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Postharvest Physiology, Biochemistry and Quality Management of Chili Plum (*Spondias purpurea* var. Lutea): A Review

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Abstract

The chili plum (*Spondias purpurea* L.) is an exotic fruit with a climacteric pattern of respiration belonging to the Anacardiaceous family and is consumed in both the fresh and processed states. The fruit has a yellow pulp, pleasant aroma, sweet sour taste with vitamin A content being higher than cashew, guava, some papaya and mango cultivars. However, a relatively short shelf-life of 5-6 days at ambient temperatures for harvested fruits and widespread incidence of fruit-fly infestation are two major limitations for increased utilization of this fruit. Fruits can be successfully stored up to 14 days at 12.5^{o}C. Fruits stored at 12.5^{o}C and then subsequently transferred to 30-32^{o}C ripened normally with a shelf-life of 4 days. Storage at temperatures below 9-10^{o}C resulted in the occurrence of chilling injury damage accompanied by an inhibition of ripening. Chili plums have a caloric density of 74 kcal/100 g^{-1} edible portion which is significantly higher than the 39 to 58 kcal/100g^{-1} for peach, apricot, and mango and cherry. The higher caloric density is attributed to its total carbohydrates of 19.1% and fructose, glucose and sucrose which together account for 65% of the soluble matter. Unlike the other fruits, chili plum retains a fair amount of starch in the mesocarp. It is a moderate source of potassium (250 mg/100g^{-1} edible portion) and an excellent source of vitamin C (48 mg/100g^{-1} edible portion). Analysis of volatile flavour compounds showed 2-hexenal to be the main flavour compound present.

Keywords: chili plum, postharvest, respiration, ethylene, packinghouse

1. Introduction

The chili plum (*Spondias purpurea* L) is a member of the Anacardiace family, native to Mexico with a distribution inclusive of the Caribbean, Central America and Peru and Brazil, where it is also known as ‘Lapa’, ‘Job’, ‘Moyo’, ‘Sta Roseno’, ‘Jismoyo’ and ‘De Cocer’ (Barbeau, 1994; Mohammed, 2011; Maldonado-Astudillo et al., 2014). It is a is a tropical species whose physiological, anatomical, and agronomic plasticity allows it to adapt to a wide range of soil conditions and is tolerant of varying altitudes and temperatures (Maldonado-Astudillo et al., 2016). The yellow chili plum has high commercial potential due to its relatively low production cost and its adaptability to tropical soils (Avitia-Garcia et al., 2000). The fruits are consumed fresh with high acceptability throughout the Caribbean and Latin America, and demonstrated its potential for commercial largescale production (Bridgemohan, 2008). Information about the particular characteristics of different genotypes is sparse, mainly because their cultivation is still largely based on traditional agricultural practices such as backyard gardens, hedgerows, and small farms (Cuevas, 1994), as well the tendency of the crop to be prolific in the wild (Maldonado-Astudillo et al., 2014). Fruits are seasonal, highly perishable, often displayed for sale in heaps under ambient conditions at roadside outlets and municipal markets and susceptible to fruit fly infestations and fruit-rot fungi in the field. The postharvest quality of the fruit is influenced by the plant genotype and its edaphoclimatic conditions. The fruits are pre-disposed to several pre-and postharvest factors such as the maturity index (phenological, physical or chemical), the harvest method

(manual or mechanical), transport and storage conditions (temperature, humidity, ventilation, etc.) and the conditions of product manipulation.

Currently, there is heightened interest in the consumption of functional foods (fruits and vegetables) associated with the prevention of chronic diseases. The beneficial effects of chili plums are amplified due to the presence of bioactive compounds (vitamins, enzymes, carotenoids, flavonoid, and phenols) which are rich in antioxidant properties (Hooper & Cassidy, 2006; Isabelle et al., 2010). The utilization of the fresh fruit or processed forms must be explored in order to penetrate international markets since the fruits have the capacity to neutralize harmful molecules such as free radicals, that interact and destabilize important macromolecules like proteins, nucleic acids and lipids causing degenerative diseases (Niva, 2007). Accordingly, this review focusses on all aspects of the physiology and biochemistry of yellow chili plums and to examine the various preharvest and postharvest management procedures to optimize quality. This information is critical for the realization of the full potential of this fruit which has unique flavour and is a rich source of nutraceuticals.

2. Morphology and Structure

The tree attains a height of 3-10 m with a grayish smooth bark. The trunk is stout, with thick spreading branches and the leaves are pinnate 2.5-6.5 cm long with 5-23 leaflets. The flowers are solitary or fascicled in the axils of the fallen leaves. The plant is deciduous and defoliate during the early dry season as it enters the pre-floral stage. Flowering is stimulated by water stress (Bridgemohan & Mohammed 2019). This characteristic has been attributed to its specific mechanism of defoliation which gives the plant the ability to spontaneously grow in, and adapt to poor soils (Avitia et al., 2000; Maldonado-Astudillo et al., 2014). The mature fruit is a smooth and shiny ellipsoid drupe that measures 2.5–4.0 cm in length and 1.5-2.5 cm in diameter. At different stages of maturity, fruits are consumed fresh, frozen, or processed into diverse food products such as pickles and candies. The epicarp is thin, has a smooth to semi-smooth texture, and upon maturity, can acquire a yellow colouration. The fruits have a thick and fibrous endocarp with the mesocarp giving the fruit its flavour quality characteristics which ranges from sour to sour-sweet coupled with a delectable aroma. This unique feature is embraced by consumer appeal and acceptance. The moderate levels of vitamin C, potassium and calcium together with antioxidant compounds such as phenols and carotenoids contribute to the chemo-preventive potential of chili plum fruits (Sameh et al., 2018).

2.1 Worldwide Importance and Economic Value

Yellow chili plum is a tropical fruit with increasing acceptance in both national and international fruit markets (Bridgemohan, 2008). Mature trees produce several thousand fruits with an average yield per tree ranging from 40-50 kg (Barbeau, 1994). Koziol & Macia (1998) examined the economic aspects of chili plum production in Ecuador and reported 1800 hectares are under cultivation with production of 4,500 metric tonnes of fruits. They estimated 10,000 metric tonnes would be required for industrial processing of the fruit to be commercially viable. Production levels below 4,500 metric tonnes per year would therefore limit commercial possibilities to processing at the level of a cottage industry or small industrial scale.

Mature-green harvested chili plum fruits ripen in 4-5 days and have a short shelf life of an additional 2-3 days. Tree-ripened fruits have a shelf life of just 1-2 days after harvesting. The limited shelf-life combined with the fruits susceptibility to physical damage during transport make international exportation of fresh chili plums very challenging and restricted. Macia & Barfod (2000) described the sale of fresh chili plums to have impacted negatively to the income of small-scale producers in Ecuador because the majority of the profits are absorbed by middlemen who purchase and transport fruits in bulk to markets in the larger cities. Macia & Barfod (2000) argued that for the assurance of any economic potential for chili plum, farmers would have to focus on "value-added products" rather than on the sale of fresh fruits. Macia & Barfod (2000) justified this claim by highlighting an example whereby small farmers were paid US$0.10 – 0.30 per kg for fresh chili plums whereas a 300g jar of chili plum jam could be sold for US$1.45. With production costs for such a jam roughly estimated at US$1.00 per kg, the small farmers profit could increase to about US$3.83 per kg of processed chili plum fruits.

2.2 Culinary Uses, Nutritional Value and Health Benefits

The yellow chili plum pulp is an important source of potassium and copper (Tiburski et al., 2011). The antioxidant activity and total phenolic values scored 17.5mmol TEAC g^{-1} and 260 mg galic acid/100g, respectively, and is higher than most tropical fruits. Five carotenoids were identified, β-cryptoxanthin, lutein, zeinoxanthin, α and β carotene, with β-cryptoxanthin being dominant and accounting for the high level of pro-vitamin A activity in the pulp (Tiburski et al., 2011). The carotenoid content is lower than that of mango (*Mangifera indica* L.) var. Tommy Atkins (50.5 g^{-1}) whose most abundant carotenoid is violaxanthin, red guava (*Psidium guajava*) (56.6–69.7 g^{-1}) or tomato (*Solanum lycopersicum)* (43.9 g^{-1}), with lycopene being the most

abundant carotenoid in these last two species (Rodríguez-Amaya, 1999; Tiburski et al., 2011). The main carotenoid in the pulp of chili plum is cryptoxanthin, (17.08 g^{-1}) and zeinoxanthin (3.52–5.47 g^{-1}), although, carotene, phytoene, phytofluene, and cryptoflavin have also been identified as precursors (Hamano and Mercadante, 2001; Rodríguez-Amaya, 1999; Tiburski et al., 2011). In fruits of the *Spondias purpurea* ecotype 'Cuernavaqueñaa', the total carotenoid content increases during ripening, with a greater concentration present in the epicarp (skin) compared to the pulp. A similar pattern was found in other fruits, including yellow chili plum (Rodríguez-Amaya, 1999). Recently, Sameh et al. (2018) reported that plants belonging to the genus *Spondias* were widely used in traditional medicine due to their beneficial therapeutic effects. Diverse pharmacological activities include cytotoxic, antioxidant, ulcer protective, hepatoprotective, anti-inflammatory, antiarthritic, and antidementia effects. These attributes are supportive of their potential to treat various degenerative diseases (Sameh et al., 2018).

The importance of phenolic compounds in *Spondias* fruit are natural antioxidants against chronic-degenerative diseases, but this compound also has a tendency to induce browning during processing and affects the flavour of the fruit (Engels et al., 2012; Filgueiras et al., 2001; Tiburski et al., 2011). A 100g portion of yellow plum pulp can provide more than 37% of the recommended daily allowance of vitamin A. The chili plum fruit weight is distributed in the seed (34%), peel (8%) and pulp (50-58%) (Leung & Flores, 1961; Winton & Winton, 1935). Chili plum fruits according to Koziol & Macia (1998) have the highest energy value when compared to other popular fruits such as apricot, cherry, peach and mango and this is attributed principally to its higher concentrations of total carbohydrates. The total concentrations of the three sugars sucrose (5.9-7.2g/$100g^{-1}$ edible portion), fructose (2.5g/$100g^{-1}$) and glucose (2.0g/$100g^{-1}$) account for 65% of the total soluble solids measured as ^{o}Brix. The fibre content is uncharacteristically low (0.2-0.7g/$100g^{-1}$), while there is a considerable amount of starch in the unripe fruit (8.4g/$100g^{-1}$) which is about 4 times higher than the ripe fruit. The free sugars and starch are easily fermentable and have an advantage for the development of an effervescent wine (Koziol & Macia, 1998). Despite the low pectin content (0.22g/$100g^{-1}$), it is still sufficient for making a jam without the addition of more pectin. Given the acidity of the fruit (pH of 3.3) and the formation of jams without additional gelling agent, it is most likely that native pectins are of the high methoxyl type, that is, with degrees of esterification in excess of 50% (Mitchell et al., 1978).

Chili plums are a moderate source of potassium (100-300 mg per serving) (Guthrie, 1979). Thus 100g edible portion of chili plums would provide 63% of the potassium requirements for children 4-6 years old, 44% for children 7-10 years old, 16% for adolescents 11-14 years old, 12% for adolescents 15-18 years old and 10% for adults (Koziol & Macia., 1998).

The vitamin C content of chili plums according to Koziol & Macia (1998) is the highest compared to apricot, cherry, peach, mango. Accordingly, a 100g edible portion would provide 98-123% of the recommended dietary allowance (RDA) for children 1-14 years old and 82% of the RDA for people over 14 years old. The nutritional composition of the fruit includes: protein (0.9%), fat (0.24%), ash (0.7%), total carbohydrates (18.1%), calcium (17 mg), iron (0.30 mg), sodium (9 mg), phosphorus (42 mg), zinc (20 mg), carotene (119 mg), thiamine (84 mg), riboflavin (40 mg), niacin (1.0 mg), citric acid (30 mg), malic acid (110 mg), oxalic acid (30 mg), tartaric acid (20 mg) (Koziol & Macia., 1998).

3. Postharvest Physiology

3.1 Fruit Growth, Development and Maturation

Filgueiras et al. (2001) reported that fruit weight of chili plums increased from 13.62g to 14.05g when predominantly green to 15.91g when ripened or predominantly yellow. They also reported that fruit length and width are greatest when fruits are ripe. The percentage seed however decreases during growth and development with fruits at the yellow stage having 18.4% while at the earlier stages of development when predominantly green it is 20.58 – 23.63%. Fruit pulp density increase with fruit maturity at the yellow ripe stage (81.58%) compared to the green stages of development (75.71-79.41%).

3.2 Respiration and Ethylene Production

Sampaio et al. (2007) investigated the respiratory activity and associated changes in chemical constituents during maturation of chili plums. They reported that the pre-climacteric was marked by the initial production of 24.4 ml $kg^{-1} h^{-1}$ CO_2 and initial oxygen absorption of 25.5 ml $kg^{-1} h^{-1}$. The minimum evolution of CO_2 was 11.0 ml $kg^{-1} h^{-1}$ while the minimum absorption of O_2 was 15.5 ml $kg^{-1} h^{-1}$ after 102 and 108 hours, respectively, after harvest thereby confirming a climacteric minimum. The maximum liberation of CO_2 of 54.2 ml $kg^{-1} h^{-1}$ and O_2 absorption of 49.0 ml $kg^{-1} h^{-1}$ occurred at 186 hours after harvest thereby defining the climacteric peak. They also calculated the respiratory quotient of chili plums (RQ) at the pre-climacteric, climacteric minimum,

climacteric peak and post-climacteric to be 0.96, 0.63, 1.11 and 0.59, respectively. Accordingly, the RQ value of 1.11 at the climacteric peak represented the oxidation of carbohydrates while the 0.96 accounted for the oxidation of proteins and the 0.63 and 0.59 indicated consumption of lipids.

In previous studies, Graham et al. (2001) reported an overall suppression of respiration for chili plums stored at 4-5 ^{o}C and 9-10 ^{o}C, but at 20-21 ^{o}C and 30-31 ^{o}C respiration increased inducing ripening in 3-4 days (Figure 1A and 1B). At 20-21 ^{o}C, immature and half-ripe fruits exhibited a more pronounced climacteric peak in comparison to mature-green and turning or breaker fruits. However, they did not observe the respiratory climacteric in fully-ripened fruits compared to fruits harvested immature, mature-green, turning or ripe. In more recent studies, Vargas et. al. (2017) observed no significant differences in CO_2 production among the different ripening stages, with the average rate of respiration being 1.11 ml kg h^{-1}. In an earlier study, Pérez et al. (2004) did not observe significant differences in the respiration rate of Mexican plum fruit at three different ripening stages: green, ½ yellow, and ¾ yellow. Similar responses were articulated by Kohatsu et al. (2011) on *Spondias purpurea* fruit grown in Brazil. In contrast, Dantas et al. (2016), Montalvo-González et al. (2011), Pareíra et al. (2000) and Osuna et al. (2011) reported a climacteric behavior in Mexican plums grown in Mexico and Brazil. Maldonado-Astudillo et al. (2014) indicated that due to the variation in the behavior of CO_2 production, it is difficult to determine if Mexican plum is a climacteric fruit although physical and biochemical changes during ripening suggest it is a climacteric fruit. They also reported an increase evolution of ethylene for immature, mature-green and turning fruits with the occurrence of a peak in ethylene production between days 3 to 5 at 20-21^{o}C followed by a rapid decline thereafter. Peak ethylene production preceded peak CO_2 production rates for immature and turning chili plums. Vargas et. al. (2017) also claimed that ethylene production increased at ripening from 3.92 to 9.43 µl kg h^{-1}, with the highest production observed in the fully ripe stage. Likewise, Montalvo-González et al. (2011) detected a significant increase in ethylene production during ripening of yellow chili plum under different light conditions. In general, an increase in ethylene production acts as a trigger to fruit ripening, inducing the autocatalytic production that causes changes in colour, texture, aroma, flavour, and other biochemical, physiological and physical attributes of the fruit (Hiwasa-Tanase & Ezura., 2014) that the consumer consider important for consumption.

C_2H_4 production rates also increased significantly ($P<0.001$) for immature, mature-green and turning fruits, peaking between 3-5 days of storage at 20-21^{o}C followed by a rapid decline (Figure 1C). Peak C_2H_4 production rates preceded peak CO_2 production rates for immature and turning fruits (Graham et al., 2001).

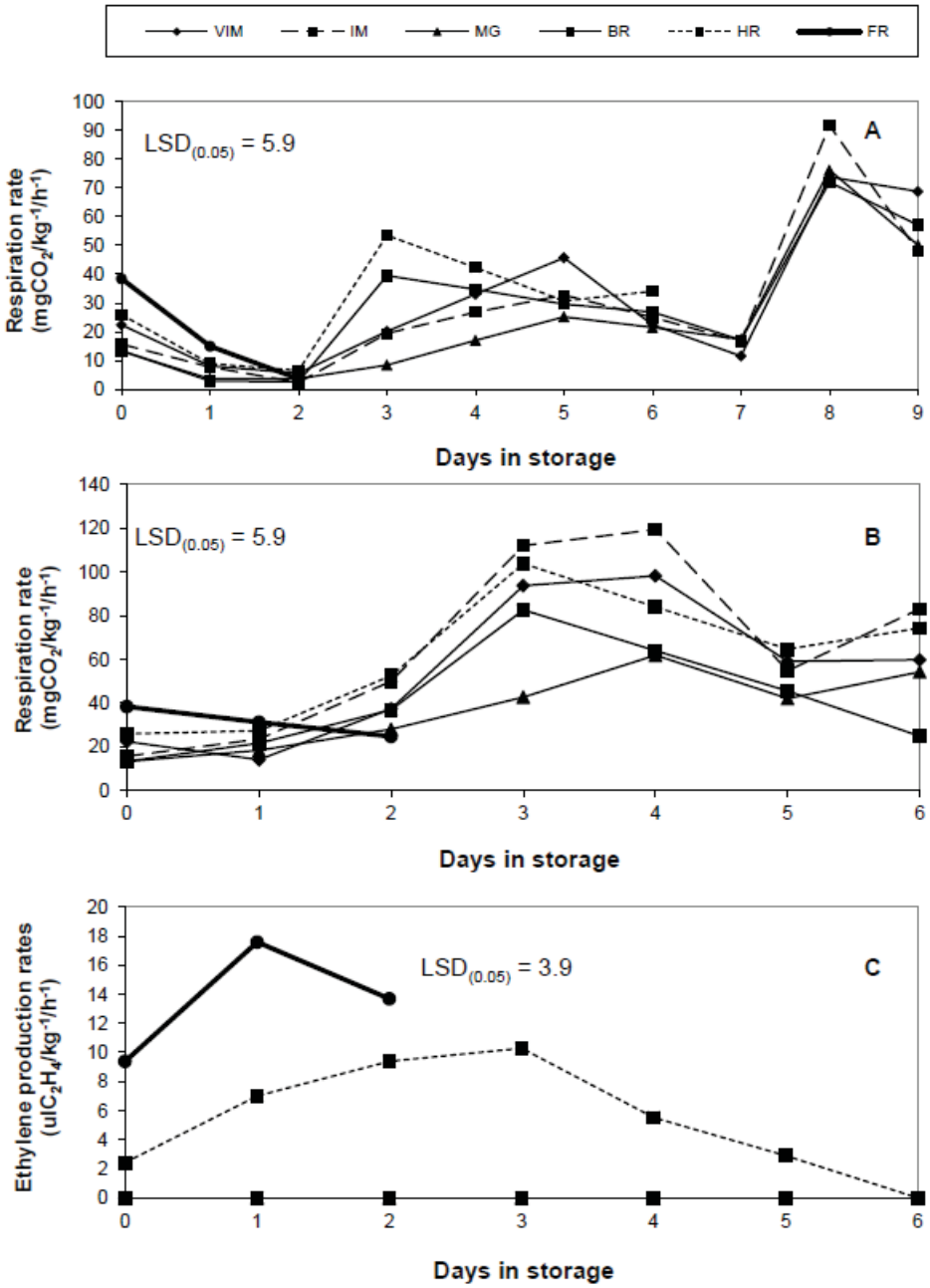

Figure 1. Changes in the respiration and ethylene production rates of chili plum fruits during storage

(A) Fruit stored at 9°C, (B) fruit stored at 20°C and (C.) fruit stored at 20°C. VIM= very immature fruit, IM= immature fruit, MG= mature-green fruit, BR= breaker fruit, HR = half-ripe fruit and FR = full-ripe fruit. Levels of significance were ($P<0.001$) for respiration and ethylene production rates respectively (Graham et al., 2001).

3.3 Physicochemical Changes during Ripening

Physicochemical changes of the mature-green chili plums during storage at 27.8-29.2°C was reported by Sampaio et al., (2007). They attributed that total soluble solids (TSS) increased from 9.1 °Brix at the mature-green stage to 13.7 °Brix at the climacteric peak. At the same time, total titratable acidity (TTA) decreased during maturation and ripening from 1.35% initially to 1.31% at pre-climacteric, 1.0% at climacteric peak and eventually to 0.8% at the post-climacteric stage. This decrease in organic acids as ripening progressed suggested the involvement of the acids as a source of energy during respiration (Coombe, 1976; Kader, 1986). Noteworthy, is the total acid content of 1.30% and 1.49% in ripened chili plum fruits in earlier studies reported

by Oliveira et al. (1999) and Bora et al. (1991). In their study, Sampaio et al. (2007) commented on the TSS-TTA ratio as increasing significantly at each stage in the climacteric curve while the opposite occurred for vitamin C content. Previously, Graham et al. (2001) provided evidence of increased total sugars and reducing sugar content, increased pH values and vitamin C content as ripening progressed. There was also increased softening in turning fruits compared to immature and mature-green fruit, and decreased TTA in more mature fruits over time. They concluded these changes to the breakdown of complex polysaccharides and subsequent conversion into simple sugars which normally occurs in many ripening fruits. In the same study, it was also articulated that fruits stored at 30-31°C had lower total and reducing sugars content than those stored at 20-21°C after 5 days. Fruits stored at 30-31°C had higher rates of respiration and consequently a much faster depletion of soluble sugars.

The behavior of chlorophyll pigments of chili plums during ripening decreased while carotenoids exhibited a continuous decrease as a result of changes in pH, presence of the oxidation system and the activity of chlorophyllase (Sampaio et al., 2007). The sequence of colour changes of the chili plum peel showed a transformation from an initial dark green to light green at the climacteric minimum; from light green to orange yellow during the climacteric rise in respiration and maintenance of an orange yellow colour during the climacteric peak and senescence.

Colour parameters in the epicarp of Mexican plum showed significant ($P<0.05$) changes among the four ripening stages (Vargas et al., 2017), similar to that reported by Dantas et al. (2016). The colour measurements of the green stage revealed that the epicarp was green (ho=108.9), dull (C*=26.4) and slightly bright (L*= 39.9) compared to the ½ green stage which was close to yellow (ho=93.7), less dull (C*=34.4) and brighter, and in the ¾ ripe stage, where fruits displayed an angle hue approaching orange (ho= 75.3) with a chromaticity of (C*= 43.4) and enhanced brightness (L*= 50.3). Colour changes in plum could be related to a decrease in chlorophyll levels and an increase in carotenoid biosynthesis. Soluble solids (SS) significantly increased from the green stage (5.8°Brix) to the ripe stage (23.9°Brix). Alia-Tejucal et al. (2012) reported maximum values of 17.3 °Brix after 67 harvestings. Elsewhere, Montalvo-González et al. (2011) and Tiburski et al. (2011) found SS for yellow plums varied between 14.9 ^{0}Brix to 15.0^{0}Brix. An increase in the SS as fruit ripening progressed has been reported by several authors (Dantas et al., 2016; Bautista-Banos et al. 2003; Perez et al. 2004; Osuna et al. 2011). Titratable acidity (TA), both in pulp and epicarp, was higher in the green stage and significantly decreased as the fruit ripened from 0.48% to 0.27% in pulp and from 0.42% to 0.23% in the epicarp (Vargas et. al. 2017). These results are in support with those reported by Dantas et al. (2016), Díaz-Pérez et al. (1999), Filgueiras et al. (2001) and Pérez et al. (2004) who got a decrease of titratable acidity as fruit ripening progressed. Alia-Tejacal et al. (2012) obtained values between 0.2 and 2.0% citric acid, while Tiburski et al. (2011) reported values of 1.46 % of citric acid in yellow plum. Flavour index (FI) significantly increased with ripening both in pulp (from 12.35 to 87.62) and epicarp (from 18.47 to 105.16) (Vargas et al. 2017). The flavor index increased as a result of a decrease in TA and an increase in SS. Dantas et al. (2016), Filgueiras et al. (2001) and Pérez et al. (2004) reported a similar behavior. On the contrary, Alia-Tejucal et al. (2012) confirmed a high variation on the FI (3.0 to 63.2) which they attributed to the wide genetic diversity of the evaluated fruits.

The ½ green and the ¾ ripe fruit stages contained higher total phenols concentrations in the pulp, 89.21 and 77.7 mg GAE 100 g^{-1} respectively, compared to the green and fully ripe stages (Vargas et al., 2017). However, the fully ripe stage showed the highest total phenols concentration in the epicarp (190.6 mg GAE 100 g^{-1}). Filguerias et al. (2001) quantified the highest concentration of total phenols in the ripe stage of *Spondias purpurea* and obtained values between 160 and 240 mg GAE 100 g^{-1}. Vieira et al. (2011) reported total phenols in plums of 55.0 ± 2.1, 70.92 ± 1.31 mg GAE 100 g^{-1}, respectively. Tiburski et al. (2011) reported total phenols concentration in pulp of yellow plum (*Spondias mombin*) of 260 mg GAE 100 g^{-1}. In the case of Mexican plum 'Cuernavaqueña', both the pulp and the epicarp are consumed, therefore the sum of the total phenol concentration of the pulp and the epicarp in ripe fruit make approximately 239 mg GAE 100 g^{-1} (Vargas et al., 2017). Phenols in *Spondias purpurea* have a natural antioxidant function and its consumption provides benefits against chronic and degenerative diseases (Filguerias et al., 2001; Tiburski et al., 2011). Flavonoids concentration in the pulp significantly increased from the green stage to the ¾ ripen and the fully ripe stages, from 17 to 23-22 mg QE 100 g^{-1} (Vargas et al., 2017). They indicated that the highest concentration of total flavonoids was observed in the epicarp of ripe plums with 214 mg QE 100 g^{-1}. The total flavonoids content in ripe Mexican plum 'Cuernavaqueña' when the pulp and the epicarp flavonoids concentrations were added resulted in a total of approximately 245 mg QE 100 g-^{1} which is higher than the content reported for papaya (63.2 mg QE 100 g^{-1}), grape (55.9 mg EQ 100 g^{-1}), açaí (70.1 mg QE 100 g^{-1}) and strawberry (21.8 mg QE 100 g^{-1}) (Zielinski et al., 2014).

3.4 Maturity Indices and Quality Components

The stage of maturity at which chili plums are harvested impacts significantly on their ultimate flavour and shelf life. Harvest maturity has a direct effect on the fruits' flavour components, physiological deterioration, susceptibility to physical injuries, resistance to moisture loss, susceptibility to invasion by rot organisms, market life and ability to ripen (Mohammed, 2015). Maturity of chili plums is determined by skin colour changes and fruit size and shape. Chili plums are usually classified as immature dark green (MI), mature light green (M2), slightly turning (light cream yellow) or breaker (M3) and tree ripe (uniform yellow) (M4). Immature dark green (M1) fruits may fail to ripen or ripen abnormally (Bridgemohan, 2008; Bridgemohan & Mohammed, 2019)). Apart from being highly prone to physical damages, immature fruits lack a fully developed surface cuticle thereby increasing their susceptibility to moisture loss. Immature chili plums have the lowest total soluble solids content and the highest acids compared to other stages of fruit maturity. Mature green (M2) and breaker (M3) fruits, moreso the latter than the former, are ideal as a fresh fruit desert or for processing into sweet or sour pickles and are also associated with a higher level of consumer acceptance. At different stages of maturity, fruits are consumed fresh, frozen, or processed into diverse food products such as pickles and candies. In situ tree ripen fruits (M4) which have the highest level of consumer acceptance also have a shortened shelf life due to flesh softening which render them conducive to, physical damage, bird damage and secondary microbial infections. Usually, by the time M4 fruits reach the consumer they become overripe, with poor eating quality and high postharvest losses (Bridgemohan, 2008).

3.5 Pre-harvest Factors Affecting Fruit Quality

Maintaining the nutritional status is an important factor in quality evaluation of chili plums at harvest and during storage. Excessive nitrogen application to the plant delays fruit maturity, induces poor colour development, and inhibits ground colour change from green to yellow. However, nitrogen soil deficiency leads to small fruit development accompanied by poor flavour and unproductive trees (Barbeau, 1994; Bridgemohan & Mohammed, 2019).

Since the chili plum seeds are not fertile, the plant is mainly propagated through large cuttings, 60-180 cm long and selection of cuttings is based on the most vertical new growths 1-2 years old, at the end of the dry season. However excess water at planting time could jeopardize plant growth as the wounds do not heal causing the cuttings to rot. With this technique, production begins the following year, climaxing after 4-5 years (Barbeau, 1994; Bridgemohan & Mohammed, 2019).

Ripe chili plums are highly vulnerable to fruit flies (*Anastrepha* spp). Other important pests include mites and birds. Fungal attacks are another limitation as infested fruits become covered with a grey ash-like dust which also spreads rapidly along the branches thereby reducing fruit size, appearance, taste, flavour and overall marketability and display quality (Barbeau, 1994, Bridgemohan, 2008).

3.6 Postharvest Management Affecting Quality

3.6.1 Temperature Management

Management of fruit temperature and relative humidity is paramount for extending the shelf life of fresh chili plums. Removal of field heat following harvest could be expedited by room cooling (9-10^{o}C, 85-95% r.h) or by hydrocooling. Chili plums should be stacked in the refrigerated rooms with air spaces between pallets and room walls to ensure good air circulation. Transit vehicles must be cooled before loading the fruits. Delays between cooling after harvest and loading into transit vehicles should be avoided. Maintaining the cool chain throughout the handling system is essential to optimize quality and shelf life (Mohammed, 2015).

3.6.2 Physical Damage

Chili plums have a thin skin and are eaten with the skin intact. Physical injuries resulting from abrasions due to over packing, punctures arising from sharp protrusions within harvesting containers or directly from harvesters' finger nails or from impact and vibration bruising during transportation and distribution must be controlled and reduced. Such physical injuries compromise fruit appearance, accelerate water loss, provide sites for fungal and bacterial invasion and stimulate respiration and ethylene production (Mohammed, 2015).

3.6.3 Transpiration

Fresh weight losses in chili plums is related to stage of maturity at harvest, storage temperature and storage duration. Graham et al. (2001) reported that immature fruits had higher percentages fresh weight losses irrespective of storage temperature and duration than mature green and turning or breaker fruits. This could be attributed to differences in cuticular thickness of the epidermal epicarp which offered more protection against

moisture losses as fruit maturity progressed. Graham et al. (2001) also demonstrated that fruits stored at 20-21°C and 30-31°C exhibited more moisture losses compared to fruits stored at 4-5°C and 9-10°C. They implied that this is due greater respiratory activity at the higher versus lower storage temperature. However, chili plums regardless of temperature and stage of maturity showed accelerated increases in percentage fresh weight losses as storage time advanced. Chili plum fruits with higher percentages fresh weight losses appeared to have a rougher skin surface rather than shriveled surface. These were notably less juicy, with thinner edible epicarps (Figure 2).

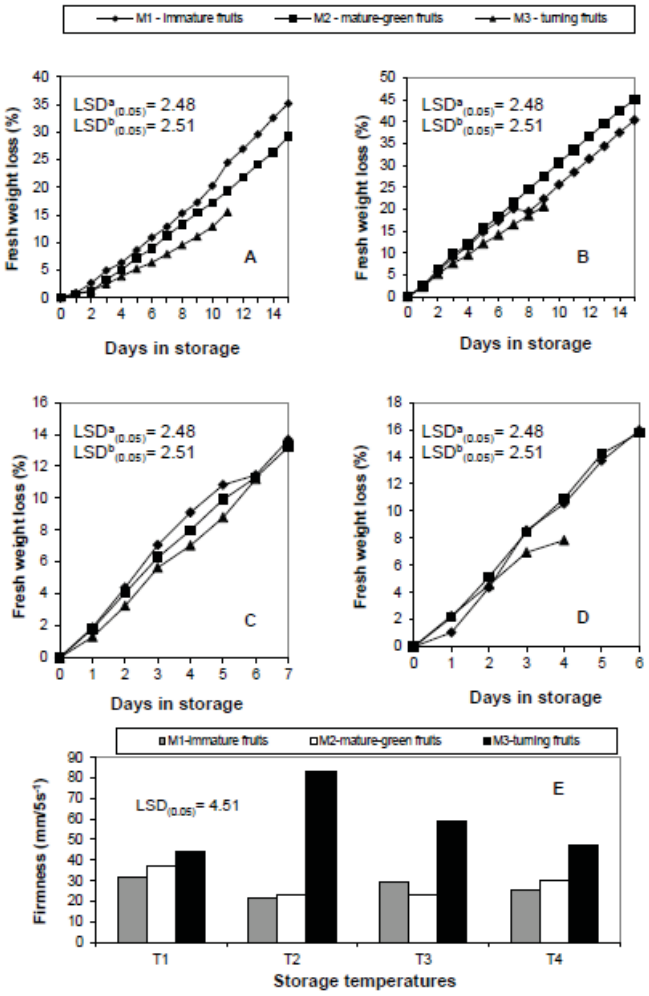

Figure 2. Changes in fresh weight losses and firmness of chili plums during storage

(A) Fruits stored at 4-5°C, (B) fruits stored at 9-10°C, (C.) fruits stored at 20-21°C, (D) fruits stored at 30-31°C and (E) firmness. LSD: over time[a] while across maturity stages and temperatures on the same day[b](Graham et al., 2001)

3.6.4 Atmosphere

Notable benefits were obtained as a result of the modified atmosphere created within the sealed packages when chili plums were packaged in low density polyethylene (LDPE) or high density polyethylene bags and stored under refrigerated conditions. The high relative humidity surrounding fruits in the saturated moisture environment within sealed packages led to a reduction in fruit weight losses and chilling injury symptoms development while enhancing overall appearance. Accordingly, fruits acquired a longer shelf life and were marketed over longer periods. Evidently, the fruits had a more acceptable taste, were juicier, firmer with thicker epicarps. (Graham et al., 2001).

3.7 Physiological Disorders

3.7.1 Chilling Injury (CI)

Chili plums are very sensitive to storage under refrigerated conditions (Avitia-García et al., 2000; Mohammed, 2015). Chilling injury alters the function of plasma membranes and their associated enzymes, as well as the general metabolic activities of cells (Maldonado-Astudillo et al., 2014). Graham et al. (2001) stored fruits at three different stages of maturity under refrigerated conditions. They reported chilling injury symptoms such as skin pitting in fruits at the immature green, mature green and turning or breaker when fruits were stored at 4-5^{o}C and to lesser extent at 9-10^{o}C, after 4 days only. Pitting was extensive in the immature green fruits and least in the turning or breaker fruits. Mature-green fruits kept at 9-10^{o}C for 15 days and subsequently transferred for 2 days at 20-21^{o}C had moderate chilling injury damage. Immature green fruits on the other hand, showed severe CI damage. Mature green fruits stored continuously for 15 days at 4-5^{o}C and then transferred to 20-21^{o}C for 1 day had severe CI symptoms with tiny pits coalescing into larger depressed areas with a definite brown discolouration. Similar occurrences were noted in mature green fruits stored at 9-10^{o}C for 15 days upon subsequent transfer to 20-21^{o}C for 2 days.

3.7.2 Other Physiological Disorders

Chili plum fruits are receptive to heat injury when exposed to temperatures over 35^{0}C for extended periods. Heat injury symptoms include development of scalds accompanied with surface discolouration and water-soaked translucent epicarps. Usually fruits would show symptoms of heat injury with the presence of hard lumps of unripen flesh directly underneath the skin (Mohammed, 2002; 2015).

3.7.3 Pathological Damage

Graham et al. (2001) reported that the shelf life of chili plums stored at 20-21^{o}C and 30-31^{o}C were terminated by decay after 8 days, with the incidence of decay being more prevalent and rapid in mature green and breaker fruits compared to immature green fruits (Table 1). The major cause of fruit decay was attributed to stem end rot caused by a fruit-rot fungus of the *Phoma* species. In another experiment Graham et al. (2001) stated that fruits stored at 30-31^{o}C ripened in 3-4 days and remained marketable for an additional 2 days, beyond which fruits were completely decayed.

While fruits stored at 15^{o}C and 12.5^{o}C initiated ripening after 10 days, excessive softening was obtained eventually resulting in decay amounting to 65%. Fruits stored at 12.5^{o}C maintained a dark-green skin colour without evidence of decay up to 14 days. During this period fruits had a TSS of 11% and were still firm. Upon transfer to 30-31^{o}C for 4 days following 14 days of storage at 12.5^{o}C fruits ripened uniformly and were highly acceptable according to organoleptic evaluations.

Table 1. Interaction effects of day x temperature x maturity on decay incidence of chili plums stored for 8 days at 20-21^{o}C and 30-31^{o}C

Storage duration (days)	Decay (%)					
	20-21 (^{o}C)			30-31(^{o}C)		
	M1	M2	M3	M1	M2	M3
4	0.0a[x]	0.0a	13.3ab	0.0a	20.0bc	13.3ab
5	0.0a	0.0a	13.3ab	6.7a	20.0bc	26.7c
6	13.3ab	13.3ab	40.0d	53.3e	73.3f	86.7g
7	46.7de	73.3f	86.7g	100h	100h	100h
8	100h	100h	100h	100h	100h	100h

[x]Means followed by the same letter(s) are not significantly different (P<0.05). M1 = immature fruits, M2 = mature-green fruits and M3 = turning fruits. Graham et al. (2001).

3.7.4 Insect Pests and Their Control

A major threat to the expansion of chili plums as a fresh fruit regionally and extra regionally is the high susceptibility to the Caribbean fruit fly (*Anastrepha* spp.) (Barbeau,1994). Graham et al. (2001) found live fruit fly larvae in chili plums subjected to a hot water treatment at 45^{o}C for 10 and 15 minutes respectively after 6 days of storage at 20-21^{o}C. Their investigation showed that while live larvae of variable sizes were found in control fruits, the larvae in heat-treated fruits at 45^{o}C, for 10 and 15 minutes were smaller. Dead larvae and eggs were detected in fruits treated at 45^{o}C for 20 minutes and at 50^{o}C for 10, 15 and 20 minutes respectively. However, the heat treatment at 45^{o}C for 20 minutes was more successful than the 50^{o}C regime since the former

accounted for no heat injury symptoms as opposed to the latter treatment where scalding manifested as brown coloured necrotic lesions (bronzing) dominated the surface of affected chili plum fruits.

3.8 Postharvest Handling Practices

3.8.1 Harvest Operations

The harvesting of fruits at physiological maturation to stimulate the process of ripening is essential in order to avoid potential losses during storage and market display (Bautista-Banos et al., 2003; Vanegas, 2005). When harvested at an unripe stage, the fruits may experience deleterious changes in colour and firmness, and they will typically fail to develop any of the optimal quality characteristics so important for commercialization (Mohammed, 2015). On the other hand, when fruits are harvested during the half-ripe stage they will usually tolerate longer periods of storage, while at the same time, achieve a quality that is both acceptable to consumers and is similar to the one attained by fruits that were allowed to ripen completely on the plant (Pérez et al., 2004; Souza, 2008). Chili plums fruits are borne in clusters and fruits in any one cluster may differ in maturity due to intra-plant competition for assimilates (Bridgemohan and Mohammed, 2019). The stem end of each fruit is attached with a fragile and extended pedicel. Harvesting of fruits is normally conducted manually in order to select fruits at physiological maturity. The abscission layer becomes weakened as the fruit begins to turn and upon ripening it is easily dislodged by strong winds and birds (Bridgemohan & Mohammed, 2019). The fragile nature of the pedicel induces physical damages at the stem ends during harvesting operations, eventually resulting in stem end rots and multiple infections, particularly when the severity of injuries is associated with split stem ends, rupturing of the pericarp and release of substrates. Abrasions, microscopic punctures and compression damages also accompany fruits during harvesting and are sites for secondary infections. Harvested fruits should be packed in well ventilated, shallow, light coloured containers to optimize quality and reduce postharvest losses associated with physical damages outlined above. Fruits should be placed in shaded environments during delays between harvest and transport to the packinghouse in order to minimize heat stress (Bridgemohan & Mohammed, 2019).

3.8.2 Packinghouse Practices

Due to the climacteric nature of chili plums, fruits must be pre-cooled to remove field heat via room cooling or hydrocooling. It is imperative that fruits are washed in chlorinated water (100-150 ppm) followed by a rinse and air drying. Fruits placed on packing lines should be equipped with conveyor belts that are well-padded to minimize bruising. Sorting and grading must be exercised prior to packaging to attain uniformity in size and fruit maturity to suit market requirements. Sanitation protocols must be monitored and implemented throughout all packinghouse operations. Since fruits are eaten with the unpeeled skin every effort must be made to ensure they are sufficiently sanitized to eliminate occurrences of food borne diseases (Mohammed, 2015).

3.8.3 Control of Ripening and Senescence

Changes from the typical green skin colour to a light yellow colour as well as the reduction of flesh firmness are the best visual indicators of chili plum fruit ripening and is a good predictor of potential shelf life (Mohammed, et al., 2016; Bridgemohan & Mohammed, 2019). Changes in skin colour and flesh firmness are directly related to the stage of maturity at harvest and controlled by temperature. Mature green and breaker stage fruits will ripen properly without exogenous ethylene application. Generally, ethylene application to fruits harvested at the turning or breaker stage will only ripen the fruit more uniformly without speeding up the rate of ripening. Adequate air circulation and maintaining a relative humidity of 90-95% are recommended to achieve uniform fruit temperature and prevention of fruit shriveling during the ripening process (Mohammed et al., 2016). Physiologically mature fruits stored at 28-30^{o}C would ripen in 3-4 days whereas at 15-17^{o}C would ripen in 10-11 days. At even lower storage temperatures at 9-10^{o}C it would require 14-15 days for full ripening. However, storage of fruits at 4-5^{o}C would extend shelf life up to 15 days but chilling injury and decay would occur. Graham et al. (2001) indicated that chili plums could be successfully stored up to 14 days at 12.5^{o}C and when fruits are subsequently transferred to 30-31^{o}C fruits ripened normally with an additional shelf life of 4 days. Mohammed (2002; 2016) found that chili plums harvested or taken from storage where ripening was already initiated (breaker) would tolerate chilling temperatures of 5-6^{o}C and maintain good organoleptic quality (Figure 3).

Effects of 1-methylcyclopropene (1-MCP) on the shelf life and the quality of Mexican plums were investigated by Garcia et al. (2011). 1-MCP was applied at 100, 200 or 300 nL L^{-1} for 12 hours at ambient temperature inside sealed experimental chambers of 0.512 m^3, and compared to an untreated control. After the 1-MCP application, fruits were stored under simulated marketing conditions (22 ± 2 °C; 70 ± 10 % RH) up to 9 days. 1-MCP at rates of 100, 200 or 300 nL L^{-1} were able to extend shelf life and to maintain the quality of Mexican plum yellow

fruits up to 3 additional days compared to the control, thus achieving 9 and 7 days of shelf life for ripe and ¾ ripe fruits, respectively. 1-MCP decreased the respiration rate and the weight loss of ripe fruits, but not in ¾ ripe fruits. 1-MCP delayed also the development of external colour and maintained fruit firmness, without affecting its total soluble solids content.

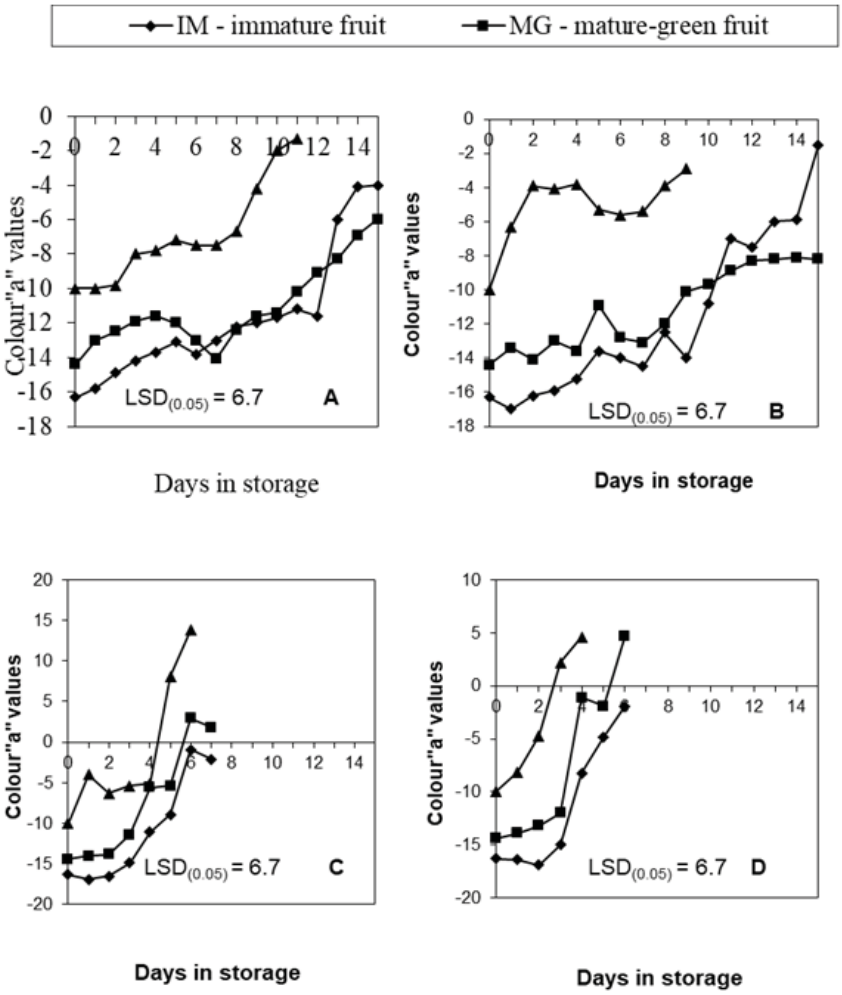

Figure 3. Colour changes ("a" values) in chili plums stored for 15 days

(A) Fruit stored at 4°C, (B) fruit stored at 9°C, (C.) fruit stored at 20°C and (D) fruit stored at 30°C. Level of significance (P<0.001) (Graham et al., 2001).

3.8.4 Storage and Shipping Conditions

Chili plum fruits harvested at full mature or slightly turning with a light yellow skin colour, that are blemish-free and subjected to a hot water treatment at 45°C for 20 minutes should be selected for overseas markets. Prior to shipment, fruits should be stored in shallow, ventilated one-ply cardboard cartoons at 12.5°C and 90-95% relative humidity. These conditions should be maintained throughout shipment for a maximum 7-8 days to allow display at overseas retail outlets at 20-22°C for another 4-6 days (Mohammed 2002). At display outlets fruits could be repackaged into smaller portion sizes in sealed low density polyethylene bags in order to achieve marketable fruits of over 90-95% (Mohammed, 2002, 2016).

3.9 Processing

3.9.1 Fresh-cut Processing

Fresh-cut technology has only been applied at the cottage level where slits are made with a knife at 2-3 locations on the fruit flesh and then sprinkled with salt and pepper as an appetizer or snack. Seal-packaged with 10-15 fruits per bag (LDPE), these snacks are popular at bazaars, cafeterias, sport events and other public functions as

ready-to-eat items. Alternatively, some vendors would make longitudinal face slices on either side of each fruit and serve with other fresh-cut fruits in stretch-wrapped styrofoam containers. Fresh-cut chili plums are also used as a stuffing in baked or curried fish (Mohammed, 2002).

3.9.2 Other Processing Practices

The processing and utilization of chili plums into a diverse range of value-added products were extensively investigated and described by Sammy (1994). Chili plums selected at the mature green and ripe stages were transformed into high sugar products, such as jams, jellies, fruit cheeses, preserves, candies, cordials and squashes. Value-added food products such as salted pickles, canned in brine and in sauces were also articulated by Sammy (1994). Other processing initiatives included production of fermented items such as wines and yogurt derived from ripe fruits, as well as dried and dehydrated products either whole or in slices (Sammy, 1994) all of which are currently manufactured in several cottage industries throughout the Caribbean. For the high sugar products, a concentration of 60-70% sugar is required, which through its osmotic effect, prevented microbial spoilage. For high salted products, the fruits were immersed in pure, granulated uniodized salt (1-3%) for flavour, plus vinegar containing 4-6% acetic acid for flavour and preservation purposes. Salt (2.5-8%) was also used as a selective agent facilitating the growth of lactic acid bacteria during the preparation of fermented chili plum pickles. For fermented chili plum pickles, mature green fruits sanitized in a chlorine dip (50ppm) were allowed to ferment for 2-3 weeks in a brine solution made up of 25-50g salt and 50ml vinegar per litre of water. The fermented plums were submerged in fresh brine and flavoured and acidified. The final stage involved pasteurizing at 85^{o}C for 15-20 minutes or by using a combination of chemical preservation with sodium benzoate (0.1% w/w) or potassium sorbate (0.1% w/w) or potassium metabisulphite (0.03% w/w) and pasteurization (Sammy, 1994). For dried and dehydrated products Sammy (1994) identified the use of fully mature, ripe firm fruits to be blanched for 5-15 seconds in boiling sodium hydroxide (10-20 g l^{-1}) to roughen the skin and accelerate the drying procedure. This was followed by dipping the treated fruits for 10-15 minutes in a 0.5% sodium metabisulphite solution (5gl^{-1}) to induce preservation and prevent brown discolorations. Afterwards, fruits were then soaked for 12-18 hours in a sugar solution containing two parts of sugar and one part of water by weight to withdraw moisture out of the fruit via osmosis and then dried to a moisture content of 12-14% (Sammy, 1994).

3.10 Medicinal Benefits and Ago Industrial Waste Utilization

In their studies, Vargas-Simon (2018) confirmed the climacteric pattern of respiration and further explored the nutraceutical values and medicinal potential of the plum *Spondias purpurea*. Vargas-Simon (2018) claimed that fruits have diuretic and antispasmodic properties and are commonly used as an antihistaminic. The extract from the tree bark is recommended for stomach upset such as dysentery, among other conditions. It has a strong larvicidal activity; the endocarp, seminal coat, and seed have significant amounts of cellulose, useful for phosphate ions flocculation from wastewaters.

4. Conclusion

The highly perishable nature coupled with very short seasonality of production, are major challenges confronting the availability and marketing of fresh chili plums. The low flesh to seed ratio makes it imperative to select only fruits at the mature green and breaker or turning stages of maturity for consumption as a fresh fruit or utilization into value-added products. Temperature management supplemented with atmospheric control to reduce or alleviate chilling injury damages and at the same time ensure proper ripening and optimum eating quality are achievable. As a potential export fruit, the use of hot water or hot moist air treatments must be explored to counteract problems associated with fruit fly infestations. The significance of enzymes on fruit softening warrants further investigation in order to counteract the rapidity of textural changes during ripening. Obtaining suitable packaging requirements to reduce physical damages would be beneficial in order to secure the best quality fruit for development into value-added product.

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A Behavioral Assessment of College Students' Knowledge, Awareness, and Consumption on Snack Foods That May Contain Probiotics

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Abstract

With the increasing variety of snack foods containing probiotics infiltrating the market, it is important that consumers become more aware and knowledgeable about these products. The aim of the current study was to investigate potential consumers' behavior by assessing knowledge about probiotics, awareness of snack foods containing probiotics, and frequently consumed snacks among student college departments within a university setting. Participants included 125 college students (n = 34 male, n = 91 female), all 18 years and older, and evaluated via a 19-item questionnaire using descriptive statistics, one-way analysis of variance (ANOVA) and Gabriel's post hoc test. Level of significance was set at $p \leq 0.05$. There was a statistically significant difference in knowledge about probiotics among the student college departments, $p = 0.012$. Specifically, students in the College of Health and Human Services (CHHS) were statistically significantly more knowledgeable than those in the Science, Technology, Engineering and Mathematics (STEM) college, $p = 0.010$. There was no statistically significant difference in awareness of snack foods containing probiotics, $p = 0.262$. On average, participants' knowledge about probiotics was low (48.1%) and awareness of snack foods containing probiotics was very low (2.5%), though, a majority of participants (94.1%) were aware that yogurt may contain probiotics. Overall, these findings should guide food product developers and marketers to create products that are relevant and messages that enhance consumers' knowledge and awareness to the existence of the probiotics in that product.

Keywords: awareness, college students, knowledge, probiotics, snacks

1. Introduction

As a result of a shift in meal times and growing clinical evidence supporting the importance of probiotics, snacking and probiotic consumption have increased (Clarke, Black, Stussman, Barnes, & Nahin, 2015; Douglas & Sanders, 2008; Kant & Graubard, 2015; Kumar, Vijayendra, & Reddy, 2015; Piernas and Popkins, 2010). According to Piernas and Popkins (2010), there was a 26% increase in snacking between the years 1977 and 2006. From 2007 to 2012, the use of probiotics and prebiotics had a fourfold increase with approximately 3 million more adults consuming probiotics (Clarke et al., 2015). To keep up with these demands, food product developers are investigating a number of innovative and novelty snack foods to be potential carriers for probiotics (Champagne, Gardner, & Roy, 2005). In order for any food product to be classified as "probiotic" it must contain live microorganisms which, when administered in adequate amounts, confer a health benefit to the host (Lahtinen, 2012). Though not all the time, but often, fermented foods (primarily an anaerobic process [meaning that it happens without oxygen] in which microorganisms [e.g. bacteria or yeasts] convert sugars in food to other compounds like alcohol or organic acids, while also producing energy for themselves) serve as the main carriers for probiotics (Chilton, Burton, & Reid, 2015; Stanton, Ross, Fitzgerald, & Van Sinderen, 2005). While fermented dairy-based foods, including yogurts, kefir, and other cultured drinks, are the most common probiotic foods available, there is a growing demand for non-dairy alternatives (Champagne et al., 2005; Heller, 2001; Payahoo, Nikniaz, Mahdavi, & 2012; Stanton et al., 2005). Non-dairy fermented foods that have been examined with respect to their potential in carrying probiotics include sauerkraut, pickles, fruit and vegetable juices, soy milk, pudding, chocolate, cereals, and dry sausages (Douglas & Sanders, 2008; Kumar et al., 2015). Probiotics have been demonstrated to help with *Clostridium difficile*, onset and relapse of pouchitis, ulcerative colitis, bacterial vaginosis, oral cavity diseases and stimulation of the immune system (Derikx et al., 2016;

Gionchetti et al., 2012; Goldenberg et al., 2017; Guillemard, Tondu, Lacoin, & Schrezenmeir, 2010; Haukioja, 2010; Tachedjian, Aldunate, Bradshaw, & Cone, 2017). These various health benefits, in addition to the research supporting probiotics strain stability and convenience, may help explain for the increase in probiotic consumption over the years (Champagne et al., 2005; Kumar et al., 2015).

According to literature (Al-Nabulsi et al., 2014; Stancak and Heuberger, 2009), although there is a wide range of probiotic choices commercially available, consumers may lack awareness and knowledge about probiotics. Awareness implies consciousness of something, while knowledge represents clear and certain mental apprehension (Rettie, 2003). In other words, a person can be aware of probiotics, but not know what they are, or vice versa (Christiansen & Maglaughlin, 2003). One study found that more patients were familiar with the term "probiotics" (43%) than they were knowledgeable about probiotics (20%), such that they could correctly define the term from a list of responses (Betz, Uzueta, Rasmussen, Gregoire, Vanderwall, & Witowich, 2015). And still, consumers have a difficult time identifying probiotic foods. When Stanczak and Heuberger (2009) asked participants to identify food sources that may contain probiotics (e.g., yogurt and milk), 54.6% instead answered "don't know." Another study performed by Al-Nabulsi et al. (2014) found that a large number of participants (97.5%) did not consume probiotics because they were "unaware" of what they are. While probiotic yogurt accounts for 78% of all probiotic sales worldwide, it is also considered as one of the most common foods that consumers associate with probiotics (Granato, Branco, Nazzaro, Cruz, & Faria, 2010; Payahoo et al., 2012; Stancak and Heuberger, 2009). It is, therefore, important to further study consumers' extent of awareness and knowledge about probiotics, as it may unintentionally minimize consumers' options and sales among food product companies.

Furthermore, focusing on consumers' awareness and knowledge are two behavioral data points that link to behavioral segmentation which is essential in tailoring products to meet the needs of consumers (Fieldboom, 2018). Behavioral segmentation stems from marketing segmentation which allows companies to divide the market into groups or segments based on distinct needs or wants (Djokic, Salai, Kovac-Znidersic, Djokic, & Tomic, 2013). By assessing a consumers' awareness to the existence of a product and knowledge of its full benefits, it will help determine what promotional materials and messaging are needed (Sunderland, 2017). And so, further research is needed to identify consumers' awareness toward snack foods that have been found to be potential carriers of probiotics and knowledge about probiotics. Additionally, identifying most frequently consumed snacks is a predictable assessment that allows researchers to keep up-to-date with commonly snacked foods and their positioning as a potential probiotic product. The current study will focus on college students, due to its convenience in sampling. The purpose was to better understand consumer behaviors to allow marketers to develop messages that enhance knowledge and awareness of probiotics which may ultimately optimize consumer options.

2. Method

2.1 Hypothesis, Participant Characteristics, and Sampling Procedure

The current study assessed students among college departments within a university setting by analyzing their knowledge about probiotics, evaluating their awareness of snack foods containing probiotics, and determining their top five frequently consumed snacks per week using a beverage and snack questionnaire (BSQ). The current study hypothesized that there would be no statistically significant difference in the target population's (i.e., student college departments) knowledge about probiotics, or awareness of snack foods containing probiotics.

Institutional Review Board (IRB) approval (1237262-2) was obtained prior to commencement of the study. Participants (n = 34 male, n = 91 female) were recruited through snowball and convenience sampling and screened based on the inclusion criteria for their status as college students and at least 18 years of age. A multiple-choice questionnaire of 19-items was administered to eligible participants via version 2018 of Qualtrics. An informed consent was obtained from each participant and all participants were completely anonymous.

2.2 Demographics and Knowledge about Probiotics

The demographic questions included information about gender, age, ethnicity, education level, and college (i.e., arts, business administration, education, engineering, health and human services, liberal arts, natural sciences and mathematics, and continuing and professional education). To assess knowledge about probiotics, the participants were asked to answer nine multiple choice questions, plus an additional question on familiarity, addressing basic concepts of probiotics.

2.3 Awareness of Snack Foods Containing Probiotics

To assess the participants' awareness of snack foods containing probiotics, they were asked to select how many visually represented snacks they believe may contain probiotics. There were eleven pictures in total of snack foods that have all been examined with respect to their potential in carrying probiotics (Douglas & Sanders, 2008; Kumar et al., 2015). The included items were sauerkraut, chocolate, cereal, vegetable juice, fruit juice, pickles, yogurt, cheese, pudding, soy milk, dry sausages and one option listed as "none of the above."

2.4 Beverage and Snack Questionnaire (BSQ)

The BSQ was adapted from the Supplemental Nutrition Assistance Program Education (SNAP-ed) Evaluation Framework Interpretive Guide which the Fred Hutchinson Cancer Research Center (2010) developed; instruments were similar to those described by Cock et al. (2016). The BSQ assessed participants snack frequency with a reference period of one week. The six frequency categories were: never or less than one per week; one per week; two to four per week; five to six per week; one per day; two or more per day. The BSQ included a total of 35 beverage and food items which were selected according to the snack categories commonly illustrated on grocery store websites. The beverage items included the following: fruit and vegetable juices, carbonated beverages, coffee, hot tea, iced tea, drinks such as energy, sports, powdered mixes, milk substitutes, dairy, smoothies, hot cocoa as well as cocktails, beer/wine/spirits. Snack food items included the following: chips, nuts/trail mixes, cookies, crackers, popcorn, dips/spreads, granola bars/snack bars, dried meats, fruit snacks, pretzels, dried fruit/dried vegetables, pudding/gelatin, yogurt, fruit cups/fruit sauces, rice cakes, snack cakes, gums/mints, candies, chocolate and ice cream.

2.5 Questionnaire Validity

Prior to distribution of the questionnaire, an expert panel of credentialed faculty members reviewed the survey items and provided feedback. Good internal validity was demonstrated by the expert panel results.

2.6 Data Analysis

Data from the current study was analyzed using the International Business Machine (IBM) Statistical Package for the Social Sciences (SPSS), Version 24. Descriptive statistics (i.e., frequency and mean scores) were used to analyze all variables for demographic data, frequency of consumed snacks, knowledge about probiotics, and awareness of snack foods containing probiotics. One-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences ($p \leq 0.05$) among the student college departments, age (18-50 years old), and ethnicity regarding knowledge about probiotics and awareness of snack foods containing probiotics. The Gabriel's post-hoc test was used to confirm exactly which student college department had significant differences. The independent samples *t*-test was used to determine whether there was a statistically significant difference ($p \leq 0.05$) between the means in gender and degree level on knowledge about probiotics and awareness of snack foods containing probiotics.

3. Results and Discussion

3.1 General Demographic Characteristics of the Study Population

In the current study, participants were recruited between the months of June and July in the year of 2018 from several college departments within a university setting and was based on convenience sampling. Specifically, the participants (n = 125) were college students, mainly undergraduates (79.0%) as presented in Figure 1, and predominantly female (Figure 2). Age groups of the participants ranged from 18-50 years old, with most of them being between the 21-24 years old range (Figure 3). With regards to their ethnicity, most of the participants were Hispanic/Latino (31.2%) followed by White American/Caucasian (28.0%), Asian/Native Hawaiian/Pacific Islander (25.6%), African American/African/Black/Caribbean (8.0%), and other (7.2%). Participants were originally surveyed and categorized according to their college department (Figure 4). In order to appropriately perform statistical analysis, the categories were repositioned so that population size was more equally distributed across all categories. The college departments that were smaller in numbers were combined into categories of similar college departments, while larger college departments were able to remain as their own category. In brief, the revised college department categories consisted of 32.8% College of Health and Human Services (CHHS), 24.8% Science, Technology, Engineering and Mathematics (STEM) fields which amalgamated the following college departments: College of Engineering (COE) and College of Natural Sciences and Mathematics (CNSM), 24.8% College of Liberal Arts (CLA), and 17.6% non-science (College of the Arts [COTA], College of Business Administration [CBA], and College of Education [CED]).

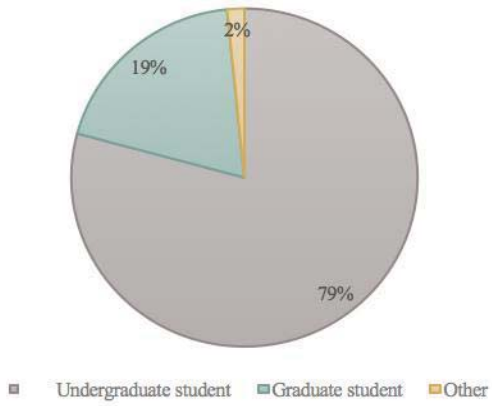

Figure 1. Demographic characteristics of students' degree level

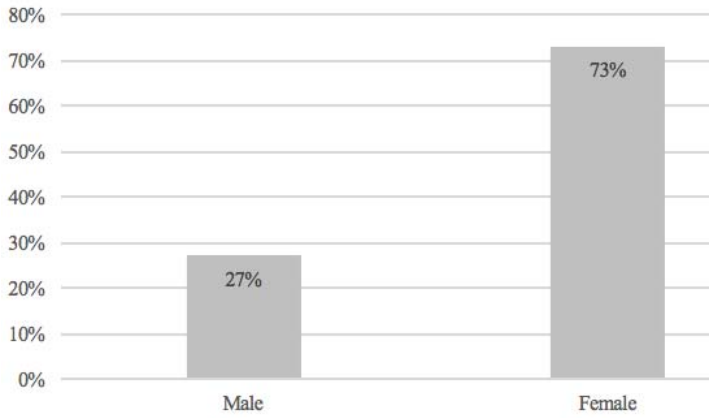

Figure 2. Demographic characteristics of students' gender

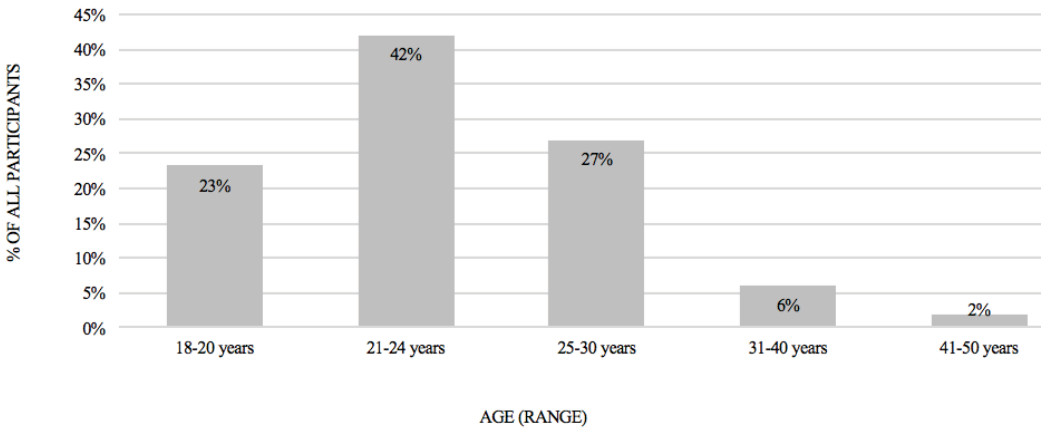

Figure 3. Demographic characteristics of students' age ranges from 18-50 years' old

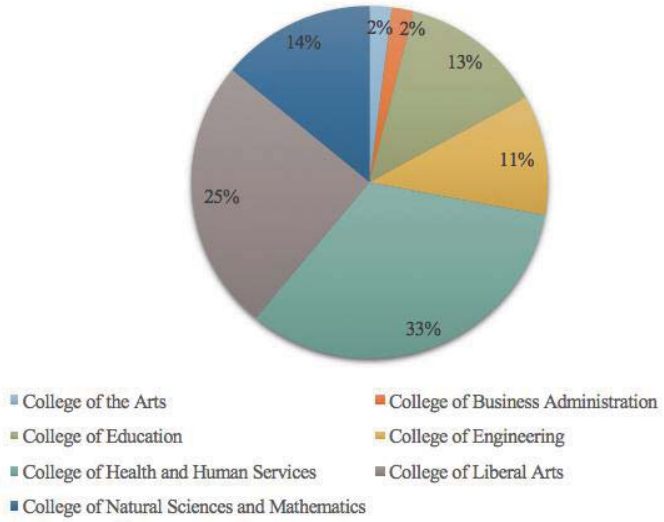

Figure 4. Demographic characteristics of students' college department categories

3.2 Knowledge about Probiotics

There was a statistically significant difference in knowledge about probiotics on the different college departments, $p = 0.012$. More specifically, Gabriel's post-hoc analyses revealed that CHHS students were statistically significantly ($p = 0.010$) more knowledgeable about probiotics than students in STEM. A similar trend was observed in the study by Al-Nabulsi et al. (2014) in which probiotic knowledge was significantly influenced by the students' study major and particularly higher among health science students compared to those in basic sciences, engineering and others. Payahoo et al. (2012) also found significant differences across majors, in which pharmacy and nutrition reported a higher level of knowledge than other majors. These differences in knowledge about probiotics across the college departments reject the null hypothesis, and may be due to the differences in curriculum and exposure to updated nutritional findings from faculty as well as colleagues.

While there were no statistically significant differences *(*$p = 0.616$) between ethnicity on the students' knowledge about probiotics, there was a significant difference found among the age ranges ($p = 0.006$). To illustrate, Gabriel's post-hoc analyses revealed that 25-30 year olds were statistically significantly *(*$p = 0.033$) more knowledgeable about probiotics than 21-24 year olds. Another statistically significant effect was found in degree level ($p = 0.006$, $r = 0.25$; Pearson's correlation is denoted by r in which 0.10 - 0.29 indicates a small effect), in which graduate students scored higher on knowledge about probiotics than undergraduate students. It could be implied that differences in educational levels may be related to additional courses taken by graduate students and not undergraduate students. There was also a statistically significant effect of gender ($p = 0.035$, $r = 0.13$; indicating a small effect), by which females scored higher on knowledge about probiotics than males. Furthermore, the effect size indicated that beyond statistical significance, there was a meaningful difference between knowledge about probiotics on degree level and gender. Al-Nabulsi et al. (2014) similarly found a significant difference in knowledge about probiotics and gender, by which female students' knowledge was better than male students; yet, did not find a statistically significant difference ($p = 0.784$) between knowledge about probiotics and educational level. The differences between gender may be related to the differences in perception of healthiness and willingness to try functional foods (Ares & Gambara, 2007). Ares and Gambaro (2007)) further found that women were able to perceive healthiness better than men when considering concepts about functional foods, such as probiotic yogurt.

Moreover, it was observed that the majority of participants correctly defined the term probiotics (96.8%) and its health benefit (84.0%) (Figure 6). Yet surprisingly, only 69.6% were able to accurately identify probiotic food sources. It was also observed that students were less knowledgeable about probiotic questions that were more specific. This may imply that consumers are least likely to choose a food containing probiotics, despite their ability to define the term. For this reason, it is important for food product developers to create transparent messages and clearly label food products if probiotics are present in them. This is especially important for novel or non-dairy products in which consumers would not expect probiotics to exist in.

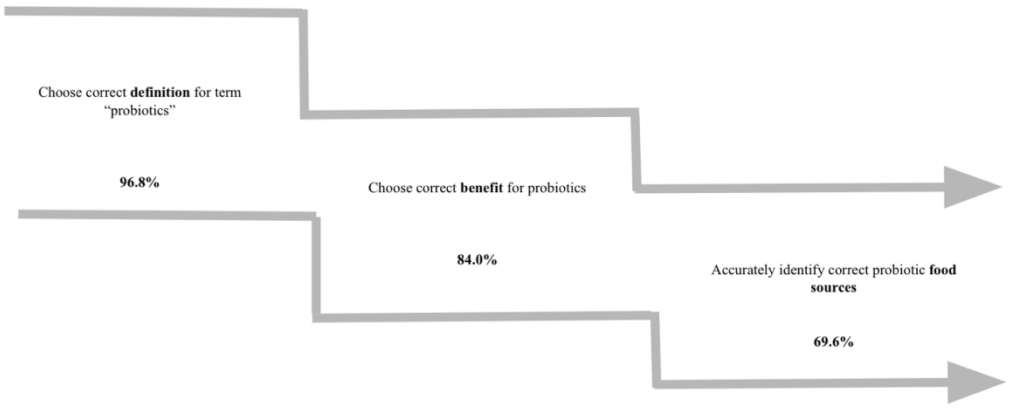

Figure 5. The correct response (%) for each question on knowledge about probiotics

When comparing the current study results to other studies, differences were found. To illustrate, participants sampled from the general public, were less able to identify the term probiotics (43.7%) compared to the current study that sampled students (96.8%) (Stanczak & Hueberger, 2009). This difference may be environmental (i.e. students vs. non-students) in which the participants from the study, being students, may have been more up-to-date on research and informed about certain topics related to probiotics. Meanwhile, when comparing the current study to student-based studies, there were contrasting results. The high percentage (96.8%) of those that correctly defined the term probiotics in the current study was similarly high among medical science students (83%), yet drastically lower among other college students (7.0%) (Al-Nabulsi et al., 2014; Payahoo et al., 2012). This may be due to differences in questions used and other influencing factors such as level of degree.

3.3 Awareness of Snack Foods Containing Probiotics

In the current study, there was no statistically significant effect of the different college departments on awareness of snack foods containing probiotics (p = 0.262), which failed to reject the null hypothesis. There were no statistically significant differences in age range (p = 0.097), ethnicity (p = 0.067), degree level (p = 0.077) or gender (p = 0.144) on awareness of snack foods containing probiotics. Gender, however, did represent a small-sized effect (r = 0.13), indicating a type II error (also known as a "false finding") as there seems to be a meaningful difference in awareness between genders. In contrast, Al-Nabulsi et al. (2014) found a statistically significant difference in awareness between genders, in which females were significantly more able than males to correctly identify yogurt (p = 0.033) and milk (p = 0.044) as commonly available forms of probiotic products. Briefly, experimental data showed that participants were most aware of yogurt (94.4%) containing probiotics, followed by cheese, soy milk, sauerkraut, vegetable juice, pickles/pudding/fruit juice, chocolate, cereal, and dry sausages (Figure 7). These results are consistent with a study by Payahoo et al. (2012) in which most of the students (72.9%) reported yogurt and other dairy products as commonly available sources of probiotics. Interestingly, Al-Nabulsi et al. (2014) found that college students who were aware of probiotics obtained their information mostly from college courses, followed by television commercials, television programs, healthcare providers and magazines or newspapers. This may emphasize that college students may be more likely to be aware of foods containing probiotics than the general public considering school was considered the main source of information.

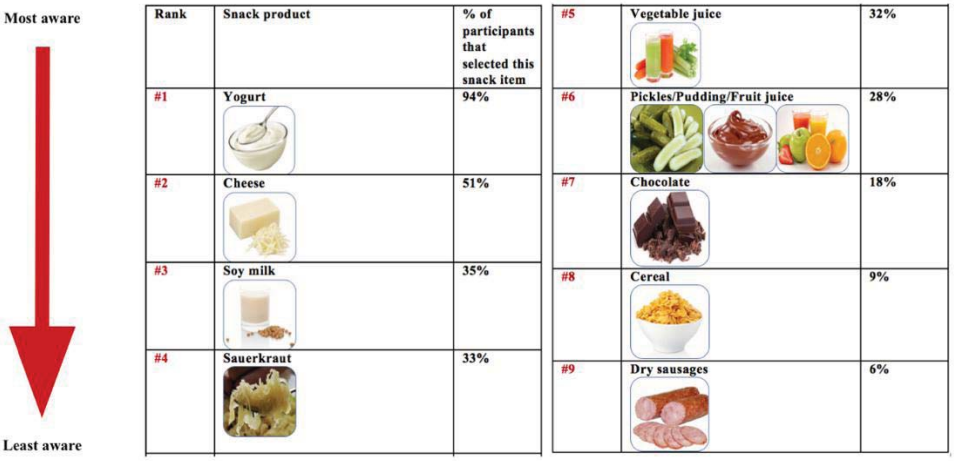

Figure 6. Awareness of snack foods containing probiotics ranked in order from most to least aware

3.4 General Knowledge about Probiotics and Awareness of Snack Foods Containing Probiotics and Familiarity

Overall, the experimental data indicated that there was a lack of knowledge about probiotics and awareness of snack foods containing probiotics among college students. Out of all the questions assessing their knowledge, on average, students answered 48.1% of the questions correctly. In relation to questions of awareness, on average, students only answered 2.5% of the options provided on snack foods containing probiotics. Both awareness and knowledge scores were low and demonstrate the need for educating and developing marketing tactics to raise knowledge and awareness. Furthermore, most respondents were "somewhat familiar" (40.8%) followed by those that were "familiar" (34.5%), "very familiar" (12.8%), "extremely familiar" (10.4%), and "not familiar" (1.5%). Those that were "not familiar" was defined as not having heard of the term probiotics before. This percentage was very small compared to another study who found 32.9% of participants that had not heard of the term probiotics before (Chin-Lee, Curry, Fetterman, Graybill, & Karpa, 2014). This may be due to differences in level of education, by which all of the current study participants were current college students compared to those that had varied levels of education, some not having obtained a high school diploma/GED (25.6%).

3.5 Beverage and Snack Questionnaire (BSQ) per Week

From the data analysis, it was observed that the five most frequently consumed snack foods using the BSQ per week were nuts (73.6 %), > coffee (70.4 %), > dairy (69.6 %) > granola (66.4 %), > chips (60.8%) (Figure 5). The popularity of salty snacks, including nuts and chips, can be supported by other research. To illustrate, Piernas and Popkins (2010) showed that between 1977 and 2006, there was an increased intake in salty snacks, chips, and nuts. In 2014, salty snacks (i.e. nuts, chips, popcorn, and crackers) were also found to be ranked the top category of snack foods (Bartelme, 2016). This may be related to consumers valuing the taste of salty snacks more than their nutrition (Mintel, 2015). It is interesting to note how snacking selections have changed over time. In contrast, in 1980, Khan did not find nuts to be a common snack among college students, and that fruit was a most common snack. Yet, Piernas and Popkins (2010) research showed that intake of nuts had increased, while other snacks such as fruit have slightly declined since 1977. The results from past research and the current study demonstrates the importance of keeping up-to-date on research and assessing consumers' behavior in relation to the positioning and marketing of snack foods, especially those with added benefits such as probiotics. Snacking selections may also differ from region to region, as in the example of fruit being the most common snack in Brazil, despite its decline in the U.S (Duffey, Rivera, & Popkin, 2014).

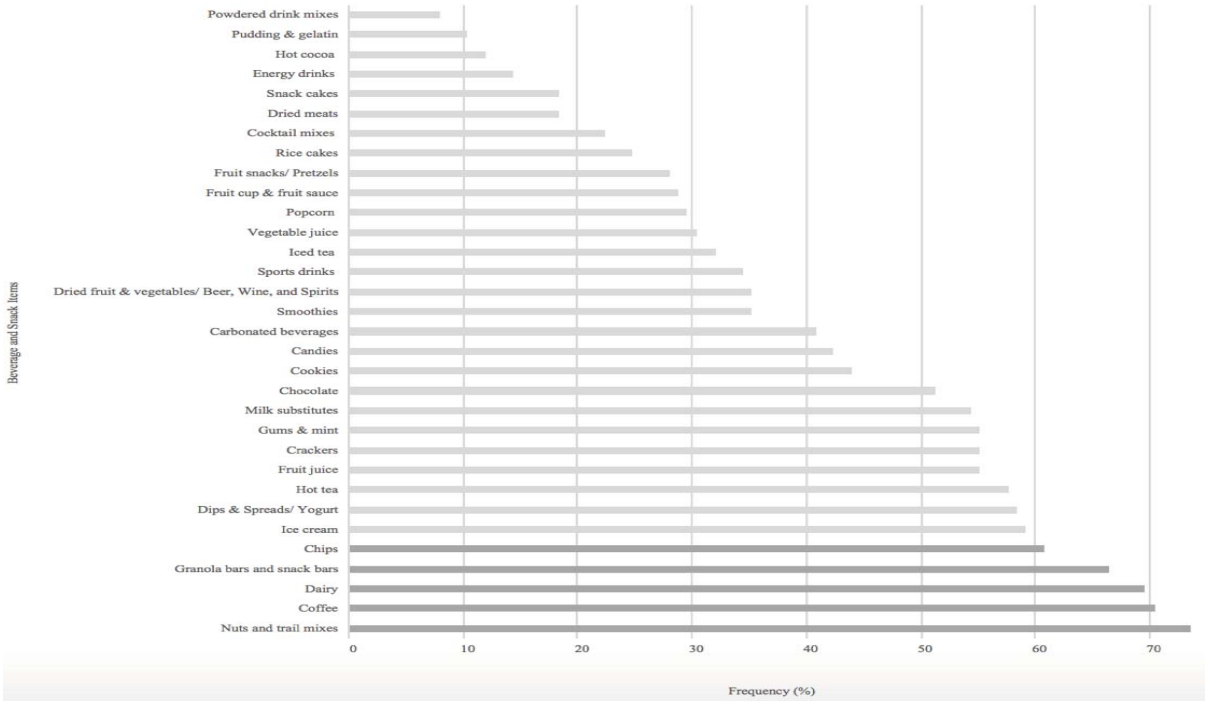

Figure 7. Most frequently consumed beverages and snacks (bottom to top)

3.6 Limitations

It is important to consider the limitations of the current study since the sample size was small and not representative of the entire college student population or general public. This may limit the analysis of students' current knowledge and awareness, compared to using a greater and more diverse sample. Using the BSQ was another limitation, as it relies on the participant's memory rather than the actual frequency intake. Also, the questionnaire was not tested for reliability which is important for internal consistency.

3.7 Future Recommendations

As researchers continue to study which foods may source probiotics, nuts and other frequently consumed snacks could be the focus according to the experimental results of the current study. It also supports the idea of behavioral segmentation, in which food product developers create innovative items based on the studied consumer behaviors. In relation to differences in knowledge about probiotics, an adjustment in curriculum or increase in the number of basic food science and nutrition courses offered may increase and balance the level of knowledge among students within various college departments. To help increase awareness of snack foods containing probiotics, marketing may focus on enhancing recognition by utilizing advertisements, public relations, and promotion to increase the impact and knowledge about probiotics. In conclusion, the results of the current study may help food product developers and marketers create messages that are relevant and will resonate with the consumer to increase the impact of their product in terms of potential health benefits.

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An Assessment of Total Polyphenolic Content and Antioxidant Potential of Mauby Bark Extracts (*Colubrina arborescens*) Brewed for Different Lengths of Time

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Abstract

Mauby bark (*Colubrina arborescens)* is commonly used to make a beverage,"Mauby", in the Caribbean and is believed to possess antiglycemic, antilipidemic, and anticarcinogenic properties. However, limited studies have been conducted to substantiate the compounds present that may confer these benefits. Therefore, the objectives of this research were to quantify the total polyphenolic content and evaluate the antioxidant capacity of Mauby bark extracts brewed in water at 30, 45, and 60 minutes. In the extracts, the Total Flavonoid Content (TFC) ranged from 1.93 - 3.17 mg CE/mL and the Total Phenolic Content (TPC) ranged from 2.10 mg ± 0.11 GAE/mL (45 minutes) - 2.36 mg ± 0.067 GAE/mL (30 minutes). Moreover, their antioxidant activity was assessed using the 2,2 Diphenyl 1-Picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays. The DPPH scavenging activity observed from Mauby extracts ranged from 75% ± 4.02 (30 minutes) to 83% ± 0.66 (60 minutes) and the FRAP values ranged from 6.29± 0.84 (30 minutes) to 6.90 ± 1.54 mM $FeSO_4$ equivalents/ 0.2 mL Mauby extract (45 minutes). Although, polyphenolic content at 30 minutes was greater than 60 minutes of brewing for TFC ($p < 0.001$) and TPC ($p = 0.002$), the scavenging activity was greater at 60 minutes than 30 minutes ($p = 0.014$) while antioxidant power was not affected by brewing time ($p = 0.736$). In summary, brewing the bark at 60 minutes was observed to provide the highest antioxidant activity.

Keywords: beverage, Caribbean, Ethnomedicine, tree bark

1. Introduction

Mauby bark (*Colubrina arborescens)* (Figure 1) which belongs to the *Rhamnaceae* family (i.e. Buckthorn) is indigenous to the tropical regions of Central America and Florida (Johnston, 1971) where it is widely consumed as a beverage believed to possess medicinal properties (Alleyne, Roache, Thomas, & Shirley, 2005). Previous research (Alleyne et al., 2005; Smith, 2012) suggests that Mauby bark extracts may reduce the prevalence of hypertension and diabetes. Additionally, Mauby bark extracts have been used through ethnomedicinal practices to alleviate inflammation due to rheumatism and possesses diuretic properties (World Agroforestry Centre [WAC], 2017). Conventionally, Mauby bark extract is produced by brewing the bark and extracting the liquid which is believed to be rich in polyphenolic compounds attributing to its hypothesized medicinal benefits (Barbados Pocket Guide, 2011). Notably, polyphenols are extensively studied due to their ability to reduce or prevent chronic illnesses in humans which may be applicable to studies involving Mauby bark extracts.

Figure 1. Mauby bark

Photo Courtesy: J. Embola and C.Rock

Polyphenols are molecules synthesized in plants to provide protection against ultraviolet radiation (UV) and pathogens (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004; D'archivio et al., 2007). Additionally, polyphenols act as dietary antioxidants by scavenging free oxygen radicals to reduce the risk of acquiring chronic illnesses related to oxidative stress (OS) (Manach et al., 2004; D'archivio et al., 2007). To illustrate the role of dietary polyphenolic intake among individuals with diabetes, flavonoids found in plants can decrease the rate of oxidative damage through inhibiting the oxidation of β-cells and stabilizing reactive oxygen species (ROS) (Patel, Kumar, Laloo, & Hemalatha, 2012). Mauby bark and its associated beverages are widely consumed and commercialized throughout the Caribbean, but are not extensively studied as exemplified by limited studies. More research is needed to substantiate the compounds present that may confer these benefits regarding its polyphenolic and antioxidant properties. Therefore, the dissemination of Mauby bark benefits is hindered until analyzed and verified. Furthermore, the use of ethnomedicinal tree barks established globally in commercial food applications is novel in the Unites States. Thus, the overall objective of this experimental study was to quantify the total polyphenolic content and assess the antioxidant potential of Mauby bark extract using different brewing times to provide information that may explain attributes relative to its proposed health benefits.

2. Materials and Methods

2.1 Mauby Extract Preparation

First, Mauby bark (obtained by a third party vendor) was ground into a fine powder using a blender. Next, twenty grams (20 g) of Mauby bark powder was added to 250 mL of boiling water at 100°C with 5 g of boiling chips for 30 minutes. Furthermore, the same quantity of powder was used for 2 additional batches and brewed for 45 minutes and 60 minutes respectively. The treatments selected were similar to times Mauby bark is brewed by native Caribbean consumers. Once the Mauby bark samples were brewed for their respective times, the extracts were centrifuged at 4,000 revolutions per minute (RPM) for 10 minutes at 4°C using a Thermo Scientific Sorvall ST 8 R centrifuge and diluted (1:10) for subsequent analyses.

2.2 Total Flavonoid Content (TFC) of Mauby Extracts

The TFC of Mauby bark extracts were measured according to the methodology described by Marinova, Ribarova, and Atanassova (2005). First, a catechin standard curve was created from a stock solution of a concentration of 100 mg/100 mL to make five catechin standard solutions of varying concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL). Next, 1 mL of each standard as well as diluted Mauby extracts were pipetted into 10 mL volumetric flasks, containing 4 mL of deionized distilled water (DDH_2O). Furthermore, 0.3 mL of 5% Sodium Nitrate ($NaNO_2$) was added to each flask and held to incubate at room temperature (~ 25° C) for 5 minutes. Then, 0.3 mL of 10% Aluminum Chloride ($AlCl_3$) was added to each flask followed by 2 mL of 1M Sodium Hydroxide (NaOH). Next, 6.4 mL of DDH_2O was added to each flask to acquire a final volume of 10 mL and mixed

thoroughly. The absorbance of each sample extract, which was a blue hue after addition of all reagents, was measured at a wavelength of 510 nm using an Ultraviolet (UV) spectrophotometer (Thermo Scientific AquaMate 8000 UV-Vis Spectrophotometer). Last, TFC results were expressed as mg catechin equivalents (CE)/ mL Mauby bark extract. All samples were analyzed in triplicate and averaged for statistical analysis.

2.3 Total Phenolic Content (TPC) of Mauby Extracts

The TPC of Mauby bark extracts were measured according to the methodology described by Marinova et al. (2005). First, a gallic acid standard curve was created from a stock solution with a concentration of 100 mg/mL to make five gallic acid standard solutions of varying concentrations (0.02, 0.04, 0.06, 0.08 and 0.10 mg/mL). Next, 1 mL of each sample as well as diluted Mauby bark extracts were added to 25 mL volumetric flasks containing 9 mL of DDH_2O. After that, 1 mL of Folin-Ciocalteu (FC) reagent was added to the flask and mixed thoroughly. Furthermore, the samples were incubated at room temperature for 5 minutes then 10 mL of 7% Sodium Bicarbonate (Na_2CO_3) solution was added to the mixture in each flask. Subsequently, the flasks were incubated for 90 minutes at ambient room temperature (23˚C). Next, the absorbance of each sample extract, which was orange-yellow in hue, was measured at a wavelength 750 nm using an UV spectrophotometer (Thermo Scientific AquaMate 8000 UV-Vis Spectrophotometer). Lastly, the TPC results were expressed as mg gallic acid equivalents (GAE)/mL Mauby bark extract using a standard curve. All samples were analyzed in triplicate and averaged for statistical analysis.

2.4 Determination of the Free Radical Scavenging Activity of Mauby Bark Extract

The free radical scavenging activity of Mauby bark extract was quantified according to a methodology described by Priyanka, Kadam, Ghule, and Aparadh (2013). First, 0.1 mM DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) was dissolved in 90% methanol in a beaker protected from light. Next, 1 mL of Mauby bark extract was added to 3 mL of the DPPH radical solution. Additionally, a control was made using 1 mL of methanol and 3 mL of the DPPH● radical solution. Then, the solution was mixed and incubated at room temperature for 30 minutes in the dark. Afterwards, the DPPH● radical solution was analyzed using a UV Spectrophotometer (Thermo Scientific AquaMate 8000 UV-Vis Spectrophotometer) at a wavelength of 517 nm. Next, the free radical scavenging activity was calculated as the percentage of free radicals able to be scavenged by the extract (Equation 1). Last, all sample extracts were analyzed in triplicate and averaged for analysis.

$$\text{Inhibition of DPPH activity (\%) in Mauby} = (A_{control} - A_{sample} / A_{control}) * 100 \quad (1)$$

2.5 Determination of the Ferric Reducing Antioxidant Power (FRAP) of Mauby Bark Extract

The total antioxidant power of Mauby bark extract was measured according to Abbasian et al. (2013) with slight modifications. First, Ferrous Sulfate ($FeSO_4$) standards (0, 3, 6, 9 and 12 mM) were prepared to determine the antioxidant power of Mauby bark extract via a standard curve. Next, the FRAP reagent was made fresh as needed by combining three reagents in a 10:1:1 ratio namely: 300 mmol/L of acetate buffer (pH 3.6); 10 mmol/L of 2,4,6-tripryridyl-s-triazine (TPTZ) dissolved in 40 mmol/L hydrochloric acid (HCl), and 20 mmol/L of ferric chloride ($FeCl_3$) dissolved in DDH_2O. Then, 3.8 mL of the FRAP reagent was added to 200 µL of Mauby bark extract into dark test tubes and incubated at 37°C in a temperature-controlled water bath for 5 minutes. Next, the absorbance of each sample extract was measured at an absorbance at a wavelength of 593 nm to test the total antioxidant capacity of reagents using a UV spectrophotometer (Thermo Scientific AquaMate 8000 UV-Vis Spectrophotometer). Last, the results were expressed as mM $FeSO_4$ / mL Mauby extracts. All samples were analyzed in triplicate and averaged for analysis.

2.6 Statistical Analysis

All numerical data of this study was analyzed using the International Business Machine (IBM) Statistical Package for the Social Sciences (SPSS), Version 24. Moreover, the following analyses were used: one-way analysis of variance (ANOVA) to assess significance of polyphenolic quantity at 30, 45 and 60 minutes and Tukey's Honest Significance Difference (HSD) post-hoc to determine which time interval was significant upon significance of ANOVA. Lastly, the mean and standard deviation (SD) was completed for each chemical assay.

3. Results and Discussion

3.1 Total Flavonoid Content (TFC) of Mauby Extracts

The polyphenolic content of Mauby bark extracts was analyzed using the TFC and TPC assays. Specifically, flavonoids are a subcategory of polyphenols that comprise of six different classes: anthocyanins, flavanols, flavanones, flavones, flavonols, and isoflavones (Dai & Mumper, 2010; Scalbert & Williamson, 2000). The TFC assay signifies the total concentration (mg/mL extract) of flavonoids in Mauby bark extracts as opposed to the

individual sub classes which were previously mentioned. Notably, the mean TFC in Mauby bark extracts (Figure 2) in this present study ranged from a low of 24.13 ± 2.13 mg CE/g (60 minutes) to a high of 39.63 ± 1.38 mg CE/g (30 minutes). After 30 minutes, there was a significant reduction in the total quantity of flavonoids suggesting that prolonged exposure to heat destroys polyphenolic compounds. Results from Tukey's HSD post-hoc suggest a significant ($p < 0.001$) difference in flavonoid content between brewing at 30 minutes to both 45 minutes and 60 minutes respectively, and a significant ($p = 0.018$) difference between brewing at 45 minutes and 60 minutes.

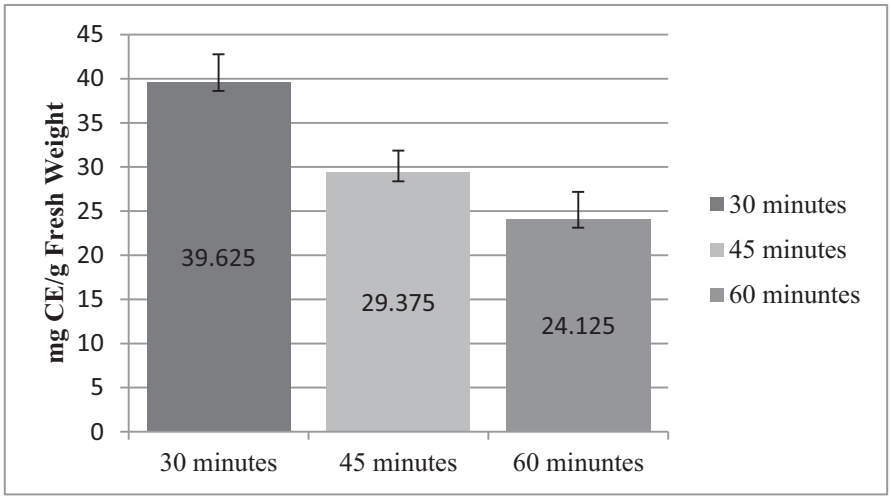

Figure 2. TFC results for Mauby extracts

Table 1. Tukey's HSD post-hoc analysis for TFC

Treatment Time	P Value
Between 30 minutes and 45 minutes	< 0.001
Between 30 minutes and 60 minutes	< 0.001
Between 45 minutes and 60 minutes	0.018

$p \leq 0.05$ is significant

Mauby bark extract was observed to have a mean TFC of 31.04 mg CE/g. Bark extracts observed from other studies (Baliga, Pai, Bhat, Palatty, & Boloor, 2011; Koti & Ashok, 2010) have similar TFC content to Mauby bark extract. For instance, *Mimusops elengi* Linn., is a bark indigenously used in India and other South Asian countries as a diuretic. Additionally, *M. elengi* Linn can be used to treat cardiovascular disease (CVD) and contains antidiabetic and anticarcinogenic properties (Baliga, et al., 2011; Koti & Ashok, 2010). The TFC content of *M. elengi* Linn was observed to have 23.55 ± 0.80 mg Quercetin Equivalents (QE)/g (Mathur & Vijayvergia, 2017). Another study (Dróżdż & Pyrzynska, 2018) investigated the polyphenol content of oak bark (*Quercus robust* L.) extracts which has been used to treat skin diseases and found the TFC to range from 35.1 ± 0.2 mg CE/g to 38.0 ± 1.1. Additionally, oak bark is commercially available as a supplement that is rich in tannins, a subdivision of polyphenols, and is used extensively for its anti-inflammatory properties (Dawid-Pać, 2013).

3.2 Total Phenolic Content (TPC) of Mauby Extracts

From the experimental results, the mean TPC in Mauby bark extracts (Figure 3) ranged from a low of 26.21 mg ± 1.38 GAE/g (45 minutes brewing time) to a high of 29.5 ± 0.88 mg GAE/g (30 minutes brewing time). Results from Tukey's HSD post-hoc suggest a significant ($p = 0.002$) difference in phenolic content between brewing at 30 minutes to both 45 minutes and 60 minutes respectively. However, no significant ($p = 0.995$) difference was observed between brewing at 45 minutes and 60 minutes. In contrast, *Cinnamomum zeylanicum* and *Pinus maritima* bark extracts have been observed by researchers to have higher TPC than Mauby bark which has a mean value of 27.29 mg GAE/g. In contrast, in a similar analysis of polyphenolic content, researchers (Ghitescu et al., 2015) observed that increases in time resulted with increased total polyphenolic extraction for spruce bark for up to 60 minutes using ethanol and water for extraction. However, a temperature of 50˚C was used and the

authors (Ghitescu et al., 2015) noted that increased time at high temperatures may initiate polyphenolic degradation. This may explain the decrease in TPC and TFC in Mauby bark after 30 minutes brewing at an extraction temperature 100˚C.

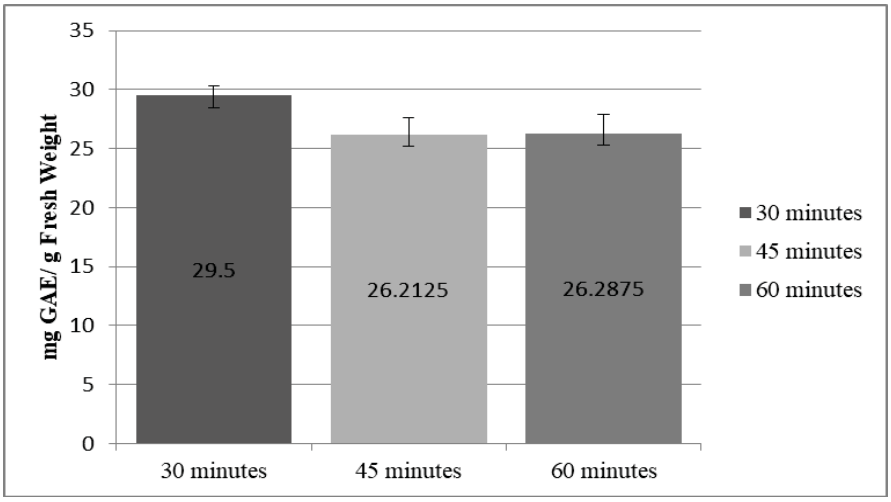

Figure 3. TPC results for Mauby extracts

Table 2. Tukey's HSD post-hoc analysis for Total Phenolic Content

Brewing Time	P Value
Between 30 minutes and 45 minutes	0.002
Between 30 minutes and 60 minutes	0.002
Between 45 minutes and 60 minutes	0.995

$p \leq 0.05$ is significant

Notably, the TPC of *C. zeylanicum* and *P. maritima* were 309 ± 0.05 mg GAE/g and 360 ± 0.04 mg GAE/g (Dudonné, Vitrac, Coutiere, Woillex, & Mérillon, 2009). *C. zeylanicum* is a spice also known as Ceylon Cinnamon that is beneficial in the treatment of diabetes, CVD and hypertension through lowering blood glucose, blood pressure and serum cholesterol (Ranasinghe, Pigera, Premakumara, Galappaththy, & Constantine, 2013). *P. maritima,* sometimes referred to as French maritime pine bark, has further been observed to be a potent scavenger of ROS produced from hydrogen peroxide (H_2O_2; Cho, Yun, Packer, & Chung, 2001). In the food industry, supplements derived from procyanidin of *P. maritima* bark are among the most popular and extensively researched tree bark applications (Kähkönen et al., 1999).

In this study, a temperature of 100°C was used for polyphenolic extraction with noticeable declines in polyphenolic content as brewing time progressed. When compared to a temperature of 50°C used for polyphenolic extraction of the barks, *C. zeylanicum* and *P. maritima*, resulted in a higher TPC content. Therefore, temperatures above 50°C may be responsible for the decrease in TPC of Mauby bark. A comparison of TPC content of Mauby Bark to other known tree barks revealed that the TPC content of scotch pine bark (*Pinus sylvestris)* was 76 ± 2.9 mg GAE/g, willow bark (*Salix caprea)* was 75.5 ± 1.5 mg GAE/g, silver willow bark *(Salix alba)* was 58.6 ± 0.9 mg GAE/g and lastly, aspen bark (*Populus tremula)* was 32.1 ± 0.2 mg GAE/g (Kähkönen et al., 1999). While, it is true that some bark extracts have markedly higher TPC values than the Mauby bark extract, the bark extract of silver birch (*Betula pendula*) was observed to have 2.0 ± 0.1 mg GAE/g (Kähkönen et al., 1999). It is to be noted that the majority of the barks listed have applications as nutraceuticals.

3.3 2,2 Diphenyl 1-Picrylhydrazyl (DPPH) Free Radical Scavenging Activity of Mauby Bark Extract

The free radical scavenging activity of Mauby bark extract was measured by its ability to donate hydrogens to the DPPH● radical, thereby inhibiting it. Free radicals in the body which accumulate from diets low in antioxidants contribute to the oxidation of biomolecules such as DNA (Francisqueti et al., 1992). The inhibition of free radical scavenging capacity results from DPPH (Figure 4) ranged from 75 ± 4.02 % (30 minutes brewed) to 83 ± 0.66% (60 minutes brewed) inhibition. A significant (p = 0.038) increase in free radical scavenging activity was observed between samples that were brewed for 30 minutes and 45 minutes, and between samples

brewed for 30 minutes and 60 minutes ($p = 0.014$) (Table 3). These results suggest that as brewing time increases, the antioxidant activity of Mauby bark extract also increases. Therefore, the results suggest that brewing Mauby bark for prolonged times may allow for donation of electrons more effectively. Furthermore, data about other bark extracts as powerful antioxidants by scavenging free radicals were published in literature. The DPPH % inhibition of *C. zeylanicum* bark and *P. maritima* bark were 84.43 ± 3.48% and 94.51 ± 0.01% respectively (Dudonné et al., 2009).

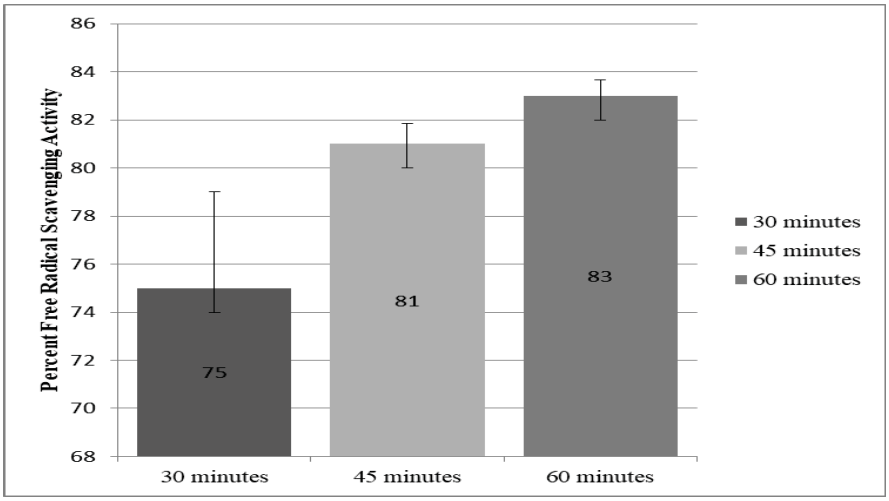

Figure 4. DPPH results for Mauby extracts

Table 3. Tukey's HSD post-hoc analysis for DPPH

Brewing Time	P Value
Between 30 minutes and 45 minutes	0.038
Between 30 minutes and 60 minutes	0.014
Between 45 minutes and 60 minutes	0.697

$p \leq 0.05$ is significant

3.4 Ferric Reducing Antioxidant Power (FRAP) of Mauby Bark Extract

The antioxidant power of Mauby bark extracts derived from brewing times of 30, 45 and 60 mins to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) was measured by the FRAP antioxidant assay. The ability of Mauby Bark extracts to reduce ferric iron directly relates to the antioxidant power of Mauby bark extract. The FRAP results (Figure 5) ranged from a low of 6.29 ± 0.84 mM $FeSO_4$ (30 minutes) to a high of 6.90 ± 1.53 mM $FeSO_4$ (45 minutes) with no significant (p=0.736) difference between time brewed and antioxidant power. For this reason, it can be concluded that 60 minutes appears to be the ideal time to brew Mauby bark due to the highest increase in free radical scavenging activity in the DPPH assay and similarly antioxidant power among all the time intervals. The mean antioxidant power of Mauby bark extracts when converted to gram quantity (0.105 mM $FeSO_4$/g) were slightly weaker than other barks. For example, the FRAP value of apple tree (*Malus domestica)* bark was 0.343 mM $FeSO_4$/g when samples were extracted at 60°C (Withouck et al., 2017). Apple tree bark also contains the polyphenols such as phloretin which has been observed to contain antioxidant, anti-inflammatory, anticarcinogenic, antimutagenic and immunosuppressive properties (Xü, Lü, Qü, Shan, & Song, 2010). Differences in FRAP values may be explained by geographic location and the ratio of antioxidant phytochemicals present within bark (Abuashwashi, Palomino & Gómez-Serranillos, 2016). Conversely, when FRAP values are measured in mL quantities as Mauby is most commonly consumed as a beverage, Mauby bark extracts were observed to be more potent than tea extracts in FRAP values. The FRAP values for white tea (0.73 mM $FeSO_4$/mL), green tea (0.50 mM $FeSO_4$/mL and black tea (0.38mM $FeSO_4$/mL; Al-Obaidi & Sahib, 2015) are all markedly lower than Mauby extracts. However, there is limited research expressing FRAP values for bark extracts as mM $FeSO_4$ equivalents which allows these results to enhance existing literature.

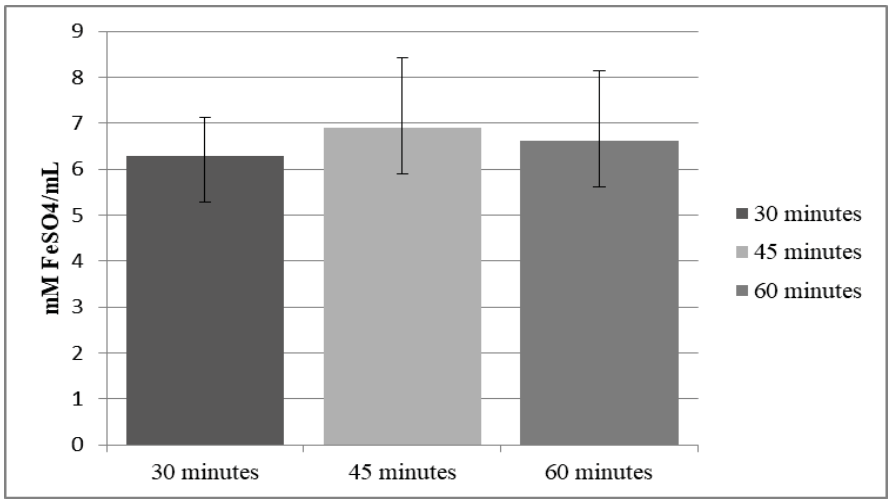

Figure 5. FRAP results for Mauby extracts

4. Conclusion

The experimental results of this study indicated that Mauby bark contains polyphenols that exhibit free radical scavenging activity that may protect against naturally occurring oxidative damage within the human body commonly implicated in the development of chronic illnesses. Evidently, for both TFC and TPC analyses, brewing of the bark for 30 minutes resulted in the highest quantity of polyphenols suggesting that increased exposure to heat will degrade polyphenols present in Mauby bark. The observed decrease in polyphenolic content however did not affect free radical scavenging activity at increasing brewing times suggesting that upon exposure to heat, the phenolics and flavonoids may be converted to other intermediate compounds or other compounds may be present in Mauby bark which may result in the higher antioxidant activity as brewing times increased. The brewing time recommended for Mauby Bark to provide the greatest antioxidant benefits is 60 minutes. Although at 30 minutes, the TPC and TFC values were greater than at 60 minutes, the free radical scavenging activity was found to be significantly higher at 60 minutes thus capable of performing as a more efficient antioxidant in the body. Results from this research can be used as a basis for product development of Mauby bark extracts infused into beverages to be sold in the United States of America. Further research is needed to characterize the specific polyphenolic compounds and derivatives that are responsible for the antioxidant capabilities of Mauby bark using techniques such as Mass Spectrometry (MS) and High Performance Liquid Chromatography (HPLC).

Author Contributions

J. Embola and C. Rock performed the experiments and collected data. J. Embola conducted data analysis and interpreted results. C. Rock, L. Wang, W. Reiboldt, S. Aliabadi and S. Ahmed reviewed and edited the manuscript that was written by J. Embola and C.Rock.

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A Comparison of the Polyphenolic and Free Radical Scavenging Activity of Cold Brew versus Hot Brew Black Tea *(Camellia Sinensis, Theaceae)*

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Abstract

Recently, a new trend called cold brewing gained popularity in the tea and coffee beverage industry. Cold brew and hot brew black tea may have different sensory qualities and antioxidant levels because of their polyphenolic properties and brewing processes. The objectives of this study were to determine antioxidant properties and polyphenolic content of commercial brands of cold brew and hot brew black tea. The total phenolic content of the cold brew tea was determined to be 0.19 mg/mL gallic acid equivalents/100 g and hot brew tea was 0.43 mg/mL gallic acid equivalents/100 g when assayed by Folin-Ciocalteu's reagent method. The total flavonoid content of the cold brew tea was 0.40 mg/mL catechin equivalents/100 g and hot brew was 1.01 mg/mL catechin equivalents/100 g. Moreover, antioxidant capacity of cold brew and hot brew black tea was analyzed where their ability to scavenge DPPH radicals was 86.3% and 88.1% respectively. There was a significant difference in total phenolic content between hot brew and cold brew (p = 0.004). Similarly, there was a significant difference in total flavonoid between cold brew and hot brew (p = 0.004). Additionally, there was a significant difference in DPPH scavenging activity between cold brew and hot brew (p = 0.016). Overall, it can be concluded that although cold brew tea contained a lower amount of phenolics and flavonoids as compared to hot brew tea, they both were able to scavenge DPPH radicals in nearly same capacity.

Keywords: antioxidants, cold brew, hot brew, tea

1. Introduction

1.1 Consumption Trends and Tea Types

Teas are classified as the following: black, green, oolong and white tea, which are generally processed using the standard steps: withering, rolling, fermentation (oxidation) and drying (firing) with slight variations based on the type (Shinde, Das, & Datta, 2013). All tea varieties come from leaves of the *Camellia Sinensis* (L.) plant (Carloni et al., 2013). However, black tea, the main focus of this research, is a fully fermented type (Shinde et al., 2013). Black tea is the most consumed (80%; Nie, Dong, Bai, & Xia, 2014) and produced (78%; Ruxton & Mason, 2011) variety in the world (Striegel, Kang, Pilkenton, Rychlik, & Apostolidis, 2015).

1.2 Cold Brewing in Tea and Coffee Industry

Recently, a new process of cold brew has gained popularity in the beverage industry (Refermat, 2017). Until major coffee franchises introduced cold brew coffee in the United States (U.S.) in 2015, it was a small market niche with which only few consumers were familiar (Kline, 2016). Especially in the coffee industry, cold brew is drawing the attention of a new generation referred to as millennials (aged 18-34; Fry, 2016) in that many of them consider it as their drink of choice (Strand, 2017). Importantly, successful outcomes of cold brew method illustrate the trends toward healthier foods (Kline, 20106). With increased consumer demands, new frequent innovations and products such as unique tea products similar to cold brew coffee continue to expand the beverage industry (Tea Association of the U.S.A. Inc., 2017).

1.3 Innovation of Cold Brew Tea Product

Cold brew tea products are produced using low temperature (15 ^{o}C) or cold water (4 ^{o}C) and have the benefits of traditional tea as it relates to established literature on their antioxidant properties (He, Liu, & Huang, 2011).

Other specific benefits include the convenience of preparation while enjoying a freshly brewed tea taste (Balentine et al., 2004). Moreover, the cold brew method minimizes the bitterness and astringency of caffeine since less tannins are extracted from the tea leaves as compared to hot brewing (Dobos, 2017). As a consequence, cold brew tea began to spark the interest since it can create smoother and sweet iced tea also (Tea Association of the U.S.A. Inc., 2017).

1.4 Oxidative Stress and Tea Antioxidants

Tea is known to contain plant compounds which are believed to prevent chronic diseases (Koutelidakis et al., 2016). Some of these polyphenols in tea include catechins or flavan-3-ols, theaflavins, thearubigins and proanthocyanidins (Chen, Qu, Fu, Dong, & Zhang, 2009) and have been implicated in preventing cancer, obesity, type 2 diabetes and cardiovascular disease (CVD; Fernando & Soysa, 2015). Mechanisms include inhibiting lipoprotein oxidation, decreasing oxidative stress (OS) and controlling inflammation biomarkers (Koutelidakis et al., 2016). Polyphenols also have been shown to decrease serum glucose, triglyceride and low-density lipoprotein (LDL)/high-density lipoprotein (HDL) plasma cholesterol ratio and to improve plasma antioxidant level, which may help to lower the risk of CVD (Pan et al., 2013).

1.5 Importance of Tea Brewing Methods Research

There is an abundance of research on tea and antioxidants; however, there is a limited research on comparing antioxidant activity in cold versus hot brew black tea; in particular commercially made cold brew black tea. Specifically, in literature, there were some studies that analyzed antioxidant levels in different tea types by using various brewing times and temperatures. However, there were limited studies that tested antioxidants in black tea, which is the most consumed and produced tea type globally. The caveat with these studies is that cold-water tea infusions were prepared by using the teas that are supposed to be brewed with hot water brewing methods. This error can affect test results on antioxidant properties and quality of teas. Therefore, it is important to study the polyphenolic content as well as the antioxidant capacity of the cold brew and hot brew black tea, as they could have different antioxidant levels when utilizing the proper techniques or protocols in their brewing methods. In the same manner, it is important to consider that antioxidant properties of certain teas are best acquired with the inscribed (hot water) conditions. In addition, it is important to study the polyphenolic content and antioxidant power of cold and hot brew black tea as cold brew preparation is receiving a great attention of consumers such as millennials recently and the consumers can be more knowledgeable about amount and quality of tea antioxidants that are available in the products. The overall objective of this study was to compare the polyphenolic content and free radical scavenging activity of cold brew and hot brew black teas.

2. Method

2.1 Tea Brewing

For both cold brew and hot brew black tea, 2 g of each tea sample respectively were brewed in 230 mL of cold (4 ^{o}C) and hot (100 ^{o}C) water for 5 minutes as prescribed on the commercial package.

2.2 Antioxidant Capacity Determination

2.2.1 Determination of the Free radical Scavenging Activity of Black Tea

The free-radical scavenging activity of the prototype was measured with reference to Priyanka, Kadam, Kadam, Ghule, & Aparadh (2013). First, 1 mL of each extract was added to 3 mL of 0.1M DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) radical solution. The DPPH radical was dissolved in 90% methanol, shaken, and incubated at ambient temperature (about 23 ^{o}C/73 ^{o}C) for 30 minutes with minimal light exposure. Then, analysis of DPPH was conducted using a UV Spectrometer (Thermo Scientific AquaMate 8000 UV-Vis Spectrophotometer) at a wavelength of 517 nm. Free radical scavenging activity was calculated as the percentage (%) of free radicals scavenged by the extracts (Equation 1). Also, the blank was 1 mL of methanol to 3 mL of DPPH.

$$\%\text{DPPH of Sample} = A_{blank} - A_{sample} / A_{blank} \text{ X } 100 \quad (1)$$

2.3 Polyphenolic Content Determination

2.3.1 Phenolic Content of Black Tea

Total phenolics were measured since they contribute to the antioxidant capacity and potential of cold brew and hot brew black tea products as described by Marinova, Ribarova, & Atanassova (2005). One mL of each of the sample extracts as well as a series of gallic acid standard solutions (0.02, 0.04, 0.06, 0.08 and 0.10 mg/mL) were added to 25 mL volumetric flasks, containing 9 mL of deionized distilled water (DDH_2O). Subsequently, 1 mL of Folin-Ciocalteu's (FC) reagent was added to the mixture and shaken. After 5 minutes, 10 mL of 7% sodium

bicarbonate (Na_2CO_3) solution was added to the mixture in each flask. The solution in each flask was diluted to volume (25 mL) using DDH_2O and was mixed. Following that, the flasks were incubated for 90 minutes at room temperature and the absorbance of the mixtures were read at 750 nm using an Orion Aquamate 8000 UV spectrometer. Then, the total phenolics of the cold brew and hot brew black tea were expressed as gallic acid equivalents (GAE)/100 g fresh weight. Last, all samples were analyzed in triplicate.

2.3.2 Flavonoid Content of Black Tea

A standard colorimetric assay as described by Marinova et al. (2005) with slight modifications was used to quantify total flavonoid content. First, one mL of a series of catechin standard solutions (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) was added to 10 mL volumetric flasks containing 4 mL of DDH_2O respectively. Then, to each flask, 0.3 mL of 5% sodium nitrate ($NaNO_2$) was added. After 5 minutes, 0.3 mL of 10% aluminum chloride ($AlCl_3$) was added to each flask, followed by 2 mL of 1M sodium hydroxide (NaOH). The mixture in each flask was filled to volume (10 mL) using DDH_2O and was mixed well. Using an Orion Aquamate 8000 UV spectrometer, the absorbance of the samples was read wavelength at 510 nm. Finally, the total flavonoid contents were expressed as mg catechin equivalents (CE)/100 g of tea. All samples were analyzed in triplicate.

2.4 Data Analysis

All numerical data of this study were analyzed by using the International Business Machine (IBM) Statistical Package for the Social Sciences (SPSS), Version 24. Mean and the standard deviation (SD) of the each chemical test were used. For all chemical tests above mentioned, Mann-Whitney tests (non-parametric test) were used to compare the differences in mean phenolic content, flavonoid content and antioxidant capacities of the cold brew versus hot brew black tea. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1 Total Phenolic Content

Figure 1 shows the results of gallic acid standard curve equation ($y = 3.995x + 0.0137$, $r^2 = 0.9917$) for total phenolic content in our experiment. In this study, the total phenolic content was lower in cold brew black tea (0.19 mg/mL gallic acid equivalents (GAE)/100 g or 43.7 mg/230 mL GAE/100 g and mean absorbance value was 0.78 ± 0.04) than hot brew black tea (0.43 mg/mL GAE/100 g or 98.9 mg/230 mL GAE/100 g and mean absorbance value was 1.74 ± 0.10; Table 1). Moreover, the findings showed that there was significant difference in total phenolic content between cold brew and hot brew ($p = 0.004$; Table 2).

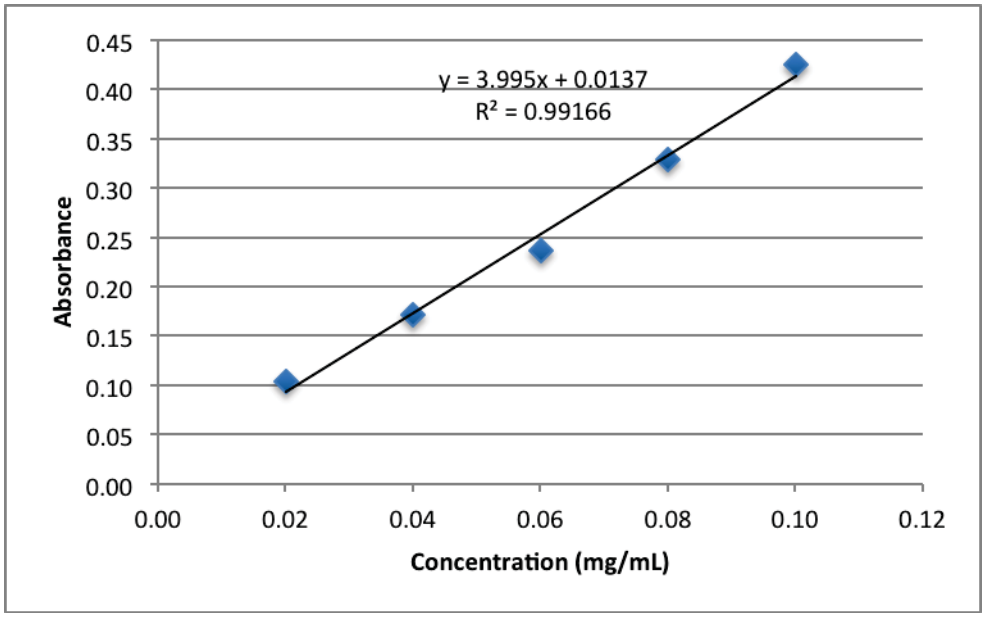

Figure 1. Standard curve for total phenolics content

3.2 Total Flavonoid Content

Figure 2 shows the results of catechin standard curve equation ($y = 0.2465x + 0.0204$, $r^2 = 0.9787$) for total flavonoid content in our research. In our study, the total flavonoid content was lower in the cold brew tea (0.40

mg/mL catechin (CE) equivalents/100 g of extract or 92.0 mg/230 mL CE/100 g and mean absorbance value was 0.12 ± 0.01) than hot brew tea (1.01 mg/mL CE/100 g or 232 mg/230 mL CE/100 g and mean absorbance value was 0.27 ± 0.02; Table 1). Additionally, the results showed that there was a significant difference in total flavonoid between cold brew and hot brew (p = 0.004; Table 2).

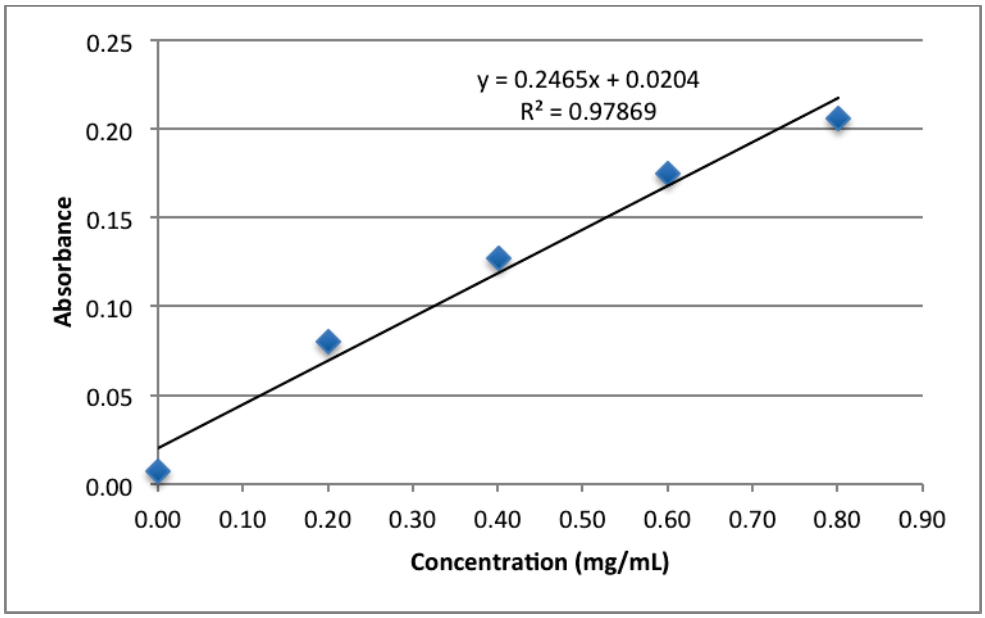

Figure 2. Standard curve for total flavonoids content

3.3 Determination of the Free Radical Scavenging Activity of Tea

In addition, in this study, antioxidant capacity of cold brew and hot brew black tea was analyzed by DPPH chemical assay. To illustrate, the ability of cold brew and hot brew black tea to scavenge DPPH radical was 86.3% (mean absorbance value was 0.61 ± 0.06) and 88.1% (mean absorbance value was 0.53 ± 0.01) respectively (Table 1). The high percentages show high power of fighting off of free radicals by the tea antioxidants. Moreover, the results indicated that there was a significant difference in DPPH scavenging activity between cold brew tea and hot brew tea (p = 0.016; Table 2).

Table 1. Polyphenolic content results of cold versus hot brew black tea

Tea brew type	Total phenolic content (mg/mL gallic acid equivalents (GAE)/100 g or mg/230 mL GAE/100 g)	Total flavonoid content (mg/mL catechin (CE) equivalents/100 g of extract or mg/230 mL CE/100 g)	DPPH: Free radical scavenging activity (%)
Cold Brew Tea	0.19 OR 43.7	0.40 OR 92.0	86.3
Hot Brew Tea	0.43 OR 98.9	1.01 OR 232	88.1

Table 2. Mann- Whitney U- test results comparing polyphenolic properties of cold versus hot brew black tea

Antioxidant test	Cold brew mean ± SD	Hot brew mean ± SD	*P- value*
Phenolic Test	0.78 ± 0.04	1.74 ± 0.10	0.004
Flavonoid Test	0.12 ± 0. 01	0.27 ± 0.02	0.004
DPPH Radical	0.61 ± 0.06	0.53 ± 0.01	0.016

4. Discussion

4.1 Total Phenolic Content

In our study, cold brew tea had lower phenolic content and may be due to the fact that cold water extracted less phenolics from tea leaves than hot water. Another reason for more efficient extraction of tea phenolics by hot brew could be because tea polyphenols (phenolics) are more water soluble in hot water than cold water. This also showed that cold brew may need more time to extract more phenolics from tea leaves at that temperature. A study (Lantano, Rinaldi, Cavazza, Barbanti, & Corradini, 2015) investigated total phenol content of tea extraction made with hot water (12 g tea in 1 L of water of specific temperature), cold tea (tea in 4 ^{o}C water ± 1 for 12 hours) and ice tea (tea in 80 ^{o}C water and adding ice after required time of infusion and removing tea leaves). The researchers (Lantano et al., 2015) found that the total phenol content (mean ± SD expressed as mg/g leaves) for hot black tea was 14.2 ± 0.1, for cold tea was 20.4 ± 0.1 and for ice tea was 14.8 ± 0.2 (Lantano et al., 2015). However, the results of the study performed by Lantano et al. (2015) were not similar to ours as it was revealed that cold brew tea had higher phenolic content than hot brew tea. This could be due to the fact that they prepared the cold-water tea infusions after brewing the tea in 80 ^{o}C water. Therefore, brewing conditions such as time and temperature could affect the total phenolic content of tea infusions. Another reason could be that we used a commercially available cold brew black tea product in our study.

In addition, another study by Carloni et al. (2013) determined that total phenols of hot brew (90 ^{o}C for 7 minutes) was (10.7 ± 4.0 mM GAE) in black crush-tear-curl (CTC) tea and (14.9 ± 5.9 mM GAE) in black orthodox tea respectively. This study found a lower amount of total phenols than in the present study and it could be because of use of different black tea brands that were from various parts of the world and use of variety of brewing times and temperatures. Moreover, another study (Abbasian et al., 2013) investigated the total polyphenolic compounds of six medicinal plants and ten commercial tea brands and found that the total phenols (mg GAE/L) of a popular commercial brand of black tea sample was 98.05 ± 11.37. In comparison, the numerical results of the total phenols of black tea in their study (98.05 ± 11.37 mg GAE/L) were comparable with our study results (0.43 mg/mL GAE/100 g or 98.9 mg/230 mL GAE/100 g) for hot brew black tea. This could be because of use of same tea brand with the similar tea quality standards to make hot brew tea in both studies.

4.2 Total Flavonoid Content

Our results showed that cold brew tea had lower flavonoids content than hot brew tea and it may be due to the fact that extraction of tea flavonoids from tea leaves was more efficient and rapid with hot brew than cold brew technique. This could be due to the fact that solubility of tea polyphenols (flavonoids) in hot water was higher than in cold water. Moreover, it also suggests that cold brewing method may need longer infusion time to extract more tea flavonoids from tea leaves. On the other hand, a study found that total flavonoid content for black orthodox tea was 6.60 ± 1.52 mM CE (Carloni et al., 2013). This study had different results than our study and it could be because of use of black tea that was made from different processing methods such as orthodox versus CTC and use of different brewing conditions (different brewing time and temperature and weight of tea used). Furthermore, a study (Abbasian et al., 2013) investigated the total flavonoid content of six medicinal plants and ten commercial tea brands. This study (Abbasian et al., 2013) found that a popular commercial tea brand had the highest total flavonoid content when compared to other tea brand samples. To elaborate, it was observed that the total flavonoid content (mg CE/L) of this popular commercial brand of black tea sample was 230.72 ± 15.20. According to these results, the total flavonoid content of this specific brand of black tea used in their study was very similar results of our study for hot brew black tea. This could be due to the use of similar brewing times, techniques and temperatures to prepare the teas and use of teas from similar parts of the world to produce the black tea type.

4.3 Determination of the Free Radical Scavenging Activity of Tea

Experimentally, we found that cold brew and hot brew black tea both were able to scavenge DPPH radicals in very similar capacity even though there were lower total phenolic and flavonoid content in cold brew tea than hot brew tea. In other words, our results revealed that cold brew tea and hot brew tea were almost effective in same extent in antioxidant activity. Bhuyan et al. (2013) analyzed a total of 60 CTC black tea samples from six regions from India. According to Bhuyan et al. (2013), the mean value of the DPPH scavenging activity was 86.73% for Barak Valley, 86.37% for Terai region, 77.57% for Dooars region, 82.68% for Upper Assam region, 80.95% for North Bank region and 80.92% for South Bank region. As a result, this study's (Bhuyan et al., 2013) results for DPPH assay of black tea samples matched our results. This could be possibly because of use of same tea type in the both studies.

4.4 Importance of Findings

In current literature, there are many studies on tea and antioxidants; however, there are very few studies comparing antioxidants in hot brew black tea versus cold brew black tea, especially commercially made cold brew black tea. In our study, even though cold brew tea had lower phenolics and flavonoids compared to hot brew tea, both cold and hot brew teas were able to scavenge DPPH radical at the same capacity. This could show that there is a certain bioavailability of tea antioxidants (phenolics and flavonoids) in tea regardless of the amount of antioxidants in tea drink. A limitation of our study was that there might be slight differences in the antioxidant properties of cold and hot brew teas since they might have been originally acquired from various tea suppliers to formulate the products. Additionally, the results do not reflect the bioavailability of the antioxidants in a biological system (i.e. *in vivo*). The repositioning of tea consumption as cold brew tea as an alternative to the traditional hot brew tea makes it necessary and useful for the consumers to know more about the amount and quality of the tea antioxidants that they can acquire by consuming cold brew versus hot brew tea for achieving desired health benefits. Overall, according to the results of our study, it can be concluded that cold brew tea had lower phenolics and flavonoids as compared to hot brew tea. However, both cold and hot brew teas were able to scavenge DPPH radicals at nearly the same capacity. In the food industry, the cold brew tea method can be used to make ready-to-drink cold brew tea in cans and bottles, instant tea and coffee powders that are high in polyphenols and have high antioxidant capacity. As a result, consumers can enjoy tea and coffee instantly and conveniently by using low temperature or cold water with similar antioxidative benefits.

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Dr. Cheryl Rock and Chathuranga M. Magammana designed the study, collected the data, interpreted the results, wrote and edited the manuscript. Dr. Long Wang and Dr. Virginia Gray assisted in drafting the manuscript.

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Product Development Considerations of Flaxseed Supplementation for the Aging Population: A Pilot Study

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Abstract

Obesity, cardiovascular disease, and vulnerability among older adults highlight a critical need for a careful consideration of effective and preventive dietary interventions. Consuming flaxseed, along with a well-balanced diet, has been shown to significantly improve weight, waist circumference, blood pressure, serum lipids, plasma glucose levels, and inflammatory biomarkers. Although flaxseed exhibits anti-inflammatory and antioxidant properties, little is known regarding its consumer acceptability among older adults. The objective of this study was to investigate the acceptability of a bagel with 23% flaxseed in individuals 50 years and older using a 9-point Hedonic rating scale, Paired Preference test, and Food Action (FACT) rating scale. There were no significant differences between the control and flaxseed bagel in sensory attributes and FACT ratings in 20 participants (69.0 ± 6.3 years old). Age was significantly associated with the overall acceptability of the flaxseed bagel (p = 0.004). Appearance, color, flavor, and texture were strongly correlated (p < 0.01) to overall acceptability in both bagels. Further exploration of consumer acceptance of flaxseed products among older adults is needed; clinical trials may also shed light on potential health impacts of regular flax consumption.

Keywords: aging population, bagels, consumer acceptance, flaxseed

1. Introduction

Demographic changes in the United States have long indicated that it is an aging society. Between 2014 and 2060, the United States population is projected to increase from 319 million to 417 million, with 1 in 5 Americans expected to be 65 years old or over by 2030 and 1 in 4 expected to be 65 years old or over by 2060 (Colby & Ortman, 2015). The baby boomer cohort, individuals born post-World War II between 1946 and 1964, is largely responsible for this growth in the older population as the first of this generation turned 65 in 2011(Colby & Ortman, 2014). Consequently, this profound shift presents challenges to health care, nutrition services, and food supply systems for older individuals. An increase in longevity has been positively associated with chronic conditions that sometimes translate into functional disability and need for assistance (Buttorff, Ruder, & Bauman, 2017).

Although illnesses and disease are common in older adults, no specific disease is inevitable in older age. Many diseases correlated with aging can be prevented, or at least delayed by greater awareness to health care strategies and maintenance. Interestingly, older individuals score higher in practicing health-promoting behaviors particularly in nutrition and health responsibility than any other age groups, suggesting that this population is eager to make lifestyle changes to preserve their health and freedom into advanced age in contrast to some who may not want to sacrifice taste and habits (Becker & Arnold, 2004). Moreover, older consumers report that they regularly note the nutrition information on food labels when buying functional foods (Annunziata, Vecchio, & Kraus, 2015). This suggests many older consumers are health-oriented, have an interest in eating foods that will preserve well-being, and may prefer consuming a food item with health benefits.

Flaxseed (*Linum usitatissimum*), accessible in many countries around the globe, has been drawing the attention of today's food industry due to its nutritive properties and versatility as a food ingredient. Flaxseed provides many nutrients, including omega (ω)-3 polyunsaturated fatty acids (PUFAs, 28.7%), lignans (3.5%) including

secoisolariciresinol diglucoside (SDG), and dietary fibers (27.3%; U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory, 2016). Several human trials have shown that consuming 28–40 g of flaxseed daily, along with a well-balanced diet, has a significant improvement in health outcomes such as waist circumference, weight, blood pressure, serum lipids (total cholesterol, triglycerides, low density lipoprotein-cholesterol [LDL-C], very low density lipoprotein-cholesterol [VLDL-C], and high density lipoprotein-cholesterol [HDL-C] levels), plasma glucose levels, and inflammatory biomarkers (Caligiuri, Aukema, Ravandi, & Pierce, 2014; Edel et al., 2015; Ricklefs-Johnson, Johnston, & Sweazea, 2017; Saxena & Katare, 2014; Torkan, Hassan Entezari, & Siavash, 2015). Thus, incorporating flaxseed into the diet of older adults may provide a continuous preventive mechanism, which is cost effective compared to modern drug therapy.

Analyzing current dietary practices in to elucidate foods commonly consumed by the aging population can help direct product development of flaxseed-containing products. Carbohydrate intake of older adults showed an increase from 43% of total kilocalories (kcal) in 1977 to 49% of total kcal in 2010, which may be due to an increase in bread, grain, and dessert consumption as dominant calorie sources (Johnston, Poti, Popkin, & Kenan, 2014). In a year-long study analyzing 12 daily choices of foods supplemented with 30 g milled flaxseed among older individuals, bagels were the popular choice to be ordered (37%) compared to other food options with cinnamon raisin as most favorable compared to sunflower sesame and plain bagels; this suggests bagels as a possible candidate for fortification (Austria et al., 2016). Thus, enriching cinnamon raisin bagels with flaxseed that are regularly consumed by older adults might be beneficial to increase ALA, lignan, and dietary fiber intakes. However, there has been limited investigation on the consumer acceptability of flaxseed among older adults.

The overall goal of this study is to evaluate the acceptability of a flaxseed bagel among individuals 50 years and older. The specific objectives are to evaluate the degree of likability in sensory attributes (i.e., appearance, color, flavor, aroma, texture, and overall acceptability) utilizing the 9-point Hedonic rating scale, compare the preference for two bagels (control vs. flaxseed) using a Paired Preference test, and examine the acceptance of the bagel by measuring the frequency of the consumer desire to consume the bagel by applying the Food Action (FACT) rating scale.

2. Method

2.1 Subject Characteristics and Sampling Procedure

The pilot study was approved (1221541-2) by the Institutional Review Board. Subjects were then recruited, and the procedures were explained, and the consent form was read to and signed by each participant. Candidates were screened, and demographic data were collected using a questionnaire. Exclusion criteria included individuals who were <50 years old and were allergic to wheat. An honorarium in the form of a $5 coffee gift card was awarded to participants who completed the sensory experiments.

2.2 Bagel Formulation

Clinical trials suggest consuming 30 g of flaxseed has positive health outcomes (de Oliveira et al., 2017; Saxena & Katare, 2014; Torkan et al., 2015; Vuksan et al., 2017); therefore, a cinnamon raisin bagel with 30 g flaxseed (23%) was created and evaluated against a control bagel (0% flaxseed). Ingredients for the flaxseed and control bagel were obtained in the North American market (Table 1). Honey, active dry yeast, and water at a temperature between 38 °C to 43 °C were combined in a bowl. Bread flour, flaxseed mill, and salt were combined with the yeast mixture in a standing mixer for 10 minutes. Raisins and ground cinnamon were added to the dough until combined. The dough was formed into a ball, placed in a bowl, and loosely covered with plastic wrap until it doubled in size. The dough was divided into an average of 130 g portions and formed into a smooth ball. The center of the dough was pinched by hand to make a center hole of approximately 5 centimeter wide. The diameter of the bagel was roughly 10 centimeters. The formed bagels were placed onto a baking sheet lined with parchment paper and sprayed with oil and proofed for approximately 45 minutes, placed under the broiler at 260 °C for one minute on each side, and removed from the oven. Bagels were placed in boiling water at 100 °C for one minute on each side, removed from the water, and drained on a wire rack. Each bagel was placed on a baking tray lined with parchment paper sprayed with oil and baked in a convection oven for 30 min at 191 °C. All samples were cooled, packaged in a sealed bag, and frozen immediately at –18 °C. The control bagels underwent the same process excluding the flaxseed as an ingredient. Bagels were thawed at room temperature (i.e., 23 °C) 16 hours prior to sensory evaluation and cut into half and then quarters, with eight pieces from one bagel. One eighth of the flaxseed bagel and control bagel were placed in a 60-milliliter plastic portion cup separately and held for at least 1 hour before evaluation.

Table 1. Formulation of bagels

Ingredients	Control	Flaxseed
Organic flaxseed meal	0.00 g (0.00%)	30.00 g (23.00%)
Unbleached bread flour	72.37 g (55.49%)	42.37 g (32.49%)
Iodized salt	1.22 g (0.94%)	1.22 g (0.94%)
Active dry yeast	0.61 g (0.47%)	0.61 g (0.47%)
Organic honey	3.02 g (2.32%)	3.02 g (2.32%)
Organic ground cinnamon	0.74 g (0.57%)	0.74 g (0.57%)
Seedless raisin	10.33 g (7.92%)	10.33 g (7.92%)
Water	42.13 g (32.30%)	42.13 g (32.30%)
Total	130.41 g	130.41 g

2.3 Sensory Evaluation

Consumer tests were performed in a sensory evaluation laboratory. The assessment occurred under controlled conditions—a comfortable and quiet area without distractions (i.e., isolated booths) under fluorescent lighting and controlled temperature. During the sensory evaluation, panelists were provided with two coded food items in a random order, the flaxseed bagel (#518) and the control bagel (#496). Water was supplied to cleanse the palate between samples.

2.4 Measures

Bagels were assessed using the 9-point Hedonic scale, Paired Preference test, and the FACT rating scale. The 9-point hedonic scale is a tool that assesses a food item based on sensory attributes (i.e., appearance, color, flavor, texture, and overall acceptability; Girardot, Peryam, & Shapiro, 1952). Score values included the following: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely. The Paired Preference test established whether or not the flaxseed bagel was preferred over the control bagel (Thurstone, 1927). Each subject was requested to indicate the sample code they preferred the most. The FACT rating scale, a 9-point scale, measures consumer acceptance by having participants indicate the frequency of eating the food product (Schutz, 1965). The FACT scale values are as follows: 9 = I would eat this every opportunity that I had, 8 = I would eat this very often, 7 = I would frequently eat this, 6 = I like this and would eat it now and then, 5 = I would eat this if available but would not go out of my way, 4 = I do not like this but would eat this on an occasion, 3 = I would hardly ever eat this, 2 = I would eat this if there were no other food choices, and 1 = I would eat this only if forced.

2.5 Statistical Analysis

Statistical analysis was conducted utilizing the International Business Machine (IBM) Statistical Package for the Social Sciences (SPSS), Version 25. Descriptive statistics, frequencies, percentages, and variances were performed on demographics of survey participants as well as for all scorecard ratings. Prior to comparisons, skewness and kurtosis values were analyzed for normality. Paired samples *t*-tests were used to evaluate differences in means for sensory characteristics between the control and flaxseed bagels. Associations between demographic characteristics (gender and age group of panelists) and overall acceptability and FACT ratings for two food items were tested using chi-square (χ^2) test for independence analysis. Correlation coefficients between overall acceptability and other sensory attributes were calculated and reported. All analysis tests performed used a significance of $p < 0.05$.

3. Results

3.1 General Demographic Characteristics of the Study Population

A total of 20 subjects, 25% male and 75% female, completed the sensory evaluation. The age of participants

ranged from 54 to 76 years (69.0 ± 6.33). Age was normally distributed, with skewness of –.975 (*SE* = 0.51) and kurtosis of 0.23 (*SE* = 0.99). Among these participants, 25% were 50–64 years old, 55% were 65–74 years old (young-old), and 20% were 75–84 years old (old-old). Participants were all non-Hispanic whites (100%).

3.2 9-point Hedonic rating

No significant differences were detected in sensory attributes including appearance (6.95 vs. 6.65), color (7.00 vs. 6.55), flavor (6.75 vs. 5.90), aroma (6.70 vs. 6.35), texture (6.45 vs. 6.45), and overall acceptability (7.00 vs. 6.05) for the control bagel compared to the flaxseed bagel, respectively (Table 2).

Table 2. Palatability factor, overall acceptability, and food action (FACT) rating of control bagels vs. bagels containing flaxseed (*n* = 20)

	Control	Flaxseed	
Palatability factor	*M(SD)*	*M(SD)*	*p*
Appearance[a]	6.95(1.050)	6.65(1.725)	0.453
Color[a]	7.00(1.026)	6.55(1.820)	0.324
Flavor[a]	6.75(1.251)	5.90(1.971)	0.105
Aroma[a]	6.70(1.525)	6.35(1.981)	0.439
Texture[a]	6.45(1.605)	6.45(1.504)	1.000
Overall acceptability[a]	7.00(1.026)	6.05(2.038)	0.078
FACT rating[b]	5.20 (1.281)	4.90(2.075)	0.527

[a]Measured on a scale from 1 to 9, with 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely (Girardot et al., 1952).

[b]Measured on a scale from 1 to 9, with 9 = I would eat this every opportunity I had; 8 = I would eat this very often; 7 = I would frequently eat this; 6 = I like this and would eat it now and then; 5 = I would eat this if available but would not go out of my way; 4 = I do not like this but would eat it on an occasion; 3 = I would hardly ever eat this; 2 = I would eat this if there were no other food choices; 1 = I would eat this only if forced (Schutz, 1965).

A χ^2 test of independence was performed to examine the associations between demographic characteristics (i.e., gender and age group of panelists) and overall acceptability. The relationship between gender and overall acceptability and FACT ratings was not significant (Table 3). In contrast, there was a significant relationship between age group (50–64 years, young-old, and old-old) and overall acceptability of the flaxseed bagel (p = 0.004), while there was no association between age group and overall acceptability of the control bagel (Table 4).

Table 3. Relationship between gender of panelists and overall acceptability and food action (FACT) rating of bagels

		Male (*n* = 5)	Female (*n* = 15)	
		M(SD)	*M(SD)*	*p*
Overall acceptability[a]	Control	7.20(0.837)	6.93(1.100)	0.848
	Flaxseed	5.80(1.095)	6.13(2.295)	0.121
FACT rating[b]	Control	5.60(0.548)	5.07(1.438)	0.486
	Flaxseed	4.40(1.517)	5.07(2.251)	0.087

[a] Measured on a scale from 1 to 9, with 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely (Girardot et al., 1952).

[b] Measured on a scale from 1 to 9, with 9 = I would eat this every opportunity I had; 8 = I would eat this very often; 7 = I would frequently eat this; 6 = I like this and would eat it now and then; 5 = I would eat this if available but would not go out of my way; 4 = I do not like this but would eat it on an occasion; 3 = I would hardly ever eat this; 2 = I would eat this if there were no other food choices; 1 = I would eat this only if forced (Schutz, 1965).

Table 4. Relationship between age group of panelists and overall acceptability and food action (FACT) rating of bagels

		50–64 years (n = 5)	65–74 years (n = 11)	75–84 years (n = 4)	
		M(SD)	*M(SD)*	*M(SD)*	*p*
Overall acceptability[a]	Control	7.00(0.707)	7.00(1.183)	7.00(1.155)	0.132
	Flaxseed	6.40(2.510)	6.00(1.612)	5.75(2.986)	0.004
FACT rating[b]	Control	5.20(0.447)	5.45(1.508)	4.50(1.291)	0.078
	Flaxseed	5.20(2.490)	5.00(2.049)	4.25(2.062)	0.089

[a] Measured on a scale from 1 to 9, with 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely (Girardot et al., 1952).

[b] Measured on a scale from 1 to 9, with 9 = I would eat this every opportunity I had; 8 = I would eat this very often; 7 = I would frequently eat this; 6 = I like this and would eat it now and then; 5 = I would eat this if available but would not go out of my way; 4 = I do not like this but would eat it on an occasion; 3 = I would hardly ever eat this; 2 = I would eat this if there were no other food choices; 1 = I would eat this only if forced (Schutz, 1965).

Table 5 showed that for the control bagel, flavor was strongly correlated (r = 0.907, p < 0.01) to overall acceptability, which was followed by texture (r = 0.787, p < 0.01), appearance (r = 0.778, p < 0.01), and color (r = 0.704, p < 0.01). Aroma was moderately associated with overall acceptability for the control bagel (r = 0.599, p < 0.01). For the flaxseed bagel, appearance was most strongly correlated (r = 0.785, p < 0.01) with overall acceptability, which was followed by flavor (r = 0.758, p < 0.01), color (r = 0.754, p < 0.01), and texture (r = 0.749, p < 0.01). In addition, overall acceptability and frequency of eating were strongly correlated (r = 0.887, p < 0.01) for the flaxseed bagel.

Table 5. Correlation of sensory attributes and food action (FACT) rating to overall acceptability of bagels

Sensory attributes	Overall acceptability	
	Control	Flaxseed
Appearance	0.778**	0.785**
Color	0.704**	0.754**
Flavor	0.907**	0.758**
Aroma	0.599**	0.413
Texture	0.787**	0.749**
FACT rating	0.761**	0.887**

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

3.3 Paired Preference test

Half (50%) of the participants preferred the flaxseed bagel while the other half (50%) preferred the control bagel.

3.4 FACT rating

There was no difference in how often the participants would be willing to eat the control bagel (5.20 ± 1.28, I would eat this if available but would not go out of my way) compared to the flaxseed bagel (4.90 ± 2.08, I do not like this but would eat it on an occasion), displayed in Table 3. Among participants, 25% rated flaxseed bagels as "I like this and would eat it now and then," followed by "I would frequently eat this" (20%), and "I would eat this if there were no other food choices" (15%). There was no significant relationship between demographic characteristics (gender and age group) and FACT ratings in two bagels (Table 3).

4. Discussion

The present findings demonstrate that milled flaxseed can be successfully incorporated into a cinnamon raisin bagel as there were no significant differences in sensory attributes between the two bagels, overall acceptability, preference, and FACT ratings. These results are in disagreement with Ramcharitar, Badrie, Mattfeldt-Beman, Matsuo, and Ridley (2005) that concluded there were significant differences in sensory attributes, overall acceptability, and FACT ratings between a control muffin and a flaxseed muffin among individuals from all ages.

However, the same study (Ramcharitar et al., 2005), as well as findings from Aliani, Ryland, and Pierce (2012) suggest that consumer acceptance of flaxseed bagels was higher among older adults when compared to younger adults. This supports the overall findings of this study, as the mean sensory attributes for the flaxseed bagel were between 5.90 (neither like nor dislike) and 6.65 (like slightly), which was deemed to be acceptable. The gender of the panelists had no relationship with overall acceptability or FACT ratings of the control vs. flaxseed bagel; this finding aligns with results from studies evaluating the acceptability of flaxseed muffins and bagels among individuals from all ages (Aliani et al., 2012; Ramcharitar et al., 2005).

Findings also demonstrated that there was a significant relationship between age group (50–64, 65–74, and 75–84 years old) and overall acceptability of flaxseed bagels. Mean values for overall acceptability were lower for those in the 75–84 years old individuals (neither like nor dislike) when compared to individuals age 50–74 years (like slightly), which aligns with the results of a study that showed higher mean values for overall acceptability in flaxseed bagels for individuals ages 35–64 years (like slightly) when compared to younger individuals (16–34 years, neither like nor dislike; Aliani et al., 2012). This may suggest that segments within the aging population have a different acceptance of flaxseed. For example, factors, such as physiological variables (i.e., chewing efficiency, taste, olfactory, and trigeminal stimuli), life-courses (i.e., experiences encountered during a lifetime), socioeconomic situation, and other social and psychological variables may influence preferences and consequently food choices of older adults (Doets & Kremer, 2016; Kamphuis, de Bekker-Grob, & van Lenthe, 2015; van der Zanden, van Kleef, de Wijk, & van Trijp, 2014).

The hedonic test results suggest that appearance was most strongly correlated to overall acceptability, which was followed by flavor, color, and texture for the flaxseed bagel. This has implications for guiding product developers toward appropriate strategies that will enhance the appearance, flavor, color, and texture to improve consumer acceptability for bagels among older adults. It was also found that overall acceptability and frequency of eating were strongly correlated, which aligns with results of a similar study among 89 untrained panelists (Ramcharitar et al., 2005). This confirms that the FACT rating scale can be considered another measure of acceptability; the more the bagel is liked, the more likely it is to be consumed more often, leading to increase demand. To achieve better sensory acceptance scores, different flavored bagels should be considered for future sensory evaluations as blueberry flavored bagels were rated most eaten compared to other flavors (Aliani et al., 2012).

4.1 Limitations

Although literature deemed the sample size adequate for a pilot food product sensory evaluation, results are unlikely to be representative of the population of older adults. Findings should be tested in different geographical locations since consumer preferences may vary significantly in different cultural contexts. In addition, freezing of samples may have affected the appearance, color, flavor, aroma, texture, and overall acceptability of the bagels; therefore, it may be suggested to explore other storage options.

5. Conclusion

Our study provides promising avenues for future research. While there were no significant differences in sensory attributes and intended frequency of eating in both bagels, product developers may consider improving appearance, flavor, color, and texture of a food product supplemented with flaxseed to increase overall acceptance and intended frequency of eating the product. Future experiments should additionally analyze other flavored bagels (i.e., blueberry bagels) as a promising alternative for fortification with flaxseed for use in sensory evaluations among different segments of the older population.

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Effect of Processing Methods on the Physicochemical, Mineral and Carotene Content of Orange Fleshed Sweet Potato (OFSP)

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Abstract

The effect of processing methods on the physiochemical, mineral, vitamin C and carotenoid content of orange fleshed sweet potatoes were investigated. The processing methods used were boiling, steaming, roasting, frying and microwaving. The result of the proximate composition showed that the roasted orange fleshed sweet potatoes (OFSP) had the highest ash content ranging from 0.32-0.99%, crude protein 0.96-3.12%, crude fiber 0.50-3.40% and carbohydrate content 13.98-40.10% with a decrease in the moisture content from 83.10% - 49.25%. Fat content of the fried OFSP ranging from 0.96-6.01% was higher than the other samples. Steaming method enhanced the vitamin C content of the OFSP when compared to other processing method, while carotenoid losses were higher after frying 2.59mg/g, than after microwaving 3-91%, roasting 4.73mg/g, boiling 4.60mg/g and steaming 2.68mg/g. Mineral analysis showed that the boiled orange flesh sweet potatoes (OFSP) had zinc, copper and magnesium content higher than the other heat treated samples with 6.21mg/g, 4,164mg/100g and 479.88mg/100g respectively. Sensory analysis results showed that there were no significant ($p<0.05$) differences in the sensory scores of the orange-fleshed sweet potatoes. The study therefore showed that roasting and frying made available more protein, fat, ash and carbohydrate content, while boiling made available more minerals.

Keyword: orange flesh, sweet potatoes, processing, chemical, mineral. carotenoid

1. Introduction

Sweet potato (*Ipomoea batatas)* is a dicotyledonous starch, sweet tasting, and tuberous vegetable (John, 1998).It is a very important crop in the developing world and the seventh most important food crop in the world and fourth in tropical countries. They are also a main food crop of the tropical and subtropical areas and therefore can provide a nutritional advantage to the people of rural and urban regions by enhancing their production and increasing consumption (Woolfe, 2008).

Depending on the flesh color, sweet potatoes are rich in β-carotene, anthocyanin, total phenolic, dietary fiber, ascorbic acid, folic acid and minerals (Woolfe, 1992). Orange fleshed sweet potatoes (OFSP) are bred as a tool for the global fight against vitamin A deficiency in areas that lack vitamin A rich food materials (Degras, 2003). On dry matter basis, the non-carbohydrate macronutrient composition of the edible tuberous roots includes 1.4-8.6% protein, 3.4-5.9%, crude fibre, 0.3-1.9% lipid and 1.5-6.3% ash (Degras, 2003). They are good source of Vitamin A, Vitamin C, B-vitamins, potassium and copper (FAO, 2007). The pro-vitamin A β-carotene pigment is known to be responsible for the yellow to orange coloration of the flesh of tuberous roots of sweet potato varieties (Rodriguez-Amaya and Kimura, 2004). The orange fleshed sweet potato is a seasonal crop, perishable and cannot be stored for long period of time unless preserved in some way.

Cooking could make food palatable, digestible and microbiologically safe, with many chemical, physical and sensory changes occurring during cooking which has a great influence on the final organoleptic properties as well as the nutritional value of the cooked food including texture, color and aroma development (Erdman *et al*., 1994). The Orange-fleshed sweet potato passes through different processing methods before consumption such as blanching, cooking, frying and steaming which may result in nutrient loss as it is difficult to assess the nutritional value of the final product in the form in which potato is consumed. Cooking has been reported to either be beneficial or detrimental to the nutrient content of food (Chukwu*et al*., 2010), as it helps to improve nutrient bioavailability, destroy toxins, microbes and anti-nutritional factors in food (Erdman *et al*., 1994). There is

little or no information on the effect of different cooking methods on nutrient retention of orange fleshed sweet potatoes. Therefore proper attention must be taken to establish an appropriate method that would best retain the nutrient content of food, especially orange fleshed sweet potato as food processing techniques are possible means of reducing or increasing nutrient levels. Knowledge on how the cooking methods affect the level of nutrient content could help establish the best cooking method that maintain the integrity of the components.

Therefore the objective of this work is to evaluate the effect of different cooking methods on the physicochemical, vitamin C, carotene content and sensory properties of orange-fleshed sweet potato.

2. Materials and Methods

2.1 Materials

2.1.1 Sample Preparation

The orange fleshed sweet potato (OFSP) (*Ipomoea batatas*L) variety used for this study was harvested after three (3) months of planting from the Rukpokwu farm site of the Nigerian Stored Products Research Institute (NSPRI), Mile 4, Port Harcourt Rivers State, Nigeria. After harvest, good and healthy tubers were selected and 1.5kg weight each was used for the different treatments.

2.2 Methods

2.2.1 Oven-Roasting (130^{O}C for 12min)

1.5kg samples of sweet potato were washed with clean water to remove dirt and other foreign materials. They were peeled using a knife, cut into cubes of about 1.5 cm, washed using distilled water and placed in a pre-heated oven at 130^{o}C for baking until ready to eat (12minutes). Samples were left to cool at room temperature and then milled. The samples were stored until required for further analysis.

2.2.2 Frying

Freshly harvested 1.5kg weight of orange-fleshed sweet potato was peeled, sliced using knife to about 1.5cm thickness and washed using distilled water. Frying was carried out in a frying pan having a capacity of 2 L oil for the frying of 200 gram of potatoes at a given time, which is 1:20 (w/v). The potato strips were fried in vegetable oil at about 120°C for 5 min. Fried samples were allowed to cool at room temperature, mashed using a mortar and stored for further analysis.

2.2.3 Boiling

Freshly harvested 1.5kg Orange-fleshed sweet potato were peeled, washed with distilled water, sliced to 1.5 cm thick and boiled at 100^{o}C in clean water for about 15min until tender, drained and cooled at room temperature. Mashed and stored for analysis.

2.2.4 Microwave Cooking

Orange fleshed sweet potato varieties (1.5kg) were sliced to about 1.5 cm thickness. The sliced pieces were cooked in a microwave oven (Samsung model) for 5minutes at 600W power. The samples were left to cool, mashed and stored for analysis.

2.2.5 Steaming (Moist heat)

1.5kg Orange fleshed sweet potato varieties were sliced to about 1.5 cm thickness. The sliced pieces were steamed in a steam pot at 100^{o}C for 10min.The samples were left to cool, mashed and stored for further analysis.

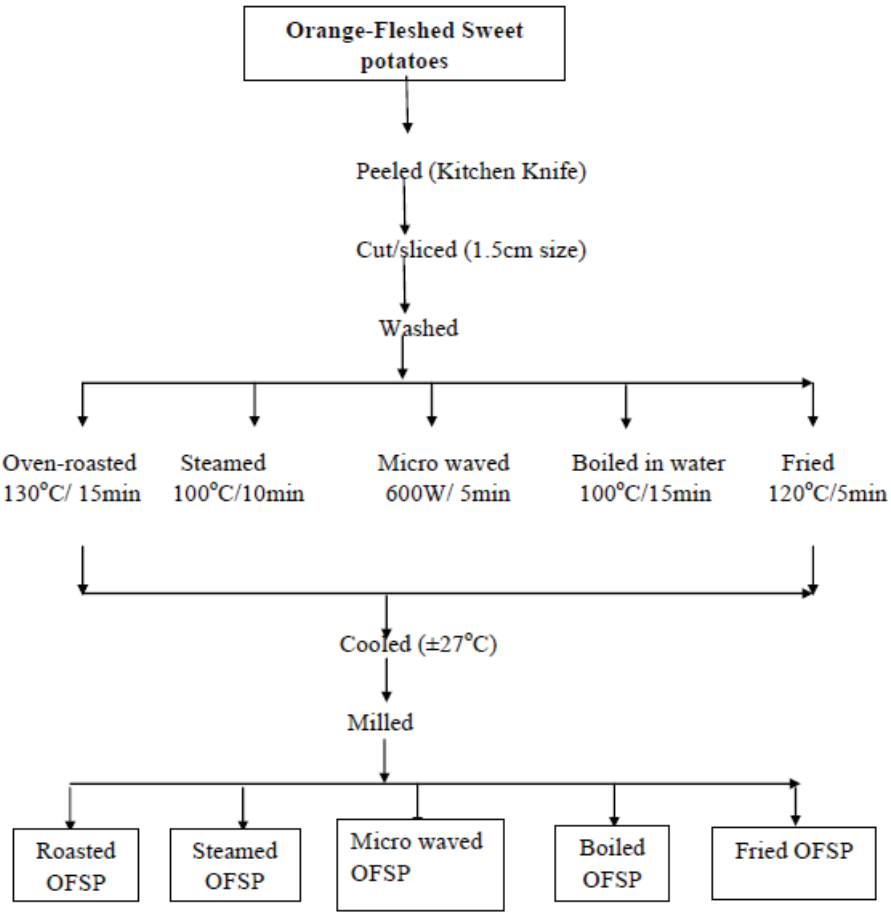

Figure 1. Flow chart for the processing of Orange-fleshed sweet potatoes using different processing method

2.3 Sensory Analysis

The sensory attributes of the heat processed orange fleshed sweet potatoes was done using simple nine point hedonic tests as described by Larmond (1991). This was done using a 20-member panellist comprising of students of the Department of Food Science and Technology, Rivers State University, who were used to consuming potatoes and where neither sick nor allergic to potatoes as at the time of the sensory testing. Scale used was 1 and 9 representing dislike extremely and like extremely respectively. The attributes evaluated include color, taste, texture, crispness and general acceptability.

2.3.1 Proximate Analysis

The moisture, ash, fat, protein, crude fibre and carbohydrate content were determined according to a procedure described by (AOAC, 2012), while total carotenoid was determined by the method of Harbone (1973)

2.3.2 Mineral Determination

The Mineral content of the samples was determined using the wet method as described by Onwuka (2005). Calcium, iron, zinc, potassium and magnesium element content was determined by atomic absorption spectrophotometer.

2.3.3 Statistical Analysis

Analyses were done in triplicate. Mean scores were analyzed statistically using analysis of variance and least significant difference (LSD) testwhich was defined at ($p<0.05$) (Ihekoronye and Ngoddy, 1985).

3. Results and Discussion

3.1 Proximate Composition of Orange-Fleshed Sweet Potato (OFSP) Samples Cooked with Different Methods

Table 1 shows the proximate composition of orange-fleshed sweet potatoes (OFSP) from different cooking

methods. Moisture content ranged from 49.25-83.10% with boiled sample recording the highest and roasted sample the lowest. Moisture result revealed that there was a significant ($p<0.05$) difference in moisture content with the boiled sample having the highest. This is expected as the boiling process provided for the starch granules in potato to imbibe and retain more water. This high moisture content reduced their shelf life and storability. Moisture content is a key to determining when a food substance is safe to be packaged. Robert *et al.*, (2017) also observed reduced moisture for roasted ripe plantain samples from 22.70-14.42% while Thomas *et al*.(2017) reported increase in moisture content from 66.79-69.50% for boiled yellow yam and reduced moisture for roasted yam samples (57.85%). Roasting has been showed to reduce the moisture content of food materials than other thermal processing methods, owing to the dry technique where the food samples are heat treated without immersing in water liquid. It has been known that dry heat dehydrates foods causing water loss while the increase in moisture of steamed sample was due to the moist heat employed in the process.

Ash content of the OFSP ranged from 0.32-0.99% with roasted sample recording the highest, while fried and boiled OFSP samples had the lowest. There was also a significant ($p<0.05$) increase in the ash content of heat processed OFSP from 0.63% in the raw sample to 0.99% for roasted sample, 0.71% for steamed sample and 0.85% for micro waved sample, while ash decreased significantly ($p<0.05$) for boiled and fried OFSP samples respectively. Ash content of a food gives an indication of the mineral composition of the food sample (Genah *et al*. 2012). Water losses occurring during roasting may have resulted in higher ash content in roasted and fried plantain (Thomas *et* al., 2017) which was also applicable in the present study. The lower ash contents obtained for the boiled OFSP sample may be due to loss of minerals into the cooking water by leaching. To support this finding, previous work by Robert *et al.*, (2017) showed that there was an increase in the ash content of yellow yam after roasting (0.63-1.42%) while a decrease was observed by Thomas *et al*. (2017) for boiled plantain (3.31-3.14%).

Fat content of the OFSP samples ranged from 0.96-6.01% with fried sample recording the highest and steamed sample the lowest. There was also a significant ($p<0.05$) increase in the fat content from 1.49% in the raw sample to 6.01% for fried sample and 3.15% for roasted sample, while a decrease in fat content was observed for boiled (0.97%), micro waved (1.42%) and steamed (0.96%) respectively. The fat in the boiled, micro waved and steamed samples may have melted as a result of the high heat applied thus causing a reduction in the fat content (Tsado *et al*., 2015). The low fat content of the roasted samples would enhance the storage life of the product. This is in agreement with the findings of Thomas *et al*., (2017) who reported that low fat content resulting from roasting, lowered chances of rancid flavor development. Previous work by Robert *et al.*, (2017) shows that there was an increase in the fat content of OFSP after frying (0.27-7.12%) and for roasting (0.46%) with a decrease in boiled yellow yam (0.27%). Thomas *et al*., (2017) also reported 0.14-15.06% for fried ripe plantain. The high fat content of the fried OFSP sample may be due to oil absorption of the sample during the frying process on the food after water is partially lost by evaporation (Saguy and Dana, 2003).

Protein content of the OFSP samples treated with different heat processing ranged from 0.91-3.12% with the roasted sample recording the highest and steamed OFSP the lowest. There was also a significant ($p<0.05$) increase in the protein content from 1.48% in the raw sample to 3.12% for roasted sample, 2.02% for micro waved sample and 1.83% for fried samples, while a decrease in protein content was observed for boiled (1.11%) and steamed (0.91%) OFSP respectively. Roasting improved the protein content of the OFSP by 52.5%, microwave by 26.7% and frying by 19.1% while boiling reduced protein by 25% and steam by 38.5%. The changes in the protein content of OFSP samples could also be attributed to leaching of nitrogen–containing compounds to the cooking medium (Saguy and Dana, 2003) as observed for boiled and steamed samples). The study showed that boiling is better than steaming in reducing protein loss. This may be because of higher latent heat of steam than boiling water, which leads to higher temperatures treatment and heat penetration during cooking, causing protein denaturation. (Richardson and Finley, 2000).

Crude fiber content ranged from 0.50-3.40% with the roasted OFSP sample recording the highest and microwaved OFSP sample lowest. Fiber content was found to increase for roasted sample (3.40%), fried sample (3.2%) and steamed OFSP (0.73%), while a decrease was observed for boiled (0.53%) and micro waved 90.50%) samples respectively. Thomas *et al.* (2017) reported an increase in the crude fiber content of roasted plantain (1.80-2.67%) and fried plantain (4.54%) respectively, while Robert *et al*. (2017) also reported the same increase for roasted yam (0.17-0.20%), fried yam (0.42%) and a decrease in crude fiber for boiled yam (0.13%).

Carbohydrate content of the orange fleshed sweet potatoes ranged from 13.98-40.10% with roasted sample recording the highest and boiled OFSP sample the lowest. There was a significant ($p<0.05$) increase in the carbohydrate content from 19.75% in the raw sample to 40.10% for roasted sample, 26.17% for micro waved sample and 22.16% for fried samples, while a decrease in carbohydrate content was observed for boiled 13.98% and steamed 17.07% respectively. Gouado*et al*.(2011) showed that there were losses in the carbohydrate content

of sweet potato after boiling and frying. They deduced that these losses might be as a result of diffusion of free sugar from food to oil/water during frying and boiling.

Table 1.Proximate analysis of orange-fleshed sweet potato processed with different methods

Method	Moisture%	Ash%	Fat%	Crude protein%	Crude fiber%	% CHO
Control	75.95±3.89^{b}	0.63±0.17^{b}	1.49±0.33^{c}	1.48±0.24^{bc}	0.70±0.11^{cd}	19.75±3.05^{cd}
Roasted	49.25±2.05^{d}	0.99±0.16^{a}	3.15±0.10^{b}	3.12±0.74^{a}	3.40±0.01^{a}	40.10±1.37^{a}
Fried	66.45±0.49^{c}	0.32±0.04^{c}	6.01±0.06^{a}	1.83±0.07^{bc}	3.20±0.18^{b}	22.16±0.25^{c}
Boiled	83.10±0.57^{a}	0.32±0.12^{c}	0.97±0.01^{d}	1.11±0.04^{c}	0.53±0.02^{de}	13.98±0.65^{e}
Micro waved	69.05±0.21^{c}	0.85±0.04^{ab}	1.42±0.00^{c}	2.02±0.01^{b}	0.50±0.02^{e}	26.17±0.14^{b}
Steamed	79.65±1.06^{ab}	0.71±0.04^{b}	0.96±0.03^{d}	0.91±0.05^{c}	0.73±0.06^{c}	17.07±1.07^{de}
LSD	4.72	0.23	0.34	0.81	0.18	3.84

Results are expressed as mean ±SD, n=2. Values in the same column having different superscript are significantly different at $p<0.05$.

3.2 Mineral Composition (mg/100g) of Orange Fleshed Sweet Potatoes as Affected by Processing Methods

Table 2 shows the mineral composition of orange fleshed sweet potatoes as affected by processing methods. Zinc content of the orange fleshed sweet potatoes ranged from 5.22-6.22mg/100g with the boiled sample recording the highest and the roasted sample the lowest. Results showed that roasting reduced zinc content by 6.5%, steam by 3.5% while the heat process by boiling increased zinc content by 11%, frying by 1% and micro wave by 9.3%. Zinc plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells (Huang and Kirschke, 2016).

Copper content ranged from 2.08-4.16mg/100g with the control sample recording the lowest and the boiled sample the highest. There was an increase in the copper content of the orange fleshed sweet potatoes after processing from 2.08mg/100g in raw sample to 4.16mg/100g in boiled sample, 3.69mg/100g in micro waved sample, 3.40mg/100g in fried sample, 3.29mg/100g in roasted sample and 3.27mg/100g in steamed sample respectively. Boiled OFSP sample had an increased copper content significantly ($p<0.05$) different from all others.

Magnesium content ranged from 428.28-479.99mg/100g with control sample recording the lowest and boiled sample the highest. An increase in the magnesium content of the orange fleshed sweet potatoes was also observed for boiled sample with 479.99mg/100g and fried sample 475.49mg/100g, roasted 473.84mg/100, micro waved 464.42mg/100 and steamed 438.24mg/100 respectively. There was a significant difference ($p<0.05$) in the magnesium contents of the orange fleshed sweet potatoes treated with different processing methods. This is in agreement with the findings of Robert *et al.* (2017) who observed an increase in the magnesium content (0.19-0.39mg/100g) of boiled plantain.

Phosphorus content of the orange fleshed sweet potatoes ranged from 0.25-0.40mg/100g with the micro waved sample recording the highest and the control sample the lowest. There was an increase in the concentration of phosphorus from 0.25mg/100g in the raw sample to 0.42mg/100g in the steamed sample followed by micro waved sample with 0.40mg/100g. Heat processing had no significant ($p>0.05$) effect on the potassium content of the orange fleshed sweet potatoes.

Potassium content reduced from 3288.24 -2816.48mg/100g with the micro waved sample recorded the lowest and the control the highest. Frying and roasting methods had 4.15% loss in potassium, while micro waved processing incurred 16.75% loss in these minerals. The minimal loss in potassium in the roasted sweet potatoes could be due to increased concentration of minerals in the pulp through loss of water during the roasting process. A surface crust is readily formed around the food material during roasting, resulting in the sealing of the intracellular spaces. This could be the reason for the minimized losses that might have arisen as result of possible volatilization. Potassium is a vital mineral and electrolyte for the body. The adequate intake (AI) for potassium is 4,700mg in healthy individuals. Sweet potatoes therefore can be an alternative food to support its intake

Calcium content ranged from 177.92-280.50mg/100g with roasted orange flesh sweet potato sample recording the lowest and boiled OFSP the highest.Calcium content of the orange fleshed sweet potatoes was affected by boiling and steaming with concentrations of 280.50mg/100g and 210.00mg/100g respectively. While the other heat treatment employed (roasting, frying, and microwaving) caused a significant ($p<0.05$) decrease in the calcium content of the orange fleshed sweet potatoes. To support the findings of the present study, USDA(2009) reported that sweet potatoes are high in minerals such as potassium, calcium, magnesium, phosphorus, and iron.

Table 2.Mineral composition (mg/100g) of orange fleshed sweet potatoes as affected by processing methods

Samples	Zn	Cu	Mg	P	K	Ca
Control	5.59^{d}	2.08^{f}	428.28^{f}	0.25^{f}	3,288.24^{b}	236.40^{b}
Roasted	5.22^{f}	3.29^{d}	473.84^{c}	0.28^{e}	3,157.20^{a}	177.92^{f}
Fried	5.65^{c}	3.40^{b}	475.49^{b}	0.34^{d}	3,157.20^{a}	187.84^{e}
Boiled	6.22^{a}	4.16^{a}	479.99^{a}	0.38^{c}	3,061.08^{c}	280.50^{a}
Micro waved	6.17^{b}	3.69^{c}	464.42^{d}	0.40^{a}	2,816.48^{e}	178.70^{d}
Steamed	5.40^{e}	3.27^{e}	438.24^{c}	0.42^{b}	2,951.04^{d}	210.00^{c}

Results are expressed as mean ±SD, n=2. Values in the same column having different superscript are significantly different at $p<0.05$

3.3 Total Carotenoid and Vitamin C Content of Orange-Fleshed Sweet Potatoes (OFSP) Processed with Different Methods

Figure 2 shows the total carotenoid and vitamin C content of OFSP processed from different methods. Total carotenoid ranged from 2.59-5.29mg/g with control sample recording the highest and fried OFSP sample as the lowest. Carotenoid losses were higher after frying 2.59mg/g , steaming 2.68mg/g, micro waved 3.91mg/g, boiling 4.60mg/gand roasting 4.73mg/g respectively. It is known that longer cooking time can increase the extractability and probably improve the bioavailability of carotenoid content from the vegetable matrix. According to Mayer-Miebach *et al*., (2005), the presence of oil increases the trans-cis isomerization during a short time treatment of foods. Thus, extensive losses during frying of the orange fleshed sweet potatoes may be probably due to the presence of oil.

Vitamin C content of the OFSP samples ranged from 0.01-0.20mg/100g with the steamed sample recording the highest and roasted sample the lowest. The result showed that processing method affected the Vitamin C content of the samples. Solubility and leaching may be the main reasons for the high loss of ascorbic acid during cooking. Different studies havefound a decrease of ascorbic acid by boiling up to75% (Gould and Golledge, 1989; Pattersen, 1998; Schnepf and Driskell, 1994).Some authors reported about losses of 3.7% by boiling different vegetables while others have found increases of 18% by pressure steaming(Booth and Bradford, 1963; Brubacher, 1966).Steaming method was found to increase the vitamin C content of the orange fleshed sweet potatoes from 0.08-0.20mg/100g while losses were more for roasting method with 0.01mg/100g. Loss as a result of boiling is justified since vitamin C is water-soluble and heat labile (Egerg*et al*., 1977). Thus vitamin C is easily leached into the boiling medium.

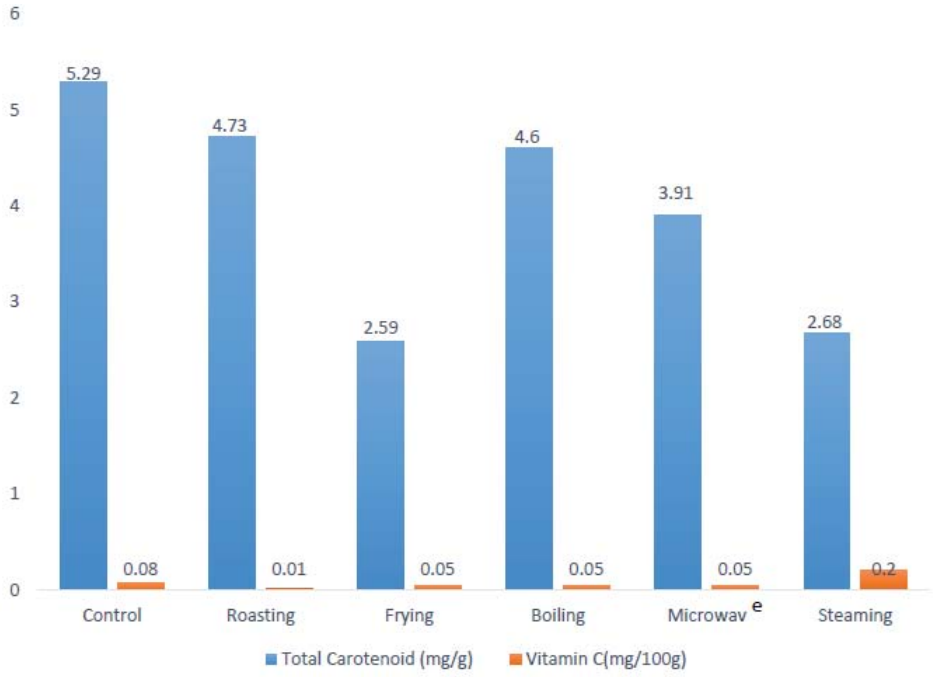

Figure 2. Carotenoid and Vitamin C content of Orange-fleshed sweet potato as affected by processing method

3.4 Sensory Analysis of Orange-Fleshed Sweet Potatoes (OFSP) Processed with Different Methods

The results of the sensory evaluation of orange fleshed sweet potatoes processed with different heat treatment method is shown in Table 3.

The mean sensory scores for the orange-fleshed sweet potato samples showed that fried, boiled, micro waved and roasted orange fleshed sweet potatoes were highly acceptable by the panelists

Color ranged from 5.10-6.35 with roasted sample as least preferred and steamed sample as most preferred. Results show that the roasted sample was significantly ($p<0.05$) different from all others. Roasting had an effect on the color of the OFSP due to crust that was formed on the roasted potato.

Taste of the OFSP samples ranged from 4.70-5.75 with the boiled sample having the least value and fried sample as most preferred.

Mouth feel of the processed OFSP ranged from 3.95-4.10 with the boiled sample as the least and micro waved and steamed samples as the highest, while texture ranged from 4.75-5.55 with the fried sample as the most preferred.

Overall acceptability of the OFSP ranged from 5.15-6.15 with steamed having the least and roasted sample as most preferred. Samples with different cooking methods showed no significant ($p>0.05$) difference in taste, mouth feel, texture and overall acceptability from each other.

Table 3. Sensory analysis of orange-fleshed sweet potatoes processed with different methods

Methods	Color	Taste	Mouth feel	Texture	OverallAcceptability
Fried	6.20^{a}	5.75^{a}	5.00^{a}	5.55^{a}	5.90^{a}
Boiled	6.10^{a}	4.70^{a}	3.95^{a}	4.95^{a}	5.50^{a}
Roasted	5.10^{b}	5.95^{a}	5.30^{a}	5.20^{a}	6.15^{a}
Micro waved	5.45^{a}	5.35^{a}	4.10^{b}	4.75^{a}	5.20^{a}
Steamed	6.35^{a}	4.85^{a}	4.10^{a}	4.75^{a}	5.15^{a}
LSD	3.02	3.07	3.02	2.79	2.54

Values in the same column having different superscript are significantly different at $p<0.05$

4. Conclusion

This study has shown that processing methods has a significant effect on the proximate, mineral and sensory properties as well as in vitamin C and total carotenoid content of Orange-fleshed sweet potatoes. Roasting enhanced the ash, protein, crude fibre and carbohydrate content of the orange-fleshed sweet potato more than other processing treatment while fat content was increased when the OFSP were fried. Vitamin C losses were observed for roasted and steamed samples while this was minimal for fried, boiled and micro waved OFSP samples. At the same time, Carotenoid losses were observed in all methods with roasting having the least loss. The study also revealed that boiling of orange-fleshed sweet potatoes resulted to an enhancement in the minerals such as zinc, copper, magnesium and calcium contents, while sensory analyses gave acceptable products with the various methods.

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Study of Four Onion Varieties Drying Kinetics in an Oven and a Solar Greenhouse

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Abstract

Onion production (*Allium cepa* L.) in Senegal reached 390 000 tons in 2016. Due to post-harvest losses, annual demand (150 000 and 250 000 tons) is being met through imports. This work consists in proposing a drying process at a lower cost to overcome this dependence and preserve the quality of the product. The optimization of local onion varieties drying in an oven and in solar greenhouse, as well as the physicochemical characterization of the products were carried out. The moisture of fresh onion bulb varies between 85.56 ± 0.60 and 89.13 ± 0.69 (%). To obtain a moisture ≤ 8.89 ± 0.16 (%) ensuring stability, the optimal drying conditions in the oven are 60° C / 6H (Galmi Violet) and 7H (Safari, Gandiol F1 and Orient F1). Under these conditions, the content of polyphenols in g equivalent of gallic acid / 100 g db increases (0.111 ± 0.0040 to 0.312 ± 0.0041 before drying, 0.546 g ± 0.0117 to 0.837 ± 0.0091 after drying). Optimum solar drying in a greenhouse is obtained between temperatures of 35 to 65° C / 8H-9H. From a perspective of sustainable development, the perspective is the modeling of drying kinetics in a solar greenhouse.

Keywords*: Allium cepa* L., Local varieties, drying, optimal conditions, moisture, water activity, polyphenols

1. Introduction

Senegalese agriculture, particularly rainy and seasonal agriculture, contributed about 18% of GDP in 2015 (National Agency for Statistics and Demography [ANSD]) and is one of the key levers for ensuring food security. Horticultural is the most dynamic sub-sector of this Senegalese agriculture, with a growth of between 5% and 10% since 2004.(ANSD, 2014; Direction de l'Horticulture [DH], 2015). This performance is mainly driven by the growth of the onion sub-sector which represents 60% of horticultural production (DH, 2016).

In fact, onion (*Allium cepa* L.), a very popular vegetable in Senegal, with an annual consumption of between 150,000 and 250,000 tons (ARM, 2016) represents 25% of household expenditure. The most common use of onion in households across the country is fragmenting the bulb into pieces that are incorporated into recipes for flavor development. However, despite a record production of 390,000 tons in 2016, meeting household demand over the year remains dependent on imports due to significant post-harvest losses due to the high *moisture* content of the onion. Thus, to reduce post-harvest losses, developing the dehydration process seems to be an excellent opportunity but the process requires a lot of energy.

Many studies on food products drying have shown that drying efficiency and kinetic characteristics depend on the drying conditions and the types of products (varieties and their degree of maturity, shape, thickness, composition), but also on the electric or solar drying mode. Among these studies can be mentioned those on the onion (Ahmed-Zaid, 1999; Albitar, Mounir, Besombes, & Allaf, 2011; Anwar & Tiwari, 2001; Kiranoudis, Maroulis, & Marinos-Kouris, 1992; Krokida, Karathanos, Maroulis, & Marinos-Kouris, 2003; Sarsavadia, Sawhney, Pangavhane, & Singh, 1999), the pepper (Anwar & Tiwari, 2001; Lhendup, 2005) and the beef

(Lhendup, 2005; Tom, 2015).

On the other hand, the use of solar drying, a major lever to overcome the energy constraint, preserves the quality of products despite the variability of climatic conditions (Boughali, 2010; Jannot, 2006; Mendez Lagunas, 2007).

These findings and the absence of data in the literature on local varieties justify the initiation of this research.

The objective of this study is to optimize the drying of four onion varieties in an oven and in solar greenhouse and to compare solar drying, which reduces the energy bill, to electric convective drying. Optimal drying conditions are determined by studying the impact of this process on some major biochemical and physicochemical parameters.

2. Materials and Methods

2.1 Materials

2.1.1 Plant Material

The local onion is collected in the cooperative of the locality of RAO in Saint-Louis (Senegal). The varieties studied are Galmi violet, Safari, Gandiol F1 (Gandiolais) and Orient F1 (Orient) with a maturity of 85% of leaves falling at harvest.

2.1.2 Analysis Equipment

Analysis equipment: an oven with ventilation (Memmert brand), a solar greenhouse equipped with a ventilation system to regulate the ambient air temperature and humidity, sensors for temperature and humidity readings, capsules in pyrex, drying racks, a scale (Denver instrument brand with a reliability rate of 0.0001g), a thermohygrometer (Voltcraft brand with a precision of 1°C and 3.5%), a water activity meter (Rotronic HP 23 brand), a pH meter (HI 23 brand), a burette, a spectrophotometer (Specord brand), a mineralizer, a distiller and laboratory glassware.

2.1.3 Graphical and Statistical Representation Tools

Data exploitation is carried out with both the R version 3.4.0 (Team R Core, 2017) software for the comparison test between the two methods of drying, the analysis of variance and the concordance of the measurements, and the Excel version 2016 software as a tool for scientific calculations for graphic representations.

2.2 Methods

The peeled onions are washed in chlorinated water 100 ppm (0.1 mg / L water), rinsed three times with clean water, dewatered and finely chopped with a chopper to neglect the deformation of the product during the drying process.

The thickness of the samples is in the range of 1.7 mm.

2.2.1 Kinetics Study

The tests are conducted in an oven in the temperature range of 50° C to 70° C with a step of 5° C to determine the optimum temperature / time to obtain stable products.

Ten grams are taken from the chopped onions of three different bulbs, and spread in pyrex cups. The experiments are carried out with ventilation at a fixed air velocity of 2.4 m/s and a relative humidity between 10 and 15% (Babalis & Belessiotis, 2004; Clemente, Frías, Sanjuan, Benedito, & Mulet, 2011; Kiranoudis et al., 1992; Krokida et al., 2003; Sarsavadia et al., 1999).

Figure 1. (a) Photo of the oven (b) cups for monitoring kinetics (c) trays for producing onion powder samples

Regarding solar greenhouse drying, the four varieties are dried simultaneously (one variety per drying rack). Each rack is squared in four parts of equal size (0.74 X 0.71 m) on which three kg of onion are spread in monolayer. Inside the solar greenhouse, removable room sensors make it possible to follow the evolution of the temperature and the relative humidity, two determining parameters for the drying. During solar greenhouse drying, the relative humidity varies between 10-60% and the temperature varies between 35° C and 65° C.

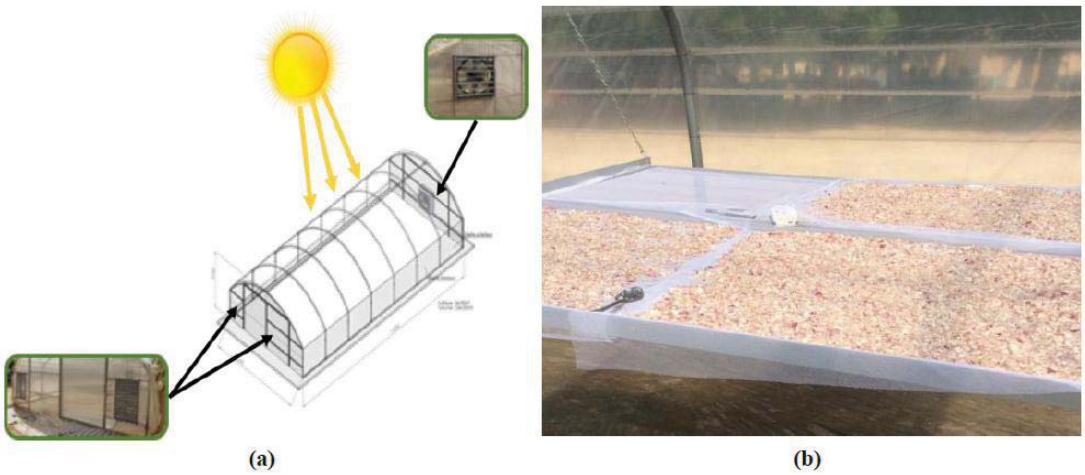

Figure 2. (a) Photo of the solar greenhouse from the outside with the ventilation and air extraction system and (b) photo of a drying rack with the onions distributed in a thin layer

Both in the oven and in the solar greenhouse, the experiments are performed in triplicate and the monitoring of the weight loss takes place every hour.

2.2.2 Physico-Chemical Analyses

The various biochemical and physicochemical analyses performed on raw materials as well as onion powders obtained by dehydration in an oven and in a solar greenhouse are the pH according to NF V76-122: 1994, NF

EN 1132, titratable acidity according to NF V 05-101 January 1974, European Standard EN 12147 December 1996), the moisture content with reference to standard NF ISO 712: 2009), the activity of water according to standard NF EN ISO 17025). In addition, the polyphenols, the most important functional elements to be preserved during the drying of the four onion varieties, are evaluated by the Folin-Ciocalteu reagent spectrophotometric assay method in a basic medium at 760 nm. The total polyphenol content is expressed in gallic acid equivalent.

All moisture measurements and characterization analyses are performed in triplicate to ensure repeatability.

2.2.3 Statistical Analyses

The evaluation of the reproducibility and the repeatability of the measurements is made by the numerical method which is the LIN coefficient (Lawrence & Lin, 1989).

The Lin's concordance coefficient varies between -1 and 1, where the values -1, 0 and +1 respectively mean perfect discordance, zero concordance and perfect match.

The Student's parametric test is used for the comparison of the:

- characteristics of onion varieties (water activity, titratable acidity, pH and polyphenol content) before drying;
- stability moisture of the oven-dried samples and those dried in a solar greenhouse.

All statistical analyses are performed with a significant threshold of $p < 0.05$.

3. Results and Discussion

3.1 Characteristics of Onion Varieties before Drying

The physical and chemical characteristics of the samples before drying are presented in table 1.

All varieties are marked by a high moisture content and Aw, pH and polyphenols values are almost identical. Only the acidity of the Safari variety seems to stand out (9.23 mEq / 100g db) from that of the other varieties (between 4 and 6 mEq / 100g db).

The two most important criteria for the stability of food products, namely the moisture content (%) and the water activity of fresh onions are respectively for:

- Galmi violet 85.56 ± 0.60 / 0.945 ± 0.01;
- Safari 88.11 ± 0.61 / 0.950 ± 0.001
- Gandiol F1 86.99 ± 0.10 / 0.940 ± 0.001;
- Orient F1 89.13 ± 0.69 / 0.947 ± 0.009.

Table 1. Major characteristics of onion samples before drying

Variety	Galmi Violet	Safari	Gandiol F1	Orient F1
Moisture (%wb)	85.56 ± 0.60	88.11 ± 0.61	86.99 ± 0.10	89.13 ± 0.69
Water Activity	0.945 ± 0.011	0.950 ± 0.001	0.940 ± 0.013	0.947 ± 0.009
Polyphenols (g EAG /100g db)	0.111 ± 0.0040	0.134 ± 0.0065	0.162 ± 0.0016	0.312 ± 0.0041
Titrable Acidity (mEq / 100g db)	6.12 ± 0.00	9.23 ± 0.00	4.51 ± 0.02	6.13 ± 0.38
pH at 10%	6.42 ± 0.03	6.29 ± 0.06	6.35 ± 0.03	6.37 ± 0.03

Legend: Equivalent Gallic Acid (EAG)

3.2 Optimal Drying Conditions

The Lin coefficients obtained for the measurement concordance test for all oven drying and solar greenhouse drying kinetics vary between 0.9993555 and 0.9999317 with a confidence interval of [0.9991869; 0,9999431]. This indicates that there is a perfect match between the three measurements made for each test.

The results of the statistical test for the comparison of the oven drying kinetic data to that in solar greenhouse are between [-0.44906 - 0.73362] for Student's parameter (t), [0.4697 - 0.9572] for the pvalue and [24 - 26] for the degree of freedom (df). Therefore, regardless of the oven drying temperature, p> 5% values show that there is no significant difference between oven drying and solar greenhouse drying kinetics.

The stability of dried fruits and vegetables is guaranteed with a moisture content of 8 ± 2% or less and an Aw between 0.5 and 0.6 to avoid any microbial activity (Bernard & Carlier, 1992; ESA, 2004; Faiveley, 2003; Le Meste & Chiotelli, 2002). These values serve as a reference to determine the optimal drying conditions taking

into account the evolution of the physicochemical characteristics after drying.

3.2.1 Optimal Drying Conditions in the Oven

Figure 3 shows the evolution of the moisture content of Galmi Violet, Safari, Gandiol F1 and Orient F1 varieties dried in an oven at different temperatures.

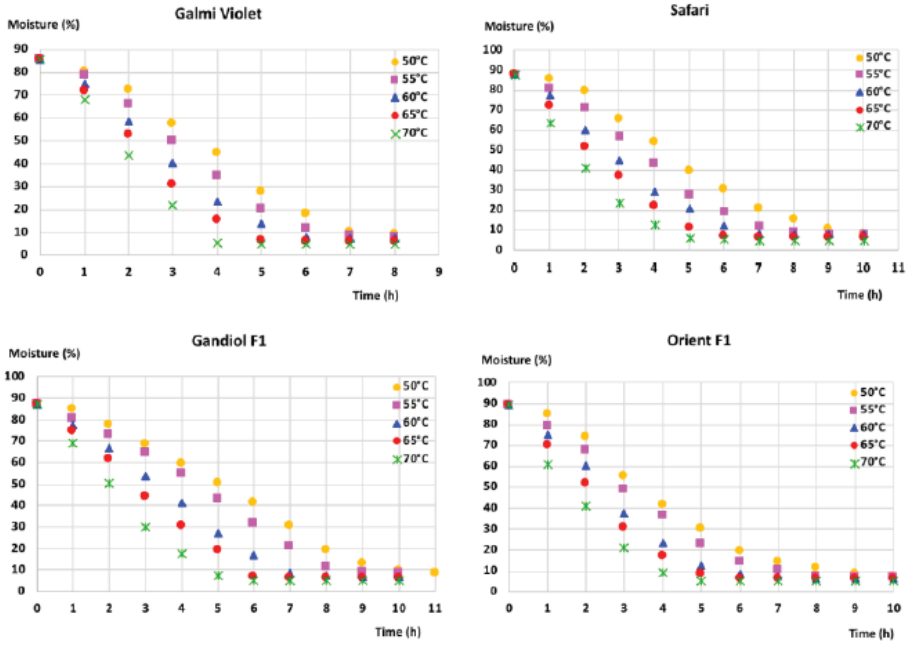

Figure 3. Evolution of moisture content of oven-dried onion varieties at different temperatures

The optimal drying time in the oven, which makes it possible to reach the stability moisture, changes inversely with the increase in temperature. Over the temperature range between 50° C and 70° C, the results (Figure 3) obtained for the four varieties are as follows:

- the initial moisture content of Galmi Violet (85.56 ± 0.60%) decreases to a moisture stability of between 8.89 ± 0.16 and 5.23 ± 0.34 (%) for an optimal time between 8H and 4H depending on the drying temperature. At each increase in temperature (+5° C), the drying time decreases (-1H);
- with an initial moisture content of 88.11 ± 0.61 (%), the stability moisture of the Safari variety, between 8.14 ± 0.52 and 6.30 ± 0.26 (%) according to the drying temperature; is reached for an optimal time between 10h and 5h. The drying time decreases (-1H) at each increase in temperature (+ 5 ° C) except from 55° C to 60° C where the time step is (-2H);
- the Gandiol F1 variety with an initial moisture content of 86.99 ± 0.10 (%) has a stability moisture of between 8.68 ± 0.33 and 7.70 ± 0.39 (%) depending on the drying temperature. The optimal drying time varies between 11H and 5H with a pitch of (-2H) for each increase of a step of 5° C in the temperature range of 50° C to 60° C and (-1H) for that ranging from 60° C to 70° C;
- for the Orient F1 variety with an initial moisture content of 89.13 ± 0.69 (%), the stability moisture is between 8.54 ± 0.41 and 5.02 ± 0.24 (% db) depending on the drying temperature. The optimal drying time is between 9H and 5H with a variation of (-1H) for each temperature increase of a step of 5° C in the range 50° C to 60° C, of (-2H) in the range of 60° C to 65° C and no variation in that of 65° C to 70° C.

For each variety, the optimal temperature and the optimal drying time are determined taking into account changes in moisture content and water activity

(Bonazzi, Dumoulin, & Bimbenet, 2008; Charreau & Cavaille, 1991; Jeantet, Croguennec, Schuck, & Brulé, 2008; Jiménez Elizondo, 2011) , as well as the impact of the process on polyphenols (Ali, Bordia, & Mustafa, 1999; Lombard, Peffley, Geoffriau, Thompson, & Herring, 2005; Yang, Meyers, van der Heide, & Liu, 2004). These are constituents of therapeutic interest (Ali, Thomson, & Afzal, 2000; Griffiths, Trueman, Crowther, Thomas, & Smith, 2002; Zohri, Abdel-Gawad, & Saber, 1995).

3.2.2 Optimal Drying Conditions in a Solar Greenhouse

Figure 4 shows the evolution of the moisture content of sun-dried onion varieties at varying temperatures during drying.

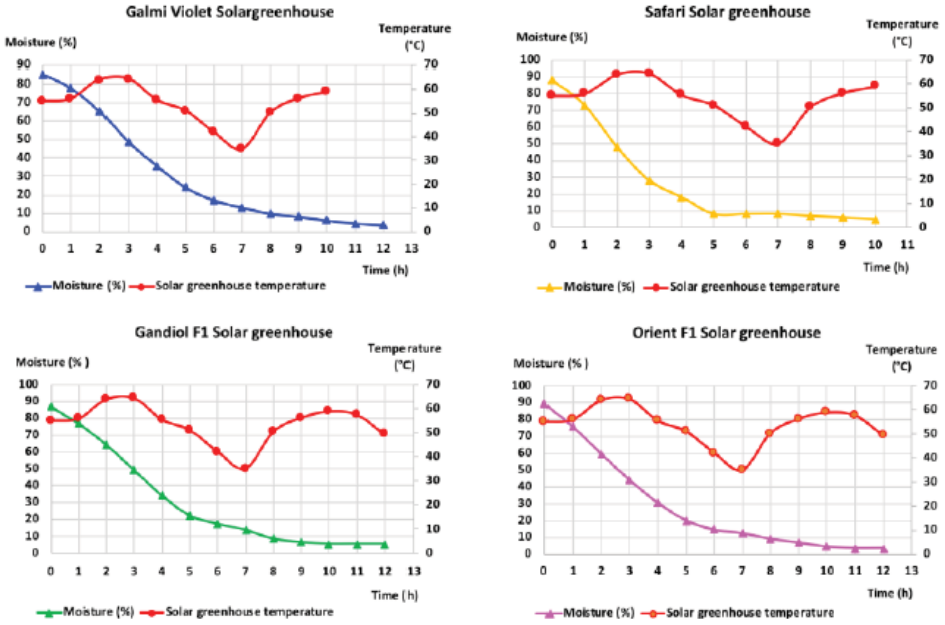

Figure 4. Evolution of the moisture content of the four sun-dried onion varieties at varying temperatures during drying

The drying of onions in a solar greenhouse unlike the oven depends on the sun. Thus during the tests the temperature and humidity in the solar greenhouse varied in the respective ranges of 35-65° C and 10-60%. The drying kinetics of the four varieties are essentially identical.

Figure 4 shows that the stability moisture values are reached from 8H solar greenhouse drying and that from 10H the values are almost stable. Table 2 displays the different moisture contents for drying times from 8H to 10H.

Table 2. Drying time in the solar greenhouse and moisture content of dried samples

	Moisture (% db)		
	Drying time		
Variety	8H	9H	10H
Galmi Violet	9.89 ± 3.034	8.23 ± 2.004	6.23 ± 2.465
Safari	6.88 ± 2.107	5.88 ± 0.195	4.88 ± 0.088
Gandiol F1	8.54 ± 2.620	6.49 ± 2.253	5.45 ± 0.954
Orient F1	9.7 ± 4.18	6.89 ± 1.045	4.66 ± 1.193

The moisture content is inversely proportional to the drying time in the solar greenhouse and the elimination of water is different depending on the variety. These results are compared with Aw and polyphenol values to determine the optimal drying time.

3.3 Physico-chemical Characterization of the Samples after Drying

Water activity (Aw), titratable acidity, and pH are characteristics of the environment as important in the stabilization of food products as the moisture content. To avoid any microbial activity, an Aw between 0.5 and 0.6 is necessary. Moreover, the more acidic the medium (pH less than 4.5), the more it is unfavorable to chemical and biochemical degradation reactions (Bernard & Carlier, 1992; Charreau & Cavaille, 1991; Faiveley, 2012).

3.3.1 Characterization of Dried Samples in an Oven

3.3.1.1 Evolution of Water Activity

The evolution of water activity for the four varieties (Figure 5) shows that the initial Aw values between 0.940 ±

0.01 and 0.950 ± 0.001 decreases with increasing drying temperature. The initial Aw values are divided by a factor between 1.70 and 2.78 for each temperature step of + 5° C in the oven. Aw reaches values between 0.362 ± 0.003 and 0.447 ± 0.069 at 60 ° C. On the other hand, the Aw values of the samples for the 65° C and 70° C temperatures remain relatively stable in this range, except for the Orient F1 variety at 65° C (0.497 ± 0.002).

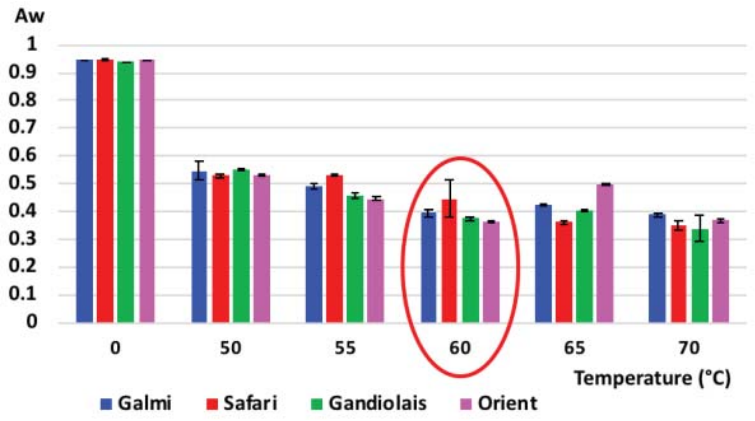

Figure 5. Water activity of the samples after optimal drying

3.3.1.2 Evolution of Titratable Acidity and pH

The monitoring of titratable acidity and pH at 10% of oven-dried samples (***Figure***) indicates that with increasing drying temperature, the initial values of titratable acidity and pH at 10% ranging respectively between 4.51 ± 0.02 and 9.23 ± 0.00 mEq / 100g (db) and 6.29 ± 0.06 and 6.42 ± 0.03, change inversely for the four varieties. The multiplicative factor for titratable acidity is between 0.84 and 2.53 while the pH is divided by a factor in the range of 1.12 to 1.28. However, over the temperature range of 50° C to 70° C, the difference is not significant for both titratable acidity and pH (all p values for Student's test are greater than 0.05).

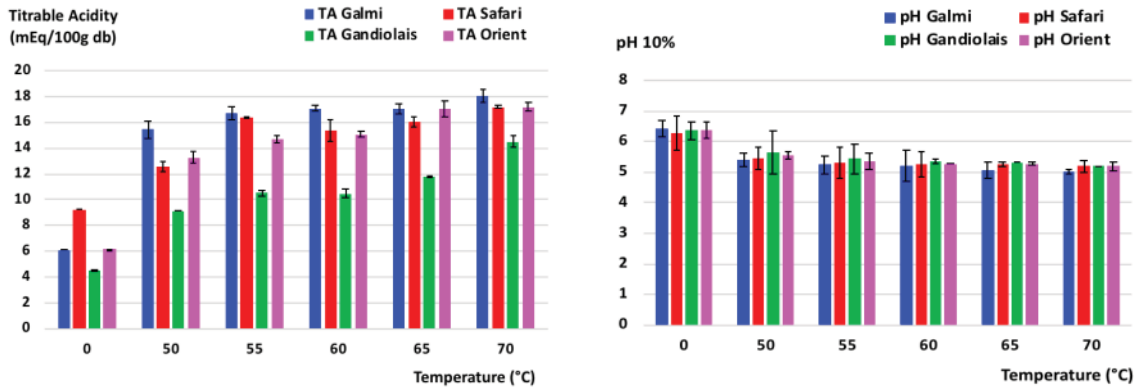

Figure 6. Titratable acidity and pH of the samples after drying in an oven at different temperatures

3.3.1.3 Evolution of the Polyphenol Content

The initial levels of total polyphenols (Table 1) of the four varieties, ranging from 0.111 ± 0.0040 to 0.312 ± 0.0041 g EAG / 100g (db), increase with drying temperature (Figure 7Figure). The increase in total polyphenol content is greatest at a temperature of 60° C with 0.546 ± 0.0117 g EAG / 100 g (db) for Galmi Violet; it is 0.837 ± 0.0091 g EAG / 100g (db) for Safari, 0.694 ± 0.0173 g EAG / 100 g (db) for Gandiol F1 and 0.691 ± 0.0162 g EAG / 100 g (db) for Orient F1. This effect of temperature on polyphenol content is similar to those found in the literature (Ali et al., 1999; Lombard et al., 2005; Yang et al., 2004).

Nevertheless, from 65° C, a decrease of about 0.3% to 37% is observed. This decrease is accentuated at 70° C, showing the negative impact of temperature on polyphenols.

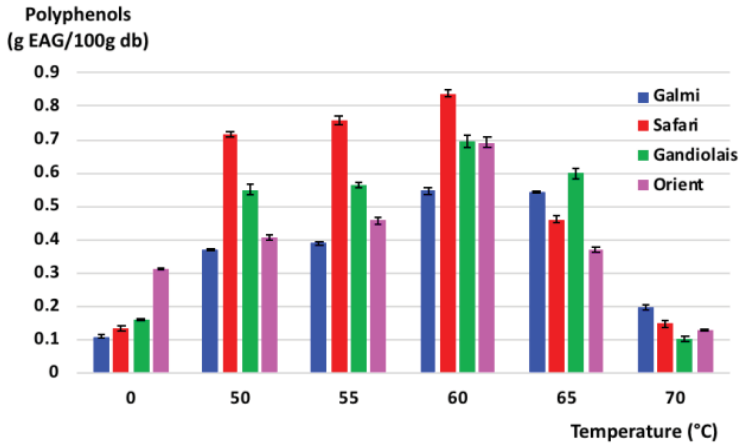

Figure 7. Polyphenol content after drying in an oven at different temperatures

The temperature range 50° C to 65° C with an optimal drying time between 6H and 11H makes it possible to obtain characteristics (Aw and moisture content) ensuring the stability of the product. However, the concern to maintain the functional properties of the polyphenols and to reduce the energy consumption makes it possible to determine the best drying time-optimal temperature pair.

Thus, the 60° C temperature with an optimal time of 6H for Galmi Violet and 7H for the other three varieties, is the best time/temperature pair because the polyphenol content is at its maximum. The products dried under these optimal conditions in the oven have a water activity of about 0.4 and a moisture content respectively for Galmi Violet, Orient F1, Safari and Gandiol F1 of 7.96% ± 0.42; 7.17 ± 0.63; 8.42% ± 0.05 and 8.67 ± 0.15%.

With these moisture and Aw values, the biochemical and physicochemical reactions and the development of the microorganisms responsible for the perishability of the products are then inhibited (Bernard & Carlier, 1992; Faiveley, 2003).

3.3.2 Characterization of Samples after Drying in a Solar Greenhouse

In the solar greenhouse drying conditions with the respective temperature and humidity ranges in the greenhouse of 35-65 ° C and 10-60%, the initial water activity of the samples decreases after 8h drying (Table 1). The water activity values of the four dried onion varieties ranged from 0.577 ± 0.007 to 0.675 ± 0.041 (Table 3). These results are in the range to avoid any microbial activity (Bernard & Carlier, 1992; Charreau & Cavaille, 1991; Faiveley, 2012).

This decrease in the water activity of varieties continues with the increase in drying time in the solar greenhouse. Thus, for a drying time of 9H, the values of the water activity of the samples are between 0.505 ± 0.005 and 0.550 ± 0.018 while at the end of 10H of drying, they are between 0.415 ± 0.012 and 0.491 ± 0.006.

Table 3. Water activity of solar greenhouse dried samples at different drying times to achieve stability moisture values

	Water Activity (Aw)		
	Drying time		
Variety	8H	9H	10H
Galmi Violet	0.577 ± 0.007	0.538 ±0.003	0.469 ±0.012
Safari	0.589 ±0.003	0.505 ± 0.005	0.477 ± 0.000
Gandiol F1	0.675 ± 0.041	0.55 ± 0.018	0.415 ± 0.012
Orient F1	0.617 ± 0.008	0.536 ± 0.005	0.491 ± 0.006

The stability moisture of samples dried in a solar greenhouse at different times (Table 2) shows that the optimal drying time is 9H for Galmi Violet and Orient F1 varieties and 8H for Safari and Gandiol F1 varieties. At these optimal times, the values of the water activity (Table 3) between 0.536 ± 0.005 and 0.675 ± 0.041 ensure the absence of any microbial activity. The characteristics of the products dried under these optimal conditions are presented in Table 4.

Table 4. pH, titratable acidity and polyphenol content of solar greenhouse dried samples under optimal conditions

Variety	Optimal drying time (H)	pH (at 10%)	Titrable acidity (mEq / 100g db)	Polyphenols (g /100g db)
Galmi Violet	9	5.52 ± 0.14	47.43 ± 5.035	0.530 ± 0.003
Safari	8	5.56 ± 0.03	48.60 ± 1.018	0.720 ± 0.003
Gandiol F1	8	5.35 ± 0.03	34.89 ± 0.198	0.505 ± 0.009
Orient F1	9	5.48 ± 0.10	48.760 ± 2.432	0.607 ± 0.005

At these optimal times of 9H for the Galmi Violet and Orient F1 varieties and 8H for the Safari and Gandiol F1 varieties, the characteristics of the solar greenhouse dried products (Table 4), compared to the initial values (Table 1), reflect that:

- the titratable acidity increases with the drying temperature whereas the pH at 10% changes inversely for the four varieties. The titratable acidity of the varieties that are dried in solar greenhouse ranges from 34.89 ± 0.1 to 48.760 ± 2.322 mEq / 100g (db). As for pH, the values are in the range of 5.35 ± 0.03 to 5.56 ± 0.03;
- polyphenol contents also increase with drying. The initial values of polyphenols of Galmi Violet (0.111 ± 0.004 g EAG / 100g db), Safari (0.134 ± 0.0065 g EAG / 100g db) Gandiol F1 (0.162 ± 0.0016 g EAG / 100g db) and Orient F1 (0.312 ± 0.0041 g EAG / 100g db) varieties are respectively multiplied by a factor of 5.48; 5.37; 3.10 and 1.94 after drying in the optimal conditions of solar greenhouse.

Photos of onion powders obtained after drying in an oven at temperatures of 60° C, 65° C and 70° C and those obtained after drying in a solar greenhouse in 8H, 9H and 10H time are shown in the Figure 8.

Figure 8. Onion powders obtained after drying in optimal conditions in an oven [(a) 60° C, (b) 65° C and (c) 70° C] and solar greenhouse [(d) 8H, (e) 9H and (f) 10H]

The higher the temperature and the drying time increase, the darker the color of the powders obtained is.

The impact of temperature on polyphenols (Ali et al., 1999; Lombard et al., 2005; Yang et al., 2004), constituents of therapeutic interest (Ali et al., 2000; Griffiths et al., 2002; Zohri et al., 1995), associated with the phenomenon of crusting when the removal of water is done too quickly and browning dried products (Figure 8), allow to avoid temperatures above 65° C and times greater than 10H for drying onions. On the other hand, too long exposure times consume not only a lot of energy, but can also affect the quality of the product. Therefore, the best temperature / time pairs are 55 to 65° C / 6H to 8H with an optimum at 60° C.

As for the results obtained by drying in the greenhouse presented in Table 2, Table 3 and Table 4, they show that moisture and Aw stability are obtained without browning the products after 8 hours to 9 hours of drying with a temperature in the greenhouse varying from 35 to 65° C during the day. Moreover, in this solar greenhouse temperature range, the polyphenol contents of the four onion varieties increase after drying. These results are comparable to those obtained in the oven.

The results of the parametric test of Student confirm that there is no significant difference between oven drying and solar greenhouse drying kinetics under the study conditions because all p values are > 5%.

4. Conclusion

The research carried out in the framework of this study made it possible to optimize the dehydration process of onion bulbs using two different energy sources. The ideal drying ranges are 55° C to 65° C / 6H to 8H in an oven and 35 to 65° C / 8H to 9H in a solar greenhouse to obtain products with low moisture content (≤ 8%).

Reducing high moisture content and water activity in the onions by drying in the oven as well as in solar greenhouse thus ensures the stability of the dried products. In addition, although the drying time in solar greenhouse is greater than that in the oven, the impact of drying on the evolution of the polyphenol content is substantially identical regardless of the energy source used. These results guide the choice towards the solar source for the management of post-harvest losses through the dehydration of onions.

However, lack of control of solar greenhouse drying temperatures can affect the nutritional and organoleptic quality of dried onions. The Establishment of the desorption isotherms and the modeling of the drying kinetics is thus necessary to control the parameters and ensure the regularity of the quality of the finished product. A study of the stability of onion powders including the monitoring of the re-humidification and color changes during storage should be considered. The reconstitution of dried onions and the sensory analysis by the consumers will be the next stages to be explored for a possible vulgarization of the products.

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Evaluation of a Real Time PCR Assay and a ELISA Method for the Detection of Walnuts and Almonds Allergen Traces in Food Products

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Abstract

Food allergens are a well acknowledged issue in food industry and are regulated by legislation. The presence of allergens can either origin from the raw material or due to contamination during production. Allergen information on packaging is mandatory although it cannot be accurate in the case of contamination therefore warnings are used. The purpose of the study is the development and validation of a SYBR Green Real Time Polymerase Chain Reaction method using specific primer pairs based on Jug r 1, Jug r 3, and Jug r 4 allergen-coding sequences to improve the sensitivity of Real Time Polymerase Chain Reaction techniques for detection of walnut and almond traces in commercial food products and its comparison with ELISA methodology in terms of detection ability. A total of 100 samples were collected from local markets and were analyzed by Real Time Polymerase Chain Reaction (RT-PCR) and ELISA methods. The results indicated that 16 samples (16%) were found positive in walnut traces and 18 samples (18%) were found positive in almond traces by Real Time Polymerase Chain Reaction of which Elisa identified 14 samples (14%) positive in walnut traces and 15 samples (15%) positive in almond traces. Among them, 4 samples (25%) that contained walnut traces and 6 samples (33.3%) that contained almond traces had no allergen declaration on their label. The improved accuracy of Real Time Polymerase Chain Reaction underlines the importance of this method for allergen detection and quantification in the food industry

Keywords: Real Time PCR, ELISA, food allergens, legislation

1. Introduction

1.1 Introduce the Problem

Among the most serious food safety problems that raise concerns from consumers are food allergies. These are the clinical manifestation of an immunological process in which certain food ingredients (mainly proteins) or their metabolic derivatives, act as antigens and stimulate the production of antibodies against them (Sampson, 2004). Undeclared allergens as contaminants in food products pose a major risk for sensitized persons.

Tree nuts are extensively used mostly due to their high nutritional value but at the same time they are well-known allergens, included in the list of the 14 major allergens as described in E.U. Food Information Regulation No.1169/2011. The allergenic action of tree nuts is quite intense and severe health consequences can be caused even by traces. The importance of tree nuts allergies is related to not only to the severity of the reactions, but also to the prevalence in the general population which is 0.2% to 0.7%. (Emmett, Angus, Fry, & Lee, 1999).

The origin of allergen can be traced to the raw material but there is also a chance that allergens can end up in foodstuffs by cross-contamination when allergenic ingredients are also processed in the same factory. Allergen information on food packaging is mandatory although it cannot be accurate in the case of contamination therefor warnings are used. In cases, a common practice in food industry is the use of warning phrases such as *"This product may contain traces of .....",* which are not required by E.U. legislation but can act as a precaution.

Unnecessary labelling of food products with such claims prevents the susceptible customer to purchase a safe product. Nevertheless, hidden allergens can end up in food products by cross-contamination when they are processed in the same factory with allergenic ingredients. The non- identification of allergenic ingredients can cause life-threatening situations since even small traces are unsafe for very susceptible individuals. Considering the varying sensitivity among the allergenic patients, appropriate thresholds for allergenic ingredients are difficult to be determined. Thus, reliable, specific and sensitive detection and quantification methods for food allergens are necessary to ensure compliance with food labelling and to improve consumer protection.

Test procedures which can be performed in a short time are preferred. Enzyme-Linked Immunosorbent Assays (ELISAs), lateral flow tests and dipsticks are very popular tests to obtain the first information about the presence of an allergic substance. Besides ELISA, Polymerase Chain Reaction (PCR)-based methods can be performed in second instance to increase certainty. (Semi)-quantification of the allergen can be achieved by both ELISA and PCR-based methods.

PCR based methods amplifying specific DNA sequences offer alternative tools to the detection of allergenic or marker proteins for the species (Goodwin 2004; Poms & Anklam 2004). In most cases, DNA presents a more stable analyte compared to proteins and is less affected by denaturation (Poms & Anklam,2004). Nevertheless, the lack of available reference materials, and the absence of official methods for allergen detection and quantification, can be major constrains.

Enzyme-linked immunosorbent assay (ELISA) is the most frequently used method for the detection of allergens in complex food matrices (Costa, Carrapatoso, Oliveira, & Mafra, 2014) while both Conventional (Yano et al., 2007) and Real Time-PCR have been used to detect walnut in food samples using different dye methods and target sequences, such as Jug r 2 (Piknová, Pangalio & Kuchta 2007) and Jug r 3 (Costa, Oliveira, & Mafra, 2013). Real TimeT-PCR technology has been extensively evaluated in food allergens. Conventional and Real-time PCR methods for the detection of soybean, sesame, mustard, peanut, hazelnut and almond have been recently compared (Pancaldi, Paganelli, Righini & Carboni, 2005).

The aim of study is the development and validation of a SYBR Green Real Time PCR method using specific primer pairs based on Jug r 1, Jug r 3, and Jug r 4 allergen-coding sequences to improve the sensitivity of Real Time-PCR techniques for detection of walnut *(Juglands regia*) and almond (*Amygdalus communis L*.) traces in commercial food products and its comparison with ELISA methodology in terms of detection ability.

2. Materials and Methods

2.1 Food Products

A total of 100 samples of widely consumed products, namely 20 cereal based products, 17 chocolates, 20 biscuits, 13 wafers and 30 snacks were collected from the Greek market during the period of September 2017 to March 2018. The samples were separated in 4 categories according to the allergen declarations on their packaging. The first category (category I) included 15 products that had as ingredients walnuts and/or almonds (10 with walnuts and 5 with almonds) while the second category (category II) included 35 products which bared the indication “May contain traces of walnuts and/or almonds” (15 about walnut traces and 20 about almond traces). Finally, there were 29 products with indication “may contain traces of nuts” but without further information (category III) and 21 products without any tree nut allergen indications (category VI) (Table 1). Raw walnut and almond kernels were used as “positive” controls while peanuts, sesame and hazelnut kernels as negative controls

Table 1. Categorization of samples

	Number of samples	
	walnuts	almonds
Category I: Tree nuts as ingredients	10	5
Category II: May contain traces of walnuts and/or almonds”	15	20
Category III: “May contain traces of nuts”	29	
Category IV: No tree nut allergen indications	21	

2.2 Genomic DNA Extraction and Quantification

NucleoSpin Food kit (Macherey-Nagel, GmbH & Co. KG, Germany) USA) was used for the DNA extraction of all samples. The extraction method was applied according to the manufacturer’s instructions with some modifications. About 100 mg of each sample were used for the extraction, after grinding in liquid nitrogen. The

sample was incubated with the Lysis Buffer and the Proteinase-K overnight at 65°C. After the lysis, the precipitation with absolute Ethanol and the washing steps DNA was eluted duplicate in order to increase the concentration. DNA concentration was determined spectrophotometrically. All samples were tested neat and diluted 10^{-1} in dH_2O.

2.3 Real Time PCR

2.3.1 Real Time PCR Assay for Walnuts

The protocol was an in-house established Real Time-PCR assay (Yano et al., 2007). Real Time PCR targets the Chloroplast maturase *matK* gene amplifying a 120 bp fragment. Reactions were performed in a 25 μL final volume, containing 12.5 μL of Master Mix ((KAPA SYBR GREEN Fast qPCR, KAPA BIOSYSTEMS). 0.9 μM of each primer, and 7.5 μL of eluted DNA to make up for 25 μL. Amplification conditions consisted of a 10 min initial denaturation step at 95°C, followed by 40 cycles of 15 s denaturation at 95°C, 60 s annealing and elongation at 60 °C.

2.3.2 Real Time PCR Assay for Almonds

The protocol was an in-house established Real Time-PCR assay (Koppel et al., 2010). Real Time PCR targets the *Pru av 1* gene amplifying a 129 bp fragment. Reactions were performed in a 25 μL final volume, containing 12.5 μL of Master Mix ((KAPA SYBR GREEN Fast qPCR, KAPA BIOSYSTEMS). 0.9 μM of each primer, and 7.5 μL of eluted DNA to make up for 25 μL. Amplification conditions consisted of a 10 min initial denaturation step at 95°C, followed by 40 cycles of 15 s denaturation at 95°C, 60 s annealing and elongation at 60 °C.

2.3.3 Standard Curves for Real-Time PCR Analysis

Along with the description of subjects, give the mended size of the sample and number of individuals meant to be in each condition if separate conditions were used. State whether the achieved sample differed in known ways from the target population. Conclusions and interpretations should not go beyond what the sample would warrant.

2.3.4 Repeatability and Reproducibility of Assays

For the evaluation of the repeatability and reproducibility of the method, 5 samples at a concentration of 1.5 ng/μL were randomly chosen as PCR templates and amplified in triplicate in an experiment, performed 3 times.

2.4 ELISA Method

A sandwich ELISA was performed using the commercially available Kit VERATOX (NEOGEN) and according to the manufacturer's instructions.

3. Results and Discussion

3.1 Real Time PCR Assay

The specificity of the primer pair was confirmed by Real Time PCR amplification of the peanuts, sesame and hazelnut kernels which were used as negative controls. No amplification signal was observed for any of them even at threshold cycle (CT) values higher than 35. All five levels of positive controls produced fluorescence curves except the blank control. Real Time-PCR runs were acceptable only when the negative control had an undetectable Ct, the KC2=7.0 ng/100 mg of food and KC3=0.7 ng/100 mg had Cts between 25 and 27, and the efficiency of the PCR was 90-100%. The intrinsic detection limit of the improved method was 0.007 ng. The practical detection limit of the improved Real Time PCR was determined by amplifying different-percentage walnut and almond mixtures including 10, 1, 0.5, 0.1, 0.05, 0.01 (wt/wt) and traces of walnut and almond. All samples, even traces, produced a fluorescence curve.

3.2 Repeatability and Reproducibility of Assays

The results showed that the coefficient of variation values for both intra-experimental and inter-experimental data ranged from 0.45 to 0.80% and 0.23 to 0.71% respectively, which is a strong indication of good repeatability and reproducibility.

3.3 Food Testing

By using the RealTime PCR method all 15 products of Category I, containing walnuts or almonds as ingredients were identified. Furthermore, all 15 products declared to contain “traces” of walnuts (Category II) and 13 (65%) of the products declared to contain “traces” of almonds (Category II) were found positive in the tested allergens. In total 28 (80%) of the products of category II were found positive in the tested allergen traces. A significant 27.6% of the products of category III was found positive in the tested tree nut traces (4 for almonds and 4 for

walnuts). Finally, a 33.3% of the products which had no allergen claim was found positive in the tested tree nut traces (2 products for almonds and 5 for walnuts). Analytical sensitivities of the Real Time -PCR assay tested is shown in Table 2.

Table 2. Results of the positive samples in walnuts and almonds for Real Time-PCR assay of the products of Categories III and IV

		Walnuts			Almonds		
Product	Real Time-PCR	No of samples	DNA yield (ng/100 mg food) Mean Value	Ct Mean Value	No of samples	DNA yield (ng/100 mg food) Mean Value	Ct Mean Value
Wafers	Positive	2	0.172	26.12	2	0.122	27.12
Snacks	Positive	7	0.060	28.50	4	0.056	28.58

When ELISA method was applied, again all 15 products of Category I, containing walnuts or almonds as ingredients were identified. The performance of ELISA Method differentiated in the case of Category II where 57.1% of which were found positive in tree nut traces, in particular 10 (66.7 %) of the products with traces indications for walnuts and 10 (50.0 %) of the products with almond traces indications. Positive in the tested tree nuts traces was found the 20.7% of the products of category III but 2 for almonds and 4 for walnuts. Finally, ELISA method detected tree nut traces in 9.5 % of the products of category IV (1 for each tree nut allergen tested). (Table 3)

Table 3. Results of the positive samples in walnuts and almonds for ELISA assay of the products of Categories III and IV

		Walnuts		Almonds	
Product	ELISA	No of samples	Mean Concentration (ppm)	No of samples	Mean Concentration (ppm)
Wafers	Positive	1	41.44	2	53.52
Snacks	Positive	6	49.35	1	58.03

The two methods were equally efficient in detecting walnuts and almonds in the products where these were used as ingredients, proofing their detection ability of the tested allergens. In the case of the products with labelling warmings for traces of a particular allergen, PCR assay was proofed to be more sensitive and identified walnut traces in 33.3% more products than ELISA and also 15% more products with almond traces. For the products of category III Real Time-PCR assay and ELISA the same positive samples in the case of walnuts while when products were tested for almonds Real Time -PCR identified twice as many positive samples as ELISA did. For the last category PCR identified almost 5 times more products that contained walnut traces than ELISA did and almost 2 times more products containing almond traces. Finally, there is an indication that PCR was more sensitive in detecting walnut traces rather than almond ones. (Table 4).

Table 4. Percentage of positive products in walnut and almond traces by Real Time -PCR and Elisa assays per product category

		Positive samples (%)	
		PCR	Elisa
Category I:	walnuts	100	100
	almonds	100	100
Category II:	walnuts	100	66.7
	almonds	65.0	50.0
Category III:	walnuts	13.8	13.8
	almonds	13.8	6.9
Category IV:	walnuts	23.8	4.8
	almonds	9.5	4.8

Brežná et al 2006, referred similar results. It was found that the intrinsic detection limit of their method was 0.24 ng walnut DNA. Using a series of model pastry samples with defined walnut contents, a practical detection

limit of 0.01% walnut content was estimated. Practical applicability of the PCR method was tested by the analysis of 13 food samples (bakery and confectionery products), out of which two cakes (15,4%) were found to contain walnuts although they were not adequately labelled. Also, Costa et al 2013 using Real Time PCR assay found that the relative limit of detection (LOD) for walnuts, varied from 0.005% to 0.001% in both batter and sponge cakes. The absolute LOD was 1 pg for walnut DNA (1.6 DNA copies) in both mixtures, evidencing that the performance of the method was not affected by the heat processing. Finally, Doi et al, 2008 developed a novel sandwich enzyme-linked immunosorbent assay (ELISA) for the detection and quantification of walnut soluble proteins in processed foods. The recovery ranged from 83.4 to 123%, whereas the intra- and inter-assay coefficients of variation were less than 8.8 and 7.2%, respectively similar with our results. This study showed that the proposed method was a reliable tool for detection in the presence of hidden walnut proteins in processed foods.

4. Conclusions

As demonstrated, the presented Real Time- PCR assay is highly sensitive and selective, which makes it suitable for the detection of small amounts of the tested tree nut traces in food samples. More specifically, it has been used for the direct detection of allergenic substances in food, using technologies like SYBR Green, and hydrolysis TaqMan probes. Real Time-PCR is rapid, in addition, no post-PCR processing is necessary, and both amplification and detection are performed in a single closed tube, thus minimizing the risk of carry over or cross-contamination. Quantification is another potential advantage of Real Time-PCR protocols, which nevertheless needs to be further evaluated, in order to reach any definite conclusions regarding the improvement of detection of allergen traces. Moreover, it can also be useful for monitoring the effectiveness of the cleaning processes in the production units of the food industry. On the other hand, ELISA method is less sensitive and more laborious but for the time being is more affordable and easy to use as it is a well-established method. Finally, the importance of proper allergen labelling is underlined since both over precaution and insufficient labelling were found. Over precaution prevents allergic people from consuming safe products while insufficient labelling is exposing them in serious health risks

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Effect of Soy Concentrate, Oat (*Avena sativa*) Flour and Chia Seeds (*Salvia hispanica*) as a Partial Substitute of Wheat Flour (*Triticum aestivum*) on Protein Content, Dietary Fiber Content, Textural Shelf Life and Organoleptic Properties of Breadsticks

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Abstract

Few studies have examined the effects on nutrient contents and organoleptic properties of substituting wheat flour by protein dense ingredients as are soy protein concentrate, oat and chia in bakery formulas. The objective of the study was to assess the effect of adding soy protein concentrate, oat and chia to two breadsticks formulas proposed to provide at least 10 % of the daily recommended value of protein for an adult. Thirty three percentage of wheat flour were substituted by 3:1 oat:chia (BO) and 1:1 oat:chia (BC) composite flours. The analyzed parameters were wheat-meal fermentation time, moisture, protein, insoluble and soluble dietary fiber, firmness, organoleptic acceptance, and preference. Results revealed that both formulas contributed the minimum wished protein content, had higher dietary fiber content than only wheat flour breadsticks and had an acceptable texture, flavor, and appearance. It is recommended to continue formula BO because it turned out to be the favorite in the preference test.

Keywords: soy protein concentrate, oat, chia, protein content, dietary fiber, breadstick

1. Introduction

In recent years obesity, diabetes, and other chronic diseases have been increasing among the poor population of Mexico, this is partially due to an important growth in sugar consumption, refined carbohydrates and fat, most likely found in higher concentrations in cheap convenience foods. On the other hand, protein intake has declined in all regions (Rivera et al., 2002), especially in those with less economic resources.

There are many definitions of functional foods, but this category encompasses potentially helpful products including any modified food or food ingredients that may provide a health benefit beyond that of the traditional nutrient it contains. In this respect, the addition of dietary fiber at a determined concentration in a food product could turn it into a functional food since there is a growing number of studies proving the beneficial effects of dietary fiber consumption. It has been found that this type of fiber may participate in the regulation of the gastrointestinal motility, influence glucose, and lipid metabolism, promote faecal output, stimulate bacterial metabolic activity and contribute towards maintaining the equilibrium of the colon ecosystem and integrity of intestinal mucosa (Gibson & Williams, 2001).

Oat (*Avena sativa*) differentiates itself from other cereals because it contains β-glucan, a type of dietary fiber, and it contains the best amino acid composition profile among all the cereal grains in addition to overall high protein content (Salehifar & Shahedi, 2007). In the same way, chia (*Salvia hispanica L*) is a seed that has been gaining attention for its high content of soluble and insoluble dietary fiber, and its high protein content which also has a complete amino acid profile (Inglett, Chen, Liu & Lee, 2014). Coorey, Tjoe and Jayasena (2014) studied the effect of chia in chips and observed that until 10% generated a good quality dough with important effects on protein content, dietary fiber and calcium content. Likewise, soy (*Glycine max*) protein is well known for supplying all nine essential amino acids, being easily digested by humans, and equaling the protein quality of

milk, meat, and eggs (Singh, Kumar, Sabapathy & Bawa, 2008).

Bearing all this in mind, it can be concluded that there is the opportunity to develop new affordable snacks that fight hunger deliciously and conveniently, but that act as functional foods by providing less refined carbohydrates, substituting them with dietary fiber, and provide more protein to the diet of the Mexican population.

Thus, the objective of the study was to evaluate the effect of incorporating soy concentrate, oat, and chia in breadsticks formulation that could serve as healthy snacks by providing 10% of the daily recommended protein consumption value for an adult with an average weight of 70 kg (5.6 g of proteins) per serving size (60 g) (Committee on Dietary Reference Intakes, 2005). The present study pretends to reach that protein while keeping texture, flavor, and appearance considered as acceptable by potential consumers.

2. Materials and Methods

2.1 Materials

Soy protein concentrate 65%, was given by Nutrigrains®. The other ingredients were obtained at the local market: Medium strength commercial soft and white wheat flour (Selecta®, Guadalupe, N.L., Mexico), oat flakes (Granvita®, Zapopan, Jal., Mexico), oat flour (Granvita®, Zapopan, Jal., Mexico), chia (O3 Chía Premium®, Guadalajara, Jal., Mexico), jalapeño pepper (San Ambrosio®, Ciudad Mante, Tamps., Mexico), sun dried tomatoes in olive oil (Bella Sun Luci®, Chico, CA), salt (La Fina®, Ciudad de Mexico, D.F., Mexico), honey (Vitareal®, Azcapotzalco, D.F., Mexico), olive oil (Carbonell®, Madrid, España), skimmed milk (Alpura Light®, Cuautitlán Izcalli, Edo. Mex., Mexico), calcium propionate (Alkem, Monterrey, N.L., Mexico), instant yeast (Tradi-Pan®, Toluca, Edo. Mex., Mexico), and dried basil (McCormick®, Mexico City, D.F., Mexico).

2.2 Methods

2.2.1 Breadsticks Preparation

Breadsticks were prepared as recommended by Atkinson (2004). The formulations were three, control and two treatments, the first substituting white wheat flour in 35% by oat and chia in 3:1, respectively (BO), and the second treatment substituting white wheat flour in 35% by oat and chia in 1:1 (BC) (Table 1). For the treatments, jalapeño peppers and sun-dried tomatoes were previously chopped with a kitchen knife in tiny pieces. Likewise, oat was slightly minced in a food processor (Traditions Mod.72588R, China) for 40 s or until getting small pieces. All the ingredients for the control were mixed in a Hobart mixer (Hobart, Mod. 8-120T, Troy, OH) at low speed (60 rpm) for 3.5 min. A similar mixing procedure was used for both treatments, except that all the ingredients (excluding jalapeño pepper, sun-dried tomato and dried basil) were mixed at low speed in the same Hobart mixer for 2.5 min. Then, jalapeño pepper, sun-dried tomato and dried basil were added to the mixer and the dough was mixed at low speed for 1 min more.

Each dough was formed into a ball and placed in a lightly oiled bowl. It was covered with plastic wrap and left to rise for 15 min at room temperature and 85% relative humidity (RH) in a fermentation cabinet (National MFG. Co. Lincoln, NE). After that, the dough was stretched and thinned with a roller pin until reaching a thickness around 1.0 cm and cut with a kitchen knife in 1.3 cm x 28.0 cm pieces. Then, each piece was slightly twisted and placed on a pan covered with a wax baking sheet. Pans were introduced to the fermentation cabinet, and pieces were left to rise for another 10 min at room temperature and 85% relative humidity (RH). Immediately, the pans were taken to a convection oven (Electrolux, Stockholm, Sweden) and baked for 20 min and 150°C. Finally, breadsticks were left to cool down for about an hour or until reaching room temperature and packaged in cellophane bags.

2.2.2 Flour Mixes Properties Measurement

Flour mixes (including white wheat flour, minced oat, oat flour and chia) were evaluated using Pelshenke assay as determined by American Association of Cereal Chemists (AACC, 2000, Method 56-50.01, 7).

2.2.3 Chemical Composition of Breadsticks

Moisture content (n = 3 per treatment) was analyzed as determined by AACC (2000, Method 44-15.02, 8). Crude protein (n = 3 per treatment) was analyzed by the Kjeldahl test as determined by AACC (1999, Method 46-12.01, 9). Total dietary insoluble and soluble fibers (n = 3 per treatment) were determined using the commercial kit Total Dietary Fiber provided by Megazyme®, following approved methods 32-45-01 (2000) and 32-50.01 (2010) of the AACC.

Table 1. Formulations employed to produce control 100% white wheat flour (Control), 65% white wheat flour and 35% oat-chia substitution (3:1) (BO), and 65% white wheat flour and 35% oat-chia substitution (1:1) (BC) breadsticks

Ingredients	Control	BO	BC
Wheat flour	100	65.65	65.65
Oat Flake	-	10.10	4.04
Soy concentrate	-	10.10	10.10
Oat flour	-	8.08	8.08
Chia	-	6.06	12.12
Jalapeño pepper	6.94	6.94	6.94
Sun dried tomato	6.24	6.24	6.24
Salt	0.51	0.51	0.51
Honey	1.05	1.05	1.05
Olive oil	1.05	1.05	1.05
Fluid skim milk	-	1.00	1.00-
Calcium propionate	0.75	0.75	0.75
Instant yeast	0.51	0.51	0.51
Dried basil	0.21	0.21	0.21

2.2.4 Measurement of Firmness during Shelf Life

Firmness of breadsticks was measured after baking at days 0, 1 and 8 using TA.XT plus texture analyzer (Stable Micro Systems Ltd, Surry GU7 1JG, England) and a modified version of the American Institute of Baking (AIB) Standard Procedure for White Pan Bread Firmness Measurement (AIB, 2015). Firmness was measured on five breadsticks per treatment. The measures were taken on the widest parts of the breadsticks not containing blisters. A texture profile analysis (compression test) was executed with a flat-ended cylindrical probe, a 5 mm distance, and pre-test, test and post-test speeds of 10 mm/s, 1 mm/s, and 10 mm/s, respectively.

2.2.5 Organoleptic Evaluation

Two organoleptic tests were applied the same day that breadsticks were baked, the first test evaluated the acceptance of the breadsticks' attributes, while the second was a preference test. Both organoleptic tests were applied at room temperature to the same 30 bread consumers (18 women and 12 men) whose ages were between 18 and 35 years old. In the acceptance test, consumers assigned a value from 1 (dislike extremely) to 7 (like extremely) for each one of the following attributes: texture, crunchiness, flavor, and appearance. In the preference test, consumers chose their favorite formulation (Control, BO or BC).

2.2.6 Statistical Analysis

It was used to analyze the characteristics of the breadsticks with oat and chia concentration as the independent variables, and the protein content and firmness, after 0, 1 and 8 days of storage as the dependent variables. The JMP 5 software (SAS, SAS Campus Drive Building T Cary, NC) was used for the analysis of variance (ANOVA) at a constant significant effect ($p < 0.05$). Differences in the acceptability of the treatments' attributes caused by the different oat and chia concentrations were assessed by ANOVA using the same statistical program. Preference differences were evaluated by a binomial test carried out in Microsoft Excel 2013 software (Microsoft Corporation, Redmond, WA). Both organoleptic tests had as null hypothesis that there was no difference in the acceptance and preference of the treatments, while the alternative was that a difference does exist in their acceptance and preference.

3. Results and Discussion

3.1 Characterization of Flour Mixes

The Pelshenke values decreased as the wheat flour concentration was reduced and the content of chia was increased. The Pelshenke values for control, BO, and BC were 50±6.6 min, 45.7±4.5 min, and 23.7±6.5 min, respectively. The Pelshenke test is a simple fermentation-based assay that measures gluten quality (Serna-Saldívar, 2013) since part of the wheat flour was replaced for more water-binding substances in the treatments it could be expected that their Pelshenke values would be lower. Fibers and proteins are water-binding substances present in higher proportion in some ingredients such as soy concentrate, oat (flour and grain) and chia. Comparing the control against the treatments, control has a higher Pelshenke value than the treatments. This is due to the partial replacement of wheat flour with soy concentrate, oat, and chia. Soy protein concentrates

contain polysaccharides that absorb a significant amount of water. Adding soy proteins, either in the form of flour or concentrate, to wheat flour dilutes the gluten proteins and starch while exhibiting a strong water-binding power that provides some resistance to dough expansion (Jideani, 2018).

Likewise, the higher Pelshenke value for BC in comparison with BO is due to a higher content of chia instead of oat. De-husked oat meal contains a considerable amount of crude fiber (2.1% dry weight basis) in comparison with wheat flour (0.4% dry weight basis) (Chang & Sosulski, 1985), but chia has even a bigger content of crude fiber (25.55% dry weight basis) (Coorey et al., 2014) which translates into a higher water binding capacity, and thus a faster precipitation of the dough ball in the Pelshenke test. This have effect on water absortion of mixes and control absorbed 22.66% (14% basis) until BO and BC absobed 29.30 and 29.01%, respectively.

3.2 Composition

3.2.1 Moisture

Moisture content is higher in treatments in comparison with control (Table 2) as it was expected since, as discussed above, soy concentrate, oat and chia act as water-binders that reduce the water released during baking. Numerous authors report an increase in moisture content in baked goods that include soy proteins (Mishra & Chandra, 2012; Zhang, Albrecht, Bomser, Schwartz & Vodovotz, 2003) oat products (Kurek, Wyrwisz, Piwińska & Wierzbicka, 2016; Lee & Inglett, 2006), and chia seeds (Rendón-Villalobos, Ortíz-Sánchez, Solorza-Feria & Trujillo-Hernández, 2012; Silveira Coelho & Salas-Mellado, 2015).

3.2.2 Protein

Protein content was also significantly higher ($p<0.05$) in treatments than in control due to the addition of soy concentrate, oat, chia and skim milk (Table 2). Protein content increased from 5.80% in control to 9.73% and 10.4%, in BO and BC treatments, respectively. According to Ndife, Abdulraheem and Zakari (2011) substituting 7% of the whole wheat flour with soy flour (39.4% protein) resulted in a bread with 9.44% protein. Addition of soybean protein to cereal products could not only be effective to increase their protein content but also their protein quality since it contains a considerable concentration of lysine, an essential amino acid missing in cereals (Singh et al., 2008). Oat differentiates itself from other cereals by its high relative content of lysine, according to Chang and Sosulski (1985) meal from domestic groats contains 15.3% protein and 4.1 g of lysine per 100 g of groats. Likewise, chia is a seed that characterizes for being a good source of protein, since its protein content is higher than that of other traditional crops such as wheat and corn, and its amino acid profile has no limiting factors in the adult diet (Ayerza & Coates, 2011).

Table 2. Moisture, protein and dietary fiber composition (%) of 100% white wheat flour (Control), 65% white wheat flour and 35% oat-chia substitution (3:1) (BO), (3) 65% white wheat flour and 35% oat-chia substitution (1:1) (BC), dry basis breadsticks

Treatment	Moisture[2]	Protein[1,3]	Total Dietary Fiber[1]	Insoluble Dietary Fiber[1]	Soluble Dietary Fiber[1]
Control	5.33 ± 0.01^{c}	5.80 ± 0.50^{b}	3.99	2.56 ± 0.05^{c}	1.43 ± 0.17^{c}
BO	9.00 ± 0.01^{b}	9.73 ± 0.52^{a}	7.39	3.04 ± 0.04^{b}	4.35 ± 0.05^{b}
BC	9.70 ± 0.05^{a}	10.4 ± 0.51^{a}	12.50	5.92 ± 0.07^{a}	6.58 ± 0.07^{a}

Means with different letters in each column are statistically different ($p < 0.05$). Each value is the average and standard deviation of three observations.

[1] Values provided in dry weight basis.

[2] Moisture taken on storage day 0.

[3] N*6.5.

3.2.3 Insoluble and Soluble Dietary Fiber

Incorporating soy concentrate, oat and chia in the treatments increased ($p<0.05$) their insoluble and soluble dietary fiber content (Table 2) in comparison with control as it was expected, especially for BC since it includes the highest chia concentration. According to Bednar, Patil, Murray, Grieshop, Merchen and Fahey (2001) soy flour contains 15.4 g/100 g of total dietary fiber (TDF), 14.7 g are insoluble dietary fiber (IDF), and 0.7 g are soluble dietary fiber (SDF), it could be expected that TDF for soy concentrates would be lower since in its production process carbohydrates, mainly sugars and soluble carbohydrate material, are extracted from defatted soy flour in order to increase its protein content (Jideani, 2018). In the same way, oat has a TDF content of 37.7

g/100 g, 33.9 g are IDF and 3.8 are SDF (Bednar et al., 2001). Likewise, Vázquez -Ovando, Rosado-Rubio, Chel-Guerrero and Betancur-Ancona (2009) reported that defatted chia flour obtained by dry processing contains 56.46 g/100 g of TDF, most of its content represented by 53.45 g of IDF and 3.01 g of SDF. Results presented in Table 2 show that SDF content was higher than IDF in both treatments, obtaining 4.35 g and 6.58 g of SDF for BO and BC, respectively. Several authors have reported an increase in the TDF content of baked goods by incorporating oat products (Kurek et al., 2016; Gularte, de la Hera, Gómez & Rosell, 2012) and chia seeds (Rendón-Villalobos et al., 2012; Silveira Coelho & Salas-Mellado, 2015).

3.3 Firmness

Table 3 shows the values for breadstick firmness on storage days 0, 1 and 8. On day 0 there were not a significant difference in firmness among control, BO, and BC ($p>0.05$). On day 1 a significant difference in firmness ($p<0.05$) is detected among control, BO and BC, being this last the one with the highest firmness and BO the treatment with less firmness. This trend continued on day 8, in which the difference ($p<0.05$) among treatments increases especially for BC.

Table 3. Firmness (N) of breadsticks 100% white wheat flour (Control), (2) 65% white wheat flour and 35% oat-chia substitution (3:1) (BO), (3) 65% white wheat flour and 35% oat-chia substitution (1:1) (BC) stored at room temperature for 8 days. Values correspond to mean ± standard deviation

	Day 0	**Day 1**	**Day 8**
Control	5.44 ± 0.68^{a}	$5.50 \pm 0.58^{a,b}$	5.44 ± 0.68^{b}
BO	2.16 ± 0.24^{b}	4.22 ± 0.79^{b}	4.24 ± 1.11^{b}
BC	2.16 ± 0.40^{b}	6.77 ± 0.88^{a}	10.35 ± 0.82^{a}

Means with different letters in each column are statistically different ($p < 0.05$). Each value is the average and standard deviation of three observations.

Less firmness or greater softness of treatments in comparison with control on day 0 is due to the addition of soy concentrate, oat, and chia. As previously discussed, the higher protein and dietary fiber content of BO and BC contributed to a stronger water binding capacity that trapped more moisture inside the product. BO kept a relative low firmness through time due to its higher content of oat; it has been reported that oat starch has a higher water absorption than other cereals, thus keeping bread fresher for longer periods of time (Salehifar & Shahedi, 2007). Also, oat has a high content of β-glucan, a hydrocolloid that at certain concentrations (around 1% flour basis) prevents changes in the water activity (aw) values of the crumb, a reduction in aw values is associated with an increase in firmness of crumb (Lazaridou, Duta, Papageorgiou, Belc & Biliaderis, 2007).

On the other hand, surprisingly BC firmness increased through time, even though in the formulation chia seeds replaced starch, main component that causes staling of bakery products through retrogradation, probably this ingredient interfered with the size and distribution of the air cells within the breadsticks structure, a similar result was reported by Luna Pizarro, Lopes Almeida, Sammán and Chang (2013).

3.4 Organoleptic Evaluation

3.4.1 Acceptance

In this study, attributes acceptance of products after tasting them was evaluated using a seven-point hedonic scale. Results of organoleptic evaluation (Figure 1) for overall texture acceptance show that control, BO, and BC were accepted since all of them were given a score above 4.0. Also, they reveal that there is a significant difference ($p<0.05$) in this attribute among treatments being control (6.27) the most accepted, followed by BC (5.83) and BO (5.50). A decrease in texture acceptance because of increasing the substitution levels of wheat flour with buckwheat-chia flours has been reported by Divyashree, Ashwath Kumar, Sharma, Semwal and Umesha (2016).

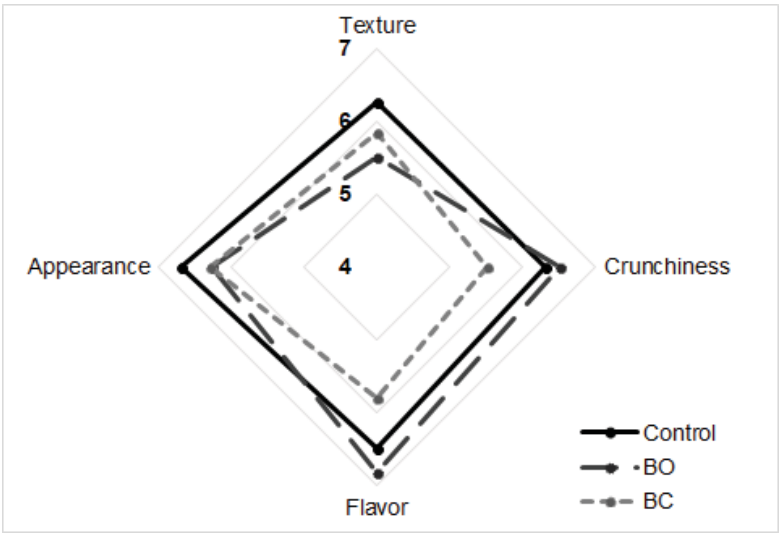

Figure 1. Comparison of organoleptic acceptance of breaksticks made with 100% white wheat flour (Control), (2) 65% white wheat flour and 35% oat-chia substitution (3:1) (BO), (3) 65% white wheat flour and 35% oat-chia substitution (1:1) (BC)

Results for crunchiness (Figure 1) indicate that all the treatments had an acceptable crunchiness, but that there is a significant difference ($p<0.05$) among them. BO got the highest score (6.5) for crunchiness acceptance, control obtained the second-best score (6.3), and finally, BC was evaluated with the lowest score (5.5). Comparing these results with overall texture acceptance, it's concluded that crunchiness didn't play an important role in texture acceptance. Probably other texture attributes such as firmness, cohesiveness or grainy are more decisive on consumers texture acceptance. Flavor acceptance values (Figure 1) reveal that consumers accepted control, BO, and BC and that there is a significant difference for this attribute ($p<0.05$). According to consumers, BO had a slightly most acceptable flavor (6.83), followed by control (6.5), and lastly by BC (5.80). No adverse effect on flavor by incorporating oat flour at levels of 10% and 20% in bread has been reported by Salehifar and Shahedi (2007). Likewise, lower scores in flavor acceptance of bread incorporating whole chia flour in comparison with only wheat flour bread have been reported by Luna Pizarro et al. (2013). Scores for appearance acceptance (Figure 1) indicate that the consumers accepted all the breadsticks, and that there is not a noticeable difference ($p>0.05$) in their appearance. Results obtained for this attribute were 6.70, 6.30 and 6.27 for control, BC, and BO, respectively.

3.4.2 Preference

Organoleptic evaluation revealed a statistical preference of breadsticks prepared with oat-chia substitution 3:1 (70%), over control (17%) and breadsticks including oat-chia substitution 1:1 (13%) (Figure 2).

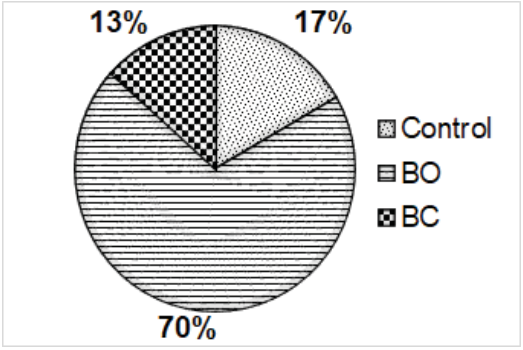

Figure 2. Preference results (%) for 100% white wheat flour (Control), 65% white wheat flour and 35% oat-chia substitution (3:1) (BO) and 65% white wheat flour and 35% oat-chia substitution (1:1) (BC) breadsticks

4. Conclusion

Results of this study indicate that substitution of white wheat flour with oat-chia blend and soy protein

concentrated improve protein content by 67.75%. Breadstick with 10% soy concentrate, 6% chia seed, and 18% oat improve total dietary fiber content by 85.21%, interestingly soluble dietary fiber was improved twice. The breadstick produced with this grains blend has the highest preference, even higher than 100% white wheat flour breadstick. According to the results obtained, it is recommended to follow up with BO since it remained softer longer, it received the best score in flavor and crunchiness acceptance, and was preferred over BC. Further studies are needed to determine fat content. The evaluated breadsticks could serve as the base for the development of functional breadsticks, or other baked goods, that could help to diminish the increasing incidence of chronic diseases in Mexico.

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Disclosure statement

The authors declare no conflict of interest.

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Development of Probiotic "*creamy requeijão"* Formulations Containing *Lactobacillus* Strains

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Abstract

Functional foods, with emphasis on probiotics, are products that, besides possessing adequate nutritional value, stimulate physiological and metabolic activities in the body. Thus, these benefits combined with greater awareness of the population in the search for food reeducation boost the consumption of this kind of food. "*Creamy requeijão"* is a dairy derivative that has a matrix with physicochemical characteristics suitable for its use as a potential carrier of probiotic microorganisms. Thus, this study aimed to the evaluation of probiotic "*creamy requeijão"* formulations containing, individually six *Lactobacillus* strains, as follows: *L. plantarum* ATCC 8014, *L. acidophilus* ATCC 4356, *L. delbrueckii* UFV H2B20, *L. fermentum* ATCC 9338, *L. casei* ATCC 7469 and *L. paracasei* SP11. The results revealed that during the storage of the formulations for a period of 65 days at 5 °C the cells remained viable at levels above 10^8 CFU.g $^{-1}$, which allows to classify these formulations as functional foods. In addition, the consumption of only 1 g per day of this food would be enough to attend the requirements of Brazilian legislation regarding the consumption of food with probiotic claims.

Keywords: functional foods, lactic acid bacteria, dairy products

1. Introduction

Probiotics are defined as "living microorganisms which, when consumed in adequate amounts, confer health benefits to the host by modulating the microbiota and preventing infection" (FAO, 2002). Besides promoting the maintenance of a healthy gastrointestinal tract microbiota, they confer resistance to colonization by pathogens, as well as help in the lactose digestion in intolerant individuals and promote activation of the immune system (Kechagia *et al.*, 2013; Kerry *et al.*, 2018). Dairy products are the main vehicles of probiotic microorganisms, being the pioneers in this category (Sanchez et al., 2009). The global market for probiotics was valued at $ 42.66 billion in 2016 and some projections indicate that this sector could reach $ 64 billion in 2022 (*www.marketsandmarkets.com*).

Several functional foods can be found currently in the market. Fermented milks and yogurts are the most traditional, but the development of probiotic cheeses have been highlighted, because it is a matrix that presents higher fat content and higher pH. These characteristics favor the maintenance of microorganism integrity during food storage and in its passage through the gastrointestinal tract (Stanton *et al.*, 1998; Kechagia *et al.*, 2013). In this context, "*creamy requeijão"*, which is a typical Brazilian dairy product accepted by the population, has not been explored yet as a vehicle for probiotic microorganisms. According to the Pattern of Identity and Quality of "c*reamy requeijão"* (PIQ), this food must present at least 55% of fat in the dry extract and the maximum of 65% of moisture (Brazil, 1997).

The probiotics microorganisms commonly found in commercial formulations comprise lactic acid species belonging to the genera *Lactobacillus, Bifidobacterium, Streptococcus and Enterococcus*, as well as yeasts such as *Saccharomyces boulardii* (Kerry *et al.*, 2018). In this scenario, the use of *Lactobacillus* species in the human diet is relevant since the beginning of the 20th century, being considered a promising group of microorganisms for the formulation of probiotic foods (Stiles & Holzapfel 1997; Singh *et al.,* 2011).

The National Agency of Sanitary Vigilance in Brazil recommends that, in order to ensure the beneficial effect of probiotics, a minimum of viable cells of these microorganisms should be consumed in the range of 10^8 to 10^9

CFU.g $^{-1}$ per day (Brazil, 2008). On the other hand, the International Association of Probiotics (2000) recommends that daily intake of these functional foods should be equal to or greater than 10 ^{7} CFU.g $^{-1}$. However, the Brazilian Normative Instruction No. 28/2018 does not establish maximum or minimum levels regarding the concentration of viable cells per gram in this sort of products (Brazil, 2018).

Studies involving the incorporation of probiotic microorganisms into different food categories have been developed with emphasis on goat milk ice cream supplemented with *L. acidophilus,* and *B. lactis* (Akalin *et al.*, 2018), chocolates containing *L. paracasei* and *L. acidophilus* containing prebiotic carbohidrate such as inulin (Konar *et al.*, 2018); *Minas cheese* containing *L. acidophilus* LA 14,,and *B. longum* BL 05 (Lollo *et al.* , 2015) and cream cheese containing *B. animalis* subsp. lactis DSM 10140, and *L. reuteri* DSM 20016 (Speranza *et al.*, 2017). In these studies, the authors considered the mentioned foods as suitable matrices for incorporation and maintenance of probiotic species.

Considering the characteristics of "*creamy requeijão"* as a vehicle for probiotic microorganisms, based on the consumer's acceptance of this product and the consumer interest regarding functional foods, the present study aimed to evaluate the development of a probiotic "*creamy requeijão"* formulation containing different *Lactobacillus* species, considering the cell maintenance when the product was stored for 65 days / 5 ° C, as well as its physicochemical composition.

2. Material and Methods

2.1 Micro-organisms and Inoculum Preparation

Six *Lactobacillus* strains were evaluated, consisting of: *L. plantarum* ATCC 8014, *L.acidophilus* ATCC 4356, *L. delbrueckii* UFV H2B20, *L. fermentum* ATCC 9338, *L. casei* ATCC 7469 and *L. paracasei* SP11 (Nestlé), maintained at -20 ° C in glycerol 20% (v/v). The activation of these strains was performed by inoculating 2 mL of the stock cultures in 198 ml of Man Rogosa & Sharpe - MRS medium (De Man, Rogosa & Sharpe, 1960) previously sterilized, followed by incubation for 24 h at 37 ° C. Subsequently, successive replications were performed in order to obtain a higher inoculum volume, followed by concentration of the cells so that a high amount of *Lactobacillus* could be reached in a small volume of inoculum.The cells were then suspended in 50 ml of reconstituted skim milk (10%) and transferred to two sterile Erlenmeyer flasks, followed by incubation at 37 ° C for 2 h, following methodology proposed by Drunkler (2009), with adaptations.

2.2 Preparation of Probiotic "creamy requeijão" Formulations

The probiotic "*creamy requeijão"* was prepared by taking 9 L of pasteurized milk (3% fat) heated to 82 ° C and added with 85% food grade lactic acid (Purac), diluted (1:10), with slow stirring until coagulation was observed. Afterwards, the clotted milk was cooled at room temperature (23 ± 2 ° C), and the curd was separated and pressed in a properly hygienized nylon sieve. Butter cream was then added to this curd, in amount equivalent to 50% of its weight, followed by manual homogenization and heating to 85 ° C. After, sodium chloride (1.3% w/w) sodium citrate and polyphosphates (1% (w/w), diluted in water at 80ºC, were added, followed by homogenization for approximately 5 min and cooling at 50°C.

The obtained product was then characterized regarding its physicochemical properties, and a fraction of it was used to incorporate the *Lactobacillus* strains, and then evaluated regarding its microbiological aspects, and cell viability.

One portion of the product (control) was packed while still hot in a properly sterilized polypropylene pot, sealed with PVC film (Wyda Pratic), and stored at 5 ± 1 ° C. The remaining product was cooled to 50 °C and portioned in 6 fractions. Each fraction was inoculated, individually, with *Lactobacillus* strains according to methodology described by Drunkler (2009), in order to get an initial cell concentration of approximately 10^{10} to 10^{13} CFU.g $^{-1}$ followed by cooling and stored at 5 ± 1 ° C. Samples of each one were collected after 7, 30, 45 and 65 days for characterization.

2.3 Analytical Methods

2.3.1 Determination of Cellular Viability

The population of viable cells (CFU.g $^{-1}$) in the samples of potential probiotic "*creamy requeijão",* was determined by pour plate method, as described by Silva, Junqueira & Silveira (2001).

2.3.2 Determination of pH, Titratable Acidity, and Lipid Content

The pH was determined by the electroanalytical method in digital pH meter (GEHAKA® PG1800) employing 2 g of sample at room temperature (23,0 ± 2,0 ° C) diluted in 20 mL of water. The titratable acidity, expressed as lactic acid (%), and the lipid content was determined according to Zenebon *et al* (2008).

2.3.3 Determination of Total Dry Extract (TDE) and Fat Content in Dry Extract (FDE)

The TDE and FDE content in the "*creamy requeijão"* was determined by drying 3 g of the samples in a microwave oven (Brastemp mod. BMP 40E SAAB), according to the methodology described by Van Dender (2000).

2.4 Microbiological Analyzes

Microbiological characterization of the samples was performed, in relation to the presence of total and thermotolerant coliforms, *Staphylococcus spp* and *Salmonella,* according to Silva (2001).

3. Results and Discussion

3.1 Characterization of "cream requeijão" Regarding the Cellular Viability

The results showed in Figure 1 demonstrate that the populations of the strains *L. plantarum* ATCC 8014, *L. delbrueckii* UFV H2B20 and *L. fermentum* ATCC 9338 decreased 3,0 log cycles when the respective formulations were stored at 5 ° C for 65 days. On the other hand, formulations containing the strains *L. casei* ATCC 7469, *L. paracasei* SP11 and *L. acidophilus* ATCC4356 showed a decrease of only one cycle under the same conditions.

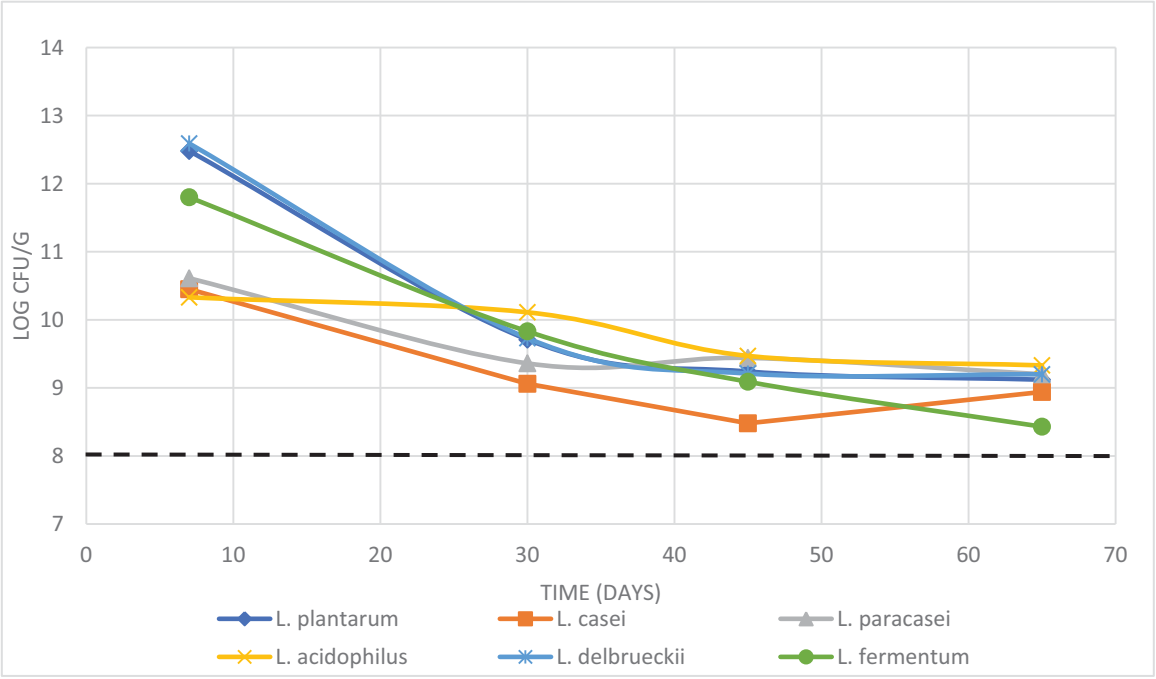

Figure 1. Profile of the *Lactobacillus* strains viability in "*creamy requeijão"* storaged for 65 days at 5 ° C. Dashed line corresponds to the minimum amount of probiotic cells to be consumed according to Brazilian law

According to Saad, Cruz & Faria (2011) there are factors that may interfere in the survival of probiotic strains, incluiding the quality of the raw material (considering that milk may contain residues of antibiotics and pesticides and its pH, lipid and salt content), as well as the strain used and storage conditions, such as temperature and oxygen level in the medium.

The results obtained in the present study are similar to those reported by Speranza *et al* (2017) in their research with cream cheese formulations supplemented with *Bifidobacterium animalis* subsp. y*actis* DSM 10140 and *L. reuteri* DSM 20016, stored for 28 days at 4ºC. These results demonstrate the potential of dairy products as a matrix for carrying probiotic microorganisms.

On the other hand, lower results were reported by Konar *et al* (2018) studying sugarless white chocolate as a matrix, inoculated with a *L. paracasei* strain, showing that the initial cell concentration was 10^9 CFU.g^{-1}, and after 60 days storage at room temperature, the population decreased to 10^6 CFU.g^{-1}. The authors pointed out that this result should be due to the low water activity in the matrix studied. Similarly, Teixeira (2012), studying "*creamy requeijão"* containing *L. acidophilus* and *B. bifidum*, reported that these strains were maintained at

stable viability levels after storage for 15 days at 10ºC. After this period there was a decrease in the *Lactobacillus* population, equivalent to two log cycles, reaching a cell population of 4.0 x10 ^{7} CFU.g $^{-1}$, and 2.0 x10 ^{8} CFU.g $^{-1}$ of B. *bifidum*. The incorporation of *Bifidobacterium animalis* subsp. *Lactis* Bb-12 and prebiotic compounds (inulin and oligofructose) in "*creamy requeijão"* formulations were also evaluated by Drunkler, (2009), and the results showed that the cells remained viable at about10^{6} CFU.g $^{-1}$ for 60 days storage.

However, regarding the daily intake of probiotic foods, it should be emphasized that is necessary to consume 10^{6-7} CFU. g^{-1}, which is contained in 100 g of the product, according to the National Agency of Sanitary Vigilance recommendation (Brazil, 2008). Therefore, it becomes unfeasible, considering that the average *"creamy requeijão"* consumption by the population is 30g per day. In this context, it is worth mentioning that in only 1 g of the different formulations evaluated in the present work was verified a viable cell population greater than 10 ^{8} CFU after 65 days of storage at 5 ° C. This result demonstrates the technical feasibility of using "*creamy requeijão"* as a vehicle of the probiotic strains.

"Creamy requeijão" is a product of high acceptance in the different social classes in Brazil, so it can easily be inserted into the daily diet of the population, with an additional benefit of having the probiotics in its formulation. Therefore, it can contribute to the gastrointestinal health of consumers, and presents potential to be marketed, due to the benefits on daily consumption of functional foods.

3.2 Phisicochemical Characterizations of the "creamy requeijão" Formulations

As showed in Table 1, the pH values in the probiotic "*creamy requeijão"* (6.20 ± 0.10 to 6.70 ± 0.13) were lower than the control formulation (6.44 ± 0.17 to 6.73 ± 0.18), after the storage for 65 days at 5 ° C.

Table 1. pH values and standard deviation of the probiotic "*creamy requeijão"* formulations evaluated during 65 days at 5 ° C

	Formulations						
Time (days)	**Control**	**LP**	**LC**	**LI**	**LA**	**LD**	**LF**
7	6.65±0.18	6.40±0.16	6.44±0.19	6.40±0.16	6.41±0.17	6.20±0.10	6.22±0.19
30	6.73±0.18	6.49±0.17	6.30±0.17	6.42±0.18	6.39±0.15	6.22±0.15	6.40±0.19
45	6.44±0.17	6.51±0.19	6.39±0.10	6.70±0.13	6.46±0.18	6.32±0.16	6.52±0.17
65	6.51±0.19	6.58±0.13	6.44±0.12	6.69±0.15	6.54±0.11	6.41±0.19	6.69±0.14

LP = Lactobacillus plantarum ATCC 8014; LC= Lactobacillus casei ATCC 7469; LI= Lactobacillus paracasei SP11; LA= Lactobacillus acidophilus ATCC 4356; LD= Lactobacillus delbrueckii UFV H2B20; LF= Lactobacillus fermentum ATCC 9338.

Drunkler (2009) reported that the pH decreased was not significantly altered (p> 0.05) in symbiotic "*creamy requeijão"* formulations in relation to control formulation after 60 days of storage at 5ºC. This observation was also verified in the present work, where the pH values after 65 days of the control formulation (6.51 ± 0.19) and the respective probiotic formulations remained between 6.41 ± 0.19 to 6.69 ± 0.15.

The results in Table 2 show that the lactic acid content was higher in the formulations containing the *Lactobacillus* strains, ranging from 0.43% to 0.30% after 7 days at 5 ° C and 0.28% at 0.24% after 65 days in relation to the control formulation (0.17% after 7 days at 5 ° C and 0.12% after 65 days). This observation might be due to the production of lactic acid that occurred during the strains preactivation step in reconstituted milk at 10% at 37 ° C for 2 h, prior to the inoculation in the curd.

Table 2. Values of lactic acid contents and standard deviation in the different probiotic "creamy requeijão" formulations stored for 65 days at 5 ° C

	Formulations						
Time (days)	**Control**	**LP**	**LC**	**LI**	**LA**	**LD**	**LF**
7	0.17±0.07	0.35±0.08	0.30±0.09	0.38±0.08	0.30±0.08	0.43±0.09	0.43±0.09
30	0.12±0.09	0.23±0.09	0.30±0.09	0.32±0.09	0.29±0.07	0.23±0.08	0.27±0.09
45	0.14±0.09	0.26±0.08	0.29±0.08	0.33±0.09	0.29±0.09	0.26±0.09	0.28±0.08
65	0.12±0.08	0.25±0.09	0.28±0.09	0.24±0.08	0.28±0.09	0.28±0.09	0.24±0.09

LP = *Lactobacillus plantarum* ATCC 8014; LC= *Lactobacillus casei* ATCC 7469; LI= *Lactobacillus paracasei* SP11; LA= *Lactobacillus acidophilus* ATCC 4356; LD= *Lactobacillus delbrueckii* UFV H2B20; LF= *Lactobacillus fermentum* ATCC 9338.

3.4 Moisture, Total Dry Extract and Fat in Dry Extract in Probiotic "cream requeijão"

The results shown in Table 3 demonstrate that the content of fat in the dry extract, and the humidity of the "cream requeijão" formulations attend the minimum standard described in the Technical Regulation - Administrative Rule 359 (Brazil, 1997). These values consist of fat content in the dry extract (FDE) should be higher than 55% and the maximum Total Dry Extract (TDE) content of 65%.

Table 3. Physicochemical characteristics of probiotic "*creamy requeijão"* formulations after 65 days at 5 ° C

Formulation	Moisture (%w/w)	TDE (%w/w)	FDE (%w/w)
Control	60.00	40.00	67.22
***L. acidophilus* ATCC 4356**	62.58	37.42	65.52
***L. plantarum* ATCC 8014**	62.00	38.00	63.81
***L. paracasei* SP11**	64.15	35.85	65.52
***L. casei* ATCC 7469**	64.95	35.05	66.30
***L. delbrueckii* UFV H2B20**	64.03	35.97	66.49
***L. fermentum* ATCC 9338**	63.45	36.55	65.82

TDE = Total dry extract; FDE = Fat in dry extract

3.5 Microbiological Characterization of the Probiotic "creamy requeijão"

The microbiological characterization of the different "*creamy requeijão"* formulations, stored for 65 days at 5°C, reveals that all formulations are in agreement with the microbiological standards stablished in Technical Regulation - Administrative Rule 359 (Brazil, 1997).

4. Conclusions

In the present study, it has been demonstrated that "*creamy requeijão"* is a suitable vehicle for carrying strains of probiotic microorganisms without changing the characteristics of this dairy food. The formulations evaluated had a population above 10^{8} CFU $g.^{-1}$ of viable cells after 65 days at 5 ° C. In this context, it is emphasized that the consumption of only 1 g of these formulations is enough to attend the daily intake requirements of products with probiotic claim, according to Brazilian law.

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Hypercholesterolemia Risk Related to Consumption of Palm Oil Produced in Côte d'Ivoire

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Abstract

The purpose of this work is to determine the consumption pattern estimated from quantity and frequency of consumption of palm oil produced in Côte d'Ivoire in order to assess risk of hypercholesterolemia related to these oils. To achieve this objective, a cross - sectional survey was conducted with 417 randomly sampled people in seven district capitals of Côte d 'Ivoire. This investigation shows that average of crude and refined R1 and refined R2 palm oil consumed are 24.52 mL, 25.88 mL and 24.13 mL per person per day, respectively. In addition, datas on consumption frequency of different palm oils indicate that refined palm oils are most prevalent in population's dishes. Daily consumption frequency of crude and refined palm oils varies between 7.43 % and 85.40 %. These oils contain 32.95 % to 48.04 % palmitic acid (hazard). For a bioavailability of 100 % palmitic acid, the risk assessment for hypercholesterolemia indicates that 26.02 %, 25.80 % and 21.73 % of surveyed populations ingest higher quantities of palmitic acid. Those are greater than the recommended rate Anses (National Agency for Food Safety, Environment and Labor) during consumption crude and refined palm oils. 26,020, 25,800 and 21,730 cases of increase in serum cholesterol per 100,000 inhabitants after consumption crude, R1 and R2 oils. Concerning a bioavailability of 11 %, risk of hypercholesterolemia is 0 %; 0.02 % and 0.03 % respectively for the consumers of crude, R2 and R1 palm oils. Hypercholesterolemia risk varies from the mode of consumption and oils types.

Keywords: hypercholesterolemia, palm oil, risk

1. Introduction

Fats play a crucial role in many processes related to cell survival, growth and differentiation. They constitute one of the main energy sources (900 kcal / 100 g) for human consumption. In addition, fats are also carriers of vitamins, essential fatty acids and other minor constituents, beneficial to health (De Kock, Degreyt, Gibon & Kellens, 2005; Lecerf, 2011).

Dietary oils are from vegetable and animal origin. Vegetable oils are originate from palm oil and derivatives, soybean, rapeseed, sunflower, cotton, peanut and olive oils. Indeed, palm oil is the vegetable oil the most produced and consumed worldwide (Battaglia, 2010; Jacquemard, 2011). It is a major component of food security in Asian and tropical countries due to palm climate adaptation capacity and oil productivity (Jacquemard, 2011). Palm oil is very popular in Africa and occupies a prominent place in people diet of. According to Food and Agriculture Organisation (FAO), Côte d'Ivoire is the second largest producer in West Africa with an estimated production of 370,000 ton in 2014 (Faostat, 2017).

Palm oil presents many nutritional advantages because of its macronutrients (unsaturated fatty acids 50 %, saturated fatty acids 50 %) and micronutrients (carotenoids, vitamin E, phytonutrients) composition (Lecerf, 2013). In addition, numerous studies related to palm oil qualitative description have shown its beneficial effects for humanity (Mondé et al., 2010; Selvaduray et al., 2012; Wong & Radhakrishnan, 2012).

However, Go et al. (2014) and Sun et al. (2015) reported that there has been growing health concern about palm oil due to the link between saturated fatty acids, particularly palmitic acid, and coronary heart disease. Therefore, the imperative is to assess the health risk due to the consumption of palm oils. This paper deals with the

quantitative assessment of the population's exposure to the hypercholesterolemia risk due to the consumption of different palm oils produced in Cote d'Ivoire. To achieve this, consumption survey on crude and refined palm oils was first conducted in seven (7) district capitals of Côte d'Ivoire. Next, a chemical analysis of palmitic acid content of these oils was performed. The exposure estimation model was analyzed by the Monte Carlo simulation method to determine hypercholesterolemia risk related to these oils. The knowledge level of exposure of the populations in Côte d'Ivoire will allow on the one hand to better control the impacts of the crude and refined palm oils on the health of the populations and on the other hand, to ensure the safety of these consumers.

2. Methods

2.1 Equipment

Material used in this study consisted of crude palm oil (traditionally produced) and refined one (produced by refining industries in Côte d'Ivoire). In addition, food survey tools such as photographs and questionnaire were used to collect information.

2.2 Data Collection

Studies were conducted from November 2016 to February 2017 in seven Côte d'Ivoire areas (Abidjan, Abengourou, Dabou, Daloa, Korhogo, Man and Yamoussoukro). These cities were chosen because they are areas of district capitals. They also describe the way in which crude and refined palm oils are consumed in urban areas in the North, South, Center, East and West of Côte d'Ivoire.

2.3 Sampling

Interviewed people was taken via a systematic random sampling technique and this number of respondents was 417 (Giezendanner, 2012).They were interviewed about their crude and refined oils consumption. Respondent's number chosen was proportional to its demographic size (quota sampling). Frequency and quantities consisted of men and women having at least 18 years old obtained using the following equation:

$$N = t^2 \times [p\ (1\text{-}p)\ /e^2] \quad (1)$$

With n: the size of the sample,

e: the margin of error (5 %),

t: the margin coefficient deducted from the confidence rate (1.96),

p: the proportion of the elements of the mother-population that has a given property (50 %).

In addition, crude and refined (brand R2 and R1) palm oil samples were collected from trading areas and near the resellers and producers of crude palm oil. A non-probability sampling method called "snowball" was used to collect the samples from these seven areas (N'Deye, 2001). In each area, 500 mL oils samples were taken from containers usually used for trading. The sample were wrapped in aluminum foil sheets and stored in a thermos shielded from light at temperature varying from 25 °C to 30 ° C for transport and storage in laboratory (Johnston & Sabin, 2010).

2.4 Questionnaire for Crude and Refined Palm Oils Coconsumption

As part of this work, a questionnaire on the usual consumption of crude and refined palm oils was established. This questionnaire concerned frequency and quantities of palm oils consumption by respondents.

Frequency questionnaire is used to evaluate the usual consumption of certain foods. It consists of a food or foods list with associated consumption frequency categories (in number of times per day, per week, per month, etc.). Method used herein is Cade, Thompson, Burley, & Warm (2002) one in which a respondent checks or ticks frequency that most closely approximates his usual consumption. Frequency list used in this investigation consisted of different frequency categories (never, at least once a day, once a week, two to three times a week, every two weeks, once a month). In addition, determination of quantities of palm oil consumed was made by asking respondents to either describe quantity of oil purchased and the price or, to identify from a list of photographs exhibiting different volumes of oil, one that comes closest to the amount commonly consumed.

2.5 Fatty acid Profile

Fatty acid composition was determined by analysis of fatty acid methyl esters using gas chromatography (GC) according to standard NF EN ISO 5508 (1995). This method involves extraction and esterification of fatty acids. For that, 20 mg of oil fraction was solubilized in 1 mL of TBME (tert-butyl methyl ether). After stirring and filtration with a 0.45 µm filter of, 100 µl of filtered solution were put in insert. Then 50 µl of TMSH (0.5 M

trimethylhydroxide hydroxide in methanol) were added for more volatile fatty acids. Latter are identified by comparison of their chromatograms with those of pure controls analyzed under the same conditions. Chromatograph used is of the Varian type (varian CPG, Sydney, Australia), equipped with a flame ionization detector (FID).

2.6 Exposure Estimation and Risk Assessment of Hypercholesterolemia Related to Palm Oil Consumption

According to Codex Alimentarius definition, exposure estimation is quantitative and / or qualitative assessment of probable intake of hazards (biological, chemical, physical) from food. It consists of quantifying level of chemicals, microorganisms or toxins human populations, subgroups of population and individuals are exposed, in terms of magnitude, duration and frequency.

In this work, exposure assessment consisted of determining amount of palmitic acid ingested during crude and refined palm oils consumption. To define this exposure, amount of palm oil ingested distribution (Q), palmitic acid concentration distribution (C), and frequency of consumption (F) distribution of these oils were determined from investigations and chemical analyzes. Thus, exposure estimation was determined according to following equation:

$$I = C \times Q \times F \quad (2)$$

With: I = amount of distribution of palmitic acid ingested (g); C = concentration distribution of palmitic acid in palm oils (g / 100 g);

Q = amount of distribution of palm oil ingested per person (g); F = frequency distribution of consumption of palm oils (d^{-1}).

Data obtained from different distributions relating to variables C, Q and F were resampled via Bootstrap method, which increased original sample size to 20,000. The quantity I of palmitic acid ingested as a function of C, Q and F was estimated using Monte Carlo simulation. In total, 1,500 iterations were performed. Each simulation is a numerical calculation corresponding to a possible situation, more or less probable, of a real system. Results of this simulation represent amounts of palmitic acid ingested during crude and refined palm oils consumption. Following Anses recommendations described by Guy-Grand, (2017), Recommended Nutritional Intake (RNI) in palmitic acid must not exceed 8 % of 2,000 Kcal/day requirements of an adult. This RNI corresponds to a daily consumption of 17.78 g of palmitic acid. Thus, by reporting different amounts of palmitic acid ingested by individuals at threshold of RNI (17.78 g / day), two groups of consumers are distinguished. On the one hand, proportions of populations ingesting quantities of palmitic acid at doses lower than or equal to the threshold (17.78 g / day). They are not likely to increase their serum cholesterol levels after palm oils consumption of "Probability P1". This probability P1 is obtained by projecting limit dose of palmitic acid (17.78 g) on cumulative density function described by different iterations. On the other hand, we observe a proportion of populations in the event of probable increase of serum cholesterol level (case where ingestion of quantity of palmitic acid content in oils is greater than 17.78 g / day) " Probability P2 ". This probability describes risk of hypercholesterolemia related to the ingestion of palmitic acid contained in crude and refined palm oils. P2 is defined according to equation (3):

$$P2 = 1 - P1 \quad (3)$$

P1: Proportions of populations not subject to a probable increase of their blood cholesterol (hypercholesterolemia) after crude and refined (R1 and R2) palm oils consumption.

P2 "Risk": Proportions of populations with a probable increase of their blood cholesterol (hypercholesterolemia) after crude and refined (R1 and R2) palm oils consumption.

2.7 Bioavailability of Palmitic Acid Due to Crude and Refined Palm Oils Consumption

Berry, (2009) reported that all palmitic acid in palm oils is bioavailable as a result of their consumption.

Indeed, studies based on chronic consumption of saturated fatty acid by humans, precisely, mimicking the current dietary intake of palmitate, have shown same deleterious metabolic effects of palmitic acid consumption, regardless its position within triglycerides. More precisely, palmitic acid has the same digestibility, absorption rates and has the same effects on plasma lipid concentrations regardless its position (Berry, 2009).

However, according to May & Nesarretnam (2014), bioavailability of palm oil palmitic acid is a function of this fatty acid position in triglyceride. In palm oil, 87 % of fatty acids in position 2 are unsaturated (oleic acid and linoleic acid), whereas only 11 % of palmitic acid are in position 2. However, pancreatic lipase hydrolyzes fatty acids in positions 1 and 3, become free. They can form soaps in calcium presence. This reduces their absorption, while 2-monoglyceride persists, absorbed as such, and is therefore better bioavailable (May & Nesaretnam,

2014).

Thus, in this work, quantitative assessment of hypercholesterolemia risk related to ingestion of crude and refined palm oils palmitic acid consumed will consider both cases: On the one hand, assessment of hypercholesterolemia risk taking into account bioavailability of all (100 %) palmitic acid contained in red and refined palm oils produced in Côte d'Ivoire. On the other hand, estimation of hypercholesterolemia risk related to bioavailability of 11 % palm oils palmitic acid.

3. Results

Assessment of hypercholesterolemia risk was defined by results obtained from consumption frequency, palm oil consumed quantities distribution and distribution of palmitic acid concentration in crude and refined oils.

3.1 Frequency of Oils Consumption

Different oils consumption frequencies are shown in Figure 1. Crude palm oil is less common in diet of ivestigated populations with 7.43 % daily frequency. Majority of surveyed population (49.26 %) consumed crude palm oil at least once a month. Moreover, rafined palm oil R1 and R2 are most present in dishes of this population investigated. These people consumed on daily palm oils R1 and R2 respectively at 85.40 % and 77.72 %.

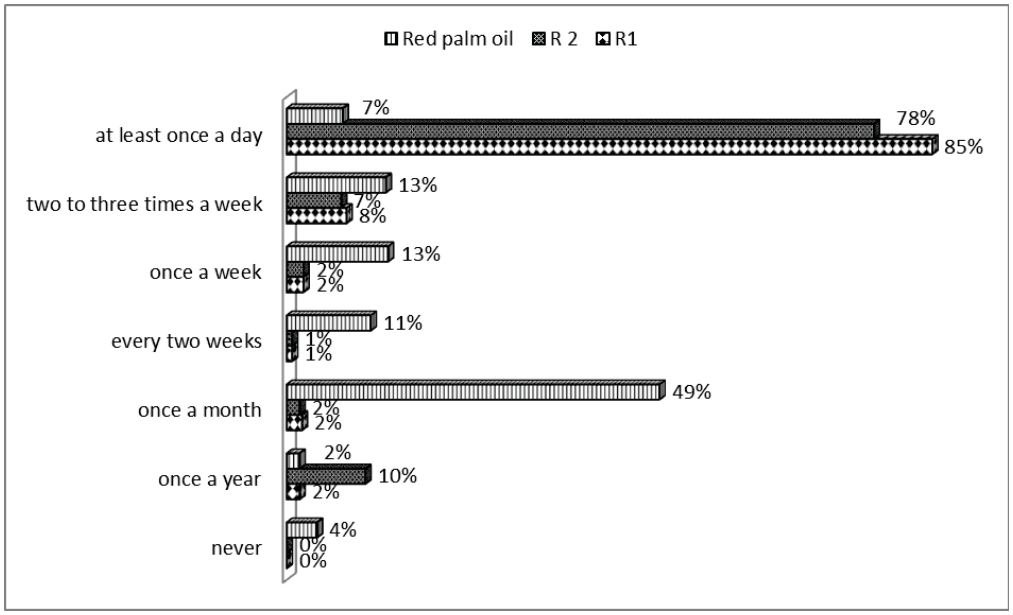

Figure 1. Frequency of crude and palm refined oils consumption

3.2 Quantity of Oils Consumed

Figure 2 depicts the distribution of crude and refined palm oil quantities consumed. Minimum daily quantities of crude and refined palm oils consumed by investigated population are in range from 0 to 30 mL / day. Maximum amounts are in a range from 90 to 125 mL / day. Quantities average consumed are between 23 and 25 mL per individual per day.

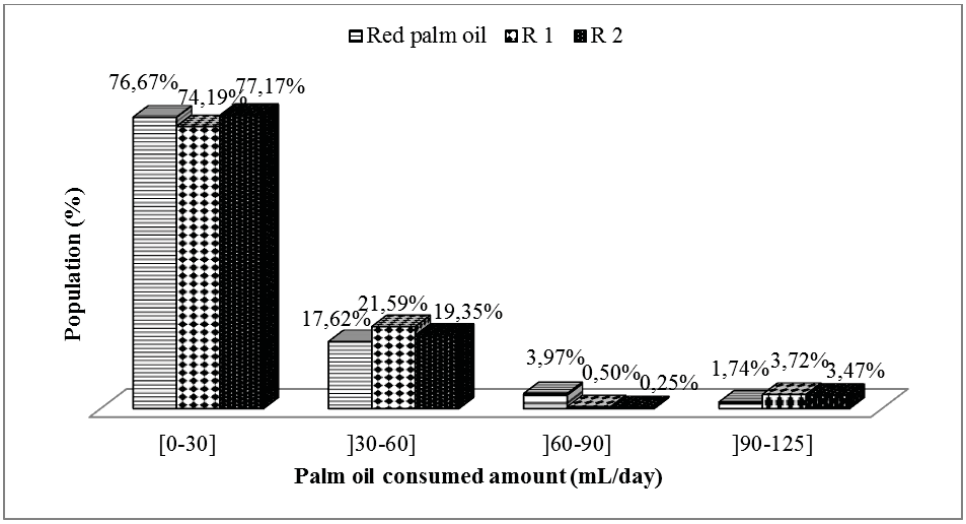

Figure 2. Distribution of crude and refined (R1 and R2) palm oils quantities consumed daily

3.3 Fatty Acid Profile of Oils Samples

Saturated Fatty Acid (SFA) and Unsaturated one (UFA) of crude and refined palm oils samples collected from different areas are listed in Table 1. Crude palm oils samples composition in saturated fatty acid and unsaturated one are ranges from 41.38 to 54 % for SFA and from 46.00 to 58.62 % for UFA. R1 and R2 palm oils contain percentages of SFA and UFA. For SFA, they are 41.54 and 41.21 % for R1 and R2 respectively. Concerning UFA, values establish at 58.46 and 58.78 %. In addition, palmitic acid, which represents the chemical hazard studied, is present at levels ranging from 32.95 to 48.58 % in artisanally produced crude palm oil. Palmitic acid contents varied from one locality to another. Thus, crude palm oil from Dabou contains the highest palmitic acid namely 48.58 %, while, samples from Man present the lowest value (32.95 %). Refined palm oils R1 and R2 contain respectively 35.29 % and 34.98 % palmitic acid.

Table 1. Fatty acid content of crude and refined palm oils

		Crude palm oils							**refined palm oils**	
Cities		Abidjan	Daloa	Korhogo	Yakro	Abengourou	Man	Dabou	R1	R2
FATTY	C14: 0	0.56	0.57	0.71	0.43	0.76	0.58	0.55	0.54	0.54
ACIDS (%)	Myristate									
	C16:0 Palmitate	34.74	37.04	37.41	34.38	39.66	32.95	48.04	35.29	34.98
	C16:1n-7c Palmitoleate	0.08	0.11	0.1	0.07	0.12	0.07	0.11	0.11	0.12
	C18:0 Stearate	6.97	5.55	6.91	8.18	5.15	7.39	5.02	5.24	5.22
	C18:1n-9c Oleate	49.05	45.21	45.25	46.57	41.3	50.58	35.41	48.09	48.28
	C18:1n-7c vacenate	0.48	0.41	0.51	0.42	0.66	0.5	0.65	0.65	0.67
	C18:2n-6c Linoleate	7.38	10.4	8.36	9.08	11.56	7.19	9.55	9.61	9.72
	C18:3n-3 α-Linolenate	0.31	0.28	0.31	0.41	0.31	0.28	0.28	-	-
	C20:0 Arachidate	0.43	0.43	0.44	0.45	0.48	0.46	0.39	0.47	0.47
TOTAL FATTY	**AGS**	**42.70**	**43.59**	**45.47**	**43.44**	**46.05**	**41.38**	**54.00**	**41.54**	**41.21**
ACIDS (%)	**AGI**	**57.30**	**56.41**	**54.53**	**56.55**	**53.95**	**58.62**	**46.00**	**58.46**	**58.78**

3.4 Risk of Hypercholesterolemia Linked to Consumption of Crude and Refined Palm Oils (Bioavailability of Palmitic Acid to 100%)

Figures 3, 4 and 5 show the ingestion probability as a function of the palmitic acid ingested amount for crude, R1 and R2 oils, based on the simulations results carried out by Monte Carlo method. This data consider a 100 % of bioavailability of palmitic acid contained in crude, R1 and R2 oils.These figures show that proportions of populations that ingest palmitic acid quantities at doses less than or equal to the Recommended Nutritional Intake (RNI) during consumption of crude and refined R1 and R2 palm oils are respectively 73.98 %, 74.20 % and 78.27 %. These "positive" probabilities indicate that about 74 % to 78 % of populations in Côte d'Ivoire

consume crude and refined palm oils at rational quantities respecting Recommended Nutritional Intake. But, 21.73 %, 25.50 % and 26.02 % of this Ivorian population consume refined (R2, R1) and crude palm oils at doses exceeding Recommended Nutritional Intake.

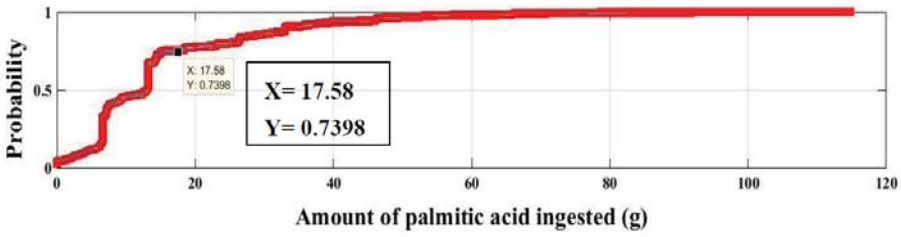

Figure 3. Risk of hypercholesterolemia at 100% bioavailability of palmitic acid for crude palm oil

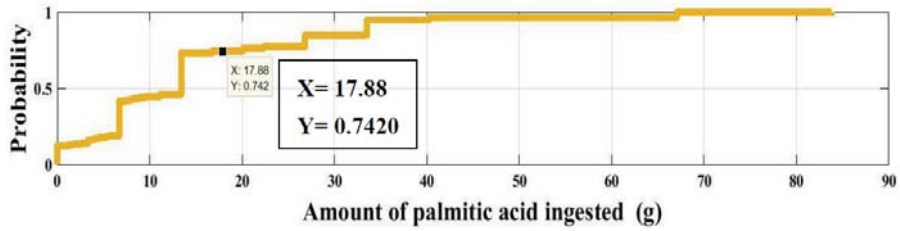

Figure 4. Risk of hypercholesterolemia at 100% bioavailability of palmitic acid for refined palm oil (R1)

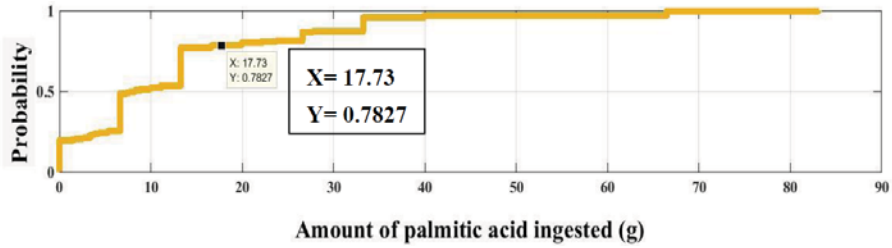

Figure 5. Risk of hypercholesterolemia at 100% bioavailability of palmitic acid for refined palm oil (R2)

3.5 Hypercholesterolemia Risk Related to Consumption Crude and Palm Refined Oil (Bioavailability of Palmitic Acid at 11 %)

In the same mood, Figures 6, 7 and 8 describe hypercholesterolemia risk related to ingestion of palmitic acid contained respectively in crude and refined (R2 and R1) palm oils at 11 % bioavailability. Considering that only 11 % palmitic acids of crude and refined palm oils are bioavailable after their ingestion, hypercholesterolemia risk is very low for refined palm oils (R2 and R1) and is almost zero for crude palm oil.

Positive probabilities that is proportion of populations ingesting amounts of palmitic acid at levels less than or equal to the Recommended Nutritional Intake are 100 %, 99.98 % and 99.97 % respectively for consumption of crude, R2 and R1 palm refined oils. These proportions indicate that per 100,000 people, representing the whole or 99,970 to 99,980 of these people will probably be not subject to increase in serum cholesterol levels after consumption crude, R2 and R1 refined palm oils. However, 20 to 30 people will be at risk of hypercholesterolemia after consuming these oils.

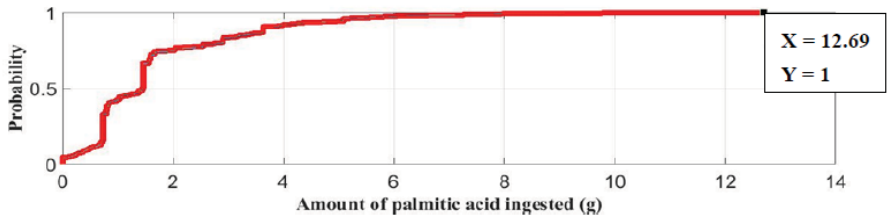

Figure 6. Risk of hypercholesterolemia at 11% bioavailability of palmitic acid for crude palm oil

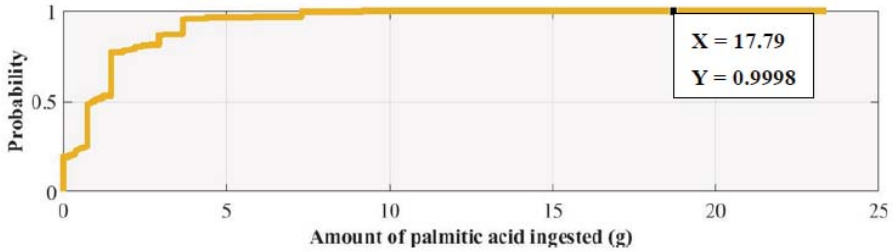

Figure 7. Risk of hypercholesterolemia at 11 % bioavailability of palmitic acid for rafined palm oil (R2)

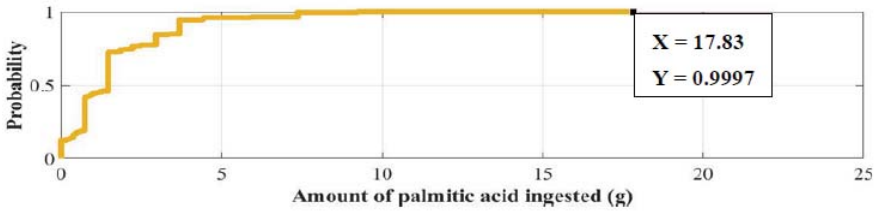

Figure 8. Risk of hypercholesterolemia at 11 % bioavailability of palmitic acid for rafined palm oil (R1)

4. Discussion

Results of food surveys have shown that crude palm oil is less common in investigated populations diet. Indeed, daily frequency is only 7.43 %. This daily crude palm oil consumption frequency is lower than that observed in Congo, which is 11.4 % (Moutoula, Mananga, Elenga, & Kinkela, 2016). Most represented crude palm oil in investigated population is at least once a month, which is 49.26 %. This monthly consumption frequency of crude palm oil is similar to that observed in Yaoundé, Cameroon (approximately 44 % per month) (Rebena, 2016). However, crude palm oil consumption frequency and that of Yaoundé seem to be lower than those of children from 6 to 36 months old in Benin. Nearly 70 % of these children consume crude palm oil at least of once a week (Hounkpatin, 2011). Indeed, crude palm oil is an excellent vitamins A and E source (Morin & Pagès-Xatart-Parès, 2012; Sen, Khanna, & Roy, 2006). Regular consumption of this oil can cover consumer's nutritional needs including vitamins A and E.

Moreover, refined oils (R1 and R2) are the most present in dishes of population investigated. These people consume R1 and R2 palm refined oils on a daily basis at 85.40 % and 77.72 %, respectively. This daily consumption frequency for refined palm oil in Côte d'Ivoire reflects world demand since according to Guilaume-Gentil et al. (2016), palm oil is most produced and consumed vegetable oil in world over the last ten years (Guilaume-Gentil et al., 2016). Similarly, it is the cheapest food oil in world (Jacquemard, 2011), so accessible to all markets. In addition, refined oils are most used for frying food in Côte d'Ivoire (Cheyns, 2001).

Ditribution of crude and refined palm oil consumption quantity show that minimum amounts vary between 0 and 30 mL and maximum are between 90 and 125 mL per day. Most consumed quantities are those between 0 and 30 mL referring to 74.19 % to 77.17 % of studied population. The least consumed amounts range from 90 to 125 mL per day (1.74 % to 3.47 % of population). The mean consumed quantities are between 23 and 25 mL per

person per day. These average quantities are slightly lower than those consumed in Ghana as described by Ofosu-Budu and Sarpong (Ofosu-Budu & Sarpong, 2013), saying that each individual in Ghana consumes between 30 and 33 mL of palm oil a day. In fact, the quantities of crude and palm refined oils consumed are the same within study population. In addition, refined palm oil consumed amount has decreased.

According to the work of Cheyns in 2001 (Cheyns, 2001) about Côte d'Ivoire people crude palm oil consumption, refined palm oils were consumed four times, in quantities than crude palm. Therfore, the cousumed quantity reduction of palm oil can be due to awareness campaigns on diseases risk related to a diet rich in oil including obesity, diabetes, high blood pressure and cardiovascular disease.

Regarding chemical analyses, saturated fatty acid (SFA) and unsaturated one (UFA) contents in studied artisanally produced crude palm oils ranged from 41.38 % to 54 % (SFA) and from 46.00 % to 58.62 % for UFA. These values are close to those reported by Mondé et al. in 2008 (Mondé et al., 2008), with SFA levels varying between 40 % and 52 % and from 48 % to 60 % for UFA. Nevertheless, this composition of SFA and UFA differs from that observed by N'goran et al. in 2017 (N'Goran et al., 2017) with lower proportions of SFA (34.8-39.3 %) in crude palm oil collected in Côte d'Ivoire four districts. Also, UFA content (58.6-64.3 %) of these four areas is higher than that obtained in this work. This difference in saturated and unsaturated fatty acid content in artisanally produced edible crude palm oil is thought to be due to the variety of palm oil seeds used in production of these oils or to type of soil on which palms grow, and climate change. Indeed, according to Boyer's work in 2010 (Boyer, 2010) on vegetable oils, it appears that these are triglycerides, whose composition depends on plant nature, its growing conditions, soil and season. Likewise, R1 and R2 refined palm oils have more or less identical SFA and UFA contents. R1 palm refined oil contains SFA 41.54 % and UFA 58.46 %. While R2 oil contains 41.21 % and 58.78 % respectively in SFA and UFA. These results are close to those obtained by Chatigre in 2014 (Chatigre, 2014). His work revealed that Dinor's (palm refined oil) contained 44.09 % SFA and 55.91 % UFA.

In addition, palmitic acid, which represents hazard studied, is present at levels ranging from 32.95 % to 48.04 % in artisanally produced crude palm oil. Dabou's crude palm oil has highest palmitic acid content with a value of 48.04 %. While, Man has the lowest one (32.95 %). These palmitic acid contents are close to those described by Lecerf with values ranging from 39.3 % to 47.5 % (Lecerf, 2013). Rafined palm oils R1 and R2 contain respectively 35.29 % and 34.98 % of palmitic acid. These results are close to those obtained by Chatigre in 2014 (Chatigre, 2014), which revealed the presence of 37.97 % of palmitic acid in Dinor. Indeed, palmitic acid is a fatty acid that is part of cell membrane constituents. It assures very important roles in humans, namely, energetic expenditure, membrane elasticity and viscosity. It also carries fat-soluble vitamins such as vitamins A, D, E and K. Besides, it can cause LDL and HDL cholesterol levels increase after an excessive ingestion (Legrand, 2007; Seghier, 2014).

Moreover, probabilities indicate that about 74 % to 78 % of populations in Côte d'Ivoire consume crude and refined palm oil at rational quantities respecting recommended nutritional intake.

Comparing these probabilities to 100,000 inhabitants, we find that approximately 73,980, 74,200 and 78,270 consumers of oils in differents forms are not at hypercholesterolemia risky level. However, risk determination, that is, probabilities of serum cholesterol increases cases after palm oil consumption indicate that approximately 26.02 %; 25.50 %; 21.73 % of populations investigated ingest quantities of palmitic acid greater than the rate recommended by Anses respectively for R1, R2 and crude palm oils. That represents 26,020, 25,800 and 21,730 cases for 100,000 inhabitants. Risk of hypercholesterolemia is high considering that palmitic acid of crude and refined palm oils produced and consumed by population in Côte d'Ivoire is 100 % bioavailable. Nevertheless, precipitation of palmitic acid by formation of calcium soap (Lecerf, 2013) would lead to a reduction of palmitic acid bioavailable level which would reduce hypercholesterolemia risk due to palm oils consumption.

However, when palmitic acid bioavailability is get to 11 %, Positive probabilities that proportion of populations ingesting palmitic acid amounts at levels less than or equal to recommended nutritional intake are 100 %, 99.98 % and 99.97 % respectively for consumption of crude, R2 and R1 refined palm oils. However, 20 to 30 people per 100,000 will be at hypercholesterolemia risk after consuming refined palm oil. Indeed, palm oil consumption impact on markers of cardiovascular risk (cholesterol especially), depends on several parameters such as quantities consumed, and nutritional context (linoleic acid and cholesterol content of diet, total lipid intake) (Lecerf, 2013). Also, it is related to consumption frequency and bioavailability of the hazard.

In conclusion, the fundamental role of oils in body is to provide fatty acids giving the necessary energy for good body functionning. But, ingestion of saturated fatty acids such as palmitic acid can lead serum cholesterol (LDL and HDL) increase. This hypercholesterolemia depends on various parameters, namely consumption frequency,

palm oil quantity consumed, palmitic acid concentration in oils and its bioavailability.

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Effect of Lactic Acid Bacteria Starter Cultures on Vitamin and Oligosaccharide Composition of Milk Extracted from Three Common Bean (*Phaselous Vulgaris* L) Varieties

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Abstract

Fermented foods have in recent times attracted consumer interest mainly due to perceived health benefits of probiotic microorganisms. This study characterized changes in the concentrations of selected B-complex vitamins and oligosaccharides of common bean milk during fermentation by a common dairy starter culture, YF L-903 (*Streptococcus thermophilus* + *Lactobacillus Bulgaricus subs Debulgaricus*), and three probiotic cultures namely ABT (*Lactobacillus acidophilus La-5* + *Bifidobacterium animalis Bb-12* + *Streptococcus thermophilus*), Yoba (*Lactobacillus rhamnosus yoba* + *Streptococcus thermophilus*), and Yoba Fiti (*Lactobacillus rhamnosus* GR1 + *Streptococcus thermophilus*). Bean milk was prepared from three common bean varieties. It was found that, apart from thiamine (vitamin B1) and riboflavin (vitamin B2), fermentation with each of the mixed cultures caused significant increase in the vitamin B complex. Significant reductions ($p<0.05$) in the oligosaccharides concentration of the bean milks were observed upon fermentation. Highest reduction in the oligosaccharide sugars of 77.8% was found in milk from pinto bean variety fermented with ABT culture. These findings suggest that LAB probiotic cultures have a potential for improving biosynthesis of vitamins and removal of the verbascose, stachyose and raffinose oligosaccharides, thus making the product more digestible and the nutrients more bioavailable.

Keywords: common bean, bean milk, fermentation, vitamin biosynthesis

1. Introduction

In developing nations dietary deficiencies, especially in vitamins is reported to cause various health disorders (UNICEF, 2011). However, in developed nations consumers are concerned with their recommended dietary intake and usually use vitamins as supplements to promote health and prevent chronic diseases (Burgess, Smid, & Sinderen, 2009; Fortmann et al., 2013). Although the major role of food in the body is to provide adequate nutrients to meet daily metabolic requirements, recent findings suggest that food may regulate several functions beyond the predictable nutritional benefit (Stanton et al., 2001). Therefore, fermented foods, particularly those fermented with probiotic cultures have in recent times attracted the interest of the consumers, due to their perceived health benefits including bioavailability of nutrients such as vitamins (LeBlanc et al., 2010) and reduction or improved oligosaccharides digestion (Difo et al., 2015).

Vitamins are generally classified into two groups, the fat soluble vitamins (A, D, E and K) and the water soluble vitamins which include a series of B-vitamins, vitamin C and biotin (Burgess et al., 2009). The B-complex or B group vitamins is comprised of thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), biotin (H or B7), folate (M, B9 or B11) and cobalamin (B12) (Capozzi et al., 2012). Owing to their water solubility the B group vitamins play an important role in cellular metabolism of fats and protein (pyridoxine and riboflavin) and carbohydrates (thiamine), where they act as coenzymes, principally as carriers of a specific chemical group (Baku & Dickerson, 1996). These molecules are normally present in a number of foods, but can easily be destroyed or removed during food processing which potentially explains why their deficiency is rather common in a large population (Capozzi et al., 2012). Thus, food industries have been subjected by laws in the country of their operations to fortify certain foods with specific B-complex vitamins (LeBlanc et al., 2010).

Prokaryotes, including some lactic acid bacteria (LAB) utilize B-vitamins to meet their nutritional requirements (Snell, 1993). However, production of these vitamins by LAB has also been established (LeBlanc et al., 2011). This natural ability for vitamin B-complex biosynthesis by LAB has the potential to be utilized, either to harness the natural biosynthetic pathway of these microorganisms in fortification of fermented foods or to replace costly chemical synthesis of such foods (Burgess et al., 2009).

Oligosaccharides in common beans are about 31 to 76% of the total sugars and are known to cause flatulence and discomfort in the stomach (Campos et al., 2009; Campos et al., 2013). Traditional processing methods such as soaking and de-hulling followed with thermal treatment can eliminate most of these oligosaccharides in common bean but require a lot of energy, which is costly, particularly in developing countries (Nakitto, Muyonga, & Nakimbugwe, 2015). Little research has been done on the role of combined processing methods such as soaking, de-hulling, fermentation and steaming on nutritional quality and production of nutritious fast cooking common bean product (Nakitto et al., 2015), yet fermentation could sequestrate oligosaccharides (Kort et al., 2015). Therefore, this study was carried out to develop a milk product from beans and to characterize changes in the concentrations of the B-complex molecules and oligosaccharides sugars of common bean milk.

2. Materials and Methods

2.1 Bean Collection and Storage

Local varieties of dry pinto beans, red haricot beans and yellow kidney beans were procured from a trader in Nairobi County, Kenya. One packet containing 2 Kg of each of the bean variety was bought, wrapped in a kraft paper and transported to the food technology workshop in the School of Food and Nutritional Sciences of the Jomo Kenyatta University of Agriculture and Technology (JKUAT). The dry beans were stored in sealed plastic containers at room temperature (20 to 25°C) until use.

2.2 Bean Milk Preparation and Fermentation

Bean milk was prepared using methods of Anino et al. (2019). Briefly, 100 g of common beans was rinsed and soaked in a 1 L of deionized water for 16 h at room temperature (23°C). Water was drained off and the seeds dehulled by hand and ground in a blender (MBLR4314/WH, Mika, Dubai, UAE) for 3 min at 550W with 1L boiling water. The resulting slurry was filtered through 2 layers of muslin cloth to allow only water soluble common bean milk to pass through. The strained milk was heated in a heavy bottom pan to 100°C for 20 min, stirring frequently to prevent sticking. The heat treated bean milk was placed at room temperature (20 to 25°C) and left to cool for 2 hours and thereafter stored at 4°C.

With regards to fermentation method used by Mani, Palou, & López (2014) was adopted with slight modifications. Briefly, LAB strains contained in a common dairy starter culture, YF L-903 (*Streptococcus thermophilus* + *Lactobacillus Bulgaricus subs Debulgaricus*), and three probiotic cultures namely ABT (*Lactobacillus acidophilus La-5* + *Bifidobacterium animalis Bb-12* + *Streptococcus thermophilus*), Yoba (*Lactobacillus rhamnosus yoba* + *Streptococcus thermophilus*), and Yoba Fiti (*Lactobacillus rhamnosus* GR1 + *Streptococcus thermophilus*) were used to ferment the bean milks. Each of the four starter cultures was prepared to yield equal amount of fermented milk for the three different bean milk varieties as follows. A 0.5 g of each of the starter culture was inoculated in 500 ml of raw milk. The inoculated bean milk was incubated at 45°C in a Heratherm microbilogical incubator until a pH ≤4.3 was attained. The fermented bean milk was placed at room temperature (20 to 25°C) and left to cool for 2 hours and thereafter stored at 4°C. Eppendorf tubes of 5 ml fermented milk were taken in triplicates to determine the concentration of B-vitamins (thiamine, riboflavin, niacin, pyridoxine and folic acid) and eppendorf tubes of 10 ml oligosaccharides (raffinose, stachyose and verbascose).

2.3 Determination of Thiamine, Riboflavin, Pyridoxine and Folic Acid

Extraction of thiamine, riboflavin, pyridoxine, niacin and folic acid was based on the modified methods of Chase et al. (1993); Ekinci & Kadakal (2005) and Kamman, Labuza, & Warthesen (1980). The extractions were carried out in triplicates by adding 20 ml of deionized water to 5 ml of bean milk (dilution factor, F, = 5 ml). The mixture was homogenized using a homogenizer at medium speed for 1 min. The homogenized mixture was centrifuged for 15 min at 1500 rpm and Sep-Pak C_{18} (500 mg) cartridges method of Cho et al. (Cho, Ko, & Cheong, 2000) was used to extract the water-soluble vitamins. The extracts were filtered through a 0.45 μm micropore membrane FP 30/45 CA-S filters (Schleicher and Schuell, Darmstadt, Germany). A 0.45 μm of the filtrate was injected with a syringe into HPLC column (20A Series, Shimadzu Co-operation, Kyoto, Japan) C_{18} 150 mm x 4.6 mm with a flow rate of 0.1 mol L^{-1} and KH_2PO_4 (PH 7.0)-methanol, 90:10, as mobile phase (0.7 mL min^{-1}) in isocratic mode. The vitamins were identified by comparing their retention times and UV-visible

spectra with those of standards stored in a data bank at 266 nm for riboflavin, 282 nm for folic acid, 234 nm for thiamine, 324 nm for pyridoxine and 261 nm for niacin.

2.4 Determination of Oligosaccharides

The methods of Brenes et al. (2003) and Campos et al. (2009) were modified to determine three forms of oligosaccharides; raffinose, stachyose and verbascose in triplicates. A10 ml sample of common bean milk was homogenized in aqueous ethanol (100 ml, 80%, v/v) and placed in a Soxhlet at 80^{o}C for 60 min. The ethanol extracts was recovered, concentrated under vacuum, and the water phase frozen and lyophilized. A 7 mg sample of the extracted oligosaccharides was re-dissolved in 1 ml of deionized water, filtered and subjected to HPLC analysis. Standard curves were determined by injecting 20 µl of raffinose, stachyose and verbascose standards into HPLC column (20A Series, Shimadzu Cooperation, Kyoto, Japan) connected to a refractive index detector fitted with a Zorbax NH_2 pre-column (4.6 x 12.6 mm, 5 µm) and Zorbax column (250 x 4.6 mm). A 20 µl of the extracted oligosaccharides was also injected into HPLC column to obtain peak areas. Water/acetonitrile (65:35) was used as mobile phase at 1 ml/min. Column and detector temperatures were maintained at 25°C.

2.5 Statistical Analysis

All data were subjected to two way full factorial ANOVA using STATA/SE 12.0 software for windows to identify significant treatment effects. Comparison among means for different groups was made using Bonferroni least significant difference (LSD) test at $p \leq 0.05$.

3. Results and Discussion

3.1 Vitamin Concentration of Fermented Bean Milk

Table 1. Vitamin concentration in milk extracted from red haricot (RH), yellow kidney (YK) and pinto (P) common bean varieties fermented with LAB probiotic starter cultures; ABT (*Lactobacillus acidophilus La-5 + Bifidobacterium animalis Bb-12 + Streptococcus thermophilus*), YF L-903 (*Streptococcus thermophilus + Lactobacillus Bulgaricus subs Debulgaricus*), Yoba (*Lactobacillus rhamnosus yoba + Streptococcus thermophilus*) and Yoba Fiti (*Lactobacillus rhamnosus* GR1 + *Streptococcus thermophilus*)

	Thiamine (mg/100g)			**Riboflavin (µg/100g)**			**Niacin (mg/100g)**			**Pyridoxine (mg/100g)**			**Folic acid (mg/100g)**		
	RH	YK	P	RH	YK	P	RH	YK	P	RH	YK	P	RH	YK	P
NF	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	88.1±1.6^{c}	52.2±3.4^{b}	40.0±4.7^{ab}	0.5±0.0^{a}	0.4±0.0^{a}	0.5±0.1^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.3±0.0^{a}	0.4±0.0^{b}	0.4±0.0^{bc}
Yoba	ND	ND	ND	27.8±0.5^{a}	57.7±3.0^{b}	49.9±9.1^{b}	1.2±0.1^{b}	2.3±0.0^{c}	1.3±0.0^{b}	0.3±0.0^{c}	0.2±0.0^{b}	0.5±0.0^{c}	0.4±0.0^{b}	0.6±0.1^{cd}	0.6±0.1^{cd}
YF L-903	ND	ND	ND	50.1±0.2^{b}	60.1±10.6^{b}	33.9±4.7^{ab}	3.7±0.4^{d}	2.8±0.2^{cd}	3.0±0.4^{cd}	0.2±0.0^{b}	0.2±0.0^{b}	0.2±0.0^{b}	0.5±0.0^{c}	0.9±0.1^{c}	0.6±0.1^{cd}
ABT	ND	ND	ND	433.0±63.7^{d}	1121.9±15.9^{f}	670.9±26.1^{e}	2.8±0.2^{cd}	2.2±0.1^{c}	2.8±0.1^{cd}	1.0±0.1^{f}	0.5±0.1^{de}	1.0±0.0^{f}	0.6±0.0^{d}	0.9±0.1^{c}	0.7±0.1^{de}
Yoba Fiti	ND	ND	ND	23.8±0.6^{a}	27.8±1.1^{a}	21.2±0.1^{a}	3.6±0.3^{d}	2.4±0.4^{c}	3.0±0.8^{cd}	0.4±0.0^{d}	0.3±0.0^{c}	0.4±0.1^{cd}	0.5±0.0^{c}	0.8±0.0^{c}	0.5±0.0^{c}
SE	0.01			8.8			0.26			0.01			0.01		
P	<0.01			<0.01			<0.01			<0.01			<0.01		

NB: Results are means ± standard deviation (SD). Different superscript letters within the same column and row indicate statistical significance (Bonferroni, $p<0.05$); NF, non-fermented; ND, not detectable.

The effects of fermentation on vitamin concentration of common bean milk extracted from the three bean varieties are shown in Table 1. There were no significant inter-varietal differences in the contents of thiamine, niacin and pyridoxine in raw bean milk, while the differences in riboflavin and folic acid were significant ($p<0.05$). Thiamine was not quantifiable in bean milk fermented with any of the cultures. This is consistent with the results of (Granito et al., 2002) who observed notable losses in thiamine after natural fermentation of lentils and red beans.

The starter cultures had different effects on the riboflavin content of beans (Table 1) which is consistent with previous reports that LAB strains can produce or utilize individual vitamin B molecules, depending with the genome of the fermenting microorganism (Burgess et al., 2009). Fermentation with Yoba Fiti culture significantly reduced ($p<0.05$) riboflavin in milk extracted from the three bean varieties by 46.7% to 73%. An earlier study by Elmadfa et al. (2001) showed that most probiotic strains of lactobacilli consume riboflavin thereby decreasing its bioavailability. Additionally, riboflavin biosynthesis has been shown to occur when the four genes; ribG, ribB, ribA and ribH are present in the microbes genome (Bacher et al., 2015). However, absence of the ribG is previously reported for *L. rhamnosus* GR1*, L. rhamnosus yoba, L. bulgaricus* and *S. thermophilus* (Thakur et al., 2015; Valle et al., 2014). On the hand, the bean variety from which milk was extracted had great influence on the riboflavin concentration of the fermented milk. For example, fermentation

with Yoba significantly reduced riboflavin content in milk extracted from red haricot but not in milk extracted from the other two varieties, while fermentation with ABT caused great increases of this vitamin in milk extracted from all the varieties, especially in milk extracted from yellow kidney beans (>2000% increase). *Bifidobacterium animalis Bb-12* and *L. acidophilus La-5* which are the fermenting bacteria in the mixed probiotic ABT culture contain the four gene operons needed to catalyze biosynthesis of riboflavin (Thakur et al., 2015). Thus, appropriate selection of species and/or strains is essential in increasing riboflavin of fermented bean milk.

Fermentation caused significant increase ($p<0.01$) in niacin concentration of the milk extracted from the three bean varieties. The niacin values in milk extracted from red haricot beans fermented with Yoba and L-903 cultures increased from 0.5±0.0 mg/100g to 1.2±0.1 mg/100g and 3.7±0.4 mg/100g respectively (Table 1). The highest increase in niacin concentration in milk extracted from the three bean varieties, an increase of 640% was found in milk extracted from red haricot beans fermented with YF-L903 culture. Increase in niacin values of cheese and yoghurt fermented with lactic acid producing bacteria was earlier reported (Gu & Li, 2016). These strains may be useful in enriching niacin composition of bean milk and could be exploited for other legumes.

With regards to pyridoxine the highest concentration was quantified in milk fermented with ABT culture (Table 1). Similarly, the highest increase of 900% was found in milk extracted from red haricot and pinto beans fermented with ABT cultures. A previous study by Vajaranant & Fields (1989) reported increase ($p<0.05$) in pyridoxine values of corn meal (0.52±0.0 to 0.72±0.1 mg/100g) fermented with different strains of *Bacilus licheniformis*. Similarly, fermentation of soy with different species and strains of *Streptococcus thermophilus, Lactobacilluis helveticus and Bifidobacterium longum* was previously reported to cause significant increases in pyridoxine concentration (Champagne et al., 2010). The biosynthesis of pyridoxine was previously reported to depend on the microbial ecological niche (Qaidi et al., 2013). This could imply that *L. acidophilus* and *B. animals* have got specific metabolic properties that make them more efficient in the biosynthesis of folic acid than other LAB (Table 1).

Milk extracted from yellow kidney beans and pinto beans had statistically higher ($p<0.05$) folic acid of 0.4±0.0 mg/100g than those extracted from red haricot beans which contained 0.3±0.0 mg/100g. Folic acid was significantly higher ($p<0.05$) in corresponding fermented bean milk than the non-fermented milk (Table 1). This agrees with earlier studies which reported increase in folic acid in milk fermented with *L. rhamnosus* (Hugenschmidt et al., 2010), *S. thermophilus* (Lai~no, LeBlanc, & Savoy, 2012), *L. acidophilus* (Lai~no et al., 2014), and *B. animalis* (Pompei et al., 2007). Milk extracted from yellow kidney beans exhibited relatively higher increase in folate than the other two bean varieties. Additionally, the highest increase in folic acid in each milk category was found in those fermented with ABT culture, an increase of (75 to 125%). *L. acidophilus* strains is reported to contain folate biosynthesis cluster which converts 6-hydroxymetheyl-7,8-dihydropterin (DHPP) to folic acid biosynthesis precursor parabenzoic amino acid (pABA) (Gu & Li, 2016). However, *L. rhamnosus* and *S. thermophilus* use an alternative biosynthesis pathway (KEGG, 2014) which could be less efficient in the biosynthesis of folic acid (Table 1).

3.2 Oligosaccharides Concentration of Fermented Bean Milk

Table 2. Oligosaccharides concentration (mg/100g) in milk extracted from red haricot (RH), yellow kidney (YK) and pinto (P) common bean varieties fermented with LAB probiotic starter cultures; ABT (Lactobacillus acidophilus La-5 + Bifidobacterium animalis Bb-12 + Streptococcus thermophilus), YF L-903 (Streptococcus thermophilus + Lactobacillus Bulgaricus subs Debulgaricus), Yoba (Lactobacillus rhamnosus yoba + Streptococcus thermophilus) and Yoba Fiti (Lactobacillus rhamnosus GR1 + Streptococcus thermophilus)

	Raffinose (mg/100g)			**Verbascose (mg/100g)**			**Stachyose (mg/100g)**		
	RH	YK	P	RH	YK	P	RH	YK	P
NF	0.4 ± 0.0^{d}	0.4 ± 0.0^{d}	0.4 ± 0.0^{d}	0.2 ± 0.0^{b}	0.4 ± 0.0^{d}	0.3 ± 0.0^{c}	3.4 ± 0.1^{g}	4.2 ± 0.2^{h}	3.6 ± 0.4^{gh}
Yoba	0.2 ± 0.1^{abc}	0.2 ± 0.0^{b}	0.3 ± 0.0^{c}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	1.0 ± 0.0^{b}	1.3 ± 0.1^{bcd}	2.3 ± 0.4^{ef}
YF L-903	0.2 ± 0.0^{ab}	0.2 ± 0.1^{abc}	0.2 ± 0.0^{b}	0.2 ± 0.1^{abc}	0.1 ± 0.1^{ab}	0.1 ± 0.0^{a}	2.6 ± 0.4^{f}	1.6 ± 0.1^{de}	1.1 ± 0.2^{acd}
ABT	0.1 ± 0.1^{ab}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.9 ± 0.2^{ab}	1.0 ± 0.1^{ab}	0.8 ± 0.0^{a}
Yoba Fiti	0.2 ± 0.0^{ab}	0.2 ± 0.1^{abc}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.1^{a}	0.1 ± 0.0^{a}	1.6 ± 0.2^{bcde}	1.1 ± 0.1^{abc}	1.0 ± 0.1^{ab}
SE		0.02			0.02			0.12	
P		<0.01			<0.01			<0.01	

NB: Results are means ± SD. Different superscript letters within the same column and row indicate statistical significance (Bonferroni, $p<0.05$); NF, non-fermented.

There are various types of oligosaccharide sugars, including raffinose, verbascose and stachyose. These sugars

are known to cause flatulence and are partly the reason for low consumption of common beans and its associated products (Paredes & Harry, 1989). Table 2 shows the effects of fermentation on oligosaccharides concentration of common bean milk extracted from three bean varieties. A narrow range of values have been reported for raffinose in legumes. For instance, Difo et al. (2015) found that *racemosa* seeds contained 1.9±0.0 mg/100g of raffinose sugars while Akinyele & Akinlosotu (1991) reported concentration of 2.0±0.0 mg/100g in cowpeas (*Vigna unguiculata)*. Thus, the concentration of 0.4±0.0 mg/100g contained in non-fermented bean milk was far much lower than the previously reported values for most legumes. Similar to the results of Da et al., (2006) stachyose (3.4±0.1 to 4.2±0.2 mg/100g) was the most abundant oligosaccharide sugar in non-fermented bean milk (Table 2). The highest reduction in raffinose concentration (75%) was recorded in pinto bean milk fermented with ABT culture. A previous study by Granito & Alvarez (2006) reported similar results when black beans varieties were fermented with lactic acid bacteria. The reduction in raffinose could be attributed to the utilization of the oligosaccharides for energy by the microorganisms. The current finding is of great interest as it suggests that fermentation could be used to reduce flatulence causing raffinose.

Fermentation with Yoba, ABT and Yoba Fiti cultures caused significant decreases ($p<0.05$) in verbascose concentration of the milk extracted from red haricot beans ($p>0.05$) (Table 2). Fermented milk extracted from yellow kidney and pinto beans were also found to contain statistically lower ($p<0.05$) verbascose values on average (0.1±0.0 mg/100g) than non-fermented milk (0.4±0.0 mg/100g) and (0.3±0.0 mg/100g) respectively, a 75% reduction in verbascose concentration in milk extracted from yellow kidney beans. This agrees with earlier reports which had shown reduction in verbascose values of common beans upon fermentation (Starzynska, Bozena, & Mickowska, 2014). LAB contains α galactosidase enzyme which potentially enables them to utilize verbascose sugars. However, there was variation in the utilization rate of verbascose (Table 2) among the fermenting LAB which could be due to differences in the expression of the α-galactosidase enzyme.

Fermentation triggered significant reduction ($p<0.05$) in stachyose values for the three bean milk, with the highest reduction of 77.8% observed in milk extracted from pinto bean variety fermented with ABT culture. Stachyose could have been hydrolyzed by α-galactosidase into sucrose and galactose, and the latter metabolized through the galactose-utilization system (Da et al., 2006). Additionally, significantly higher ($p<0.05$) stachyose value was found in milk extracted from red haricot beans fermented with YF L903. This could be an indication that the ability of fermenting microorganisms to hydrolyze bonds in oligosaccharide moieties is dependent on enzymatic properties of the bacterial strain and the efficiencies of the α-galactosidase activity of that particular strain. Thus, appropriate selection of the fermenting culture is a necessity in reducing stachyose in fermented bean milk.

4. Conclusion

Fermentation with each of the four cultures increased pyridoxine, niacin and folic acid concentrations of the three bean milks. However, thiamine was non-quantifiable in fermented milks while riboflavin values were lowered for all the fermenting cultures, except ABT culture. This implies that combination of probiotic strains of *Lactobacillus acidophilus La-5 + Bifidobacterium animalis Bb-12 + Streptococcus thermophilus* could be exploited for natural fortification of riboflavin in bean milk. It was also observed that fermentation significantly lowered the oligosaccharide compositions of stachyose, raffinose and verbascose. Thus, fermentation of bean milk with any of the four cultures could be utilized for removal of the flatulence causing oligosaccharides.

Authors' contribution

CA and AO participated in bean milk development and experimentations. SI and JM participated in conceptualization of the research design and performing the experiments. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Availability of data and materials

All the data and material supporting the conclusion of this work are included within the manuscript. Additional information may be provided on request by the corresponding author.

Consent for publication

All authors have agreed to submit the manuscript in its current form for publication in the Journal of Food Research.

Ethical approval and consent to participate

Not applicable. No tests, measurements or experiments were performed on humans as part of this work.

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Analysis of Health Risk Factors in the Vegetable Production Chain in the City of N'Djamena-Chad

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Abstract

Several market gardeners have settled in the city and supply urban markets with fresh vegetables throughout the year. Despite their nutritional importance, market gardening products may carry health risks. The objective of this study is to identify and analyse the potential risk factors that could lead to the appearance of microbiological and physicochemical hazards in the production chain of fresh vegetables from these market gardening operations. The work was carried out in 5 permanent market gardening sites in the city of N'Djamena (Chad, Africa) and involved 96 market gardeners surveyed. Data related to production methods were collected. Standard methods were used to carry out microbiological analysis tests on 15 samples of vegetables and fruits taken from 3 sites.The results of the survey show that urban market gardening in N'Djamena is dominated by two plant species: lettuce (*Lactuca sativa*) and rocket (*Eruca sativa*). It is geared towards the production of leafy vegetables. The health risks associated with the conditions of production are numerous and real: the proximity of roads, the use of dirty water for irrigation, the overdose of chemical fertilizers (urea) and pesticides, and finally the unhygienic harvesting and transport. The high-water content of fresh vegetables and the lack of processes for the elimination of pathogenic microorganisms also do not guarantee the sanitary quality of the vegetables produced and can thus increase the risk of foodborne infections. The results of the microbiological evaluation showed the presence of germs pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Aeromonas spp*. and *Salmonella sp.* in vegetable and fruit. Therefore, the best strategy to obtain a healthy product is to educate producers on good agricultural practices including reasoned fertilization, clean water, treated wastewater, approved pesticides.

Keywords: food, market gardening, fresh vegetables, health risks, N'Djamena

1. Introduction

In Chad, as in many other countries, there is the practice of agriculture around cities. Urban populations, because of new lifestyles and cultural mixing, are looking for a diversification of their consumption, mainly on fresh, perishable products: vegetables, fruits, animal products (Temple & Moustier, 2004). Although it is constituted by a variety of agricultural and pastoral activities that can take place within the limits or periphery of urban agglomerations (Smith et al., 2004), market gardening has taken an important part in this type of agriculture. Many market gardeners have settled in cities and around cities and are supplying urban markets with fresh fruits and vegetables throughout the year. This form of exploitation of the environment represents a major challenge in terms of employment, living environment, waste management and the supply of fresh produce to cities (Moustier, 1990), thus creating an important base improvement of the food and nutritional situation of urban populations in these areas. At the microeconomical level, it is an important source of income for the poorest households in urban areas (Golhor, 1995).

The city of N'Djamena in Chad, like most cities in developing countries is home of several basins of production of fresh vegetables. Market gardening has played an important role in this agriculture. Market gardening provides city dwellers with fresh vegetables, which have become almost indispensable in their daily diet (Nazal

et al., 2017). In contrast to the seasonal production of food crops in the countryside, urban and peri-urban vegetable production is used throughout the year using intensive production techniques (irrigation, organic and mineral fertilizers, plant protection products, etc.) on small areas. As a result, this production ensures a constant supply of various vegetables to meet the demands of the urban population and, as such, contributes to food security. In addition, the activity provides regular income to producers of various origins.

However, these various assets that militate in favour of its valuation, market gardening in the city of N'Djamena, is marginalized in urban planning policies. This is why land is today a major constraint to its development. This climate of land insecurity coupled with the flooding of some production areas for a large part of the year, does not reassure producers to invest in modern and sustainable tools, leading to major changes in their farming practices. To maintain and increase their production in the face of strong consumer demand, market gardeners are forced to intensify their production, use pesticides and wastewater in some sites. The practices of this type of production system involve economic and health risks that must be prevented and controlled (UNDP, 1996). It is not uncommon also to note that certain practices of harvesting and transporting market garden products are done in unsatisfactory conditions in view of the rules of hygiene. These poor practices could introduce pathogens into the products and thereby expose consumers to a possible hazard.

Consumers expect to be protected from the risks present all along the food chain, from the primary producer to the consumer: often referred to as "from farm to table" (FAO/OMS, 2003). Therefore, our study proposes to identify and analyse all the relevant risk factors that could lead to the appearance of microbiological and physicochemical hazards on fresh vegetables from vegetable farms in urban and peri-urban areas of N'Djamena to take preventive measures to have healthy products.

2. Methods

This study was conducted in N'Djamena, the capital of Chad. Located in the center-west of the country, at the confluence of the rivers Chari and Logone, the city of N'Djamena, which concentrates nearly 40% of the urban population, knows a perpetual growth characterized by a strong galloping urbanization. Microbiological analyzes were carried out at the Food Sciences and Nutrition Research Laboratory (LARSAN). After an exploratory study in the study area and documentary research on market gardening systems in N'Djamena, the various market gardening sites were listed and the large sites serving the major markets were selected for considering sampling:

- Access to water: sites were selected to include different water sources;
- The importance of the site: this criterion considers the number of producers operating on the site or its importance in terms of area;
- And finally, the permanence of market gardening activities in the site.

This allowed to identify 5 vegetable production sites (figure 1) as follows: Djamba Ngato Airport (1), Djamba Ngato Base (2), Sabangali (3), Habena-Double lane (4) and finally Habena-Komé (5).

For microbiological analyzes, a total of 15 samples of vegetables and fruits were randomly collected on 3 sites, or 5 units per site. They were stored in coolers at 4 °C and transported aseptically to LARSAN Laboratory.

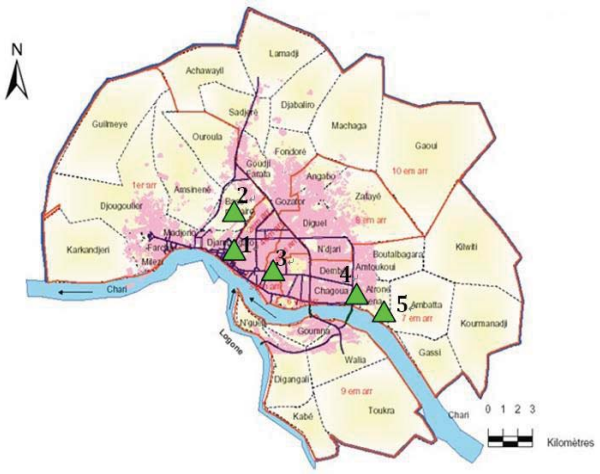

Figure 1. Location of the market gardening; zones surveyed 1. Djamba Ngato Airport; 2. Djamba Ngato Base; 3. Sabangali; 4. Habena-Double lane; 5. Habena-Komé

The microbiological evaluation was performed according to standard methods. Also supports relating to these

standards are used (Leyral & Vierling, 1991; AFNOR, 2002). *Escherichia coli*, *Aeromonas spp., Staphylococcus aureus* and *Salmonella sp*. germs were sought. Vegetable samples are taken at maturity. Samples of 5 units corresponding to a test portion of 100 g were made. Then, after grinding with a Blender® type mill at 1500 rpm, 10 g of each ground material are suspended in 90 ml of tryptone-salt solution containing 0.03 g / l of tween 80 ; then the revivification is done for 45 min at laboratory temperature (25 ° C). The yellow colonies on the agar Hektoen and colonies with metallic reflections were suspicious of *E. coli* after 18 to 24 hours of incubation at 37°C. The colonies of *S. aureus* presented yellow color on Chapman agar. *Aeromonas spp*. was carried out on Pril-xylose-Ampicillin (PXA) agar, clear red colonies are suspected of *Aeromonas spp*. For the Salmonella search, 25 g of sample were homogenized in 225 ml of buffered peptone water. The identification was made by API 20E (BioMérieux, France).

Data collection is based on individual questionnaire surveys, 96 questionnaires were administered to randomly selected producers representing 40.9% of the total agricultural population listed (Table 1). The data collected focused on production techniques, input management, water use and the geographical location of this activity.

The collected data were entered on Excel XLSTAT software, processed and analyzed by descriptive statistics to determine averages and frequencies. Statistical analysis was performed using the chi-square test (χ^2) for comparison of two variables. Differences were considered significant when $P \leq 0.05$.

Table 1. Distribution of market gardeners surveyed by production site

Site	Total number of producers surveyed	Number of producers surveyed
Djamba Ngato Airport	42	18
Djamba Ngato Base	113	46
Sabangali	35	12
Habena Double line	25	11
Habena-Komé	20	9
TOTAL	235	96

3. Results

3.1 Types of Vegetables Produced

This study identified 11 vegetable species in the study area (Table 2). However, 2 species were identified as the main vegetables grown by market gardeners: lettuce (*Lactuca sativa*) and rocket (*Eruca sativa*). Indeed, in the various production sites studied, all market gardeners produce at least lettuce or rocket. Some produce both at the same time. Other plant species are grown at low proportions. They are grown for self-consumption or for certain expenses specific to each market. The number of species cultivated per market gardener varies from 1 to 4. The local names of the different vegetables produced and their uses are shown in the table below (Table 2).

Table 2. Vegetables encountered in the different production perimeters

Scientific Name	Family Name	English Name	Local Name (Local arabic)	Consumed Body
Lactuca sativa	*Asteraceae*	Lettuce	*Salade*	Leaf
Eruca sativa	*Brassicaceae*	Rocket	*Djir-djir*	Leaf
Hibiscus sabdariffa	*Malvaceae*	Sorrel	*Karkandji*	Leaf, fruit
Daucus carota	*Apiaceae*	Carrot	*Carotte*	Root
Brassica oleracea	*Brassicaceae*	Cabbage	*Chou*	Leaf
Allium cepa	*Liliaceae*	Onion	*Bassal*	Bulb
Hibiscus esculentus	*Malvaceae*	Okra	*Daraba*	Fruit
Petroselinum sativum	*Umbellifers*	Parsley	*Persil*	Leaf
Basella alba	*Basellaceae*	Spinach	*Épinard*	Leaf
Apium glaveolens	*Umbellifers*	Celery	*Céleri*	Leaf
Capsicum annuum	*Solanaceae*	Green Pepper	*Poivron*	Fruit

Depending on the organs consumed (Table 2), vegetable production is oriented towards the production of leafy vegetables (63, 6% of species).

3.2 The Geographical Location of the Production Sites

The survey showed that the vegetable production sites in the study area are located either along the waterways (Habena-Double Lane and Habena-Komé) or in empty state areas (Djamba Ngato-Aéroport and Djamba

Ngato-Base) or in fenced and unopened plots (Sabangali). All market gardening perimeters are located not far from the roads. The average distance between the road and crops is estimated at 30 m with a minimum distance of 50 cm at Djamba Ngato-Airport (Figure 2a) and a maximum distance of 100 m at Habena-Komé.

3.3 Cultural Practices in Urban and Peri-urban N'Djamena

Agricultural practices are considered as all the processes and ways of acting of the farmers (Milleville, 1987) implementing a technical operation. In this study carried out in the city of N'Djamena, it was limited to the analysis of agricultural practices that could have repercussions on the hygienic quality of market garden products and thus the health of the consumers, namely: crop irrigation, improving soil fertility and phytosanitary protection of plants.

3.3.1 Crops Irrigation

In the study area, surface waters (river), groundwater (boreholes, wells) and sewage (sewage) are the sources of water supply for market gardeners. Producers at the same production site use the same source of water. Thus, those in Habena-Komé use surface water; groundwater is used in Habena-Double lane, Sabangali and Djamba Ngato-Base, and producers in Djamba Ngato-airport use wastewater (Figure 2b). As a result, the surveys show that most of the sites (71.9%) draw their irrigation water from the water table (borehole or open well). Sewer water is used by 18.8% of market gardeners (Figure 2b). Although the city of N'Djamena is crossed by two rivers, only 9.4% of producers irrigate with water from the river. Our survey showed that for the water supply to the plants, all growers use manual watering with a watering can (Figure 2b).

3.3.2 Improvement of Soil Fertility

Soil fertilization is done through organic and inorganic fertilizers. Cow dung is identified as the main source of organic matter used by 18.8% of market gardeners in vegetable production. This organic fertilizer is brought once at the beginning of the cycle. Its application is direct without composting. It was observed the practice of burying crop residues in the soil during plowing.

If the use of organic fertilizers is not widespread, the supply of mineral fertilizers is systematic in all sites. Urea, a mineral fertilizer composed mainly of nitrogen, is the main source of inorganic material used by market gardeners in vegetable production. Spreading on crops is in solid form (Figure 2c). All gardeners use at least once per cycle.

Figure 2. Diversity of health risks. Legend: a. Proximity of the road to Djamba Ngato-Airport; b. Use of wastewater at Djamba Ngato-Airport; c. Spreading Urea (Habena Double-way); d. Phytosanitary treatment with Perfect Killer (Habena Double-way)

3.3.3 Phytosanitary Protection of Crops

Chemical control is the only method used by market gardeners in the study area. In order to meet growing demand and achieve economically viable production levels, market gardeners use several types of pesticides (Figure 3).

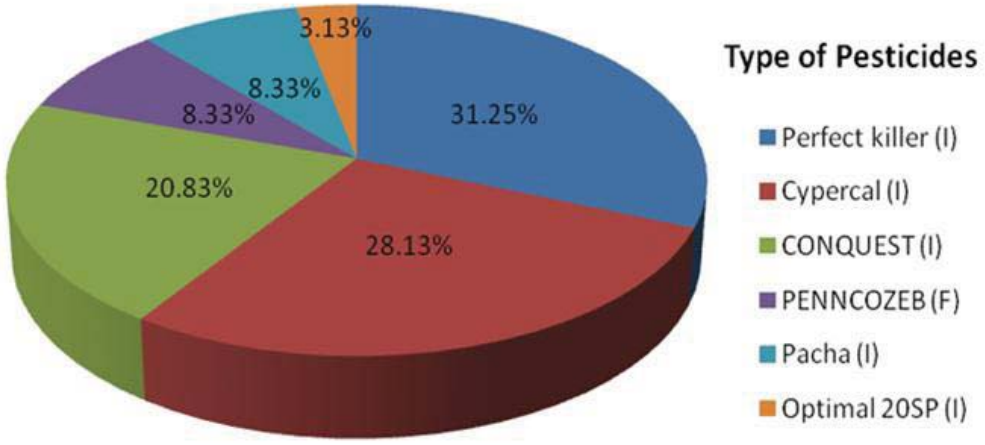

Figure 3. Phytosanitary products used in market gardening (I: Insecticide, F: Fungicide)

The insecticide (Perfect Killer; Cypercal; Conquest; Pacaha et Optimal 20 SP) are the pesticides used. However, Perfect Killer is the most represented pesticide ($P<0.001$), while the fungicide is only represented by Penncozeb 80 and is used by only 8.33% of producers located in Habena (difference not significant $x^2= 3.2$, ddl= 1; $P> 0.05$). This study identified a single mode of use of these phytosanitary products in the production system: treatment by a hand-held sprayer (Figure 2d). It consists of dissolving the product with water inside a 1.5l bottle before starting the operation. The measure used to apply pesticides on farms is the bottle cap (two caps per 1.5L bottle). The number of applications per cycle varies according to the producers' experiences. The treatment is done whenever there are insects on the plant. Finally, the harvest is made without respect of the withdrawal period (the last treatment is made one to two weeks before the harvest of the vegetables).

3.4 Harvest and Transport of Finished Products

The harvest is the end of the cultivation period and the beginning of the preparation for the market. Fresh vegetables for market or own consumption are harvested by hand.

These freshly harvested vegetables are soaked in irrigation water before being packed in fully or partially closed bags. Transportation to the markets is done using motorcycles.

3.5 Evaluation of the Microbiological Quality of Market Gardening Products

Table 3 shows the prevalence of pathogens isolated in market garden products. Microbiological analysis of vegetables shows a high burden of pathogenic germs (Table 3). Lettuce and sorrel show high microbial loads with proportions of *Escherichia coli* and *Salmonella spp.* respectively 46.7% and 7% ($x2 = 39.459$, ddl = 1, $p = 0.001$).

Table 3. Prevalence of isolated pathogens in market garden produce

Analyzed part	Market garden products	Number of germs UFC/g per sample (%)			
		E. coli	*Aeromonas spp.*	*Salmonella sp.*	*S. aureus*
Leaf	Lettuce	7 (46.7)	2 (13.3)	1 (7)	3 (20)
	Rocket	1 (7)	0 (0)	0 (0)	0 (0)
	Celery	2 (13.3)	1 (7)	0 (0)	1 (7)
	Parsley	5 (33.3)	0 (0)	0 (0)	0 (0)
	sorrel	7 (46.7)	0 (0)	1 (7)	0 (0)
	Spinach	1 (7)	0 (0)	0 (0)	0 (0)
	Cabbage	6 (40)	0 (0)	1 (7)	0 (0)
Fruit	Okra	2 (13.3)	0 (0)	0 (0)	1 (7)
	Green beans	3 (20)	0 (0)	0 (0)	3 (20)
	Green pepper	2 (13.3)	0 (0)	0 (0)	2 (13.3)
Root / bulb	Carrot	3 (20)	0 (0)	0 (0)	3 (20)
	Green onion	3 (20)	0 (0)	0 (0)	5 (33.3)

4. Discussion

The study made it possible to analyze, in all the selected production sites, the relevant risk factors that could lead to the appearance of microbiological and physicochemical hazards on fresh vegetables. These main factors identified, which could be at the source of the risks to human health, are grouped in three (3): the environment of the farms, the agricultural practices and finally the harvesting and the transport. This study first shows a variety of vegetables produced by market gardeners, dominated, on occasion, by lettuce and rocket. Depending on the organs consumed, market gardening is geared towards the production of leafy vegetables. The species cultivated for their leaves are the most represented compared to those cultivated for their fruit, root or bulb.

The results of our research conducted in five (5) vegetable production basins of the city of N'Djamena reveal that behaviours are not without consequences on the health of consumers. First, the proximity of roads is a source of contamination by heavy metals, pathogens, dust and impurities. With regard to irrigation, the origin, type and quality of water, the type of plant, the method of irrigation and the type of irrigation (measure of exposure of the edible part of the plant to water) and the period between the last irrigation and the harvest (possible disappearance of microorganisms for example by photodegradation, degradation by soil microorganisms, transformation by the plant, etc.) play an important role (Allende & Monaghan, 2015; AFSCA, 2009; Uyttendaele et al., 2015). Three main risk factors related to irrigation water used in production basins in the study area were identified: water source, irrigation method and type of plants. The results of the investigation showed that the producers of the same production site use the same source of water. This is why in our study area, the health risks vary according to the geographical location of the production sites. Wastewater is known to be more susceptible to contamination by pathogens than groundwater and surface water. The reuse of partially or untreated wastewater in agriculture is widespread in African cities (Cissé et al., 2002) and according to FAO (2007), 200 million urban farmers worldwide would use wastewater, untreated or partially treated. The use for irrigation of sewage collected on the sewer system, exposes the production to health risks via certain metals and metalloids (copper, molybdenum, nickel, selenium, and zinc) which certainly are essential to the good plant growth, but are toxic at high concentrations. Qadir et al. (2000) found that in the case of irrigation with raw sewage, leafy vegetables accumulated certain metals such as cadmium in larger quantities than leafless vegetables. Sharma et al. (2007) concluded that sewage irrigation increased the contamination of the edible parts of vegetables by cadmium, lead and nickel, and that this poses long-term potential health risks. In addition to the risks they pose to consumers, the heavy metals they contain can also increase plant susceptibility to disease and pests, generally resulting in excessive pesticide use that is responsible for plant residues of pesticide residues in quantities greater than the acceptable limits. The risk of contamination of crops following sprinkler irrigation is also higher than the cases where a drip system is used. Our survey showed that all growers use sprinkler as their only irrigation method. On the other hand, the degree of risk is not the same from one culture to another. In fact, leafy vegetables do not carry the same risk as other types of vegetables. Pazou Yehouenou et al. (2010) reported in their study the presence of heavy metals and nitrates in vegetable crops at various concentrations. To intensify their productions, gardeners use a large amount of fertilizers. The overdose practices observed highlight the risks of over-fertilization nitrogen leading to a build-up of nitrates in the leaves of vegetables. This cannot remain without consequences on the health of consumers. Nitrates as such are not dangerous to health, but ingested by humans, they are degraded by a bacterium and turn into nitrites. Historically, nitrates and their derivatives have

been implicated in the occurrence of acute intoxication, methemoglobinemia, in newborns and in the occurrence of long-term cancers, particularly digestive (Testud, 2004 ; Vilaginès, 2003). The use of pesticides reduces crop losses due to pests and stabilizes yields. However, their uncontrolled use can be a source of harm to human health and the environment (Kanda et al., 2014). The residues of pesticides inevitably constitute risks of poisoning in the short, medium or long term for humans (Mondedji et al., 2015). In our study area, a variety of pesticides are used inappropriately, and the harvesting of vegetables is done without respect of safety time. They leave, inevitably, residues that could harm the health of consumers. Other studies (Farag and al., 2011; Touré et al., 2015; Bakary et al., 2019) mentioned in their work the risks associated with the use of pesticides that can lead to metabolic diseases such as cancer. While the use of these products is often necessary for producers to achieve their production objectives, it is important to remember that pesticides are toxic and their use can only be accepted or encouraged if they are fully controlled. Use and risks to human health and the natural environment that may be affected (Devillers et al., 2005). Pesticide leaves, inevitably, residues that could harm human health and the environment. This mortgages the quality of vegetables because horticultural products must meet strict quality standards, particularly with regard to maximum pesticide residue limits. Integrated pest management reduces the number of chemical interventions and produces healthy foods that meet established standards (Colignon et al., 2000). Biopesticides could be an alternative to the misuse of synthetic pesticides. Among new crop protection technologies, the use of effective and less toxic botanical insecticides would be an alternative to the use of synthetic pesticides in the control of insect pests (Cloyd 2004; Charleston et al., 2005, Shannag and al., 2014). In terms of harvesting and transportation, there are four (4) potential risks that can affect the hygienic quality of finished products:

• Although this manual may occur during this stage, contamination due to poor hygiene.

• After cutting, the release of cellular liquids plant offers a favourable nutrient medium for the growth of microorganisms exposing consumers to possible danger.

• Rinse water from freshly harvested vegetables is also a potential source of pathogen contamination. If these agents survive on the products, they can threaten the health of the consumer and cause food poisoning.

• During transportation to urban markets, fresh vegetables may also be contaminated by lack of consistent packaging and unsuitable means of transport. This contamination can be physical as well as microbiological.

The results of the microbiological analyze show that the high levels of bacteria indicating fecal contamination coincide with the sites where the producers irrigate with the wastewater. Indeed, the site most loaded with pathogenic bacteria was that of Djamba Ngato where isolated bacterial species were isolated from the samples with a high contamination in proportion of *E.coli* and *S. aureus*. Other authors (Barro et al., 2007, Mayoré et al., 2018) indicated that *E. coli* is an indicator of faecal contamination. For *S. aureus*, this germ is commonly involved in food poisoning due to the production and their toxins are responsible for animal and human disease (Pereira et al., 2017). The presence of *E. coli* and *S. aureus* in vegetables is thought to be due to the unsanitary environment, the use of dirty water and the poor hygienic practices of the staff. Meldrum et al. (2016) in UK also isolated *E. coli* and *S. aureus* in samples of vegetables such as salads and in sauces used for the preparation of salads. Also, several authors (Baba-Moussa et al., 2006; Tidjani et al, 2016; Doutoum et al., 2019) have found these germs in food. The identification of these pathogens confirms the direct contamination of vegetables produced by irrigation sewage or by open defecation. In principle, the raw fruits are not likely to allow the growth of pathogenic microorganisms when they maintain the integrity of their envelope. But if they are poorly sorted, transported, stored in poor hygienic conditions and poorly cleaned, they constitute a source of microbial contamination (ACIA, 2012). *Aeromonas spp*. was found with a proportion of 13.33% in salads. The presence of these bacteria in the purified effluents, sometimes at concentrations higher than those of faecal coliforms, poses a problem of sanitary interest (Maalej et al., 2002). Vegetables are often watered by river water or effluents. For salmonella, they were found only in two vegetable samples. These results corroborate with those of Traoré et al. (2015), who also identified salmonella in salads but with a high proportion (50%).

5. Preventive Measures

In urban and peri-urban vegetable farms in the city of N'Djamena, the mechanisms by which vegetables may be contaminated are complex (Table 4). Their high-water content, the absence of a lethal process such as cooking to eliminate pathogenic microorganisms, also do not guarantee the sanitary quality of the vegetables produced and can thus increase the risk of intoxication. As a result, the prevention of contamination of vegetables by pathogenic microorganisms (*Escherichia coli*, *Aeromonas hydrophila, Aeromonas sobria, Salmonella spp., Staphylococcus aureus*) and residues of harmful chemicals (heavy metals), is the most effective way to ensure the safety of these products generally consumed raw. Several authors (Atolaye et al., 2007 ; Katemo Manda et al.,

2010 ; Djibrine et al., 2018)) indicated that heavy metals are among the main pollutants in the environment, with a high potential for toxicity in animal species. Special precautions then deserve to be taken before the consumption of vegetables and fruits. The washing of vegetables and fruits with drinking water and their disinfection would be necessary to prevent food poisoning and protect the health of consumers. This can be accomplished by means of fundamental preventive approaches, such as good agricultural practices (reasoned fertilization, clean water, treated wastewater, approved phytosanitary products, among others.) and the implementation of the system HACCP (Hazard Analysis and Critical Control Point).

Table 4. Potential Risks of Contamination of Leafy Vegetables and Preventive Measures

Production stage	Risks / Potential dangers	Prevention
Place of production	Eventual contamination by heavy metals, pathogenic germs, dust and impurities due to the proximity of roads.	– Maintain the sites; – Set up a protection hedge.
Inorganic fertilization	Possibility of introduction of heavy metals due to non-compliance with fertilizer use requirements	Determine the right quantity and the best adapted product as well as the optimal date and the good location of the input (reasoned fertilization)
Irrigation	Possible contamination by pathogenic microorganisms present in the water and by the presence of heavy metals.	Treat sewage water
Phytosanitary protection	Application of unapproved products that exceed the maximum pesticide residue limit and pre-harvest timelines	Use pesticides registered for cultivation; – Scrupulously respect the instructions for use; – Respect the dosages of the active ingredients and the deadlines before harvesting; – Use biopesticide and integrated pest management
Harvest	Introduction of pathogenic microorganisms attributable to unhealthy producers or rinsing water; Wash hands before harvest	Rinse with fresh water fresh vegetables once harvested
Transport	Contamination by pathogenic micro-organisms, chemicals and / or foreign objects (sand, insects, plant debris, stones, ...)	Transport the products in transport vehicles and containers intended to receive the products Destroy vegetables with diseases, damaged ...

6. Conclusion

This study has made the possible to better understand the risk factors for microbiological and physicochemical hazards in the N'Djamena urban and peri-urban vegetable production chain and to draw the consequences for human health. The results show that the environment of the production basins, the current farming practices and the transport of fresh vegetables to markets contribute to the deterioration of the sanitary quality of the vegetables. In fact, practices of over-fertilization with mineral fertilizers, inappropriate or inappropriate use of plant protection products, the use of wastewater noted during the surveys, are not likely to guarantee the hygienic quality of the products resulting from the urban and peri-urban vegetable farms. The analysis of the microbiological quality of the fresh vegetables produced and fruits highlighted the presence of pathogenic microorganisms in the samples analyzed. The risks of intoxication of consumers are large and real. But the consumer has no way of detecting the presence of dangerous substances in food and it completely depends on the seriousness and responsibility of all members of the production and distribution chain. A combined action of different actors in urban agriculture is essential for sustainable food security in urban and peri-urban areas in the Sahelian zone such as N'Djamena. In perspective, it will be necessary to consider another study for in-depth knowledge of hazards in the production chain of vegetable crops.

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Assessment of the Risk of Microbial Contamination of an Urban Crop in the City of Daloa (Côte d'Ivoire): Case of Lettuce (*Lactuca sativa* L.)

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Abstract

The growing population of the city of Daloa, together with its growing urbanization, has a diversified need for food resources. Urban and periurban production of lettuce is a food resource for its people. This study was designed to assess the risk of microbial contamination. For the conduct of the study, first a survey was carried out on different sites of urban production to know the technical process of production. Then, mature lettuce, ready for sale and then consumed from a site, was subjected to microbiological characterization according to the standards in force. Microbiological analyzes revealed a high contamination. For mesophilic aerobic germs, the charges in CFU/g ranged between $1.7x10^7$ and $6.7x10^7$. The loads in CFU/g for yeasts and molds ranged from $3.4x10^5$ to $9.3x10^5$. As for fecal coliforms and enterobacteria, their loads ranged between $1.2x10^5$ to $2.8x10^5$ CFU/g for the first and $7.2x10^5$ to 10^6 CFU/g for the second. These samples were contaminated with both *Escherichia coli*, *Staphylococcus aureus* c + and *Salmonella* sp. The loads in *E. coli* and *S. aureus* c+ ranged between $9.4x10^4$ and $1.8x10^5$ for the first and $4x10^3$ to $1.1x10^4$ CFU/g for the second. *Salmonella* sp. was found with loads ranging from $6.1x10^4$ to $8.2x10^4$ CFU/g. Empirical production process would increase the risk of microbial contamination. It is necessary to produce healthy lettuce for Daloa's consumers.

Keywords: contamination, *Lactuca sativa*, Daloa, Côte d'Ivoire

1. Introduction

Rapid urbanization and the strong concentration of urban populations have spawned a new form of agriculture, practiced in the urban and periurban areas of the major cities of West Africa. In Côte d'Ivoire, like other African countries, this new agriculture, dominated by market gardening, is booming (Dongmo *et al*., 2005; Soro *et al*. 2008; Ba & Aubry, 2011). This urban and periurban agriculture is a revenue and employment generating activity practiced by urban vulnerable groups; thus contributing to the food security of the populations (Gomiero *et al*., 2011; Koffi *et al*., 2012; Loudit *et al*., 2017; Ba & Cantoreggi, 2018). Daloa, the third most populous city in Côte d'Ivoire after Abidjan and Bouake, covering an area of 5.305 km^2 and an estimated population of more than 288 000 in habitants, with its many administrative changes (commune, sub-prefecture, department and region, university town), is also confronted with this new form of agriculture (RGPH, 2014; Zah, 2015). This agricultural activity often practiced in the city center, faces enormous difficulties such as the thorny problem of urban pollution and the proximity of garbage and waste disposal sites of all kinds. Unfortunately, crops are being grown in difficult contexts, marked in particular by the lack of financial means for the supply of drinking water and synthetic fertilizers for soil fertilization; this often pushes market gardeners to use wastewater for irrigation and animal manure as fertilizer for the soil. These farming practices could favor a high contamination of soil and

production tools by microorganisms, some of which may be harmful to human health according to several studies (Koffi *et al*., 2012; Pereira *et al*., 2013; Woldetsadik *et al*., 2017). Productions resulting from these cultivation practices, especially market garden produce, contaminated by pathogens can be dangerous for the consumer (Koffi *et al*., 2011; Alio *et al*., 2017). Based on the work of Patterson *et al*. (2010) and those of Blaak *et al*. (2015), the consumption of such cultures constitutes a potential risk factor for infection. Contamination of vegetables is one of the potential risks of infection with enteropathogenic bacteria such as *Salmonella* and *Escherichia coli*. This contamination occurs from an environmental, animal or human source at the time of planting, harvesting or handling vegetable prior to consumption (Cobbina *et al*., 2013; Wognin *et al*., 2013). Considered as a contamination of plant surfaces, recent work has shown that certain pathogens such as *salmonella* are able to infect and multiply in the mesophyll of certain plants such as lettuce (Kroupitski *et al*., 2009; Guchi *et al*., 2010; Pelletier *et al*., 2011; Schikora *et al*., 2011). In Daloa, lettuce is cultivated in urban, peri-urban areas on marshy sites and in shallows at the very heart of some of the central districts of the city. Thanks to its incomparable nutritional richness and the fibers it contains, lettuce (*Lactuca sativa L*.) is of paramount importance in the dietary habits of the population and also for the proper functioning of the organism (Zhang *et al*., 2009; Berger *et al*., 2010; Pereira *et al*., 2013). It is one of the most popular and most consumed vegetables in the world, often the main ingredient in salads (Koffi *et al*., 2011; Maffei *et al*., 2013). However, to our knowledge, no exhaustive study of the production route, the microbiological quality of the urban production lettuce in Daloa has not been the subject of any scientific study. In addition, there is little data available on urban lettuce producers. The overall objective of this study was to assess the risk of microbial contamination of lettuce produced in the city of Daloa. The information obtained can be used to sensitize producers to improve the route and sanitary quality of lettuce (*Lactuca sativa* L.) produced in the city of Daloa or in other cities where urban and periurban agriculture were practiced.

2. Method and Material

2.1 Presentation of the Study Site

The study area is the town of Daloa, located in the central west of Côte d'Ivoire between 6°3' north latitude and 6°27' west longitude. The study site is a low-lying geographic coordinates 06°27'33.52" west longitude and 06°53'35.92'' latitude north, located in the center of the city. **Figure 1** below shows the geographical location of the study area.

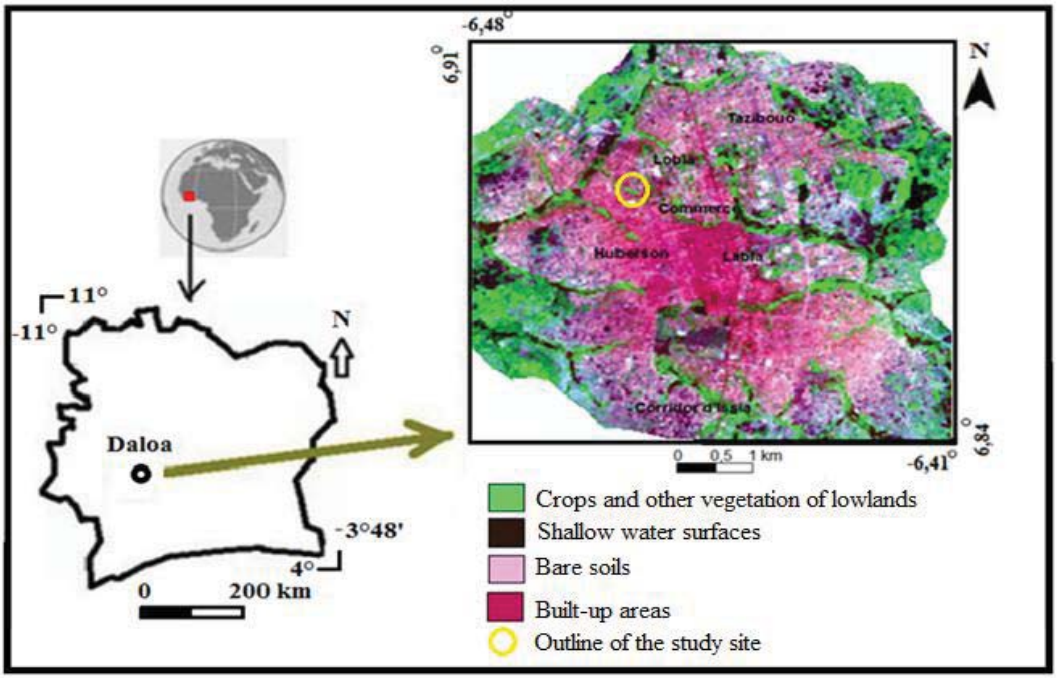

Figure 1. Map of the city of Daloa presenting the study site

2.2 Diagnosis and Characterization of Urban Production of Lettuce in Daloa

A survey with a questionnaire was developed to collect information on urban lettuce production sites in the city

of Daloa. This survey first gave information on the profile of the producers (gender, age, nationality, level of study) and then on the technical itinerary of the urban production of lettuce (source of irrigation water, type amendment). The survey was conducted from 25-10-2017 to 28-12-2017 at four urban and periurban production sites in the city of Daloa. The people surveyed are of two kinds, of all levels of study and of all social strata. In total, the survey covered 31 lettuce producers.

2.3 Microbiological Characterization

2.3.1 Sampling

On the site, three different lettuce beds are a study block. For each block, were selected three different samples composed of three feet of lettuce at maturity taken at random on the three boards. These harvested samples are packaged in stomacher sachets. Samples once taken are stored in a cooler with dry ice and transported to the laboratory for analysis. These analyses were done at the Laboratory of Host-Microorganisms and Evolutions Interactions (LIHME) of University Jean Lorougnon Guede.

2.3.2 Methods of Analysis

Several methods were used in this study. Buffered Peptone Water (BPW) broth was used in the preparation of stock solutions as described in ISO 6887-4: 2011. Decimal dilutions were performed with Tryptone Sel broth as recommended in ISO 6887-1: 1999. Plate Count Agar (PCA) was used to count mesophilic aerobic flora at 30°C for 72 h as recommended in NF/ISO 4833: 2003. Enterobacteria count was performed at 37°C for 24 h on Violet Red Neutral Bile Glucose (VRBG) agar according to ISO 21528-2: 2004. Violet Neutral Bile Lactose (VRBL) agar was used for fecal coliforms count at 44°C for 24 h as described in ISO 4832: 2006. For the search, isolation and enumeration of *Salmonella* sp, media Buffered Peptone Water (BPW), broth Rappaport of Vassiliadis Soya Broth and Hecktoen Enteric Agar were used as described in the reference standard NF/ISO 6579:2002 Amd 1: 2007. Baird-Parker Agar with Telluride Egg Yolk and 0.2% Sulphamethazine served the identification of *Staphylococcus aureus* at 37°C for 48 h according to the French standard NF/ISO 6888: 2004. Rapid'E coli 2 agar served the isolation and enumeration of *Escherichia coli* at 44°C for 24 to 48 h as recommended in standard NF/ISO 16140: 2013. Yeasts and molds were counted with Sabouraud agar chloramphenicol 25°C for 5 days according to the NF/ISO 16212: 2011 standard. The different culture media used were prepared according to the manufacturers' instructions.

2.3.2.1 Preparation of the Stock Solution and Decimal Dilutions

Twenty-five grams (25 g) of lettuce's sample was aseptically transferred to an Erlenmeyer flask containing 225 mL of sterile (BPW) medium to prepare the stock solution. Everything is carefully mixed, taking care to soften the leaves with the fingers 2 to 3 minutes. After a 1 hour rest on the bench at room temperature, the stock solution was decimally diluted in sterile Tryptone Salt medium up to 10^6.

2.3.2.2 Inoculations and Incubations

Research of mesophilic aerobic germs, yeasts and molds, fecal coliforms, enterobacteria and *Escherichia coli*

According to the prescriptions of the standards adopted, the pour plate method was applied. Thus, one milliliter (1 mL) of the diluted lettuce sample to be analyzed is aseptically transferred to a sterile Petri dish and mixed with 20 mL of the respective agar. After solidification, the dishes are inverted and incubated at temperatures as given in the respective standard. Three Petri dishes were inoculated per dilution. The characteristic colonies according to the different media are then counted taking into account the calculation standard (NF/ISO 7218: 2007).

Research of Staphylococcus aureus

The surface spreading method was used for the detection of *S. aureus*. It consisted of taking 0.1 mL of the stock solution or a dilution of the lettuce sample to be analyzed, using a sterile pipette, and transferring to a Petri dish containing the Baird Parker agar medium already poured and solidified. The dilution is spread on the agar using a spreader rake. These manipulations are all performed under aseptic conditions near the Bunsen burner flame. Petri dishes are then inverted and incubated at 37°C for 48 h. Two plates of Petri were seeded by dilution. Black colonies with a clear halo (action of lecithin) and an opaque zone (action of lipase) are counted (15-150 characteristic colonies) taking into account the dilution.

Highlighting *Salmonella sp*

It is done in three stages. Pre-enrichment is performed by incubating the stock solution at 37°C for 24 h. The enrichment consisted of taking 0.1 mL of the stock solution (pre-enriched) and transferred to a tube containing

10 mL of Vassiliadis Rappaport previously prepared and sterilized. After homogenization, the tube is incubated at 42°C for 24 h. Finally the isolation was carried out from the enrichment medium incubated on a solid selective medium: Hecktoen agar. A drop is taken using a Pasteur pipette and then seeded by streaks on the surface of the Hecktoen agar. The dish is incubated at 37°C for 24 h, and sometimes even for 48 h, in the absence of characteristic colonies after the first incubation. On Hecktoen agar, the typical *Salmonella* colonies observed are green or blue with a black center**.**

Enumeration

The number of Colony Forming Units per milliliter of sample (CFU/g) from the number of colonies obtained in the Petri dishes is carried out according to standard NF/ISO 7218: 2007.

$$N = \frac{\Sigma Ci}{(N_1 + 0.1N_2)\, d \cdot V}$$

ΣCi: Sum of characteristic colonies counted on all retained Petri dishes;

N1: Number of Petri dishes retained at the first dilution;

N2: Number of Petri dishes retained at the second dilution;

d: Dilution rate corresponding to the first dilution;

V: Inoculated volume (mL);

N: Number of microorganisms (CFU/g).

Standards for assessing the microbiological quality of lettuce

The microbiological quality assessment standards for lettuce are taken from the "Microbiological Criteria for Foodstuffs Guidelines for Interpretation of 2015 of Luxembourg"; supplemented by the normative reference of the microbiological criteria of human foods (C.E. n° 2073/2005).

Statistical analyses

Statistical analyzes were conducted with the Statistica, 99 Edition. The different parameters analyzed were then subjected to an analysis of variance (ANOVA) with the software Statistica, 99 Edition. For this purpose, a single-factor ANOVA and Duncan's multi-extended tests were used. ANOVA was used to test, on the one hand, the variability between the different samples. As for Duncan's test, he later made it possible to first locate the differences between the samples and then the differences between them. Statistical differences with P-values under 0.05 were considered significant.

3. Results

3.1 Characteristics of Urban Producers of Lettuce and Diagnosis of Their Production Process

The profile of the urban lettuce producers of the different sites investigated is summarized in **Table 1**. Lettuce is produced by both genera. It is dominated by the female gender (58.1 %) against 41.9 % for the male gender. The age of the producers varies between 30 and 60 years. They are mostly Ivorian (58.1 %) and have a low level of education**.**

Table 1. Profile of urban producers and characteristics of lettuce production

		Frequency	Percentage (%)
Age (years)	< 30 years	7	22,6
	30-60 years	14	45,2
	> 60	10	32,3
Gender	Male	13	41,9
	Female	18	58,1
Nationality	Ivorian	18	58,1
	Burkinabe	8	25,8
	Malian	5	16,1
Level of study	Illiterate	25	80,6
	Primary	3	9,7
	Secondary	3	9,7
Agricultural inputs	Surface water	31	100
	Poultry manure + chemical fertilizers	15	48,4
	Poultry manure + cow dung + chemical fertilizers	16	51,6

3.2 Technical Process of Urban Lettuce Production

The technical process of producing lettuce from the different study sites investigated is summarized in the diagram below (Figure 2). According to the survey, the quantities of chemical or natural fertilizer used are not quantified, so the earth planks for the crops are made by punching.

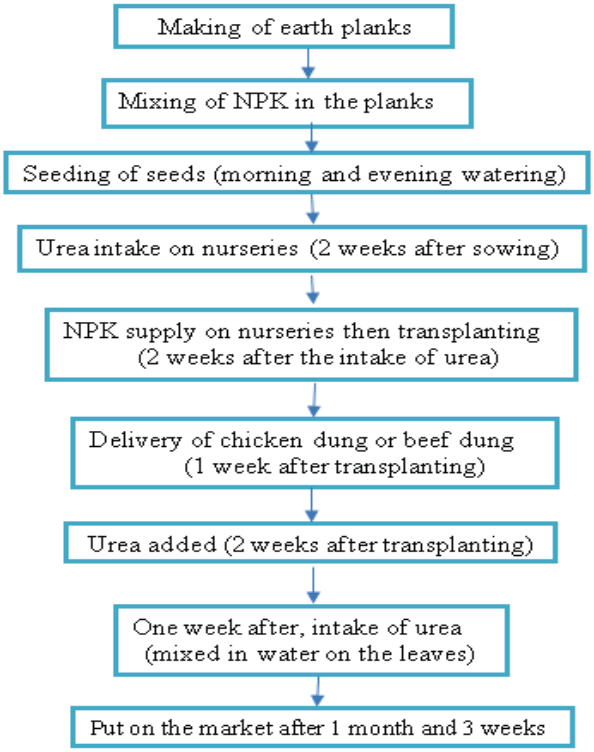

Figure 2. Urban production process of lettuce (*Lactuca sativa* L.)

3.3 Microbiological Characteristics of the Lettuce Produced

3.3.1 Presence of Microflora in Lettuce

The microbiological study assessed the level of microbiological contamination of lettuce produced in urban areas of Daloa. Microbiological analyzes made it possible to count microorganisms; major alteration microfloras and/or floras suggestive of a deficit in good production and hygiene practices of lettuce produced in urban areas in Daloa. These are fungal flora (yeasts/molds), mesophilic aerobic germs, fecal coliforms and enterobacteria. All samples from the study site were heavily contaminated by these different microfloras. In addition, all charges (CFU/g) of the flora were all well above the expected microbiological quality standards. The CFU/g load for yeasts and molds ranged from $3.4x10^5$ to $9.3x10^5$ while the standard predicts 10^4 (Figure 3). The loads of the fungal flora of the different study blocks were unequally distributed and therefore statistically different ($p > 0.05$) from one block to another. For mesophilic aerobic germs, the CFU/g load ranged from $1.5x10^7$ to $6.3x10^7$ while the standard indicated 3.10^6 (Figure 4). As for fecal coliforms and enterobacteria, their loads ranged between $1.2x10^5$ to $2.8x10^5$ CFU/g for the first and $7.2x10^5$ to $1.1x10^6$ CFU/g for the second while the standards are 10^2 CFU/g and 10^4 CFU/g respectively (Figures 5 and 6). These microfloras varied from one block to another and therefore statistically different ($p > 0.05$). The B12 block samples were more contaminated by the mesophilic aerobic germs ($6.3x10^7$ CFU/g), those of the B5 block contained the highest yeast and mold load ($9.3x10^5$ CFU/g); the B8 block samples contained both the largest fecal coliforms and enterobacteria load ($2.8x10^5$ CFU/g) for the first and (10^6 CFU/g) for the second.

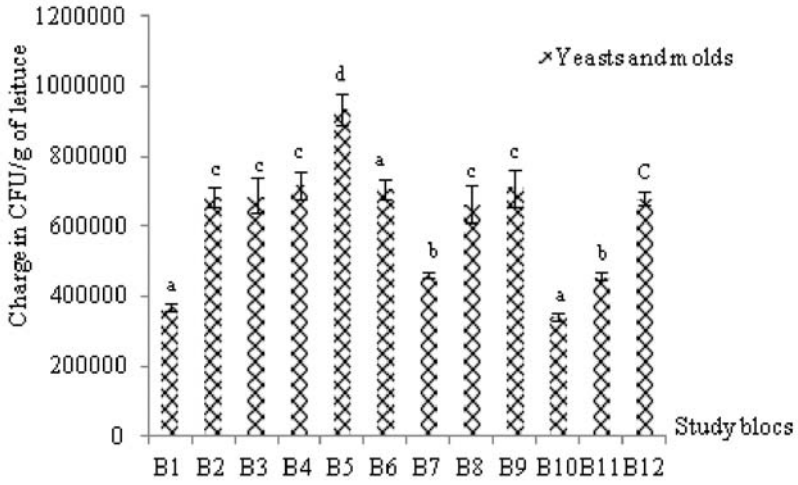

Figure 3. Numbers in CFU/g of yeasts and molds in lettuce according to the study blocks. Values with the same letters are not significantly different (P>0.05)

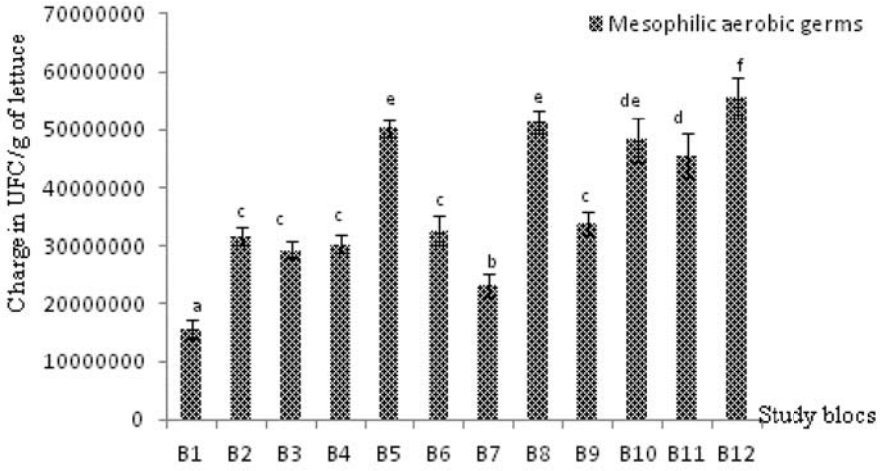

Figure 4. Numbers in CFU/g of mesophilic aerobic germs in lettuce according to the study blocks. Values with the same letters are not significantly different (P>0.05)

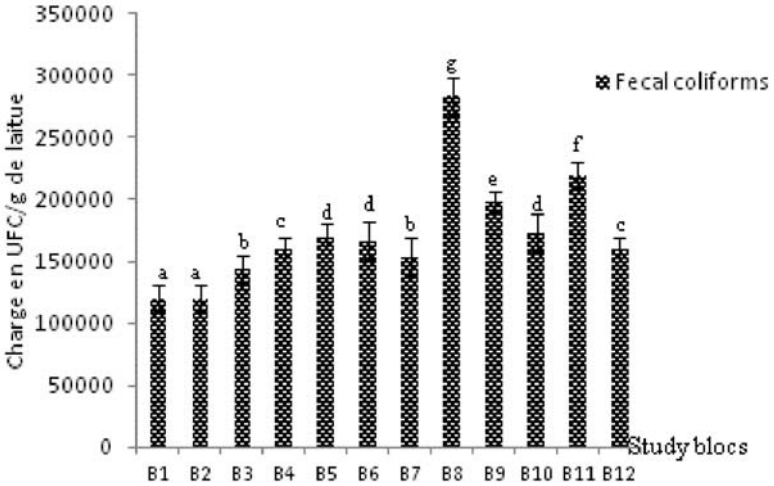

Figure 5. Numbers in CFU/g of fecal coliforms in lettuce according to the study blocks. Values with the same letters are not significantly different (P>0.05).

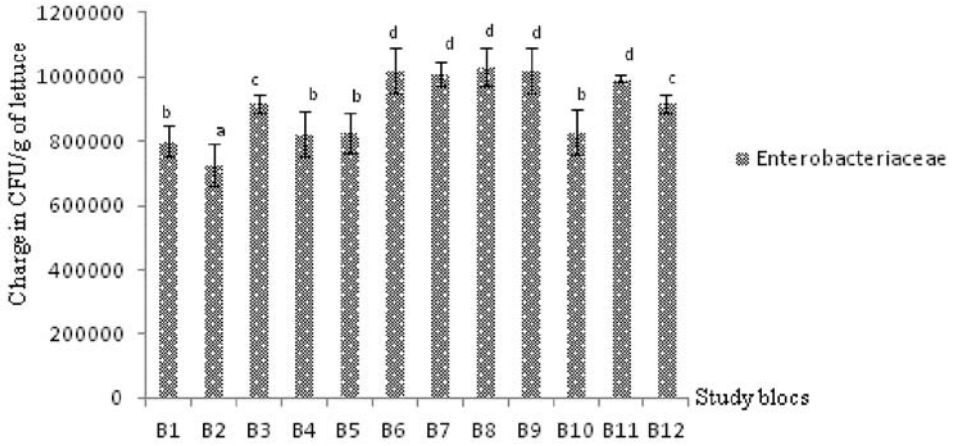

Figure 6. Numbers in CFU/g of enterobacteriaceae in lettuce according to the study blocks. Values with the same letters are not significantly different (P>0.05)

3.3.2 Presence of Pathogenic Species

Lettuce from the investigated site was contaminated with pathogenic bacterial species including *Escherichia coli*, *Salmonella* sp and *Staphylococcus aureus* coagulase positive. Worse, all the samples from the twelve study blocks contained all three species at the same time, with heavy loads exceeding the microbiology standards for fresh vegetables. The CFU/g number for *E. coli* ranged from $9.4x10^4$ to $1.8x10^5$, whereas the standard predicts 10 to 10^2 (Figure 7). For *S. aureus* coagulase +, the charges in CFU/g ranged from $5.9x10^4$ to $1.4x10^5$ while the standard indicated 10^2 (Figure 7). Where all the standards in food microbiology on *Salmonella* sp are a total absence in 25 g of food, there were loads of $6.1x10^4$ to $8.2x10^4$ CFU/g for the lettuce samples of the different study blocks (Figure 7). The charges for *E. coli*, *Salmonella* sp and *S. aureus* coagulase positive from the different study blocks were unevenly distributed from one block to another. These loads were therefore statistically different (p> 0.05) in the different blocks.

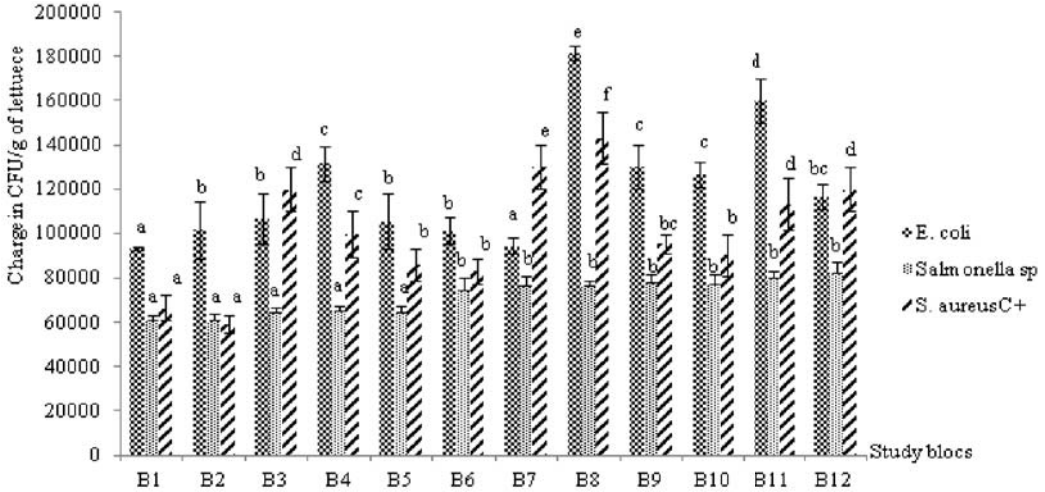

Figure 7. Numbers in CFU/g of pathogenic bacterial species in lettuce according to the study blocks. Values with the same letters are not significantly different (P>0.05)

4. Discussion

The production of lettuce for the consumption of the people of the city of Daloa is urban. This activity was practiced by adults aged between 30 and 60, then dominated by the female gender (58.1 %) against (41.9 %) for men. These data differ from other data provided in other similar studies. Indeed, the works of Ackerson & Awuah (2010) and those Kenmongue *et al*. (2010) carried out in Ghana and Cameroon respectively, urban and periurban market gardening was dominated by men and the majority of producers were between 24 and 68 years old. Urban and periurban production of lettuce farmers in Abidjan was also dominated by men (83 %) according to

Koffi *et al*. (2012). If the age of producers between 30 and 60 years could be explained by the arduous nature of market gardening, the sudden dominant appearance of women could have a purely local explanation. In fact, in agricultural areas dominated by cash crops (coffee or cocoa); men's main activity, food crops such as vegetable crops are left to women. Urban lettuce producers in Daloa were predominantly Ivorians (58.1 %), unlike those in Abidjan who were dominated by non-nationals (88.7 %) in a similar study (Koffi *et al*., 2012). The preponderance of nationals in this study is explained by the fact that agriculture is still the main activity in the deep country (Daloa) unlike the big capitals like Abidjan where the activities are much diversified. The high rate of illiterate producers (80.6 %) results from the high rate of illiteracy among the populations in the study area (61.9 %) according to (RGPH, 2014). In addition, a study on market gardening in Bouake (central Côte d'Ivoire) revealed that this activity was practiced by people with no education (Fondio *et al*., 2011). The main inputs for production were either poultry manure or a mix of dung, cow dung and chemical fertilizer. In addition, all producers (100 %) used surface or runoff water for watering lettuce. These same risky practices have been noted in other studies. In a study of the diversity and dynamics of *Salmonella* isolated from lettuce in Niger, soil amendments were mainly cow dung and chemical fertilizer (Alio *et al*., 2017). Exclusive use of surface water or gutters was also reported. In studies on perceptions of risk of contamination of urban and periurban crops, in Accra (Ghana), (Keraita *et al*., 2008) as in Belgium (Holvoet *et al*., 2014a), the majority of producers also used surface water or sewage for watering vegetables. The technical process of lettuce production practiced by urban producers was therefore empirical and unconventional or unknown. Some crucial provisions on the boards for a good production (width, length and spacing between boards) were royally ignored by these producers. In addition, no initial disinfection of the plants was carried out to avoid feathers, due among other things to fungi, insects and nematodes. In addition, soil amendments (fed with poultry and cow dung) were not processed and were used without precautions. All these facts are due to the low level of education of producers. The survey revealed that only about 20% were educated. Amponsah-Doku *et al*. (2010), Soendjojo (2012) and Woldetsadik *et al*. (2017) also reported empirical or artisanal production techniques in their studies of urban and periurban market gardeners. The informal nature of this agriculture, the high level of illiteracy and the lack of training programs on good practices in urban farming could justify the behavior of producers who were not aware of the risks of contamination from gardening practices. These farming practices would make lettuce susceptible to contamination, including microbial contamination.

Microbiological analyzes have shown the contamination of lettuce produced at the study site. The lettuces of the different blocks were heavily contaminated by different flora including mesophilic aerobic germs, total coliforms, enterobacteria and yeasts and molds. The same flora had also been identified in urban production lettuce in other works (Mohammad *et al*., 2013). Similarly, Koffi *et al*. (2011), Mngoli & Ng'ong'ola-Manani, (2014) and Akusu *et al*. (2016) isolated enterobacteria in lettuce in similar studies in Côte d'Ivoire, Malawi and Nigeria, respectively. The strong presence of these microfloras would translate into a marked deficit of good production and hygiene practices in the study site. High loads of these microfloras were also reported in other studies. Fecal coliforms were found in lettuce at production sites with loads ranging from 10^3 to 10^5 CFU/g and 10^3 to 10^4 CFU/g respectively, in Ghana in the works of Cobbina *et al*. (2013) and Ethiopia those Woldetsadik *et al*. (2017). Mesophilic aerobic germs ($3x10^5$ to $1.1x10^6$ CFU/g) and fungal flora ($2x10^3$ to $2x10^4$) were reported in lettuce grown in Romania (Soendjojo, 2012). The site of this study, located in downtown Daloa and in shallow water, regularly receives wastewater, sewage and gutter water on a continuous basis without any treatment. In addition, these waters are either directly used for watering or they communicate directly in the surface waters of the lowlands that were used for this purpose. These waters, once contaminated would therefore be the source of contamination of lettuce produced. Several studies have reported this form of contamination. For Koffi *et al*. (2011) in Côte d'Ivoire, Abbou *et al*. (2014) in Morocco and Holvoet *et al*. (2014b) in Belgium, irrigation water from urban vegetable crops was the main source of microbial contamination of production. Coagulase positive *Staphylococcus aureus* has been reported in work in lettuce in Brazil (César *et al*., 2015). In the present study, the presence of enterobacteriaceae could be due to the precarious hygienic conditions in which these leafy vegetables are grown as already stated by other authors Amoah *et al*. (2007) and Koffi *et al*. (2012) after work on vegetables produced in urban areas or periurban. The urban lettuce producers of the investigated site used large quantities of poultry droppings (sometimes fresh), and beef dung as fertilizer for soil fertilization. This agricultural practice would favor permanent fecal contamination, hence the strong presence of fecal germs such as *E. coli*, *Salmonella* sp. The presence of these species has already been reported in several similar studies (Petterson *et al*., 2010; Schikora *et al*., 2011; Koffi *et al*., 2012; Jensen *et al*., 2015; Traoré *et al*., 2015). In a similar study in Ghana, the load of *E. coli* counted in lettuce on an urban site ranged from 10^3 to 10^4 CFU/g (Cobbina *et al*., 2013). In New Zealand, *S. aureus* was counted in lettuce from various production systems with feeds ranging from 10^2 to 10^3 CFU/g (Wadamori *et al*., 2016). *Salmonella* sp (0 to 10^4) loads have been found

recently in urban lettuce production in Ghana (Abakari *et al*., 2018). The high contamination of lettuce is also due to soil that has been heavily contaminated by the presence of dumpsites in the area, surroundings in addition to wastewater. Indeed, human excrement, farm animals such as oxen, sheep, other animals (lizards, migratory birds, dogs and cats) are constantly straying there. Their excreta are carried by rain runoff to soils, wells and other water sources used for watering. The urban lettuce of the investigated site would be a source of microbiological hazards, which would cause multiple infectious diseases such as diarrhea, gastroenteritis, typhoid and paratyphoid fevers. Its consumption constitutes a real risk of infection or a source of food poisoning which can lead to a public health problem.

5. Conclusion

Urban agriculture is a major source of lettuce (*Lactuca sativa* L.) for urban populations, as is the case in Daloa. The assessment of the risk of microbial contamination revealed that consumption of lettuce (*Lactuca sativa* L.) from urban production in the city of Daloa would pose a risk to the health of consumers. High loads of microflora reflecting a deficit of good production and hygiene practices such as fecal coliforms, enterobacteria, mesophilic aerobic germs, yeasts and molds have been discovered. Pathogenic bacterial species such as *Escherichia coli*, coagulase positive *Staphylococcus aureus* and *Salmonella* sp were detected in all samples from the 12 blocks of the investigated site. The consumption of this lettuce would present a real danger to the health of consumers. The profile of producers, the upstream empirical production route and the difficult production conditions would increase the risk of microbial contamination and even other types of contamination. Thus, the competent authorities must raise awareness and raise awareness of the health risks to consumers. Establishing regulations for urban agriculture could limit the risk of contamination. An organization of urban agriculture with good training in agricultural production routes would contribute to the food security of urban populations and create jobs for urban vulnerable groups. Health education would be needed to prevent the health risks of consuming urban lettuce and to prevent possible foodborne infections. Good communication about these identified hazards would help the well being of consumers.

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