10 ± 37.29^{bc}	$47.96 \pm 0{,}32^{b}$
Fin Carre		83.23 ± 2.70^{c}	385.40 ± 39.63^{ab}	$51.06 \pm 0{,}59^{g}$
Napoli		81.05 ± 1.06^{abc}	515.70 ± 84.87^{d}	$48.93 \pm 0{,}70^{cd}$
Fin Carre Lemon		82.20 ± 2.18^{bc}	464.80 ± 102.36^{bcd}	$48.25 \pm 0{,}63^{bc}$
Sweet Gold		79.62 ± 2.41^{ab}	505.90 ± 44.66^{cd}	$49.71 \pm 0{,}82^{ef}$
Fin Carre Strawberry		81.92 ± 3.37^{bc}	441.00 ± 50.31^{bcd}	$50.20 \pm 0{,}49^{f}$
Jadro		84.32 ± 1.64^{c}	302.20 ± 80.37^{a}	$47.18 \pm 0{,}56^{a}$
Biscoteria		77.93 ± 3.31^{a}	366.20 ± 107.62^{ab}	$49.23 \pm 0{,}47^{de}$
Bella		79.65 ± 2.47^{ab}	374.70 ± 70.94^{ab}	$48.05 \pm 0{,}79^{b}$
ANOVA	F-value	7.488	9.111	40.135
	P-value	0.000	0.000	0.000

From the different brands of wafers, the products Fin Carre group and Sweet Gold were examined as more crispy due to their higher MSP, TCP and MV values.
In conclusion, this study can lead further investigations on wafer quality in terms of crispness which is generally difficult to determine, by using obtained test conditions and acoustic parameters from acoustical-mechanical measurements. Although, sensory test methods were carried out to determine crispness of wafers, using fast and reliable methods such as acoustical-mechanical tests in industry is important. With this method conditions and parameters, quality of wafer can be distinguished by means of simple and reproducible test.

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Extraction and Characterisation of Cellulose Nanocrystals from Pineapple Peel

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Abstract

The potential of pineapple peel as a source of cellulose nanocrystals was evaluated. Peels skin from fresh-cut fruit was used as raw material. These residues were purified to remove pigments, lipids and hemicellulose, and a bleaching process for delignification was carried out for 4-6 h. All resulting products were characterised for their lignin, hemicellulose, cellulose and ash contents using standard techniques. Dry matter at the end was low (ca. 50%) compared with the raw material (ca. 90%). The process applied resulted in ca. 20% (m/m) of purified cellulose (ca. 80% purity), with ineligible levels of lignin and hemicellulose present, especially when using 6h of bleaching. The purified cellulose was subject to acid hydrolysis for nanocrystal extraction with two testing times, 30 and 60 minutes. These cellulose nanocrystals had small sizes (< 1000 nm), with high variability and negative zeta potential values. The time of extraction did not affect the nanocrystals' chemical and physical properties. The use of 6 h of bleaching treatment during purification was shown to be more effective than 4 h. Pineapple peel was demonstrated to be a good source of cellulose for the production of cellulose nanocrystals.

Keywords: Cellulose; CNC; Pineapple peels

1 Introduction

In the last decade, there is a great deal of research focusing on the use of cellulosic-rich wastes. Cellulose fibre is the most abundant renewable material, constitutes the major component of plant fibres, and is a natural hydrophilic polymer. Plant/vegetable fibres consist of cellulose, hemicellulose and lignin. Hemicellulose is a branched multiple polysaccharide polymer, composed of different types of sugars including glucose, xylose, galactose, arabinose and mannose. Lignin is a highly cross-linked phenolic polymer. Both hemicellulose and lignin are amorphous polymers, whereas cellulose is a semi-crystalline polymer. There are two types of linkages between hemicellulose groups and lignin. One is an ester-

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Nomenclature

CNC	Cellulose Nanocrystal
PP	Pineapple Peel
TPP	Treated Pineapple Peel
TPP4	Treated Pineapple Peel with a bleaching treatment of 4 hours
TPP6	Treated Pineapple Peel with a bleaching treatment of 6 hours
PS	Particle Size
PI	Polydispersity Index
ZP	Zeta Potential
NP	Nanoparticles

type bond between the hydroxyl of lignin and the carboxyl of uronic acid in hemicellulose. This linkage is sensitive to alkali solutions. Hence, starting from raw fibres, an alkali treatment can be applied to hydrolyse and remove the hemicellulose, soluble mineral salts and other components (Deepa et al., 2011). The second linkage is of an ether-type, and is formed between the hydroxyls of lignin and those of carbohydrates. Other linkage types are insensitive to alkali solutions, but an acid hydrolysis can be applied to disrupt these amorphous domains. This treatment releases individual rod-like rigid nanocrystals, named cellulose nanocrystals (CNCs) (Aspler et al., 2013), and introduces negative charges to their surface due to the formation of sulphate ester groups. Cellulose nanocrystals possess high mechanical strength, high surface area and aspect ratio, non-toxicity, biocompatibility and biodegradability, making them an excellent candidate for pharmaceutical applications (Lin & Dufresne, 2014). Because of their properties, CNCs could be used for numerous applications, such as the preparation of composite materials, regenerative medicine and drug delivery (Qiu & Hu, 2013).

The majority of the studies of extraction of CNCs from food industry wastes and natural sources are very recent, owing to the interest generated in such systems. Studies were made on the extraction from wood, sisal, coconut husks, agave fibres, bananas, rice husks, soy hulls, mango seeds, sweet potato residue, garlic skins, pineapple leaves, mengkuang and capim mombaça (Cherian et al., 2010; Deepa et al., 2011; Flauzino Neto, Silverio, Dantas, & Pasquini, 2013; Henrique, Silvério, Neto, & Pasquini, 2013; Johar, Ahmad, & Dufresne, 2012; Prozil, Evtuguin, Silva, & Lopes, 2014). However, pineapple processing residues have not been explored until now, and this fruit is one of the most important commercial fruits of the world. It is a typical tropical fruit, with a total global annual production between 16 and 19 million tonnes. Generally, pineapple is consumed as fresh fruit, or is processed into salads, fruit cocktail and jam/conserves, or canned. During pineapple fruit processing, the wastes (peel and stems mainly), ca. 50% (w/w) of total pineapple weight, are removed and discarded. In fact, pineapple peel consists of cellulose, hemicellulose, lignin and pectin, and represents the largest portion of pineapple wastes (30–42%, w/w) (Hu, Wang, & Huang, 2013). The composition is ca. 42 % fibre, ca. 9% protein and 1.5% lipids.

Hence, the aim of this study was to evaluate the potential of using pineapple peel residues as a source of CNCs, and to characterise them in terms of physical properties.

2 Materials and Methods

2.1 Purification of pineapple residues

Pineapple residues were provided from a fresh-cut fruit processing company. The peel was immediately frozen after processing procedures

(Fig. 1a), while still at the company production line. Initially, the pineapple peels (PP) were dried and milled (Fig. 1b). They were treated with a 2% (w/w) NaOH solution for 4 h at 100 °C under mechanical stirring, and then washed several times with deionised water until the alkali was completely removed, and finally dried at 50 °C for 12 h in an air-circulating oven (Fig. 1c). After this treatment, the resulting fibres were bleached with a solution made up of equal parts (v:v) of acetate buffer (27 g NaOH and 75 mL glacial acetic acid, diluted to 1 L with distilled water) and aqueous sodium chlorite (1.7 wt% $NaClO_2$ in water). This bleaching treatment was performed at 80 °C for 4 h or 6 h (Fig. 1d & e). The bleached fibres were washed repeatedly in distilled water, until their pH became neutral, and subsequently dried at 50 °C for 12 h in an air-circulating oven. The material which resulted after this purification was the treated pineapple peel (TPP), and this was subsequently bleached for 4 h (TPP4) and 6 h (TPP6).

2.2 Extraction of cellulose nanocrystals (CNCs)

After the chemical treatment described previously, TPP4 and TTP6 dried material was milled with a blender, and the nanocrystals extracted by acid hydrolysis. The hydrolysis was performed at 50 °C, for either 30 min or 60 min, under vigorous and constant stirring. For each gram of TPP, 20 mL of a solution of H_2SO_4 64% (w/w) was used. Immediately following the hydrolysis, the suspension was diluted 10-fold with cold water to stop the hydrolysis reaction, and centrifuged twice for 10 min at 7000 rpm to remove the excess acid. The precipitate was then dialysed with tap water to remove non-reactive sulphate groups, salts and soluble sugars, until a neutral pH was achieved (5–7 days). Subsequently, the resulting suspension of the dialysis process was sonicated for 5 min at 70% intensity in a VCX 130 ultrasonicator (Sonics & Materials, Newtown, USA), with sample tubes immersed in an ice bath to prevent heating. The colloidal suspension was stored in a refrigerator at 4 °C, with the addition of some drops of chloroform to avoid any bacterial growth until the freeze-drying pro-

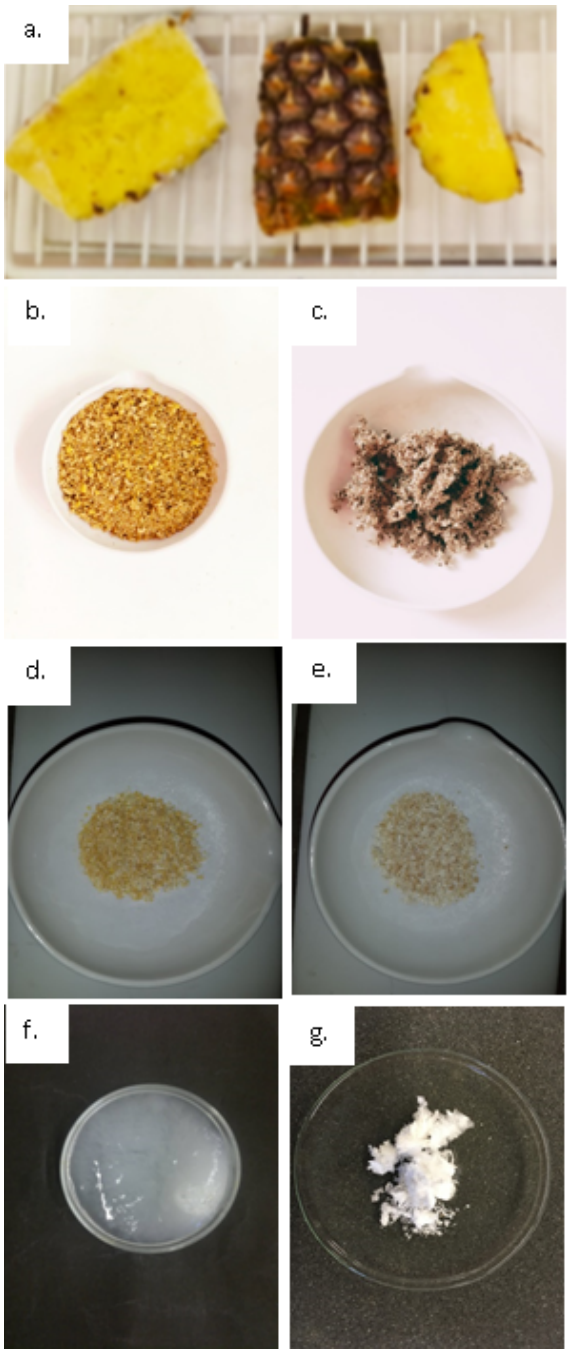

Figure 1: Images of fresh pineapple peel residues (a), dried and milled pineapple peel PP (b), after alkali drying and milling treatments (c), TPP4 (after 4h bleaching), drying and milling treatments (d), TPP6 (after 6h bleaching), drying and milling treatments (e), CNC30 colloidal and dialysed suspension after 30 min acid hydrolysis (f), and lyophilised CNC30 (g).

cess. The freeze-drying process was performed using a Vacuum Freeze Drier (Model FT33, Armfield, UK), under a vacuum pressure of 100 millitorr; the temperature in the freezing chamber was -46 ˚C, and the temperature in the sample chamber was 15 ˚C. The cellulose nanocrystals were labelled as CNC30 or CNC60, depending on the time of extraction by hydrolysis (30 and 60 minutes, respectively).

2.3 Product chemical characterisation

The chemical composition of the dried PP and TPP was measured as follows. The lignin content was determined according to the standard method of the Technical Association of Pulp and Paper Industry TAPPI T222 om-88. The holocellulose (α-cellulose + hemicellulose) content was estimated by the acid chlorite method (Browning, 1967). The α-cellulose content was determined by treating the holocellulose with a potassium hydroxide solution (Browning, 1967). The hemicellulose % was determined by subtracting the α-cellulose content from the holocellulose. An average of three replicates was calculated for each sample.

2.4 Fourier transform infrared spectroscopy (FTIR)

An IRPrestige-21 infrared spectrophotometer (Shimadzu, Japan) was used to obtain spectra for PP, TPP and CNC30. The KBr disk (ultrathin pellets) method was used to measure the IR spectra. Samples were ground and mixed with KBr (sample/KBr ratio = 1/100) to prepare discs. The experiments were carried out using the wavenumber range of 500-4000 cm^{-1}, with a resolution of 4 cm^{-1} and a total of 32 scans for each sample.

2.5 Particle size and charge measurements by dynamic light scattering (DLS)

Particle size (PS), polidispersity index (PI) and zeta potential (ZP) were measured using dynamic light scattering (DLS) with a ZetaSizer NanoZSP (Malvern, UK). Particle sizes were measured by taking into account the first order result from a DLS experiment as an intensity distribution of PS. The intensity distribution was weighted according to the scattering intensity of each particle fraction or family. Data was validated only if the cumulants fit error was <0.005. Zeta potential was measured using Laser Doppler Anemometry (*LDA*). All analyses were carried out with an angle of 90˚ at 25 ˚C.

2.6 X-ray diffraction (XRD)

X-ray diffraction (XRD) was carried out using a Rigaku Geigerflex D/max Series diffractometer with Cu Kα radiation, between 20-70˚, with a 2θ step size of 0.02˚ and a time of 10 s per step. The crystallite size was estimated from the FWHM (full width at half maximum) value of the 100 % cellulose peak at 22.3˚, using the Scherrer equation:

$$D = \frac{K\lambda}{h_{1/2} cos\theta} \quad (1)$$

where D = average size of the crystallites, K = Scherrer constant (0.94 for spherical crystals), λ= wavelength of radiation (1.54056 $\mathring{A}$), $h_{1/2}$ = FWHM, and θ = Bragg angle (the peak position = 2θ).

2.7 Scanning Electron Microscopy (SEM)

Morphology of PP, TPP and CNC was evaluated by Scanning Electron Microscopy (SEM) using a JEOL-5600 Lv microscope (Tokyo, Japan). Briefly, a small amount of freeze-dried sample was placed on a metallic stub with carbon tape and coated with gold/palladium using a Sputter Coater (Polaron, Bad Schwalbach, Germany). SEM was operated at the high vacuum mode, with a potential of 15-20 kV. All analyses were performed at room temperature (20 ˚C).

3 Results and Discussion

3.1 Extraction process and physical properties of CNC

Pineapple peel was obtained from a local fresh-cut fruit production company which is dedicated to the processing of ready-to-eat fruit, such as melon, mango and pineapple. The dried and milled pineapple peel (PP) contains several components (Fig. 1b), such as pigments, proteins and lipids. In Table 1, the chemical composition of each product generated is depicted. In terms of dry matter, a decrease of ca. 90% to 50% occurred after the bleaching process. This is a consequence of the occurrence of the lost of cellulose during the process, since no increase on cellulose content occurs after bleaching treatment. These results can be related to the breakdown of cellulose chains during bleaching and the loss of material during the filtering processes and mass transfer.

Lignin is the major component of the PP at ca. 29%, followed by cellulose (ca. 17%) and hemicellulose (ca. 16%) (Table 1). These values accord with those found by a few other authors that characterized pineapple peel (Raji, Jibril, Misau, & Danjuma, 2012; Thirawan, Karnnasuta, & Srinorakutara, 2017). In the samples purified by the alkali process, a bleaching was performed during two times, 4 and 6 h (TPP4 and TPP6). With the bleaching process, the delignification of the samples is supposed to occur (Cherian et al., 2010). For the two times of study, a high decrease in lignin was detected, which proved the efficiency of delignification treatment. The hemicellulose % was determined by subtracting the α-cellulose content from the holocellulose. Hemicelullose % decreased, as is supposed to happen with the first alkaline treatment. Holocellulose present in the bleached samples only contained cellulose and very low hemicelluloses' content, ca. 54% of α-cellulose and increased to more than 80% of cellulose after bleaching (data not shown). When calculating the % of cellulose in the initial samples' masses, the values were shown to be maintained or slightly increased (Table 1). Nevertheless, the TPP products were used to extract the cellulose nanocrystals. At the end of hydrolysis, samples were dialyzed, and a gelly and alkaline suspension was obtained as shown in Fig. 1f.

The colloidal suspensions of TPP4 and TPP6 were analysed by DLS to determine the CNC particle size in suspension and charge (Table 2). The sizes obtained were high since crystals' length is also measured, not only the width, using a DLS scatter that is dynamic, and agglomeration may occur in solution. Nevertheless, smaller CNCs were obtained for TPP6 than for TPP4.

The polydispersity index (PI) indicates the variation in the distribution of the particle size. Aa high polydispersity shows the existence of particle families with different sizes, which may mean the occurrence of aggregation (Hanaor, Michelazzi, Leonelli, & Sorrell, 2012). In general, all samples showed PI values much higher than 0.3, which indicates a polydisperse distribution of CNCs. On the other hand, zeta potential (ZP) can give us an indication of whether repulsion between adjacent, similarly charged particles in dispersion will occur or not. When ZP is high (whether they are positive or negative values) means stability between the particles, whereas when the potential is low, particles tend to coagulate/flocculate as attraction exceeds repulsion in the dispersion. The ZP values where ca. 30 mV in the CNCs extracted from TPP4, which means a moderate stability. In the case of the CNCs extracted from TPP6, this value decreased but is still moderate. In both cases, the values are negative, which is a result of the acid hydrolysis, in which sulphuric acid removed the amorphous regions in the cellulose fibres, leaving only the highly ordered crystalline regions intact, resulting in negatively charged, sulphonated nanoparticles. Due to the relatively low surface charge, this method required ultrasonication to disperse and stabilize the CNC suspension.

The colloidal suspensions with the lowest sizes, i.e. those originating from TPP6, were freeze-dried and a white and light powder was obtained as shown in Fig. 1g.

Table 1: Chemical characterisation in % (w/w) (means±SD) of the products obtained during samples processing

Samples	Lignin	Cellulose	Hemicellulose	Ash
PP	28.9±0.390	16.9±2.02	15.8±2.02	3.92±0.721
TPP4	3.40±0.103	21.8±0.793	3.91±0.983	2.48±0.201
TPP6	2.48±0.041	21.0±1.23	2.79±1.03	2.00±0.510

PP-Pineapple peels residues; TPP-Treated and bleached residues 4 h (TPP4) and 6 h (TPP6)

Table 2: Chemical characterisation in % (w/w) (means±SD) of the products obtained during samples processing.

Purification time (h)	CNC (hydrolysis time)	Particle size (nm)	Polidispersity index	Zeta potential (mv)
TPP4	30	2291±1556	0.891±0.0792	-29.8±3.83
	60	970.1±242.8	0.859±0.1297	-32.7±1.32
TPP6	30	657.8±188.7	0.611±0.1319	-13.8±1.35
	60	1455±91.15	1.000±0.0381	-15.3±3.99

TPP4-Treated pineapple residues after 4h bleaching; TPP6-Treated pineapple residues after 6h bleaching

3.2 FTIR spectra

The powders of the PP, TPP4, TPP6, and CNCs obtained from TPP6, were analysed by FTIR and compared (Fig. 2). The presence of **peaks 1** and **4** at 3310 and 1640 cm^{-1}, respectively, in all samples shows that during the purification, cellulose is present and was not removed during the purification and CNC extraction processes (Sheltami, Abdullah, Ahmad, Dufresne, & Kargarzadeh, 2012; Sun, Xu, Sun, Fowler, & Baird, 2005). **Peak 2** at 2900 cm^{-1} is from the C-H stretching vibration, is also present in all samples as expected, and was observed in other similar samples (Alemdar & Sain, 2008; Sheltami et al., 2012). **Peak 3** at 1700 cm^{-1} originates from the acetyl and ester groups in hemicellulose, or carboxylic acid groups in the ferulic and p-coumeric components of lignin. The existence of this peak shoulder was also reported in other works with wheat straw, rice husks and soy hulls (Alemdar & Sain, 2008; Flauzino Neto et al., 2013; Sun et al., 2005). It should disappear with extraction, since hemicellulose is removed from PP with the purification process. Indeed, this peak is more pronounced in PP than TPP4 and TPP6, and disappears in the CNC spectra. **Peaks 6** and **7** at 1060 and 897 cm^{-1}, respectively, correspond to the cellulose C-O stretching and C-H vibrations of cellulose, and appeared in all of the spectra, growing with extraction (Alemdar & Sain, 2008; Sheltami et al., 2012). **Peak 5** at 1205 cm^{-1} is only present in the CNC spectra, and it is not common to see in other CNCs extracted from lignocellulosic materials. It can be attributed to the S-O vibration, due to the esterification reaction, as reported in soy hulls (Flauzino Neto et al., 2013) (Lu, Gui, Zheng, & Liu, 2013; Lu & Hsieh, 2012).

3.3 X-ray diffraction measurements

The CNCs obtained from the two purification treatments, TPP4 and TPP6, were evaluated regarding their degree of crystallinity. Both intra- and intermolecular hydrogen bonding occurs in cellulose via hydroxyl groups, which results in various ordered crystalline arrangements. The x-ray diffractograms obtained (Fig. 3) are typical of cellulose I, the more widespread crystalline form of the 4 existent cellulose polymorphs, with well-defined crystalline peaks around 22° and 35° (Klemm, Heublein, Fink, & Bohn, 2005). The

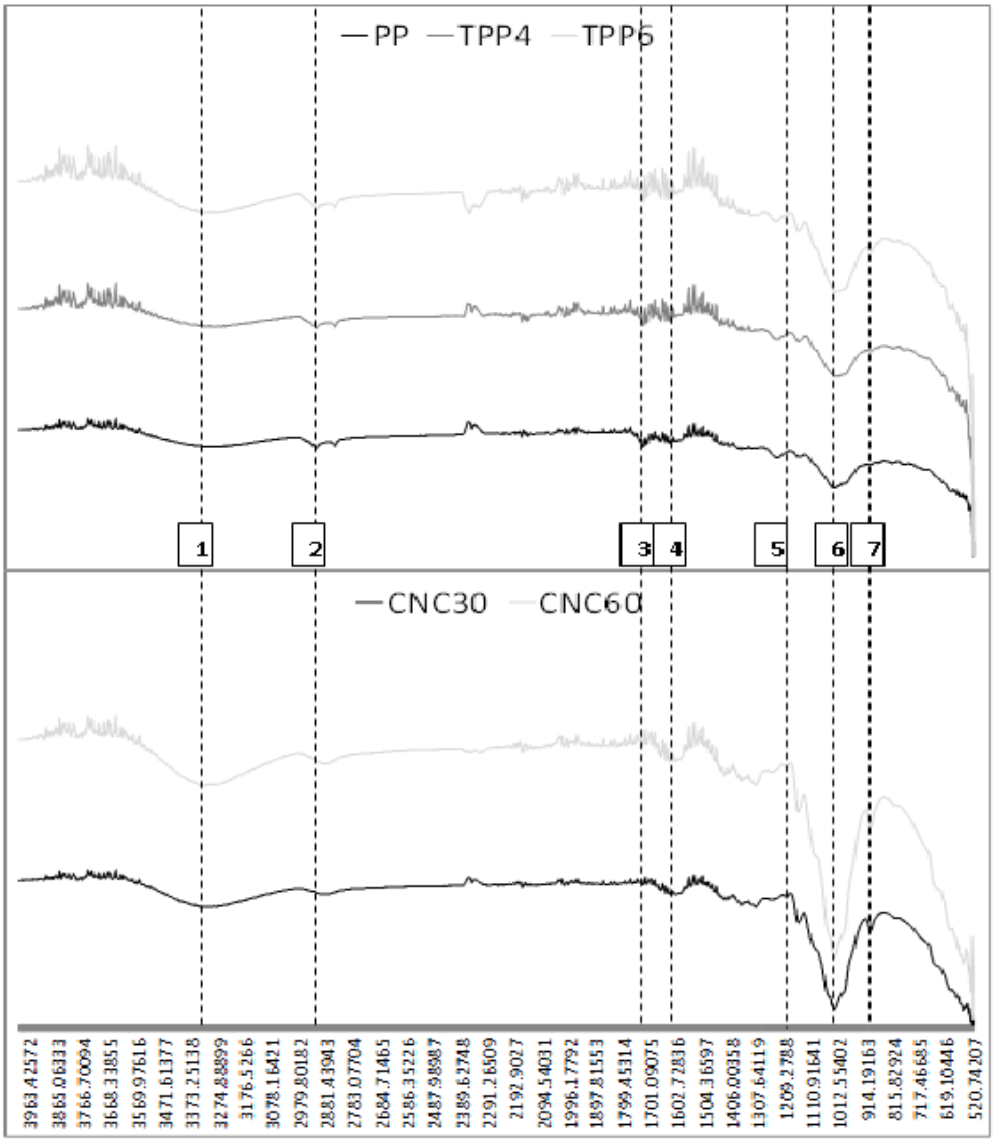

Figure 2: FTIR spectra of dried and milled pineapple peel (PP), after 4h bleaching, drying and milling treatments (TPP4) or after 6h bleaching (TPP6) and cellulose nanocrystals (CNCs) obtained from 6 h of purification (TPP6) and after 30 min (CNC30) and 60 min (CNC60) of acid hydrolysis. 1- 3310 $cm^{-}1$; 2 – 2900 $cm^{-}1$; 3 – 1742 $cm^{-}1$; 4 – 1640 $cm^{-}1$; 5 – 1307 $cm^{-}1$; 6 – 1060 $cm^{-}1$; 7 – 897 $cm^{-}1$.

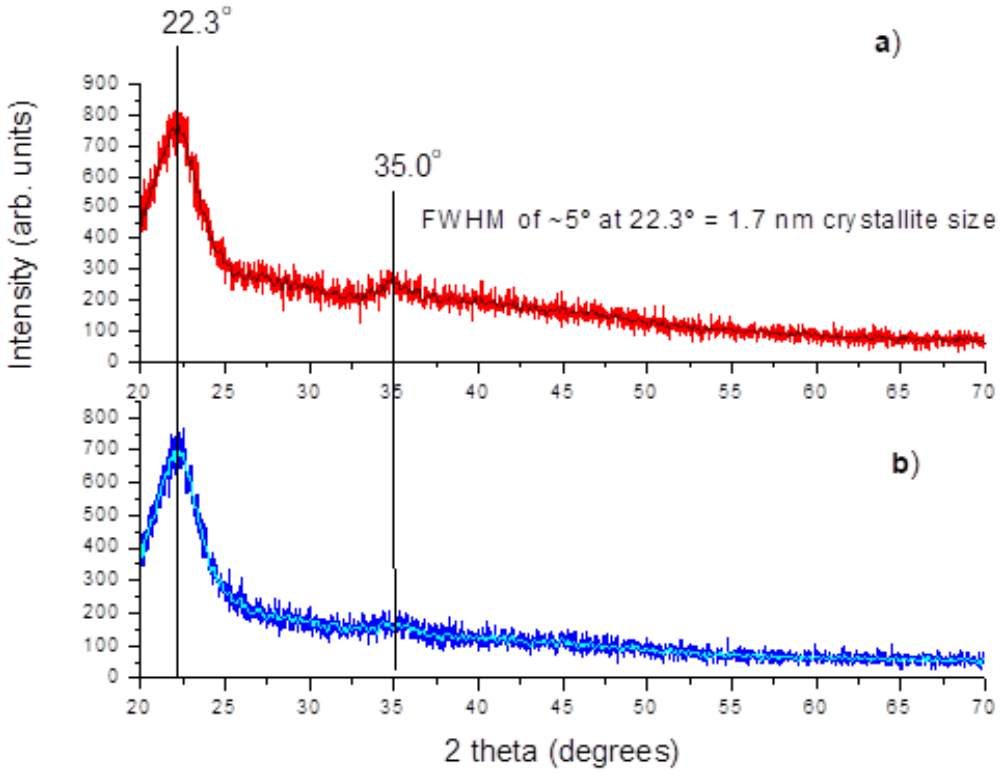

Figure 3: X-ray diffraction patterns of CNCs obtained from hydrolysis for 30 min of TPP6 (a) and TPP4 (b)

major peak, which is related to the crystalline structure of cellulose I, was seen for all samples at 22.3˚, with a much smaller peak at 35.0˚, and both indicate a poorly crystalline material, made of nanoscale crystals. The background "hump" around 20-30˚ also indicates the presence of some amorphous material. The peaks are slightly more pronounced in the CNC obtained from TPP6, which again shows the importance of using a higher bleaching time during purification to better remove lignin and hemicellulose. The average cross-sectional dimension of the elementary cellulose crystallites was estimated from their X-ray diffractograms by applying the Scherrer equation, as described in Section 2. The Scherrer equation is unreliable for dimensions lower than 100 nm, as the broadening becomes excessive, but it can still be used to get a very approximate estimate of the average crystallite size. For both samples in Fig. 3, with a FWHM of ∼5˚, the value for the crystallite size was found to be only 1.7 nm.

3.4 Morphology

In Fig. 4a and b, the raw fibres have become narrow fibrils, with a reticular structure, after the chemical-purified treatment. This indicates that the purification process and bleaching did not provoke the breaking of the cellulose chains. In Fig. 4b, the release of cellulose microfibers can be clearly seen. Fig. 4c and d show the CNC samples. In Fig 4c, a drop of the colloidal suspension was put on the carbon tape and left to dry at room temperature. After covering with gold, the samples were observed and aggregations of small entities were identified. CNCs which are freeze dried appear as an aggregation of irregular

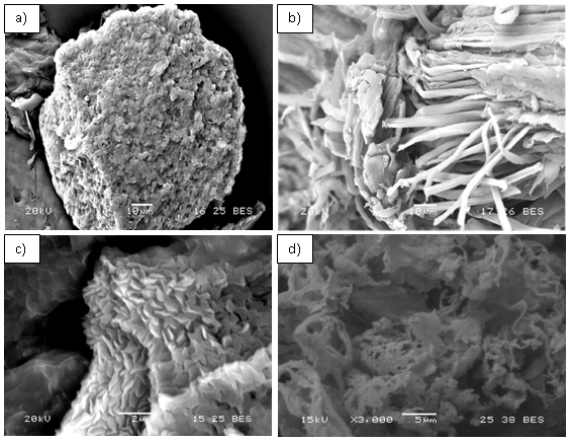

Figure 4: Micrographs of pineapple peels residues PP 1000x (a), TPP6 1000x (b), colloidal suspension CNC30 4000x (c) and freeze-dried CNC30 3000x (d)

rod-like assemblies of CNCs. However, individual NPs cannot be distinguished, as they are far too small for the resolution of the SEM method, which has a limit of around 30 nm. This was also seen with CNCs extracted from sweet potatoes residues (Lu et al., 2013).

4 Conclusions

The purification process is important in the treatment of wastes for cellulose extraction, since pigments, waxes, hemicellulose and lignin are effectively extracted from the residues, while cellulose is not removed. The best conditions for extraction include a 6 h bleaching process. The time of bleaching was shown to be important for the purification of cellulose, and this was reflected by the composition, the physical properties and FTIR spectra. Indeed, the pineapple peel that was bleached for 6 hours had lower hemicellulose and lignin contents, and the CNCs produced from TPP6 (6 h) were marginally more crystalline than those produced with TPP4 (4 h). In terms of hydrolysis of the extracted cellulose, the time used was not shown to be important, and 30 minutes was shown to be effective in the hydrolysis process. Even with low yields, the cellulose extracted had ca. 80% purity. Pineapple peel was shown to be a suitable cellulose source for the extraction of CNC.

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Quality Assessment and Shelf Life Modeling of Pulsed Electric Field Pretreated Osmodehydrofrozen Kiwifruit Slices

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Abstract

The objective of this work was to investigate the potential use of pulsed electric field (PEF) in combination with osmotic dehydration (OD) as a pre-freezing step and to evaluate the effect on quality characteristics and shelf life of frozen kiwifruit. Peeled kiwifruit was subjected to PEF (1.8 kV/cm), sliced and treated in OD-solution (containing glycerol, maltodextrin, trehalose, ascorbic acid, calcium chloride, citric acid, sodium chloride; 1/5 ($w_{fruit}/w_{solution}$)) for 30 and 60 min at 35 °C. Combined, PEF only and OD only treated samples as well as nontreated and blanched (80 °C, 60 s) samples were frozen and stored at constant (-5, -10, -15, -25 °C) and dynamic temperature conditions (-18 °C-3 d, -8 °C-2.5 d, -15 °C-3 d). Quality of frozen samples was evaluated by means of drip loss, colour, texture, vitamin C and sensory evaluation (1-9 scale); and shelf life (SL) was calculated. Nontreated and blanched samples presented high drip loss and tissue softening (instrumentally measured as F_{max} decrease). The tissue integrity was well retained in all osmotically pretreated samples. PEF pretreatment caused increase of fruit whiteness (increase of L value) and yellowness (a and/or b value increase); SL calculation was based on colour change. All OD samples had high vitamin content (24.6 mg/100 g fresh material compared to 138-154 mg/100 g osmodehydrated material); PEF led to 93% (of the initial) vitamin retention; blanched samples showed the lowest retention (86.9% of the initial) (criteria for SL calculation). OD and combined PEF-OD treatment increased the shelf life of frozen kiwifruit (up to 3 times; based on sensorial criteria). The developed kinetic models for colour change, vitamin loss, and sensory quality deterioration were validated at dynamic temperature conditions. PEF pretreated OD (at significantly shorter time, 30 min compared to 60 min) kiwifruits retained optimum quality and sensory characteristics. PEF and OD could be used as a preprocessing step of good quality, longer shelf life kiwi sliced frozen products.

Keywords: *Actinidia deliciosa*; Freezing; Pulsed electric field; Osmotic dehydration; Quality; Shelf life

1 Introduction

Kiwifruit (*Actinidia deliciosa*) is an important source of vitamin C, chlorophyll, lutein,

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carotenoids (beta-carotene) and phenolic compounds with high antioxidant capacity. The antioxidant capacity of the fruit is strongly related to the vitamin C content and the content of phenolic compounds including phenolic acids and flavonoids, and is dependent on the species and cultivar of Actinidia (Leong & Shui, 2002; Du, Li, Ma, & Liang, 2009; Latocha, Krupa, Wolosiak, Worobiej, & Wilczak, 2010; Krupa, Latocha, & Liwinska, 2011). However, the shelf life of kiwifruit is short and processing should be employed to achieve satisfactory commercial life of kiwi products (Kaya, Aydm, & Kolayli, 2010; Orikasa et al., 2014). Main quality characteristics of kiwifruit such as colour, firmness and flavour are negatively affected by conventional processing techniques (drying, canning, and freezing and modified atmosphere packaging) (Stanley, Wegrzyn, & Saleh, 2007). Freezing of kiwifruit leads to significant cellular damage, and subsequent loss of product quality (texture degradation, colour alteration and nutritional loss) (Talens, Escriche, Martinez-Navarrete, & Chiralt, 2003).

Osmotic dehydration (OD) can be applied as a pretreatment before freezing (dehydrofreezing) to improve and maintain desirable quality characteristics such as colour, flavour and texture, mainly due to the reduction of freezable water and properties of functional impregnated solutes. OD occurs at mild temperatures (up to 50 °C) and requires less energy compared to other dehydration methods. OD is achieved by immersion in hypertonic sugar and/or salt solutions of high osmotic pressure/low water activity. Diffusion phenomena take place with two simultaneous counter current flows: a water flow from the food to the outer solution (water loss) and a simultaneous flow of solute from the solution to the plant tissue (solid gain). However, cellular membranes exert a high resistance to mass transfer thus slowing down the OD rate. Various approaches have been reported in the literature (such as pretreatment of the material prior to osmosis, the composition and the concentration of the osmotic solutes in the osmotic solution, the mixing parameters) to influence the rate of diffusion during OD (Rastogi, Eshtiaghi, & Knorr, 1999; Ferrando & Spiess, 2001). It is necessary to identify methods which enhance rate of transfers with minimal alteration in quality (Ade-Omowaye, Taiwo, Eshtiaghi, Angersbach, & Knorr, 2003; Ade-Omowaye, Talens, Angersbach, & Knorr, 2003).

Among the emerging nonthermal processes of interest, pulsed electric field (PEF) shows promise for inducing cell membrane permeabilisation, called electroporation of food plants (such as apples, carrots, mangos, and red bell peppers). PEF processing is used to increase permeability of the cell membrane and, in the case of expression and extraction, enhances mass transport out of the cells leaving the product matrix largely unchanged (Knorr, Geulen, Grahl, & Sitzmann, 1994; Knorr & Angersbach, 1998). Disruption of the cells can be achieved by applying PEF of different intensities (for particular products) across the cell medium or plant tissue (Ade-Omowaye, Taiwo, et al., 2003; Ade-Omowaye, Talens, et al., 2003; Amami, Vorobiev, & Kechaou, 2005, 2006; Lebovka, Bazhal, & Vorobiev, 2001; Tedjo, Taiwo, Eshtiaghi, & Knorr, 2002). The mechanism of electropermeabilisation of cell membranes induced by PEF is not yet well elucidated. However, different researchers indicate that the cell walls seem not to be seriously affected by this treatment and the product overall retains its quality (Jemai & Vorobiev, 2002; Lebovka et al., 2001; Amami et al., 2006). The effect of PEF treatment, preceding OD, on the osmotic behaviour of certain plant tissues has been reported (Ade-Omowaye, Taiwo, et al., 2003; Amami et al., 2005; Parniakov, Bals, Lebovka, & Vorobiev, 2016). Dermesonlouoglou, Giannakourou, and Taoukis (2016) studied the impact of PEF on mass transfer of osmotically dehydrated kiwifruit.

OD of kiwifruit has been reported in literature, mostly, to describe the structural, and physicochemical changes which occur during the process (Bressa, Dalla Rosa, & Mastrocola, 1997; Escriche, Garcia-Pinchi, Andres, & Fito, 2000; Gianotti, Sacchetti, Guerzoni, & Dalla Rosa, 2001; Cao, Zhang, Mujumdar, Du, & Sun, 2006; Tocci & Mascheroni, 2008; Castro-Giraldez, Tylewicz, Fito, Dalla Rosa, & Fito, 2011; Tylewicz, Fito, Castro-Giraldez, Fito, & Rosa, 2011; Panarese, Tylewicz, Santagapita, Rocculi, & Dalla Rosa, 2012). Dehydrofreezing technology has been applied in kiwifruit, and the volatile profile, phe-

nolic content and sensory attributes have been studied (Talens et al., 2003; Tylewicz et al., 2011). However, the OD process effect on frozen kiwifruit quality and shelf life through a systematic kinetic analysis of the quality loss has not been reported. Neither has the freezing response of PEF treated plant tissues been studied, nor the combined effect of PEF and OD on frozen kiwifruit quality investigated.
The objective of this study is to investigate the potential use of PEF in combination with OD as a prefreezing step and to evaluate the effect on quality characteristics (drip loss, texture, colour, firmness, vitamin C) and shelf life of frozen kiwifruit. Quality deterioration in the whole temperature range of practical interest (from -5 to -25 °C as well as dynamic conditions) was investigated and kinetically modelled.

2 Materials and Methods

2.1 Pulsed electric fields

Whole peeled kiwifruit "Hayward" was subjected to different PEF process conditions. PEF treatment was conducted in a versatile pilot scale system for food processing (Elcrack-5kW, DIL, Germany). It comprises a pulse generator module and liquid handling system and can also be operated as stand-alone research equipment. Kiwifruits were treated in a 80*100*50 mm (gap*length*depth), 400 ml volume stainless steel batch chamber for cell disintegration in tap water. The inlet temperature of the chamber was around 20 °C, and the outlet temperature rise due to the PEF treatment did not exceed 5 °C. In the experimental set 250 pulses at field strength intensity of 1.8 kV/cm (with 15 μs pulse width at a frequency of 300 Hz) were applied, with average specific energy input of 42.3 kJ/kg. The selection of PEF conditions was based on a previous comparative study of mass transfer phenomena occurred during subsequent OD by Dermesonlouoglou, Zachariou, Andreou, and Taoukis (2016).

2.2 Osmotic dehydration

PEF treated and fresh, non-treated samples were cut into flat discs (43.5±0.8mm diameter, 6.83±0.03mm thickness), and then partially dehydrated by immersion in a hypertonic solution of glycerol (30%), high-DE (Dextrose Equivalent) maltodextrin (20%), trehalose (10%), ascorbic acid (2.0%), calcium chloride (1.5%), sodium chloride (1.0%) and citric acid (0.2%) (w/w). Glycerol, maltodextrin and trehalose have been used as the main osmotic solutes (Dermesonlouoglou, Giannakourou, & Taoukis, 2007; Dermesonlouoglou, Zachariou, et al., 2016; Dermesonlouoglou, Pourgouri, & Taoukis, 2008; Parniakov et al., 2016). Glycerol, a low molecular weight sugar alcohol which is easily digested, non-toxic and recognized as a safe additive (Regulation EC No 1333/2008), is used as a humectant to control aw without % limitation. Trehalose, a non-reducing dissacharide which is available as a food ingredient and has low sweetness (Treha 16400, Cargill), has been reported to exert a protective role during freezing of membranes and proteins (Ferrando & Spiess, 2001). The high-DE maltodextrin used (Glucidex, Dehydrated Glucose Syrup 47, Roquette) is an effective osmotic agent which has a very low sweet taste compared to sucrose. The OD solution also comprised citric acid and ascorbic acid to reduce the pH and hinder extended surface discolouration, calcium chloride to minimize tissue damage during processing, and sodium chloride to enhance mass transfer kinetics as well as to improve the sensory characteristics (particularly to balance the sweet taste) of the final osmodehydrated kiwifruit product (Dermesonlouoglou et al., 2016; Dermesonlouoglou, Zachariou, et al., 2016).
OD treatment was conducted at 35 °C (T) for 30 (PEFOD1 sample) and 60 min (PEFOD2 sample) (t). The solution to sample ratio was 5:1(w:w) to avoid significant dilution due to water removal, thereby causing localized reduction of the osmotic driving force during the process. The experimental OD procedure was the same for non-treated (OD) and PEF treated samples (noted as PEFOD1, PEFOD2). Two different types of samples were used as "control" samples: non-treated and blanched by direct immersion of

samples in hot water at 80 °C for 60s (Dermesonlouoglou et al., 2008).

Analysis of physicochemical properties

Water content (X_w) and total solids (X_s) were measured in fresh, blanched and PEF pretreated samples to determine the compositional changes induced by OD. The water loss (WL: g water/g initial dry matter or g w./i.d.m.) and solid gain (SG: g solids/g initial dry matter or g s./i.d.m.) occurring during OD were calculated (Dermesonlouoglou et al., 2007, 2008). Moisture content was determined gravimetrically after drying at 105 °C for 24 h (WTB BINDER 7200, Type E53, Germany). Water activity (a_w) was monitored (Aqua LAB 4TEV, Decagon Devices, Inc., U.S.A.).

2.3 Freezing

All samples were quick frozen at -40 °C with forced air convection [convection coefficient h=11 W/m^{2}K, Sanyo MIR 553, Sanyo Electric Co, Ora-Gun, Japan], packed in pouches from BOPP-PE laminate film which is used for commercial frozen vegetable products, and kept at this temperature for a short period of time before being distributed to controlled temperature cabinets.

2.4 Storage and quality loss determination

Packages of frozen kiwifruit samples were stored in controlled temperature cabinets (Sanyo MIR 153, 253 and 553, Sanyo Electric Co, Ora-Gun, Japan) at constant (-5, -10, -15 and -25 °C used as "control frozen" samples) and dynamic conditions (consisting of several repeated cycles of three isothermal steps -18 °C-3d, -8 °C-2.5d, -15 °C-3d) in temperature programmable control cabinets monitored by type T thermocouples (CR10X,Campbell Scientific, UK).
Selected quality parameters (drip loss, texture, colour, vitamin C, and sensory characteristics) were evaluated at appropriate time intervals (Constant conditions -5 °C: 8, 16, 29, 41, 57 days; -10 °C: 8, 29, 57, 100, 132, 176, 201, 258 days; -15 °C: 16, 41, 57, 100, 176, 258 days; Dynamic conditions: 16, 41, 57, 111, 158, 213 days) in order to model their rate of change versus storage time at all constant temperatures.

Drip loss

Frozen slices were each put into a covered tare 100ml beaker which were defrosted for 24 h at 4 °C, put on a sieve for 15 min to allow the excess water to drip off and were weighed to measure the drip loss of the kiwifruit slices during thawing (Talens et al., 2003). Triplicates of all the differently treated slices were determined.

Texture

Texture (at least eight sampling times per treatment) of kiwifruit was determined with a texture analyzer (TA-XT2i of Stable Micro Systems, England). The test was performed on a non-lubricated flat platform, using a knife probe, where the samples were cut at a fixed rate (0.5mm/s) and depth (1/3 of the initial). The maximum peak force (F_{max}, N) was the mechanical parameter considered, and was expressed as firmness. Triplicates of all the differently treated slices were measured at each sampling time.

Colour

Quantification of the colour change was based on measurement of CIELab values (L: illuminance; a: red saturation index; b: yellow saturation index) (CIE, 1978) using a hand-held dual-function reflected-light CR-200 Minolta Chromameter® with diffuse illumination/0° viewing angle (Minolta Co., Japan). Three replicates of each measurement were conducted on the uniform green part of ten kiwifruit samples after thawing. Instrumentally measured colour was found to be adequately described by total colour change $\Delta E = \sqrt{(L-L_0)^2+(a-a_0)^2+(b-b_0)^2}$, where L_0, a_0 and b_0 values are L, a and b values measured at zero storage time.

Vitamin C

Vitamin C (L-ascorbic acid) was determined using a high performance liquid chromatography method previously detailed in Giannakourou and Taoukis (2003). The results were expressed in mg L-ascorbic acid/100g fresh material (f.m.) or osmodehydrated material and plotted versus time. All measurements were conducted in duplicate.

Sensory attribute

Trained assessors were chosen for the assessment of the sensory attributes of kiwifruit samples (International Organization for Standardization, 2012). Assessors (8 members of the trained sensory panel; female 5 and male 3; ages 22-44) were asked to score for overall visual appearance, overall texture (including firmness, consistency, juiciness), flavour and overall acceptability of kiwifruit samples, in appropriate forms with descriptive terms reflecting the organoleptic evolution of quality deterioration. Rating was assigned separately for each parameter on a 1–9 descriptive intensity scale (9 being the highest quality score and 1 the lowest) (Lim, 2011; Dermesonlouoglou et al., 2008, 2016; Tsironi, Dermesonlouoglou, Giannakourou, & Taoukis, 2009).

2.5 Statistical analysis

Analysis of variance (ANOVA) and Tukey multiple range tests (α=0.05) were used to determine statistically significant differences (STATISTICA®) between the different storage temperatures and treatments, concerning physico-chemical parameters, the rates of colour change, vitamin C, and sensory quality loss.

3 Results and Discussion

3.1 Pulsed electric fields and osmotic dehydration

In Table 1, physico-chemical parameters (initial water content X_W, water activity a_W, pH, water loss-WL, and solid gain-SG) of kiwifruit slices before and after blanching, PEF and/or OD processing are presented. The OD pretreatment caused a decrease in the water content ($p<0.05$), pH and water activity, with a corresponding increase in the total solids. The application of blanching as well as PEF processing did not significantly change the level of water, total solids, pH and a_W ($p>0.05$).
During OD pretreatment, a high rate of mass exchange was achieved. The water loss (WL) and solid gain (SG) values for OD time 30 and 60 min were calculated as 1.78 and 1.99 g w./g i.d.m., and 0.208 and 0.248 g s./g i.d.m., respectively. Application of PEF significantly enhanced the rate of transfers during OD. WL was calculated as 1.68 and 2.37 g w./g i.d.m. for OD time 30 and 60 min (PEFOD1 and PEFOD2 samples), respectively, and SG as 0.550 and 0.636 g s./g i.d.m. for OD time 30 and 60 min (PEFOD1 and PEFOD2 samples), respectively.
Osmotic pretreatment substantially lowered a_w to a level of 0.89(PEFOD2)-0.92(PEFOD1 & OD samples) (compared to 0.95 for non-pretreated samples), mainly due to the hypertonic solution ingredients used in this particular case. The final aw value for PEFOD processing was achieved at the shorter OD time. 30 min OD time for PEF pretreated samples was found to be equivalent to 60 min OD time.

3.2 Quality loss determination at isothermal conditions

Drip loss and texture

In Figs 1(a and b) the drip loss and firmness values for non-treated, blanched, OD, PEF, and PEF pretreated OD kiwifruit samples stored at isothermal temperature conditions T=-15 °C, and non-isothermal conditions T_{eff}=-13.3 °C (representatively) were demonstrated. Non-treated and PEF pre-treated samples suffered from higher drip loss and tissue softening at zero storage time. Respective values, measured at zero storage time for all kiwifruit samples were presented in Table 2. The tissue integrity was retained with storage time for all osmotically pretreated, combined with PEF (PEFOD2 and PEFOD1) or not, samples (low drip loss showing a decrease with storage time and increased firmness not showing any clear tendency). On the

Table 1: Physico-chemical parameters of kiwifruit slices before and after blanching, PEF and/or OD processing

Sample	X_w (g w/g i.f.m.)	a_w	pH	WL (g w./g i.d.m.)	SG (g s./g i.d.m.)
Non	0.858±0.003a	0.951±0.014a	3.39±0.18ab	-	-
OD	0.765±0.007b	0.927±0.018a	3.28±0.09b	1.99a	0.248a
blanched	0.862±0.011a	0.955±0.017a	3.42±0.11ab	-	-
PEF	0.856±0.010a	0.947±0.016a	3.53±0.07a	-	-
PEFOD1	0.740±0.006b	0.923±0.022ab	3.30±0.06b	1.68b	0.550b
PEFOD2	0.684±0.005c	0.892±0.048b	3.27±0.11b	2.37c	0.636b

± represent standard deviation of triplicates. Different superscript letters indicate significantly different means ($p<0.05$) within a column (differences between treatments)

other hand, blanched samples showed high drip loss and increased firmness compared to non-treated and PEF pretreated samples. Blanching pretreatment seemed to protect partially kiwifruit from tissue collapse.

The influence of fruit firmness on kiwifruit acceptability is significant. The firmness of a kiwifruit strongly influences its sensory quality at the moment of consumption, influencing the perception of aroma intensity, sweetness, acidity and ripeness. It has been reported that fruits with softer core are perceived as having a more intense flavour, a more sweet and fruity taste and stronger ripe fruit characteristics (Stec, Hodgson, Macrae, & Triggs, 1989). Osmodehydrated kiwifruits with firmness values >0.5N (up to 1.5N) were characterized as "not soft", and at the same time as "having a more intense flavour, and a more sweet taste (acceptable and desirable)" according to sensory analysis conducted (data not shown). All OD treated kiwifruit samples presented increased firmness compared to non-treated ones showing that one of the main goals of the application of the osmotic dehydration had been achieved. Delicate plant tissues, with high water content (such as cucumber, strawberry), treated by osmotic dehydration have been reported to have improved texture characteristics (Dermesonlouoglou et al., 2008, 2016). PEF application caused a significant decrease in firmness. All PEF treated OD samples (compared to OD samples) had lower firmness values at zero storage time leading to softer fruit tissue. Firmness values increased with OD time (from 30 to 60 min) for PEF pretreated OD samples (from PEFOD1 to PEFOD2 sample). However, the effect of applied pretreatments of PEF and OD on kiwifruit texture (drip loss and firmness) could not be mathematically modelled.

Colour

Colour is an important quality index for fresh-cut kiwifruit products. Fresh kiwifruit is characterized by the green colour of its flesh. The colour changes in kiwifruit during ripening (towards more yellowness and less greenness) are due to the loss of chlorophylls which no longer mask the carotenoids (Montefiori, McGhie, Hallett, & Costa, 2009).

The results of this study showed that the colour of kiwifruits was significantly different immediately after the application of PEF pretreatment. In PEF and blanched pretreated OD kiwifruits, L- and a-values were higher, but b- values did not show significant differences at the zero storage time. Respective values, measured at zero storage time for all kiwifruit samples are presented in Table 2. The effect of PEF and blanching on colour, compared to non-treated kiwifruit was an increase in fruit lightness (L increase) and a decrease in green colour (a increase; b did not show significant differences). The PEF effect on OD treated kiwifruit colour was an increase in fruit lightness (L increase), and a decrease in yellow colour (b decrease; a did not show significant differences).

The total colour change of all kiwifruit samples during storage was expressed by ΔE values. The

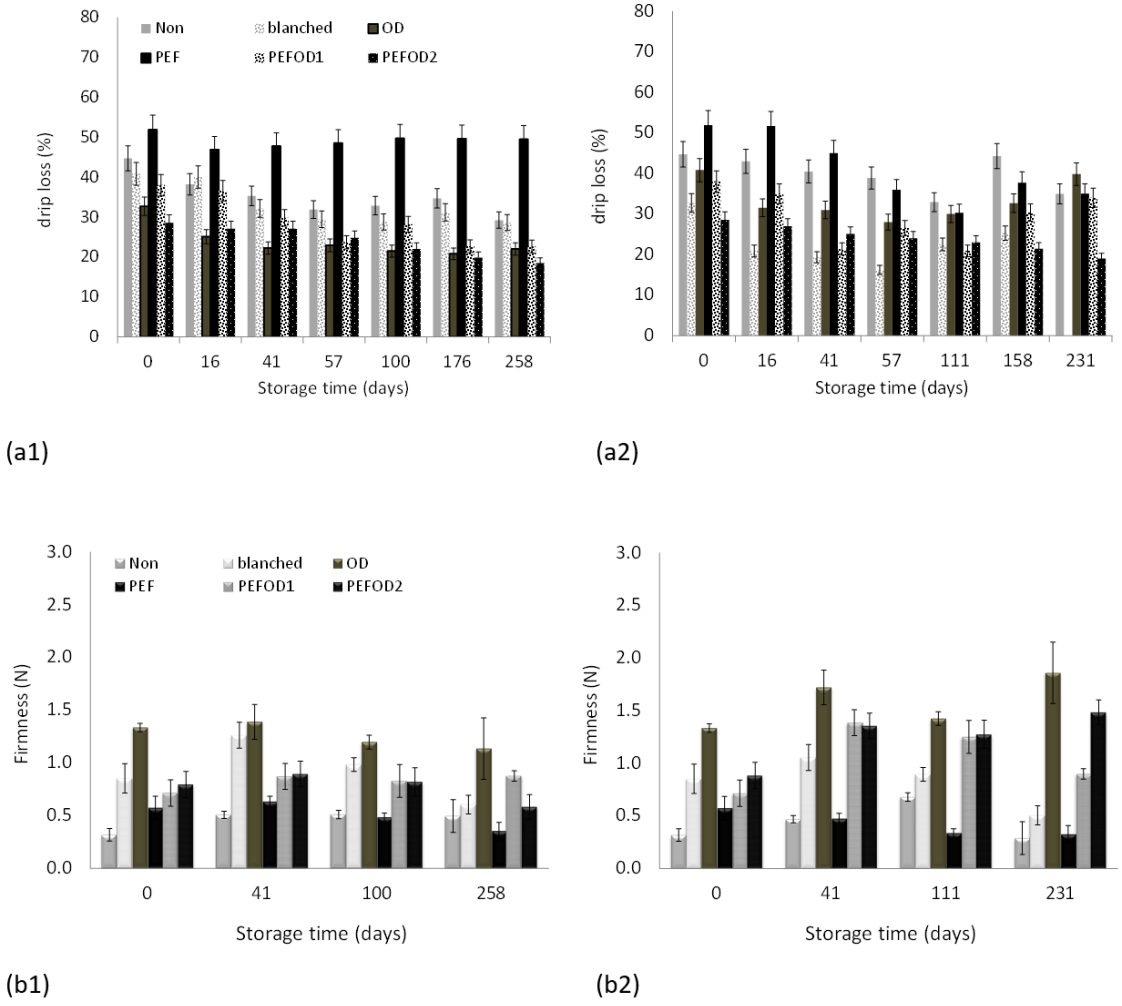


Figure 1: (a) Drip loss (%), and (b) firmness (F_{max}, N) versus time t for non pretreated, blanched, OD-, PEFOD1-, PEFOD2-pretreated kiwifruit slices stored at constant temperature conditions T=-15 ˚C (index 1), and dynamic conditions T_{eff}=-13.3 ˚C (index 2) (error bars indicate the standard deviation from replicates)

Table 2: Quality parameters of kiwifruit slices before and after blanching, PEF and/or OD processing

Sample	Drip loss (%)	Firmness (Fmax, N)	Colour (L0-, a0-, b0-values)	Sensory scores for colour, texture, flavour, overall impression	Vitamin C (mg/100 g f.m. or OD.m.)
Non	44.7±5.2a	0.32±0.06a	42.46±0.66a -9.25±0.53a 21.63±1.67a	7.3ab, 6.5a, 7.0a, 6.5ab (±0.5)	24.6±2.4a
OD	32.7±3.0b	1.33±0.04d	44.39±1.28ab -8.91±1.10ab 22.47±2.62a	8.0b, 8.0bc, 7.5a, 7.5b (±0.3)	138±11b
blanched	40.8±4.2a	0.85±0.01c	47.50±5.44bc -7.97±0.95bc 22.01±0.11a	6.0c, 6.0a, 6.5ab, 6.0c (±0.3)	22.9±3.2a
PEF	51.9±4.8c	0.57±0.04b	48.49±1.79cd -7.00±0.60c 21.80±2.50a	5.8c, 5.8a, 5.5b, 6.0c (±0.2)	21.4±2.8a
PEFOD1	38.4±3.1ab	0.72±0.08c	54.91±0.11e -8.25±0.29ab 31.98±0.08b	7.5ab, 7.5c, 7.0a, 7.0ab (±0.4)	140±9b
PEFOD2	28.5±2.8b	0.79±0.08c	51.42±0.89d -6.95±0.62c 28.83±0.59b	7.5ab, 7.3c, 7.0a, 7.5b (±0.4)	154±13b

± represent standard deviation of triplicates. Different superscript letters indicate significantly different means ($p<0.05$) within a column (differences between treatments)

normalized version of ΔE (describing the exponential decrease of $\frac{\Delta E_{max}-\Delta E}{\Delta E_{max}}$ as follows) has been preferred so as to better show the comparative effect of the different osmotic pretreatments on the rate of colour change, and also to the best fit the experimental data (Eq. 1):

$$\frac{\Delta E_{max} - \Delta E}{\Delta E_{max}} = exp(-{k_{colour}}^{t}) \qquad (1)$$

where, ΔE is the ΔE-value after storage time t (days), ΔE_{max} is the asymptotic value of ΔE-value observed after a period of storage, defined as ΔE_{max} =6.0, for non-treated and blanched samples, 8.0 for OD samples, and 10.0 for PEF, PEFOD1, and PEFOD2 samples, and k_{colour} the apparent rate of colour deterioration (days^{-1}). The temperature dependence of colour change was described by the Arrhenius Equation (Eq. 2):

$$\ln k = \ln k_{ref} - \left(\frac{E_a}{R}\right)\left(\frac{1}{T} - \frac{1}{T_{ref}}\right) \qquad (2)$$

where, k_{ref} is the reaction rate constant at a reference temperature (days^{-1}) T_{ref} (K) (in this study -18 °C), E_a the activation energy of the estimated action (J/mol) and R the universal gas constant (J/mol·K).

In Figs 2(a1 and a2), the colour change (calculated using Eq. 1) for non-treated, blanched, OD and PEF pretreated OD kiwifruit samples stored at isothermal temperature conditions T=-15 °C, and non-isothermal conditions *Teff*=-13.3 °C (representatively) are presented. PEF pretreatment accelerated the increase in lightness (L value increase) and yellowness (a value increase and/or b value increase), at zero storage time as well as during storage (data not shown). Increasing L, a and b values respectively indicate an increase in fruit lightness or whiteness, a decrease in green colour, and an increase in yellow colour. For example, L, a, b values of non pretreated and pretreated kiwifruit samples stored at -10 °C for two different representative storage times were measured as Non: 47.48, -7.63, 18.81; OD: 45.03, -7.82, 22.70; PEF: 50.36, -6.95, 26.89; PEFOD1: 50.80, -7.11, 23.83; PEFOD2: 48.21, -5.95, 24.34, for 29 days, and Non: 43.84, -3.61, 16.42; OD: 43.61, -3.96, 24.00; PEF: 53.45, -6.05, 27.12; PE-

FOD1: 52.86, -6.31, 29.03; PEFOD2: 52.59, -6.91, 28.55, for 176 days. PEF treated samples showed the highest colour change rate values k_{colour} at all temperatures studied, followed by non-treated, blanched and OD treated samples (the last two showed similar colour change rates). On the other PEF pretreated OD samples had lower k_{colour} values which positively affected the colour change of osmodehydrated kiwifruit tissue. Activation energy E_a values ranged from 77.49(PEF sample) to 97.77(Non-treated sample) kJ/mol (Table 3).

Vitamin C

Vitamin C content is the most important nutritional attribute of kiwifruit (Du et al., 2009). In this study, the zero storage time vitamin C content of kiwifruit was measured to be 24.6 mg/100 g f.m. (Table 2: Respective values, measured at zero storage time for all kiwifruit samples). The vitamin C retention for PEF and blanched pretreated kiwifruit samples were 93.0% and 86.9% of the zero storage time values, respectively. The vitamin C retention of osmotically dehydrated red bell peppers was reported to vary from 89.6 to 96.5%. Those samples subjected to 50 pulses, at a field strength of 2 kV/cm, had the least retention whilst those treated with 1 pulse, at the same field strength, had the highest retention according to Ade-Omowaye, Talens, et al. (2003). During OD, the vitamin C increased to 138 mg/100 g osmodehydrated material (at OD time 60 min). For PEF pretreated samples, the vitamin C content of osmodehydrated samples varied from 140 to 154 mg/100 g osmodehydrated material (at OD time 30 and 60 min). In this study, the vitamin C increase observed during osmotic dehydration is due to the enrichment of the osmotic solution with ascorbic acid. This increase was more pronounced for longer OD times and with PEF pretreatment. Ade-Omowaye, Taiwo, et al. (2003), Ade-Omowaye, Talens, et al. (2003) reported improved mass transfer during drying as a consequence of PEF pretreatment and suggested minimal loss of vitamin C due to electric field treatment (red bell peppers, 2 kV/cm, 1-50 pulses). Taiwo, Angersbach, Ade-Omowaye, and Knorr (2001) reported that using field strengths >1.0 kV/cm depleted the vitamin content. All the PEF treated samples had vitamin C content lower than the nontreated due to faster leaching (as a result of permeabilized cells) into osmotic solution.

In Figs 2(b1 and b2) the vitamin C loss for non-treated, blanched, OD and PEF pretreated OD kiwifruit samples stored at isothermal temperature conditions T=-15 °C, and non-isothermal temperature conditions T_{eff}=-13.3 °C (representatively) are shown. Vitamin C content change with storage time was mathematically described by a first-order reaction as follows (Eq. 3).

$$\frac{C_{vitC}}{C_{vitC0}} = exp(-k_{vitC}t) \tag{3}$$

where, C_{vitC} is the vitamin C concentration (mg/g initial dry mass) at time t, $C_{vitC,0}$ is the initial concentration of vitamin C, and k_{vitC} is the reaction rate constant. The temperature dependence of colour deterioration was described by the Arrhenius equation (Eq. 2). OD and/or PEF pretreated kiwifruit samples gave the lowest k_{vitC} values, whilst PEF, blanched and non-treated samples gave the highest ones. Activation energy E_a values, calculated by Eq. 2, varied from 59.45 to 67.78 kJ/mol for the pretreated samples. The larger E_a value, 81.16 kJ/mol calculated for the non-treated samples indicates the rate of vitamin C loss has a higher dependence on temperature change compared to pretreated samples (Table 4).

Sensory attributes

The descriptive tests for kiwifruit samples showed significant effects of the osmotic pretreatment. Based on results, blanched and PEF pretreated frozen samples compared to non-treated samples suffered from detrimental texture, colour and flavour deterioration at zero storage times and during their storage. The tissue integrity was well retained for osmotically pretreated samples, followed by PEF pretreated samples (PEFOD1 and PEFOD2). PEF pretreated samples were characterized by a colour change (green to yellow). Flavour and taste of all pretreated samples were judged as “pleasant and acceptable” despite differing from the fresh kiwifruit characteristics. The pretreated samples were preferred in all attributes, including taste, and they showed

Table 3: Estimated kinetic parameters [k_{colour}, k_{ref} (days^{-1}) at T_{ref}=-18 °C, E_a, kJ/mol] for colour change of non, blanched, OD, PEF, PEFOD1, and PEFOD2 pretreated kiwifruit slices stored at constant and dynamic temperature conditions

Colour change	**k(d^{-1})**				***Ea*** **(kJ/mol)**	$\boldsymbol{k_{ref}}$ **(d^{-1})**	**Adjusted R^2**	**RMSE**
	-5 °C	**-8 °C**	**-15 °C**	**Dynamic* (*Teff*, °C=-13.3 °C)**				
Non	0.044±0.0061^{ab}	0.012±0.0012^{ab}	0.0080±0.00082^{a}	0.009 (0.988) (Exp.) 0.009 (Fitted)	97.77±30.77^{a}	0.0040±0.0015^{a}	0.8197	0.1430
Blanched	0.028±0.0081^{bc}	0.012±0.0012^{ab}	0.0070±0.00072^{a}	0.008 (0.955) (Exp.) 0.008 (Fitted)	97.43±15.32^{a}	0.0043±0.0008^{a}	0.9486	0.0354
OD	0.031±0.0061^{bc}	0.011±0.0012^{ab}	0.0060±0.00082^{a}	0.006 (0.995) (Exp.) 0.007 (Fitted)	94.24±14.86^{a}	0.0033±0.0008^{a}	0.9486	0.0354
PEF	0.054±0.0041^{a}	0.024±0.0122^{a}	0.0140±0.0022^{bc}	0.010 (0.986) (Exp.) 0.017 (Fitted)	77.49±9.77^{a}	0.0087±0.0016^{b}	0.9680	0.0148
PEFOD1	0.020±0.0071^{c}	0.009±0.0012^{b}	0.0040±0.00082^{cd}	0.006 (0.952) (Exp.) 0.005 (Fitted)	92.52±0.57^{a}	0.0024±0.0001^{a}	0.9999	5.5×10^{-5}
PEFOD2	0.015±0.0041^{c}	0.006±0.00512^{b}	0.0030±0.00032^{d}	0.004 (0.985) (Exp.) 0.004 (Fitted)	92.43±8.13^{a}	0.0017±0.0003^{a}	0.9835	0.0107

± represent standard error based on the statistical variation in the kinetic parameters of models – regression analysis. Different superscript letters indicate significantly different means ($p<0.05$) within a column (differences between treatments). Different superscript numbers indicate significantly different means ($p<0.05$) within a row (differences between temperatures)

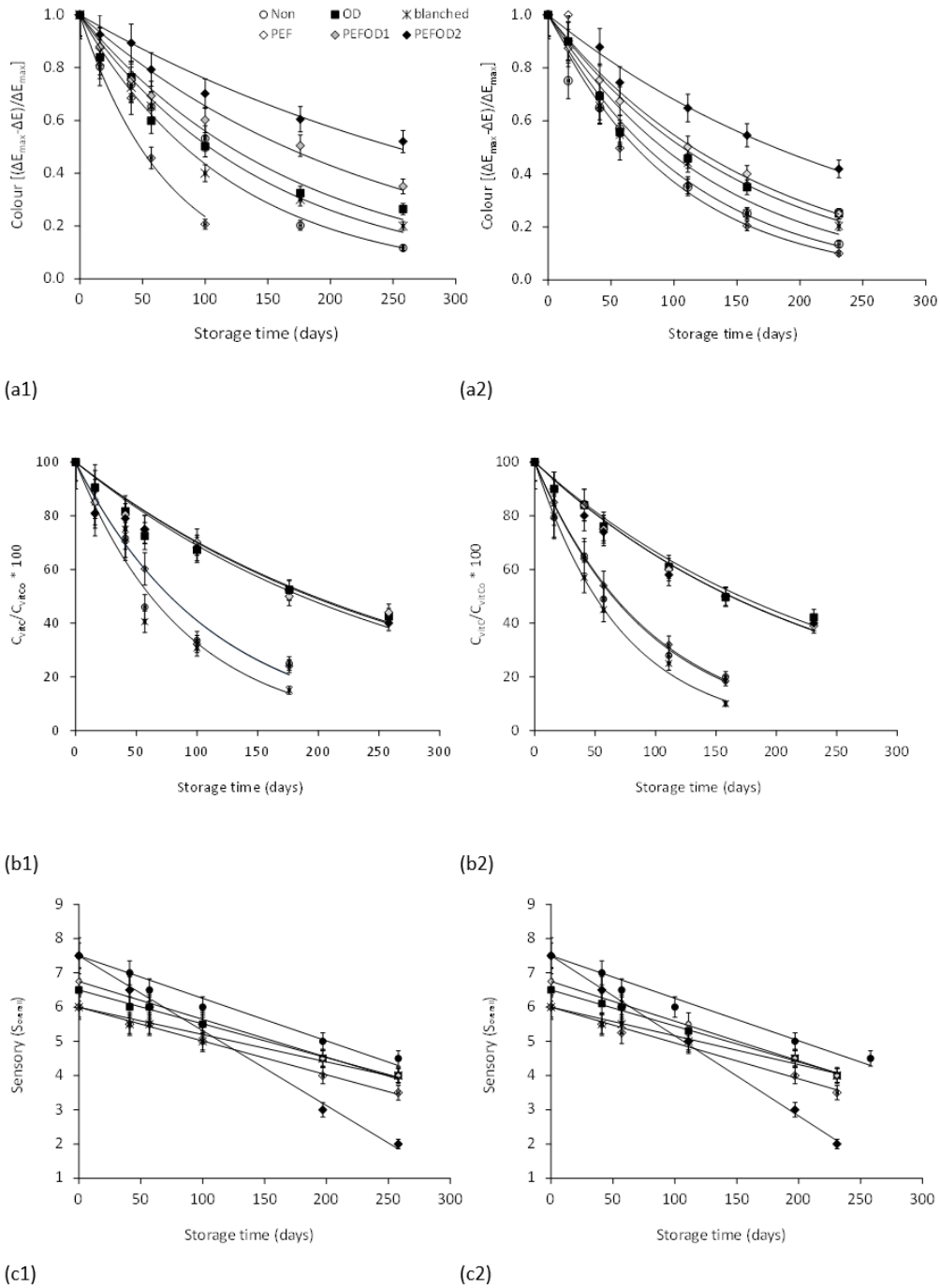
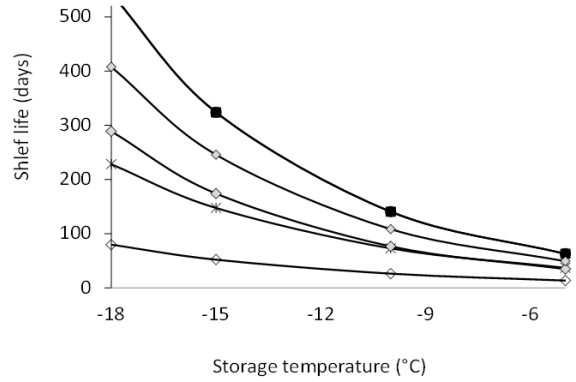
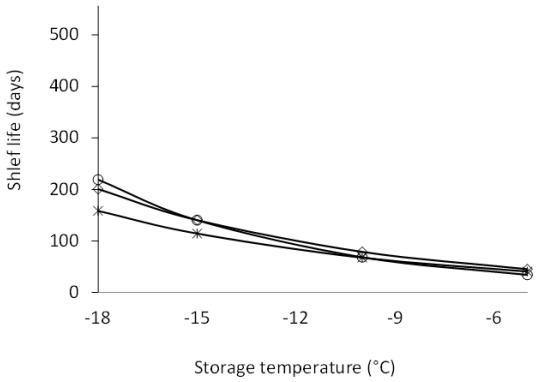


Figure 2: (a) Colour [(ΔE_{max}-ΔE)/(ΔE_{max})], (b) vitamin C (C_{vitC}, mg/100 g), and (c) sensory quality (scoring for overall quality, $S_{overall}$) versus time t for non pretreated, blanched, OD-, PEFOD1-, PEFOD2-pretreated kiwifruit slices stored at isothermal temperature conditions T=-15 ˚C (index 1), and nonisothermal conditions T_{eff}=-13.3 ˚C (index 2) (lines represent the first and zero order fit, respectively and error bars indicate the standard deviation from replicates)



(a)



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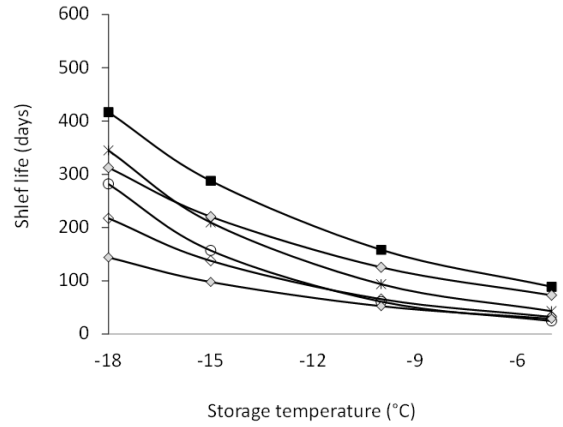

(c)

Figure 3: Shelf life (SL, d) non, blanched, OD, PEF, PEFOD1, and PEFOD2 pretreated kiwifruit slices stored at isothermal and non-isothermal temperature conditions based on (a) colour ((ΔE_{final}= 5), (b) vitamin C ($C_{vitC,final}$=0.30*($C_{vitC,0}$, samples: non, blanched and PEF pretreated) and (c) sensory evaluation (($S_{overall,final}$=5.0/9.0)

Table 4: Estimated kinetic parameters [k_{vitC}, k_{ref} (days^{-1}) at T_{ref}=-18 °C, E_a, kJ/mol] for vitamin C loss of non, blanched, OD, PEF, PEFOD1, and PEFOD2 pretreated kiwifruit slices stored at constant and dynamic temperature conditions

Vitamin C loss	**k(d^{-1})**				**E_a (kJ/mol)**	**k_{ref} (d^{-1})**	**Adjusted R^2**	**RMSE**
	-5 °C	**-10 °C**	**-15 °C**	**Dynamic* (T$_{eff}$, °C=-13.3 °C)**				
Non	0.0370±0.00301^{a}	0.0160±0.00082^{a}	0.0090±0.00203^{a}	0.011 (0.999) (Exp.) 0.011 (Fitted)	81.16±9.48^{a}	0.0055±0.0010^{a}	0.9723	0.0140
Blanched	0.0310±0.00501^{ab}	0.0160±0.00202^{a}	0.0110±0.00202^{a}	0.014 (0.990) (Exp.) 0.013 (Fitted)	59.45±10.17^{a}	0.0076±0.0014^{a}	0.9116	0.0308
OD	0.0120±0.00201^{c}	0.0060±0.00012^{b}	0.0040±0.00042^{b}	0.004 (0.977) (Exp.) 0.005 (Fitted)	63.05±3.87^{a}	0.0027±0.0005^{b}	0.9862	0.0053
PEF	0.0280±0.00201^{b}	0.0140±0.00102^{a}	0.0090±0.00013^{a}	0.011 (0.999) (Exp.) 0.010 (Fitted)	65.15±9.05^{a}	0.0060±0.0010^{a}	9621	0.0124
PEFOD1	0.0130±0.00041^{c}	0.0050±0.00042^{b}	0.0040±0.00023^{b}	0.004 (0.990) (Exp.) 0.004 (Fitted)	67.78±15.18^{a}	0.0024±0.0009^{b}	0.7578	0.0949
PEFOD2	0.0120±0.00151^{c}	0.0070±0.00052^{b}	0.0040±0.00063^{b}	0.004 (0.974) (Exp.) 0.005 (Fitted)	63.15±0.01^{a}	0.0028±0.0001^{b}	0.9972	0.0010

± represent standard error based on the statistical variation in the kinetic parameters of models - regression analysis. Different superscript letters indicate significantly different means ($p<0.05$) within a column (differences between treatments). Different superscript numbers indicate significantly different means ($p<0.05$) within a row (differences between temperatures)

an increased stability with storage.
The scores for the selected sensory properties (colour, texture, flavour-data not shown; Respective values, measured at zero storage time for all sensory properties were presented in Table 2) as well as overall sensory quality during storage were kinetically modelled using zero-order reaction kinetics (Eq. 4).

$$S = S_0 - k_{sensory}t \quad (4)$$

where, S and So are the scores for sensory attributes evaluated at time t and zero respectively (Table 2) and $k_{sensory}$ the apparent rate of quality deterioration based on sensory evaluation (days^{-1}). The temperature dependence of colour deterioration was described by the Arrhenius equation (Eq. 2).
In Figs 2(c1 and c2) the scores for the overall sensory quality versus time for non-treated, blanched, OD and PEF pretreated OD kiwifruit samples stored at isothermal temperature conditions T=-15 °C, and non-isothermal temperature conditions T_{eff}=-13.3 °C are representatively shown. Kiwifruit samples gave similar $k_{sensory}$ values. However, scores at time zero S_0 were higher for pretreated samples [7.5(OD), 7.25(PEFOD2), 7.0(PEFOD1), 6.5(non-treated), 6.0(blanched and PEF)]. E_a values varied from 79.39(PEFOD1 sample) to 109.32(blanched sample) kJ/mol (Table 5).

3.3 Shelf life determination

The end of shelf life for all samples was correlated to colour change, ΔE_{final}= 5, vitamin C retention, $C_{vitC,final}$=0.30*$C_{vitC,0}$, and sensory scoring $S_{overall,final}$= 5.0/9.0. Based on these limits, indicating the end of shelf-life, the Arrhenius equation (Eq. 2) can be combined with Equations 1, 3, and 4 to develop predictive models that allow shelf-life determination at any temperature, based either on colour change (Eq. 1) or vitamin C retention (Eq. 3) or sensory scoring (Eq. 4). The shelf life values calculated for temperatures from -5 to -18 °C are shown in Figs. 3(a, b and c). The rejection of non-treated, PEF and PEFOD1 samples was mainly due to instrumentally measured colour loss. The instrumentally measured colour loss was well correlated with the sensory evaluation of colour, especially at low temperatures <-15 °C (data not shown). The rejection of OD and PEFOD2 samples was due to the sensory scoring for the overall quality of the final kiwifruit product, and taking into account the scoring for all studied sensory characteristics. As far as the blanched kiwifruit samples were concerned, the shelf life determining criteria was found to be the vitamin C retention. Based on the above mentioned criteria for each sample, the shelf life at the reference temperature of -18 °C could be calculated: 79 days for PEF, 158 days for blanched, 173 days for non-treated, 211 days for PEFOD2, 288 days for PEFOD1, 378 days for OD samples. The respective shelf life values at -10 °C were: 26 days for PEF, 40 days for blanched, 44 days for non-treated, 76 days for PEFOD1, 125 days for PEFOD2, and 158 days for OD samples. According to results, the application of osmotic dehydration and PEF pretreatments significantly increased the stability of kiwifruit slices during their frozen storage.

3.4 Validation of the predictive models in dynamic conditions

To demonstrate the integrated effect of the temperature variability on quality, the term, effective temperature T_{eff} is introduced (T_{eff}= -13.3 °C; calculated in this study) as previously detailed in Giannakourou and Taoukis (2003). The models developed from isothermal experiments were validated at dynamic conditions (fluctuating temperatures from -8 to -18 °C). The results of validation tests showed that the developed kinetic models can be adequately used at variable temperature conditions, within the same range and duration of temperature fluctuations, to predict the kiwifruit quality and consequently the remaining shelf life. For this purpose, the quality change rates derived from the models were compared to the respective experimental values [Figs. 2(a2, b2 and c2)]. The models predicted satisfactory results, as the relative error (RE) values, calculated using Eq. 5:

$$\%RE = \frac{k_{exp} - k_{fitted}}{k_{exp}} \times 100 \quad (5)$$

Table 5: Estimated kinetic parameters [$k_{sensory}$, k_{ref} (days^{-1}) at T_{ref}=18 °C, E_a, kJ/mol] for sensory quality loss of non, blanched, OD, PEF, PEFOD1, and PEFOD2 pretreated kiwifruit slices stored at constant and dynamic temperature conditions

Sensory quality loss	**k(d^{-1})**				***E*$_a$ (kJ/mol)**	***k*$_{ref}$ (d^{-1})**	**Adjusted R^2**	**RMSE**
	-5 °C	**-10 °C**	**-15 °C**	**Dynamic* (T$_{eff}$, °C=-13.3 °C)**				
Non	0.047±0.0041^{a}	0.0143±0.000612^{a}	0.0097±0.00032^{ab}	0.012 (0.971) (Exp.) 0.011 (Fitted)	90.17±17.53^{a}	0.0052±0.0019^{ab}	0.8295	0.1153
Blanched	0.054±0.0021^{b}	0.0131±0.00062^{a}	0.0080±0.00043^{a}	0.0084 (0.979) (Exp.) 0.009 (Fitted	109.32±31.05^{a}	0.0038±0.0013^{a}	0.8439	0.1534
OD	0.045±0.0011^{b}	0.016±0.0022^{a}	0.0124±0.00073^{c}	0.012 (0.989) (Exp.) 0.014 (Fitted)	84.10±13.87^{a}	0.0066±0.0028^{ab}	0.7690	0.1076
PEF	0.045±0.0071^{b}	0.0155±0.00042^{a}	0.0098±0.00032^{b}	0.010 (0.989) (Exp.) 0.012 (Fitted)	87.26±15.90^{a}	0.0055±0.0017^{ab}	0.8897	0.0675
PEFOD1	0.042±0.0011^{b}	0.0140±0.00052^{a}	0.0111±0.00073^{c}	0.010 (0.989) (Exp.) 0.012 (Fitted)	79.39±18.08^{a}	0.0059±0.0023^{ab}	0.7377	0.1325
PEFOD2	0.112±0.0091^{c}	0.036±0.0042^{b}	0.022±0.0012^{d}	0.023 (0.997) (Exp.) 0.026 (Fitted)	93.31±20.07^{a}	0.0118±0.0038^{b}	0.8915	0.0756

± represent standard error based on the statistical variation in the kinetic parameters of models – regression analysis. Different superscript letters indicate significantly different means ($p<0.05$) within a column (differences between treatments). Different superscript numbers indicate significantly different means ($p<0.05$) within a row (differences between temperatures)

where within the 20% limit of acceptance for sensory evaluation (%RE:-10.0-+4.2; all samples), vitamin C loss (%RE:-20.0-+9.8; all samples) and instrumentally measured colour change (%RE:-20.0-+11.8; apart from PEF samples where %RE>20)., These models adequately predict the quality deterioration of frozen kiwifruit slices.

4 Conclusions

PEF and OD could lead to adequate mass exchange, adequate decrease of a_w and improved quality characteristics of processed kiwifruit. The optimum processing conditions were OD: T_{OD}= 35 °C, t_{OD}= 60 min, and PEFOD1: E_{PEF}= 1.8 kV/cm (250p), T_{OD}= 35 °C, t_{OD}= 30 min to have a_w decrease from 0.95 to 0.92, acceptable colour, increased firmness, low drip loss and high vitamin C content. Increase of OD time from 30 to 60 min for PEF pretreated kiwifruit slices did not lead to better final product quality and sensory characteristics. The shelf life calculation was based on different criteria for each sample. The shelf life determining criteria were found to be colour change (expressed by ΔE_{final}= 5) for blanched, PEF as well as PEFOD1 samples, sensory quality (expressed by the score given for the overall quality and acceptability) for OD and PEFOD2 samples, and vitamin retention for blanched samples (C_{vitC}=0.30$C_{vitC,0}$), respectively. Based on this study, it could be suggested that (i) combined PEF and OD may have a potential as a preprocessing step in the manufacture of sensitive frozen fruit products, and (ii) the application of PEF alone as a prefreezing step may not be recommended.

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Optimization of Osmotic Dehydration of Chestnut (*Castanea sativa* Mill.) Slices Using Response Surface Methodology

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Abstract

Osmotic dehydration of chestnut slices in sucrose was optimized for the first time by Response Surface Methodology (RSM). Experiments were planned according to a three-factor central composite design (α=1.68), studying the influence of sucrose concentration, temperature and time, on the following parameters: volume ratio, water activity, color variation, weight reduction, solids gain, water loss and normalized moisture content, as well as total moisture, ash and fat contents.
The experimental data was adequately fitted into second-order polynomial models with coefficients of determination (R^2) from 0.716 to 0.976, adjusted-R^2 values from 0.460 to 0.954, and non-significant lacks of fit. The optimal osmotic dehydration process conditions for maximum water loss and minimum solids gain and color variation were determined by the "Response Optimizer" option: 83% sucrose concentration, 20 °C and 9.2 hours. Thus, the best operational conditions corresponded to high sugar concentration and low temperature, improving energy saving and decreasing the process costs.

Keywords: Chestnut; *Castanea sativa* Miller; Osmotic Dehydration; Response Surface Methodology; Physicochemical properties

1 Introduction

Chestnut production is of great economic importance for some countries. In 2012, the main world producer was China, representing about 82.5% of the total production, followed by Europe, with 6.4% (Food and Agriculture Organization of the United Nations, 2014). Portugal accounts for about 15% of the European production (Food and Agriculture Organization of the United Nations, 2014), with the North area, mainly Trás-os-Montes region, contributing to 80.5% of the national production and representing 87.4% of chestnut national production area (30586 ha) (Instituto Nacional de Estatística, 2014).
Being a seasonal product, some problems may arise during chestnuts' storage, compromising its availability and quality throughout the year. One way to mitigate this problem is to use different post-harvest technologies such as low temperature storage or convection drying. However, a promising technology to preserve perishable

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Nomenclature

RSM	Response Surface Methodology
OD	Osmotic dehydration
WR	Weight Reduction (g g^{-1} fresh chestnut)
SG	Solids Gain (g g^{-1} fresh chestnut)
WL	Water Loss (g g^{-1} fresh chestnut)
NMC	Normalized Moisture Content
a_w	Water activity
M_0	Sample mass before osmotic dehydration (g)
M	Sample mass after osmotic dehydration (g)
m_0	Initial mass of the solids in chestnut sample (g)
m	Mass of the solids in chestnut sample after osmotic dehydration (g)
W, L, t	Axial dimensions (Width, Length and thickness, respectively (mm))
L^*, a^*, b^*	Color parameters (CIELab)
X	Moisture ratio
CCD	Central Composite Design

items and make them available to distant regions of a production area throughout the year is osmotic dehydration (OD) which is a simple and a low cost method (Rastogi, Raghavarao, Niranjan, & Knorr, 2002; Shi & Le Maguer, 2002). Furthermore, with this OD technology an interesting chestnut snack may be produced. As chestnut is naturally gluten-free, snacks of this nut may be a good option for celiac patients.

Osmotic dehydration occurs by immersion of the food in osmotic solutions. During this process, the cellular structure of the food allows water loss, while a gain of solute occurs simultaneously (Rastogi, Raghavarao, & Niranjan, 1997). Both mass flows are affected by diverse factors, including the nature of the food and its geometry, the composition and concentration of the osmotic solution, and several methodological parameters such as temperature, contact time and agitation (Kaymak-Ertekin & Sultanoglu, 2000; Singh, Kumar, & Gupta, 2007; Tonon, Baroni, & Hubinger, 2007).

Diverse statistical and mathematical techniques have been applied to optimize and improve the development of these processes, combining and analyzing the role of different factors such as temperature, solute concentration and time, while minimizing analyses' error and the amount of necessary experiments. Response Surface Methodology (RSM) is one of these techniques, aiming to optimize response-variables of interest by studying the influence of a defined number of independent variables. Besides having the advantage of analyzing the effects of independent variables, this methodology generates a mathematical model that describes the chemical or biochemical processes under study (Anjum, Tasadduq, & AlSultan, 1997). In particular, RSM has been applied to osmotic dehydration studies of some fruits and vegetables, including apples (Azarpazhooh & Ramaswamy, 2012), bananas (Atares, Gallagher, & Oliveira, 2011), carrots (Changrue, Orsat, Raghavan, & Lyew, 2008), cherry tomatoes (Derossi, Severini, Del Mastro, & De Pilli, 2015), figs (Vasconcelos, Andrade, Maciel, Guerra, & Vasconcelos, 2012), green peppers (Ozdemir, Ozen, Dock, & Floros, 2008), kiwi (Cao, Zhang, Mujumdar, Du, & Sun, 2006), peaches (Yadav, Yadav, & Jatain, 2012), plums (Koocheki & Azarpazhooh, 2010) and strawberries (Changrue et al., 2008). Generally, three factors are studied, namely, temperature, time and concentration of the osmotic solution (Azarpazhooh & Ramaswamy, 2012; Cao et al., 2006; Changrue et al., 2008; Koocheki & Azarpazhooh, 2010; Vasconcelos et al., 2012; Yadav et al., 2012).

Regarding chestnut, most studies of OD have

been focused on whole fruits of Spanish chestnut varieties, and primarily on diverse osmotic agents and temperatures (Chenlo, Moreira, Fernández-Herrero, & Vázquez, 2006b, 2006a, 2007; Moreira, Chenlo, Chaguri, & Oliveira, 2007; Moreira, Chenlo, Chaguri, & Fernandes, 2008; Moreira, Chenlo, Chaguri, & Vazquez, 2011; Moreira, Chenlo, Chaguri, & Mayor, 2011). The osmotic agents studied included sodium chloride (17 to 26.5%), glucose and sucrose (40 to 60%) at different concentrations and submitted to several temperatures in the range of 25 and 65 °C.
Nevertheless, none of these studies performed the optimization of the OD process taking into account several factors and responses simultaneously. Thus, the aims of our work were: i) to evaluate by RSM the role of the three main parameters, temperature, time and concentration of the osmotic solution (sucrose), in affecting some physicochemical properties of chestnut slices; and ii) to optimize these parameters for the industrial production of an interesting chestnut based snack in the near future. Sucrose was the first osmotic agent to be tested by RSM because it is more common to use this compound in OD processes of fruits than sodium chloride that can induce high blood pressure (Appel et al., 2012). Furthermore, sucrose is also cheaper than glucose.

2 Materials and Methods

2.1 Plant material

Castanea sativa Miller (European chestnut) fruits, variety Longal, were acquired in Bragança (NE Portugal) in November 2013, and stored in cold chambers (4±1 °C) until the osmotic dehydration experiments were performed. Before doing these experiments, chestnuts were carefully unshelled and sliced (approximately 4-6 mm of thickness).

2.2 Osmotic Dehydration (OD)

The osmotic solutions were prepared with food-grade sucrose and potable water. The OD experiments were carried out in 1L beakers. For each condition, 70 g of fresh sliced chestnuts were added to 700 mL of sugar solution and mixed with a magnetic stirrer at 310 rpm in a temperature controlled water bath. At specific times, the dehydrated chestnut slices were removed from the solution, drained, and gently cleaned with absorbent paper to remove any sugar solution in excess. For each condition, the assays were performed in duplicate.
In order to adequately follow the OD kinetics, several parameters were analyzed, namely weight reduction (WR), solids gain (SG), water loss (WL) and normalized moisture content (NMC). These were determined according to the following equations (Eq. 1 to 4) (Lerici, Pinnavaia, Rosa, & Bartolucci, 1985):

$$WR = \frac{M_0 - M}{M_0} \tag{1}$$

$$SG = \frac{m - m_0}{M_0} \tag{2}$$

$$WL = WR + SG \tag{3}$$

$$NMC = \frac{1 - \frac{m}{M}}{1 - \frac{m_0}{M_0}} = \frac{X}{X_0} \tag{4}$$

where M_0 and M represents the total mass of sample before and after OD, respectively; m_0 and m are the mass of the solids before and after OD, respectively; and X_0 and X correspond to the moisture contents of the samples before and after the OD treatment, respectively.

2.3 Physicochemical characterization

Volume

The three axial dimensions (Width, W; Length, L; and thickness, h) of all chestnut slices were measured using a digital caliper, before and after the OD experiments. Volume was calculated by the following equation:

$$V = Area\ of\ the\ elipse \times h = \pi \times \frac{W}{2} \times \frac{L}{2} \times h \tag{5}$$

The volume was calculated by considering the dimensions before (V_0) and after (V) OD, enabling the calculation of the volume ratio ($\frac{V}{V_0}$).

Color

Color analyses were carried out on chestnut slices before and after being subjected to OD. A Minolta CR-400 colorimeter was used, in CIE*Lab* color space, through the coordinates *L**, *a** and *b**, using the Spectra Magic Nx software (version CM-S100W 2.03.0006, Konica Minolta Company, Osaka, Japan), as already described in previous work (Delgado, Pereira, Baptista, Casal, & Ramalhosa, 2014).
In order to analyze the color changes due to the OD process, the total color difference (ΔE^*) was calculated according to:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (6)$$

All color determinations were made on 20 slices, before (the color of fresh chestnuts was considered as reference) and after the OD process, each time in duplicate.

2.4 Water activity (a_w), moisture, ash and crude fat contents

Water activity was determined by means of a LabSwift- a_w instrument (Novasina AG, Lachen, Switzerland). The instrument was calibrated with three water activity standards, namely 11% (a_w =0.112), 58% (a_w =0.587) and 84% (a_w =0.845).
Moisture, ash and crude fat contents were determined using standard procedures (Association of Official Analytical Chemists, 1995) in duplicate on samples of each osmotic dehydration assays (n=4). All reagents were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Crude fat was determined on 5 g of sample, using petroleum ether for 24 h in a Soxhlet apparatus (P Selecta, Abrera, Barcelona). Moisture was determined on 5 g of sample at 105 °C in an oven (Memmert UNB 500, Schwabach, Germany), until constant weight, while total ash was obtained by incineration at 550 °C (Lenton Thermal Designs Ltd, Hope Valley, United Kingdom). The ash and crude fat contents were expressed on g 100 g^{-1} dry matter.

2.5 Experimental design and statistical analysis

In order to determine the effect of selected operational parameters in the above mentioned chestnut properties, as well as to establish the best conditions to perform OD of chestnut slices, the Response Surface Methodology (RSM) was used through Minitab® software (USA). A one block with an α-value equal to 1.68 and a central composite design (CCD) was constructed to investigate the influence of the following three independent factors: sucrose concentration, temperature and time. The response variables were a_w, $\frac{V}{V_0}$, ΔE^*, *WR*, *SG*, *WL*, *NMC*, moisture, ash and crude fat contents. Each variable was coded at five levels: -1.68, -1, 0, +1 and +1.68. The correspondence between coded and uncoded variables is indicated in Table 1. Each point of the CCD was carried out in duplicate.
The relationship found between the dependent variables (a_w, $\frac{V}{V_0}$, ΔE^*, *WR*, *SG*, *WL*, *NMC*, moisture, total ash and crude fat contents) and the operational variables was established by the following second order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i>j}^{k} \beta_{ij} X_i X_j \quad (7)$$

where Y is the predicted dependent variable; β_0 is a constant that fixes the response at the central point of the experiment (constant); β_i are the regression coefficients for the linear effect terms; β_{ij} are the quadratic effect terms; β_{ii} are the interaction effect terms of variables i and j; X_i and X_j are independent variables (X_1 – sucrose concentration; X_2 – temperature; X_3 – time); and k the total number of independent factors (k=3).
Twenty experiments, with six replications in the central point (Experiments 1, 5, 14, 15, 19 and 20), were performed (Table 2). In order to limit the influence of systematic errors, the sequence of the experiments was randomized. The experiments performed in the central point allowed an estimate of experimental error, whereas the other experiments allowed the calculation of the regression coefficients of the model. The adequacy of the models was assessed through the coefficient of determination (R^2), the adjusted-R^2 (adj-R^2) and the analysis of variance (ANOVA).

Table 1: Independent variables and their coded and uncoded values for optimization

Coded value	Sucrose concentration (%, w/v) X_1	Temperature (°C) X_2	Time (h) X_3
-1.68	53	20	0.8
-1	60	30	2.5
0	70	45	5.0
1	80	60	7.5
1.68	87	70	9.2

Furthermore, the lack of fit of the models was used to check the quality of second-order polynomial models. If the p-value of the lack of fit is less than 0.05, evidence exists that the model does not accurately fit the data.
In order to obtain useful information about the behavior of the system within the experimental design, response surface plots were generated for different interactions of any two independent variables, while holding the value of a third variable constant. Furthermore, at the end an optimization of the osmotic dehydration process was performed by using the "Response Optimizer" option of Minitab® software, in order to define the levels of the independent variables that would give maximum water loss and the lowest solids gain and ΔE^* (these response-variables are very important to achieve a product that will be well accepted by consumers). The optimization procedure picks several starting points from which to begin searching for the optimal factor settings, being displayed as the global solution, which corresponds to the "best" combination of factor settings for achieving the desired responses. In more detail, the optimization is accomplished by:

- obtaining the individual desirability for each response. The individual desirability will be closer to one, if the response is closer to the defined target (in the present work, our goal was to maximize water loss and minimize solids gain and ΔE^*);

- combining the individual desirabilities to obtain the combined or composite desirability. This measure is the weighted geometric mean of the individual desirabilities for the responses. In the present work, all individual desirabilities were equally important, so they had the same weight. In the present work the weight used was 1 (we placed equal emphasis on the target and the bounds). The composite desirability has a range of zero to one. One represents the ideal case, while zero indicates that one or more responses are not inside their acceptable limits;

- at the end, Minitab employs a reduced gradient algorithm with multiple starting points that maximizes the composite desirability to determine the numerical optimal solution. At the end, the optimal input variable settings were tested to confirm if the optimal response was observed.

3 Results and Discussion

The coefficients of the second-order response surface models relating response variables with sugar concentration, temperature and time are described in Table 3. For the volume ratio and a_w, the model results are not shown because the p-value of the lack of fit was lower than 0.05 (0.001 and 0.003, respectively), suggesting that the models developed did not represent accurately the observed results. A good fit between the experimental data and the predicted values by the model is obtained when high R^2 and adj-R^2 (near 1) are achieved together with a p-value for the lack of fit higher than 0.05, indicating that the variation between samples was only due to the factors selected for the model and

the pure error (Minitab® software). Nevertheless, $\frac{V}{V_0}$ varied between 0.841 and 1.39 (Table 2) showing that in some situations chestnuts (slices) submitted to OD may shrink or increase volume due to solution absorption. Concerning a_w (Table 2), this parameter varied between 0.849 and 0.935, a range that is normally encountered for dried foods or with high concentrations of solutes (Pereda et al., 2005; Moreira, Chenlo, Torres, & Vázquez, 2007).

3.1 Color variation (ΔE^*)

Values of color variation predicted by the mathematical model were similar to the experimental data (Table 2), yielding a good fit with a R^2 of 0.976 and an adj-R^2 of 0.954 (Table 3), meaning that the experimental data may be predicted with great accuracy. Moreover, the model was good because the lack of fit was non-significant (p=0.717). Concerning the linear model coefficients, temperature and time were found to be significant model terms on color variation of chestnut slices, whereas the sucrose concentration was not a significant model term. This can be a direct consequence of Maillard reactions taking place when temperature and time increase. Regarding the quadratic terms, the temperature was the only parameter that had a significant effect ($p<0.05$). The results also showed that the interaction between temperature and time was significant for ΔE^*, yielding the following recalculated model taking into account only the significant terms:

$$\Delta E^* = 10.2 + 4.49X_2 + 2.02X_3 + 1.27X_2^2 + 0.866X_2X_3 \quad (8)$$

Furthermore, temperature had a higher effect on ΔE^* than time due to its higher linear coefficient (4.49 *versus* 2.02). In fact, as shown in Table 2, the maximum of the color variation was 21.7 at experiment 13 performed at the highest temperature (70 °C). Fig. 1 shows the effect of temperature and time on chestnut slices color variation for a sucrose concentration of 70%. At low temperatures, time had little effect on color variation, which remained quite low. On contrary, the highest variation in ΔE^* occurred when the highest temperature and time were applied. These results showed that, in some situations, an OD process may change chestnut slices color, with ΔE^* values higher than 12, an indicative value referred to by Cecchini, Contini, Massantini, Monarca, and Moscetti (2011). Color variation might be a negative point because color is one of the most important parameters for consumers' acceptance (Andrés-Bello, Barreto-Palacios, García-Segovia, Mir-Bel, & Martínez-Monzó, 2013). These color changes should be mostly due to non-enzymatic browning reactions, namely Maillard and caramelization reactions that are favored by high temperatures (Ajandouz, Desseaux, Tazi, & Puigserver, 2008). On the other hand, enzymatic browning, due to polyphenoloxidase activity, might be difficult to develop due to the high ionic strength of the medium.

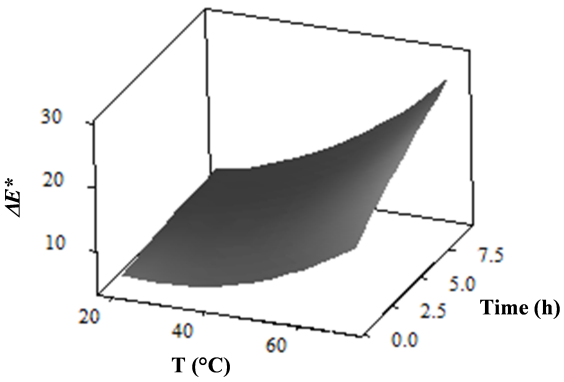

Figure 1: Response Surface plot for ΔE^* as a function of T (°C) and time (h) at sucrose concentration of 70%

3.2 Moisture content

By analyzing Table 2, the values of moisture content predicted by the model were also in good agreement with the experimental data, yielding a good R^2 (0.872), an adj-R^2 of 0.756 and a non-significant lack of fit (p=0.094) (Table 3). The minimum and maximum moisture contents on osmotic dehydrated product were 11.4 and 36.3 g of water 100 g^{-1}, obtained with a sugar concentration of 80% (w/v), 60 °C and 7.5 h, and 60% of sucrose, 30 °C and 2.5 h, respectively. The

Table 2: CCD with experimental and predicted values for the studied properties

Experiments	Levels of Coded variables[a]			Experimental values[b,c,d]										Predicted values						
	X1	X_2	X_3	V/V_0	a_w	ΔE^*	***Moisture***	***Ash***	***Fat***	***WR***	***SG***	***WL***	***NMC***	ΔE^*	***Moisture***	***Ash***	***Fat***	***WR***	***SG***	***WL***	***NMC***
1	0	0	0	0.905	0.897	11.0	18.7	1.42	1.70	0.062	0.131	0.193	0.508	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553
2	-1	1	-1	0.997	0.909	13.5	27.4	0.95	1.82	-0.009	0.101	0.092	0.744	13.2	27.2	1.07	1.62	-0.010	0.125	0.113	0.737
3	1	-1	-1	0.875	0.918	5.97	16.8	1.56	2.07	0.052	0.157	0.209	0.457	5.92	19.1	1.69	1.96	0.069	0.092	0.227	0.518
4	-1	-1	-1	0.900	0.933	6.27	36.3	1.47	2.15	0.005	0.002	0.007	0.987	5.92	33.7	1.52	1.96	0.026	0.034	0.058	0.914
5	0	0	0	0.845	0.896	11.0	21.2	1.04	1.80	0.061	0.109	0.170	0.575	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553
6	0	0	-1.68	1.06	0.921	6.10	33.1	1.59	1.84	0.032	0.017	0.049	0.898	6.91	25.0	1.51	1.88	0.030	0.086	0.170	0.677
7	1	1	1	0.976	0.856	17.1	11.4	0.78	1.17	0.090	0.175	0.265	0.308	18.9	7.13	0.842	1.36	0.102	0.248	0.283	0.192
8	1	-1	1	0.841	0.886	8.83	14.8	1.17	1.83	0.090	0.142	0.232	0.403	8.22	13.7	1.30	1.70	0.138	0.157	0.227	0.369
9	-1.68	0	0	1.39	0.935	10.1	34.9	1.15	1.54	-0.082	0.073	-0.010	0.948	10.3	32.6	1.04	1.66	-0.064	0.093	0.028	0.886
10	-1	-1	1	0.946	0.928	7.90	30.0	1.12	1.97	-0.015	0.079	0.064	0.814	8.22	28.2	1.13	1.70	-0.043	0.099	0.058	0.765
11	0	-1.68	0	0.922	0.908	5.63	33.4	1.63	1.89	0.005	0.031	0.036	0.908	6.31	25.9	1.57	1.95	0.060	0.065	0.123	0.702
12	0	0	1.68	0.959	0.878	13.1	17.0	0.89	1.55	0.006	0.193	0.199	0.462	13.7	15.8	0.855	1.44	0.030	0.196	0.170	0.429
13	0	1.68	0	0.992	0.872	21.7	16.3	0.84	1.64	-0.029	0.230	0.201	0.442	21.4	14.9	0.798	1.37	-0.0003	0.217	0.217	0.404
14	0	0	0	0.848	0.889	11.6	23.8	1.13	1.76	-0.020	0.145	0.125	0.647	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553
15	0	0	0	0.895	0.892	8.89	16.4	1.21	1.59	0.017	0.189	0.207	0.447	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553
16	1	1	-1	0.935	0.849	12.4	14.7	1.20	1.64	0.038	0.189	0.226	0.400	13.2	12.6	1.23	1.62	0.034	0.183	0.283	0.341
17	1.68	0	0	0.971	0.921	10.3	14.3	1.50	1.81	0.099	0.141	0.239	0.388	10.3	8.17	1.32	1.66	0.124	0.189	0.312	0.220
18	-1	1	1	1.17	0.857	20.2	28.8	0.61	1.46	-0.171	0.202	0.031	0.783	18.9	21.7	0.676	1.36	-0.078	0.190	0.113	0.588
19	0	0	0	0.900	0.889	9.99	19.9	1.09	1.65	0.021	0.153	0.174	0.539	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553
20	0	0	0	0.867	0.888	9.28	21.7	1.17	1.47	0.038	0.121	0.159	0.590	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553

[a] X_1 – Sugar concentration (%); X_2 – Temperature (°C); X_3 – Time (hours).
[b] Average of two values after performing osmotic dehydrations regarding *WR, SG, WL, NMC* and Moisture content.
[c] Average of four values after performing two osmotic dehydrations regarding a_w, ash and fat contents.
[d] V/V_0 – Volume ratio; ΔE^* - Total color difference; *WR* – Weight reduction; *SG* – Solids gain; *WL* – Water loss; *NMC* – Normalized moisture content.

Table 3: Coefficients of the second-order polynomial models (with all terms) and model statistical parameters

Term	ΔE^*		Moisture		Ash		Fat		*WR*		*SG*		*WL*		*NMC*	
	Coefficient	***p***	**Coefficient**	***p***	**Coefficient**	***p***	**Coefficient**	***p***	**Coefficient**	***p***	**Coefficient**	***p***	**Coefficient**	***p***	**Coefficient**	***p***
Constant	10.3	0.000	20.4	0.000	1.183	0.000	1.66	0.000	0.0296	0.032	0.141	0.000	0.170	0.000	0.553	0.000
X_1	-0.234	0.386	-7.28	0.000	0.0834	0.045	-0.0172	0.711	0.0560	0.000	0.0288	0.010	0.0848	0.000	-0.198	0.000
X_2	4.49	0.000	-3.26	0.011	-0.229	0.000	-0.172	0.003	-0.0178	0.049	0.0455	0.001	0.0277	0.037	-0.0885	0.011
X_3	2.02	0.000	-2.73	0.026	-0.195	0.000	-0.128	0.018	-0.00985	0.242	0.0326	0.005	0.0227	0.077	-0.0741	0.026
X_1^2	0.0381	0.883	0.984	0.357	0.0188	0.608	0.0147	0.745	-0.00622	0.439	-0.00844	0.365	-0.0146	0.221	0.0267	0.357
X_2^2	1.26	0.001	1.07	0.318	-0.0149	0.683	0.0478	0.303	-0.0134	0.112	-0.000023	0.998	-0.0134	0.259	0.0291	0.318
X_3^2	-0.185	0.479	1.14	0.291	-0.0129	0.724	0.0221	0.627	-0.00255	0.748	-0.00896	0.338	-0.0115	0.330	0.0308	0.291
X_1X_2	-0.595	0.109	0.562	0.690	0.0357	0.471	-0.0320	0.600	0.0195	0.089	-0.0196	0.131	-0.000169	0.991	0.0153	0.690
X_1X_3	-0.108	0.757	-0.0582	0.967	-0.0143	0.771	-0.0200	0.742	0.0341	0.008	-0.0258	0.056	0.00827	0.596	-0.00158	0.967
X_2X_3	0.866	0.028	0.798	0.573	-0.00249	0.959	-0.0534	0.386	-0.0160	0.152	0.00310	0.801	-0.0129	0.411	0.0217	0.573
Lack of fit		0.717		0.094		0.477		0.146		0.589		0.260		0.094		0.094
R^2	0.976		0.872		0.882		0.716		0.885		0.882		0.872		0.872	
Adj-R^2	0.954		0.756		0.775		0.460		0.781		0.775		0.757		0.756	

X_1 – Sugar concentration (%); X_2 – Temperature (°C); X_3 – Time (hours). When $p<0.05$ the coefficient is significant.
ΔE^* - Total color difference; *WR* – Weight reduction; *SG* – Solids gain; *WL* – Water loss; *NMC* – Normalized moisture content.

only significant terms of the model were the linear ones of all factors, namely sucrose concentration, temperature and time. Considering these significant terms, the recalculated model was as follows:

$$Moisture\ content = 20.5 - 7.28X_1 - 3.26X_2 - 2.73X_3 \quad (9)$$

Our results showed that temperature and time had a significant negative effect on moisture content (Fig. 2A). Indeed, these are the main determinants on any drying procedure, being in agreement with Noshad, Mohebbi, Shahidi, and Mortazavi (2012), who reported that an increase of temperature and time promoted a decrease of moisture content in quince. Chenlo et al. (2007) also observed that an increase in the temperature made the dehydration of chestnuts more intense, reaching lower values of moisture content. Furthermore, sucrose concentration also had an important role on moisture content (Fig. 2B), with the high sucrose concentrations yielding the lowest moisture contents. Indeed, sucrose concentration had a higher role than temperature and time, as observed by the highest coefficient of the former (-7.28 *versus* -3.26 and -2.73); however, as the temperature increased (Fig. 2B), moisture content also decreased slightly.

3.3 Ash content

The total ash values varied between 0.61 and 1.63 g 100 g^{-1} of dry matter (Table 2). The predicted values were in agreement with experimental data, yielding a good R^2 (0.882), an adj-R^2 of 0.775 and a non-significant lack of fit (p=0.477). Again, just the linear terms were significant, yielding the following recalculated model:

$$Ash\ content = 1.176 + 0.0834X_1 - 0.229X_2 - 0.195X_3 \quad (10)$$

Due to the highest absolute values of the coefficients, temperature and time had a higher effect on ash content than sucrose concentration. By analyzing Fig. 2C, we can observe an inverse behavior between ash content and temperature, with the same being verified with time. So, the highest ash content was found at the lowest temperature and time. When comparing the osmotically treated samples (0.61 to 1.63 g ash 100 g^{-1} of dry matter) with fresh chestnuts (1.76 g ash 100 g^{-1} of dry matter), the ash content decreased after the osmotic treatment. This could be due to the diffusion of sucrose to the interior of chestnut or to the output of water from the fruit to the osmotic medium, increasing the dry matter. Furthermore, other possible explanations are as follows: the removal of the shell and pellicle when slicing chestnuts may greatly increase the mass transfer rate of minerals due to the disappearance of adhesive substances and other components in the endocarp that protect the fruit (Moreira, Chenlo, Chaguri, & Oliveira, 2007) and can also be due to the high osmotic pressure at high sucrose concentrations that may break the cellular walls (Sacchetti, Gianotti, & Dalla Rosa, 2001), promoting the transfer of some minerals to the osmotic medium.

3.4 Crude Fat

The crude fat of chestnut slices contents varied between 1.17 and 2.15 g 100 g^{-1} of dry matter (Table 2). A reasonable R^2 of 0.716 and an adj-R^2 of 0.460 were obtained, showing that this model can reasonably predict the experimental data. As desired, the lack of fit was not significant (p=0.146) (Table 3). Only temperature and time were significant factors, yielding the following recalculated model:

$$Fat\ content = 1.72 - 0.172X_2 - 0.128X_3 \quad (11)$$

By the analysis of the response surface plot (Fig. 2D), we can predict that crude fat decreases as temperature and time increase. This decrease on fat content could be related with the output of fat to the osmotic medium due to the breakage of cell walls due to the high osmotic pressure (Sacchetti et al., 2001) and/or high temperatures and time. Diffusion of sucrose to the interior of chestnut or the output of water from the fruit to the osmotic medium might also have increased the dry matter, decreasing the fat content in dry basis. In fact, all OD products presented lower crude fat contents than fresh chestnut (3.3%, dry basis), supporting the hypotheses described above.

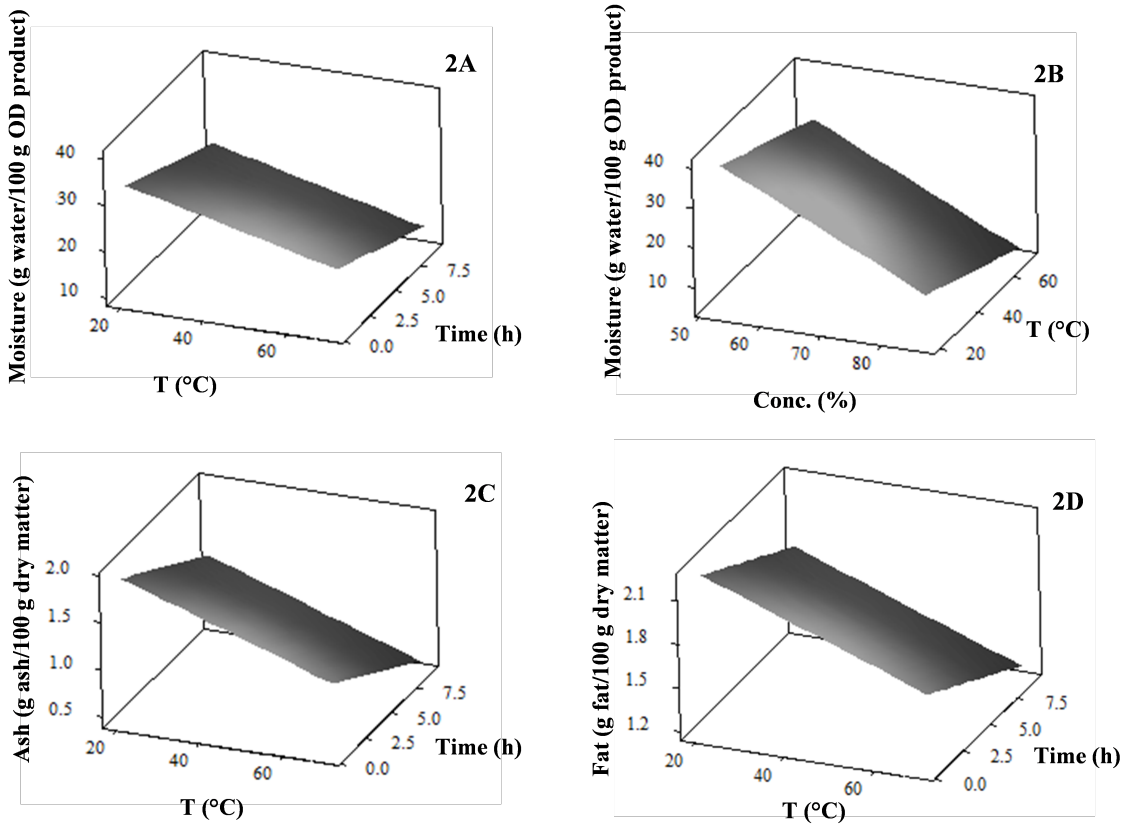

Figure 2: Response Surface plots: (A) Moisture content as a function of T (°C) and time (h) at sucrose concentration of 70%; (B) Moisture content as a function of sucrose conc. (%) and T (°C) at a contact time of 5.0 hours; (C) Ash content as a function of T (°C) and time (h) at sucrose concentration of 70%; (D) Fat content as a function of T (°C) and time (h) at sucrose concentration of 70%

3.5 Weight Reduction (*WR*)

For *WR*, the linear terms of sucrose concentration and temperature were found to be significant variables (Table 3), as well as the interaction between sucrose concentration and time. The R^2, adj- R^2 and p-value for the lack of fit of the predicted model were 0.885, 0.781 and 0.589, respectively, suggesting that the fitted model predicted well the experimental data. The recalculated model with only the significant terms is the following:

$$WR = 0.0145 + 0.0560X_1 - 0.0178X_2 + 0.0341X_1X_3 \quad (12)$$

The response surface plot of sugar concentration and temperature on *WR* is shown in Fig. 3A. The use of high temperature and high sucrose concentration would give the highest values of *WR*. When comparing the effect of the interaction of sugar concentration with time (Fig. 3B), we could conclude again that the highest *WR* were obtained at high sucrose concentrations and contact times. Chenlo et al. (2007) when performing osmotic dehydration of chestnut using glycerol solutions also stated that the *WR* increased with glycerol solution concentration. Moreover, our results are in agreement with previous studies performed on plums (Koocheki & Azarpazhooh, 2010) where an increase in WR

of the fruits is promoted by an increase of sucrose concentration and temperature. Nevertheless, at the highest sugar concentration, increasing the contact time from 2.5 to 7.5 h caused an increase of only 20% on *WR*. On the other hand, at the lowest sucrose concentration (60%), an increase of time did not cause an increase on *WR*. Furthermore, in some runs (ex. 2, 9, 10, 13, 14 and 18), negative *WR* values were observed due to the occurrence of a case-hardening effect that may induce some rigidity of the external cell layers and form a barrier to sucrose transfer, as suggested by Lee, Tham, and Wong (2014).

3.6 Solids Gain (*SG*)

Solids gain is an important factor to consider in OD, since it is intended to be the minimum as possible. Nevertheless, solids gain should be enough for preservation but not so high to induce changes of sensorial and nutritional properties. Only the model's linear terms were significant, and the lack of fit of the model was not significant (p=0.260). The R^2 and adj-R^2 were 0.882 and 0.775, respectively, showing a good adjustment between the experimental data and the values predicted by the model (all terms). When considering only the significant terms, the model obtained was:

$$SG = 0.129 + 0.0288X_1 + 0.0455X_2 + 0.0326X_3 \quad (13)$$

Fig. 3C represents the response surface plot, showing the role of sucrose concentration and temperature on *SG*. Generally, increasing temperature always favored the *SG* increase. At high temperatures, the effect of sugar concentration was almost negligible. Thus, the lowest *SG* would be obtained at low sucrose concentrations and temperature.

Considering the sucrose concentration and time (Fig. 3D), the lowest *SG* was obtained when applying low concentrations of sucrose and contact times, in line with observations by Chenlo et al. (2006a) for osmotic dehydration of whole chestnuts using glucose solutions. Nevertheless, even when high sucrose concentrations and times were used, *SG* was always lower than 0.25. These results were in agreement with Koocheki and Azarpazhooh (2010), and Uddin, Ainsworth, and Ibanoglu (2004), who also observed an increase in *SG* of plums and carrots when temperature, time and sucrose concentration increased during osmotic dehydration. This could be attributed to the increased mass transfer of sugar molecules due to possible membrane swelling/plasticizing effect, enhanced by the effect of temperature and contact time, which might increase cell membrane permeability to sucrose molecules (Lazarides, Gekas, & Mavroudis, 1997).

3.7 Water Loss (*WL*)

Beyond the *SG*, another main mass flux that is taking place is *WL*. During an OD process the water removal must be greater than solute acquisition (Chenlo et al., 2006a, 2007). A good fit between experimental and predicted values was obtained (Table 3), with a R^2 of 0.872 and an adj-R^2 of 0.757. The lack of fit of the model (all terms) was not significant (p=0.094). In terms of *WL*, only sugar concentration and temperature (linear terms) were significant, yielding the following recalculated model:

$$WL = 0.143 + 0.0848X_1 + 0.0277X_2 \quad (14)$$

By analyzing the response surface plot (Fig. 3E), high temperatures and sugar concentrations promoted *WL* that was equal to 0.30. Moreover, the role of sucrose concentration was more significant than temperature, with a higher coefficient for the former. These results were in agreement with Park, Bin, Brod, and Park (2002) and Uddin et al. (2004) who also observed an increase in *WL* with the increase in sucrose concentration. In general, *WL* in osmotic dehydrated chestnuts was favored by increasing sugar concentration and temperature. These results were in agreement with Chenlo et al. (2006a), Cao et al. (2006), Eren and Kaymak-Ertekin (2007), Koocheki and Azarpazhooh (2010), Rodrigues and Fernandes (2007) and Uddin et al. (2004) for chestnuts, kiwifruit, potato, plums, melons and carrots, respectively. Indeed, when temperature increases the water diffusion rate might also increase (Kim, 1990) and it will promote faster *WL* through swelling and plasticizing of the cell membrane, as well as by the better transfer characteristics

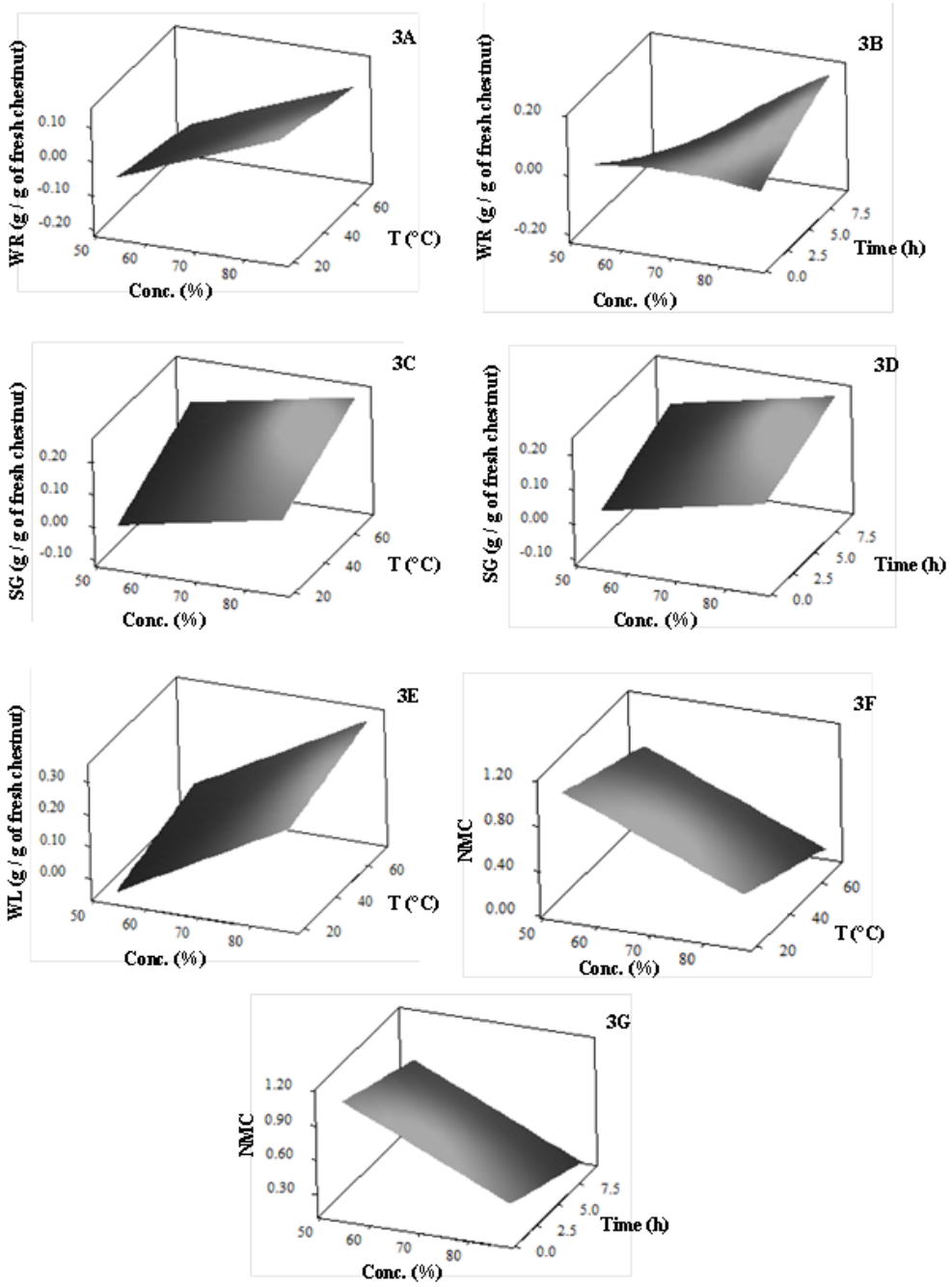

Figure 3: Response Surface plots: (A) *WR* as a function of sucrose concentration (%) and T (°C) for a time of 5.0 hours; (B) *WR* as a function of sucrose concentration (%) and time (h) at 45 °C; (C) *SG* as a function of sucrose concentration (%) and T (°C) for a time of 5.0 hours; (D) *SG* as a function of sucrose concentration (%) and time (h) at 45 °C; (E) *WL* as a function of sucrose concentration (%) and T (°C) for a time of 5.0 hours; (F) *NMC* as a function of sucrose concentration (%) and T (°C) for a time of 5.0 hours; (G) *NMC* as a function of sucrose concentration (%) and time (h) at 45 °C

of the water on product surface that might be due to the lower viscosity of the osmotic medium (Contreras & Smyrl, 1981).

3.8 Normalized Moisture Content (*NMC*)

In terms of *NMC*, the experimental and predicted values were similar (Table 2), were a R^2 of 0.872, an adj-R^2 of 0.756 and a non-significant lack of fit (p=0.094) were obtained (Table 3). The experimental values varied between 0.308 and 0.987 (Table 2), which were observed when applying simultaneously high and low sugar concentrations, temperature and time, respectively. After submitting the samples to 50% sugar concentration, at 30 °C for 2.5 h, the normalized moisture content of the samples (0.987) almost did not vary when compared to the beginning (1.0), suggesting a low water transfer of the samples to the osmotic medium and therefore ineffective drying. Again, only the linear terms were significant, with the sucrose concentration being the term with the highest negative effect. The recalculated model obtained was the following:

$$NMC = 0.612 - 0.198X_1 - 0.0885X_2 - 0.0741X_3 \quad (15)$$

By observing Fig. 3F, the lowest *NMC* values were obtained when applying the highest sucrose concentration and temperature. When considering the sucrose concentration with time (Fig. 3G), we could observe that increasing sugar concentration caused a more pronounced decrease on *NMC* than increasing contact time. By applying high sucrose concentrations, chestnut samples with only 30% of the moisture content of the beginning could be obtained.

Our results were in agreement with Chenlo et al. (2007) for osmotic dehydrated chestnut (whole fruits) with sucrose. These authors also reported that an increase in temperature caused lower values of *NMC* but the intensity of the effect was higher with the most concentrated sugar solutions. Furthermore, generally our results obtained for chestnut slices by RSM were in accordance with those observed for whole fruits (chestnuts) that were osmotically dehydrated with sucrose but where this optimization methodology was not followed, namely Chenlo et al. (2007) and Moreira, Chenlo, Chaguri, and Oliveira (2007). Furthermore, our study also showed that to obtain an osmotic dehydrated product we can apply a low energy cost process due to the low temperatures that might be involved.

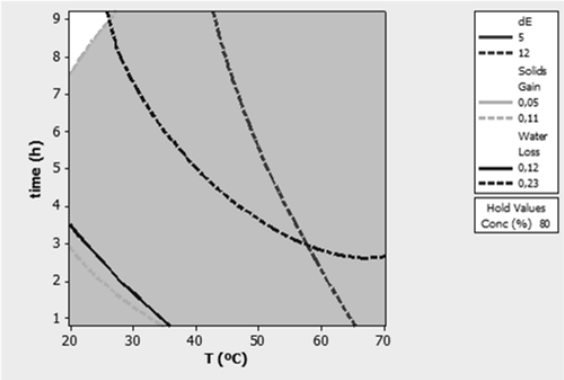

Figure 4: Combination of temperature and time to obtain ΔE^* between 5 and 12, *SG* between 0.05 and 0.11, and *WL* between 0.12 and 0.23

3.9 Optimization of solid gain, water loss and color variation

To evaluate the best osmotic dehydration conditions that optimized the responses of *WL, SG* and ΔE^* simultaneously, an optimization study was performed using the "Response Optimizer" option of Minitab® software. Our target was to obtain simultaneously high *WL* (0.12-0.23 g g^{-1} fresh matter), and low *SG* (0.05-0.11 g g^{-1} fresh matter) and ΔE^* (5-12) effects. The optimal osmotic dehydration conditions determined by the software were a sucrose concentration of 83% (w/v), a temperature of 20 °C and a duration of 9.2 hours. Fig. 4 represents the region (white area) where the values of ΔE^*, *SG* and *WL* mentioned above were obtained simultaneously. When the optimal conditions were applied, a ΔE^* equal to 7.53, a *SG* of 0.095 g g^{-1} of fresh matter and a *WL* of 0.23 g g^{-1} of fresh matter were obtained, showing that these results were within the previously defined ranges. Our results for chestnut slices were in accordance with Chenlo et al. (2006a), Chenlo et al. (2007) that

obtained the best results at low temperatures and high concentrations of the osmotic media when performing osmotic dehydration of whole fruits in glucose and sucrose solutions, respectively, and when studying osmotic dehydration kinetics without using any optimization software.

4 Conclusions

The optimal process parameters for the osmotic dehydration of chestnuts slices in sucrose solutions were determined by applying RSM. The developed models showed good correlation with the experimental data at 95% confidence level. The optimal osmotic dehydration conditions were 83% sucrose (w/v), 20 °C and 9.2 hours to achieve maximum WL and lower SG and ΔE^*. These results indicated that this process may be applied by the industry without high energy requirements and costs while not changing significantly the color of this nut, which is a characteristic valued by consumers.

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Estimating the Risk of Phthalates Exposure via Tea Consumption in the General Population

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Abstract

Four common phthalic acid esters (PAEs) levels in tea fusions samples prepared from three types of tea bags (green, black and white) of ten commercial brands were extracted from the infusions by a dispersive liquid-liquid micro extraction method and determined by GC-MS.
PAEs were not found in white tea samples. Residue levels of total phthalic acid esters (TPAEs) in black and green teas showed no significant difference (median=367.5, Interquartile range=244.7-667.5 and median=381, Interquartile range=188.7-688.2μg/kg respectively). DEHP levels in green teas were significantly higher than those in black teas (Median= 93.5 and 204 respectively). Total phthalate esters (TPAEs) levels in flavored teas were about two-fold higher than in non-flavored teas. The four commercial brands tested contain significant levels of DEHP when compared to other brands. Essential oils and essences that were added to tea for improvement of color and taste could be the main sources of PAEs contamination.
If oral absorption of phthalates were assumed to be 100%, the maximum daily exposure levels to TPAEs via tea consumption (due to consumption of 5 cups of tea prepared from the tea containing the highest levels TPAEs) were estimated to be $230e^{-4}$ μg/kg bw/Day, which are far lower than the regulation levels set by the expert panels on regularly toxicity.

Keywords: Phthalates; Tea; Exposure assessment

1 Introduction

Phthalate esters (PAEs) are dialkyl or alkyl/aryl esters of phthalic acid and are widely used as plasticizers and additives for production of many daily products including plastics, pesticides, paints and cosmetics (Del Carlo et al., 2008). PAEs do not covalently bond to the polymer molecules and easily release from plastic products (Glue et al., 2005) which leads to wide distribution of these materials in the environment. Phthalates are described as being among the most abundant environmental and food contaminants (Latini, 2005).
Di-(2-ethylhexyl) phthalate (DEHP) is the most commonly used plasticizer. Dimethyl phthalate (DEP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), and benzyl butyl phthalate

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(BBP) also widely used worldwide (Kamrin, 2009). Due to their potential risk for human health and environment, several of PAEs have been included in the priority list of pollutants of different national and international organizations (Heudorf, Mersch-Sundermann, & Angerer, 2007).

Certain phthalate esters, as well as their metabolites, may have adverse effects on human health, particularly on liver, kidney and testis. Potential endocrine disrupting effects and carcinogenic properties are also reported. Metabolic products of phthalates are shown to have developmental and reproductive toxicity, mainly on the male reproductive system. Some of the phthalates interfere with the function of the endocrine system, which is responsible for growth, sexual development and many other essential physiological functions in both sexes.

Several adverse effects due to phthalate exposure including decreased fertility, foetal defect (growth retardation and dysmorphogenesis), reduced survival of offspring, altered hormone levels, uterine damage, male reproduction abnormalities such as reduced sperm production and motility, Sertoli cell damage and Leyding cell tumors have been demonstrated in animal studies (Martino-Andrade & Chahoud, 2010; Montuori, Jover, Morgantini, Bayona, & Triassi, 2008).

Tea is the most frequently consumed beverage next to water, with about 20 billion cups consumed daily worldwide (Shen & Chen, 2008). Tea is made from the processed leaves of the tea plant (*Camellia sinensis* and *Camellia assamica*). Black, green and white teas are the most common types of tea and are distinguished on the basis their respective manufacturing methods. Black tea is fermented; green tea is dried and roasted but not fermented; white tea is made from buds and young leaves and just dried without further fermentation or other procedures (Chacko, Thambi, Kuttan, & Nishigaki, 2010). It is estimated that 2.5 million metric tons of dried tea are produced annually, of which 75% is processed as black tea consumed in many countries (Karimi et al., 2008).

Tea has received increasing attention for its beneficial health properties that include the prevention of cardiovascular diseases, skin cancer, coronary artery disease, Parkinson's disease and myocardial infarction (Qin & Chen, 2007)). However, several studies have demonstrated that tea leaves may also contain some harmful contaminants, which can be released into infusions; such as, considerable levels of heavy metals including lead, cadmium, arsenic, mercury (Han, Mihara, & Fujino, 2014; Shaltout, Abdel-Aal, Welz, & Castilho, 2013), poly-chlorinated biphenyls (PCBs), fertilizers, pesticides (Beizhen, Weihua, Liping, & Tiefeng, 2008; Shen & Chen, 2008) and poly-aromatic hydrocarbons (PAHs) (Lin, Zhu, He, & Tu, 2006)) are reported in different types of tea infusions. Contaminated cultivation soils, transportation and possessing procedures are suggested as possible sources of these contaminants.

In a recent study by Di Bella, Saitta, La Pera, Alfa, and Dugo (2004) phthalate esters concentrations were measured in tea samples from Italian markets and considerable levels of di-(2-ethylhexyl)-phthalate (DEHP), di-n-butyl-phthalate (DBP) and diethyl phthalate (DEP) were found in all analyzed samples. That was the first report about phthalate contamination in tea infusions and some subsequent studies have confirmed this contamination (Du, Ma, Qiao, Lu, & Xiao, 2016; Lu, Du, Qiao, Wang, & Xiao, 2015). In most of these studies, PAEs levels have been determined in dry tea samples but given that traditionally, tea is prepared by infusion of dry leaves in boiling water, and considering that tea is consumed in the form of infusion by consumers, it is essential to determine the dissolving ratio of PAEs from dried tea to infusion.

Easy preparation and effective advertising from tea producers during last decade has encouraged consumers to use tea bags for tea infusion. The aims of this study were to investigate the presence of 4 common phthalate esters in tea infusions made using tea bags and to study daily dietary intake of these phthalates due to tea consumption.

2 Materials and Methods

2.1 Sampling

This cross-sectional study was conducted in the autumn and winter of 2015 in Iran. In initial field

investigations, 10 famous brands of tea bag in Iran markets were identified and selected for this study. All brands had operating and hygienic licenses from Iranian Food and Drug Organization of the Ministry of Health. Eight of these brands produce only green and black tea bags. Two of them also produce white tea bags. Based on the information presented on package labels of tea bags, all teas were produced in India (Ceylon) and were packed in Iran.
Each tea bag pack usually contains 20 or 25 tea bags. For the present study, six tea bag packs (three green tea bags and three black tea bags) were purchased randomly from different supermarkets in Isfahan, Iran. All samples were produced during the prior 2 months of sampling time. Three tea bags of each pack were selected randomly, labeled and sent for immediate analysis in the laboratory.

2.2 Materials

Analytical standards of diethyl phthalate (DEP), Bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP) and benzyl butyl phthalate (BBP) were purchased from Sigma Aldrich (Sigma, St. Louis, USA, catalog number: 41320, 53008, 36735, and 36927 respectively).
The GC grade acetonitrile, carbon tetrachloride, methanol, and ultrapure water were purchased from the Merck (Darmstadt, Germany).

2.3 Sample preparation

Each tea bag contained 2±10% g dry tea. In the laboratory, tea bags were opened and dry teas were removed from the bags. Then, the dry teas and the bags were infused separately in 50 ml boiling ultrapure water for 5 min. The infusion cooled down to room temperature, was filtered and subjected to the process of extraction. To ensure the complete migration of PAEs from leaves to infusion, each tea bag was infused in 50 ml boiling water twice and the infusions were mixed before extraction.

2.4 Stock and working standard preparation

A mixed stock standard solution of 1000 ppm of all phthalate esters (DEP, DEHP, DBP and BBP) was prepared in methanol. The working standard solutions of 50, 10, 5, 1 and 0.1ppb were prepared by spiking white tea infusions with PAEs standards (phthalates-free white tea infusion were prepared by ultrapure water and confirmed by GC-MS analysis). The stock and working standard solutions were stored at 4°C.

2.5 Extraction of phthalate esters

Phthalate esters were extracted from the tea infusion samples by a dispersive liquid-liquid extraction (DLLME) method according to previous work [11] with some modifications. For this purpose, an aliquot of 5 mL of each sample was placed in a 10mL glass test tube with a conical bottom. A mixture of acetonitrile (0.75mL) as a dispersive solvent and carbon tetrachloride (50μL) as an extraction solvent were prepared and injected rapidly into the sample solution using 1mL Hamilton syringe. After that, a cloudy solution (sample+ acetonitrile/CCl_4) was formed and the analytes were extracted into the fine CCl_4 droplets. After centrifugation for 5 min at 4500 rpm, the extraction solvent was separated and precipitated to the bottom of the conical test tube (about 50μL). 2μL of precipitated phase was removed using a 10μL GC/MS microsyringe and injected into the GC system for analysis.

2.6 Analytical methods

The extracts were analyzed by gas chromatography/mass spectrophotometry using a quadruple Agilent GC-MSD (Agilent Technologies, Palo Alto, CA, USA) model 7890A coupled to a mass selective detector model 5975C inert, operated in the electron-impact mode at 70 eV. Data recording and instrument control were performed by the MSD ChemStation software (G1701CA; Version C.00.00; Agilent Technologies). Helium (99.999%) was employed as carrier gas at the flow rate of 1 mL/min. The analytes were sepa-

rated using a capillary column (HP-5, 30 m, 0.25 mm id., 0.25 μm coating thickness). The gas chromatographic conditions were as follows; Injection volume: 2 μL; split ratio: 1/10; injector temperature: 280°C. The oven temperature was programmed from 100°C (holding for 2 min), to 210°C at 10°C/min then to 250°C at 5°C/min and finally to 280°C at 30°C/min keeping the final temperature for 4 min. The MS transfer line and ion source were kept at 280°C and 230°C respectively. The MS was tuned to selective ion monitoring (SIM) mode with m/z 69, 219, and 502 for the electron impact (EI) corresponding to perfluoro tetrabutyl amine (PFTBA). Data acquisition was carried out in the full-scan mode (m/z 149) mode and results were qualified by comparison with the NIST and Wiley's library spectral data bank (G1035B; Rev D.02.00; Agilent Technologies).

2.7 Method validation

The validation was performed according to International Conference of Harmonization (ICH) recommendations for linearity, range, accuracy and precision, limit of detection (LOD), limit of quantification (LOQ) and relative recovery (Ermer & Miller, 2006).

2.8 Statistical analysis

Experiments were repeated at least three times (using real and independent samples) and the results are expressed as mean±SD. Data were analyzed by Student t-test and analysis of variance (ANOVA) with significance level defined as $p < 0.05$ using GraphPad-Prism 5 software (GraphPad-Prism Software Inc., San Diego, USA).

3 Results

3.1 Validation of the method

This is a highly sensitive, selective and accurate analytical method for phthalate detection and determination in aqueous solutions. A good resolution was achieved for phthalates separation in this method. All four phthalate esters leave the column at 11.4, 14.3, 15.4, 20.6 min as shown in Figure 1.
Quantification was done using the external calibration method showing linear correlations with $R^2 > 0.98$ for all the target analytes from the range of 1 to 1000 ppb. Other method validation parameters are presented in Table 1.

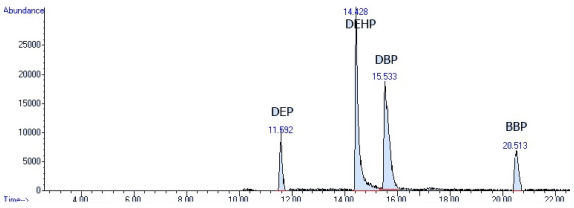

Figure 1: A typical chromatogram of phthalate esters obtained by DLLME-GC/MS under SIM data acquisition mode (m/z: 149)

3.2 PAEs residues in tea samples

Minimum, maximum, median and Interquartile range (IQR) of PAEs levels in different types of tea bags from all brands are presented in Table 2. Statistical analysis (shapiro-wilk normality test) had shown that the data do not have a normal distribution, therefore non-parametric statistical tests were used for analysis of data. No amount of any of the phthalates were detected in white tea samples. Application of the Mann-Whitney test confirms that DEHP and DnPB in green tea samples were significantly higher than black teas but total phthalic acid esters (TPAEs) levels showed no significant differences between black and green teas (Figure 2). Black tea bags from all brands contain considerable levels of different PAEs but in just some of green tea bag brands significant levels of PAEs have been detected (Figure 3-A). In the case of DEHP, this issue is much more obvious and analysis of DEHP in green teas from different brands indicated that four of the tea bag brands contain significantly higher DEHP levels when compared to other six brands (Figure 3-B). Although TPAEs levels showed no significant differences between green and black tea bags but its concentration in

Table 1: Method validation parameters for determination of phthalate esters by DLLME extraction and GC/MS analysis

Factor	**R2**				**LOD (ppb)**				**LOQ (ppb)**			
PAEs	DEP	DEHP	DBP	BBP	DEP	DEHP	DBP	BBP	DEP	DEHP	DBP	BBP
Value	0.99	0.99	0.99	0.98	0.03	0.02	0.04	0.05	0.11	0.09	0.13	0.17
Factor	RSD%								Recovery %			
	Within days				Between days							
PAEs	DEP	DEHP	DBP	BBP	DEP	DEHP	DBP	BBP	DEP	DEHP	DBP	BBP
Value	6.3	6.8	4.5	7.2	7.6	6.6	6.5	9.7	96.1	88.7	91.2	87.6

flavored teas was significantly higher than non-flavored teas (Figure 4).

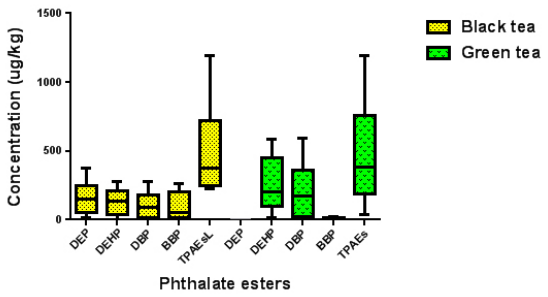

Figure 2: PAEs levels in green and black teas. Data are presented as median (max, min)

4 Discussion

Tea plants grow in certain areas of the world, notably in tropical and subtropical areas that have adequate rainfall, acid soils and good drainage. It is estimated that about 80% of dry teas are produced in the India and China and then exported to other countries (Shah & Pate, 2016). Therefore, the findings of the present study could be generalized to tea samples used in other countries.

None of the white tea samples contained PAEs residues, indicating that common theories about soil contamination and bioaccumulation of these contaminants in plant proposed for other contaminants such as PAHs and PCBs (Lin et al., 2006) cannot be applied for plasticizers contamination in tea. It is obvious that processing procedures are definite sources of this contamination. In a study by Mohamed et al., machines or paper coatings during the production steps are proposed as potential sources of plasticizers residues in tea (Mohamed & Ammar, 2008). Lo Turco, Di Bella, Potorti, Fede, and Dugo (2015) claim that paper, silk or nylon bags are important sources of phthalate contamination in teas, but the results of the present study showed no detectable amounts of PAEs in infusion of tea-free bags in boiling water.

Several reports exist about the use of essential oils and synthetic essences as color, taste and flavors by tea producers. In a study by Di Bella et al. (2004), significant levels of phthalate esters have been detected in essential oils. Likewise, the findings of the current study showed higher levels of PAEs in flavored teas than in non-flavored teas. These findings are in consistent with some previous studies (Lo Turco et al., 2015). On the other hand, PAEs levels in some tea brands are significantly higher than other brands indicated that some essential oils and synthetic essences have been added to tea by certain tea producers to improving the tea taste and color, is the main source of phthalates residues in the tea.

It is generally believed that green tea has numerous health benefits and is safer than black tea (Cabrera, Artacho, & Gimenez, 2006; Gupta, Saha, & Giri, 2002), however, our results show that about 50% of green tea samples contained significant levels of PAEs, specifically the more hazardous ones (EDHP).

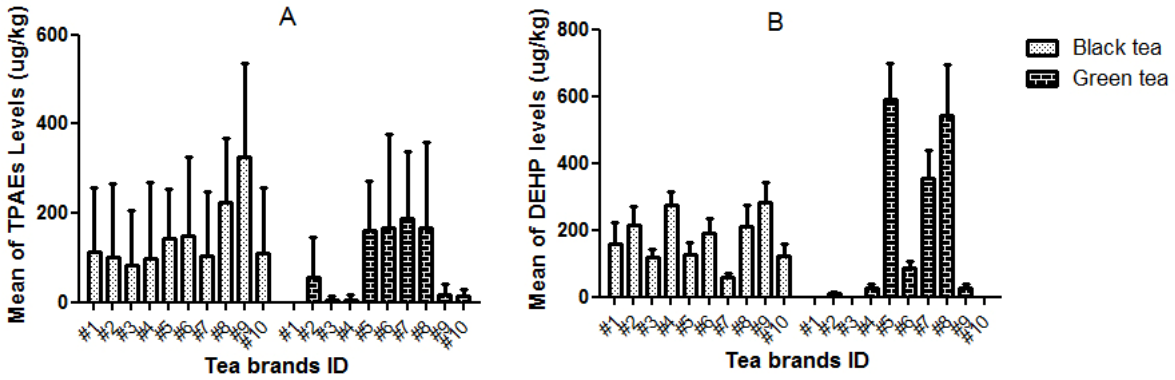

Figure 3: PAEs levels in green and black teas from different brand. A) TPAEs levels, B) DEHP levels. Data are presented as mean±SD from three independent analysis

Table 2: PAEs levels in different types of tea bags (μg/kg dry tea)

	Black tea				**Green tea**			
	Min.	Max.	Median	IQR.*	Min.	Max.	Median	IQR.*
DEP	ND	373	149.5	55-230.5	ND	ND	ND	ND
DEHP	ND	281	135.5	40.7-187.5	ND	590	204	98.5-397-7
DBP	ND	276	93	13.7-143.2	ND	585	169	25.5-289.2
BBP	ND	259	55	17.2-175	ND	21	10	3.7-14.7
TPAEs	ND	1189	376.5	244.7-667.5	ND	1196	381	188.7-688.2

* Interquartile range

4.1 Human exposure estimates to PAEs from tea consumption

The findings of the current study show considerably high amounts of phthalates in Indian teas consumed in several countries. In most populations tea is used much more frequently than coffee and more attention should be paid to the quality of tea bags used by the general population. This is of special concern for those at higher risk for adverse health effects of phthalates.

Tea is consumed in the form of infusion, therefore, in the present study, we determined the dissolved ratios of PAEs from dried tea to infusion leading to exact estimation of human exposure to PAEs via tea consumption

The amount of tea consumption is the key determinant in exposure assessment. Among tea drinkers, tea drinking habits are quite different. In some countries, as Turkey, tea is a very popular drink and its per capita consumption is about 7.5 kg, whereas in some other like Mexico, this amount is about 0.003 kg (FAO, 2015). Even in members of a family, one person drinks several cups of tea and the other one does not. Some of tea drinkers like heavy and dark tea infusion, prepared from higher amounts of dry tea and some other in its light form. In this study, human exposures were estimated based on preparation of a cup of tea using a tea bag (common form of tea bag usage) according to the following equation:

$$\text{Human daily exposure}(\mu\text{g/kg bw/d}) = \frac{C(ppm) \times V(ml/time) \times F(times/D)}{\text{Body weight (kg)}} \quad (1)$$

Where C is the concentration of PAEs in the tea infusion samples (ppb), V the volume of tea conception per time (ml/time), F the frequency

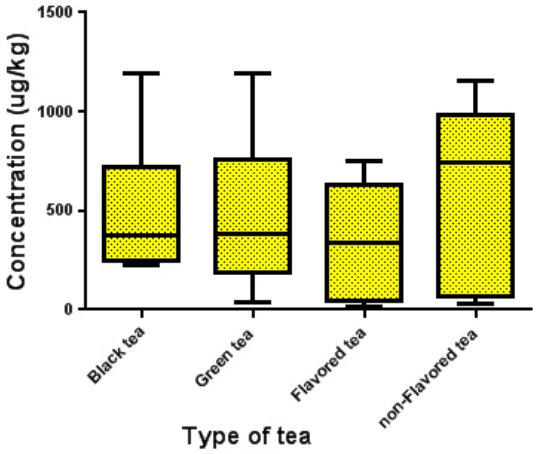

Figure 4: TPAEs levels in green, black, flavored and non-flavored tea bags. Data are presented as median (max, min)

of tea conception (times/Day), and *abs.* the absorption rate.
Concentration of PAEs in the tea infusion samples are presented in Table 2. Volume of tea consumption per time was considered 250ml (volume of a typical tea cup). Three ranges were defined for frequency of tea conception per day. Some previous studies suggested that 100 % of the daily orally ingested phthalates, such as DEHP and DINP, are absorbed (United States Consumer Product Safety Commission, 2010). Based on this information, human exposure to PAEs from tea consumption were estimated and presented in Table 3.

4.2 Risk assessment

According to the Environmental Protection Agency (EPA) guideline, the hazard indices (HIs) were calculated as following equation (Zheng et al., 2007):

$$HIs = \frac{\text{Daily exposure level}}{\text{Regulation level [e.g.,TDI,MRL,ADI]}} \quad (2)$$

Different expert panels in Europe and America, such as the US Food and Drug Administration, European Food Safety Authority (EFSA) based on animal studies recommended values for human exposures to PAEs that are summarized in table 4 (Yen, Lin-Tan, & Lin, 2011). The EPA also has established a maximum admissible concentration (MAC) value of 6 μg/L for DEHP in water (Farahani, Norouzi, Dinarvand, & Ganjali, 2007).

4.3 Study limitations and strengths

The main limitation of this study is that we only measured phthalates in tea samples, and not the level in the human body. We analyzed 10 common brands of teabags used in Iran, but considering nearly similar results in all brands, and given that all samples were from teas imported from India (Ceylon), contamination of other tea brands with the same country of origin is highly probable and further investigation is recommended.

5 Conclusions

We found considerably high levels of phthalates in teabags used in Iran, prepared by Indian (Ceylon) imported tea. However, comparison of the estimated human exposure to PAEs from tea consumption with their permissible levels indicates that the HIs for phthalates of all teabags were far below 1, which implies that the daily exposure levels are below regulation levels. Therefore, the usual habit of tea drinking, even 5 cups of tea/day, would not have harmful consequences for human health. However, it should be considered that tea consumption is not the only means of exposure to phthalates and several other sources for human exposure have been identified. The current findings could be useful in comprehensive phthalates risk assessment programs.

Table 3: Human exposure estimates to PAEs from tea consumption (μg/kg bw/Day). Estimated from mean concentration of PAEs in tea samples))

	TPAEs			DEHP		
	Up to 1 cups/d	Up to 3 cups/d	Up to 5 cups/d	Up to 1 cups/d	Up to 3 cups/d	Up to 5 cups/d
Black tea	2,60E-03	7,80E-03	1,30E-02	2,90E-03	8,70E-03	1,30E-02
Green tea	2,30E-03	6,90E-03	1,15E-02	4,60E-03	1,38E-02	2,30E-02
Flavored tea	6,20E-03	1,86E-02	3,10E-02	5,20E-03	1,56E-02	2,60E-02
non-Flavored tea	2,90E-03	8,70E-03	145 e-4	2,90E-03	8,70E-03	1,30E-02

Table 4: Recommended exposure values to PAEs by different expert panels

PAE	Expert panels	Value (μg/kg bw/Day)	MRL/TDI/RfD
DEP	ATSDR	7	MRL, acute oral exposure
		5	MRL, chronic oral exposure
	US-EPA	0.8	RfD, chronic exposure
DnBP	ATSDR	0.5	MRL, acute oral exposure
	US-EPA	0.1	RfD, chronic exposure
BBP	US-EPA 0.2		RfD chronic exposure
	EU-CSTEE 0.2		TDI
DEHP	US-EPA	0.02	RfD chronic exposure
	EU-CSTEE	0.050	TDI

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Effect of Storage Time and Gamma Irradiation on the Chemical Properties of Olive (*Olea europaea*) Oils

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Abstract

The objective of this study was to investigate the effects of gamma irradiation at doses of 0, 1, 2 and 3 kGy, and storage time of olive fruits for 0, 30 and 45 days on changes in chemical properties of olive oil during storage periods of 0, 6 and 12 months. The initial acidity value (AV), peroxide value (PV), Thiobarbituric Acid (TBA), phenolic content, iodine value (IV) and saponification value (SV) of virgin olive oil obtained from olives fruits immediately after harvest (at day zero) were 1.04%, 3.06 mEqO_2 kg^{-1}oil, 0.025 mg MDA kg^{-1} oil, 314.71 mg gallic acid kg^{-1} oil, 93.38 gI_2 100 g^{-1} oil and 194.88 mg KOH g^{-1} oil, respectively. In general, the AV and PV of olive oil was increased by gamma irradiation, while the phenolic and IV of olive oil was deceased by gamma irradiation and storage time. The TBA value and SV of olive oil was not significantly ($p>0.05$) changed by gamma irradiation.

Keywords: Olive oil; Gamma irradiation; Olive fruit storage; Oil storage; Chemical properties

1 Introduction

Virgin olive oil (VOO) is one of the essential elements of the Mediterranean diet. The nutritional value of olive oil arises from high levels of oleic acid and minor components, such as phenolic compounds (Artajo, Romero, Morello, & Motilva, 2006).

In households and restaurants, olive oil is stored for various periods of time, under different temperature and light conditions depending on the consumption rate. It is well known that some factors affect oil properties. Among the factors that significantly influence the preservation of olive oil quality are the extraction methods, the packing materials and the storage conditions (Cecchi, Passamonti, & Cecchi, 2010). Moreover, the storage conditions and the extractive technology cause further differences (Vekiari, Papadopoullou, & Kiritsakis, 2007). Therefore, before consumption of the oil, its initial components and properties can change significantly in many different ways (Cecchi et al., 2010; Jenisová, Branisa, Jomová, & Porubská, 2014). However, many aspects still require further investigation, due to the complexity and variable composition of olive oil. (Jenisová et al., 2014). This text describes some of them and shows a vision of research on olive oil for the near future, bringing together those aspects that are more relevant for better understanding and protection of this edible oil (Garcia-Gonzalez & Aparicio, 2010).

Food irradiation is increasingly recognized as a method for reducing post harvest food losses, ensuring hygienic quality, and facilitating a wider trade in foodstuffs.

Irradiation can also slow down the ripening or maturation of certain fruits and vegetables (Sadecka, 2010). In general, any modifications caused by treatment with doses up to 10 kGy are considered harmless to human health by WHO,

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FAO and IAEA (Hong, Kim, Cho, & Park, 2010). The effect of irradiation on locally stored foods is of utmost importance and an insight into these aspects of storage will help in understanding the shelf life of foods as well as its effects on sensitive nutrients (Al-Bachir & Zeinou, 2014). Very few studies have addressed the evolution of the quality parameters and shelf-life during irradiation and prolonged time. Even though it is well known that, in the traditional producing countries, in the better vintages, people usually purchase great amounts of olive oils which are then stored for years (Baiano, Terracone, Viggiani, & Del Nobile, 2014). The present study investigated the changes of chemical parameters of olive oil from gamma irradiated and non-irradiated Kaissy cultivar, an important Syrian cultivar, stored after irradiation for 0, 30 and 45 days at ambient temperature before extracting the oil, and during 12 months of olive oil storage.

2 Materials and Methods

The studied olive cultivar was Kaissy, the most widespread in Syria. The good quality olive fruits of that were mature and firm were harvested from the crop year 2008/2009, from the trees grown in grove located in the countryside near Damascus (Deer Al Hajar, Syria), using conventional agriculture practices. The olive fruits were weighed according to the sampling plan and transferred into polyethylene pouches for irradiation. Each pouch of olive fruits (1 kg) was considered as a replicate. The samples were then divided into four groups: group 1 (control) and groups 2, 3 and 4 were irradiated with 1, 2 and 3 kGy of gamma irradiation.

2.1 Irradiation process

Samples of olive fruits were exposed to gamma radiation doses at 0, 1, 2 and 3 kGy in a ^{60}CO package irradiator (ROBO, Techsnabexport, Moscow, Russia). Irradiation was carried out in the stationary mode of operation with the possibility of varying dose rate (10.846 to 3.921 kGy h^{-1}) depending on the location and the distance from the source (10 to 40 cm). The samples were irradiated in place (15 cm from source) with a dose rate of 9.571 kGy h^{-1}. The irradiations were carried out at room temperature (20 – 25 °C) and atmospheric pressure. The absorbed dose was determined using alcoholic chlorobenzene dosimeter (Al-Bachir & Zeinou, 2014).

2.2 Oil extraction

The oils from control and irradiated olive fruits were extracted from olives stored at ambient temperature for 0, 30 and 45 days after irradiation using a mechanical and physical processes (Blatchly, Delen, & O'Hara, 2014). Olive fruits were crushed with hummer crusher and slowly mixed for about 30 min at 27 °C. Then, the past mixed was centrifuged at 3000 rpm for 3 min without addition of water to extract the oil. Finally, the oils were decanted and immediately transferred into dark glass bottles and stored at room temperature (20 – 25 °C). Chemical analysis of oils extracted from irradiated and non-irradiated olive fruit samples were performed immediately after irradiation, and after 6 and 12 months of storage.

2.3 Determination of total phenol content of olive oil extracts

Phenolic compounds were isolated from olive oil by a 3-time extraction of solution of oil in hexane with water mixture (60:40. v/v). The Folin-Ciocalteau reagent (Merck Schuchardt OHG. Hohenbrunn, Germany) was added to a suitable aliquot of the combined extracts, and the absorption of the solution was measured at 725 nm using UV-VIS spectrophotometer (PG Instrument Limited, England). Results were expressed in milligrams of gallic acid per kilogram of oil (Gutfinger, 1981).

2.4 Chemical analysis of oils

Acid value (AV) in terms of (Oleic acid %), peroxide value (PV) in terms of mEq O2 kg^{-1} oil, iodine value (IV) in g I_2 100 g^{-1} and saponification (specification) value (SV) in term of mg KOH g^{-1} oil sample were determined according to standard methods (Official Methods of Analysis, 2010). TBA number (Thiobarbituric acid) in

mg MDA kg^{-1} sample was measured according to IUPAC direct method (International Union of Pure and Applied Chemistry, 1992).

2.5 Statistical analysis

Treatments were distributed in a completely randomized design with three replicates. Data were subjected to the analysis of variance test (ANOVA) using the SUPERANOVA computer package (Abacus Concepts Inc, Berkeley, CA, USA; 1998). The p-value of less than 0.05 was considered statistically significant. The degree of significance was denoted as: $p<0.05$*, $p<0.01$** (Snedecor & Cochran, 1988).

3 Results and Discussions

3.1 Effect of gamma irradiation and storage time on acid value of olive oil

The acid values (AV) in terms of percent of free fatty acid FFA (% oleic acid) of oils obtained from olives treated at 0, 1, 2 and 3 kGy of gamma irradiation and stored at ambient temperature for 0, 30 and 45 days before extraction and stored at ambient temperature for 0, 6 and 12 months after extraction are presented in Table 1. The AV of oil extracted from olives immediately after harvest (at day zero) was 1.04%. The AV of olive oil was not significantly ($p>0.05$) changed by gamma irradiation, while the AV of olive oil was significantly ($p<0.05$) changed by storage time. The AV of the analyzed oils extracted immediately after irradiation at 0, 1, 2 and 3 kGy and stored for 0, 6 and 12 months ranged from 0.94% to 1.23% and did not exceed the standard limit of 2.0%, indicating that these oils could be labeled as virgin olive oil quality (International Olive Council, 2015)
Data presented in Table 1 indicate that the AV of oil extracted after 30 days of harvest was 9.15%. The AV of olive oil was significantly ($p<0.01$) decreased by gamma irradiation, while the AV of olive oil was not significantly ($p>0.05$) changed by storage time. The AV of the analyzed oils extracted from olive fruits stored for 30 days of irradiated olives at 0, 1, 2 and 3 kGy and stored for 0, 6 and 12 months ranged from 5.31% to 9.97% and exceed the standard limit of 3.3%, indicating that these oils could not be labeled as ordinary virgin olive oil quality (International Olive Council, 2015).
The results of this study show that the AV of oil extracted after 45 days of harvest was 13.28%. The AV of olive oil was significantly ($p<0.01$) decreased by gamma irradiation, while the AV of olive oil was significantly ($p<0.05$) increased by storage time. The AV of the analyzed oils extracted after 45 days of irradiated olives at 0, 1, 2 and 3 kGy and stored for 0, 6 and 12 months ranged from 6.92% to 15.22%. Oxidation of oils generates a multitude of compounds, such short chain acids, formed by the oxidation of aldehydes. Under proper harvest conditions, very little hydrolysis of triglycerides is seen. However, there are a number of bad harvest practices that lead to hydrolysis, including improper storage conditions and presence of pests or mold. Therefore, such bad practices lead to off-tasting oil. One of the primary international standards for olive oil is the level of free fatty acids that arises from hydrolysis of the triglycerides in the olives prior to processing (Blatchly et al., 2014).

3.2 Effect of gamma irradiation and storage time on peroxide value of olive oil

The peroxide value (PV) of oils is an important indicator of deterioration of fats. As oxidation takes place the double bonds in the unsaturated fatty acids are attacked forming peroxides. This parameter is expressed as a meq O_2 kg^{-1} oil (Enujiugha, Olotu, Malomo, & Sanni, 2012).
Table 1 shows PV in terms of meq O_2 kg^{-1} of all studied samples . The PV of oil extracted from olives immediately after harvest was 3.06 meq O_2 kg^{-1} oil. The PV of olive oil was significantly ($p<0.05$) affected by gamma irradiation. The PV of oil extracted after 30 days of harvest was 4.36 meq O_2 kg^{-1} oil. The PV of oil extracted after 45 days of harvest was 3.67 meq O_2 kg^{-1} oil. The AV of olive oil was not significantly ($p<0.05$) affected by gamma irradiation nor by storage time. The PV of the analyzed oils extracted after 0, 30 and 45 days of irradiated olives at 0, 1, 2

Table 1: Effect of the extraction time and gamma irradiation on acid value (free fatty acid) (%) and peroxide value (mEqO_2 kg^{-1} oil) of olive oil

Treatments		Control	1 kGy	2 kGy	3 kGy	P-Value
Type		**Acid value Free Fatty Acid (%)**				
0 days	**0 months**	1.04 ± 0.08^{aB}	1.04 ± 0.08^{aB}	1.09 ± 0.02^{aB}	1.08 ± 0.04^{aA}	**NS**
	6 months	0.99 ± 0.1^{bB}	0.94 ± 0.02^{cB}	1.04 ± 0.03^{aC}	1.05 ± 0.02^{aA}	**
	12 months	1.15 ± 0.02^{aA}	1.20 ± 0.09^{aA}	1.23 ± 0.02^{aA}	0.97 ± 0.05^{bB}	**
	P-Value	*	**NS**	**	*	
30 days	**0 months**	9.15 ± 0.54^{aA}	8.96 ± 0.39^{aB}	7.10 ± 0.40^{bB}	5.31 ± 0.05^{cA}	**
	6 months	8.64 ± 0.15^{aA}	8.77 ± 0.17^{aB}	7.25 ± 0.27^{bAB}	5.31 ± 0.17^{cA}	**
	12 months	9.76 ± 0.86^{aA}	9.97 ± 0.56^{aA}	7.96 ± 0.44^{bA}	5.36 ± 1.35^{cA}	**
	P-Value	**NS**	*	*	**NS**	
45 days	**0 months**	13.28 ± 0.84^{aB}	13.13 ± 0.26^{aB}	9.72 ± 0.34^{bB}	6.92 ± 0.15^{cB}	**
	6 months	14.52 ± 0.15^{aA}	14.94 ± 0.58^{aA}	10.38 ± 0.19^{bAB}	6.97 ± 0.08^{cB}	**
	12 months	14.68 ± 0.07^{aA}	15.22 ± 0.39^{aA}	11.10 ± 0.62^{bA}	7.44 ± 0.34^{cA}	**
	P-Value	*	*	**	*	
		Peroxide value (mEqO_2/kg Oil)				
0 days	**0 months**	3.06 ± 0.03^{bC}	3.06 ± 0.03^{bB}	3.14 ± 0.06^{aB}	3.19 ± 0.04^{aC}	*
	6 months	4.05 ± 0.12^{aB}	4.51 ± 0.51^{aA}	4.39 ± 0.20^{aA}	4.32 ± 0.12^{aB}	**NS**
	12 months	4.99 ± 0.31^{aA}	4.99 ± 0.18^{aA}	4.68 ± 0.27^{aA}	4.70 ± 0.11^{aA}	**NS**
	P-Value	**	**	**	**	
30 days	**0 months**	4.36 ± 0.05^{bB}	4.26 ± 0.05^{bA}	4.25 ± 0.07^{bB}	5.49 ± 0.08^{aA}	**
	6 months	4.83 ± 0.56^{aAB}	4.09 ± 0.45^{aA}	5.03 ± 0.60^{aA}	5.11 ± 0.46^{aAB}	**NS**
	12 months	5.36 ± 0.51^{aA}	4.30 ± 0.26^{bA}	$4.42\pm0.27\text{b}^{AB}$	4.70 ± 0.17^{bB}	*
	P-Value	*	**NS**	*	*	
45 days	**0 months**	3.67 ± 0.16^{aB}	3.61 ± 0.08^{aB}	3.60 ± 0.05^{aC}	3.60 ± 0.03^{aC}	**NS**
	6 months	4.37 ± 0.09^{aA}	4.39 ± 0.04^{aA}	4.39 ± 0.13^{aA}	4.37 ± 0.07^{aA}	**NS**
	12 months	4.50 ± 0.13^{aA}	4.29 ± 0.60^{aAB}	4.09 ± 0.17^{aB}	4.05 ± 0.20^{aB}	**NS**
	P-Value	3.06 ± 0.03^{bC}	3.06 ± 0.03^{bB}	3.14 ± 0.06^{aB}	3.19 ± 0.04^{aC}	*

^{abc} Mean values in the same column not sharing a superscript are significantly different.
^{ABC} Mean values in the same row not sharing a superscript are significantly different.
NS: not significant.
* Significant at $p<0.05$.
** Significant at $p<0.01$.

and 3 kGy and stored for 0, 6 and 12 months ranged from 3.06 to 5.49 meq O_2 kg^{-1} oil (Table 1) being lower than the maximum value (20 meq O_2 kg^{-1} oil), which is accepted as the limit for extra-quality virgin olive oil category (International Olive Council, 2015). The challenge is to produce high quality olive oils. According to the literature, hydroperoxides, the initial products of oxidation, are comparatively unstable, are a very sensitive indicator of the early stages of oxidative deterioration and a good guide to the evaluation of olive oil quality (Vekiari et al., 2007). Onyeike and Acheru (2002) reported the peroxide value of castor, coconut, diakanut, groundnut, melon, bean and palm kernel seed oil to be 22.7, 40.0, 40.0, 20.0, 21.3, 23.3 and 20.0 mg g^{-1} of sample, respectively. These values were generally higher than those reported by Okogeri and Okoro (2014) who reported the ranges of peroxide values of unadulterated palm kernel oil to be 3.35, 16.00 and 2.07- 6.80 meq kg^{-1}, respectively that are also higher than the values reported for groundnut oil (Yusuf, Olaniyan, Atanda, & Sulieman, 2014). Abiodun, Akinoso, Olosunde, Adegbite, and Omolola (2014) determined a PV of 11.0 mg g^{-1} for *Thaumatococcus danielli*, which in a typ-

ical fruit plant. The difference in the value must have resulted from the different methods used for determination as well as the variation in the species. Regarding the effect of gamma radiation on the peroxide values, present results are in general agreement with those obtained from pumpkin (El-Aziz & El-Kalek, 2011), and sesame oil (Al-Bachir, 2016). It should be noted that the PV characterizes the quantity of peroxides formed in the oils are as intermediates of oxidative reactions under irradiation and high temperatures (Uquiche, Jerez, & Ortiz, 2008).

3.3 Effect of gamma irradiation and storage period on TBA value of olive oil

The thiobarbituric acid value (TBA) of oil extracted from olive fruits stored for 0, 30 and 45 days were 0.025, 0.025 and 0.026 mg MDA kg^{-1} oil, respectively (Table 2). The TBA of olive oil extracted from olive fruits stored for 0 and 45 days was not significantly ($p>0.05$) affected by gamma irradiation, while the TBA of olive oil extracted from olives stored for 30 days was significantly ($p>0.01$) changed by gamma irradiation. Also, the TBA value of olive oil extracted from olives immediately after harvest (at day zero) did not significantly ($p>40.05$) changed by storage time, while the TBA of olive oil extracted from olives stored for 30 and 45 days was significantly ($p<0.01$) changes with storage time. The TBA of the analyzed oils extracted from olives stored for 0, 30 and 45 days of irradiated olives at 0, 1, 2 and 3 kGy and stored for 0, 6 and 12 months ranged from 0.022 to 0.027 mg MDA kg^{-1} oil (Table 2). Some significant changes were observed during the creation of TBA in olive oil due to irradiation and storage, but the changes were within the standard limits given by the International Olive Council (International Olive Council, 2015). Our results, related to the TBA analyses are consistent with previous reports, which also reveal a slight difference in TBA value due to irradiation at 1, 2 and 3 kGy, and storage for 12 months of almond (Al-Bachir & Zeinou, 2014), pistachios (Al-Bachir, 2015b), and peanuts (Al-Bachir, 2015a).

3.4 Effect of gamma irradiation and storage period on phenolic content of olive oil

It has been noted that phenols and polyphenols are substances with natural antioxidant properties and their presence in olive oils has been associated to their general quality, improving storability, nutritional value and sensorial properties (Fernandes-Silva, Gouveia, Vasconcelos, Ferreira, & Villalobos, 2013). The concentration of total phenols of olive oil extracted from irradiated and non-irradiated fruits at different periods of extraction and storage time are presented in Table 2. The initial concentration of total phenolics in the olive oil extracted from olive fruits stored for 0, 30 and 45 days were 314.71, 194.53 and 154.43 mg gallic acid kg^{-1} oils, respectively. The phenolic content of oil extracted from olive fruits stored for 0, 30 and 45 days was significantly ($p<0.05$) changed by gamma irradiation and storage. This data indicate that gamma irradiation is capable of affecting the phenolic composition in olive oil. In general, the concentration of total phenolics in the olive oil decreased with increasing irradiation dosed and storage time. Storage time has a much greater influence on reducing the phenolic contents in olive oil comparing to the applied irradiation doses.

In agreement with our study, Antonio et al. (2011) reported that storage time (up to 60 days) had a much higher influence on the variation, with irradiation (up to 0.54 kGy) being a minor contributor to change the phenolic content in chestnut during storage.

Nothing was reported on the effect of gamma irradiation on this property of olive oil. However, the effect of the irradiation treatments on phenolic contents was reported for other plants and spices. Our finding was in agreement with that of Koseki et al. (2002) who reported that gamma irradiation, up to 30 kGy, decreased total phenolics in dehydrated rosemary. Gamma irradiation treatment have been shown to either increase or decrease the total phenolics content of plant materials, which is dependent on the dose delivered and the raw materials used. Khattak, Simpson, and Ihasnullah (2009) reported that gamma irradiation at dosage levels of 1, 2, 4

Table 2: Effect of gamma irradiation and storage period on TBA value (mg MDA kg^{-1} oil) and total phenolics (mg gallic acid kg^{-1} oil) of olive oil

Treatments		Control	1 kGy	2 kGy	3 kGy	P-Value
Type		**TBA value (mg MDA/kg Oil)**				
0 days	**6 months**	0.022 ± 0.001^{aA}	0.023 ± 0.001^{aB}	0.023 ± 0.001^{aA}	0.023 ± 0.001^{aB}	**NS**
	12 months	0.026 ± 0.001^{aA}	0.024 ± 0.001^{aA}	0.023 ± 0.001^{aA}	0.024 ± 0.001^{aA}	**NS**
	P-Value	**NS**	*	**NS**	*	
30 days	**0 months**	0.025 ± 0.001^{aC}	0.025 ± 0.001^{aA}	0.026 ± 0.001^{aA}	0.025 ± 0.001^{aA}	**
	6 months	0.022 ± 0.001^{bB}	0.023 ± 0.001^{aAB}	0.023 ± 0.001^{aB}	0.024 ± 0.001^{aB}	**
	12 months	0.023 ± 0.001^{bA}	0.023 ± 0.001^{aB}	0.023 ± 0.001^{aB}	0.024 ± 0.001^{aB}	**NS**
	P-Value	**	**NS**	**	**	
45 days	**0 months**	0.026 ± 0.01^{aA}	0.024 ± 0.001^{aA}	0.027 ± 0.001^{aA}	0.024 ± 0.001^{aB}	**NS**
	6 months	0.023 ± 0.01^{aB}	0.024 ± 0.001^{aA}	0.024 ± 0.001^{aA}	0.024 ± 0.001^{aA}	**NS**
	12 months	0.024 ± 0.01^{bC}	0.025 ± 0.001^{abA}	0.026 ± 0.001^{aA}	0.026 ± 0.001^{aAB}	*
	P-Value	**	**NS**	**NS**	*	
		Total phenolics (mg gallic acid/kg Oil)				
0 days	**6 months**	281.56 ± 3.12^{aA}	276.32 ± 5.28^{aA}	255.24 ± 13.18^{bB}	258.21 ± 11.67^{bB}	*
	12 months	165.72 ± 3.43^{aB}	164.50 ± 4.70^{aB}	164.24 ± 3.49^{aC}	171.15 ± 3.55^{aC}	**NS**
	P-Value	**	**	**	**	
30 days	**0 months**	194.53 ± 5.56^{aA}	192.16 ± 1.97^{aA}	184.26 ± 4.48^{bA}	179.35 ± 2.46^{bA}	**
	6 months	168.47 ± 1.77^{aB}	164.77 ± 1.37^{abB}	162.90 ± 3.66^{bB}	160.04 ± 3.49^{bB}	*
	12 months	136.56 ± 3.11^{aC}	139.53 ± 0.71^{aC}	136.72 ± 1.51^{aC}	138.22 ± 2.46^{aC}	**NS**
	P-Value	**	**	**	**	
45 days	**0 months**	154.43 ± 4.56^{aA}	151.68 ± 4.26^{aA}	150.38 ± 0.90^{abA}	145.04 ± 0.60^{bA}	*
	6 months	143.88 ± 3.54^{aB}	138.21 ± 3.18^{bB}	133.10 ± 2.74^{bcB}	138.72 ± 1.69^{abB}	*
	12 months	121.62 ± 1.56^{abC}	123.29 ± 1.48^{aC}	119.68 ± 1.37^{bC}	120.12 ± 1.70^{bC}	*
	P-Value	**	**	**	**	

^{abc} Mean values in the same column not sharing a superscript are significantly different.
^{ABC} Mean values in the same row not sharing a superscript are significantly different.
NS: not significant.
* Significant at $p<0.05$.
** Significant at $p<0.01$.

and 6 kGy increased the yield of total phenolics in *Nelumbo nucifera* rhizome. In contrast to this results, it has been found that gamma irradiation with doses of 5, 10, 15, 20 and 25 kGy did not have any significant change in the total phenolic contents of olive leaves (Aouidi, Ayari, Ferhi, Roussos, & Hamdi, 2011). When compared with respective control seed materials, irradiation (with 2, 4 and 6 kGy) significantly ($p<0.05$) increased the total phenolic contents. This might be due to the increased extractability of phenolics by depolymerisation and dissolution of cell wall polysaccharides after irradiation (Bhat, Sridhar, & Tomita-Yokotani, 2007).

3.5 Effect of gamma irradiation and storage time on iodine value of olive oil

The iodine value (IV) (degree of un-saturation) of olive oils obtained from olives stored at ambient temperature for 0, 30 and 45 days were found to be 93.38, 88.44 and 83.89 g I_2 100 g^{-1} oil, respectively (Table 3). The values support that the oil is unsaturated but not highly. The IV of olive oil was significantly ($p<0.05$) changed by gamma irradiation and storage time. In general, the doses of gamma irradiation used (1, 2 and 3 kGy) and storage time of oils (6 and 12 moths) significantly ($p<0.05$) decreased the iodine value of olive oil. The decrease in IV upon irradiation

Table 3: Effect of gamma irradiation and storage period on iodine number (g I_2 100 g^{-1} oil) and saponification value (mg KOH. g^{-1} oil) of olive oil

Treatments		Control	1 kGy	2 kGy	3 kGy	P-Value
Type		**Iodine number (g I_2 100 g^{-1} Oil)**				
0 days	**0 months**	93.38 ± 0.46^{aA}	90.33 ± 4.30^{aA}	90.26 ± 1.92^{aA}	90.75 ± 0.15^{aA}	**NS**
	6 months	82.43 ± 1.41^{abB}	83.63 ± 0.76^{aB}	81.80 ± 0.72^{abC}	80.80 ± 0.99^{bC}	*
	12 months	84.17 ± 1.65^{aB}	84.32 ± 0.59^{aB}	84.30 ± 0.23^{aB}	83.47 ± 0.35^{aB}	**NS**
	P-Value	**	*	**	**	
30 days	**0 months**	88.44 ± 1.70^{aA}	84.40 ± 1.95^{abAB}	80.88 ± 6.03^{bA}	79.87 ± 0.92^{bB}	*
	6 months	84.65 ± 0.75^{bB}	85.14 ± 1.57^{bA}	84.41 ± 0.30^{bA}	87.40 ± 1.27^{aA}	*
	12 months	81.58 ± 1.94^{aB}	79.88 ± 3.36^{aB}	80.30 ± 1.17^{aA}	80.33 ± 1.06^{aB}	**NS**
	P-Value	**	*	**NS**	**	
45 days	**0 months**	83.89 ± 0.46^{aAB}	83.71 ± 1.37^{aAB}	81.94 ± 1.55^{abB}	80.61 ± 1.02^{bB}	*
	6 months	86.78 ± 1.11^{aA}	86.56 ± 0.82^{aA}	83.90 ± 1.87^{bAB}	83.57 ± 1.08^{aB}	**
	12 months	82.80 ± 2.49^{bB}	81.38 ± 2.69^{bB}	85.99 ± 1.56^{bA}	91.87 ± 2.94^{aA}	**
	P-Value	*	*	**	**	
		Saponification value (mg KOH. g^{-1} Oil)				
0 days	**0 months**	194.88 ± 1.08^{aBB}	196.74 ± 1.29^{aA}	197.06 ± 0.76^{aA}	195.66 ± 2.03^{aA}	**NS**
	6 months	193.38 ± 0.46^{aB}	192.43 ± 1.34^{aB}	191.60 ± 0.86^{aB}	192.93 ± 1.28^{aA}	**NS**
	12 months	196.07 ± 0.79^{aA}	195.55 ± 0.97^{abA}	195.79 ± 1.09^{bA}	194.79 ± 1.03^{abA}	**NS**
	P-Value	*	*	**	**NS**	
30 days	**0 months**	195.03 ± 1.20^{aA}	194.28 ± 1.85^{aA}	194.23 ± 0.37^{aA}	191.57 ± 0.98^{bA}	*
	6 months	192.69 ± 1.27^{aB}	193.44 ± 1.04^{aA}	193.88 ± 1.70^{aA}	191.85 ± 1.44^{aA}	**NS**
	12 months	194.99 ± 0.89^{aA}	194.88 ± 0.65^{abA}	195.14 ± 0.26^{abA}	194.29 ± 0.59^{bB}	**NS**
	P-Value	*	**NS**	**NS**	**	
45 days	**0 months**	197.32 ± 1.62^{aA}	196.37 ± 2.52^{aA}	194.76 ± 1.01^{aA}	194.50 ± 1.99^{aA}	**NS**
	6 months	193.74 ± 0.57^{aB}	194.00 ± 0.51^{aA}	193.90 ± 0.91^{aA}	193.93 ± 0.54^{aA}	**NS**
	12 months	194.13 ± 0.48^{aB}	193.70 ± 0.31^{aA}	193.80 ± 0.38^{aA}	194.43 ± 0.67^{aA}	**NS**
	P-Value	**	**NS**	**NS**	**NS**	

^{abc} Mean values in the same column not sharing a superscript are significantly different.
^{ABC} Mean values in the same row not sharing a superscript are significantly different.
NS: not significant.
* Significant at $p<0.05$.
** Significant at $p<0.01$.

or storage could be attributed to some loss in the un- saturated fatty acids of olive oil by irradiation and the formation of peroxide compounds. These results are in agreement with those obtained by several investigators who reported a decrease in the IV of soybean sesame, peanut oil upon irradiation (Afify, Rashed, Ebtesam, & El-Beltagi, 2013). Radiation probably broke some double bonds and induced oxidation processes in fatty acids resulted in its saturation (Anjum, Anwar, Jamil, & Iqbal, 2006). These results also agree with other studies, which found that, the unirradiated samples had highest iodine values, suggesting saturation of oils as a results of irradiation (El-Aziz & El-Kalek, 2011).

3.6 Effect of gamma irradiation and storage period on saponification value of olive oil

Table 3 shows the saponification value (SV) data reported as mean value of oils obtained from olive fruits treated at 0, 1, 2 and 3 kGy of gamma irradiation and stored at ambient temperature for 0, 30 and 45 days before extraction and stored oil at ambient temperature for 0, 6 and 12 months after extraction. Initial SV for olive oil obtained from fruits stored at ambient temperature for 0, 30 and 45 days were 194.88, 195.03 and 197.32 mg KOH g^{-1} oil, respectively. The

applied irradiation doses did not cause any significant change in the SV of olive oils. The current study demonstrated that storage time caused significant ($p<0.05$) changes in the SV of olive oil obtained from non-irradiated olives and stored at ambient temperature for 0, 30 or 45 days. The SV of the analyzed oils extracted after 0, 30 and 45 days of irradiated olives at 0, 1, 2 and 3 kGy and stored for 0, 6 and 12 months ranged from 191.57 to 197.32 mg KOH g^{-1} oil. These values fall within the recommended Codex for virgin olive oil (Codex Alimentarius, 2003). Regarding the effects of gamma radiation on the SV of olive oil, results of this study were in contrast with a previous study, where SV of irradiated pistachio was increased upon irradiation (Al-Bachir, 2015b). Whereas, a decrease in SV was found in gamma irradiated almonds (Al-Bachir & Zeinou, 2014). Therefore, it can be concluded that the effect of gamma irradiation on SV of oils is affected by plant species.

4 Conclusion

The results of this study demonstrated that the acid values of the oil extracted immediately after harvest were lower than 2% which is the limit set for "virgin olive oil quality". While the AV of oil extracted from olive fruit stored for 30 days exceed the standard limit of 3.3%, indicating that these oils could not be labeled as ordinary virgin olive oil quality (International Olive Council, 2015). The AV of olive oil was significantly ($p<0.05$) decreased by gamma irradiation. Also, phenol content in olive oil was significantly ($p<0.05$) decreased by gamma irradiation and storage time.

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Effects of Heat Treatment by Immersion in Household Conditions on Olive Oil as Compared to Other Culinary Oils: A Descriptive Study

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Abstract

The objectives were to evaluate the properties of refined (ROO) and extra-virgin olive oil (EVOO) in their natural state (fresh) and after heating, while comparing them with each other and with refined soybean (SBO) and refined sunflower seed oil (SFO). The methodology was designed to simulate, in controlled laboratory conditions, the home-frying process, while evaluating fatty acid profile (fatty acid methyl esters were separated by gas chromatography), concentration of phenolic compounds (Gallic acid dosage), antioxidant activity (DPPH), and production of polar compounds (thin layer chromatography) before and after heating to 200 °C for six minutes. It was observed that, before and after heating, SBO and SFO are rich in polyunsaturated fatty acids (FA) and ROO and EVOO are rich in monounsaturated FA. Fresh or heated, ROO and EVOO do not have trans FA, which are present in SBO and SFO, and increase in SBO after heating (+ 32.8%). The concentrations of phenolic compounds are always higher in olive oils, despite the decrease that occurs after heating (-7.5% in the ROO and -24.6% in EVOO). Antioxidant activity is greater when olive oils are fresh and remains present in EVOO after heating. The concentration of polar compounds was similar for all oils after heating. In conclusion, ROO and EVOO are the richest in monounsaturated FA even after heating, with no production of saturated or trans FA. Despite losing some antioxidant activity, heated EVOO remains richer in monounsaturated FA than ROO, SBO and SFO in the fresh version. All oils suffer similar rates of degradation.

Keywords: Olive Oil; Thermic Treatment; Oxidation stability; Phenolic Compounds; Mediterranean Diet; Oils

1 Introduction

Vegetable oils are widely used in home food preparation, either fresh as seasoning of cold dishes or added to the preparations that will be cooked, baked or fried by immersion. Oils from different sources such as olive, soybean, sunflower seed, canola and corn germ, among others can be found in the market, and the composition of each has differences that can impact the user's health. In respect to olive oil, studies conducted over the past few decades have demonstrated many health benefits due to the high content of oleic acid (18:1n-9), and the presence of α-linolenic acid (18:3n-3), antioxidants and free radical scavengers (Guasch-Ferre et al., 2014; Schwingshackl, Christoph, & Hoffmann, 2015; Schwingshackl & Hoffmann, 2014a, 2014b).

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Nomenclature

ROO	refined olive oil	SBO	soybean oil
EVOO	extra-virgin olive oil	SFO	sunflower seed oil

There are several different presentations of olive oil in the market. Extra-virgin is obtained from the fruit of the olive tree (*Olea europea* L.) by mechanical means only, without any treatment other than washing, filtrating, decanting, and centrifuging (Codex, 2015). The minor components from the olive fruit, including phenolic compounds remain in the final product, unlike other vegetable oils that are subjected to refining processes (Ballus, 2014; Shahidi & Zhong, 2005). The non-extra virgin olive oil is a blend of refined olive oil and virgin olive oil. The "Mediterranean" food standard, recognized by science as one of the healthiest, has in olive oil one of its main components, according to the Second International Conference on Olive Oil and Health (Lopez-Miranda et al., 2010). The main benefits of this food recognized by science are: it reduces LDL-cholesterol (LDL-c), increases HDL-cholesterol (HDL-c)/LDL-c, reduces LDL-c oxidability, improves glucose metabolism, helps blood pressure control and endothelial function, promotes antithrombotic environment, has a favorable effect against obesity, promotes less activation of fasting and post prandial nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB), reduces age-related cognitive decline and Alzheimer's disease (Lopez-Miranda et al., 2010).

In Brazil, the olive oil consumption of about 200 milliliters *per capita* per year is quite low as compared to other countries. In Greece it is 26 liters, in Spain 12 liters, and in Portugal 7 liters (Nogueira-de-Almeida, Ribas Filho, de Mello, Melz, & Almeida, 2015). One possible reason for this low consumption is the combination of high price compared to other vegetable oils, and the idea that its use should be restricted to finishing cold dishes. There is, in fact, the belief that, once heated, olive oil would suffer changes in its lipid profile, thereby degrading and losing its beneficial health properties. Additionally, even recognizing that olive oil may be the best option when used fresh, it is commonly believed that after heating, it becomes worse than other oils in relation to nutritional quality (Nogueira-de-Almeida et al., 2015). Oil can be heated in many ways, but the most common process is by frying, which leads to multiple reactions that generate mostly non-volatile, chemical compounds, i.e., they remain in the oil, affecting their physical properties (Katragadda, Fullana, Sidhu, & Carbonell-Barrachina, 2010). The quality of the foods cooked by this method is dependent on: frying conditions, temperature, time, type of food, and volume and type of oil used (Katragadda et al., 2010). The formation of aldehydes and volatile compounds during frying depends mainly on the process temperature. The trend towards the production of volatile compounds also depends on the fatty acids' composition (Katragadda et al., 2010). Heating also modifies many properties of oils, such as the profile of fatty acids, antioxidant capacity, and nutritional quality (Nogueira-de-Almeida et al., 2015; Valderas-Martinez et al., 2016). Some of these can be evaluated by laboratory indicators, such as the composition of fatty acids, the antioxidant capacity, the amount of phenolic compounds, and degradation (Allouche, Jimenez, Gaforio, Uceda, & Beltran, 2007; Ballus, 2014; Cicerale, Conlan, Sinclair, & Keast, 2009; Daskalaki, Kefi, Kotsiou, & Tasioula-Margari, 2009; Freire, Ferreira, & Mancini-Filho, 2013; Nunes, de Souza, Correa, & da Costa e Silva, 2013; Santos, Cruz, Cunha, & Casal, 2013). This study intended to describe properties of two types of olive oil in their natural state and after heating at home-

frying simulation, while comparing them to other oils used for cooking.

2 Methods

Four options of the most used oils for home cooking in Brazil were defined for the study: refined olive oil (ROO), characterized as a blend of refined olive oil and virgin olive oil; extra-virgin olive oil (EVOO) characterized as a mixture of extra-virgin olive oils; refined soybean oil (SBO) and refined sunflower seed oil (SFO).

Some parameters were defined considering that the main objective of the study was to evaluate the effects of heat treatment: temperature, type, and duration of heating. We chose to mimic, in controlled laboratory conditions, the process of home-frying, since it is one of the most common and more drastic heat treatment used in food preparation. Therefore, we researched products available in the Brazilian market that indicated on the label their suitability for frying and manufacturer's instructions on the correct way of preparation. Temperature and heating time were set at 200 °C for 6 minutes, an estimated average time to prepare 1 portion of food used as a reference: potatoes, nuggets, chicken fingers, fish, squid, croquette, polenta, meat, churros, and vegetables. These products were used only to obtain references for the methodology. The trials were conducted only with the oils, and no foods were prepared.

About 5 liters of oil were used for each heat treatment. All samples were obtained, by donation or purchased in supermarkets in the city of São Paulo, within the expiration date as described by the manufacturers on the label. The same batch as described by the manufacturer on the label was used for each sample, which were coded with 3 non-sequential and random numbers, making it impossible to identify them at the time of the analytical methodology. The samples were weighed, and after setting the fryer to 200 °C they were deposited into the tanks and the temperature monitored to reach the target value of the heat treatment. The temperature was measured for each sample at time zero, and thereafter at every minute until reaching 200 °C. Once the target temperature was reached, it was measured every minute to establish the specific range of the heat treatment of each sample as detailed in Table 1. After the heat treatment, the samples were immediately placed in amber vials with no head-space in order to prevent oxidation, and cooled in a freezer until the temperature reached below -10 °C.

Determination of fatty acids was carried out on a single sample of each oil, and the other tests in a set of three. All measurements were performed at the Instituto de Tecnologia de Alimentos (Food Technology Institute) (ITAL) of the State of São Paulo, with the exception of the polar compounds, which were measured by the Oils and Fats Laboratory of the Food Engineering School of Unicamp.

The following properties were measured before and after heat treatment for each sample:

Antioxidant activity: determination of antioxidant activity based on the ability of the sample to reduce 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, as described by Brand-Williams, Cuvelier, and Berset (1995).

Phenolic compounds: Concentration of phenolic compounds expressed as gallic acid equivalents, as proposed by Kim, Chun, Kim, Moon, and Lee (2003).

Determination of fatty acid composition: Conducted according to the Ce 1a-13 methodologies, CE 1h-05 and 996.06 of the American Oil Chemists' Society (American Oil Chemists' Society, 2009).

Determination of polar compounds: Column chromatography using methodology standardized by the American Oil Chemists' Society (2009).

3 Results and Discussion

The results were expressed using the fresh values, in the case of fatty acids (in which only one

Table 1: Conditions of Heat Treatment

Samples	Time to reach the 200°C goal temperature	Temperature variation during heat treatment of 200°C for 6 minutes
Soybean oil	5 minutes and 21 seconds	201.97°C ± 2.13
Sunflower seed oil	6 minutes and 3 seconds	200.9°C ± 1.72
Refined olive oil	6 minutes and 16 seconds	199.27°C ± 2.08
Extra-virgin olive oil	5 minutes and 22 seconds	200.47°C ± 1.78

sample of each oil was measured), and as means and standard deviations for phenolic compounds, gallic acid and polar compounds (where determination was performed on three samples of each type of oil), being grouped in tables. A comparison between the characteristics of each of the oils at time zero and after heat treatment was presented in a descriptive way.

3.1 Results

The results are depicted in Tables 2 and 3.
Table 2 demonstrates that the four oils studied are different in their composition when fresh, and the differences remain after heating. SFO is the poorest in saturated fatty acids and this profile persists after heating. Olive oils (ROO and EVOO) are the richest in monounsaturated fatty acids and this profile also remains after heating. SBO and SFO are quite rich in polyunsaturated fatty acids, and SBO is especially rich in omega-3, and this profile persists after heating. There is also little change in the saturated fatty acid profile after heating, although for EVOO the amount is reduced. There is also little change in the monounsaturated fatty acid profile, although for EVOO the concentration increases. There is little change in the polyunsaturated fatty acid profile, although for EVOO there is a reduction in levels of both omega-3 and omega-6. It is important to state that this is a descriptive study, so we did not conduct statistical analysis that would be relevant to support the findings.
When fresh, olive oils have no trans fatty acids, although they are found in SBO and SFO. After heating, olive oils still do not contain trans fatty acids but they increase in amount in SBO and remain present in SFO.
The quantities of phenolic compounds are much higher in olive oils and this difference remains after heating. This process decreases the amount of these compounds in olive oils (loss of 7.5% in ROO and 24.6% in EVOO), and they become undetectable in SBO and SFO. Even considering the decrement in phenolic compounds, heated olive oils are still richer in these compounds than SBO and SFO in the fresh version.
The antioxidant activity evaluated by the DPPH method is greater in fresh olive oils and remains significant for EVOO after heating. SBO and SFO have no antioxidant activity, neither before nor after heating. Olive oils lose their antioxidant activity upon heating (100% for ROO and 26.6% for EVOO). Even considering the loss of antioxidant activity, heated EVOO is still richer in these compounds than ROO, SBO and SFO in the fresh version.
The results for the polar compounds demonstrate that all oils suffer some degree of degradation and although having different concentrations of polar compounds when fresh, after heating the values are very similar.
Table 3 depicts the individual fatty acids. The profile of SBO and SFO differs significantly from olive oils. The omega-9 content is 66% less for SBO and 47% less for SFO than for both olive oils.
Some qualitative results on the characteristics of the samples during the heat treatment have to be underscored: SBO and SFO caused a burning sensation on the researcher's eyes during the frying test, possibly due to the production of acrolein (Beauchamp, Andjelkovich, Kligerman, Morgan, & Heck, 1985) and this was more intense with SBO. ROO hardly caused any discomfort. EVOO caused a perception of burning in the mucosa of the digestive tract, possibly due

Table 2: Fatty acids, phenolic compounds, antioxidant activity and polar compounds

	Refined Olive Oil		Extra-virgin Olive Oil		Soybean oil		Sunflower seed oil	
	T 0	T 1	T 0	T 1	T 0	T 1	T 0	T 1
Saturated fatty acids (g/100g)	15.32	15.32	16.86	14.94	14.26	14.61	9.31	9.11
Monounsaturated fatty acids (g/100g)	71.73	71.85	67.40	71.53	24.24	24.98	37.52	37.84
Polyunsaturated fatty acids (g/100g)	8.44	8.25	11.22	8.89	56.26	54.88	48.61	48.52
Polyunsaturated omega 3 fatty acids (g/100g)	0.60	0.60	0.65	0.58	5.67	5.48	0.18	0.16
Polyunsaturated omega 6 fatty acids (g/100g)	7.84	7.65	10.57	8.31	50.59	49.41	48.43	48.35
Total trans isomers fatty acids (g/100g)	ND	ND	ND	ND	0.64	0.85	0.15	0.13
Non-identified fatty acids (g/100g)	0.11	0.17	0.11	0.24	0.19	0.28	ND	ND
Phenolic compounds (Gallic acid mg/100g) average (SD)	10.33 (0.56)	9.55 (0.89)	14.88 (0.22)	11.94 (0.66)	4.59 (0.35)	Traces	4.63 (0.31)	Traces
Antioxidant activity (gDPPH/Kg) average (SD)	0.35 (0.02)	ND	0.65 (0.02)	0.49 (0.03)	ND	ND	ND	ND
Polar compounds (%) Average (SD)	5.43 (0.63)	6.20 (0.37)	4.16 (0.24)	5.87 (0.63)	4.18 (0.41)	5.59 (0.67)	4.17 (0.75)	6.22 (0.85)

Table 3: Fatty acids, phenolic compounds, antioxidant activity and polar compounds

		Refined Olive Oil		Extra-virgin Olive Oil		Soybean oil		Sunflower seed oil	
		T 0	T 1	T 0	T 1	T 0	T 1	T 0	T 1
Palmitic	C 16:0	12.29	12.24	13.99	11.8	10.54	10.63	5.14	2.02
Palmitoleic (omega 7)	C 16:1	1.06	1.06	1.43	0.98	0.09	0.09	0.09	0.09
Margaric	C 17:0	0.07	0.07	0.07	0.10	0.07	0.08	ND	ND
Cis-10-heptadecanoic	C 17:1	0.14	0.14	0.14	0.16	ND	ND	ND	ND
Stearic	C 18:0	2.61	2.67	2.50	2.72	3.09	3.37	3.33	3.27
Oleic (omega 9)	C 18:1	70.29	70.37	65.60	70.12	24.16	24.89	37.27	37.60
Linoleic (omega 6)	C 18:2	7.84	7.65	10.57	8.31	50.59	49.41	48.43	48.35
Arachidic	C 20:0	0.34	0.35	0.31	0.33	0.20	0.22	0.19	0.18
Alfa linoleic (mega 3 alpha)	C 18:3	0.60	0.60	0.65	0.58	5.67	5.48	0.18	0.16
Cis-11-eicosenoic	C 20:1	0.23	0.28	0.22	0.27	ND	ND	0.16	0.15
Trans linoleic (omega 6 t)	C 18:2	ND	ND	ND	ND	0.28	0.38	0.15	0.13
Trans linolenic (omega 3 t)	C 18:3	ND	ND	ND	ND	0.36	0.47	ND	ND
Behenic	C 22:0	ND	ND	ND	ND	0.29	0.24	0.54	0.54
Lignoceric	C 24:0	ND	ND	ND	ND	ND	ND	0.11	0.11
Myristic	C 14:0	ND	ND	ND	ND	0.08	0.08	ND	ND
Not identified		0.11	0.18	0.11	0.23	0.19	0.28	ND	ND

to volatilization of spicy compounds which are typical of this type of product.

Although many oils are used in industry, often in extreme temperature conditions, it is important to understand how they behave in household use and thus provide health professionals with results that can help educate their patients.

Data presented in this study were obtained simulating household conditions and it is worth remembering that the experiments were conducted only once under controlled temperature and time. It is known that if the same oil is used repeatedly for frying, its properties are progressively lost and there is the risk of occurrence of toxic by-products (Freire et al., 2013).

The fatty acids' profile directly affects the sensory properties of oils in the parameters of texture and melting point, which impacts on the stability of the oil oxidation when exposed to light and oxygen during the shelf life of the product. Saturated fatty acids increase the melting point, providing firmer texture than polyunsaturated and monounsaturated acids, even when the oil is in liquid form. In this study, the oil with the lowest percentage of saturated fatty acids was SFO, which possibly gives it a lower melting point. The high content of oleic acid in olive oil is important because it is less susceptible to oxidation than polyunsaturated fatty acids, which predominate in other oils, therefore contributing to its stability (Owen et al., 2000). It also helps to reduce serum levels of LDL cholesterol and increase HDL cholesterol. α-linolenic acid (ALA, 18:3n-3) helps reduce the risk of heart disease, decreases blood pressure, and protects against the formation of plaques in the arteries (Cicerale et al., 2009).

Many metabolic reactions that occur physiologically in the human body cause reduction of molecular oxygen releasing reactive species known as free radicals. Due to the damage caused by such radicals in the metabolism and induction of various diseases, they must be inactivated. That role is played by antioxidants which can either be produced by the body or ingested in the diet. One way to evaluate the antioxidant capacity of oils is by the DPPH method. In this study and by this method, SBO and SFO did not show antioxidant activity, not even when fresh. Refined olive oil exhibited antioxidant activity only before heating but EVOO exhibited antioxidant activity before and after heating, with greater activity than the other oils even after being heated. Phenolic compounds such as gallic acid, which was measured in this study, are among the antioxidants found in plant foods. These compounds can reduce blood pressure and protect against the formation of atheroma plaques (Cicerale et al., 2009). EVOO is not refined, therefore containing many unsaponifiable compounds, especially phenols, which would generally be removed by refining (Caruso et al., 1999; Ragazzi & Veronese, 1973). In fact, the data demonstrated that gallic acid is present in olive oils, in higher amounts in EVOO and, after heating, can still be found in quantities higher than in the other oils studied even when fresh. Fresh SBO and SFO contain gallic acid and may, therefore, be considered as having an antioxidant effect but, when heated, even at a home process temperature and for a short time, completely lose this property. The phenolic compounds of olive oils have shown higher antioxidant properties than vitamin E in lipids and in DNA oxidation *in vitro* and *ex vivo* (Fitó et al., 2007). They are also able to prevent endothelial dysfunction, decreasing the expression of cell adhesion molecules and increasing the production of nitric oxide (Fitó et al., 2007). In addition, they inhibit platelet aggregation and improve the mRNA transcript of the antioxidant enzyme glutathione peroxidase (Fitó et al., 2007). Other potential activities include anti-inflammatory chemopreventive activity and delay in the progression of atherosclerosis (Fitó et al., 2007). Phenolic compounds play an important role in the quality of olive oils, as they contribute significantly to the oxidative stability. EVOO has remarkable resistance to oxidation, which has been related to its composition in fatty acids and high levels of natural antioxidants such as phenolic hydrophilic and lipophilic compounds (Papadimitriou et al., 2006).

Other authors have investigated the effect of heat on edible oils. Daskalaki et al. (2009) found that by frying at a temperature of 180 °C there was a 60% reduction of phenolic compounds (hydroxytyrosol derivatives) in EVOO after 30 minutes. At 100 °C for 2 hours, the decrement was of less than 20%. Amati et al. (2008) also observed

partial degradation of phenolic compounds in EVOO, but only at 180 °C. Sánchez-Muniz and Bastida (2006) considered that olive oils are the most suitable for frying because they are more resistant to heat oxidation. Santos et al. (2013) stated that, even recognizing loss of antioxidant properties, EVOO is the most stable household oil, but emphasized that it is important not to expose it to excessive temperatures, especially for long periods, which could lead to loss of its properties. Allouche et al. (2007) evaluated the decrement of antioxidants in olive oil and found that tocopherol and polyphenol were the most affected by heat treatment and decreased the most. Sanchez-Gimeno, Negueruela, Benito, Vercet, and Oria (2008) when comparing the behavior of olive oil to SFO concluded that, during frying, olive oil was the more stable to an oxidative process and was able to sustain its rich oleic acid composition. Allouche et al. (2007) found that the concentration of oleic acid, the most abundant fatty acid in olive oil, does not change with the duration of heating. Penz (2010) reported that the lipid profile of olive oil suffers little change after heat treatment. Furthermore, according to these authors, heating does not lead to a loss of phytosterol; on the contrary, the levels may increase. This may be due to an increased solubility of these compounds with increasing temperature.

The evaluation of oil degradation after heat treatment can be done by checking the production of polar compounds, which are derived from oxidation, hydrolysis or other degradation processes. Those are compounds present in the hydrophilic phase of oil, which is unwanted, since it is an oil-based product (lipophilic). The quantification of polar compounds has been the most efficient method to measure the quality of oils (Carapinha, 2012). In Portugal, for example, legislation sets the value of 25% as the maximum allowed in the oils used for frying (Carapinha, 2012). The assessment is made by the total content of these compounds, whose formation is strongly related to the primary and secondary stages of oxidation, demonstrating the oil degradation (Kalantzakis, Blekas, Pegklidou, & Boskou, 2006). The oily phase, hydrophobic, must be the major and almost total composition of oils. The results of this study demonstrate that polar compounds already appear in the fresh version at least for ROO, SBO and SFO, and may have been formed during the refining process, which includes heating steps. After the frying tests, it was noted that the 4 oils had similar concentrations of polar compounds, which demonstrates some degree of degradation always occurs when they are heated. Dobarganes (1992) found that when compared to other oils commonly used (sunflower seed, soybean, and palm oil), olive oils are the most stable, with less production of polar compounds after five hours of thermal oxidation and even after being used 15 times for frying. The fatty acid composition of olive oils favors oxidation stability, generating less polar compounds while maintaining the integrity of the lipophilic phase naturally present in olive oils. If foods were to be fried in the oils during heat treatment, the amounts of polar compounds would be different due to the water activity of the food, oil absorption and food temperature before being added to the fryer. Likewise, if the oils had been tested in successive frying, the results of polar compounds would possibly rise according to the number of re-uses (Freire et al., 2013).

In order to make recommendations based on the available scientific evidence, some authors draw conclusions about olive oil. Sacchi, Paduano, Savarese, Vitaglione, and Fogliano (2014) considered olive oil as the best option for food preparation, after observing that heating it hardly led to the production of toxic products such as acrylamide and, even when considering this possibility, the antioxidants present in olive oil would inhibit its formation. Dobarganes (1992) considered that, in the fresh version, olive oil is the best choice, especially for its organoleptic characteristics; for frying, it would also be the best option, due to its greater stability.

This is a descriptive study, which is its main limitation. The tests were made in a single sample of each type of oil or at the most in three samples, therefore statistical analysis was not possible. However, in the absence of similar studies in oils marketed in Brazil, the present data can serve as foundation for future research. Additionally, the researchers chose to conduct a descriptive study, considering the budget available to include more indicators at the expense of more samples. Another limitation is the fact that food

was not used (only the oils were heated) and this is an important issue, because the presence of moisture in the food is quite relevant during the frying process. With the inclusion of food, the process could approach that of real cooking, but on the other hand, variables related to the chosen food would be introduced and the results could not be generalized. The option therefore was for a strictly experimental study, bringing concrete information on the behavior of the oil itself.

4 Conclusions

The heating tests of this study, in conditions similar to home-frying, demonstrated that ROO and EVOO are the richest in monounsaturated fatty acids and that they retained this profile after heating, without any observed formation of saturated fatty acids. When fresh, ROO and EVOO have no trans fatty acids but these are present in SBO and SFO. After heating, ROO and EVOO also contain no trans fatty acids but they increase in amount in SBO and remain present in SFO. The quantities of phenolic compounds are higher in ROO and EVOO and this difference remains after heating. Even considering the loss of antioxidant activity, the heated EVOO is still richer in these compounds than ROO and SBO, and SFO in the fresh version. All oils suffer some degree of degradation and, whilst containing different concentrations of polar compounds when fresh, they have similar amounts after heating.

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Fermentation of Tender Coconut Water by Probiotic Bacteria *Bacillus coagulans*

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Abstract

Coconut water is currently being considered as an elixir for patients suffering from diseases like dengue and malaria as well as chikungunia to provide hydration properties to the body. It has become a popular beverage for many people owing to its palatability and high mineral content. In this study, the growth, survival and fermentation performance of the probiotic bacterium *Bacillus coagulans* in coconut water was assessed in order to produce a novel non-dairy, probiotic beverage. The species was characterized on the basis of morphology, physiology and biochemical parameters and its probiotic attributes were assessed. Batch fermentations were carried out for 2 days at a constant 37°C, thereafter the samples were subjected to microbiological and chemical analysis. The results suggested that the specie produced lactic acid and was acid and bile tolerant. The pH and titratable acidity of probiotic fermented coconut water were found to be 4.4 and 0.53 % lactic acid, respectively. The viscosity of fermented coconut water increased significantly from an initial 5.13 mPa.s to 5.35 mPa.s because of the increase in soluble solids content due to exopolysaccharide production by *B. coagulans* during fermentation. Also, the overall acceptability score of probiotic coconut water was higher than tender coconut water, suggesting its feasibility for use as a probiotic beverage.

Keywords: Probiotic non dairy beverage; Fermented coconut water; Sensory evaluation; Physico-chemical characteristics

1 Introduction

According to the World Health Organization and the Food and Agriculture Organization of the United Nations (FAO / WHO, 2001), probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". Numerous studies have highlighted the health benefits associated with consumption of probiotic bacteria. In the past decade, there has been an increase in consumer demand for functional foods such as yoghurt and other fermented dairy products supplemented with probiotic organisms (Penna, Rao-Gurram, & Barbosa-Canovas, 2007). However, dairy substrates may contain potential allergens, such as casein and they require cold storage to enhance their shelf life. Also, the cholesterol content of dairy products is high. Owing to such facts and the increasing trend of vegetarianism , the demand for novel products with non-dairy matrices has expanded (Ranadheera, Baines, & Adams, 2010). Also producing probiotic products with foods and beverages which are part of day-to-day life is encouraged. This has led to an increased demand for non-dairy probiotic foods, such as coconut aqueous extract, fruit drinks, nutrition bars, soy products and cereal-

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based products. The nutritive values and wide distribution of these raw materials are important when they are used as functional food items (Angelov, Gotcheva, Kuncheva, & Hristozova, 2006). Tender coconut water (TCW), the liquid endosperm obtained from immature green coconuts, in its natural form is a refreshing and nutritious beverage, widely consumed around the world due to its beneficial health properties (Pummer, Heil, Maleck, & Petroianu, 2001). Moreover, coconut water plays an important alternative role for oral rehydration and even for intravenous hydration of patients in remote regions (Campbell-Falck, Thomas, Falck, Tutuo, & Clem, 2000) in addition to providing protection against induction of myocardial infarction (Anurag & Rajamohan, 2003). It was identified in the late 1930s as a nutrient helping to reduce anemia in pregnancy (Jackson, Gordon, Wizzard, McCook, & Rolle, 2004) and which also helped to prevent mitochondrial toxicity induced by methanol metabolites. The major chemical constituents of coconut water are sugars and minerals and minor ones are fat and nitrogenous substances.

Interestingly, the perception and utilization of coconut water has evolved over the years owing to its unique chemical composition of sugars, vitamins, minerals, amino acids, enzymes and phytohormones that play different functional roles in the human system (Yong, Ge, Ng, & Tan, 2009). One example is the consumption of coconut water as a refreshing and hydrating beverage due to its rich mineral content of sodium, potassium, magnesium and calcium, which can replenish the electrolytes of the human body excreted through perspiration (Saat, Singh, Sirisinghe, & Nawawi, 2002). Studies have shown that coconut water has hydrating and exercise performance effects that are comparable to those of carbohydrate electrolyte sports drinks (Kalman, Feldman, Krieger, & Bloomer, 2012). Chauhan, Archana, Singh, Raju, and Bawa (2014) blended coconut water with lemon juice to develop a refreshing beverage by optimizing the pH, colour and sensory attributes (appearance, aroma, taste, consistency and overall acceptability).

Current knowledge on the fermentation of coconut water is rather limited (Kuswardani, Kusumawati, Srianta, & Sabrina, 2011), especially fermentation with probiotic bacteria. However, Dharmasena (2012) recently developed a novel non-dairy probiotic beverage with a mixture of oat meal and coconut water using probiotic *Lactobacillus plantarum Lp* 115- 400B. Although lactic acid bacteria (LAB) are the most commonly used probiotics, some spore-forming bacteria have also been exploited as probiotics due to their unique properties. Lee, Boo, and Liu (2013) studied the fermentation performance, growth patterns and survival of *Lactobacillus acidophilus* and *Lactobacillus casei* in coconut water. Prado et al. (2015) developed a non dairy fermented functional beverage using coconut water for its hydrating properties, functional health properties and nutritional benefits.

The genus *Bacillus* is the most extensively studied group of spore-forming probiotics. Other spore-formers being used as probiotic bacteria are *Paeni Bacillus polymyxa* and *Brevi Bacillus laterosporus* that were initially classified as *Bacillus* species (Cutting, 2011). There are several advantages of using spores over other non-spore forming bacteria. Spores are heat resistant and can survive harsh conditions during production and storage processes. They are also able to withstand the extreme physiological conditions such as the low pH of the gastrointestinal tract, bile salts and enzymes (Cutting, 2011). *Bacillus coagulans*, a widely used probiotic, has been shown to induce antibody production in humans. This probiotic bacterium is the most commercially available and investigated probiotic bacterium, with proven beneficial impacts on health in animal and human trials (Hawrelak, 2003).

In order to be able to exert its beneficial effects, a successful potential probiotic strain is expected to have a number of desirable properties. Bacterial characterization with good probiotic properties is of great importance in probiotic functional foods. In addition to production of lactic acid, the acid and bile tolerance are two fundamental properties that indicate the ability of probiotic microorganism to survive the passage through the upper gastrointestinal tract, particularly the acidic conditions in the stomach and the presence of bile in the small intestine (Hyronimus, Le Marrec, Sassi, & Deschamps, 2000).

The objective of the present investigation was to

assess the growth, survival and fermentation performance of probiotic bacterium *B. coagulans* in coconut water to produce a novel non-dairy probiotic beverage, which could provide both hydration as well as probiotic benefits to all individuals, especially athletes and recreationally active fitness enthusiasts.

2 Materials and Methods

2.1 Procurement and preparation of raw material

Tender coconuts of the *Cocus nucifera* type, age 5-7 weeks, were chosen for this study. These were purchased from a local market in Delhi. Tender coconut water was collected in a sterile beaker (500 ml capacity) under aseptic precautions as per method given by Acharya, Gupta, Golwala, Store, and Sheth (1965). The flask was plugged with cotton and autoclaved at 121°C at 15 psi for 15 minutes. The flask with sterile coconut water was cooled and stored at 4°C prior to the fermentation stage.

2.2 Chemicals

Sodium hydroxide, sodium chloride, hydrochloric acid, bile salts, L-cysteine, dextrose, peptone, yeast extract, beef extract, MRS agar, MRS broth, GYE broth and agar were obtained from Sigma-Aldrich (New Delhi, India). , Gallic acid, phenol, 3, 5-dinitro salicylic acid (DNS) reagent, sulphuric acid, methanol, ethanol, hexane, ether, crystal violet, Rochell's salt, bovine serum albumin (BSA) and sodium sulphite were procured from HiMedia (Mumbai, India). All chemicals employed were of reagent grade.

2.3 Procurement of Probiotic culture and Preparation of Bacterial Suspension Culture

B. coagulans MTCC 5856 strain used in the study was procured from Microbial Type Culture Centre and Gene Bank (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India. The spores of *B. coagulans* were propagated separately in sterile MRS broth in a sterile Erlenmeyer flask for up to 48 h at 37°C aerobically and then stored at 4°C until use.

2.4 Analysis of Probiotic attributes

The probiotic attributes of the species such as the ability to produce lactic acid, high acid tolerance and their ability to deconjugate bile salts were investigated (Aly, Abd-El-Rahman, John, & Mohamed, 2008).

Analysis of Probiotic attributes

To determine the tolerance of the specie to low pH, the method of Pennacchia et al. (2004) was used with slight modifications. For this purpose, active cultures were used (incubated for 16-18 h). A 0.5 ml aliquot of the bacterial culture was inoculated in 10 ml of phosphate buffered saline adjusted to pH 2.5 with 4 N HCl. Cultures were incubated at 37°C. During 0, 1, 2 and 3 h of incubation, viable microorganisms were enumerated using the pour plate technique on MRS agar plate at 37°C.

Bile salt tolerance

The tolerance capacity of *B. coagulans* for high bile concentration was checked using the method suggested by Chung, Kim, Chun, and Ji (1999). A 1 % concentration of bile salts in sterile distilled water was inoculated with 1 % active bacterial suspension and incubated at 37°C. After incubation for 4 h, viable colonies were enumerated each hour using pour plate technique.

Production of lactic acid

The qualitative test for lactic acid production by *B. coagulans* was carried out using the method as described by Demirci, Pometto, and Johnson (1993). Glucose yeast agar plates were prepared and dilutions were made from the main culture suspension. 1 ml of the bacterial suspension was pour plated from the final dilution tube. After solidification, the plates were incubated at 37°C for 48 hours. The colonies thus obtained

were transferred aseptically to 15 ml of previously sterilized and cooled glucose yeast extract liquid broth. This was incubated at 37°C for 48 hours and was then centrifuged at 2500-3000 rpm for 10 minutes. The clear supernatant was transferred to a separating funnel and extracted by using 5 ml of dilute sulphuric acid (10%) and 50 ml of ether. The ether layer was then collected, evaporated in water bath and the residue thus obtained was dissolved in 5 ml of water. To this, Uffelman's reagent (prepared by adding two drops of 1N ferric chloride to 10 ml of 1% phenol solution) was added dropwise and the colour change was observed.

2.5 Preparation of the inoculum

For preparation of the inoculum, 25 ml of sterile tender coconut water was inoculated aseptically with 1% v/v ml of bacterial suspension culture and incubated at 37°C for 12–14 h. This was then serially diluted to obtain a working culture containing 10^8 CFU/ml. 1 ml from the respective tube was pour plated onto the MRS agar media plate and the plate was incubated at 37°C for 48 h. The number of colonies between 30 - 300 were considered ideal during counting. The viable spore count was obtained by the following formula:

The viable spore count = Number of colonies per plate × Final dilution factor

2.6 Fermentation of the Tender Coconut Water

Fermentations with *B. coagulans* were carried out in 150 ml of sterile coconut water in sterile 250 ml Erlenmeyer flasks. These flasks containing sterile coconut water were inoculated with 1 % (v/v) pre-culture of the probiotic strain from the respective broth. The batch fermentations were carried out for 2 days at a constant 37°C in triplicate. After two days, the samples were taken aseptically after swirling the conical flasks gently for homogenization and these were subjected to microbiological and chemical analysis.

2.7 Analytical determination

Samples (20 ml) were taken after 2 days of fermentation, and the viability of the probiotic culture, pH, total soluble solids (°Brix), acidity and cell biomass of the probiotic coconut water were determined. Similar tests were also carried out for the tender coconut water sample. The pH of tender coconut water and fermented coconut water was measured using a digital pH meter (TOSHCON, India) at 25 °C. The total soluble solids were determined using an Abbe refractometer (AC0012, MRC Scientific Instruments, India) and the total soluble solid content was expressed as °Brix and Refractive Index at 25°C. The rheological measurements were carried out at 25°C using a controlled stress viscometer (Brookfield VIS-S2, MRC Scientific Instruments, India) equipped with a coaxial cylinder (cylinder no. 4); the radii ratio of coaxial cylinder was 1.08477. The acidity was determined by titration with standard 0.01M NaOH solution, using phenolphthalein as indicator and acidity was expressed as % citric acid (Ranganna, 1986). The biomass/cell density was determined spectrophotometrically at 540 nm using the MacFarland scale (Kandler & Weiss, 1986), both pre and post the prebiotic fermentation.

Estimation of total sugars and reducing sugars

Total sugars of tender coconut water and fermented coconut water were determined colorimetrically using the phenol-sulphuric acid method and expressed as percentage sugar (Miller, 1959). The absorbance was measured at 490 nm and expressed as glucose concentration (mg/ml). Similarly, the reducing sugars of tender coconut water and fermented coconut water were determined colorimetrically using 3, 5-dinitro salicylic acid (DNS) reagent and expressed as % (Miller, 1959). The absorbance was measured at 540 nm and expressed as glucose concentration (mg/ml).

2.8 Viable cell determination

Appropriate dilutions from coconut water samples were made using sterile peptone water (1

gl^{-1}) and pour plated onto MRS agar. Plates were then incubated at 37°C for 48 h. The experiment was performed in triplicate and the average number of colony-forming units per millilitre (CFU/ml) were determined using a Darkfield Quebec Colony Counter.

2.9 Sensory evaluation of Fermented Coconut water

Sensory quality of the fermented coconut water was measured after 7 days of fermentation using a 9 point hedonic scale, with respect to the appearance/colour, smell/odour, aroma/flavour, taste and texture/mouthfeel, and for assessment of overall acceptability of the product. Sensory evaluation was carried out by a semi trained panel consisting of 30 food scientists and technologists (between 20-45 years of age) chosen from faculty members and post graduate food technology students of the department. The samples were presented at 20 °C in the sensory evaluation laboratory. The samples were coded and presented individually to each panellist to avoid bias. Potable water to rinse between the two samples was also supplied. The panellists were asked to record their observations on the sensory sheet using the scales described above.
The research was approved by the institutional human experimentation committee or equivalent, and informed consent was obtained from the participants.

2.10 Statistical analysis

Results were expressed as mean values ± standard deviation of at least three replications. Results were statistically evaluated by ANOVA (Minitab 14) at a confidence level of 0.95.

3 Results and Discussion

3.1 Analysis of probiotic attributes

Resistance to low pH

Strains need to be resistant to the stressful conditions of the stomach (pH 1.5-3.0) so resistance to pH 3 is often used in *in vitro* assays to determine the resistance of probiotic species to stomach pH. Food usually stays in the stomach for about 3 h so this time limit was taken into account in the research (Prasad, Gill, Smart, & Gopal, 1998) Since a significant decrease in the viability of strains is often observed at pH 2.0 and below, phosphate buffered saline (PBS) was used with the pH adjusted to 3.0 to select strains resistant to low pH. Effects of low pH (at 2.5) and survivability of *B. coagulans* at 0, 1, 2 and 3 h intervals are shown in Table 1. No effect of low pH (at 2.5) on *B. coagulans* was observed, suggesting that the colonies were able to survive the low pH conditions and were tolerant to high acid. This agreed with results reported by Argyri et al. (2013) where nine strains of *Lactobacillus* showed very high resistance to low pH (*L. plantarum, L. pentosus, L. casei subsp paracasei*). Acid tolerance can be mediated by membrane ATPases as described for *L. acidophilus* by Lorca and de Valdez (2001).

Bile salts tolerance

As the mean intestinal bile concentration is believed to be 0.3% (w/v) and the residence time of food in small intestine is estimated to be 4 h (Prasad et al., 1998), this parameter was considered. Bile salts tolerance of *B. coagulans* at various time intervals are shown in Table 2. The results showed that the specie retained viability with no reduction in the cell count at 1% bile salt concentration. *B. coagulans* showed a good tolerance towards bile salts. Similar results have been reported by Jensen, Grimmer, Naterstad, and Axelsson (2012) where *Lactobacillus* species were found to tolerate gastric juices with negligible reduction in the viability.
In high bile salts concentration, most bacteria show an inability to survive, but spore formers show a better tolerance. *Bacillus* sp. as probiotics, survive the transit very well since they are in the form of spores (Duc, Hong, Barbosa, Henriques, & Cutting, 2004). Bile secreted in the small intestine reduces the survival of bacteria by destroying their cell membranes, whose major components are lipids and fatty acids and these modifications may affect not only the cell permeability and viability, but also the interac-

Table 1: Effect of low pH and bile salts on survivability of *B. coagulans*

pH	Time duration (h)	No. of viable colonies (log CFU/ml)
2.5	0	9.74 ± 0.44^{c}
2.5	1	9.71 ± 0.31^{c}
2.5	2	9.55 ± 0.05^{b}
2.5	3	9.08 ± 0.57^{a}
Bile salt concentration (%)	**Time duration (h)**	**No. of viable colonies (log CFU/ml)**
1	0	9.83 ± 0.43^{d}
1	1	9.81 ± 0.35^{c}
1	2	9.73 ± 0.21^{b}
1	3	9.69 ± 0.18^{a}
1	4	9.65 ± 0.36^{a}

Means and standard deviation for n=3; Values within columns with different superscripts were significantly different ($p<0.05$) according to Duncan's multiple test range

tions between the membranes and the environment (Gilliland, Staley, & Bush, 1984).

Production of lactic acid

B. coagulans showed positive results for lactic acid production capability. The solution turned bluish, violet to yellow which suggested the presence of lactic acid in the medium. This meant that the medium contained sugars that could be fermented by the bacterium to produce lactic acid.

3.2 Analytical determination

pH and total soluble solids

The pH of tender coconut water and probiotic fermented coconut water was found to be 5 and 4.4 respectively. Fermentation causes a rapid decrease in pH from 5.02 to 4.44. *B. coagulans* could tolerate acid medium and survive during fermentation process. The total soluble solids in tender coconut water and probiotic fermented coconut water were found to be 5.0 and 6.0 °Brix respectively, suggesting that the increase in viable cell count corresponded to the decrease in pH and sugars consumed during fermentation. Total soluble solids content was 5.0 °Brix which indicated that solids present in tender coconut water was mainly soluble solids such as sugars. An increase in total soluble solids content of the fermented probiotic coconut water was due to the increase in viable cell counts after fermentation. The refractive index was found to be 1.340 and 1.342 in tender coconut water and fermented coconut water, respectively. It showed the purity of the coconut water.

Titratable acidity

Titratable acidity in coconut water samples was found to be 0.18 % (citric acid) and 0.53 % (lactic acid) respectively. Tender coconut water showed a titratable acidity value of 0.18 % (citric acid) due to the presence of ascorbic acid. After fermentation, the titratable acidity value was 0.53 % lactic acid. Lactic acid is the major end product of the conversion of carbohydrates due to utilization of sugars present in coconut water. *B. coagulans* is a typical strain reported for lactic acid production; the thermophilic character of this strain (growth at 52°C) indicates that it is particularly adapted for industrial production of lactate without sterile conditions (Payot, Chemaly, & Fick, 1999).

Total sugars and reducing sugars

Total sugars and reducing sugar in tender coconut water was 3.96 % and 2.37 % after fermentation, which decreased to 3.15 % and 2.27 % respectively due to utilization of sugars present

Table 2: Physico-chemical characteristics of tender coconut water (TWC) and fermented coconut water (FWC)

S.No.	Parameter	TCW	FCW
1.	pH	5.02 ± 0.03^a	4.44 ± 0.12^b
2.	Total soluble solids (˚Brix) - Refractive Index	5.0 – 1.340 (RI)^a	6.0 – 1.342 (RI)^b
3.	Viscosity (mPa.s) at 25 ˚C	5.13 ± 0.04^a	5.35 ± 0.02^b
4.	% Titrable acidity	0.18 ± 0.01^a (% Citric acid)	0.53 ± 0.02^b (% Lactic acid)
5.	Biomass/Cell density at 540 nm	0.121 ± 0.02^a	0.583 ± 0.01^b
6.	Total Sugars (%)	3.96 ± 0.10^a	2.37 ± 0.07^b
7.	Reducing sugar (%)	3.15 ± 0.05^a	2.27 ± 0.02^b

Means and standard deviation for n=3; Values within rows different superscripts were significantly different ($p<0.05$) according to a paired *t-test*

in tender coconut by the species during fermentation.

Flow behaviour

Rheological parameters are good indicators of texture and important for consumer acceptance. The viscosity values of tender coconut water and fermented coconut water was 5.13 and 5.35 mPa.s at 25˚C depending upon the concentration. Total soluble solids content had a significant effect on viscosity of tender coconut water. The magnitude of viscosity of fermented coconut water increased significantly 5.35 mPa.s with the increase in soluble solid content due to exopolysaccharide production by *B. coagulans*. Several strains of *B. coagulans* have been studied for their exopolysaccharide production. The probiotic bacterium produces an exopolysaccharide (EPS) during exponential and stationary growth phases (Kodali & Sen, 2008).

Microbial exopolysaccharides are getting attention as natural thickeners. Most of the economically important bacterial EPS are produced by LAB, which are manipulated as probiotics to improve rheology and texture of fermented products.

The viscosity of tender coconut water is strongly depended on inter-molecular forces between molecules and water-solute (sugars and acids) interactions, which result from the strength of hydrogen bonds and inter-molecular spacing as both were strongly dependent on concentration and temperature. An increase in soluble solid content leads to increase in hydrated molecules and hydrogen bonding with hydroxyl groups of solute, which would enhance the flow resistance that leads to increase in viscosity of liquid. In case of tender coconut water, soluble solids was mainly due to the sugars content and in case of fermented coconut water, viable cells and exopolysaccharide played an important role in the viscosity values.

Biomass / Cell density

The biomass / cell density was determined spectrophotometrically at 540 nm. The optical densities of tender coconut water and fermented coconut water were 0.121 and 0.683 respectively. The cell density of fermented coconut water determined at 540 nm was 0.683, which was higher than 0.600 that corresponded to 10^9 CFU/mL, using the Mac Farland scale. This is ideal for probiotic beverage functionality.

Viable cell counts

In order to obtain the potential health benefits, the population of probiotics in a product, the viability of probiotic microorganisms and their ability to activate at the desired site in the alimentary canal are very important. The initial inoculum size of probiotics in the selected food item is critical. The effective daily dose of probiotics is considered to be 10^9-10^{11} CFU (Sanders, 1999). Hence, consumption of 100 ml or a g of a product bearing the therapeutic minimum (10^6-10^8 CFU/ml or g of the product), would satisfy the daily requirement. The viable cell counts for

Table 3: Evaluation of sensory properties of coconut water after 7 days of fermentation. Attribute scales: 1 - 9

Attributes	Fermented Coconut Water	Tender Coconut Water (Control)
Appearance / Colour	$7.5\pm.04^a$	$9\pm.30^b$
Smell / Odour	$7.5\pm.12^a$	$7.5\pm.10^a$
Taste	$7.5\pm.04^a$	$6\pm.14^b$
Mouthfeel	$7.5\pm.08^a$	$6\pm.21^b$
Overall acceptability	$7\pm.12^a$	$6\pm.20^b$

The experimental values within rows with different superscripts were significantly different ($p<0.05$) according to a paired *t-test*

fermented coconut water were found to 9.73 log CFU/ml (Table 1), showing that it could be used successfully as a vehicle for probiotics.
The physico-chemical characteristics of tender coconut water and fermented coconut water are summarized in Table 2.

3.3 Sensory evaluation of Fermented Coconut water

Sensory properties were chosen as the main criterion of the quality of fermented products, being the most important attribute for consumers.
According to the consensus of the panellists during sensory evaluation, the overall acceptability on a 9 point hedonic scale of fermented coconut water was found to be higher than tender coconut water. It was determined that the main descriptors that characterized the product were acidity and sweetness, with acidity being the attribute responsible for the sensory difference perceived by the panellists.
The parameter of fluid food quality related to rheological viscosity is known as mouthfeel and is defined as the mingled experience derived from the sensation on the skin of the mouth after ingestion of a food or beverage. Nevertheless, the fermented coconut water still had high concentrations of residual sugars, which would enable retention of sweetness. The evaluation parameters and their respective scores are shown in Table 3.

4 Conclusions

In this study, tender coconut water was used as the sole fermentation medium, without any additives, to ensure that it was the only raw material that regulated the growth and metabolism of the probiotic bacteria. The good adaptation of *B. coagulans* in the tender coconut water showed that if a potential probiotic strain is used as a starter culture then it might produce a fermented product with defined and consistent characteristics and possibly health-promoting properties. Fermented coconut water gives the advantages of plant-based products, and the presence of live bacteria with probiotic qualities enhances the benefits. In conclusion, the present study demonstrated good growth of probiotic *B. coagulans* in tender coconut water. These results suggest the feasibility of fermenting coconut water into a probiotic beverage, especially for its nutrition, with the health benefits of probiotics.

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Energy Pattern and Conservations of Condiment Produced from Soybean (*Glycine max*)

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Abstract

Energy being one of the largest operating expenses in most organizations especially manufacturing and processing industries leading to considerable scope for energy conservation and hence cost. Information on energy utilization and conservation pattern were obtained based on time taken, number of person involved and sources of energy using standard energy equations. A total of 445.40 ± 17.32MJkg^{-1} where thermal energy (420MJ ≈ 94%) and manual energy (25.40MJ ≈ 6%) were the only forms of energy used during production process. Conservation approach I resulted in mean energy of 72.08 ± 1.73MJkg^{-1} where electrical energy, manual energy and thermal energy accounted for 1.75MJ (3%) 7.34MJ (10%) and 62.99MJ (87%) respectively. Conservation approach II reduced the energy further to 57.24 ± 1.73MJkg^{-1} as the operation was thermal energy dependent, followed by manual and electrical energy with energy values of 48.13, 7.33 and 1.78MJ equivalent to 84.10%, 12.80% and 3.10% accordingly. Conclusively, traditional method of processing utilized highest energy (445.40MJ) followed by conservation approach I (72.08MJ) and conservation approach II (57.24MJ) was least in energy demand. Conservation approach II permits energy conservation to be 87% as compared with traditional method.

Keywords: Condiment; Conservation approach; Energy; Traditional process; Soybean

1 Introduction

Soybean (*Glycine max*) is one of the most important legumes in the world which provides vegetable protein for millions of human. It is most nutritious and easily digested food of the bean family. The soybean is considered as one of the richest and cheapest sources of protein. It is a staple in the diet of humans and animals in different corners of world today. Soybean contains 35–40% protein on a dry-weight basis (Liu, 1997); these proteins contain all amino acids essential to human nutrition but it is deficient in sulphur containing amino acids, however, soy products almost equivalent to animal sources in protein quality but with less saturated fat and no cholesterol. Its consumption has been discouraged due to its beany taste, off flavor and anti-nutritional factor such as phytate, saponin (Akande, Doma, Agu, & Adam, 2010) and lectins (Lajolo & Genovese, 2002). In spite of this occurrence, soybean has been receiving attention in Nigeria as diet. A popular product that is common in the soybean producing areas of the country is soybean *daddawa* (also referred to as *soy-daddawa* or *iru*) which is a food flavoring condiment produced from whole soybean by means of fermentation and the end product is similar in its characteristic stickiness and pungent ammonical smell to that of *iru* made from fermented locust

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bean (*Parkia biglobosa*) seeds.
The production of condiment from soybean has been reported by Oboh (2006). The processing of soybean into condiments require energy of different forms. Energy development, management and improvement must have predetermined plans and strategies and this can be achieved through a proper understanding of its utilization and consumption pattern where it is applicable (Aderemi, Ilori, Aderemi, & Akinbami, 2009). This makes energy one of the largest controllable costs in most organizations especially manufacturing and processing industries leading to considerable scope for energy conservation and hence cost (Jekayinfa & Bamgboye, 2006).
Energy conservation contributes to profitability of the industry, reduction in environmental impact, and conservation of depleting non renewable energy sources such as crude oil, natural gas and coal. It is now widely accepted fact, that current production of non-renewable energy cannot satisfy the ever increasing population and industrial needs which demands for conservations of energy by processes and at same time development of renewable energy. Energy audit is the review of the total energy used and costs, normally performed in conjunction with site investigation. It involves the classification of the energy sources and their contribution in running the factory (Jekayinfa & Bamgboye, 2006). It provides a structural review of how energy is being purchased, managed and used with the aim of identifying opportunities for energy cost saving through improved services [6]. It also gives the estimate of potential annual energy savings with implementation costs and pay back periods. Effective energy utilization in the manufacturing sector required the in-depth knowledge of energy performance of machines, and operations related to the production process as these vary from industry to industry. To reduce operating cost to a minimum, the cost of energy consumption which is prime factor under operating cost must be well monitored (Wang, 2009). Some reports on energy audit and survey have been published on processing of palm oil (Akinoso & Omolola, 2011), sugar beet production (Mrini, Senhaji, & Pimentel, 2002), cashew nut processing (Jekayinfa & Bamgboye, 2006) and palm kernel processing (Jekayinfa & Bamgboye, 2007), cowpea flour production (Akinoso, Olapade, & Akande, 2013) and condiment from locust beans (Akinoso & Adedayo, 2012), bambara nut (Anjorin, Sanusi, & Hussein, 2015) and ogiri (Anjorin et al., 2015) among others. However, energy quantification and data were not evident on condiments produced from soybean despite several researches on the legume. Therefore, the objective of this paper is to provide data on energy pattern and subsequent conservations for the processing operation of soybean condiment (*iru*).

2 Materials and Methods

Data on energy utilization and conservation were obtained on basic unit operations, quantity of fuel used, time taken, gender involved and sources of energy used (Table 1). Each process was repeated three times and obtained data were subjected to descriptive analysis using SPSS software at 5% significance level. Mean was reported for each unit operations.

2.1 Traditional Process

Method described by Oboh (2006) was adopted for traditional condiment production from soybean. One kilogram (1kg) of soybean was weighed, sorted, winnowed (as preliminary operation), washed and boiled at (100 °C to 105 °C, 760mmHg) in a cast iron pot for 12 h using air dried wood as source of fuel. The boiled water was used to soak the seeds overnight prior to dehulling by rubbing the cotyledons between palms of the hands and subsequent washing with potable water to remove the hulls. The cleaned cotyledons were boiled in a cast iron pot for another 2h using air dried wood, boiling water was drained using plastic sieve. Drained cotyledons were spread and wrapped on already cleaned banana leaves, then wrapped in a polythene bag before being placed in an air tight container for spontaneous fermentation for 4 days at ambient temperature.

Table 1: Data used in evaluating energy consumption pattern during production process and conservation approach I and II of soybean into condiment

Unit Operation	**Required Parameters**	**Production Process**	**Conservation Approaches**
Preliminary Operations	Number of persons involved	1	1
	Time taken (h)	0.23h	0.23h
First Boiling	Air dried wood consumed l	24kg	NA
	Number of Persons involved in boiling	2	NA
	Time taken for boiling (h)	12h	NA
Soaking	Number of persons involved	1	1
	Time taken (h)	0.1h	0.10h
Dehulling and Washing	Number of persons involved	2	NA
	Time taken (h)	1.78h	NA
Mechanized Dehulling	Number of persons involved	NA	2
	Time taken (h)	NA	0.33h
Washing	Number of persons involved	NA	2
	Time taken (h)	NA	0.50h
Second Boiling	Air dried wood consumed l	4kg	NA
	Number of Persons involved in boiling	2	1
	Fuel consumed l (Kerosene)	NA	0.70L
	Fuel consumed (LPG)	NA	0.31kg
	Time taken for boiling (h)	2h	3.5h
Wrapping	Number of persons involved	2	2
	Time taken (h)	0.68h	0.68h
Fermentation	Number of persons involved	1	1
	Time taken (h)	0.12h	0.12h
Drying	Number of persons involved	NA	2
	Time taken (h)	NA	1h
	Weight of charcoal (kg)	NA	1.2kg
Milling	Number of persons involved	NA	2
	Time taken (h)	NA	0.16h
Packaging	Number of persons involved	2	2
	Time taken (h)	0.25h	0.25

NA: Not Applicable

2.2 Conservation Approaches for Processing Soybean into Condiment

Soybean of 1kg was weighed, sorted, winnowed, washed and soaked for 24h. The soaked seeds were dehulled in a locally fabricated dehuller and subsequent washing with waters to remove the hulls. The cotyledons were boiled for 3.5h in an aluminum pot using kerosene in a kerosene stove as source of fuel, boiling water was drained using plastic sieve. Drained cotyledons were spread and wrapped in already sterilized banana leaves, placed in polythene bag and put in an air tight container for inherent fermentation for the period of 4 days at ambient temperature. The fermented soybean condiments were dried using coal powered drier and milled in an attrition mill prior to packaging using high density polyethylene. These procedures were repeated during conservation approach II with exception of Liquefied Propane Gas (LPG) being used as source of fuel during boiling operation.

2.3 Energy Quantification

Energy utilization during each operation was categorized as electrical, thermal, and manual energy as represented in equation (1) (2) and (3). These modes of energy were estimated using approach adopted by Akinoso and Adedayo (2012) during energy utilization pattern of processing African Locust beans (*Parkia biglobosa*) into condiment.

Manual Energy

Manual energy was estimated based on the values recommended by Goyal, Jogdand, and Agrawal (2014) as stated in equation (1):

$$E_M = 0.75 \times N \times T_a(kW) \tag{1}$$

Where 0.75 = the average power of a normal human labour in kW, N = number of person involved in the operation; and Ta = useful time spent to accomplish a given task in hours.

Thermal Energy

The thermal energy was estimated according to the heating source term used by (Rajput, 2001) which establishes that the thermal energy E is directly proportional to amount of fuel used $W(MJ)$ as expressed in equation (2):

$$E = CfW(MJ) \tag{2}$$

Where *Cf*, is the constant of proportionality which represents the calorific value (heating value) of fuel used, W is the quantity of fuel consumed and E is the quantity of energy consumed. Calorific value of typical air dried wood (15MJ/kg), (Kerosene (43.7 MJ/L), Liquefied Propane Gas (LPG) (50.35MJ/kg) and charcoal (27MJ/kg) (Akinoso & Adedayo, 2012).

Electrical Energy

Equipment using electrical energy, the rated horse power of each motor was multiplied by the corresponding hours of operation as represented in equation (3). A motor efficiency of 80% was assumed to compute the electrical input (Rajput, 2001).

$$E_p = \Delta PN \tag{3}$$

Where Ep is the electrical energy consumed in kWh= kJ, P is the rated power of motor in kW (dehuller, 4.48; Attrition Mill, 2.98 and Impulse sealer, 0.26), (Akinoso & Adedayo, 2012). N is the time spent in hours (h) during the operation, Δ is the power factor (assumed to be 0.8).

2.4 Total Energy Estimation (En)

This was calculated by summation of all energy of each unit operation involved during the production of each condiment for traditional process and conservation approaches as stated in equation (4) and (5):

Traditional Process

$$(E_n) = E_{PO}+E_{WA}+E_{FB}+E_D+E_{SB}+E_W+E_F+E_P \tag{4}$$

Where E_{PO}, E_{WA}, E_{FB}, E_D, E_{WA}, E_{SB}, E_D, E_F, E_D, E_M, and E_P are energy for preliminary

operation, washing, first boiling, dehulling, second boiling, wrapping, fermentation and packaging.

Conservation Approaches

$$E_n = E_{PO} + E_s + E_D + E_{WA} + E_B + E_W + E_F + E_D + E_M + E_P \quad (5)$$

Where E_{PO}, E_s, E_D, E_{WA}, E_B, E_W, E_F, E_D, E_M, and E_P are energy for preliminary operation, soaking, dehulling, washing, boiling, wrapping, fermentation, drying, milling and packaging respectively.

Conservation Approaches

Statistical analysis of all data was done with the Statistical Package for the Social Sciences (SPSS) (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The mean values were considered at 95% significance level using One-way Analysis of variance (ANOVA) procedure while Duncan multiple test was used to separate the means.

3 Results and Discussion

3.1 Energy Requirement during Traditional Process of Soybean Condiment

A total of 445.40±17.32 MJ/kg of energy was expended during the traditional process (Table 2). The energy intensity of this process was higher than the energy (59.82±1.40MJ/kg) utilized during processing of condiment from locust beans (*Parkia biglobosa*) (Akinoso & Adedayo, 2012); 2.38MJ/kg used for extraction of crude soybean oil (Wang, 2009); 7.20 MJ/kg for palm oil production using electrical energy (Mahlia, Abdulmuin, Alamsyah, & Mukhlishien, 2001) and 4.86 MJ/kg utilized during production of burukutu (Ibrahim, Alex, & Ierve, 2013). The differences in energy consumption can be adduced to crop physiology such as seed coat hardness, quantity of sample used, technology utilized and sources of energy.

During the operations, two major forms of energy were utilized which were thermal and manual energy, thermal energy (such as boiling operations) was estimated to be 420MJ/kg while manual energy (such as preliminary operations (weighing, winnowing and sorting), soaking, dehulling, wrapping fermentation and packaging) accounted for 25.40MJ/kg, these operations amounting to 94% and 6% respectively.

3.2 Energy Used by the Conservation Approaches

In conservation approach I where kerosene was used during boiling operation a total of 72.08±1.73MJ/kg of energy was used as against 445.40±17.32 MJ/kg consumed during the traditional process counterpart (Table 2). The drastic reduction in energy utilization can be linked to elimination of most energy demanding operation which was first boiling being replaced with 24hrs soaking, change in fuel source from firewood to kerosene with calorific value 43.7MJ/L which tend to release high proportion of energy as a function of technological advancement of appliance used as well as change in cooking pot from mild steel to aluminium and manual dehulling being substituted with mechanical dehulling.

The energy intensity of this operation was higher as compared with semi-mechanized processing of condiment produced from locust bean where 67.56±1.30MJ/kg was used [14] and energy conservation of condiments produced from bambara nut 25.64MJ was utilized [20]. However, the second boiling consumed 33.22±1.73MJ of total energy while 0.08±0.01MJ was utilized during soaking operation making the operation the most and least intensive energy consuming operations respectively. These two operations answered for 46.08% and 0.10% of the total energy input. Also, more than 75%, of the energy was utilized by the two thermal operations (second boiling and drying), preliminary operations (weighing, winnowing and sorting), soaking, washing, wrapping and fermentation calculated to be 2.92%, while mechanized dehulling, milling and packaging answered for 3.97%. In this technique, electrical energy, manual energy and thermal energy accounted for 1.75MJ, 7.34MJ and 62.99MJ com-

prising 3%, 10% and 87% respectively.
During conservation approach II where liquefied propane gas was used during boiling operation, In this process, preliminary operations, soaking and washing, accounted for 0.99MJ corresponding to 1.74%, wrapping, mechanized dehulling, fermentation, milling and packaging utilized a total of 3.98MJ amounting to 6.95%, while boiling and drying consumed 52.26MJ corresponding to 91.31%. The total estimated energy of the process accounted for 57.24±1.73MJ/kg as against 72.08±1.73MJ/kg and 445.40±17.32MJ/kg utilized during conservation approach I and traditional techniques. The total energy utilized during conservation approach II was less when compared to energy used during improved processing (semi-mechanized) of locust beans where 67.56±1.3MJ/kg was consumed [14].
The further reduction in energy consumption as compared with conservation approach I can be attributed to change in fuel source from kerosene to LPG while other materials and operations remain the same. Reduction of energy as evidenced in conservation approach II justified the use of liquefied propane gas for the heating process with calorific value 50.35MJ/kg making high proportion of its energy content to be converted to heat. However, boiling operation accounted for 18.36MJ of the total energy input while 0.08MJ was consumed during soaking making the operations most and least energy demanding operations corresponding to 32.08% and 0.14% respectively. Thermal operations (second boiling and drying) consumed a total of 52.26MJ which is more than three-quarter of the total energy input corresponding to 91.37 in percentage. Besides, mechanized dehulling, milling and packaging which represented 5.06% of the remaining energy input preliminary operations (weighing, winnowing and sorting), soaking, washing, wrapping and fermentation accounted for the smallest proportion of the energy input totaling 3.63%. It was observed that, the operation was thermal energy dependent, followed by manual and electrical energy with energy values of 48.13, 7.33 and 1.78MJ equivalent to 84.10%, 12.80% and 3.10% accordingly.

3.3 Comparison of Total Energy Usage during Processing Soybean into Condiment

In the three operations, energy utilized during preliminary operation ranged between 0.17±0.06 in conservation approach II to 0.17±0.01 in traditional process amounting to 0.29% and 0.04% respectively. There were no significant differences in energy utilized during preliminary operations of the processes ($p > 0.05$). First boiling operation accounted for a total of 378MJ in traditional process representing 84.87% of the energy usage during the operation. The operation was identified as most energy demanding operation and thus not applicable during conservation approach I and II. However, second boiling was involved during the three operations with energy values 63.00MJ, 33.22MJ, and 18.36MJ in traditional process, conservation approach I and II corresponding to 14.15%, 46.08%, and 32.08%. Significant differences were evidenced at ($P = 0.05$) during the operation.
The energy utilized during traditional process can be traced to low efficiency of energy (about 10%) from firewood as well as the calorific value (15MJ/kg) which makes firewood to utilize more quantity of firewood to attain heating process [19]. Also, manual dehulling (Hand dehulling) in traditional process was higher than conservation approaches where in both approaches locally fabricated mechanized dehuller was adopted for the operation. The energy values were 2.67±0.54 in traditional process, 1.68±0.52MJ in conservation approach I and 1.68±0.52 in conservation approach II corresponding to 0.60%, 2.33% and 2.93% of respective energy input during the operation. This could be attributed to the reduction in the dehulling time because mechanical dehuller was used for both conservation approaches which saves time as compare with traditional process that used manual method and this agreed with the findings of [7] that the duration of processing is a major parameter in estimating energy utilization.
There were significant differences between traditional process and conservation approaches but significant differences were not established between conservation approaches I and II.

Table 2: Energy utilization during production process and conservation approach I and II of producing soybean condiment

Unit Operation	Production Process Energy (MJ)		Conservation Approach I Energy (MJ)	Percentage	Conservation Approach II Energy(MJ)	Percentage
Preliminary Operations	0.17±0.01^a	0.04	0.17±0.01^a	0.24	0.17±0.06^a	0.29
First Boiling	378.00±6.93^a	84.87	NA		NA	
Soaking	0.08±0.01^a	0.02	0.08±0.01^a	0.10	0.08±0.01^a	0.14
Dehulling	2.67±0.54^a	0.60	1.68±0.52^b	2.33	1.68±0.52^b	2.93
Washing	NA		0.75±0.03^a	1.04	0.75±0.03^a	1.31
Second Boiling	63.00±4.04^a	14.15	33.22±1.73^b	46.08	18.36±1.47^c	32.08
Wrapping	1.02±0.02^a	0.23	1.02±0.02^a	1.42	0.99±0.19^a	1.73
Fermentation	0.39±0.30^a	0.02	0.39±0.30^a	0.12	0.09±0.01^a	0.16
Drying	NA		33.90±1.87^a	47.03	33.90±1.87^a	59.23
Milling	NA		0.62±0.05^a	0.86	0.66±0.14^a	1.15
Packaging	0.38±0.09^a	0.08	0.56±0.13^a	0.78	0.56±0.13^a	0.98
Total	445.40±17.32^a		72.08±1.73^b		57.24±1.73^b	

*NA= Not Applicable; Means followed by different letters across the rows are significantly different (P = 0.05) from one another

Washing was introduced during conservation approaches as a result of need to separate the hulls from the cotyledons although the operation was carried out simultaneously during manual dehulling in traditional process. Soaking, wrapping and fermentation operation of the three processes indicated that there were no significant differences between operations (P = 0.05). This is as a result that the mode of operations was the same in spite of level of processing.

Drying and milling operations consumed 33.90±1.79MJ and 0.62±0.05MJ corresponding to 47.03% and 0.86% respectively in conservation approach I and 33.90±1.79MJ and 0.66±0.14MJ corresponding to 59.23% and 1.15% respectively in conservation approach II as these operations were not applicable to production process. There were no significant differences between the conservation approaches for drying and milling at (P =0.05). Energy required for packaging in conservation approaches I and II were 1.12MJ and 0.38MJ for Energy required for packaging in conservation approaches I and II were 1.12MJ and 0.38MJ for traditional method. There was no significant difference in energy utilization during packaging at (P =0.05). This can be traced to insignificant electrical energy input that was utilized for packaging in the conservation approaches I and II. There was no significant difference in energy utilization during packaging at (P =0.05). This can be traced to insignificant electrical energy input that was utilized for packaging in the conservation approaches I and II.

4 Conclusions

Energy requirements for processing of soybean into condiments revealed that traditional processing of 1kg of soybean into condiment utilized a total of 445.40MJ where manual and thermal energy were the only forms of energy used, which amounted to 6% and 94% of the total energy input. In conservation approach I, energy was reduced to 72.08MJ with thermal, manual and electrical energy utilizing 87.38%, 10.19% and 2.43% respectively. The energy was further conserved during conservation approach II to 57.24MJ comprising of 12.80% manual, 84.10% thermal and 3.10% electrical energy. Conservation approach II permits energy conservation to be 87% as compared with traditional method in soybean processing into condiment. Also, the total energy demand depends on source of energy, unit operation under consideration, level of production, adopted technology and crop physiology. Therefore, conservation approach II should be adopted

for processing of soybean into condiment since it is efficient and less consumption of energy.

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