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Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

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Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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African Journal of Food Science

Review

Some traditional Algerian products from durum wheat

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Foods derived from durum wheat play an important role in the diet of the Algerian people, this is attributed to the semiarid climate favourable for the cultivation of this staple cereal crop. Flat bread, pasta, couscous and frik are the most common products made from durum wheat in Algeria. It is also used to produce various kinds of traditional cakes. All these products are the major products made from durum wheat in Algeria and most of them are scientifically unknown. This work is a general review on currents works on some traditional products such as flat bread, pasta, traditional cake and others. It describes for each product, raw materials used, manufacturing process and end products quality criteria.

Key words: Durum wheat, semolina, traditional product, pasta, pastry, bread, frik.

INTRODUCTION

Algeria is located in northwest Africa, in an area known as the Maghreb, "Sunset" in Arabic. The center of the country is a semi-arid highland region where the climate is conducive for the cultivation of durum wheat, making this staple cereal crop and its derived foods an important element in the Algerian people's diet (Winget and Chalbi, 2004). Flat bread, pasta, couscous and frik are the most common products made from durum wheat in Algeria. It is also used to produce various types of traditional cakes (Abecassis et al., 2013).

This paper is a summary review on some traditional Algerian products from durum wheat such as flat breads, pasta products, pastries and frik.

DURUM WHEAT FLAT BREADS

In Algeria, housewives produce two types of durum flat bread:

Kesra

This means fraction, and designate four kinds of home-made flat bread. Kesra includes four kinds of flat bread: Mathlouaa, maadjouna, rakhsis and harcha. These appellations vary throughout Algeria but recipes and flow charts are alike.

Khobz eddar

This literally mean home bread, differs from kesra by its recipe (fat, eggs and some additives) and by its flow chart for production (Dagher, 1991).

Kesra types

Mathlouaa

Mathlouaa (Figure 1a) (which means leavened or risen) is one of the most consumed flat breads in Algeria especially during fasting month of Ramadan. It is leavened bread made from fine durum semolina. Usually the leavening agent is dry yeast. It is baked on a clay pan with little spikes all over the bottom called tajine (Figure 1b). The bread is light and spongy, with typical tenacity from the semolina. The bread has a short shelf life. It is good

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Figure 1. Mathlouaa, a leavened flat bread. (a): whole and parts of bread. (b): Mathlouaa baking on tajine (clay pan with little spikes all over the bottom).

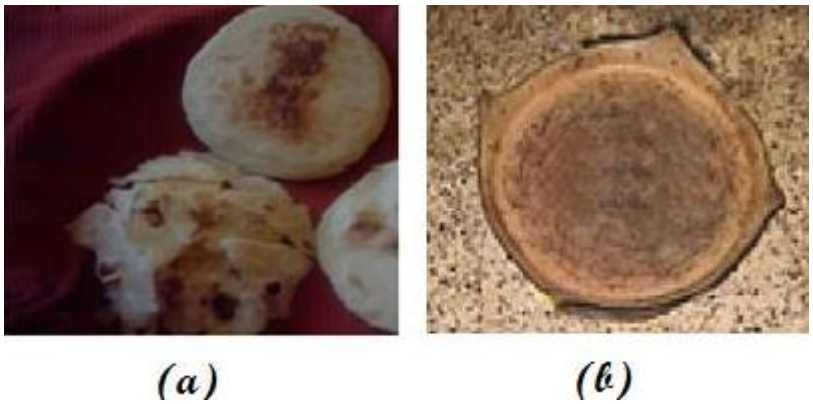

Figure 2. Maadjouna, unleavened flat bread (a): whole bread. (b): Smooth clay pan for baking maadjouna and other products.

only the day it is made (Kezih, 1998; Cheriet, 2000).

Maadjouna

Maadjouna (Figure 2a) (which means kneaded), the recipe for this type of bread is simple (fine semolina + water + salt) as its preparation (kneading, shaping, baking) is quick and easy. Households make maadjouna when they are too busy. It is baked on a smooth clay pan (smooth tajine (Figure 2b). The bread is chewy and dense, with a pleasant taste from the burned blisters on its faces. The bread has a very short shelf life. It goes to stale quickly. It is good only the hours it is made. It serves as an edible spoon (Kezih, 1998; Cheriet, 2000).

Rakhsis

Rakhsis (Figure 3) (which means soft), this bread has also a simple recipe (fine semolina + fat + water + salt) and its preparation is easy and quick (no fermentation). Rakhsis is thinner than mathloua. It is also baked on a clay pan with little spikes. The end product has a homogenous surface without blisters because fat prevent their development. With a firm crust and a compact crumb, the product is soft. Rakhsis has a long shelf life because the addition of fat retards staling. Rakhsis is full of flavor (Kezih, 1998; Cheriet, 2000).

Harcha

Harcha (Figure 4) (which means rough) is a kind of bread "kesra" which is prepared a little differently from the above kinds. Coarse semolina, fat with high rate, salt, and water are mixed and undergo a moderate kneading to form unrefined dough which lacks visco-elastic properties. The dough is fragile and must be handled with care. Then it is shaped into squares or lozenges which are baked on a smooth clay pan (Figure 2b). The end product has a rough surface, a long shelf life and it is tasty (Cheriet, 2000).

Khobz eddar

This traditional bread is prepared during major celebrations such as weddings and religious feasts. It is more

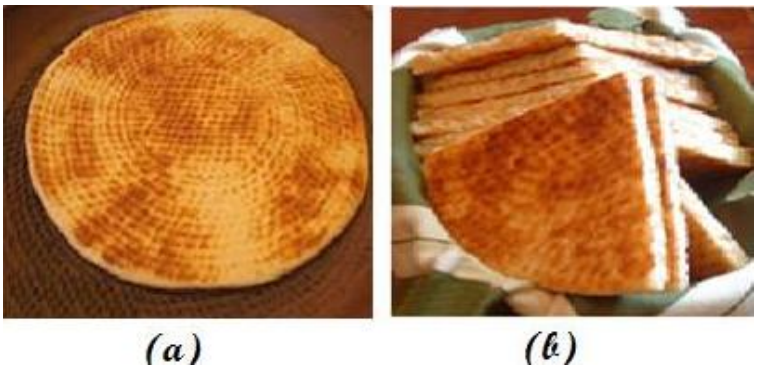

Figure 3. Rakhsis: unleavened flat bread with fat (a): hole bread. (b): slices of bread.

Figure 4. Harcha, unleavened flat bread with high fat content.

Figure 5. Khobz eddar, traditional leavened flat bread.

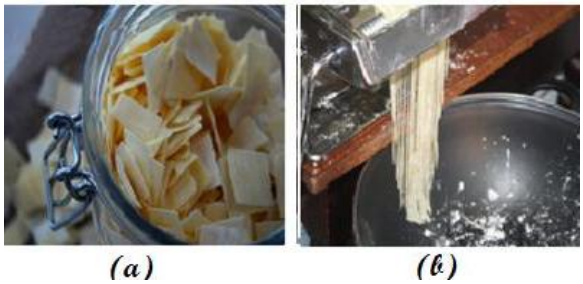

Figure 6. Two types of Algerian traditional pasta. (a): trida, (b): rechta.

challenging to make because its sophisticated recipe (fine semolina + fat + milk + salt + yeast + egg + sesame bread is baked in a bakery or kitchen oven. The end product has a crispy crust and a soft crumb and delicious (Figure 5) (Cheriet, 2000).

PASTA PRODUCTS AND COUSCOUS

Pasta products

Semolina and water are the greatest components of all seeds + water), also it required more steps than kesra: knead, flatten and set on the baking pan to leaven. The traditional pasta, which is processed by sheeting and cutting the dough into strips, squares or some other shapes (Figure 6). Pasta is consumed either fresh, or undergoes drying and stocked for a later use. Usually traditional Algerian pasta is steam cooked. Good pasta should have the following criteria: yellow amber color, smooth aspect, non-sticky, and when cooked should swell well, do not disintegrate and have a good taste (Namoune et al., 2000; Namoune et al., 2007).

Couscous

Couscous (Figure 7), the Algerian national dish is made from mixing semolina with water. Couscous preparation requires five steps: Blinding, agglomeration, shaping, steaming and drying. However in some regions of Algeria couscous does not undergo steaming and it is dried immediately. Characteristics of good couscous are: amber yellow color, uniform particle size, high sauce absorption capacity, ability to keep its integrity during steaming or sauce application, and particles are non-sticky, good taste (Derouiche et al., 2002).

PASTRIES DURUM WHEAT PRODUCTS

In Algeria, durum semolina is widely used to produce a

Figure 7. Couscous undergoing preparation in a wooden pan.

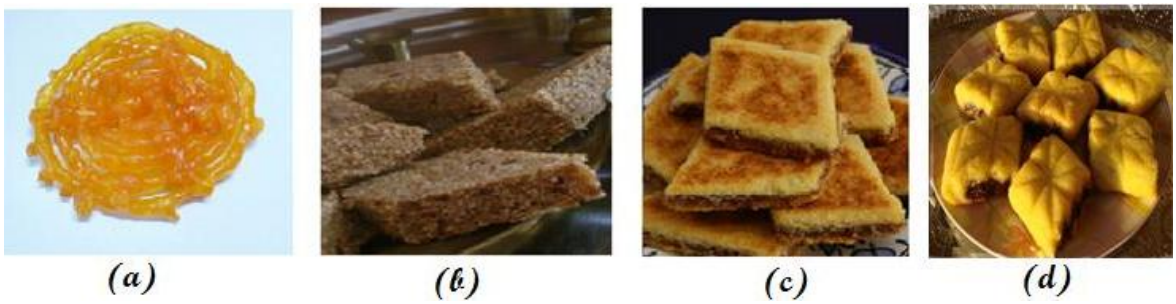

Figure 8. Some traditional Algerian pastries from durum wheat. (a): zlabia, (b): tamina, (c): braje, (d): makroud.

Figure 9. Frik whole grains (a); ground and sieved grains (b); frik soup (c).

range of pastries products (Figure 8), like zlabia, tamina, bradje, makroud and many others. Production and consumption of these pastries increase in Islamic holidays and in summer where families' celebrations are frequent. The recipes of these pastries are various and their manufacturing is different. They share a characteristic: sweet or must be eaten sweet (Ouelhi et al., 2004).

NON-PASTE DURUM WHEAT PRODUCT

Frik

Throughout Algerian eastern regions, immature durum is processed into frik (Figure 9). This is not specific to Algeria but there are similar traditions in other parts of the world. The grains are harvested during the interval between the "milk stage," and the "soft dough" stage. Wheat is cute with a sickle and the spikelets are stacked on a large sheet metal. The spikelets are then roasted. After which the grains are threshed and winnowed. The fire imparts a smoky flavor to the grain, and the heating stops the maturation of the endosperm and stops the degradation of the chlorophyll. The ideal frik grain is green with just the tip charred. The smoky, sweet and grassy quality of the grain adds a unique taste to frik dish. These green grains are more nutritious than mature grains, high in dietary fiber and low in phytic acid. Frik grains are ground and sieved and used to prepare a soup often consumed

in the Eastern regions of Algeria especially in the month of Ramadan (Ouelhi et al., 2002; Bouza and Nacer, 2006).

CONCLUSION

Traditional products cited in this work are the major products of durum wheat in Algeria. The introduced products are not the only products produced; there are many others products not cited in the work. The review of these products shows that they constitute an interesting research field. Study of these products shows: rational use of durum wheat; research and definition of specific quality criteria for these products; improving the manufacturing process in order to have products with required qualities.

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African Journal of Food Science

Full Length Research Paper

Protective effects of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on physicochemical and microbial attributes of liquid smoked silver carp (*Hypophthalmichthys molitrix*) wrapped in aluminium foil during chilled storage

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Accepted 7 November, 2013

The antioxidant and antimicrobial effects of equivalent concentrations of both garlic and ginger separately in fresh and powder form on quality attributes of liquid smoked silver carp (*Hypophthalmichthys molitrix*) during chilled storage were investigated for a period of 24 days. The control and the treated fish samples were analysed periodically for microbiological (total viable count) and physicochemical (pH, colour, water activity, free fatty acid, total volatile basic nitrogen; TVB-N, 2-thiobarbituric acid-reactive substances; TBARS) characteristics. The results showed that each spice in either fresh or powder form treatment significantly ($P < 0.05$) reduced growth of microorganisms as reflected in total viable counts (TVC) from day 6 of storage, lipid oxidation decrease as displayed in TBARS, chemical spoilage decrease as reflected in TVB-N after day 6 of storage as compared to the control samples. The results obtained from this study suggests that garlic and ginger in fresh and powder form, through their combined antioxidant and antimicrobial effects, are potentially useful in preserving liquid smoked silver carp at 4 ± 1°C.

Key words: Liquid smoke, silver carp, spices, shelf life, chilled storage.

INTRODUCTION

Silver carp (*Hypophthalmichthys molitrix*) is a prevalent freshwater fish, it has been one of the most widely cultured species all over the world due to its fast growth rate, easy cultivation and high feed efficiency ratio (Hu et al., 2008). This species is also known to provide a high content of important constituents of the human diet such as nutritional and readily digestible proteins, lipid, soluble vitamins, microelements and polyunsaturated fatty acids (Erkan and Ozden, 2007). However, distributors face challenges of deterioration during postmortem storage and processing as a result of damage mechanisms such as autolytic degradation, microbiological spoilage and lipid oxidation (Naseri and Rezaei, 2012).

Fish smoking is one of the oldest method of preservation, giving a characteristic flavour and colour to the product and increasing its shelf life. The preservative effect is due to the presence of some antimicrobial compounds in smoke such as phenols and formaldehyde (Muratore and Licciardello, 2005). Nevertheless, the conventional smoking process is commonly substituted by the use of liquid smoke (Hattula et al., 2001). Liquid smoke contains the same functional components such as phenols, carbonyls

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and acids that are found in vaporous smoke. Liquid smoke is free of harmful compounds such as polycyclic aromatic hydrocarbons (PAHs) which are ubiquitous environmental contaminants; these are commonly found in convectional smoke, also considered carcinogenic and mutagenic molecules (Alcicek, 2013). Additional advantages of liquid smoke are environmental friendliness, lower cost and easy application such as direct addition, drenching or dipping, impregnated (smoked) casings and atomization (Varlet et al., 2007). The smoking process is often coupled with other treatments, such as salting, addition of spices, packaging techniques and chilled storage, to produce synergistic effects on spoilage microorganisms and to increase shelf life (Visciano et al., 2008).

Ginger and garlic are spices, in addition to contributing taste and aroma to foods, also contain a variety of bioactive substances which are of considerable use from the standpoint of food science and technology. These may be used singly or in combination, and some act synergistically to control spoilage of foods, these have made it to be used as bio preservative (Yanishlieva et al., 2006). Recently, use of plant extracts as natural antioxidants has gained increasing interest because of the global trend of restriction in use of synthetic substances, also antioxidant rich plant extracts have potential benefits in food preservations (Uhart et al., 2006).

The aim of this study was to investigate the antioxidant as well as the antimicrobial effect of equivalent concentrations of two garlic and ginger preparations (fresh and powder form) on shelf life of liquid smoked silver carp wrapped in aluminum foil and stored at 4 ± 1°C by determining microbiological and physico-chemical differences.

MATERIALS AND METHODS

Fresh garlic bulbs (*Allium sativum*, Chinese white garlic) and fresh ginger (*Zingiber officinale*, Chinese yellow ginger) were purchased from a local market. The dry skin of both fresh garlic and ginger were removed before use; they were peeled and crushed finely by using a kitchen hand held grater (Evasolo). Garlic powder and ginger powder were purchased from a supermarket. 4 kg of produced garlic powder was the weight equivalent of 16 kg of fresh garlic, also 2 kg of produced ginger powder was the weight equivalent of 10 kg of fresh ginger, according to the manufacturer details.

Preparation of fish

fifteen silver carp *(H. molitrix)* with average weight of 815.12 ± 14.55 g were purchased from a local market (Wuxi, Jiangsu, China), transported within 1 h in sealed polystyrene foam boxes containing ice to the Food Processing Technology Laboratory at Jiangnan University, where processing was carried out, then randomly divided into 5 groups for further treatments after they were gutted, eviscerated, deboned, the scale, skin, pin bones, debris were removed, and filleted.

Sample treatment and brining with liquid smoke

C: The samples without ginger and garlic treatment; T1: 10 g/kg ginger powder was added to brine; T2: 50 g/kg of fresh ginger applied on fish for 4 h at 4 ± 1°C after brining; T3: 12.5 g/kg garlic powder was added to the brine;T4: 50 g/kg of fresh garlic was applied on the fish for 4 h at 4 ± 1°C after brining.

All the samples above were immersed in brine containing 10% sodium chloride and 1% liquid smoke solution at a ratio of 1:1(w/w) for 4 h at 4 ± 1°C.

Drying, heating and cooling of samples

All samples were air dried at 20°C for 40 min. The samples were then heated in an oven at 75°C for 80 min. After cooling at 20°C for 30 min, the smoked products were wrapped in aluminium foil, and stored at 4 ± 1°C until analysis performed in replicates on day 0, 6, 12, 18 and 24 of storage.

pH value measurement

A 1 g sample of the fish flesh was homogenized in 10 mL of distilled water and the mixture was filtered. The pH of the filtrate was measured according to the method of Vyncke (1981) using a pH meter (Mettler Toledo 320-s, Shanghai, China).

Water activity

An adequate amount of ground sample was placed in a holding cup (about one-half full) and the water activity was read (AQUA LAB CX-2, Decagon Devices Inc, Pullman, WA, USA) after equilibration at 25°C.

Color evaluation

The color of fish was measured using the L*, a*, b* mode of CIE using color meter (Model TC-P*α* G, Beijing optical Instruments Factory, Beijing, China). The measurement of L* (indicator of lightness), a* (indicator of redness), b* (indicator of yellowness) was performed in two replications (Lanier et al., 1991).

Determination of total volatile basic nitrogen (TVB-N)

TVB-N was determined according to the method of Antonacopoulos and Vyncke (1989). For total TVB-N, fish muscle (10 g) was homogenized with 6% perchloric acid (90 mL) for 1 min in an IKA T25 basic Ultra-Turrax (Janke & Kunkel, IKA Labor-technik, Selangor, Malaysia). The homogenates were filtered through a filter paper (Whatman no. 1) and filtrates alkalized by NaOH (20%). The distillate was titrated with 0.01 N HCl.

Determination of free fatty acid (FFA) content

Free fatty acid content of the fish samples was determined according to the method of Kirk and Sawyer (1991). A mixture of diethyl ether (25 mL), ethanol (70% v/v) (25 mL) and 1% phenolphthalein solution (1 mL) was prepared then neutralized with 0.1 M NaOH solution. Two grams quantities of fish samples were blended in the neutral solvent prepared above for about 20 min, and then titrated with 0.1 M NaOH with constant shaking until a pink color was formed which persisted for about 15 s. All samples were analyzed in triplicate and the free fatty acid content was expressed

Table 1. pH.

Storage time(days)	pH				
	C	T1	T2	T3	T4
0	6.07 ± 0.54^{aA}	6.1 ± 0.18^{aA}	6.15 ±0.07^{aA}	6.18 ± 0.25^{aA}	6.07 ±0.09^{aA}
6	6.25 ± 0.26^{aA}	6.23 ± 0.04^{aAB}	6.19 ±0.44^{aA}	6.2 ± 0.12^{aA}	6.13 ±0.13^{aA}
12	6.35 ± 0.08^{aA}	6.3 ± 0.14^{aAB}	6.26 ± 0.01^{aA}	6.3 ± 0.45^{aA}	6.15 ±0.21^{aA}
18	6.39 ± 0.13^{aA}	6.34 ± 0.06^{aAB}	6.29 ± 0.11^{aA}	6.32 ± 0.03^{aA}	6.2 ±0.07^{aA}
24	6.52 ± 0.12^{aA}	6.45 ±0.03^{abB}	6.33 ±0.1^{abA}	6.36 ±0.08^{abA}	6.27 ±0.16^{bA}

$^{A–B}$Means with different superscript letters in the same column indicate significant differences ($p<0.05$). $^{a–b}$Means with different superscript letters in the same row indicate significant differences ($p < 0.05$). Data are expressed as means ± standard deviation ($n = 2$).

as oleic acid equivalent.

Determination of 2-thiobarbituric acid-reactive substances (TBARS)

2-Thiobarbituric acid reactive substances (TBARS) were determined according to the method of Buege and Aust (1978). 0.5 g of fish fillet was homogenized in 10 ml of the mixture containing TBA (0.375 g/100 ml), TCA (15 g/100 ml) and HCl (0.25mol/l). The mixture was heated in the boiling water for 10 min, followed by cooling with the running tap water. The mixture was centrifuged at 3600 ×*g* for 20 min and the absorbance was measured at 532 nm (Model 135 WFZ-UV-2100, UNICOTM, Shanghai, China). The TBARS value was calculated from the standard curve of malonaldehyde and expressed as mg malonaldehyde/kg sample.

Total viable counts

10 g of each sample homogenate was diluted with 100 ml of 1% BPW at pH 7.5. Serial dilutions were made until 10^{-7} g/ml samples were obtained. A 1 ml aliquot of each dilution was placed in a Petri dish and approximately 15 ml plate count agar (PCA, Oxoid, CM325) was added. Appropriate decimal dilutions of the samples were prepared with the same diluents and 0.1 ml aliquots of appropriate dilution were spread on the agar plates. Each Petri dish was carefully shaken, in order to achieve a homogeneous distribution of the sample. After several minutes all Petri dishes were inverted and placed in an oven at 37°C for 48 h. After 48 h of incubation, all colonies were counted by hand, following the rules reported by Gilliland et al. (1976).

Statistical analysis

For data analysis, analysis of variance (ANOVA) was used. Significant differences were defined at $P < 0.05$. Comparisons of group means were obtained using Duncan's multiple range tests. All statistical analysis was performed using SPSS version 19.0 for windows software (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Changes of pH

The pH value of smoked silver carp fillet was increased ($P<0.05$) with storage period (Table 1). Its values ranged between 6.07 and 6.52 for both control and treated samples throughout the entire storage period. The limit of acceptability is usually 6.8 to 7.0 (Erkan et al., 2011). This phenomenon is presumably because of the production of basic nitrogen compounds such as ammonia, TMA, and/or other basic nitrogenous compounds caused by microbial activity or proteolytic enzymes in a food material (Fan et al., 2009).

Water activity

Changes in water activity (Aw) are shown in Table 2. Initially, the Aw for all samples was in the range of 0.84 to 0.93, which increased significantly ($P<0.05$) with storage time to a final range of 0.92 to 0.99. The control samples showed higher water activity (Aw) value than the counter-part samples during time of storage. Water activity (aw) has its most useful application in predicting the growth of bacteria, yeast and mold. The knowledge of water activity is very important factor to guarantee the required stability towards microbial spoilage of the product and to ensure safety by avoiding any threat to the health of the consumer, this is because micro-organisms generally grow best between Aw values of 0.995-0.980, while most microbes cease growth at Aw <0.900. It is also necessary for the transport of nutrients and the removal of waste materials, to carry out enzymatic reactions, to synthesize cellular materials, and to take part in other biochemical reactions (Goksoy, et al., 2009).

Color

Color is one of the most important organoleptic characteristics. It influences the acceptability of the product and plays a major role in the purchase decision and evaluation of meat quality (O'Sullivan, et al., 2003).

L* values (lightness) decreased ($P < 0.05$) with the increase of storage time. Coordinates decreased from 84.36 on day 0 to 72.23 at the end of the experiment on

Table 2. Water activity.

Storage time (days)	Water activity				
	C	T1	T2	T3	T4
0	0.93 ± 0^{bA}	0.93 ± 0.01^{bA}	0.91 ± 0^{bA}	0.91 ± 0.02^{bA}	0.84 ± 0^{aA}
6	0.95 ± 0.01^{bBA}	0.94 ± 0.01^{bB}	0.92 ± 0.03^{bA}	0.93 ± 0.01^{bB}	0.85 ± 0.03^{aBA}
12	0.97 ± 0.02^{cCB}	0.96 ± 0.02^{cCB}	0.92 ± 0^{bA}	0.94 ± 0^{cbB}	0.87 ± 0^{aBA}
18	0.98 ± 0.01^{dDC}	0.96 ± 0.04^{cC}	0.93 ± 0^{bA}	0.95 ± 0.01^{bB}	0.89 ± 0.028^{aCB}
24	0.99 ± 0.02^{aD}	0.97 ± 0.03^{aC}	0.93 ± 0.04^{aA}	0.95 ± 0.05^{aB}	0.92 ± 0^{aC}

$^{A–D}$Means with different superscript letters in the same column indicate significant differences ($p<0.05$). $^{a–d}$Means with different superscript letters in the same row indicate significant differences ($p < 0.05$). Data are expressed as means ± standard deviation ($n = 2$).

Table 3. Colour measurements.

Parameter/treatment	Storage period (days)				
	0	6	12	18	24
L*-value(lightness)					
Control	84.36 ± 0.51^{dE}	82.04 ± 0.001^{dD}	79.28 ± 0.03^{eC}	76.51 ± 0.3^{eB}	72.23 ± 0.13^{eA}
T1	83.16 ± 0.06^{dcE}	80 ± 0.85^{cD}	77.44 ± 0.62^{dC}	74.73 ± 0.57^{dB}	70.68 ± 0.45^{dA}
T2	79.28 ± 1.81^{baD}	75.35 ± 0.92^{bC}	74.22 ± 0.03^{bC}	68.43 ± 0.14^{bB}	63.95 ± 0.03^{bA}
T3	81.31 ± 0.98^{cbE}	78.53 ± 0.75^{cD}	75.74 ± 0.48^{cC}	71.82 ± 0.00^{cB}	68.15 ± 0.21^{cA}
T4	77.75 ± 0.21^{aE}	72.83 ± 0.52^{aD}	71.47 ± 0.75^{aC}	66.94 ± 0.03^{aBC}	61.95 ± 0.55^{aA}
a*-value (redness)					
Control	4.3 ± 0.28^{dA}	5.62 ± 0.91^{cB}	6.65 ± 0.03^{eB}	8.12 ± 0.25^{dC}	9.49 ± 0.01^{eD}
T1	3.58 ± 0.11^{cA}	5.16 ± 0.23^{cB}	6.42 ± 0.03^{dC}	7.69 ± 0.13^{dD}	8.98 ± 0.25^{dE}
T2	0.33 ± 0.01^{aA}	2.09 ± 0.14^{bB}	3.63 ± 0.13^{bC}	4.3 ± 0.28^{bD}	5.91 ± 0.001^{bE}
T3	2.24 ± 0.08^{bA}	3 ± 0.16^{bB}	4.37 ± 0.11^{cC}	5.52 ± 0.03^{cD}	6.93 ± 0.04^{cE}
T4	0.35 ± 0.01^{aA}	0.98 ± 0.02^{aB}	2.01 ± 0.01^{aC}	3.31 ± 0.13^{aD}	4.92 ± 0.04^{aE}
b*-value (yellowness)					
Control	27.12 ± 1.24^{dC}	25.38 ± 0.099^{dC}	22.63 ± 1.26^{dB}	21.25 ± 0.14^{dBA}	19.45 ± 0.11^{eA}
T1	24.84 ± 1.75^{dcD}	22.67 ± 1.23^{cDC}	21.51 ± 0.57^{dcCB}	19.51 ± 0.51^{cBA}	18.23 ± 0.17^{dA}
T2	21.45 ± 0.21^{bD}	20.01 ± 0.16^{bC}	18.74 ± 0.13^{baB}	16.66 ± 0.92^{baA}	15.77 ± 0.06^{bA}
T3	23.12 ± 0.96^{cbC}	21.4 ± 0.14^{cbB}	19.99 ± 0.41^{cbB}	17.93 ± 0.68^{bA}	16.59 ± 0.01^{cA}
T4	18.5 ± 0.42^{aD}	17.69 ± 0.58^{aDC}	17.49 ± 0.28^{aC}	16.07 ± 0.23^{aB}	15.12 ± 0.001^{aA}

$^{a–e}$Means with different superscript letters in the same column indicate significant differences ($p<0.05$). $^{A–D}$Means with different superscript letters in the same row indicate significant differences ($p < 0.05$). Data are expressed as means ± standard deviation ($n = 2$).

the control samples, counterpart samples decreased from 77.75-83.16 at day 0 to 61.95-70.68 at the end of the experiment, depending on the treatment. Moreover, control samples showed higher values as compared to their counterpart samples (Table 3) but that difference was small and irrelevant. Many factors may contribute to more lightness on control samples than their counterpart samples, these include the concentration and type of pigments present (Viuda-Martos et al., 2010), water content, hygroscopicity of the material dissolved in the water matrix and surface water availability, but the pigment content and the fractions of MetMb and OxyMb were the most important factors for the variation in the L* value (Karlsson et al., 2006).

The a*-value was found to increase ($P < 0.05$) in all samples as duration increased. Again the values in control samples were higher than counterpart samples with increasing storage time. The a*-value in the control samples rose from 4.3 on day 1 to 9.49 at the end of the experiment. Treated samples rose from 0.33-3.58 to 4.92-8.98, depending on the treatment (Table 3). The increase of the a*value indicated that fish meat were redder, this

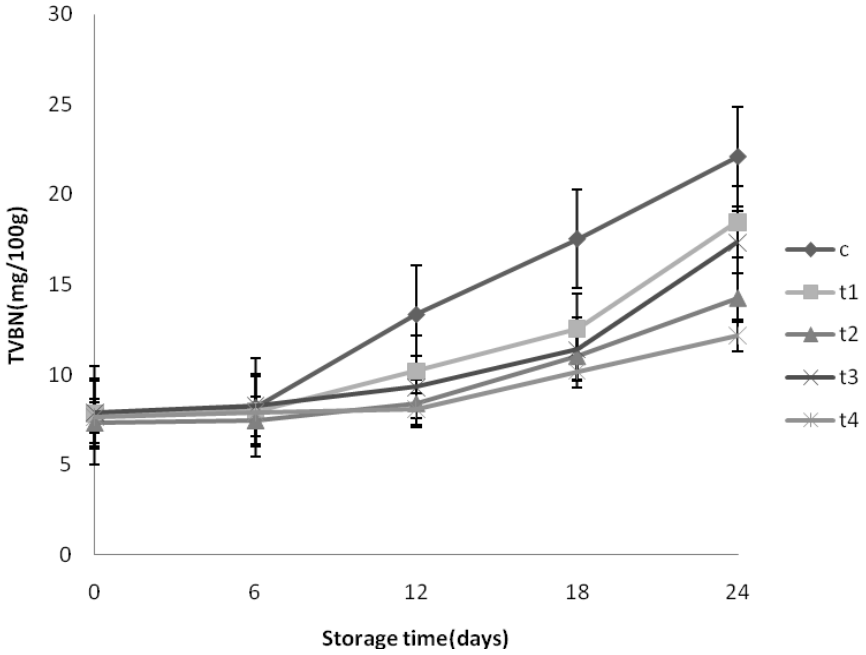

Figure 1. Total volatile basic nitrogen (mg/100 g).

could be due to less met-myoglobin formation (Faustman et al., 2010; Biswas et al., 2012). It is postulated that, spices are effective antioxidants that improve red color stability by inhibiting met-myoglobin formation in fish fillets (Allen and Cornforth, 2010). In the present study, the results do not establish the effectiveness of garlic and ginger as antioxidants and antimicrobials on red color stability.

For the yellow coordinate (b*), the value decreased ($P < 0.05$) in all samples during time of storage. Coordinates decreased from 27.12 on day 0 to 19.45 at the end of the experiment on the control samples, counterpart samples decreased from 18.5-24.84 at day zero to 15.12-18.23 at the end of the experiment, depending on the treatment (Table 3). Even though the magnitude of the differences was very small on all samples, the control samples showed higher yellowness than their counterpart samples. The differences for the yellow coordinate (b*), may be due to pH, oxidation extent, water activity, etc. in the matrix which have the greatest influence on this coordinate in many foods (Cofrades et al., 2004).

Total volatile base nitrogen (TVB-N)

The TVB-N of fish is an indicator of the freshness of the raw material (Zhou et al., 2011). Changes in TVB-N of different samples during the entire storage period are shown in Figure 1. The initial TVB-N value of silver carp was in the range of 7.31 to 7.90 mg/100 g. Similar results were also reported by Fan et al. (2009). TVB-N values of control samples showed significant ($P < 0.05$) differences from other samples on storage; as storage increased also its TVB-N value was higher than those other samples which increased more slowly than control samples after day 6 of storage. The TVB-N value of the control samples had already reached 12.32 mg/100 g on the day 12 of storage, while the values of counterpart samples did not reach this figure but was in the range of 8.08 to 10.2 mg/100 g. TVB-N values of samples T1, T2, T3 and T4 on the 24th day of storage were 18.48, 14.27, 17.36 and 12.16mg/100 g, respectively, while TVB-N value of control samples on the 24th day was 22.8mg/100 g. TVB-N values of all samples were lower than 25mg/100 g which was considered as the threshold for a good-quality fish product, high TVB-N values are unacceptable and are associated with unpleasant smell in the meat (Limbo et al., 2009). Assumably, this is because of the impact of the various treatments of TVB-N, which primarily includes nitrogen from ammonia, TMA, and dimethylamine which reflects the extent of degradation of proteins and non protein nitrogenous compounds which can be explained by proteolysis, due to enzymatic and microbial activities in the samples on storage (Erkan and Ozden, 2008). In the present study, the results establish the effectiveness of garlic and ginger as antioxidants and antimicrobials due to reduction in TVB-N on treated samples as observed, it is supposed that the spices used to treat silver carp were involved in TVB-N reduction.

Free fatty acids

The initial FFA values were in the range of 7.19 to 7.9

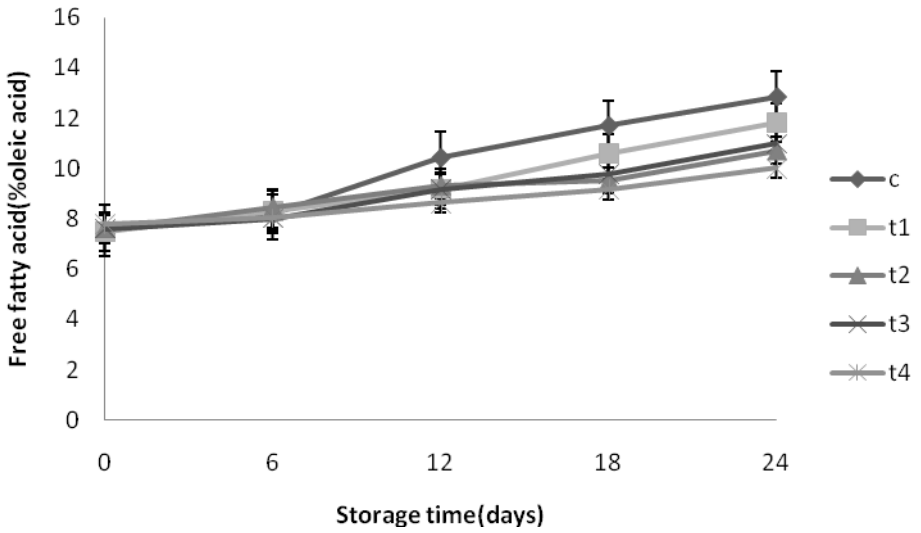

Figure 2. Free fatty acids (percentage oleic acid).

(oleic acid percentage) for all samples. FFA values increased with storage time (Figure 2); however the values in the control samples were higher than other samples during storage. Significant statistical differences were found between the treated samples and control samples ($P < 0.05$) after day 12 of storage. At the end of the storage period (day 24), values of FFA on control samples were found to be 12.83 (oleic acid percentage) which is higher than counterpart samples which were found to be 11.82, 10.72, 10.98 and 10.01 (oleic acid percentage) for T1, T2, T3 and T4, respectively. Accumulation of FFA is said to contribute to off flavor of the product and cause textural alterations by complexing with protein (Siddaiah et al., 2001). The results established the effectiveness of garlic and ginger as antioxidants which were greater in activities to inhibit the synthesis of free fatty acid in the treated samples than control samples on 24 days of storage.

Thiobarbituric acid reactive substances (TBARS)

Fish meat is particularly susceptible to oxidative changes because of the processing conditions, exposure of unsaturated fat and proteins to molecular oxygen. 2-Thiobarbituric acid (TBA) is widely used as an indicator of degree of lipid oxidation, and the presence of TBA reactive substances is due to the second stage auto-oxidation (Rezaei and Hosseini, 2008) during which peroxides are oxidized to aldehydes and ketones (Lindsay, 1991). The TBARS values of all silver carp samples increased as storage time increased, the increase of TBARS values in fish meat with increasing storage time is normal (Rong et al., 2009) (Figure 3). Significant differences were found between control samples and counterpart samples ($P < 0.05$) after day 6 of storage. The mean TBARS values of control were found to be 1.56 mgMA/kg of fish flesh which is higher than those of other samples which were found to be 1.2, 1.02, 1.1 and 0.93 mgMA/kg for T1, T2, T3 and T4, respectively, at day 24 of storage. There are two possible reasons for this phenomenon in the effectiveness of this product: 1, reduction in TBARS using garlic and ginger is related to peroxide-scavenging enzyme activity, which could reduce unsaturated fatty acid and total unsaturated fatty acid oxidation and 2, some active components in the garlic and ginger may involve desaturase and elongase activities (Mariutti et al., 2008).

Total viable counts

As shown, initial total viable counts (TVC) in all samples was in the range of 2.20 to 2.28 $\log_{10}$ CFU/g indicating very good fish quality, the value was found to increase ($p < 0.05$) but remained below 7 $\log_{10}$ CFU/g in all treatments for 24 days of storage, which is the Maximal Permissible Limit (MPL) for TVC recommended (ICMSF, 1986; Ojagh et al., 2010) in all samples. The values in the control samples were higher than their counterpart samples with storage time (Table 4). The increase of TVC in fish flesh during storage has been demonstrated by Bahmani et al. (2011). The high levels of microorganisms shown on control samples from day 6 of storage, resulted into significant differences ($P<0.05$) in TVC when compared with counterpart samples due to the strong antimicrobial activity of the organ sulfur compounds and other active components contained in garlic and ginger (Lu et al., 2011). TVC is the most common microbiological method aimed to detect and enumerate

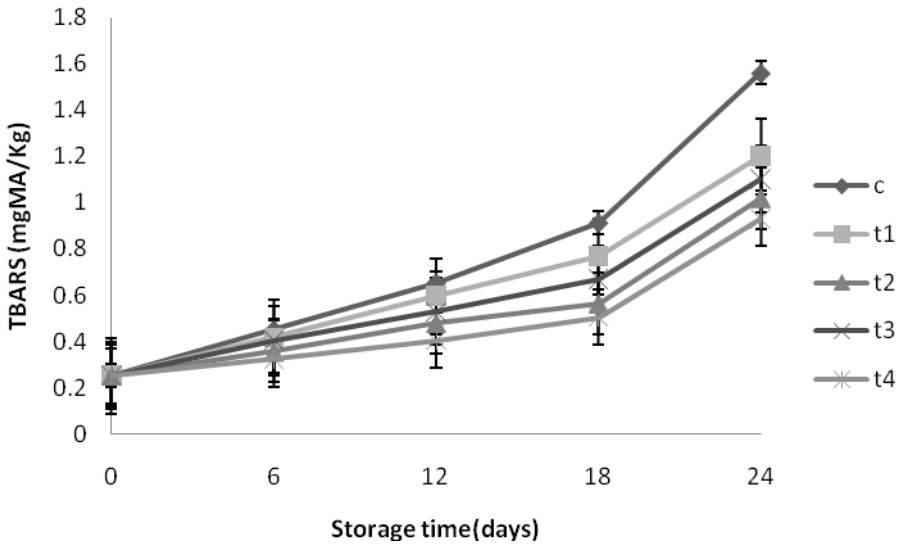

Figure 3. 2-Thiobarbituric acid-reactive substances (mgMA/kg).

Table 4. Total viable counts (log_{10} CFU/g).

Parameter	Storage time (days)				
	0	6	12	18	24
C	2.26±0.04^{aA}	3.53±0.45^{dB}	5.57±0.38^{cC}	6.12±0.11^{eD}	6.91±0.27^{cE}
T1	2.23±0.01^{aA}	3.08±0.16^{cB}	3.49±0.06^{bC}	4.16±0.18^{dD}	4.42±0.03^{bE}
T2	2.26±0.11^{aA}	2.71±0.14^{bB}	3.18±0.1^{aC}	3.72±0.07^{bD}	4.24±0.04^{baE}
T3	2.20±0.09^{aA}	2.86±0.03^{bB}	3.47±0^{bC}	3.91±0.01^{cD}	4.38±0.07^{bE}
T4	2.28±0^{aA}	2.49±0.09^{aA}	2.9±0.04^{aB}	3.44±0.17^{aC}	4±0.01^{aD}

$^{a–e}$Means with different superscript letters in the same column indicate significant differences ($p<0.05$). $^{A–E}$Means with different superscript letters in the same row indicate significant differences ($p < 0.05$). Data are expressed as means ± standard deviation (n = 2).

high proportion of the microbial population as possible. In practice, this usually means mesophilic, aerobic or facultatively anaerobic bacteria, which account for the major part of the microflora in fish. A TVC method can only provide an estimate of the microbial population based on those cells that are recoverable under the test conditions. Some viable cells may not be recoverable by any existing method, while others may only grow at low temperatures, in the presence of specific growth factors, or in the absence of oxygen (Fulford et al., 2004)

Conclusion

From the above results, it can be concluded that both garlic and ginger in fresh and powder form provide antioxidant and antimicrobial benefits to liquid smoked silver carp during chilled storage for 24 days. Therefore, it is suggested that garlic and ginger, as a natural herb, could be used to extend the shelf life of meat products, providing the consumer with food containing natural additives, which might be seen more healthful than those of synthetic origin. Further research is required to focus on understanding the mechanisms of action, in particular concentrations of active ingredients of both ginger and garlic in either powder or fresh form which applied to liquid smoked fish products.

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Short Communication

Studies on physical, chemical and rheological characteristics of pasta dough influenced by inulin

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The objective of this research was to evaluate the effects of different amounts (0, 1, 2.5 and 5%) of inulin on the physical, chemical and rheological properties of pasta dough. The results show that increasing the percentage of inulin, caused decrease in dough development time, dough stability, farinograph quality number, energy, resistance to extension as well as dough elasticity, while water absorption increased. The swelling index and the length of alveograph increased upon increasing the percentage of inulin. Increasing inulin addition to 5% caused the reduction in amount of proteins and ashes of samples, but this changes were not statistically significant (>0.05). 1 and 2.5% were generally selected as a superior treatment because they have fewer effects on dough rheological characteristics. While pasta dough containing 1% inulin was not optimum due to not having functional effects, 2.5% was accepted as a best level of inulin added to pasta dough.

Keywords: Pasta, dough, inulin, rheological characteristics.

INTRODUCTION

Inulin is an indigestible or low digestible sugar composition (from the type of oligosaccharides), which is found in more than 30,000 various plants (Kim et al., 2001). Chicory root is one of these plants that is cultivated, produced and exported by Belgium, Netherlands and French (Silva, 1996). The main reason of using chicory root as a source of inulin is the existence of glucose and long chains of fructose (Ozer et al., 2005). Inulin is a water soluble composition and its solubility depends on temperature. At 10°C, the solubility of inulin is 6%, while at 90°C, it is ~35% (Kim et al., 2001). Inulin, after getting to intestinal environment, as a source of carbon or energy causes the development and/or activity of prebiotics (beneficial intestinal bacteria containing Lactobacillus and Bifidobacterium) (Oliveira et al., 2009).

As a food additive with high nutritional value, prebiotics are considered as factors of increasing beneficial intestinal bacteria (Bifidobacterium and Lactobacillus) activity (Probert et al., 2004). Some health effects of prebiotics include: reducing the blood cholesterol level (Schrezenmeir and de Vrese, 2001), preventing the constipation and dysentery (Ozer et al., 2005), resisting infections such as salmonella (Oliveira et al., 2009), increasing the water absorption of mineral elements (manganese, magnesium and calcium) (Coudray et al., 1997), reducing the risk of osteoporosis, preventing the colonic cancer, reducing the blood glucose level (Gibson et al., 2004), treating the intestinal infections and reducing the intestinal bacteria and thus intestinal pH (Schrezenmeir and de Vrese, 2001).

In recent years, formulations of food stuffs with functional compositions especially prebiotics have been of great consideration (Fuad and Prabhasankar, 2010). Pasta has been the subject of interest of researchers because of its ease of use, low glycemic index and long shelf life (Jenkins et al., 1988). In order to enhance the

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Table 1. Chemical composition and physical specifications of inulin composition.

Composition ingredients of inulin	Amount of compositions
Moisture	Less than 3.5%
Dry matter	96±1%
Ashes	Max 0.3%
Carbohydrates	Min 99.7%
Dietary fiber	Min 90%
Sugars (glucose, fructose and sucrose)	Max 10%
Color	White
Taste	A little sweet
pH	6
Degree of polymerization	10
Aggregation	Less than 500 µ
Stability	Heat stable

Table 2. Pasta production formulation with different percentage of inulin composition.

Formula	Flour (%)	Dry gluten (%)	Inulin (%)	Total (%)
Control pasta	95.9	4.1	-	100
1	94.9	4.1	1	100
2	93.4	4.1	2.5	100
3	90.9	4.1	5	100

nutritional value of pasta, it is possible to add dietary fibers into pasta (Brennan et al., 2004). Findings of Peressini and Sensidoni (2009) showed that 5% inulin addition into flour caused a principal impact on customer attractiveness. Brennan and Tudorica (2008) found that incorporation of 10% inulin in pasta had no significant effect on pasta cooking loss; on the other hand it reduced water absorption and firmness. Manno et al. (2009) concluded inulin caused the change of starch-protein matrix in pasta structure.

According to Silva (1996) findings, inulin is a heat resistant prebiotic which can tolerate high temperatures up to 120°C. Therefore, the temperatures of pasta processing (80°C) have no significant effect on reduction of inulin content. Similar activity within flour pH is another inulin specification. The pH between 6-7 will have the best effect on inulin activity through the pasta production process. The objective of this study was to evaluate the effect of inulin on physical, chemical and rheological characteristics of pasta.

MATERIALS AND METHODS

Common wheat flour from red wheat (variety N-80-19) with ~0.650% ash, 14.3% moisture, 10.82% protein and 49.3% gluten index (provided by Karaj Zar Flour, Iran) and inulin extract of chicory root by Belgium Cosucra Company with fibruline instant brand, were prepared and stored in the warehouse for sensitive ingredients of factory with 15°C temperature until test. The chemical and physical properties of inulin are shown in Table 1. Pasta inulin with 1, 2.5 and 5% formulations were used to make prebiotic pasta dough (Table 2). Farinograph and Extensograph Brabender (with AACC approved method number (54-10) and (54-27) respectively), Chopin Alveograph (standard method (54-30A)) and ashes and proteins test (standard number (08-01) and (46-30) respectively) were used to measure the rheological properties of pasta dough (AACC, 2010). These experiments in random complete block designed by 4 replications were done, the variance of data obtained by SAS9.1 software was analyzed and the obtained mean results were compared by Duncan multiple range test (Der and Everitt, 2002).

RESULTS

The effect of inulin addition on the rheological properties of pasta dough in Farinograph are listed in Table 3. According to the results, by increasing the percentage of inulin in pasta dough, dough resistance or stability against mixing, dough development time, dough softening degree and finally, the correlation between the quality number and the rheological properties of pasta dough were decreased. The results showed that water absorption was increased by adding inulin. The effect of different percentages of inulin on the rheological properties of pasta dough in extensograph are listed in

Table 3. Result of analyzing the variance of inulin effects on pasta dough properties in farinograph.

Inulin	Water absorption	Dough stability	Degree of dough softening (after 10 min)	Degree of dough softening (after 12 min)	Farinograph number
Pasta inulin 5%	64.03±2.03^{a}	5.625±0.02^{c}	42.42±1.14^{c}	27.88±0.12^{c}	66.72±1.23^{c}
Pasta inulin 2.5%	62.47±1.78^{ab}	6.9±0.14^{b}	55.78±1.25^{c}	31.83±0.26^{b}	94.7±1.31^{b}
Pasta inulin 1%	61.13±2.32^{ab}	7.925±0.31^{a}	62.58±0.63^{b}	37.95±0.41^{ab}	107.3±1.72^{b}
Control pasta	59.85±1.34^{b}	7.6±0.26^{ab}	70±1.2^{a}	45.58±0.09^{a}	138.1±1.64^{a}

The same letter is not significantly different at P < 0.05.

Table 4. The effect of different inulin percentages on dough properties in extensograph.

Inulin	Resistance to extension (after 5 min)	Energy	Extensibility	Maximum resistant to extension	Ratio number	Maximum ratio number
Pasta inulin 5%	331± 2.41^{c}	79±0.14^{c}	127.3±1.52^{c}	454.8±3.28^{c}	2.6±0.71^{b}	3.55±0.45^{b}
Pasta inulin 2.5%	395.5± 3.25^{b}	86.25±0.14^{b}	139±1.62^{b}	482±2.76^{bc}	2.85±0.92^{b}	3.475±0.62^{b}
Pasta inulin 1%	428.5± 3.76^{ab}	92.25±0.14^{bc}	147.8±1.98^{a}	515.8±4.16^{ab}	2.925±0.56^{ab}	3.475±0.31^{b}
Control pasta	473.5± 3.92^{a}	102.3±0.14^{a}	144.5±2.21^{ab}	552.5±3.81^{a}	3.275±0.18^{a}	3.8±0.08^{a}

The same letter is not significantly different at P < 0.05.

Table 5. The effect of different inulin percentages on dough properties in alveograph.

Inulin	P value	L value	G value	W value	P/L value	I_e	P_{200}
Pasta inulin 5%	85.75± 1.17^{c}	75.25±1.51^{a}	19.75±0.67^{a}	204.8±0.14^{b}	1.15±0.02^{c}	47.42±1.08^{b}	40.65±0.47^{c}
Pasta inulin 2.5	105.5± 1.26^{b}	71.25±1.62^{a}	17.75±0.43^{ab}	219±0.14^{ab}	1.5±0.02^{c}	53.28±1.16^{a}	56.13±0.93^{b}
Pasta inulin 1%	128.3± 1.92^{a}	47.75±2.84^{b}	15.5±0.77^{bc}	224±0.14^{a}	2.7±0.06^{b}	41.72±0.87^{c}	53.45±1.04^{b}
Control pasta	142± 2.31^{a}	40.75±2.63^{b}	14.25±0.58^{c}	222.8±0.14^{a}	3.525±0.08^{a}	47.15±1.33^{b}	66.93±1.61^{a}

P: Pressure, L: length, G: swelling index, W: energy, P/L: pressure/length, I_e: extensibility index, P_{200}: the pressure in the first 4 cm. The same letter is not significantly different at P < 0.05.

Table 4. By increasing of inulin addition, the resistance to extension, dough energy, extensibility, maximum resistance to extension, ratio number were reduced. Evaluating the effect of different percentages of inulin on maximum ratio number shows that this parameter was decreased in all treatments as compared to the control pasta, by increasing of the amount of inulin.

The effect of Inulin addition on the rheological properties of pasta dough in Alveograph are listed in Table 5. Pressure value (P) and pressure/length index (P/L) were reduced by increasing the amount of inulin. On the other hand, length value (L) and swelling index (G) were enhanced. Evaluating energy (W) showed that although the pasta inulin 1% had more energy than the control pasta, this difference was statistically insignificant. The extensibility index (I_e) showed a significant decrease as compared to the control pasta by 1% inulin addition. The amount of P_{200} was reduced by increasing the percentage of inulin.

The effect of inulin addition on the pasta dough dry matter

According to Figure 1, although the content of protein is reduced by increasing the amount of inulin, this reduction is not statistically significant. Figure 2 indicates that addition of different percentages of inulin did not have a significant effect on the ash content of pasta dough. Although the amount of final product had some changes in the test samples, none of these changes were significant.

DISCUSSION

Evaluations indicate that existence of hydroxyl groups in inulin fiber structure will cause more hydrogen bonds, which will in turn, increase the water absorption (Chimrov et al., 1981). Inulin addition has a significant effect on

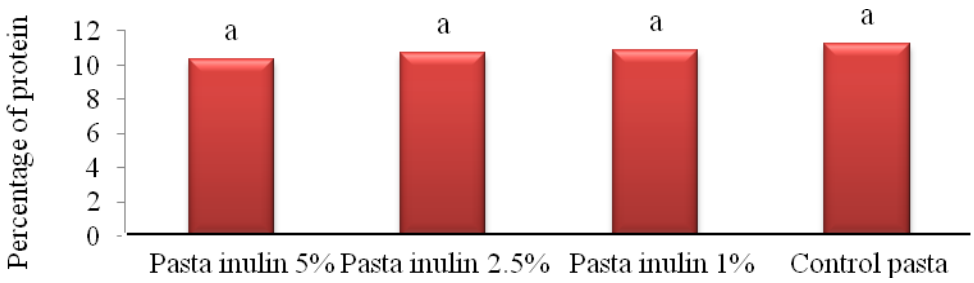

Figure 1. The effect of different percentages of inulin on pasta protein.

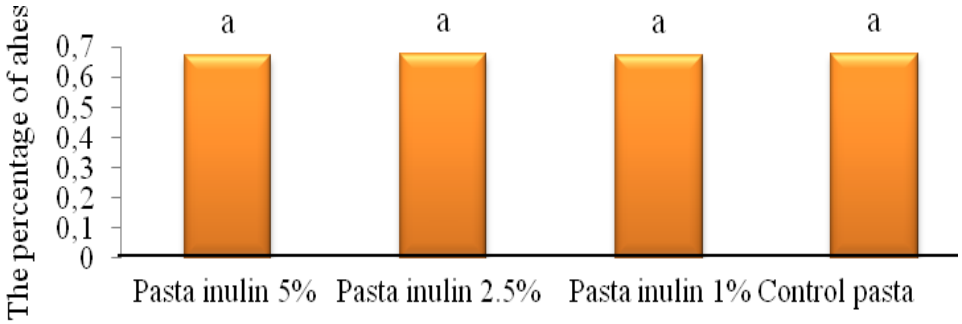

Figure 2. The effect of different percentages of inulin on pasta ashes.

pasta dough structure and would also make changes in pasta dough protein-starch bonds (Probert et al., 2004). Using the high percentages of inulin in pasta dough causes some specific changes in its organoleptic properties and consumer acceptability. Inulin addition by 1 and 2.5% percentages would cause little changes in pasta dough starch texture, while adding high percentages would cause many harsh changes in its structure and also weaken the protein-starch bonds in it. Addition of optimum percentages of inulin would cause the balance of protein-starch dehydration in the flour. Moreover, it will increase consumer attractiveness, reduce the calorie, increase the amount of indigestible fiber in it and finally increase the product's organoleptic properties (Ozer et al., 2005).

Although the addition of high percentages of more than 10% inulin cause significant decrease in pasta protein (Ozer et al., 2005), researches have shown that inulin addition by 1 to 5% will have no significant effect on reducing the pasta protein. For pasta, the minimum amount of protein is 10.5, which is also decreased by replacing the high percentages of inulin instead of flour as this reduction is not permitted below the standard range (10.5). Among the three pasta inulins of 1, 2.5 and 5%, pasta Inulin 5% is not appropriate in terms of the quality related to pasta dough properties in farinograph, because of some problems such as dough softening and reduction in dough resistance against mixing. According to the scientific articles, suggesting that pasta inulin 1% would not have functional specifications, inulin 2.5% was selected as a superior treatment among the other three experimented treatments.

The results obtained from extensograph indicated that pasta inulin (2.5%) has the highest capability among the three applied treatments and was more optimum in these properties. The results of alveograph, showed a significant correlation between the increase of the amount of inulin and rupture time, in other words, the higher the dough swelling index, the sooner it will rupture (Silva, 1996). So addition of inulin percentages more than 2.5% would cause the dough to rupture sooner, thus the high percentages are not optimum in terms of physical properties. Therefore, pasta inulin was selected as the best optimum treatment, although pasta inulin (1%) had better qualification than other percentages in alveograph.

Conclusion

Since the purpose of producing the pasta with inulin is primarily to increase its nutritional properties and then to have a high quality product, so in this research, the formulation that retains both aspects of nutritional properties and product quality according to the optimization of product process was used. The results showed that 2.5% treatment is more superior to the others. Therefore, we recommend the use of 2.5% inulin in the production formulation.

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Full Length Research Paper

Effect of moisture content on selected physical properties of shea kernel of varying slice thickness

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Bulk density, kernel density, particle porosity and shrinkage of sheanut krnel slices were determined as a function of moisture content and slice thickness in order to establish preliminary design parameters for drying in an indirect solar dryer. Solid density increased linearly while bulk density decreased non-linearly during drying as the moisture content decreased from 150 to 5 %d.b. The porosities of the kernels ranged from 60.12 -67.91, 57.05-64.87 and 56.88-62.49%, respectively, for the 5, 10 and 15 mm slices. The volumetric shrinkage of the shea slices reduced with decrease in moisture content for all the particle sizes evaluated in this study. Among the shrinkage models tested, the empirical quadratic model gave the best fit of the experimental data. It is proposed that the mechanism of shrinkage in sheanut kernel slices is linked to the removal of water that leads to structural collapse.

Key words: Density, porosity, volumetric shrinkage, sheanut kernel, slices

INTRODUCTION

The shea tree has long been reported as important livelihood tree (Maranz et al., 2003). This is because in addition to the medicinal value its leaves and roots has, the tree produces fruits with high oil content (45-50%) usually referred to as shea butter. In the processing of shea fruits to obtain butter, drying of the kernels after depulping and dehusking has been described as one of the key steps indispensable in the production process (Womeni et al., 2004). This is because sheanut kernels at harvest contain about 45-60% moisture content on a wet mass bases and are therefore prone to deterioration if not properly preserved. The kernel size varies from one locality to the other. For example, it measures 28.3 by 20.6 mm with respect to length and diameter in Chad (Mohagir, 2010), 36.63 by 27.93 mm in Nigeria (Olajide et al., 2000). In Cameroon, it measures averagely 45 by 30 mm (Bup Nde et al., 2012). Sheanut kernel is therefore one of the biggest oilseed in the world. Traditional drying of the whole kernel is carried out either under the sun or in solar dryers. This may take up to 20 days which therefore prolongs the processing time of these kernels. Drying experiments of whole kernels carried out in an electric dryer at 45°C reduced drying times to about 12 days but allowed the development of a surface coat which hindered uniform drying of the kernels (Kapseu et al., 2007). Drying for more than 12 days may equally lead to the development of moulds which contain lipases that catalyse hydrolyses thereby leading to rancidity in the resultant oil (Kapseu et al., 2007). To reduce this drying time it is advisable to dry these kernel as thin slices in solar dryers which can be cheap and easily affordable to local African women, the main processors of shea fruits to butter. The understanding and modelling of the drying behaviour of sheanut kernel slices and the development of a solar

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dryer for sheanut kernel slices requires the mastery of its physical properties such as densities, porosities and shrinkage. Although some of these physical properties have been reported in the literature for shea fruits, nuts and kernels (Olajide et al., 2000; Aviara et al., 2005; Bup Nde et al., 2012) those of sheanut kernels slices remain very scarce.

An important aspect of the study of shrinkage involves modelling so that they can be incorporated into drying models to study the effect of shrinkage on the drying behaviour of the product. Many of such models have been reported in the literature and one needs to make a judicious choice of which one best suits the product under study. The aims of this work were therefore to:

1. Determine the bulk density, solid density, porosity and volumetric shrinkage of sheanut kernel slices as a function of moisture content and slice thickness and
2. Model the volumetric shrinkage phenomenon in sheanut kernel slices.

MATERIALS AND METHODS

Sampling and sample treatment

Sheanuts obtained from Tchabal Ngaoundere were cooked in water using a laboratory oven at 80°C for 120 min (Bup Nde et al., 2012). They were then withdrawn from water, allowed to cool on a laboratory bench overnight, cracked and the kernels were cut into 5, 10 and 15 mm thick slabs using a Tommy Slicer (model Siemens, Erlangen, Germany). The sheanut kernel slices were then dried in an indirect solar dryer (drying air temperature 45 ± 5°C; airspeed 1.4 m/s, air realative humidity 55 ± 5%) for predefined periods of time and the resulting moisture contents was measured by the oven method. Results of the physical properties were then expressed as a function of moisture content.

Determination of bulk density of the kernels

The bulk density was determined using the AOAC (1980) method. This involved the filling of a 500 ml cylinder having an inner diameter of 49.54 mm with sheanut kernel slices to a height of 15 cm and weighing the contents. The bulk density ρ_b in kg/m^3 was given by:

$$\rho_b = \frac{m_b}{v_b} \quad [1]$$

where V_b is the bulk volume. These experiments were carried out for the 5, 10 and 15 mm thick slices. Each experiment was replicated four times at each particle size.

Determination of solid density of sheanut kernel slices

To determine the true or solid density of the shea kernel slices, an analytical balance (model Scout Pro SPU402, OHAUS, USA) adapted for this purpose was used. The balance was set to the specific gravity mode. A spring was attached to the balance from which the sample tied to a string of negligible weight was hung. The weight of the sample was taken in air. The sample was then immersed into a beaker of water placed on the balance and the new weight was taken. The specific gravity of the sample was then determined from the relation (as indicated in the User's Manual of the balance):

$$Specific\ gravity = \frac{weight\ in\ air}{weight\ in\ air - weight\ in\ water}$$

This was then converted to solid density given that the density of water at 22°C is 1 g/cm^3. At each moisture content, five kernel slices were used for each determination. The experiment was replicated thrice at each moisture content. These studies were carried out on 5, 10 and 15 mm thick sheanut kernel slices.

Determination of porosity

The porosity was calculated from the solid and bulk densities using the relationship given by Pabis et al. (1988). The porosity, ε, was given by the equation:

$$\varepsilon = 100\left(1 - \frac{\rho_b}{\rho_p}\right) \quad [2]$$

Determination of volumetric shrinkage of sheanut kernel slices

Volumetric shrinkage ratio was determined by measuring the volume of the kernels at each moisture content and defining the ratio V/V_0 as volumetric shrinkage ratio. V_0 is the corresponding volume of the kernels at the initial moisture content X_0, V is the corresponding volume of the kernels at moisture content X. Both empirical and fundamental shrinkage models with and without porosity terms were tested and some selected to describe the shrinkage behaviour in sheanut kernel slices. The first group of these models included empirical linear (Equation 3) and quadratic models (Equation 4):

$$\frac{V}{V_0} = a + b\frac{X}{X_0} \quad [3]$$

$$\frac{V}{V_0} = c + d\frac{X}{X_0} + e\left(\frac{X}{X_0}\right)^2 \quad [4]$$

The second group included the Vacarezza (1975) (Equation 5) and Suzuki et al. (1976) (Equation 6) models which are fundamental models without porosity:

$$\frac{V}{V_0} = q_0 + q_1\frac{X}{X_0};\quad q_0 = \frac{1}{X_0\left(\rho_s/\rho_w\right)+1};\quad and\quad q_1 = \frac{X_0\left(\rho_s/\rho_w\right)}{X_0\left(\rho_s/\rho_w+1\right)} \quad [5]$$

$$\frac{V}{V_0} = q_2 + q_3 X$$

$$q_2 = \frac{1-q_5}{X_0 - X_e - q_4\left(q_5X_0 - X_e + q_5 - 1\right)} \qquad q_3 = \frac{q_5X_0 - X_e - q_4\left(p_5X_0 - X_e + q_5 - 1\right)}{X_0 - X_e - q_4\left(q_5X_0 - X_e + q_5 - 1\right)}$$

$$q_4 = \frac{\rho_e - \left(1-X\right)\rho_e}{\rho_e} \quad \text{and} \quad q_5 = \frac{\left(1+X_e\right)\rho_0}{\left(1+X_e\right)\rho_e} \quad [6]$$

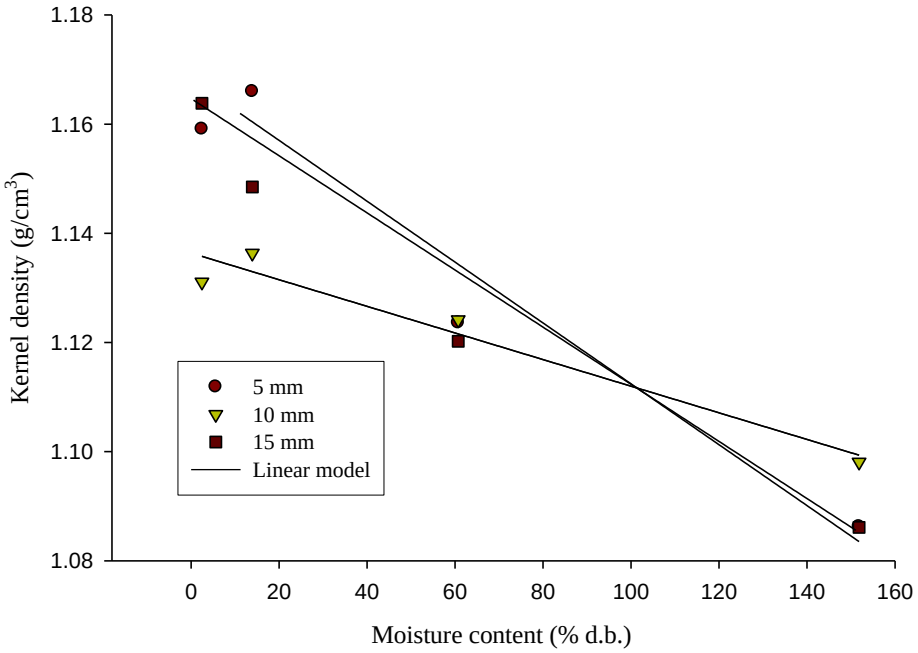

Figure 1. Influence of moisture content on the particle density of sheanut kernel slices.

The third group was the modified Perez and Calvedo model (Mayor and Sereno, 2004) (Equation 7) which included a porosity term, that is:

$$\frac{V}{V_0} = \frac{1}{(1-\varepsilon)}\left[1 + \frac{\rho_0(X - X_0)}{\rho_w(1+X_0)} - \varepsilon_0\right] \quad [7]$$

a, b, c, d, e are constants of the empirical models, q_i are parameters of fundamental models (variable), X_e is the equilibrium moisture content and p_e is the equilibrium density, p_0 is density at X_0, p_s and p_w are the densities of the dry solid and water respectively. q_5 is a constant when the equilibrium density (p_e) and the equilibrium moisture content (X_e) are known. ε and ε_0 are the porosities at moisture contents X and X_0.

Validation of models

The criteria for evaluating the reliability of the simulations were the correlation coefficients and/or the standard relative error of deviation observed on the moisture content between the experimental and theoretical results. The standard relative error (SRE) of deviation of theoretical from experimental results was determined from Equation 8.

$$SRE(\%) = \frac{100}{p}\sum_{i=1}^{p}\left|\frac{Y_{\exp} - Y_{\mathrm{mod}}}{Y_{\exp}}\right| \quad [8]$$

where Y_{exp} and Y_{mod} are the values obtained from experiments and from the model respectively. P is the number of points at which measurements were carried out.

RESULTS AND DISCUSSION

Solid density of sheanut kernel slices

The solid density increased linearly during the drying period as the moisture content decreased from 150 to 5% d.b. (Figure 1). The values ranged from 1.086-1.160, 1.098-1.131 and 1.086-1.163 g/cm^3 for the 5, 10 and 15 mm slices, respectively, in the moisture range studied. Solid density is simulated with linear equations with regression coefficients of 0.956, 0.951 and 0.963 for the 5, 10 and 15 mm thick kernels, respectively. The increase in solid density with decrease in moisture content could be due to the fact that, during the drying process, the kernels lost water and became more compact and were therefore capable of packing more regularly and closely in the measuring cylinder. This regular and closed packing could have been acompanied by a more rapid decrease in volume as compared to its mass and led to increased solid density which obviously increased with decrease in moisture content. The solid density for all the slices at all moisture contents was greater than unity, implying that the slices are heavier than water. This property can be very useful in the design of cleaning and separation equipments for the slices. No clear relation was established between solid density and particle size as the solid density was higher for the 10 mm thick slices at higher moisture content (150-100% d.b.) as compared to the 5 and 15 mm thick slices. The trends were reversed as the moisture content decreased below 100% d.b. The variation of solid density with moisture content was

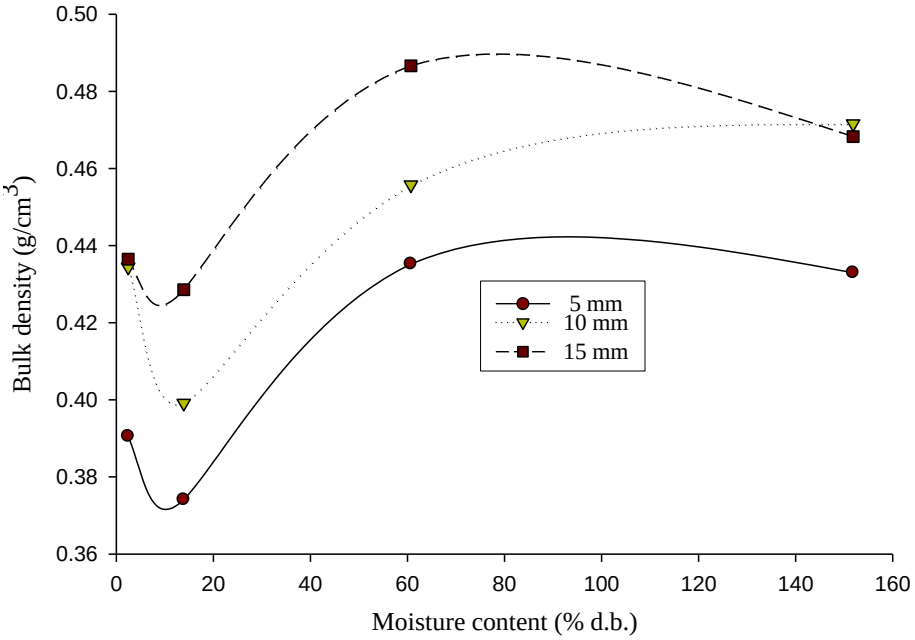

Figure 2. Influence of moisture content on the bulk density of sheanut kernel slices.

modelled with a linear equation with the regression coefficients greater than 0.95% and the SRE less than 5%.

Bulk density of sheanut kernel slices

Figure 2 shows the influence of moisture content on the bulk density of sheanut kernels slices. The bulk density of the sheanut kernel slices decreased non-linearly as the moisture content decreased from 150 to 5% d.b. In the final stages of drying, the bulk density slightly increased. The non-uniform decrease of bulk density with moisture content could be due to the fact that the bulk volume and mass of the kernels might not have changed uniformly in the course of drying. The increase in bulk density in the final stages of drying could be attributed to the fact that, at that stage, the mass of the product became constant due to the limitation of the movement of tightly bound water as observed in the drying of sheanut kernel slices (Kapseu et al., 2007). However, its volume continued to decrease due to the unbalanced pressure between the inner portion of the product and the outer environment thereby leading to an increase in the mass to volume ratio (bulk density). The bulk density decreased from 0.435 to 0.374, 0.472 to 0.399 and 0.468 to 0.429 g/cm^3 for the 5, 10 and 15 mm slices, respectively, in the moisture range studied. From Figure 2, it was equally observed that the bulk density was generally dependent on the particle size, increasing as the particle size increased.

Porosity of sheanut kernel slices

The porosity of the kernel slices increased non-uniformly with moisture content in the range 150-5% d.b. (Figure 3). The porosities of the kernels ranged from 60.12 - 67.91, 57.05-64.87 and 56.88-62.49%, respectively, for the 5, 10 and 15 mm slices. The porosities decreased with an increase in kernel size at all moisture contents. The slight decrease in porosity observed at the final stages of drying was properly due to the decrease in bulk volume at that stage as explained for bulk density above. The variation of porosity with moisture content of sheanut kernel slices observed in this work were different from trends reported by Aviara et al. (2005) for sheanuts in which porosity of the nuts increased with an increase in moisture content to a maximum value and then decreased sharply afterwards.

Volumetric shrinkage of sheanut kernel slices

The volumetric shrinkage of the shea slices reduced with decrease in moisture content for all the particle sizes evaluated in this study. It is clearly observed from Figure 4 that shrinkage of the kernel slices decreased non-linearly as the moisture content of the kernels decreased. The higher decrease in volume observed at higher moisture contents could be linked to the high elastic behaviour of the material still completely in its rubbery state. Under these conditions, they were sufficiently elastic to shrink into the space left by the evaporated water. This elasticity reduced as more and more water was evaporated from the product. The material became more rigid and led to a reduction in the rate at which the product shrunk. This suggested that shrinkage was probably caused by the volume of water removed.

As earlier mentioned, five models were tested to fit the data for shrinkage (Vacarezza, 1975; Suzuki et al., 1976); empirical linear and quadratic models and the modified

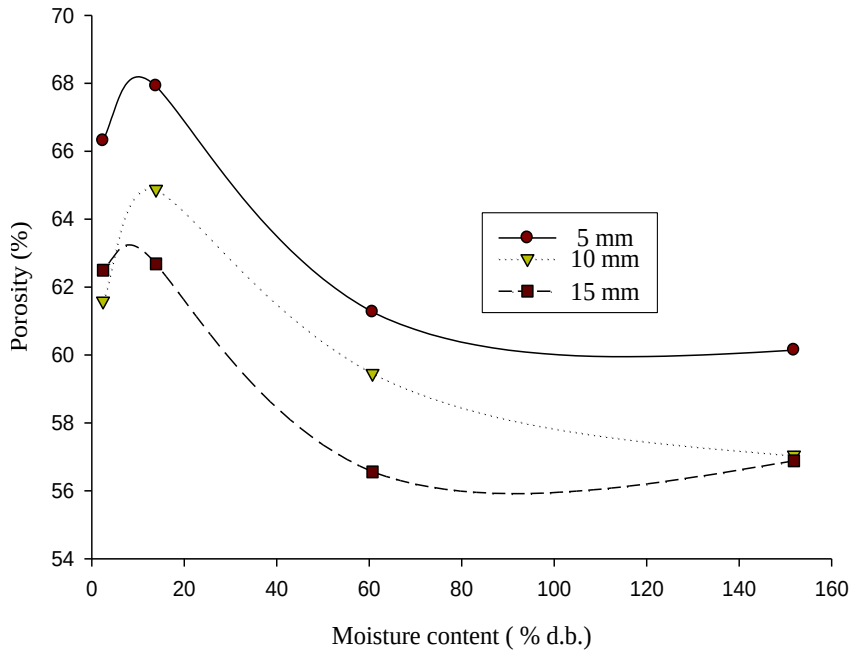

Figure 3. Influence of moisture content on the porosity of sheanut kernel slices.

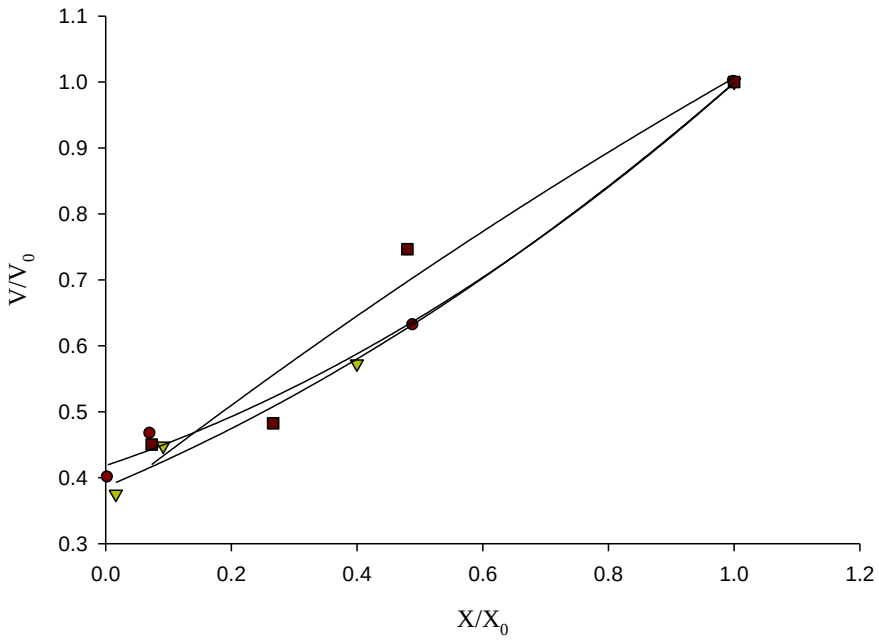

Figure 4. Influence of moisture content on the volumetric shrinkage of sheanut kernel slices.

Perez and Calvedo model (Mayor and Sereno, 2004). Of these models, the empirical quadratic model gave the best fit of the experimental data as indicated by its very high R^2 and very low SRE (Table 1). This model was therefore used to describe the shrinkage of the sheanut kernel slices. The modified Perez and Calvedo model gave very low R^2 and high SRE values indicating that it could not be used to describe shrinkage in sheanut kernel slices. The values of its constants are therefore not reported. The failure of the modified Perez and Calvado model could be attributed to the fact that, the porosity considered in this study was external porosity (voidage) instead of intrinsic porosity of the kernels. The non linear variation of volumetric shrinkage with moisture

Table 1. Model constants and R^2 and SRE values for equations used in modelling shrinkage of sheanut kernel slices.

Model / Particle size (mm)	Linear empirical				Vicarezza (1975)				Suzuki et al. (1976)		
	A	B	R^2	SRE	Q_0	Q_1	R^2	SRE	Q_5	R^2	SRE
5	0.3978	0.0038	0.982	5.59	0.4996	0.5004	0.954	12.8	0.4827	0.977	5.03
10	0.3649	0.0041	0.989	3.00	0.4996	0.5004	0.989	19.8	0.5021	0.988	17.90
15	0.384	0.0041	0.954	5.94	0.4996	0.5004	0.982	14.50	0.4847	0.999	9.75
					Quadratic						
				C	D	E	R^2	SRE			
5				0.418	0.319	0.261	0.996	2.76			
10				0.386	0.399	0.214	0.982	2.82			
15				0.367	0.735	-0.095	0.982	7.29			

content in the investigated moisture content range indicated that, during the drying process shrinkage is not essentially uniform. This assertion was buttressed by the fact that more than 60% of the samples were visually cracked after the drying experiments. Mayor and Sereno (2004) stated that surface cracking occurs during drying when shrinkage is not uniform leading to the formation of unbalanced stresses and failure of the material. A uniform shrinkage of the kernel slices could be linked to the fact that phase transition (change from a rubbery to the glassy state) in the material through out the drying process may have been highly limited or negligible. That is, if the material remained mostly in the rubbery state through out the drying process, then shrinkage almost entirely compensated for moisture lost and the volume of the material decreased linearly with moisture content. However, if the material passed from a rubbery to the glassy state (phase transition), the mobility of the solid matrix reduced and the rate and extent of shrinkage decreased significantly leading to deviations from linearity. This last phenomenon was observed for the sheanut kernel slices investigated in this work. Deviations of shrinkage from linearity especially at low moisture contents have been reported for garlic, potato and sweet potato (Ratti, 1994) and for apple and potato (Wang and Brennan, 1995). So in this work, it is proposed that the mechanism of shrinkage in sheanut kernel slices is linked to the removal of water that leads to structural collapse. Water removal becomes highly limited in the later periods of drying. This culminates into deviations from linearity which could be attributed to phase transitions. Further work is needed on the glass transition temperatures of sheanut kernel slices to verify this assertion.

Conclusion

This work reported some of the physical properties of sheanut kernel slices as a function of moisture content. Apart from the solid density whose variation was linear, bulk density and porosity varied non lineraly with moisture content and differed from those of whole large kernels in the mass range 21-28 g. Shrinkage was modelled by an empirical quadratic equation and it is proposed that the mechanism in sheanut kernel slices is linked to the removal of water that leads to structural collapse. The results of these physical properties can be employed in modelling drying processes for an eventual design of a dryer for sheanut slices.

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Full Length Research Paper

Hygienic and sanitary evaluation of minimally processed vegetables sold in public fairs in the Western Region of Paraná State, Brazil

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The microbiological quality of minimally processed vegetables sold in the public fairs of the numerous towns in the Western Region of Paraná State, Brazil, was studied. Lettuce, salad rocket, cabbage and chicory randomly collected were sent to the Food Analysis Laboratory (Lanali) in Cascavel, Brazil. The vegetables were analyzed for total and thermotolerant coliforms, aerobic mesophilic bacteria and *Salmonella* sp. Total coliforms were detected at different rates in all samples of vegetables analyzed. Cabbages from town 5 had the highest contamination rate ($3.6x10^6$ UFC/g) and the highest mean contamination rate ($4.1x10^4$ UFC/g) in terms of thermotolerant coliforms in 147 samples, at different count rates. In all the samples collected and analyzed, *Salmonella* sp. occurred in 10. Results show that vegetables sold in the public fairs in the Western Region of Paraná, Brazil were not good for human consumption due to feces-originated bacteria or high mesophilic counts. In fact, they are the possible sources of toxin and infections caused by pathogens.

Key words: Coliforms, *Salmonella*, vegetables.

INTRODUCTION

The consumption of vegetables by humans is highly relevant for a balanced diet as they contain vitamins, have health benefits and lead subsequently to healthier life style. In fact, their intake does not only reduce the development of disease, but also their fibers regulate the digestive functions of the human body (Germano and Germano, 2003). Vegetables are also a source of vitamins, especially vitamin C, B complex and A (β-carotene); while minerals, such as iron, calcium, potassium and magnesium are highly important components found in them (Philippi , 2003).

The consumption of minimally processed vegetables is highly relevant for the transmission of several infectious diseases especially those caused by irrigation with contaminated water or through the farm workers' lack of hygiene (Takayanagui et al., 2001). Since the culture of vegetables demands a moist environment, the constant irrigation of vegetables mainly leads to the formation and development of pathogenic microorganisms (Germano and Germano, 2003).

Food-transmitted diseases are mainly due to inadequate handling, lack of personal hygiene, dirtiness of the

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person who is supposed to clean the places where vegetables are prepared and sold and improper cleaning of the equipment and utensils before and after food manipulation (Vitti et al., 2010).

Since most vegetables may be consumed raw up to seven days after harvest, they may also cause many diseases. In fact, microorganism may be viable for a period between 7 and 40 days and may thus become a source of public health concern (Mogharbel, 2007).

Microbiological evaluations of minimally processed vegetables are required to verify the hygiene and sanitary conditions of food consumed by people. Verifying the presence of certain micro-organisms in vegetables is mandatory within the context of public health (Franco and Landgraf, 2005; Mogharbel, 2007).

MATERIALS AND METHODS

Current investigation was conducted between March and June 2012 at all fair stalls which sold vegetables in five different towns in the western region of Paraná. Sixty samples of all available vegetables, namely, lettuce, salad rocket, cabbage and chicory were collected in triplicates. Specific fairs were selected owing to the population´s interest in consuming products originating from these markets. The vegetables were conditioned individually in sterilized plastic bags, identified and sent to the Food Microbiology Laboratory (LANALI) of Cascavel PR, Brazil, for microbiological analysis.

Further, 25 g aliquots of each vegetable collected were retrieved and microbiologically evaluated to determine total and thermo-tolerant coliform counts (UFC/g), following methodology of Norm 62 of August 2003 (Brasil, 2003).

Total coliforms were counted in Violet Red Bile Agar and the counting of suspected colonies was done at a later phase, according to legislation. They were then inoculated in Brilliant Green Bile Lactose Broth at 2% and incubated (48h/36±1ºC) to confirm total coliforms. Thermotolerant coliforms were inoculated in EC broth and tubes were incubated (48h/44.5°C) in a warm bath under stirring (68rpm/min) for confirmatory proof.

In the case of *Salmonella* sp**.**, the materials were pre-heated in buffered peptonated water (24h/35°C) and selectively enriched in Rappaport-Vassiliadis and Selenito-Cistina broth (24h/42°C); they were then isolated in agar XLD and brilliant-green phenol-red lactose sucrose (BPLS). Biochemical identification was confirmed by tests for urease, fermentation of glucose, sucrose and lactose in TSI medium, de-carboxylation of lysine in LIA medium, H2S production, motility and production of indol by SIM medium.

Total counting of mesophilic micro-organisms was undertaken by serial dilutions in plate count agar (PCA) with plates incubated at 35ºC for 24-48 h for reading and counting of viable colonies.

Statistic analysis of variance of data evaluated was done by ANOVA and media comparison by Tukey´s test, both at 5% significance. All variables at 5% significance did not have a normal distribution but variance heterogeneity among treatments.

Box-Cox transformation was conducted with these variables to obtain normal distribution and homogeneity of variances. Results were compared with rates given by current ANVISA[1] legislation, according to RDC n. 12 of 2nd January, 2001.

RESULTS AND DISCUSSION

Whereas total coliforms at different rates were reported in ll samples, thermotolerant coliforms were registered in 147 samples of all the samples, at different rates. *Salmonella* sp. occurred in 10 samples of the total samples evaluated

Cabbages from town 5 registered the highest contamination rate ($3.6x10^6$ UFC/g), followed by lettuce, chicory and salad rocket, when total coliform rates in the different vegetables were analyzed.

Analysis of variance for total coliforms at 5% significance showed that interaction was not significant with an influence of vegetables according to their origin, except in the case of cabbages. Results were obtained by Tukey´s test of mean multiple comparison (Table 1) with a significant difference of 5% in town 4 compared to the others.

Further, significant differences (5%) were detected among the vegetables from towns 1 and 4. Tukey´s test at 5% significance (Table 1) showed that only lettuce in towns 1 and 2 differed from the others. The vegetable group of cabbage and lettuce differed from chicory and salad rocket group in town 3; only lettuce and salad rocket from towns 4 and 5 were statistically different.

Similar results were reported by Silva et al. (2007) when they analyzed the microbiological quality of minimally processed vegetables sold in Porto Alegre RS Brazil. High coliform counts were registered. Only two samples had low total coliform rates. In fact, total coliforms may be very common in food and may originate from several contamination sources such as soil and irrigation water (Mogharbel, 2007).

Analysis of thermotolerant coliforms in different vegetables revealed that cabbages had the highest mean contamination rate ($4.1x10^4$ UFC/g) for town 5, followed by lettuces. Contamination data mainly occurred as a probable result of lack in personal hygiene, handling, transport and inadequate storing of the vegetables.

In fact, there were significant differences between the towns for all the vegetable types under analysis. Results were also given by Tukey´s mean multiple comparison test, especially with regard to cabbages from town 4 when compared to the others. A statistical difference for lettuce was reported between towns 3 and 5; whereas for cabbages significant difference was reported in town 4 when compared to towns 2, 3 and 5 (Table 2).

According to Mogharbel (2007), poor hygiene conditions during food processing, production, storage and handling, coupled with lack of treatment of irrigation water from streams and rivers and transport to the consumer market are factors for the occurrence of food contamination.

Similar results were reported by Takayanigui et al. (2001) who observed high concentrations of thermotolerant coliforms in vegetables sold without any sort of treatment and related to lack of hygiene during the collection process and food selling.

Results from the microbiological evaluation of minimally processed lettuces (*Lactuca sativa* L.) and cabbages

Table 1. Comparison for the presence *Escherichia coli* (in 10,000 units) in minimally processed vegetables between the cities of origin.

Vegetable	Town				
	1	2	3	4	5
1	55.80^{aA}	84.70^{aA}	230.00^{aA}	0.19^{bAB}	41.2^{abA}
2	0.83^{aB}	0.24^{aB}	5.90^{aA}	1.28^{aA}	1.63^{aAB}
3	15.00^{aB}	0.06^{aB}	0.05^{aB}	0.03^{aAB}	0.25^{aAB}
4	0.03^{aB}	0.02^{aB}	0.03^{aB}	0.005^{aB}	0.04^{aB}

Small letters represent comparison between lines; capital letters represent comparison between columns at 5% probability.

Table 2. Comparison for the presence of *Escherichia coli* (in 10,000 units) in minimally processed vegetables between the cities of origin.

Vegetable	Town				
	1	2	3	4	5
1	55.80^{aA}	84.70^{aA}	230.00^{aA}	0.19^{bAB}	41.2^{abA}
2	0.83^{Aab}	0.24^{aB}	5.90^{aAB}	1.28^{aA}	1.63^{aA}
3	15.00^{Aab}	0.06^{aB}	0.05^{aBC}	0.03^{aAB}	0.25^{aA}
4	0.03^{aB}	0.02^{aB}	0.03^{aC}	0.005^{aB}	0.04^{aA}

Small letters represent comparison between lines; capital letters represent comparison between columns at 1% probability

(*Brassica oleracea* L.) sold in Brasília DF Brazil identified thermotolerant coliforms in some samples analyzed. In fact, 19% of the 36 lettuce samples were contaminated by thermotolerant coliforms, whereas 81% were free from any contamination, according to current legislation. Further, 22% of 60 raw lettuces and cabbages analyzed were contaminated by thermotolerant coliforms and 78% were free from any contamination.

However, a study undertaken in the Brazilian Federal District revealed that all minimally processed lettuce, cabbage and turnip samples were contaminated by thermotolerant coliforms (Almeida et al., 2012). The same result was verified in another study in Uberlândia MG Brazil, where all raw food samples were contaminated at rates above those allowed by current legislation (Bonnas et al., 2005). *Escherichia coli* was reported in 8 out of 56 vegetable samples in Porto Alegre RS Brazil (Silva et al., 2007).

Vegetables actually free from thermotolerant coliforms indicated good sanitary conditions during processing and compliance with the microbiological standards of ANVISA (Sasaki et al., 2006). The contaminated vegetables were not fit for consumption due to fecal contamination.

Table 3 shows a descriptive analysis for data on *Salmonella* sp. Cabbages and lettuces had the highest contamination (approximately 66.67%), especially those from towns 2 and 3.

Contamination by *Salmonella* sp. is a public health concern due to the possibility of infection by toxins. However, its low contamination rate when compared to that by coliforms may be related to competition by other bacteria in the environment as well as by producers´ hygiene conditions when dealing with their products (Marques et al., 2006). Takayanagui et al. (2001) reported similar results, with only 9% of vegetables contaminated by *Salmonella* sp.

Mean mesophile counts in UFC / g varied between 2.5x10 and 3.6x106. The farmer from town 5 not only had the highest mesophile rates in harvested vegetables corresponding to data on total and thermotolerant coliforms but also showed contamination related to lack of hygiene. Perhaps due to their greater leaf surface, cabbages had the highest mesophilic count when compared to rates of the other vegetables.

Interaction was not significant at 5% for mesophile counting (Table 4). Consequently, the behavior of the variable may be analyzed separately with regard to each factor (town and vegetable type) significant at 1% probability. Mesophile count at 1% significance is influenced by town and vegetable type. Tukey´s test of mean multiple comparison for mesophiles (Table 5) shows that, at 1% significance, there were significant differences among all types of vegetables and between town 4 and towns 1 and 5.

Even after minimal processing, raw vegetables may still retain most of their previous microbiota. This is a serious health issue since pathogens may be included in the microbiota. In fact, they should be stored at proper

Table 3. Comparison of minimally processed vegetables as presence of *Escherichia coli* (in 10,000 units), the cities of origin.

Vegetable	Town				
	1	2	3	4	5
1	6.73^{aA}	1.09^{aA}	1.22^{aA}	0.02^{bA}	1.03^{aA}
2	0.61^{abA}	0.05^{bB}	0.03^{bB}	0.36^{abA}	2.38^{aA}
3	0.004^{abB}	0.009^{aB}	0.02^{aB}	0.00^{bB}	0.05^{aB}
4	0.001^{abB}	0.007^{aB}	0.03^{abB}	0.00^{bB}	0.003^{abB}

Small letters represent comparison between lines; capital letters represent comparison between columns at at 5% probability.

Table 4. Comparison of minimally processed vegetables as presence of *Escherichia coli* (in 10,000 units), the cities of origin.

Vegetable	Town				
	1	2	3	4	5
1	6.73^{aA}	1.09^{aA}	1.22^{aA}	0.02^{bA}	1.03^{abAB}
2	0.61^{aA}	0.05^{aAB}	0.03^{aAB}	0.36^{aA}	2.38^{aA}
3	0.004^{abB}	0.009^{abB}	0.02^{aB}	0.00^{bB}	0.05^{abBC}
4	0.001^{aB}	0.007^{aB}	0.03^{aB}	0.00^{aB}	0.003^{aC}

Small letters represent comparison between lines; capital letters represent comparison between columns at 1% probability.

Table 5. Comparison of minimally processed vegetables as the presence of Salmonella in the cities of origin.

Vegetable	Town					Total (%)
	1	2	3	4	5	
Cabbages	1	2	2	0	1	6 (54.55)
Lettuce	0	2	2	0	1	5 (45.45)
Chicory	0	0	0	0	0	0 (0.00)
Salad rocket	0	0	0	0	0	0 (0.00)
Total (%)	1 (0.09)	4 (36.36)	4 (36.36)	0	2 (18.18)	11

temperatures so that the growth of pathogens could be inhibited.

Conclusion

Vegetables sold in the public fairs of the main towns of the western region of Paraná are improper for natural human consumption since they contain fecal bacteria or high levels of mesophiles and certain quantity of *Salmonella;* and have the possibility of causing toxin and infections.

Cabbages had the highest contamination rate compared to the other vegetables, due to their larger leaf surface.

Vegetables from town 5 had the highest contamination, with the least hygiene and sanitary care.

Effective supervision by the health authorities should be warranted to ensure that agricultural products that are consumed raw could be sold in good conditions without the addition of any special treatment.

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Full Length Research Paper

Effects of germination time on the functional properties of maize flour and the degree of gelatinization of its cookies

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Flour was produced from germinated and ungerminated maize grains, and cookies were subsequently produced from the flours. Functional properties of the flours and degree of gelatinization of the cookies were determined. The pH decreased significantly (p≤0.05) as the germination time increased. pH values ranged between 5.67 and 6.56. Bulk density (loosed and packed) showed no significant difference (p≤0.05) between ungerminated sample and sample that germinated for 24 h, but there was a significant difference (p≤0.05) between ungerminated sample and samples that germinated for 48 and 72 h. The values ranged between 0.50-0.58 and 0.70-0.79 g/mL for loose and packed density, respectively. There was a significant difference (p≤0.05) among the samples in water absorption capacity and oil absorption capacity with the ungerminated sample having the least values of 0.94 and 1.03 ml/g for water absorption capacity and oil absorption capacity, respectively. There was a significant difference (p≤0.05) in swelling power of the samples with the ungerminated maize having the highest value of 19.81 mg/g. There was a significant difference (p≤0.05) in the forming capacity with the sample that germinated for 48 h having the least value. There was a significant difference (p≤0.05) in the forming capacity with the sample that germinated for 48 h having the least value. There was no significant difference (p≤0.05) between the ungerminated flour and the sample that germinated for 48 h and foaming stability time of 15, 30 and 60 s. Degree of gelatinization of the samples ranged between 74.50-93.10, 86.20-97.40 and 30.00-84.30 at baking temperature of 140, 160 and 180°C, respectively.

Key words: Germination, gelatinization, functional properties, maize flour.

INTRODUCTION

Maize (*Zea mays* L.), the American Indian word for corn literally means “that which sustains life”. It is the third most important cereal grain of the world after wheat and rice providing nutrients for humans and animals and serving as a basic raw material for the production of starch, oil and protein, alcoholic beverages, food sweeteners and more recently, fuel (FAO, 1992). These three most important cereals have been reported to comprise at least 75% of the world’s grain production and therefore, humanity has become dependent upon cereal grains for the majority of its food supply (Imtiaz et al., 2011).

Starch is the major constituent of cereal endosperms and an important structural component in many food products made from their flours (Sasaki, 2005). Corn starch is a valuable ingredient in the food industry, being

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widely used as a thickener, gelling agent, bulking agent and water retention agent (Singh et al., 2003). Corn starch granule has a polyhedral shape and diameter between 5 and 25 µm (Ribout, 2002). On the basis of amylose and amylopectin ratio, corn can be separated into normal, waxy and high amylose (Singh et al., 2005). In addition, sugary type corn with high sugar content also exists (Singh et al., 2005). Normal starch consists of about 75 wt% branched amylopectin and about 25 wt% amylose, that is linear or slightly branched.

The effective use, attributes and consequent acceptance of cereal flours by consumers are dependent on its functional properties and the degree of starch gelatinization (Miah et al., 2001; Brou et al., 2013), Mantzari (2010) reported that due to its complexity, corn starch exhibits certain unique properties (which are not encountered in other polysaccharides) which are correlated with its physicochemical and functional properties: temperature and enthalpy, gelatinization, pasting characteristics, enzymatic susceptibility, swelling and solubility (Rubi, 2009).

Several works have been done on germination as an alternative to genetic engineering in improving the nutriative values (such as amino acids, vitamins, minerals etc.), functional (temperature and enthalpy, gelatinization, pasting characteristics, swelling, and solubility) and chemical (pH, amylose, total starch etc.) properties of maize, particularly in the developing countries (Oluwamukomi et al., 2003; Obasi et al., 2009; Eneche, 2009; Gernah et al., 2011). Typically, chemical modification could be adopted to extend the range of specific physical properties available for different uses (Nur and Purwiyatno, 2010), however, the use of chemicals are not encouraged due to their side effects, besides, these chemicals are not readily available to vast population of the developing nations who rely mostly on these cereals. FAO/WHO (1985) stated the guidelines of an ideal weaning food to be nutrient dense, easily digestible, of suitable consistency and affordable.

This study was therefore undertaken to investigate the effects of germination time on some functional properties of maize starch and the degree of gelatinization of its cookies.

MATERIALS AND METHODS

Source of material

White maize grains (TZW, 2008 harvest) were purchased from Minna Central Market, Minna and were identified at the Department of Crop Production, Federal University of Technology, Minna, Niger State, Nigeria. These were utilized for research work between June, 2009 and January, 2010.

Cleaning

The maize grains were manually cleaned to remove husks, stone, cob, damaged and coloured seeds. These were achieved through winnowing, sieving and hand picking. Subsequently, the seeds were packaged in a 10 L plastic bucket, hermetically covered and stored in a refrigerator at 10±2°C from where samples were taken for processing and analyses.

Table 1. Cookie recipe.

Parameter	Measurement
Maize flour	100 g
Baking fat	50 g
Baking powder	5 g
Sugar	45 g
Egg (raw)	2
Powdered milk	30 g

Source: Asumugha and Uwalaka (2000) with little modification.

Germination

Germination was carried out according to the method described by Ariahu et al. (1999). The seeds were washed in 5% (w/v) sodium chloride solution to suppress mould growth and soaked in tap water in ratio of 1:3 (w/v) grain for 12 h at room temperature (32±2°C), the water drained at 4 h interval after which the seed were drained and divided into four equal portions and labeled A, B, C and D, spread separately on a clean jute bag, covered with damp cotton and were allowed to germinate for 0, 24, 48 and 72 h, respectively. Water was sprinkled at 12 h interval to facilitate the germination process. At the end of germination, root hairs were removed from the germinated seeds.

Production of germinated flour

The seeds were dried at 60°C in an oven to a moisture content of 10% and ground into flour using attrition mill (globe p44 Chima). Each flour sample was passed through a 0.5 mm mesh size sieve. They were packaged in an air tight polyethylene bags, stored in plastic containers with lids and then stored in cool dry place from where samples were taken for analyses.

Production of cookies

Cookies were prepared from the flour samples using the cream-in method as described by Asumugha and Uwalaka, (2000) with little modification. Table 1 shows the cookie recipe. Fat and sugar were mixed until fluffy. Whole eggs and powdered milk were added while mixing (HR-2815 Philips Model Mixer) and then mixed for a total of about 30 min. Flour and baking powder were mixed thoroughly and added to the cream mixture and were kneaded to form dough. The dough were rolled and cut into shapes of 5 cm diameter. Baking was carried out at 140, 160 and 180°C for 25 min. Cookies were cooled and stored till when needed.

Sample analysis

Functional properties

Loosed and packed bulk densities of the flour samples were determined using the method described by Wang and Kinsella (1976), water absorption capacity (WAC) and oil absorption capacity

Table 2. Functional properties of maize flour samples.

Parameter	Germination time			
	0 h	24 h	48 h	72 h
pH	6.56±0.01^{a}	6.20±0.02^{b}	6.28±0.04^{b}	5.67±0.02^{c}
LBD (g/mL)	0.58±0.03^{a}	0.57±0.02^{a}	0.55±0.00^{b}	0.50±0.00^{c}
PBD (g/mL)	0.79±0.00^{a}	0.76±0.00^{b}	0.75±0.00^{b}	0.70±0.00^{c}
WAC (ml/g)	0.94±0.00^{d}	2.65±0.00^{c}	2.73±0.03^{b}	2.79±0.00^{a}
OAC (mg/g)	1.03±0.00^{c}	2.45±0.05^{b}	2.52±0.01^{b}	2.57±0.00^{a}
EC (%)	47.22±0.00^{c}	58.62±0.02^{b}	60.00±0.00^{b}	65.52±0.00^{a}
SP (mg/g)	19.81±0.00^{a}	19.10±0.02^{b}	18.80±0.00^{c}	15.00±0.00^{d}
FC (%)	3.10±0.00^{c}	2.50±0.02^{b}	2.00±0.00^{c}	2.50±0.00^{b}

Values are means and standard deviations of triplicate scores. Values followed by different superscript in row are significantly different (p≤0.05) from one another. LBD, Loose bulk density; PBD, packed bulk density; WAC, water absorption capacity; OAC, oil absorption capacity; EC, emulsion capacity; ES, emulsion stability; SP, swelling power; GPC, gelation power capacity; FC, foaming capacity.

(OAC) were determined by methods described by Sasulki et al. (1996), emulsion capacity and stability were determined using the method described by Yasumatsu et al. (1992), foaming capacity was determined according to the method described by Narayana and Narsinga (1992), swelling power was determined by Akpada and Miachi (2001) method and pH was determined using AOAC (2000) method.

Determination of degree of starch gelatinization

Determination of degree of starch gelatinization of cookies was determined by the method described by Marshall et al. (1993). 2 g of the sample was macerated with 100 ml distilled water in a warming blender (HR-2815 Philips model). The suspension was centrifuged at 500 rpm for 10 min and duplicate aliquots (1 ml) were diluted with water to 10 ml and treated with 0.1 ml iodine solution. The absorbance of the sample were read at 600 nm with spectrophotometer (model 2903, perkin- Elmer co. Ltd.), against a reagent blank. A further suspension of the product (2 g) was prepared in 95 ml of distilled water (instead of 100 m distilled water) as described earlier. To this suspension, 5 ml of 10 M aqueous solution of potassium hydroxide was added and mixture was allowed to stand for 5 min with gentle agitation. The alkaline suspension was centrifuged and 1 ml of duplicate aliquots was treated with 1 ml of 0.5 M hydrochloric acid and diluted with water to iodine solution (0.1 ml) and their absorbance was measured as described earlier. The degree of starch gelatinization was calculated as:

$$\frac{A_1 \times 100\ (\%)}{A_2}$$

Where A_1 and A_2 are absorbance of the iodine complex prepared from the aqueous suspension before and after alkali solubilization, respectively.

Statistical analysis

The data obtained were statistically analyzed by subjecting them to analysis of variance (ANOVA) using the completely randomized design (CRD) with comparison made between the group means using the Duncan's new multiple range test to separate the mean (at 5% probability level) using SPSS (statistical package for social scientists), version 16.0, Windows 2006.

RESULTS

Table 2 shows the functional properties of maize flour samples. The pH decreased significantly (p≤0.05) as the germination time increased. Maize grains that germinated for 72 h had the lowest pH value of 5.67. Bulk density (loosed and packed) showed no significant difference (p≤0.05) between ungerminated sample and sample that germinated for 24 h, but there was a significant difference (p≤0.05) between ungerminated sample and samples that germinated for 48 and 72 h. There was a significant difference (p≤0.05) among the samples in water absorption capacity with the ungerminated sample having the least value.

Oil absorption capacity varied significantly (p≤0.05) among the samples with germination done for 72 h having the highest value. Emulsion capacity of the flour samples also differ significantly (p≤0.05) with germination for 72 h having the highest value. There was a significant difference (p≤0.05) in swelling power of the samples with the ungerminated maize having the highest value. There was a significant difference (p≤0.05) in the foaming capacity with the sample that germinated for 48 h having the least value.

Table 3 shows the foaming stability of the maize flour samples. There was no significant difference (p≤0.05) between the ungerminated flour and the sample that germinated for 48 h at foaming stability time of 15, 30 and 60 s both having higher foaming stability than samples that germinated for 24 and 72 h at the same foaming stability time.

Table 4 shows the effect of germination time on the degree of gelatinization of cookies produced from the maize flour samples. At 140°C, the degree of gelatinization of the ungerminated sample was higher than those of germinated samples, although it was not significantly different (p≤0.05) from the sample that germinated for 72 h. At 160°C, there was a significant difference (p≤0.05) among the samples with the ungerminated sample having

Table 3. Foaming stability of the maize flour samples.

Germination time (h)	Foaming stability time (seconds)			
	15	30	60	120
0	50.90±0.000[a]	41.00±0.000[a]	25.15±0.000[a]	15.00±0.000[a]
24	40.10±0.028[b]	35.00±0.028[b]	20.15±0.000[b]	10.00±0.000[b]
48	50.57±0.000[a]	42.00±0.000[a]	25.00±0.000[a]	9.50±0.000[b]
72	30.50±0.000[c]	20.10±0.028[c]	10.00±0.000[c]	10.00±0.000[b]

Values are means and standard deviations of triplicate scores. Values followed by different superscript in column are significantly different (p≤0.05) from one another.

Table 4. Effect of germination time on the degree of gelatinization of the cookies.

Germination time (h)	Temperature (°C)		
	140	160	180
0	93.10±0.000[a]	86.20±0.283[d]	30.00±0.000[d]
24	74.50±0.000[c]	91.90±0.000[c]	40.90±0.000[c]
48	90.50±0.283[b]	93.70±0.283[b]	65.40±0.283[b]
72	92.90±0.000[a]	97.40±0.283[a]	84.30±0.000[a]

Values are means and standard deviations of triplicate scores. Values followed by different superscript in column are significantly different (p≤0.05) from one another.

the least value and the sample that germinated for 72 h having the highest value.

DISCUSSION

The decrease observed in pH might have been as a result of secretion of enzymes resulting in the hydrolysis of complex organic molecules such as phytin and protein into simpler and more acidic compounds such as phosphate and amino acids, respectively. Evans et al. (2003) reported a marked increase in alpha amylase and other amylases during cereal germination. Egwim and Oloyede (2004) reported 72 h as optimum sprouting time for maximum amylase activity in maize. Results obtained for loosed and packed densities were in line with earlier work of Gernah et al. (2011). The reduction in bulk density observed might have been as a result of reduction in weight of the flour owing to the breakdown of complex denser compounds inherent in maize into simpler ones during germination (Gernah et al., 2011). Germination increased water absorption capacity of the samples, this contrasted the work of Imtiaz et al. (2011) but in line with the work of Gernah et al. (2011). The increase observed might have been as a result of the production of compounds having good water holding capacity such as soluble sugars. According to Okaka and Potter (1997), water holding capacity depends on the water bounding capacities of food components. Germination increased oil absorption capacity in line with earlier work of Imtial et al. (2011). Giani and Bekebain (1992) reported that germination of grains enhances the oil absorption capacity due to the entrapment of oil related to the non polar side chains of proteins. The increase observed in emulsion capacity could be due to an increase in the area of stabilized oil droplet at interface which is a function of the food components (Imtiaz et al., 2011). Germination decreased the swelling power of the samples probably as a result of disruption of hydrogen atoms inherent in maize by amylases and proteases into sugars and amino acid respectively (Okafor, 1987; Egwim and Ademonom, 2009). The decrease observed in foaming capacity might have been as a result of denaturation of protein molecules during milling and germination processes. Brou et al. (2013) reported that native protein provide higher foam capacity than denatured protein.

Brou et al. (2013) reported increasing foaming stability with increasing protein content while characterizing complementary food made from maize, millet, beans and soybeans. They further reported higher protein stability for native proteins. The increase in foaming stability observed for sample that germinated for 48 h might have been as a result of bioavailability of inherent proteins which were probably bound by antinutritional factors such as phytin in the sample. Singh and Raghuvanshi (2012) reported that antinutritional factors in cereals bind to both exogenous and endogenous proteins including enzymes of the digestive tract affecting utilization of proteins. The reduction in stability observed for sample that germinated for 72 h could have been due to denaturation of protein (Brou et al., 2013). The ungerminated sample had a higher stability than the germinated samples at 120 s. The result

from the tables show that foaming stability of the samples decreased with time.

At 140°C, short germination period was not favourable probably due to the resistance of the amylose portion of the starch to the enthalpy produced at this temperature. The amylose content greatly influences the physicochemical properties of starch, such as gelatinization, retrogradation, and gelation (Parovuori, 1997; Czuchajowska, 1998; Fredriksson, 1998). Sasaki (2005) reported that starch with higher amylose content has more amorphous region and less crystal line, lowering gelatinization temperature. Germination increased the degree of gelatinization at 160°C probably due to the presence of adequate crystalline fractions in the starch molecules. The enthalpy of gelatinization reflects the loss of molecular order (Cooke and Gidley, 1992), and gelatinization temperature is considered a parameter of crystallite perfection (Tester and Morrison, 1990). At 180°C, the degree of gelatinization of the flour samples reduced remarkably probably due to disruption of the crystallite fractions of the samples.

Conclusion

The study indicated that germination has tremendous effects on the functional properties of maize flour samples considered, and on the degree of gelatinization of the cookies produced at various baking temperature. Germinated samples performed better in terms of water absorption capacity, oil absorption capacity and emulsion capacity. Sample that germinated for 48 h had the highest foaming capacity at time 15, 30 and 60 s. These are indications of improved nutritional value and functionality. Sample that germinated for 72 h performed best in water absorption capacity, oil absorption capacity and emulsion capacity. Germination resulted in cookies with better gelatinization at different baking temperature with sample that germinated for 72 h having the best result.

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Screening, identification and antagonistic activity of halo stable *Bacillus* sp. Mk22 used as probiotic in *Penaeus monodon* Fabricius, 1798

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The research of probiotics for aquatic animals is increasing with the demand for environmental friendly aquaculture. Most attempts to propose probiotic have been undertaken by isolating and selecting strains from saltpan environment. 16S rRNA gene sequencing showed that strain *Bacillus* sp. Mk22 (accession number: JF794553) was gram positive, had rod shape and was 937 nm in size. The isolated strain that was applied to *Penaeus monodon* culture under laboratory condition revealed that maximum survival rate was 92.7 ± 1.53%, wet weight was 6.9 ± 0.15 g, production was 378 ± 1.0 g and feed conversion ratio was 0.9 ± 0.03 in experiment-III compared to those of control experiment. The present study also showed that the halophilic (*Bacillus* sp.) bacterium was able to colonize both the culture in water and shrimp digestive tract. The minimum total bacterial counts ($6.0 \pm 1.0 \times 10^5$ Cfu ml^{-1}) and maximum *Bacillus* counts ($5.6 \pm 0.75 \times 10^4$ Cfu g^{-1}) were recorded in experiment-III and not in the control.

Key words: *Bacillus* sp., polymerase chain reaction, *Penaeus monodon*, probiotic, Saltpan.

INTRODUCTION

The UN FAO estimates that half of the world's seafood demand will be met by aquaculture in 2020, as wild capture fisheries are overexploited and are in decline. Shrimp (or prawn) culture is wide spread throughout the tropical world. It is in an industry set for a period with strong growing demand and is currently worth around US$10 billion. In most of the world, aquaculture industry is beset by disease outbreak, caused mostly by bacteria (especially for genome *Vibrio*) and viruses. The high density of animals in hatchery tanks and ponds is conducive for the spread of pathogens; and the aquatic environment as well as regular applications of protein-rich feed is ideal for culturing bacteria. Mostly, *Vibrio* spp. (*V. paraheamolyticus, V. harveii, V. alginolyticus*) cause major problems in aquaculture, resulting in reduced growth rate, poor feed consumption, loss of body weight and ultimately mass mortality.

A number of alternative strategies for the prevention and control of diseases have been proposed and have already been applied successfully in aquaculture, such as antibiotics, vaccines and immunostimulants. Antibiotics, one of the feed additives, were commonly used in the early 1950s (Ahilan et al., 2004). The problem of antibiotic contamination of aquaculture facilities and livestock and the indiscriminate worldwide use of antibiotics in aquaculture have led to the development of drug-resistant bacteria which are becoming increasingly difficult to control and eradicate (Hayashi et al., 1993; DePaola, 1995; Bruun et al., 2000; Sahul and Balasubramanian, 2000; Van der Waaij and Nord, 2000; Miranda and Zemelman, 2002). Subsequently, certain antibiotics such as chloramphenicol have been banned in many countries (Robert et al., 1995; FAO website – URL in reference list). As a result of resistant bacterial strains becoming

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more prevalent and difficult to treat, alternative methods of controlling the microbial environment are being investigated. One of the methods gaining recognition for controlling pathogens within the aquaculture industry is the use of beneficial or probiotic bacteria (Verschuere et al., 2000; Irianto and Austin, 2002a).

Some halophilic bacteria against aquculture pathogens are used in aquaculture. These bacteria both live in high saline and medium saline environment. Halophiles can be classified as slight, moderate and extreme microorganisms depending on their NaCl requirement. Slight halophiles require 0.2 – 0.85 M (2-5%) NaCl, moderate halophiles require 0.85 – 3.4 M (5-20%) NaCl and extreme halophilic microorganisms grow optimally above 3.4 – 5.1 M (20-30%) NaCl concentrations. Hence, in the present study, probiotic microorganisms were isolated from the saltpan environment and applied.

MATERIALS AND METHODS

Sediment samples were collected monthly from Marakkanam saltpan environment (Lat. 12° 14' 29N; Long. 79° 56' 28E) at Tamilnadu, India. Isolation of probiotic bacteria was done using well plate method in antagonistic activity. Physico-chemical character was not included in the results.

Isolation of halophilic bacteria

Isolation of halophilic bacterial was done using the halophilic agar (Hi-Media, Mumbai) (Mellado et al., 1998). Totally, 25 strains were isolated from the saltpan environment and kept at 4°C for further analysis.

Identification

16S rDNA sequencing was used to identify the beneficial bacteria. Once the DNA sequence was edited and assembled, identification was done using Microseq Analysis Software and Sequence Database and universal primers. Identification was based on the pairwise alignment algorithms and phylogenetic tree. The two shrimp pathogenic *Vibrio* strains namely, *V. parahaemolyticus* and *V. harveyi* were used to screen the antagonistic activity of the isolated halophilic bacteria. The beneficial bacteria were sequenced using the forward and reverse primer; 16S rRNA forward primer: *AGA GTT TGA TCC TGG CTC AG* and reverse primer: *ACG GCT ACC TTG TTA CGA CTT.* The sequences were assembled using Clustal W software version 1.82 (Thompson et al., 1994) available at http://www.ebi.ac.uk. Strains' morphology and their surface were observed under the scanning electron microscope (Hitachi-s-1500X-SEM).

Rearing of shrimp

Separate experiment was conducted to examine the effect of probiotic administrated to black tiger shrimp (*P. monodon*). The stocking (*P. monodon*), post larval-15 were purchased from commercial hatcheries and screened for WSSV using 2 step PCR test based on the methods of Lo et al. (1996). WSSV-negative PL was brought to the laboratory and stocked in 50 L capacity culture tanks having 35 L chlorinated filtered estuarine water. The animals were provided with proper aeration and feeding was given at 7.5% of the body weight initially; subsequent feeding was adjusted to 5 to 3.5% of the body weight per day according to the left-out, unutilized feed and increasing body weight of animals. The feed was given twice a day; 60% at dawn (6.00 a.m.) and 40% at dusk (6.00 p.m.). In the experimental tanks, the water quality parameters were maintained at the optimum range; the bottom water in the tank along with excess feed and fecal matter was siphoned out using 2 cm dia plastic hose to enhance the survival of the animals.

Experiment I

The effect of without probiotic on juveniles (*P. monodon*) was examined in experiment I. Shrimp was stocked in 50 L plastic tanks at density of 20 per tank in triplicate. The commercial pellet feed was used as a control. Water was exchanged weekly and animal behavior was observed every day.

Experiment II

The effect of commercial probiotic on juveniles (*P. monodon)* was examined in experiment II. Shrimps were stocked in 50 L plastic tank at density of 20 per tank in triplicate. The commercial pellet feed mixed with commercial probiotic contained spore of two species of *Bacillus* sp. at a concentration of 1.2×10^6 CFU/ml. Water was exchanged weekly and animal behavior was observed every day.

Experiment III

The effect of halophilic bacteria (*Bacillus* sp.) on juveniles (*P. monodon)* was examined in experiment III. Shrimps were stocked in 50 L plastic tanks at a density of 20 per tank in triplicate. The commercial pellet feed was mixed with halophilic bacteria (*Bacillus* sp.) at a concentration of 1.2×10^6 CFU/ml. Water was exchanged weekly and animal behavior was observed every day.

Survival and growth rate

At the end of each experiment, the percentage survival was determined and 10 shrimps were sampled randomly from each tank to determine wet weight, feed conversion ratio (FCR), production and survival rate with the following equation.

Production

The production rate was calculated by the following formula:

Production = Initial weight (g) / Final weight (g)

Feed Conversion Ratio (FCR)

The feed conversion was calculated using the formula:
FCR = Feed taken in (dry weight in g) / Weight gain (wet weight g)

Monitoring of bacteria

Water samples and digestive tract samples from shrimp were used to determine the counts of total bacteria and counts of the halophilic probiont bacteria (*Bacillus*). Prior to dissection or homogenation, the shrimps were rinsed with sterilized distilled water, washed with 0.1% benzalkonium chloride according to the method of Gatesoupe (1999) and then rinsed again with sterilized distilled water to remove all external bacteria. In all the experiment, the digestive tract was dissected out using sterile technique and then was homogenized. All samples were diluted serially with sterilized normal saline solution (0.85% w/v NaCl). Total counts of bacteria were determined by plating on zobal marine agar (with 1% w/v NaCl). *Bacillus* bacteria in water and digestive tract samples were cultured

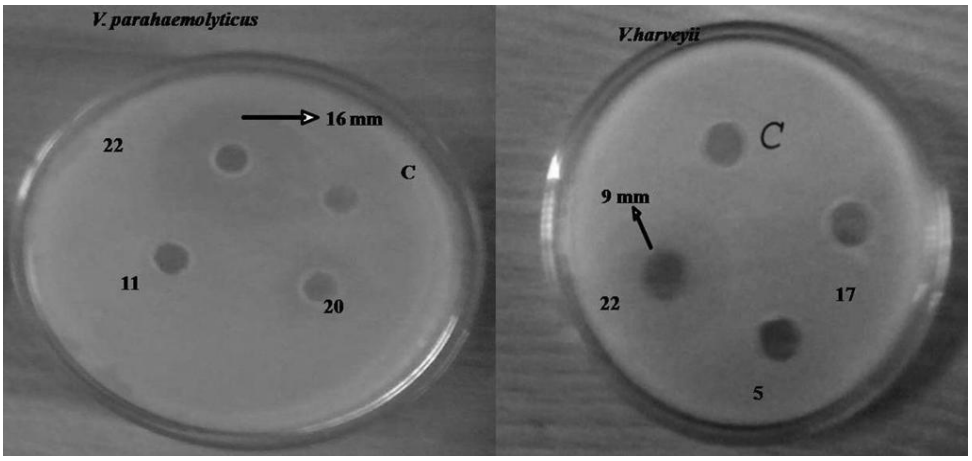

Figure 1. Experimental studies showed antagonistic activities of halophilic strain (*Bacillus* sp.)

according to the method recommended by Probiotics International Ltd. (Protexin Aquatech, Registration Dossier, unpublished pamphlet). The number of colonies on each plate was counted after incubation for 68 h at 25°C for water samples and for 72 h at 37°C for digestive tract samples. Most of the *Bacillus* spp. (Bacillus sp. MK22) cultured in fresh or marine water grow well in high and moderate salt concentration. *Bacillus* sp. had maximum cell count inside the digestive tract of shrimp for 54 h. All statistical analysis was significance; the level of '$P < 0.05$ was used for all tests. Data are reported as means ± standard deviation.

RESULTS

The present study showed that every month, the total bacterial population increased between 12 x10^6 $CFUg^{-1}$ to 45 x10^6 in saltpan environment.

A total of 25 halophilic bacterial strains were preliminary screen based on the zone of inhibition; five strains showed acceptable activities. Among these, a strain, *Bacillus* sp. Mk22 showed large clear zones around the bacterial colonies and it was selected for further studies.

The initial screening is shown in Figure 1. The strain showed maximum zone of inhibition against *V. paraheamolyticus* (16 mm) and the minimum was observed against *V. harveyii* (9 mm) (Figure 1).

The SEM images were taken to verify the size and morphology of bacterial cells. Figure 2 shows the SEM image of *Bacillus* sp: rod shape, smooth and had approximate size of 3.0 µm. The isolate, *Bacillus* sp. Mk22 was subjected to molecular level identifications.

In the Phylogenetic analysis based on a comparison of the 16S rDNA sequence, data showed that the genus *Bacillus* is phylogenetically homogeneous, forming a distinct lineage within the radiation of the bacteria.The dendrogram placed the strain *Bacillus* sp. Mk22 in a separate line of descent within the genus *Bacillus*, representing a distinct phylogenetic lineage (Figure 3).

Further, representatives of other species like *Bacillus baekryungensis*, *B. marisflavi*, *Cloacibacterium normanense*, *B. ferrariarum*, *B. aquimaris*, *Bacillus* sp., *Bacillus* sp., and *Bacillus* sp. Mk22 (JF794553.1) with 99% similarity in the genus levels formed a distinct cluster.

The maximum survival (92.7 ± 1.53%) was recorded in experiment-III and the minimum (60 ± 2.53%) was observed in experiment I (Table 1). Administration of the probiotic significantly increased survival in all treatments (generally by 20–30%) over the controls, except in experiment III, where survival was significantly ($P < 0.05$) different from that in the controls (Table 2).The maximum wet weight (6.9 ± 0.15 g) was recorded in experiment III and minimum (3.5 ± 0.10 g) was observed in experiment I (Table 1). Wet weight was significantly ($P < 0.05$) greater in treatments than in the control. The production rate was found to be higher (378 ± 1.0 g) in experiment III followed by experiment II (301 ± 1.0 g); in the control experiment, it was low (159.3 ± 1.53 g) (Table 1). Production was more ($P < 0.05$) significant in experiment III than in the control. The maximum (1.2 ± 0.28) FCR was recorded in experiment I and minimum (0.9 ± 0.03) FCR was recorded in experiment III (Table 1). There was 5% level of significance in experiment-III compared to the control.

Monitoring of bacteria

In all the experiments, Halophilc (*Bacillus* sp.) bacteria successfully colonized both the culture water and the digestive tract of the shrimp (Table 2). The maximum total count (24 ± 0.79×10^5 Cfu ml l^{-l}) was recorded in experiment I and minimum (6.0 ± 1.0×10^5 Cfu ml l^{-l}) was observed in experiment III; and the maximum *Bacillus* count (3.7 ± 0.58×10^4 Cfu g^{-l}) was recorded in experiment III and minimum (0×10^4 Cfu g^{-l}) was observed in experiment I in water (Table 2).

The maximum total *Bacillus* count (35.4 ± 0.59×10^5 Cfu ml l^{-l}) was recorded in experiment I and minimum (4.1 ± 0.47×10^5 Cfu ml l^{-l}) was observed in experiment III; and the maximum *Bacillus* count (5.6 ± 0.75×10^4 Cfu g^{-l}) was recorded in experiment III and minimum (0×10^4 Cfu g^{-l}) was observed in experiment I in the digestive tract (Table

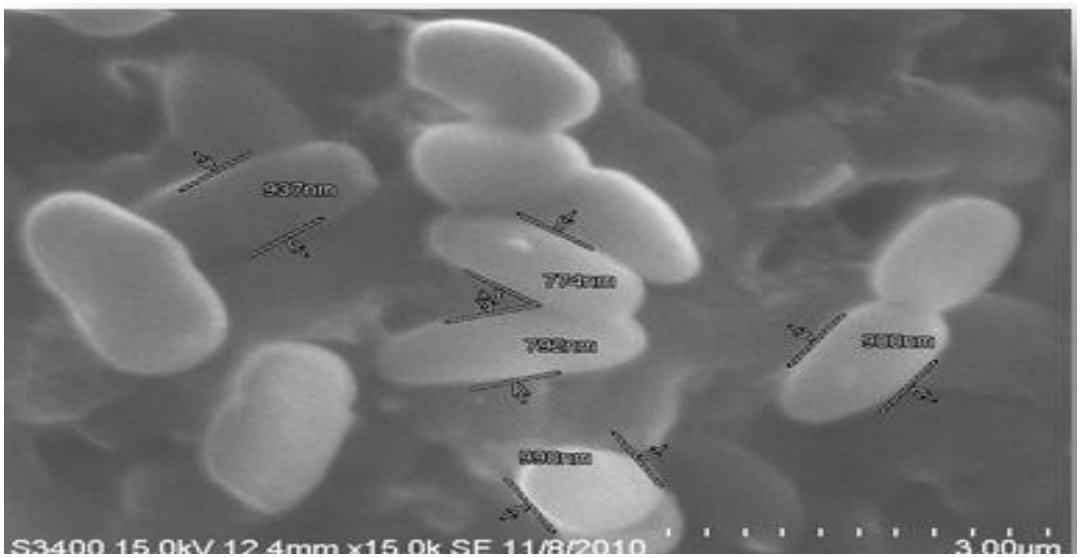

Figure 2. Scanning electron microscopy (SEM) image of *Bacillus* sp. Mk22.

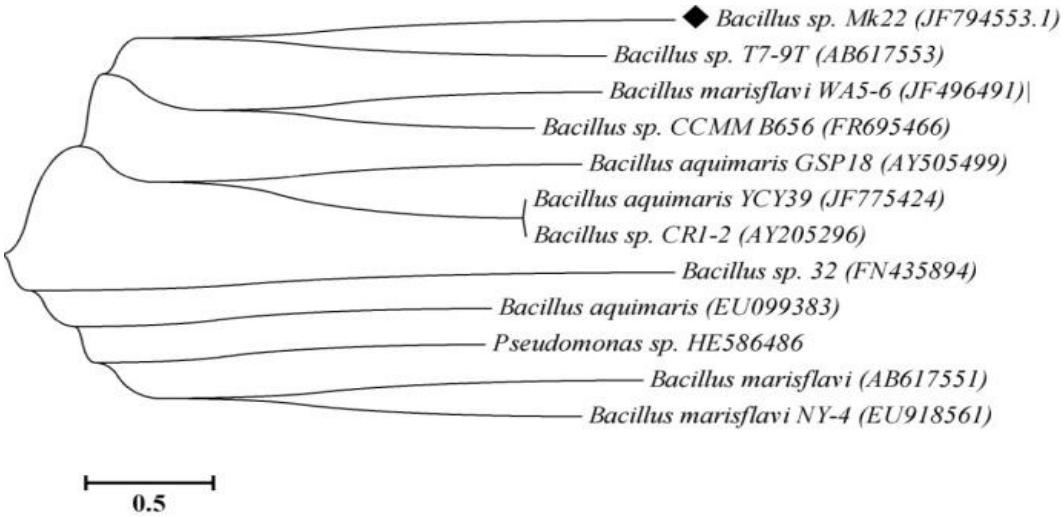

Figure 3. 16S rRNA gene sequence-based phylogenetic relationships of *Bacillus* sp. Mk22 (830 nucleotides) and closely related members of the genus *Bacillus*. The tree was constructed using the neighbourjoining algorithm. GenBank accession numbers are given in parentheses. Only bootstrap values above 50% are shown (1000 replications). Bar 0.5 substitutions per 100 nucleotides. Triangle indicates the present study strain.

Table 1. Growth and survival parameters of *P. monodon* reared with and without *Bacillus* probiotic added to water.

Experiment	Stocking density (*P. monodon*)	Survival (%)	Wet weight (g)	Final production(g)	FCR
I	60	60 ± 2.52	3.5 ± 0.10	159.3 ± 1.53	1.2 ± 0.28
II	60	83 ± 2.0	5.5 ± 0.36	301 ± 1.0	1.0 ± 0.04
III	60	92.7 ± 1.53	6.9 ± 0.15	378 ± 1.0	0.9 ± 0.03

Mean ± S.D. Exp-I (Control no probiotic provided); Exp-II- Commercial probiotic (Superbiotic) provided; Exp- III *Bacillus* sp. Mk22 provided.

Table 2. Total bacterial count and *Bacillus* count in water and in digestive tracts of *P. monodon* reared with and without *Bacillus* probiotic added to water.

Experiment	Water		Digestive tract	
	Total counts (10^5CFU ml l^{-1})	*Bacillus* count (10^4 CFU ml l^{-1})	Total counts (10^5 CFU g^{-1})	*Bacillus* count (10^4 CFU g^{-1})
I	24 ± 0.79	0	35.4 ± 0.59	0
II	8 ± 1.00	5 ± 1.00	8.7 ± 0.85	4.4 ± 0.42
III	6 ± 1.00	3.7 ± 0.58	4.1 ± 0.47	5.6 ± 0.75

Mean ± S.D. Exp-I (Control no probiotic provided); Exp-II- Commercial probiotic (Superbiotic) provided; Exp- III *Bacillus* sp. Mk22 provided.

2). The ANOVA showed 5% level of significance ($P < 0.05$) between the water and digestive tract.

DISCUSSION

Aquaculture is badly affected by parasites, fungi, bacteria, viruses and non-infectious diseases cause problems. Various toxins and other water quality stressors can also affect crustacean health. With the increasing importance of crustacean aquaculture as well as mounting pressures on fisheries, there is a need for reliable accurate means for developing the health of crustaceans (Sindermann, 1990). Particularly, vibriosis is one of the major diseases in aquatic organisms. This is usually caused by the species belonging to the genus *Vibrio* which are the natural inhabitants of estuarine and marine environments, well known for causing vibriosis in fish worldwide (Schaperclaus, 1986). Several species are known to be pathogenic to aquatic animals as well as humans. They are highly abundant in aquatic environments, including estuaries, marine coastal waters and sediments. Even though there are several remedies in controlling *Vibrio,* there exists several constraints such as the prevalence of the antibiotic resistance bacteria. In this present study we aimed to find the potential of the application of halophilic bacteria for controlling the crisis of vibriosis in the culture of *P. monodon*.

The total bacterial count varied between 12 x10^6 and 45 x10^6 CFUg^{-1}. 16S rDNA gene sequencing is a powerful tool that has been used to trace phylogenetic relationships between microorganisms and to identify bacteria from various sources, such as environmental or specimens. This technology is used today in laboratories for routine identifications, not only for slow-growing, unusual or fastidious bacteria but also for bacteria that are poorly differentiated by conventional methods.

Among the five isolates, *Bacillus* sp. Mk22 was selected for molecular level identification and it was subjected to further probiotic study. In phylogenetic analysis based on a comparison of the 16S rDNA sequencing, data showed that the genus, *Bacillus* is phylogenetically homogeneous, forming a distinct lineage within the radiation of the bacteria. The dendrogram placed the strain *Bacillus* sp. Mk22 in a separate line of descent within the genus *Bacillus*, representing a distinct phylogenetic lineage and forming a distinct cluster. Gracia et al. (1987) reported an endose spore forming gram positive *Bacillus* sp from saltpan in USA. Even though Garabito et al. (1997) proposed the new species *Bacillus salexigens* in hypersaline soils located in different soils located in different geographical areas of Spain, Yoon et al. (2004) proposed the transfer of *Bacillus* halodenitrificans a gram variable, endospore forming moderately halophilic rod isolated from a marine solar saltern of the yellow sea in Korea.

Bacillus sp. Mk22 is closely related to the *Bacillus* sp., 32 JD-2009 (FN435894); there is detection of halophilic bacteria and archaea on the extreme salt environment that attacks monuments. There was identification of halophilic bacteria from a salt marsh and two salterns in the protected ecosystem of lower Loukkos (Larache, Morocco). *Bacillus* sp T7-9T (AB617553) and *Bacillus* sp., CCMM B656 (FR695466) are all the strains isolated from the saltpan environment in South Korea (Na et al., 2011); and other species related to *Bacillus aquimaris,* with accession number EU099383, have 99% similarity in the species level. *B. aquimaris* is not only present in the saltpan environment but also in the marine environment. *B. aquimaris* is isolated from the Kumta Coast of Karnataka, India; this bacterium grows at pH 7.5- 9.5 and 40°C (Pooja and Jayaraman, 2009). *B. aquimaris* is a relatively novel marine bacterium. Recent reports are available on the culture characteristics, sequence information and phylogeny of the organism (Gontang et al., 2007; Liu and Shao, 2007). Yoon et al. (2004) reported the isolation of endospore-forming, rod shaped moderately halophilic, *B. aquimaris* (TF-12T) from seawater.

Halophilic bacterial forms produce several secondary metabolites such as bacteriocins, bacteriocin like substances and antibacterial lipopeptides and these metabolites have received considerable attention as biological control agents in pharmaceutical industry because they are generally recognized as safe (GRAS), have low toxicity, high biodegradability, and are environmental friendly (Oliveira and Pijoan, 2004). In the present study, the halophilic bacterial strain *Bacillus* sp. showed antagonistic activties against all shrimp pathogens (*V. paraheamolyticus* (16 mm) and *V. harveii* (9 mm)). Lee et

al. (2010) reported a novel analysis of *Bacillus subtilis* SC-8 antagonistic to *Bacillus cereus.* Liu et al. (2008) proved antagonistic activities of volatiles from four strains of *Bacillus* spp. and *Paenibacillus* spp. against soil-borne plant pathogens.

Several early reports are available for mode of action and application of probiotics in ponds (Sugita et al., 1998). In the present investigation, halophilic bacterial strain *Bacillus* sp. was used as probiotic for *P. monodon*. Under laboratory conditions, the result showed excellent production rate (378 ± 1.0 g), wet weight (6.9 ± 0.15g) and FCR (0.9 ± 0.03). Rengpipat et al. (2000) reported that *Bacillus* sp. will activate both cellular and humoral immune defense against shrimp pathogens in tiger shrimp (*P. monodon*). Balcazar et al. (2006) stated that the administration of a mixture of bacterial strains (*Bacillus* and *Vibrio* sp) positively influence the growth and survival of juvenile of white shrimp. The present study also revealed that *Bacillus* sp. was able to colonize both the culture water and the shrimp digestive tract. Based on the above observations, it was concluded that the strain of halophilic *Bacilus* sp. was effective in inhibiting the shrimp pathogens, like *V. paraheamolyticus* and *V. harveyi* both *in vitro* and *in vivo*. The probionts significantly reduce mortality and also do not have any pathogenic effect on the shrimp larvae. Halophilic (*Bacillus*) bacteria were administered to *P. monodon* under laboratory condition; final production, FCR, wet weight and survival were significantly higher in experiments I-III than in the control. Therefore, these bacterial probionts can be used effectively to control the shrimp pathogens and enhance production that may substitute the use of antibiotics in aquaculture.

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Heavy metal content in mixed and unmixed seasonings on the Ghanaian market

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Human exposure to some heavy metals through consumption of various seasonings in some Ghanaian markets was evaluated. The heavy metals considered were iron (Fe), zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb) and mercury (Hg). The levels of iron (Fe) , zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb) in a total of twenty two (22) mixed and unmixed seasonings were determined using flame atomic absorption spectrometry whereas the mercury levels were determined by cold vapour atomic absorption spectrometry. In unmixed seasonings, Fe content ranged from 19.4 to 971.40 mg/kg, Zn from 2.40 to 34.60 mg/kg, Cu from 0.9 to 10.10 mg/kg, Cd from below detection limit (0.01) to 0.9 mg/kg and Pb ranged from 0.6 to 1.8 mg/kg. In mixed seasonings, concentration ranged from 83.36 to 480.82 mg/kg for Fe, 1.72 to 26.78 mg/kg for Zn, 1.73 to 7.70 mg/kg for Cu and 0.63 to 1.39 mg/kg for Pb and from below detection limit (0.01) to 0.06 mg/kg for Cd. Hg was below the detection limit (0.01) in all the seasonings. The results indicated that Fe, Zn and Cu were below permissible levels whereas Pb and Cd were above permissible levels.

Key words: Toxic metals, seasonings, consumption, spectrometry, Kumasi.

INTRODUCTION

Heavy metals have bio-importance as trace elements but the biotoxic effects of many of them in human biochemistry are of great concern. They enter our bodies via food, drinking water and air (Lenntech, 2008). Iron, zinc and copper are essential metals whereas cadmium, lead and mercury have no bio-importance (Divirikli et al., 2006).

Heavy metals contamination in plants, animals and humans are due to environmental pollution through air emissions from automobile exhaust, pesticides leaching into water bodies, smelters and process wastes from mining and other industries (Ansari et al., 2004, Duruibe et al., 2007; Opuene and Agbozu, 2008).

Spices which are dried plants parts (Satter et al., 1989) can easily be contaminated by heavy metals from type of soil for cultivation, fertilizers and source of water used for irrigation (Abdullahi et al., 2008). In addition, colourants which are added to some of these flavour enhancers may contain some trace metals such as lead (Ekpo and Jimmy, 2005).

Interaction with sellers of powdered ginger and pepper (red and green) as spices in the local markets indicated that, these fruits are processed by the individual seller whereas most of the seasonings are imported. These spices may easily be contaminated by heavy metals from the soil or aerial depositions as these spices are dried on the ground or on roof tops. Moreover, commercial mills used may also introduce some amount of metals into the

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seasonings due to wear and tear of the machinery.

Heavy metals content of different spices, bouillon cubes, food condiments and aromatic herbs have been investigated in many countries (Garcia et al., 2000; Lopez et al., 2000; Divrikli et al., 2006; Ozkutlu et al., 2006; Nnorom et al., 2007). However, there is limited information on the levels of heavy metals in these seasonings on the Ghanaian markets. This work therefore seeks to bridge that gap by providing information especially to the Ghanaian populace on the levels of heavy metals of these most consumed seasonings. Information will further be provided on the sources of these seasonings and the extent to which they are contaminated with these heavy metals for future studies and effective comparative analysis. The objective of this study therefore was to evaluate human exposure to some heavy metals through consumption of mixed and unmixed seasonings in some markets in Ghana.

MATERIALS AND METHODS

Sample collection and preparation

Twenty two different powdered samples were purchased at random from local shops and hawkers from the Asafo (A), Railway (R) and Central markets (C) in Kumasi, Ashanti Region of Ghana located in the transitional forest zone about 270 km north of the national capital, Accra. The samples were purchased after preliminary investigations had indicated that they were the most consumed seasonings in the metropolis. The samples were grouped into unmixed seasonings (M) and mixed seasonings (UM). The unmixed seasonings were rosemary (*Rosmarinus officinalis)*, anise (*Pimpinella anisum*), garlic (*Allium sativum*), nutmeg (*Myristica fragrans*), prekese (*Tetrapleura tetraptera*), Senegal pepper (*Xylopia aethiopica*), ashanti pepper(*Piper guineense*), ginger (*Zingiber officinale*), red pepper (*Capsicum annuum*), green pepper (*Capsicum frutescens*). The mixed seasonings were adobo red, adobo yellow and curry. The samples were then dried using the Astell scientific dryer at 45°C for 24 h, cooled and stored in plastic bags prior to analysis.

Apparatus

All glassware and plastic containers used were washed with detergent solution followed by soaking in 10% (v/v) nitric acid overnight. They were rinsed with distilled water followed by 5% potassium permanganate, rinsed with distilled water and dried before use (Voegborlo and Akagi, 2007). Blanks and standard were prepared alongside. Analytical reagents (AnalaR) grade chemicals (BDH Chemicals Ltd., Poole. Emgland) were used throughout the study.

Digestion of samples

Digestion of samples was carried out by the method of Akagi and Nishimura (1991). About 1 g of each sample was weighed into a 50 ml digestion tube and 1 ml H_2O, followed by 2 ml HCl, 5 ml HNO_3 : $HClO_4$ (1:1) and 2 ml H_2SO4 were added. After heating at 200°C till solution was clear, samples were cooled and filtered into standard 50 ml volumetric flask and made to the volumetric mark. The digest were analyzed for Fe, Zn, Cu, Cd and Pb by air-acetylene flame atomic absorption spectrometer (Spectr AA 220, Australia). The automatic mercury analyzer model HG-5000 was for the determination of Hg.

Statistical analysis

Means and standard deviations were computed using Statsgraphics Centurion XV, 2005 Version 15 statistical software (Statpoint. Inc, USA). Where necessary, one way analysis of variance (ANOVA) was used to test if any significant differences existed between concentrations of a particular metal in a particular seasoning group using the same statistical software.

RESULTS AND DISCUSSION

Heavy metals in seasonings

Iron (Fe), zinc (Zn), copper (Cu), mercury (Hg) and lead (Hg) levels were determined in all seasonings. The mean (±standard deviation) and range of the concentration of metals in the groups of seasonings are presented in Tables 1 and 2, respectively. The results are means of three replicates. Cadmium was mostly below the detection limit (0.01 mg/kg) in unmixed seasonings but was present in most of the mixed seasonings. The levels of iron in both groups of seasonings were mostly high.

Iron is the most needed micronutrient in plants (Divrikli et al., 2006). The iron contents in the seasonings were in the range 19.4 in rosemary (UM-1) to 971.40 mg/kg in nutmeg (UM-4) for unmixed seasonings. Red pepper (UM- 14) from central market (C) was almost three times the concentration of red pepper (UM-16) from Railway market whereas red pepper (UM-15) from Asafo was almost two times the concentration for UM-16. The concentration of Fe in the various red peppers differed significantly ($p< 0.05$).

Mixed seasonings had iron levels in the range of 83.36 to 480.82 mg/kg with Adobo (yellow) (M-2) recording the least while curry (M-4) having the highest (Table 2). Four different brands of curry were analyzed. Curry (M-4) from Ghana had the highest value of Fe (480.82 mg/kg) while curry (M-5) from Nigeria had the lowest value of 149.13 mg/kg. This suggests that curry powder produced in Ghana may be a rich source of Fe. No significant difference ($p>0.05$) was found in the concentration of Fe between curry (M-3) and (M-4).

Rosemary (UM-1) had lower level of iron whereas in ginger, levels were higher than values reported in literature (Divrikli et al., 2006; Ozkutlu et al., 2006; Koc and Sari, 2009). Levels of iron in ginger (UM-8, UM- 9 and UM-10) and garlic (UM-3) were also higher than levels reported by Hashmi et al. (2007). Generally, the levels of iron in unmixed seasonings were higher than mixed seasonings. The higher Fe content in unmixed seasonings could be due to combination of the same plants parts (Nnorom et al., 2007) such as fruits whereas the Fe content in the mixed seasoning could be due to low levels of Fe in the individual spice. This is because the sellers

Table 1. Mean levels (± SD) (mg/kg) of iron, zinc, copper, cadmium and lead in unmixed (UM) seasoning.

Sample	Source	Sample code	Fe	Zn	Cu	Cd	Pb
Rosemary	China (CH)	UM-1	19.4±1.6	2.6±0.4	0.9±0.2	ND	1.5±0.2
Anise	*	UM-2	500.4±5.9	34.6±1.9	9.3±0.1	ND	1.2±0.6
Garlic	India	UM-3	58.6±8.6	14.9±1.5	3.2±0.2	ND	1.0±0.1
Nutmeg	France	UM-4	109.0±1.6	10.8±2.0	10.1±2.6	0.9±1.6	1.1±0.6
Prekese	Ghana	UM-5	24.8±0.8	2.4±0.1	2.9±0.1	ND	1.0±0.8
Senegal pepper	*	UM-6	45.5±1.0	5.1±0.9	5.5±3.9	ND	0.7±0.1
Ashanti pepper	Mali	UM-7	100.6±0.8	17.8±0.4	4.5±0.2	ND	0.6±0.1
Ginger	Ghana (GH-C)	UM-8	698.3±1.4	17.6±1.8	4.7±1.3	ND	1.8±0.6
Ginger	Ghana (GH-A)	UM-9	408.4±10.5	14.1±0.7	3.7±0.2	ND	0.9±0.3
Ginger	Ghana (GH-R)	UM-10	590.1±4.0	11.8±1.8	3.7±0.7	ND	1.6±0.5
Green pepper	Ghana (GH-C)	UM-11	94.3±8.4	19.4±0.8	1.5±0.2	ND	1.4±0.1
Green pepper	Ghana (GH-A)	UM-12	226.2±0.0	16.9±1.1	1.3±0.0	ND	1.0±0.1
Green pepper	Ghana (GH-R)	UM-13	105.9±1.1	15.4±1.1	1.4±0.0	ND	0.9±0.2
Red pepper	Ghana (GH-C)	UM-14	971.4±1.8	14.9±1.6	6.7±0.2	ND	1.4±0.6
Red pepper	Ghana (GH-A)	UM-15	614.6±1.0	9.5±0.4	7.2±0.2	ND	1.0±0.0
Red pepper	Ghana (GH-R)	UM-16	345.9±12.7	7.5±0.7	7.7±0.3	ND	1.4±0.5

*Source not known; (C), Central market; (A), Asafo market; (R), Railway market; SD, standard deviation.

Table 2. Mean level (± SD) (mg/kg) of iron, zinc, copper, cadmium and lead in a mixed (M) seasonings.

Sample	Source	Sample code	Fe	Zn	Cu	Cd	Pb
Adobo (red)	United states of America (U.S.A)	M-1	91.56±5.51	1.72±0.32	1.81±0.04	0.06±0.00	1.39±0.64
Adobo (yellow)	United states of America (U.S.A)	M-2	83.36±3.49	2.76±0.93	1.73±0.03	0.05±0.00	1.08±0.03
Curry	France (FR)	M-3	204.32±4.37	17.68±0.46	5.77±0.14	0.01±0.01	0.91±0.10
Curry	Ghana (GH)	M-4	480.82±0.95	13.67±0.28	7.70±1.72	0.02±0.02	1.05±0.16
Curry	Nigeria (NIG)	M-5	149.13±4.15	13.87±0.80	6.40±0.13	ND	0.84±0.15
Curry	South Africa (SA)	M-6	214.93±5.40	26.78±0.61	6.39±0.11	ND	0.63±0.10

purchase the same plants parts such as fruits or leaves from different sources, mix them and mill them into a single homogeneous product. The high levels of iron in all the seasonings could also be due to contamination during milling. Research indicates that grinding of spices in commercial mills contaminates them to about between 3 and 5 folds, due to wear and tear of the machine parts (Janitha et al., 1988).

Even though Fe is an essential element needed by the body, consumption of an excessive amount can lead to health effects such as enlarged liver and joint diseases (Hoffman, 2009). To safeguard the health of the public, organizations such as Nutrigold Technical (2007) and FAO/WHO have established tolerable levels known as the Recommended Daily Allowance (RDA) and Provisional Tolerable Weekly Intake (PTWI) for most elements.

In order not to exceed the RDA of 14.8 mg/day of Fe for a 60 kg body weight, a person must not consume more than 762.89 g of rosemary daily. Iron (Fe) toxicity is therefore insignificant.

Zinc plays important roles in growth and development in humans (Colak et al., 2005). Zinc deficiency is of growing concern in the developing world because consumption of plants food has inhibitory effect on zinc absorption (Divrikli et al., 2006). It is reported by Hotz and Brown (2004) that an estimated 20% of the world population was at risk of inadequate zinc intake. Delayed neurological and behavioural development in children occurs as a result of zinc deficiency (Caulfield et al., 1998). Unmixed seasonings had zinc content between 2.40 and 34.60 mg/kg (Table 1). Anise (UM-2) recorded the highest amount of Zn whereas *Prekese* (UM-5) which differed significantly ($p<0.05$) from Anise (UM-2), recorded the lowest. Ginger (UM-8) from Central market had the highest level of Zn (17.6 mg/kg) whereas ginger (UM-10) from Railway market had the lowest Zn concentration of 11.8 mg/kg. Green pepper from the various

markets, showed variation in Zn concentration though no significant difference ($p>0.05$) was observed (Table 1). Zinc level in red pepper (UM-14), purchased from Central market was two times the concentration in red pepper (UM-16) from Railway market. Concentration of zinc in red pepper from the various markets differed significantly ($p<0.05$).

Zn levels in mixed seasonings were between 1.72 and 26.7 8 mg/kg with Adobo (red) from U.S.A having the least while curry seasonings from South Africa (S.A) have the highest. The four different brands of curry analyzed showed that curry (M-6) from S.A had the highest Zn content whereas curry (M-4) from Ghana had the least. This suggests that curry from S.A may be a rich source of Zn. Though there was no significant difference ($p>0.05$) in concentration of Zn between any type of Adobo, curry (M-3) and curry (M-6) differed significantly (Table 2).

Ginger (UM-8, UM-9 and UM-10) and garlic (UM-5) had concentrations of Zn higher than that reported previously (Ozkutlu et al., 2006, Hashmi et al., 2007). Rosemary (UM-1) had lower Zn level as compared to other studies (Divrikli et al., 2006; Koc and Sari, 2009). The mean zinc content of red pepper and curry seasonings in this study ranged from 7.40 to 14.9 mg/kg and 13.67 to 26.78 mg/kg as compared to 10.40 to 35.0 and 13.65 to 29.90 mg/kg, respectively, in Nigeria (Nnorom et al., 2007; Awode et al., 2008).

Zinc levels in all samples were lower than the standard level (100 mg/kg) set by FAO/WHO (2003). The differences in Zn content from same plants parts purchased from the various markets could be due to factors such as type of soil for cultivation and drying environment. The high levels of zinc in the seasonings reflect the normal composition expected in plant derived products (Onianwa et al., 1999). Research indicates that micronntrients are generally higher in leaves than in other above ground parts in plants (Basgel and Erdemoglu, 2005). However, Zn in rosemary, a leafy spice, was quite low. This may be due to low zinc content in the soil used for cultivation.

Copper helps in iron metabolism by helping in oxygen transport as well as utilization and absorption of iron in humans (Özçelik et al., 2002). The concentration of copper in unmixed seasonings ranged from 0.90 to 10.10 mg/kg (Table 1). Rosemary (UM-1) recorded the lowest Cu concentration while nutmeg (UM-4) recorded the highest. Ginger (UM-9 and UM-10) from Asafo and Railway markets recorded similar Cu values. The concentrations of Cu in green pepper from all three markets were very close. This suggests that though samples differ in localities, their copper content may be influenced by the same factors. There was no significant difference ($p>0.05$) in Cu content between any type of ginger, red pepper or green pepper (Table 1).

The copper level in mixed seasonings was between 1.73 and 7.70 mg/kg (Table 2). The lowest level was found in Adobo (yellow) (M-2) from U.S.A and the highest in curry (M-4) from Ghana. Copper in the four brands of curry was highest in curry from Ghana followed by Nigeria, South Africa and France in that order. No significant difference was found in Cu content between any type of Adobo or curry seasonings.

Rosemary (UM-1) had lower levels of copper as compared to those reported previously in other studies (Ozcan, 2004; Divrikli et al., 2006; Koc and Sari, 2009). Ginger (UM-8, UM-9 and UM-10) garlic and nutmeg (UM-4) had higher levels of copper as compared to values reported in other literatures (Ozkutlu et al., 2006; Hashmi et. al., 2007; Krejpcio et al., 2007). The level of Cu in all groups of seasonings was below 10 mg/kg (FAO/WHO, 2003) permissible in plants.

It has been reported that presence of cadmium in food is mostly derived from various sources of environmental contamination and has no biological importance in higher organisms such as humans and plants (Adriano, 1984). Cadmium is extremely toxic even at low levels (WHO, 1989). Cadmium toxicity is characterized by chest pain, cough with foamy and bloody sputum, and death of the lining of the lung tissues due to excessive accumulation of watery fluids (Duruibe et al., 2007).

Cadmium levels in unmixed seasonings ranged below 0.01 and 0.90 mg/kg (Table 1). Nutmeg (UM-4) was found to contain the highest Cd content whereas Cd in the rest of the seasonings was below detection limit.

In Table 2, the cadmium levels in mixed seasonings ranged from below detection limit to 0.06 mg/kg. Adobo (red) (M-1) recorded the highest Cd content. The highest Cd level in the curry samples was found in M-4 from Ghana though below the permissible limit (0.20 mg/kg). There was no significant difference between any type of Adobo or curry seasoning investigated.

Cadmium was not detectable in Rosemary by Divrikli et al. (2006), while Koc and Sari (2009) reported 3.1 µg/g of cadmium in rosemary. The concentration of cadmium in pepper and ginger was reported to range from 0.25 to 1.07 mg/kg and 0.02 to 0.04 mg/kg, respectively, by Awode et al. (2008) and Krejpcio et al. (2007) but was not detected in any type of pepper or ginger in this study. Concentration in nutmeg (UM-4) was higher than reported levels (0.05 mg/kg) by Krejpcio et al. (2007). The level of cadmium in curry seasonings were lower than levels reported in literature (Nnorom et al. 2007). According to Chizzola et al. (2003), cadmium levels could be considered normally low in plants. Generally, all the seasoning samples with the exception of nutmeg were below the permissible level (0.20 mg/kg) set by the FAO/WHO (2001) for spices. Though cadmium content in the seasonings were low, its presence could be due to contamination of raw materials, technological processes or colouring agents used as they contain cadmium salts (Cabrera et al., 1995).

Spices are suspected to obtain lead during growth in lead contaminated soils or in the course of milling or other processing procedures (Woolf and Woolf, 2005).

The use of pesticides contaminated with heavy metals during the growing of herbs and spices may also be a source of lead contamination in the final product (Galal-Gorchev, 1991).

In unmixed seasonings, levels of Pb ranged from 0.6 to 1.8 mg/kg. Ashanti pepper (UM-7) from Mali recorded the least Pb level while ginger (UM-8) from GH (Railway) recorded the highest (Table 1). Ginger, red pepper and green pepper from the various markets showed differences in the concentrations of Pb. However, no significant difference ($p>0.05$) was found in Pb content between either group of seasoning. It was observed that out of the three markets, seasonings from Central had the highest Pb content. This is also evident in seasonings from Railway market which is located between two busy roads. Wheeler and Rolfe (1979) found that Pb levels in vegetation increased linearly with traffic density. Pb concentration in mixed seasonings (Table 2) was between 0.63 and 1.39 mg/kg. The highest Pb level was found in Adobo (red) (M-1) from the U.S.A and curry seasonings (M-6) from S.A had the lowest. Within the different brands of curry, Pb levels in curry (M-4) from Ghana had the highest Pb level whereas Curry (M-6) from S.A had the lowest.

Pb has been reported as non detectable in some spices and herbs (Divrikli et al., 2006; Nnorom et al., 2007). However, in other studies (Chizzola et al., 2003; Gupta et al., 2003; Koc and Sari, 2009) where Pb content was determined, the highest Pb level ranged from 2.00 to 200 mg/kg as compared to 0.37 and 2.8 mg/kg in this present study. Concentration in nutmeg (UM-4) was higher than reported levels (0.36 mg/kg) by Krejpcio et al. (2007).

The level of lead in all seasoning samples were above the permissible limit required in spices (0.3 mg/kg) by the FAO/WHO (2003). The lead content in seasonings could be attributed to the addition of lead during processing to impart colour, sweet taste or increase the weight of these products (Kakosy et al. 1996). Some studies reported that traffic density also increases the lead burden in the environment thereby increasing the lead content in vegetation (Rodriguez-Flores and Rodriguez-Castellon, 1982; Buszewski et al., 2000; Nabulo, 2004).

It must be said that though data on the rate of consumption of seasoning in Ghana is unavailable, it is unlikely that a person will consume high amounts (> 20g) of seasonings in a day. This suggest that intake of the seasonings will have negligible effects on the health of consumers. Thus, the high levels of toxic metals (Cd and Pb) suggest the need for consumption advisory.

Conclusion

The study shows that iron, zinc, copper and lead were present in all seasonings whereas only 23% of the sampled seasonings contained cadmium. Mercury was not detected in any of seasonings analyzed. The toxic metals, cadmium and lead were mostly above the FAO/WHO permissible level.

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