# African Journal of Food Science

Volume 9 Number 4, April 2015
ISSN 1996-0794

Academic Journals

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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African Journal of Food Science

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academicJournals

Vol. 9(4), pp. 170-175, April, 2015
DOI: 10.5897/AJFS2015.1276
Article Number: 42A711451562
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Review*

# Review on lactic acid bacteria function in milk fermentation and preservation

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Received 3 February 2015, Accepted 16 March 2015

**This review was conducted to find out the main function of lactic acid bacteria in milk fermentation and preservation. Lactic acid bacteria (LAB) are a group of Gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. LAB includes *Lactobacillus, Lactococus, Streptococcus* and *Leuconostoc* species. The presence of LAB in milk fermentation can be either as spontaneous or inoculated starter cultures. Both of them are promising cultures to be explored in fermented milk manufacture. LAB has a role in milk fermentation to produce acid which is important as preservative agents and generating flavour of the products. The main reasons for the fermentation practice using LAB are to increase milk palatability and improve the quality of milk by increasing the availability of proteins and vitamins. Furthermore, LAB confers preservative and detoxifying effects on milk as well. When it is used regularly, LAB fermented milks boost the immune system and strengthen the body in the fight against pathogenic bacterial infections. Thus, LAB fermentation is not only of a major economic importance, but it also promotes human health. Therefore, it was concluded that the lactic acid bacteria have a vital role in milk and milk products fermentation and preservation and this suggests the need for educating the communities about benefits of consuming fermented milk and milk products needs to be part of health education.**

**Key words:** Fermentation, lactic acid bacteria, milk, preservation.

## INTRODUCTION

Lactic acid bacteria (LABs) are industrially important organisms used for the production of milk and milk products like yoghurt, cheese, buttermilk and kefir. The species used for these applications typically belong to the group of gram-positive bacteria including the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. They are recognized for their fermentative ability and thus enhancing food safety, improving organoleptic attributes, enriching nutrients and increasing health benefits (Panesar, 2011; Liu et al., 2011; Sharma et al., 2012; Steele et al., 2013).

Due to the characteristics of milk that is highly perishable, the main purpose of milk fermentation using LAB is to prolong its shelf-life as well as to preserve the nutritious component of milk. It is also recognized that fermentation of milk using LAB will undoubtedly produce good quality of products with highly appreciated organoleptic attributes. Recently, there is a growing interest to

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develop a variety of fermented milk products for other beneficial purposes, particularly for health purposes and preventing of toxins produced by food-borne pathogens and spoilage bacteria that enter human body (Shah, 2007; Ali, 2010; Panesar, 2011; Sharma et al., 2012).

The presence of LAB in milk fermentation can be either spontaneous or inoculated starter cultures. Milk itself is known as one of the natural habitats of LAB (Delavenne et al., 2012; Wouters et al., 2002). In general, the technology of milk fermentation is relatively simple and cost-effective. On the other hand, standardized fermented milk products are produced and manufactured in large-scale production under controlled conditions and become an important industrial application of LAB as starter cultures. There are some important features of LAB starters in fermented milk products. A single potential starter culture will dominate and reduce the diversity of microorganisms in fermented milk products compared to that of products under natural fermentation.

Using lactic acid bacteria in milk fermentation and preservation is indispensable to improve milk palatability and quality. However, there is a limitation to reviewing these and other related information and thereby to delivering such synthesized and summarized data to the beneficiaries.

Therefore, reviewing sensible findings on lactic acid bacteria function in milk fermentation and preservation seems to be a milestone area to deliver combined information to the beneficiaries. Based on this outlined background, the objective of this paper was to review lactic acid bacteria function in milk fermentation and preservation and thereby to deliver combined information for beneficiaries.

Most of the related research findings of lactic acid bacteria (LAB) function in milk fermentation and preservation were reviewed. Related reports which focus on health promoting properties of LAB were also reviewed. Findings on antimicrobial and preservative property of LAB that have been reported by various scholars were also reviewed and combined.

## OUTLINED DESCRIPTION OF LACTIC ACID BACTERIA FUNCTION IN MILK FERMENTATION

LAB are widespread in nature and predominate of microflora in milk and milk products; many species are involved in the daily manufacturing of dairy products (Ayad et al., 2004). The lactic acid bacteria used in the dairy fermentation can roughly be divided into two groups of the basis of their growth optimum. Mesophilic lactic acid bacteria have an optimum growth temperature between 20 and 30°C and the thermophilic have their optimum between 30 and 45°C. Traditional fermented products from sub-tropical countries harbor mainlythermophilic lactic acid bacteria, whereas the products with mesophilic bacteria originated from western and northern European countries. The lactic acid bacteria can be mainly divided into two groups based on the end-products formed during the fermentation of glucose. Homofermentative lactic acid bacteria such as *Pediococcus, Streptococcus*, and *Lactococcus* produce lactic acid as the sole product of glucose fermentation. Heterofermentative lactic acid bacteria such as *Weissella* and *Leuconostoc* produce equimolar amounts of lactate, $CO_2$ and ethanol from glucose (Caplice and Fitzgerald, 1999; Jay, 2000; Kuipers et al., 2000).

LAB have been extensively used in food fermentation, including the production of milk products, and its proteolitic activity is very important in producing flavor compounds of end product (Moulay et al., 2013). Proteolytic system of LAB is important for the growth of microorganisms and it is involved in casein utilization within LAB cells and give contribution to the development of organoleptic properties of fermented milk products (Moulay et al., 2013; Yamina et al., 2013).

Milk fermentation process has relied on the activity of LAB, which play a crucial role in converting milk as raw material to fermented milk products. In milk fermentation industry, various industrial strains of LAB are used as starter cultures (Table 1). Starter cultures of LAB were obtained from a sequence activity and passed a process of isolation, selection and confirmation. Several behaviors as the characteristics of each individual selected strains of LAB has been established and used in the production of fermented milk products industrially. The most important properties of LAB are their ability to acidify milk and to generate flavour and texture, by converting milk protein due to their proteolytic activities (Mäyrä and Bigret, 2004). The mild acid taste and pleasant fresh are characteristics of fermented milk products such as yoghurt and cheese (Kongo, 2013; Griffiths and Tellez, 2013).

### Health promoting property of lactic acid bacteria

The high demand of fermented milk products is due to the health property generated from consumption of fermented milk products (Table 2). Fermented milk products are reported to contribute to human health through several mechanisms (Sharma et al., 2012). Certain lactic acid bacteria strains of the genera Lactobacillus, are utilized as health promoting bacteria (Saxelin et al., 2005), while certain Lactobacillus strains like *L. helveticus* are believed to produce bioactive health beneficial peptides from casein protein of milk and showed effect of antihypertensive, immune modulator activity, anticancer and calcium binding ability. *L. helveticus* is known as one of LAB which has efficient (Nouaille et al., 2003).

### Antimicrobial compounds produced by lactic acid bacteria

The preservative action of starter culture in food is

**Table 1.** The main lactic acid bacteria associated with milk and milk product fermentation.

| Species/ subspecies | Their main uses in different milk products | References |
|---|---|---|
| **Lactococcus** | | |
| *Lc. Lactis subsp. Lactis* | Mesophilic starter used for many cheese types, butter and butter milk. | Broome et al. (2003) and Wouters et al. (2002) |
| *Lc. lactis subsp. Lactis biovar diacetylactis* | Used in Gouda, Edam, sour cream and lactic butter and butter milk. | Wood (1997) and Leroy and De Vuyst (2004) |
| *Lc. Lactis subsp. cremoris* | Mesophilic starter used for many cheese types, butter and butter milk. | Weerkam et al. (1996) |
| **Streptococcus** | | |
| *Sc. thermophilus* | Thermophilic starter used for yogurt and many cheese types' particularly hard and semihard high-cook cheeses. | Broome et al. (2003) and Beresford et al. (2001) |
| **Lactobacillus** | | |
| *Lb. acidophilus* | Probiotic adjunct culture used in cheese and yogurt. | Briggiler-Marcó et al. (2007) |
| *Lb. delbrueckii subsp. Bulgaricus* | Thermophilic starter for yogurt and many cheese types, particularly hard and semihard high-cook cheeses. | Slaterry et al. (2010) |
| *Lb. delbrueckii subsp.lactis* | Used in fermented milks and high-cook cheese. | Broome et al.(2003) and Giraffa 2010 |
| *Lb. helveticus* | Thermophilic starter for fermented milks and many cheese types particularly hard and semihard high-cook cheeses | Broome et al. (2003) and Griffiths and Tellez (2013) |
| *Lb. casei* | Probiotic milk and cheese ripening adjunct culture | Briggs (2003) and Kongo (2013) |
| *Lb. plantarum* | Cheese ripening adjunct culture. | Leroy and De Vuyst (2004) |
| *Lb. rhamnosus* | Probiotic adjunct culture used in cheese | Coppola et al. (2005) |
| **Leuconostoc** | | |
| *Ln. mesenteroides subsp. cremoris* | Mesophilic culture used for Edam, Gouda, fresh cheese, lactic butter and sour cream. | Weerkam et al. (1996) and Slaterry et al. (2010) |

Lb. =Lactobacillus; Lc. =Lactococcus; Ln.=Leuconostoc; Sc.=Streptococcus, subsp.= subspecies.

**Table 2**. Health benefits when milk is fermented.

| Effect of fermentation | Changes in milk | Effect on health |
|---|---|---|
| Increase in lactic acid bacteria levels | Reduced lactose content in milk | No diarrhea and bloating<br>Improved gut health |
| | Reduced content of bad bacteria | Prevention of protection from bacterial vaginosis and fungal infections in women<br>Ability to digest remaining lactose in the fermented milk and use as energy source |
| Breakdown to shorter chain proteins | Identification of casein peptides and whey peptides with functional properties | Easier digestion<br>Some with antihypertensive effects<br>Some with pain relief effects<br>Some with immune enhancing properties<br>Some with calcium binding bone building properties |
| Increased acidity | Sharpness of taste | Prevents harmful bacterial growth in milk |

Source: http://whqlibdoc.who.int/publications/2003/9241591196.pdf and ftp://ftp.fao.org/docrep/fao/007/y5686e/y5686e00.pdf

proteolytic system (Korhonen and Pihlanto, 2003; Griffiths and Tellez, 2013). Another promising contribution of lactic acid bacteria is to use them as delivery vehicles for molecules with therapeutic value attributed

to the combined action of antimicrobial metabolites produced during the fermentation process. These include many organic acids such as lactic, acetic and propionic acids produced as end products which provide an acidic environment unfavourable for the growth of many pathogenic and spoilage microorganisms. Acids are generally thought to exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic functions (Rattanachaikunsopon and Phumkhachorn, 2010).

Some of the inhibitory compounds against other bacteria include hydrogen peroxide and bacteriocins (Oyewole, 1997). One of the arguments supporting the use of LAB fermentation is to prevent diarrheal diseases because they modify the composition of intestinal microorganisms and by this, act as deterrents for pathogenic enteric bacteria. LAB bacteria also produce fungal inhibitory metabolites. These are mainly organic acids, which include propionic, acetic and lactic acids ((Schnürer and Magnusson, 2005; Sauer et al., 2008). Thus, LAB is applied as a hurdle against non-acid tolerant bacteria, which are ecologically eliminated from the medium due to their sensitivity to acidic environment (Ananou et al., 2007). Also, fermentation has been demonstrated to be more effective in the removal of Gram-negative than the Gram-positive bacteria, which are more resistant to fermentation processing (Mensah, 1997). As such, fermented food can control diarrhoeal diseases in children (Guandalini, 2006 and Szajewska et al., 2006).

Moreover, LABs are also known to produce protein antimicrobial agents such as bacteriocins (Carolissen-Mackay et al., 1997; Aymerich et al., 2000). Bacteriocins are peptides that elicit antimicrobial activity against milk spoilage organisms and food borne pathogens, but do not affect the producing organisms. LAB also synthesizes other anti-microbial compounds such as, hydrogen peroxide, reuterin, and reutericyclin (Leroy and Vuyst, 2004). Other applications of LAB include their use as probiotics that restore the gut flora in patients suffering from diarrhea, following usage of antibiotics that destroy the normal flora (Aderiye and Laleye, 2003). In this manner, fermented food is used to prevent and to alleviate diarrhea. In addition, the consumption of food products rich in LAB helps to alleviate constipation and abdominal cramps.

Generally, bacteriocins are antimicrobial proteinaceous compounds that are inhibitory towards sensitive strains and are produced by both Gram-positive and Gram-negative bacteria (Tagg et al., 1976).

## Lactic acid bacteria as functional starter culture

Definitely, the most important application of lactic acid bacteria is their use as starter strains in the manufacture of various fermented dairy products. In particular, *Streptococcus thermophilus*, *L. lactis*, *L. helveticus*, and *L. delbrueckii subsp. Bulgaricus* are widely used as milk starter cultures. *S. thermophilus* and *L. bulgaricus* are the two bacteria required to make yoghurt and *Lactobacillus casei* is frequently found in cheeses. The proper selection and balance of lactic acid bacteria used for starter culture is critical for the manufacture of milk fermented food products with their desirable texture and flavor. Mankind exploited these bacteria for thousands of years for the production of fermented products because of ability to produce desirable changes in taste, flavor, and texture (Derek et al., 2009).

Starter cultures of LAB can be either mesophilic from the genera of *Lactococcus* and *Leuconostoc* or thermophilic from the genera of *Streptococcus* and *Lactobacillus* (Fox et al., 2004). Among species, *L. lactis, S. thermophilus* and *L. helveticus* are intensively studied. *L. helveticus* is specialized milk species and belong to the member of dairy niche species. Several cheese products are based on *L. helveticus* as starter (Slaterry et al., 2010).

## Preservative property of lactic acid bacteria

Milk is a highly perishable food raw material. Its transformation into stable milk products provides an ideal vehicle to preserve its valuable nutrients, and making them available throughout the year. It is known that while unprocessed milk can be stored for only a few hours at room temperatures, cheeses may reach a shelf-live up to 5 years (depending on variety). Fermentation with LAB is a cheap and effective milk preservation method that can be applied even in more rural/remote places, and leads to improvement in texture, flavor and nutritional value of many milk products. LAB have a long and safe history of application and consumption namely in cheese processing (Aquilanti et al., 2006; Giraffa et al., 2010).

Fermentation makes the milk palatable by enhancing its aroma and flavor. These organoleptic properties make fermented food more popular than the unfermented one in terms of consumer acceptance. The lowering the pH to below 4°C through acid production, inhibits the growth of pathogenic microorganisms which can cause food spoilage, food poisoning and disease (Ananou et al., 2007). For example, LAB bacteria have antifungal activities (Schnürer and Magnusson, 2005). By doing this, the shelf life of fermented food is prolonged. This is because the sheer overgrowth of desirable edible bacteria in food outcompetes the other non-desirable food spoilage bacteria. Thus, LAB fermented foods have lactic acid as the main preservative since lactic acid bacterial growth is accompanied by the production of lactic and acetic acids with decrease in pH and increase in titratable acidity. Using LAB fermentation for detoxification is more advantageous in that it is a milder method

which preserves the nutritive value and flavor of decontaminated food (Bata and Lásztity, 1999).

## CONCLUSION

Lactic acid bacteria are a broad group of Gram-positive organism and are mainly used as a starter strains, particularly, *S. thermophilus, L. lactis, L. helveticus*, and *L. delbrueckii subsp. bulgaricus* which are widely used as milk starter cultures. They are also widespread in nature and predominate microflora in milk and milk products. Lactic acid bacteria have an essential role in milk fermentation and preservation since lactic acid bacteria display numerous antimicrobial activities in fermented foods. This is mainly due to the production of organic acids. Therefore, lactic acid bacteria exert strong antagonistic activity against many microorganisms, including milk spoilage organisms and pathogens. In addition, some strains may contribute to the preservation of fermented milk by producing bacteriocins. Milk fermentation is profitable in terms of improving milk quality, preservation and decontamination of toxins, often found in food. Together with food safety, the nutritional and flavour profile of the products need to meet the expectations of modern consumers. Fermentation with LAB is a cheap and effective milk preservation method that can be applied even in more rural/remote places, and leads to improvement in texture, flavor and nutritional value of many milk products. Education of communities about benefits of consuming fermented milk and milk products needs to be part of health education. This technology needs to be further developed to enhance safety and ease of application in a rural poor-resource setting.

## Conflict of interests

The authors did not declare any conflict of interest.

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academicJournals

Vol. 9(4), pp. 176-181, April, 2015
DOI: 10.5897/AJFS2014.1246
Article Number: D39564351578
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Understanding local fish consumption behavior in Laguna Lake watershed area, Philippines

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Received 4 December, 2014; Accepted 26 February, 2015

**In recent years, Laguna Lake has deteriorated significantly due to agricultural, domestic and industrial waste. This paper examines local residents' fish consumption behavior in the Laguna Lake watershed area in the Philippines. The data utilized in this study was collected through the "LakeHEAD" household survey (*n*=389), conducted by the Research Institute for Humanity and Nature and University of the Philippines at Los Baños in 2012. How socio-demographic (location and education) and cognitive factors (for example, the perceptions of Laguna Lake pollution caused by domestic waste and/or heavy metals) affect consumers' behavior regarding fish consumption was examined by distributing questionnaires to the local households. According to the descriptive statistics, most of the local residents (over 80%) considered Laguna Lake as polluted by domestic waste and/or heavy metals. However, the majority of residents (over 70%) still consumed fish; bangus (*Chanos chanos*), tilapia (*Oreochromis niloticus*) and kanduli (*Arius dispar*) from the Laguna Lake. In addition, there is a strong relationship between the geographical distribution of the residents and their respective fish consumption behavior (*p* <0.001), as residents living the nearest to the lake were more likely to demonstrate a significant increase in fish consumption which is mostly due to easier access to the fish resources. It is therefore, necessary to promote public education programs focusing on food safety and illness mitigation.**

**Key words:** Education, fish consumption behavior, food safety, Laguna Lake, perception, water pollution.

## INTRODUCTION

Laguna Lake, also known as Laguna de Bay, is the largest lake in the Philippines, and is well known for the multitude of ecosystem services that it provides (Lasco and Espaldon, 2005). However, in recent years, the water quality has seriously deteriorated due to agricultural, domestic and industrial waste (Beveridge, 1984; Lasco and Espaldon, 2005; Montenegro, 2006; Su and Cervantes, 2008). As a result, there has been an increase in heavy metal concentration in the sediment and water of the lake (Cuvin-Aralar, 1990) with domestic waste being the most predominant driver of change, accounting for 70% of the deterioration of water quality

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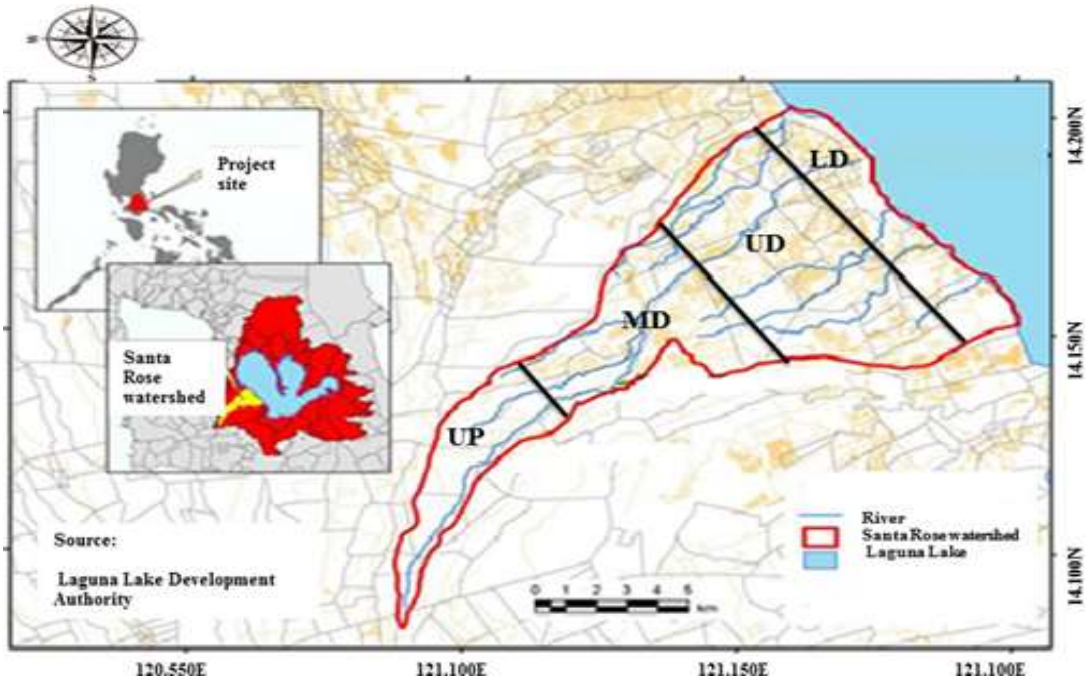


**Figure 1.** Study area: Santa Rose watershed area.

(Israel, 2008).

Fisheries are one of the most significant economic activities in the lake, with bangus (*Chanos chanos*), tilapia (*Oreochromis niloticus*) and kanduli (*Arius dispar*) being the primary production species, consumed largely by the locals, especially the lower socio-economic classes, as their price level remains low (Israel, 2008; Saguin, 2014). Bangus, tilapia and kanduli caught in Laguna Lake (Cuvin-Aralar and Aralar, 1993; Lasco and Espaldon, 2005; Chavez et al., 2006; Africa et al., 2009; Molina et al., 2011; Molina, 2012) were contaminated with heavy metals from industrial and domestic waste (Monila et al., 2011; Monila, 2012; Paraso and Capitan, 2012; Saguin, 2014), and the consumption of such catches may involve health risks for the local residents (Lasco and Espaldon, 2005; Panganiban et al., 2012).

Past research in the Laguna Lake area focused mainly on the degree of water quality and fish stock abundance levels (Chavez et al., 2006; Rosales and Rollon, 2011). There is a lack of research regarding the relationship between the Laguna Lake residents' perception of water pollution and their fish consumption behavior (Tan et al., 2010; Molina, 2012). Globally, overall consumption behavior is influenced by socio-demographic factors (such as education level, income and occupation) and cognitive factors (for example, perception and understanding of food safety) (Dosman et al., 2001; Goktolga et al., 2006; Ozilgen, 2011). People who have received a higher level of education tend to understand the food safety risks and know how to avoid or mitigate risks, such as the risk of food additives, food contamination, pesticides in food than people with less education (Dosman et al., 2001). In this study, we focused on socio-demographic factors (location and education) and cognitive factors (perception of domestic waste and heavy metals) of residents in Laguna Lake area. We analyze the following four hypotheses:

H1. Residents living in areas closer to Laguna Lake consume more fish from the lake;
H2. Residents who receive higher education consume less fish from Laguna Lake;
H3. Residents who believe that the water of the lake is polluted by domestic waste are less likely to consume fish;
H4. Residents who believe that the water of the lake is polluted by heavy metals are less likely to consume fish.

## METHODOLOGY

The research took place at the Santa Rose watershed area, which is one of the 24 sub-watersheds of Laguna Lake (Figure 1). It is located 30-40 km southeast of Metro Manila, spanning 5,543 ha, with a population of 248,890 inhabitants (Philippine Statistics Authority National Statistics Office, 2010).

### Household survey

The data utilized for this study was collected through the "LakeHEAD" household survey (n=389), conducted by the Research Institute for Humanity and Nature in Japan, and University of the Philippines at Los Banos in 2012. The villages (*barangays*) of the Santa Rose sub-watershed area were classified

**Table 1.** Descriptive statistics of socio-demographic and cognitive factors.

| **Socio-demographic factors** | | | |
|---|---|---|---|
| **Areas (n=183)** | | **Education (n=118)** | |
| 1. Upstream | 20% | 1. No formal education | 7% |
| 2.Midstream | 36% | 2. Elementary school level | 76% |
| 3. Upper Downstream | 19% | 3. Above high school level | 17% |
| 4. Lower Downstream | 25% | | |
| **Cognitive factors: the Laguna Lake is polluted by domestic wastes and/or heavy metals** | | | |
| **Domestic wastes (n=172)** | | **Heavy metals (n=124)** | |
| 1. Agree | 81% | 1. Agree | 84% |
| 2. Disagree | 19% | 2. Disagree | 16% |

| **Residents fish consumption behaviors (*n*=183)** | | | |
|---|---|---|---|
| | **Bangus** | **Tilapia** | **Kanduli** |
| Yes | 73% | 72% | 81% |
| No | 27% | 28% | 19% |

**Table 2.** Relationship between residents' fish consumption behavior and areas (n=183).

| **Area** | **Bangus** | | **Tilapia** | | **Kanduli** | |
|---|---|---|---|---|---|---|
| | **Yes** | **No** | **Yes** | **No** | **Yes** | **No** |
| Upstream | 7% | 93% | 5% | 95% | 20% | 80% |
| Midstream | 21% | 79% | 25% | 75% | 6% | 94% |
| Upper Downstream | 15% | 85% | 10% | 90% | 20% | 80% |
| Lower Downstream | 54% | 46% | 59% | 41% | 52% | 48% |

into four groups according to their geographical distribution: upstream: UP (the farthest from the lake), midstream: MD, upper downstream: UD and lower downstream: LD (the closest from the lake). A questionnaire was distributed to 389 households, comprising 11 barangays, from March until April 2012. Out of the 238 returned questionnaires (response rate = 61%), a total of 183 were usable.

### Questionnaire contents

The residents' fish consumption behavior was assessed by asking if they consumed the three most important commercial fish species (bangus, tilapia and kanduli) from Laguna Lake (1. yes, 0. no). The socio-demographic factors included the areas respondents were living in: 1. upstream, 2. midstream, 3. upper downstream, and 4. lower downstream, and education levels 1. never received a formal education, 2. elementary school level, 3. above high school level; The Philippines education system includes three levels: elementary school, high school and university. We also asked about the residents' perception about pollution of the Laguna Lake water by domestic waste (1. agree, 0. disagree) and/or heavy metals (1. agree, 0. disagree).

### Statistical analysis

Initially, using IBM-SPSS Version 22, the descriptive results of the socio-demographic and cognitive factors were summarized for the statistical analysis. Then, a logistic regression analysis was performed, in order to understand how the socio-demographic and cognitive factors under examination influence the local residents' fish consumption behavior. The statistics were tested at significant level at $p<0.05$.

## RESULTS

The descriptive statistics indicated that the majority of the locals (>80%) recognized that Laguna Lake was polluted by domestic waste and/or heavy metals (Table 1). These results correspond with the findings of Su and Cervantes (2008) that showed that the majority of respondents identified reasons of the pollution of Laguna Lake as the existence of domestic waste and heavy metals. Nevertheless, the majority of local residents (>70%) consumed fish captured in the Laguna Lake.

The consumption of bangus, tilapia and kanduli were varied among the areas under examination. The area with the highest levels of consumption was the nearest to lake (lower downstream: above 50%), and the one with the lowest levels of consumption was the one located farthest from the lake (upstream: below 10%) (Table 2).

The rest of the examined factors did not appear to

**Table 3.** Logistic regression model to predict residents' consumption behavior of bangus (*Chanos chanos*) with socio-demographic and cognitive factors as independent variables. N = 77, β = standardized coefficient, Exp (B) = odds ratio.

| Variable | *β* | Wald | Exp (B) | *P*-value |
|---|---|---|---|---|
| Areas | 0.829 | 24.016 | 2.291 | <0.001 |
| Education | 0.000 | 0.001 | 1.000 | 0.974 |
| Domestic wastes | -0.019 | 1.519 | 0.982 | 0.218 |
| Heavy metals | 0.002 | 0.128 | 1.002 | 0.720 |

**Table 4.** Logistic regression model to predict residents' consumption behavior of tilapia (*Oreochromis niloticus*) with socio-demographic and cognitive factors as independent variables. n= 77, β = standardized coefficient, Exp (B) = odds ratio.

| Variable | *β* | Wald | Exp (B) | *P*-value |
|---|---|---|---|---|
| Areas | 1.013 | 32.134 | 2.754 | **<0.001** |
| Education | 0.003 | 0.259 | 1.003 | 0.611 |
| Domestic wastes | -0.019 | 1.573 | 0.981 | 0.210 |
| Heavy metals | 0.001 | 0.035 | 1.001 | 0.852 |

**Table 5.** Logistic regression model to predict residents` consumption behaviors of Kanduli (*Arius dispar*) with socio-demographic and cognitive factors as independent variables. n = 77, β = standardized coefficient, Exp (B) = odds ratio.

| Variable | *β* | Wald | Exp (B) | *P*-value |
|---|---|---|---|---|
| Areas | 1.629 | 29.418 | 5.101 | <0.001 |
| Education | 0.001 | 0.019 | 1.001 | 0.891 |
| Domestic wastes | -0.010 | 0.365 | 0.990 | 0.546 |
| Heavy metals | 0.004 | 0.359 | 0.996 | 0.549 |

affect the residents' consumption behavior ($p$ >0.05) (Tables 3 to 5).

Furthermore, the logistic regression revealed that the residents' location affected their consumption behavior of the three commercially important fish (bangus: $p$<0.001, tilapia: $p$<0.001, kanduli: $p$<0.001). Residents living closer to the lake were more likely to consume fish, mostly due to easier access to the fish resource.

## DISCUSSION

The study concluded that fish consumption behavior is not related to the perception of domestic waste and/or heavy metal pollution directly. Fish is the primary source of animal protein for the local people and they may face health issues related to the consumption of contaminated fish, particularly if they lack understanding of the concept of food safety.

Nonetheless, it is widely accepted that heavy metals intake through fish consumption can be reduced with the utilization of various cooking methods (Atta et al., 1997; Gokoglu et al., 2004; Musaiger and Souza, 2008; Ganbi, 2010; Talab et al., 2014). The most suitable methods for reducing heavy metal concentration are grilling and baking, as these methods remove salts, along with the amino acids and proteins bonded with heavy metals (Atta et al., 1997; Gokoglu et al., 2004; Ganbi, 2010). However, in the Philippines, the most commonly used methods to cook fish, namely frying and boiling (Bayaga-Tiangson and Deveza, 2005), are not that effective in heavy metal removal. Frying, for example, does not reduce heavy metal concentration due to evaporation and loss of moisture (Musaiger and Souza, 2008; Diaconescu

et al., 2013; Talab et al., 2014). Therefore, it is necessary to promote cooking methods with increased effectiveness in heavy metals removal to the local residents. In addition, the cooking methods promoted should take into consideration the access to technology. For example, modern cooking methods such as microwaves and halogen can significantly reduce heavy metal concentration in fish (Talab et al., 2014), but the local residents may not be able to adopt them as they might not have access to microwave and halogen technology.

Additionally, fish consumption behavior was found not to be related to the level of education. Even though food safety education is included in the curriculum of elementary schools in the Philippines, specific instructions that residents can follow in their daily lives, such as how to avoid eating contaminated fish, are severely lacking (The Department of Education, 2013). Thus, residents can only rely on practical knowledge on avoiding intake of contaminated fish, regardless of educational level. Moreover, the lack of individual knowledge about the risks may make people less likely to take preventive actions. Consequently, it is necessary to promote education programs for local people and school students focusing on matters of food safety. Such programs could include, but not be limited to, increasing the numbers of food safety books available in public libraries, promotion of cooking methods to reduce heavy metals concentration in elementary schools, and distribution of children`s books on food safety.

Furthermore, as expected, we found that residents who were living closest to the lake were most likely to consume fish captured in the Laguna Lake. This implies that residents who can easily access the local fish and consume them regularly, face higher contamination risk, since Laguna Lake has been polluted. There is an urgent need for the implementation of outreach programs providing food safety information, in order to raise awareness towards fish consumption to reduce heavy metals intake.

In addition, in developing countries like the Philippines, poor households spend 30-50% of their income on rice. Moreover, the rice price crisis has had a great impact on their food consumption behaviors. The price of ordinary rice increased by 44.2%, from 21.28 peso/kg in 2006 to 30.69 peso/kg in 2009 (Ronalo et al., 2011). A family of five spends around 5.513 pesos (approximately 125US dollars) monthly to fulfill their basic food needs (The Full Year Official Poverty, 2012). As the locals face significant income constraints, they depend on fish from Laguna Lake, as these fish are the only affordable source of animal protein (Israel, 2008; Saguin, 2014) It was concluded that the increasing rice price and low income also influenced residents' food expenditure which determine their consumption behavior, turning them towards cheap commodities, such as fish. It is important, therefore, that the residents realize how risk perception affects their daily lives and health.

Lastly, food safety is closely related to concerns over public health issues all over the world, and is becoming more and more important in many developing countries. Government should take responsibility of the protection of the public and provide opportunities for locals to engage in social learning regarding food safety. However, developing countries in general lack trained personnel, as well as information about food safety, as compared to developed countries (Dewaal and Robert, 2005). Thus, practical food safety education should be promoted not only in developed countries but also in developing countries.

## Conflict of interests

The authors did not declare any conflict of interest.

## ACKNOWLEDGEMENTS

The authors would like to thank the Research Institute for Humanity and Nature, and University of the Philippines that helped in the collection of the data for this study. They thank Dr. H. Matsuda for his enormous support throughout the research and writing process. They would like to express their appreciation to E. I. Vlachopoulou for her assistance in improving the manuscript. Finally, they thank the anonymous peer reviewers for their valuable contribution to the work.

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Vol. 9(4), pp. 182-186, April, 2015
DOI: 10.5897/AJFS2015.1282
Article Number: C74914551583
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Effect of age on physico-chemical, cholesterol and proximate composition of chicken and quail meat

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Received 6 February, 2015; Accepted 9 March, 2015

**A study to evaluate the effect of age on physico-chemical, cholesterol and proximate composition of chicken and quail meat was evaluated. One hundred poultry bird comprising of 50 chickens (Harco black) and 50 quail Japanese were randomly allocated based on a completely randomized design. Birds were kept for 20 weeks and fed with compounded feeds *ad libitum*, and at 4, 8, 16 and 20 weeks, 5 birds each were randomly selected, and the thigh and breast were evaluated for the physic-chemical, cholesterol and proximate composition. Results reveal that, crude protein content was significantly higher in chicken breast (21.48) at 4 weeks and at 16 weeks in quail breast (21.93), ether extract was highest in the thigh of both chicken and quail (6.33 and 5.06) at 4 weeks of age and thereafter it decreased with increase in age of birds, respectively. Ash content was significantly (P<0.05) higher at 8 weeks for quail breast (1.73) and at 20 weeks for chicken breast (1.76) and the moisture content was significantly higher at 4 weeks for both quail (79.19) and chicken (76.51) thigh. Physiochemical analysis revealed that, the thermal and cold shortening were highest at 16 weeks of age for both quail and chicken meat (26.9 and 43.10), respectively. The cooking loss was highest (P<0.05) at eight weeks for chicken (91.30) and at four weeks for quail (90.80), water holding capacity was highest (P<0.05) (43.10) at 16 weeks in chicken meat. Cholesterol content was lowest at 16 weeks of age as compared to 4 and 20 weeks in chicken breast, whereas quail breast revealed higher values eight weeks of age. The breast of both birds (chicken and quail) had the best values for protein and ash content but highest in cholesterol content, while the thigh had the lowest cholesterol value but highest ether extracts content for both samples.**

**Key word:** Cholesterol, thigh, breast, chicken and quail meat.

## INTRODUCTION

Meat can be described as the edible flesh of domestic animals (cattle, sheep, goats, pigs, wild life and poultry). It is a food item that is not only very rich in nutrients but tastier and pleasantly more aromatic than any other type of ready to eat food. In recent times, eating of meat has been under heavy attack by Western societies because it is said to contain fat and cholesterol which can cause high blood pressure and heart attack. The data from FAO (2009) showed that Nigeria had a per capita meat consumption of 8.8 kg in 2009, meaning that Nigeria is

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among the 12 poorest nations in animal protein consumption (NIAS/ASAN Information Bulletin No. 1, 2013). In order to increase the consumption of protein in Nigeria diet, other animals of low cholesterol and fat can be introduced for example, rabbit and poultry. The poultry industry is one of the largest and fastest growing agro-based industries in the world; this could be attributed to an increasing demand for poultry meat and egg products (Bolan et al., 2010). Increasing demand for chicken meat could be due to its acceptance by most societies and its relatively low cholesterol content as compared to other domesticated livestock like cattle, sheep, goat and pigs. Eltanany and Distil (2010) reported that, at present, chicken species are considered an important source of human food around the globe, as well as a model organism for research. The consumption of chicken meat increased as a result of its low price, easy processing, high nutritive value, high protein content, readily available fats, low cholesterol content with tender and fine-fibre (Fletcher, 2002). Eating of chicken has become synonymous with eating healthy, and it is a common assumption that chicken and fish make up the gamut of healthy protein. Although, there are several alternatives to chicken that offers flavour on their own viz., rabbit, venison, bison and quail. Chicken meat contain less cholesterol and fat content as compared to red meat, but have higher cholesterol than other poultry meat like quail. In recent years, quail meat has been gaining more popularity among consumers. Generally, quails are small to medium sized birds, belonging to the same biological family of chicken and pheasants (Phasianidae), given the overall similarity in physical characteristics and behaviour (Bolan et al., 2010). Quails, most commonly bred for human consumption, belong to the species *Corturnix coturnix Japonica.* In the beginning of the 20$^{th}$ century did commercial production of quail start in Japan and from there it spread first to China and soon after to Europe. Quail meat has been known for centuries, and there are even biblical quotations of their use as a meat source. White meat, including quail meat is considered superior to red meat because it contains low fat content, low cholesterol and high amount of iron (Jaturasitha et al., 2004), consumers also acknowledge the relatively low price, the typically convenient portions, and the lack of religious restriction against its consumption. Quail is one of the leanest types of poultry and a good source of protein and minerals such as sodium, potassium and Iron. The aim of this study was to determine the physico-chemical, cholesterol and proximate status of breast and thigh of chicken meat when compared with quail meat at 2, 8, 16, and 20 weeks of age.

## MATERIALS AND METHODS

### Animal used

Fifty (50) days old chicks of Harco black and 50 days old chick of quail were purchased from reputable hatchery. They were fed *ad libitum* from day old with compounded chick mash for up to four weeks and then compounded finisher from four weeks till 20 weeks, all vaccinations necessary was given. Five birds each from chicken and quail birds at 4, 8, 16 and 20 weeks of age were randomly selected and slaughtered. Breast and thigh from both birds were cut primally and evaluated for physico-chemical, cholesterol and proximate status.

### Physico-chemical analysis

Cooking loss for both the chicken and quail birds at 4, 8, 16 and 20 weeks was measured according to the methods used (Fakolade, 2008), water holding capacity of both meat samples was determined with press method as slightly modified by Suzuki et al. (1991), the thermal shortening, was carried out by measuring the initial meat length from the final over the initial multiplied by 100. The cold shortening measured the difference between the initial weight from the final weight over the initial weight, multiplied by 100, according to Tenin et al. (2000)

### Cholesterol status

One gram of food sample was weighed into a 250 ml beaker, 50 ml of hot ethanol added, followed by the addition of 150 ml of ethanol and diethyl ether in ratio 1.1. The mixtures was placed inside a water bath set at 600°C for thorough homogenization and brought to boiling for 5 min, the mixture was filtered and the precipitate was washed once with warm 95% ethanol, and 2 ml of 40% NaOH was added and boiled on the bath for 2 h to 1/5 of the original volume. 100 ml of saturated solution of $Ca(OH)_2$ was added filtered and dried at 80°C and the precipitate was mixed with 100 ml of diethyl ether added to it to extract all the cholesterol for 30 min. The diethyl ether was later filtered off and washed with either which was later evaporated on water bath. The dry sediment was dissolved in 10 ml chloroform, 2 ml of acetic acid anhydride and 4 drops of 96% $H_2SO_4$ and placed in the dark for 30 min, prior to reading on a Spectronic 2ID Spectrophotometer. The absorbance of the sample extract as well as working standard solutions was read on a Spectronic Spectrophotometer at a wavelength of 625 nm.

$$\text{Cholesterol in mg/100 g} = \frac{\text{Absorbance of sample extract x gradient factor x dilution factor}}{\text{Weight of sample taken}}$$

### Proximate composition

The proximate composition of quail meat was determined according to AOAC method (2000). The protein content was determined by the Kjeldahl method and the lipid content was determined by the Soxhlet method. The ash content was determined by ashing the samples overnight at 550°C. Moisture content was determined by drying the samples overnight at 105°C. The carbohydrate content was calculated by difference (total mass of moisture, total fat, ash and protein substrate from the mass of the food).

### Statistical analysis

All data obtained was subjected to analysis of variance as a factorial design and when statistical significance was observed, the means was compared using the Duncan's multiple range (DMR) test (SPSS). The SAS (1999) software package was used for all statistical analysis.

**Table 1.** Feed composition of grower and layer mash for chickens and quail bird.

| Ingredient | Chick (kg) | Grower (kg) |
|---|---|---|
| Maize | 45 | 48 |
| Soya | 34 | 25 |
| Wheat offal | 13.10 | 08 |
| Palm kernel cake | - | 07 |
| Bone meal | 3 | 04 |
| Fish meal | - | - |
| Oyster shell | 4.2 | 07 |
| Lysine | 0.1 | 0.2 |
| Methionine | 0.1 | 0.2 |
| Premix | 0.25 | - |
| Grower premix | - | 0.25 |
| Layer premix | - | - |
| Salt | 0.25 | 0.4 |
| Percentage energy | 2650 ME Kcal | 2500 MEKcal |
| Percentage crude protein | 20% | 15% |

**Table 2.** Proximate composition of thigh and breast of quail at 4, 8, 16 and 20 weeks (%).

| Variable | 4 Weeks | | 8 Weeks | | 16 Weeks | | 20 Weeks | | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | Thigh | Breast | Thigh | Breast | Thigh | Breast | Thigh | Breast | |
| Protein | 17.22$^{c}$ | 20.44$^{b}$ | 20.89$^{b}$ | 21.40$^{b}$ | 19.34$^{c}$ | 21.93$^{b}$ | 18.62$^{c}$ | 24.45$^{a}$ | 1.29 |
| Ether extract | 5.06$^{a}$ | 3.40$^{b}$ | 4.00$^{b}$ | 3.70$^{b}$ | 3.93$^{b}$ | 3.90$^{b}$ | 3.90$^{b}$ | 3.35 | 0.69 |
| Ash | 0.73$^{c}$ | 1.05$^{b}$ | 0.97$^{c}$ | 1.14$^{b}$ | 1.11$^{b}$ | 1.50$^{a}$ | 1.47$^{a}$ | 1.73$^{a}$ | 0.17 |
| Moisture | 79.19$^{a}$ | 77.32$^{a}$ | 72.78$^{b}$ | 73.40$^{b}$ | 72.62$^{b}$ | 71.68$^{b}$ | 72.32$^{b}$ | 70.18$^{c}$ | 1.51 |

$^{abcd;}$Mean on the same row with different superscript are significantly different ($P < 0.05$).

**Table 3.** Proximate composition of thigh and breast of chicken at 4, 8, 16 and 20 weeks (%).

| Variable | 4 Weeks | | 8 Weeks | | 16 Weeks | | 20 Weeks | | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | Thigh | Breast | Thigh | Breast | Thigh | Breast | Thigh | Breast | |
| Protein | 18.03$^{b}$ | 18.05$^{b}$ | 19.46$^{b}$ | 21.48$^{a}$ | 20.13$^{a}$ | 20.32$^{a}$ | 20.50$^{a}$ | 20.91$^{a}$ | 1.29 |
| Ether extract | 6.33$^{a}$ | 4.17$^{c}$ | 4.83$^{b}$ | 4.11$^{c}$ | 4.80$^{b}$ | 4.05$^{c}$ | 4.70$^{b}$ | 3.90$^{c}$ | 0.69 |
| Ash | 0.73$^{b}$ | 0.96$^{b}$ | 0.98$^{b}$ | 1.05$^{b}$ | 1.23$^{a}$ | 1.31$^{a}$ | 1.27$^{a}$ | 1.76$^{a}$ | 0.17 |
| Moisture | 75.93$^{a}$ | 76.51$^{a}$ | 72.42$^{b}$ | 72.12$^{b}$ | 71.58$^{b}$ | 72.41$^{b}$ | 64.68$^{c}$ | 64.33$^{c}$ | 1.51 |

$^{abcd}$Mean on the same row with different superscript are significantly different ($P < 0.05$).

## RESULTS AND DISCUSSION

Changes in nutritional habits and increase in awareness of consumers have resulted in the growth of consumption of chicken (Vukasovič, 2011). In the present study, the proximate composition (Tables 1, 2 and 3) of quail and chicken at 4, 8, 16, and 20 weeks of age, revealed that the protein content were significantly higher in breast meat (24.45%) at 20 weeks and the least value was observed in the thigh at 4 weeks (17.22%) for quail, whereas the breast of chicken at 8 weeks had significantly higher value of 24.48% and the thigh and breast at both 16 and 20 weeks revealed no significant ($P>0.05$) difference in chicken meat. The values obtained at 4 weeks had the least value of 18.03% for thigh and 18.05% for breast of chicken meat. The results of the present study were higher than the values reported (17.48 to 18.99%) by Boni et al. (2010) who compared

**Table 4.** Cholesterol status of thigh and breast quail and chicken bird, at 4, 8, 16 and 20 weeks (%).

| Variable | Quail (mg/100 g) | | Chicken (mg/100 g) | | SEM |
|---|---|---|---|---|---|
| | Thigh | Breast | Thigh | Breast | |
| Week 4 | 64.13[b] | 67.19[b] | 62.16[c] | 70.69[a] | 2.24 |
| Week 8 | 67.90[b] | 73.70[a] | 62.78[c] | 71.30[a] | 2.24 |
| Week 16 | 62.77[b] | 69.14[a] | 61.47[c] | 69.91[a] | 2.24 |
| Week 20 | 62.33[c] | 68.24[b] | 61.45[c] | 72.45[a] | 2.24 |

[ab]Mean on the same row with different superscript are significantly different (P < 0.05).

**Table 5.** Physico-chemical properties of quail meat when compared with chicken meat at 4, 8, 16 and 20 weeks (%).

| Parameter | Chicken | | | | Quail | | | | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | 4 | 8 | 16 | 20 | 4 | 8 | 16 | 20 | |
| Thermal shorten (%) | 6.30[e] | 14.90[c] | 20.70[b] | 14.3[c] | 7.90[e] | 20.70[b] | 26.90[a] | 10.10[d] | 2.63 |
| Cooking loss (%) | 91.3[a] | 79.40[b] | 36.10[c] | 30.20[c] | 90.80[a] | 87.40[b] | 35.40[c] | 30.30[c] | 3.91 |
| Cold shortening (%) | 14.40[c] | 34.50[b] | 38.80[b] | 40.10[b] | 12.30[c] | 41.50[a] | 43.10[a] | 48.10[a] | 3.31 |
| Water holding capacity (%) | 2.60[c] | 2.40[c] | 1.60[d] | 1.30[d] | 2.20[c] | 2.60[c] | 3.70[b] | 5.60[a] | 1.81 |

[abcd]Mean on the same row with different superscript are significantly different (P < 0.05).

meat quality characteristic between young and spent quails and 14.73% for chicken and 21.76% for quail as reported by SkipThePie.org (2012), and 13.7 and 18.6% reported by Odunsi and Kehinde (2009). The protein content tends to increase from 4 to 20 weeks in both birds. Protein content of breast was higher than the thigh for both quail and chicken meat. The ether extracts decreased with the increase in age in both quail and that thigh had the higher values than the breast.

The values observed for ether extracts for both birds was lower than the values reported by Ghenchev et al. (2008) on performance and body composition of Japanese quail fed different dietary nutrients in Nigeria humid tropical environmental, and 15.75% for chicken and 4.53% quail of chicken breast and quail meat (SkipThePie.org, 2012). From the table, it is evident that the ash content increased with increase in the age of the birds but it appears that the breast at 20 weeks had the highest value as compared to other samples. However, the mineral content of the breast muscle was higher than the thigh of both birds, but quail muscles appears to have more mineral than the chicken for both thigh and breast. The values obtained were within the range of 1.44 – 1.52% reported by Boni et al. (2010). For both samples, the moisture content decreases as the age increased. This could be attributed to growth and maturity of muscle of the birds (Boni et al., 2010).

Table 3 shows the cholesterol level obtained in the thigh and breast of quail and chicken, the breast of both bird were observed to have highest cholesterol values than the thigh. But the chicken breast had highest values (P<0.05) than quail breast at 4 and 20 weeks with no significant different (P>0.05) at 8 and 16 weeks obtained in the study which were lower than the value obtained (103.62 - 207.21 mg/100g) as reported by Al–Hasani et al. (1993). The thigh had the lower cholesterol level but highest ether extract in Tables 1 and 2, while breast had low ether extract and high cholesterol level.

The physiochemical properties (Tables 4 and 5) of birds increased as the age of both birds increased. Values of cooking loss observed was higher than values (19.9 - 42.5%) reported for thigh and breast quail, chicken and geese by Akinwuni et al. (2009) and 7.36 – 23.10% cooking loss values for male and female Japanese quail reported by Odunsi and Kehinde (2009). Meat with less cooking loss will invariably give higher yield per unit cut. The thermal shortening for both bird increases from 4, 8, and 16 weeks while at 20 weeks, but reduced. The values obtained were lower than 4.04 - 47.80% reported by Akinwumi et al. (2009). Water holding capacity decreased in both birds' with increase in ages. There appear to be an inverse relationship between WHC and the cooking loss. In this study, as the cooking loss decrease with age, the water holding capacity increases in chicken but decreases in quail meat. Water holding capacity is usually caused by electrostatic repulsion between the myofibrils, or in some cases (with salts or at low or high pH) even a partial solubilization of filaments, the latter being due to the repulsions between individual molecules.

## Conclusion

Quail meat could help to increase meat consumption,

having low fat content, higher moisture and protein content, lower cholesterol level and higher water holding capacity in the breast meat at 20 weeks. This attributes suggest that quail meat is nutritious, tasty and palatable than chicken meat.

## Conflict of interests

The author did not declare any conflict of interest.

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academicJournals

Vol. 9(4), pp. 187-193, April, 2015
DOI: 10.5897/AJFS2014.1233
Article Number: 41948BD51588
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Detection of some chemical hazards in milk and some dairy products

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Received 10 November, 2014; Accepted 18 March, 2015

**Chemical contaminants in milk comprise of hazardous chemicals that may be introduced during milking, dairy processing or packaging. They possess some hazards to humans who consume milk and other dairy products. Total of one hundred and fifty (150) samples were collected; thirty each of UHT milk, yogurt, soft, hard and processed cheese. The samples were analyzed to investigate the presence of some chemical hazards. Chemical analysis indicated that tetracyclines were present in samples of UHT milk with variable percentages, while aflatoxin M1 was detected in all of the examined UHT milk samples and cheese. Sorbic acid, benzoic acid, sulphur dioxide and nitrite were detected in some samples of cheese. The potential to cause toxicological harm to the consumers is common for all of the detected chemical contaminants.**

**Key words:** Chemical hazards milk, dairy products, antibiotic, mycotoxin, preservatives.

## INTRODUCTION

The presence of chemical contaminants in milk are very harmful for the consumers and it can be a matter of public health concern because milk and dairy products are widely consumed by humans throughout the world (Jahed, 2007). They are usually classified as naturally occurring chemicals and added chemicals.

Naturally occurring chemicals include toxins that are produced by some microorganisms (e.g. mycotoxins) which can enter through animal feed and deposited some residues in milk and dairy products.Aflatoxins are groups of toxic fungal metabolites produced by certain molds of the genus *Aspergillus* growing in number of raw-food commodities. Aflatoxin M1 (AFM1) may be found in the milk of animals that are fed with aflatoxin B1 (AFB1) containing feed (Wood, 1991; Cathey et al., 1994). These toxins are very stable and may pass through quite severe processes. For this reason they may constitute a problem in processed foods (Lawley et al., 2008).

International Agency for Research on Cancer (IARC) of WHO classified AFB1 as primary and AFM1 as secondary groups of carcinogenic compounds (Cathey et al., 1994; Dragacci et al., 1995). Chronic toxicity is probably

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more important from a food safety point of view; ingestion of low levels over a long period has been implicated in primary liver cancer, chronic hepatitis, jaundice, cirrhosis and impaired nutrient conversion (Lawley et al., 2008).

The added chemicals include chemicals that are intentionally added such as permitted food additives and non-intentionally added chemicals to a food such as cleaning and sanitizing chemicals. Food and Agricultural Organization (FAO, 1995) describes preservative as any substance which is added to food and enables the physical properties and chemical composition of food to remain unaffected by microbial or other spoilage, so that the milk retains its original wholesomeness and nutritional value. The most likely preservatives to be found in milk are formaldehyde, hydrogen peroxide and neutralizers such as sodium bicarbonate.

Chemical additives such as sodium carbonate and sodium bicarbonate added to milk as neutralizers to preserve it for longer time and to prevent curdling. The continuous use of such milk may cause health hazards to the society (Iswariah and Guruswami, 1973; Satoskar et al., 1999).

Benzoic acid, sorbic acid and their salts are commonly used as chemical preservatives in food products including cheese to prevent alteration and degradation by microorganisms during storage. However, excessive addition of these preservatives may be harmful to consumers, because of the tendency to induce allergic contact dermatitis and convulsion. Therefore, the development of convenient and inexpensive analysis methods of these preservatives is of great importance for food safety (Fang et al., 2008).

The widespread use of antimicrobials in dairy cattle management may result in the presence of their residues; such residues in animal products may be toxic to humans, or may cause serious reactions in sensitive individuals (Lawley et al., 2008) and presents technological difficulties in the milk processing industry (Heeschen and Blüthgen 1991), so many countries have enacted regulations that limit the level of chemical residues in milk and dairy products; milk chemical safety is important for public health (Tenant, 1997). Tetracyclines display a wide spectrum of antimicrobial action: it has a stronger action against gram-positive bacteria and weak action against gram-negative ones. They possess exercise against mycoplasmas, chlamydiae, rickettsias, spirochetes, actinomyces, and some protozoa (Sundin, 2003).

## MATERIALS AND METHODS

### Collection of samples

UHT milk, yogurt, hard, soft and processed cheese samples were randomly collected from dairy shops and supermarkets in Giza governorate. Collected samples were transferred to the laboratory in an insulated ice box with a minimum of delay to be immediately examined.

### Chemical examination

#### Detection of Inhibitory substances in milk

Using general test (Wynther, 1927) 10 ml of milk sample were transferred aseptically into sterilized cotton pluged test tube. Ten ml of milk free from preservatives “control” were transferred to another test tube under the same condition. Two milliliters of litmus solution (10%) “as indicator” were added to both test tubes. Two test tubes were boiled in a water bath for 20 min to kill all lactic acid producing microorganisms then cooled at room temperature. 1 ml of diluted broth culture of lactic acid producing microorganisms (diluted sour milk) was added to both test tubes, then incubated at suitable temperature and kept under observation till coagulation of milk and color reduction of the litmus solution occurs. Result was recorded as positive if the difference in time was 2 h or more between the control and tested sample.

The positive samples were subjected to the following tests:

#### Detection of chemical preservatives

##### *Detection of formalin: Hehner’s test (Ling, 1963)*

Two milliliters of milk sample were mixed with 2 ml of distilled water in a test tube, and then sulphuric acid (90 % containing a trace of ferric chloride) was poured down the side of the tube. Development of violet ring at the junction between the two layers indicates the presence of formalin.

##### *Detection of Hydrogen peroxide (Pien, 1953)*

In a clean test tube, 2 ml of milk sample were added and 2 ml of HCL (1%) were added, thoroughly mixed, then 2 ml of potassium iodide (10%) were added. The tube was immersed in hot water (80-90°C) for 1 min after which the tube was quickly cooled in running water. 2 ml of starch solution (1%) were added as an indicator which develops blue color in the presence of hydrogen peroxide in the milk sample.

##### *Detection of Alkalinizer (sodium bicarbonate): (Foley et al., 1974)*

One milliliter of milk sample was pipetted into a test tube; the concentrated hydrochloric acid (0.5 ml) was added into the sample, the positive samples showed effervescence due to formation of carbon dioxide gas, when this gas was directed into lime water, it makes it turbid.

#### Quantitative analysis of tetracycline residues in milk

Analysis of tetracycline residues in milk was carried out using ELISA test kit (competitive enzyme immunoassay for the quantitative analysis of tetracycline in milk) (MaxSignal).

##### *Preparation of samples*

One and half milliliter of the cold milk samples (100C) were

centrifuged at 10000 rpm for 10 min and the upper fat layer was discarded. 50 µl of milk sample was taken then 9.95 ml of 10 mM PBS buffer (pH 7.4) was added. The tubes were vortexed for 30 s, and then 75 µl of the sample were used for the assay.

Result measurement by photometer was at wavelength of 450 nm. Standard curve was constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng./ml.

$$\text{Relative absorbance (\%)} = \frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \times 100$$

The mean relative absorbance values were used for each sample to determine the corresponding concentration of the tested drug in ng/ml from the standard curve.

**Detection and determination of preservatives in cheese**

***Detection of benzoic acid***

The test was done using a qualitative test (ferric chloride test) as described by AOAC (2000).

***Determination of sorbic acid***

Determination of sorbic acid was done using oxidation method as described by AOAC (2000).

**Preparation of standard curve**

Sorbic acid standard solution was pipetted (5, 10 and 15 ml) into separate 500 ml volumetric flask. Then each was diluted to 500 ml and mixed. Two millimeters of each solution (2 ml of $H_2O$ for blank) were pipetted into separate 15 ml test tubes, then 1.0 ml of 0.15 $H_2SO_4$ and 1.0 ml of $K_2Cr_2O_7$ solution was added to each tube and heated in boiling water bath exactly for 5 min after that the tubes were immersed in ice bath and 2 ml of thiobarbituric acid solution was added. Then the tubes were replaced in boiling water bath and heated for additional 10 min, cooled, then the absorbance (A) of each solution was determined at 532 nm against blank, using matched 1 cm cells. The absorbance was plotted against µg sorbic acid.

***Preparation of test portion***

Test sample was cut into strips and passed through food chopper for grinding. Then 300-600 test sample were blended (< 150°C) in high speed blender till homogenous mixture was obtained. Then 1.5 - 2 g of prepared test portion were weighed into distill tube containing SiC chips then 10 ml of (1 M) $H_2SO_4$ and 10 g. $MgSO_4.7H_2O$ were added. Steam distillation was done. 100-125 ml. were collected in 250 ml volumetric flask within 45 min. The prepared sample was treated as in the case of standard curve preparation and then the absorbance was plotted against µg sorbic acid/ml.

**Determination of nitrite using sulfanilic acid method (Edward, 1981)**

***Preparation of standard curve***

Into 50 ml volumetric flasks, 0 (blank), 1.0 ml, 2.0 ml, and so on up to 10 ml. of the dilute standard nitrite solution was transferred then water was added to each flask up to 40 ml and mixed. Two milliliters of the solution (3) of Griess reagent were added to each flask and made to volume (50 ml) with distilled water hich was mixed thoroughly and allowed to stand at room temperature for 1 h.

Suitable portion of each solution was transferred to separate cuvet (Blank + 10 samples); the transmittance of the blank (without nitrite) was adjusted to 100% transmittance (zero optical density) at 520 nm, then the optical density of each standard solution was measured. The optical density readings were plotted on coordinate paper against the nitrite concentration.

***Procedure***

Five grams of prepared sample were weighed in volumetric flask (225 – 250 ml) and mixed with 100 ml of water, and then the flask was heated in water bath (80°C). At the end of heating, 5 ml of saturated mercuric chloride solution were pipetted into each sample and cooled, then made to volume of 225 or 250 ml with distilled water. The solution was filtered through 12.5 cm filter paper. Ten milliliters of filtered solution were transferred to 50 ml volumetric flask. Water was added up to 40 ml and mixed with 2 ml of Griess reagent and made to volume (225 or 250 ml) with water.

The color was allowed to be developed within 40 min to 1 h at room temperature. A suitable portion of the solution was transferred to the cuvet, and the optical density was determined at wave length of 520 nm and adjusted to zero optical density with blank which contained 2 ml of reagent in 50 ml water. The sodium nitrite content was obtained in ppm through the prepared plot.

**Detection of Sulphur Dioxide in different types of cheese (AOAC, 2000)**

The test portion was placed in distilling flask, and then diluted to 400 ml with $H_2O$, and then 90 ml of HCl was added to separator which was forced into flask with gentle pressure. The flask was heated until condensation was showed in the first U-tube then the separator was removed and the heat was turned off.

One drop of methyl red was added, and titrated with NaOH (0.1 M) till clear yellow color was obtained (0.1 M NaOH =3.203 mg $SO_2$.). Similarly, the second U-tube was titrated. After titration, gravimetric determination was made by adding four drops of 1 M HCl and excess of filtered 10% $BaCl_2$ solution, then stood overnight. The precipitate was washed by decantation 3 times with hot $H_2O$ through weighed gooch, then washed with 20 ml alcohol and 20 ml ether, and then dried at 105 - 110°C (mg. $BaSO_4$ × 274.46/g. test portion = $SO_2$ µg. /g).

The blank was determined, both by titration and gravimetrically, and the results were corrected accordingly.

**Quantitative analysis of aflatoxin M1 in milk and cheese**

Using ELIZA Kit (RIDASCREEN® Aflatoxin M1 30/15) which is a competitive enzyme immunoassay for the quantitative analysis of aflatoxin M1 in milk and cheese (Riedel de-Haen, 1997).

## RESULTS AND DISCUSSION

It is evident from the obtained results that the chemical preservatives are the most prevalent type of inhibitory substances. Some unscrupulous producers tend to add

**Table 1**. Incidence of inhibitory substances in examined samples of UHT milk

| Test | No. of examined samples | Positive samples | | Negative samples | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| Wynter blyth test | 30 | 11 | 36.67 | 19 | 63.33 |
| Hydrogen Peroxide residues test | 30 | 6 | 20.00 | 24 | 80.00 |
| Formalin residues test | 30 | 0 | 0.00 | 30 | 100 |
| Alkalinizer ($Na_2CO_3$) | 30 | 2 | 6.67 | 28 | 93.33 |
| Tetracycline residues | 30 | 9 | 30.00 | 21 | 70.00 |

**Table 2.** Statistical analytical results of tetracycline residues in the examined samples of UHT milk.

| Tetracycline residues concentration (ppb.) | No. of examined samples | Positive samples | | Tetracycline residues | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Mean ± S.E.M. |
| | 30 | 9 | 30 | 10.00 | 78.71 | 23.62 ±7.01 |

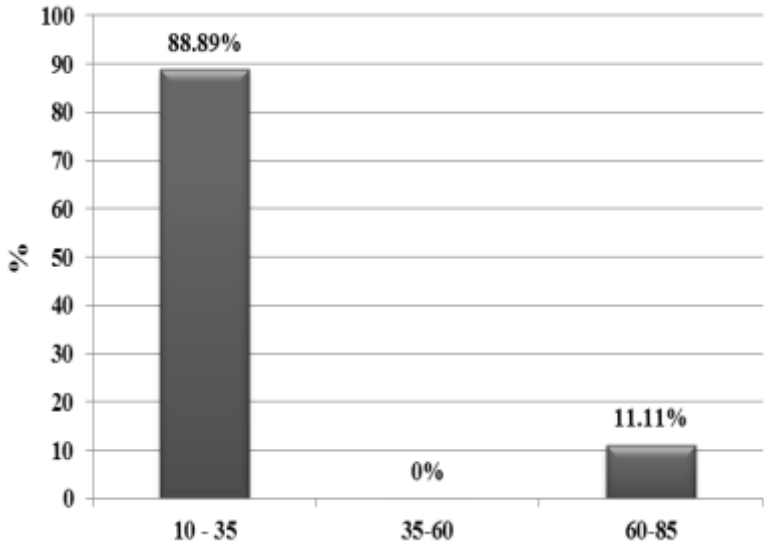


**Figure 1.** Frequency distribution of the examined samples of UHT milk based on their tetracycline residues concentration (ppb).

preservatives to milk in order to mask the neglected sanitary measures and to improve its keeping quality. Chemical preservation works either as direct microbial poisons or as acid neutralizer to prevent the microbial growth (Australian Academy of Science, 2004).

The results recorded in Table 1 show that 11 (36.67%) out of 30 examined UHT milk samples contained inhibitory substances; 6 samples (20%) contained hydrogen peroxide, 2 samples (6.67%) contained sodium bicarbonate (alkalinizer), while formalin was not detected in any of the examined samples. Tetracycline residues were detected in 9 samples (30%).

It is known that long continuous intake of sodium carbonate or bicarbonate and formalin might be harmful, as it causes damage to the gastrointestinal tract, primarily the stomach and lower oesophagus (Resmini et al., 1988). In addition, it may have a carcinogenic effect on human (Haddad and Winchester, 1990) and severe acidosis which result from rapid conversion of formaldehyde to formic acid (Mohanan et al., 2002).

Table 2 reveals that the mean value of tetracycline residues in the examined samples of UHT milk was 23.62 ± 7.01 with a minimum value of 10.00 and a maximum value of 78.71 ppb. The highest frequency distribution of tetracycline residues in the examined samples of UHT milk 8 (88.89 %) lies in the range of 10 to 35 ppb (Figure 1).

Table 3 represents the incidence of inhibitory

**Table 3.** Incidence of inhibitory substances in the examined samples of cheese.

| Type of samples | No. of samples | Benzoic acid | | Sorbic Acid | | Nitrite | | Sulphur Dioxide | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. of positive samples | % | No. of positive samples | % | No. of positive samples | % | No. of positive samples | % |
| Hard cheese | 30 | 15 | 50.00 | 6 | 20.00 | 20 | 66.67 | 0 | 0.00 |
| Soft cheese | 30 | 5 | 16.67 | 13 | 43.33 | 17 | 56.67 | 3 | 10.00 |
| Processed cheese | 30 | 0 | 0.00 | 16 | 53.33 | 29 | 96.67 | 0 | 0.00 |

**Table 4.** Statistical analytical results of sorbic acid and nitrite in examined samples of cheese.

| Types of samples | Sorbic acid (ppm.) | | | Nitrite (ppm.) | | |
|---|---|---|---|---|---|---|
| | Min. | Max. | Mean ± S.E.M. | Min. | Max. | Mean ± S.E.M. |
| Hard cheese | 9.00 | 13.30 | 2.12± 0.8 | 1.40 | 8.00 | 1.59 ± 0.31 |
| Soft cheese | 1.00 | 10.3 | 1.64 ± 0.5 | 0.40 | 3.20 | 0.67± 0.14 |
| Processed cheese | 2.00 | 14.00 | 1.71 ± 0.5 | 0.60 | 9.00 | 1.33 ± 0.027 |

**Table 5.** Statistical analytical results of aflatoxin M1 concentration (ppt.) in the examined samples.

| Types of samples | No. of examined samples | Positive samples | | Aflatoxin M1 conc. (ppt.) | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Mean±S.E.M. |
| Hard cheese | 15 | 14 | 93.33 | 3.33 | 80.00 | 22.93 ± 6.31 |
| Soft cheese | 15 | 14 | 93.33 | 14.00 | 80.00 | 62.26 ± 7.03 |
| UHT milk | 15 | 15 | 100 | 7.5 | 80.00 | 53.30 ± 7.12 |

substances in cheese samples as benzoic acid was detected in 50% of hard cheese and 16.67% of soft cheese samples but it was not detected in the examined processed cheese samples. Sorbic acid was detected in 53.33% of processed, 43.33% of soft and 20% of hard cheese samples. The nitrite was detected in all examined cheese samples (100%) with highest incidence (96.67%) in the processed cheese samples, while sulphur dioxide was detected in examined soft cheese samples with incidence of 10%, it was not detected in the rest of the examined cheese samples.

Sorbic acid was detected in the examined hard, soft and processed cheese samples with mean values of 2.12± 0.8, 1.64 ± 0.5 and 1.71 ± 0.5, respectively; while nitrite content was detected with mean values of 1.59 ± 0.31, 0.67 ± 0.14 and 1.33 ± 0.027 ppm., respectively (Table 4).

Excessive addition of these preservatives may be harmful to consumers, because of the tendency to induce allergic contact dermatitis and convulsion (Fang et al., 2008). Sorbic acid is permitted in cheese, at levels below 3000 mg/kg. At these concentrations, sorbic acid acts as an efficient fungicide (Skovgaard, 1992) while Glass et al. (1998) mentioned that addition of sorbate with concentration of 0.1% can inhibit the growth of *Staphylococcus aureus* in processed cheese slices**.**

Nitrate is used in Europe to prevent late blowing of certain cheeses due to *Clostridium butyricum* and *C. tyrobutyricum* (Skovgaard, 1992; Glaesser, 1989).

The toxic effect of $SO_2$ in human is variable; some persons may tolerate up to 50 mg/kg body weight, while others feel headache, nausea and diarrhea at this concentration (Schroeter, 1966).

According to the obtained results in Table 5, it was cleared that aflatoxin M1was detected in all examined samples (100%) of UHT milk, (93.33%) of hard and soft cheese samples, with mean values of 53.30 ± 7.12, 22.93 ± 6.31and 62.26 ± 7.03, respectively.

Results represented in Figure 2 show the highest frequency distribution of AFM1concentration in the examined soft cheese and UHT milk samples (60 and 33.33%, respectively) lies in the range 40-80 while it lies in the range 0-10 in 40 % of examined hard cheese samples.

The higher concentration of aflatoxin M1 in cheese may be attributed to the association of aflatoxin M1 with casein fractions during manufacture (Egmond, 1989) while lower levels of aflatoxin M1 in some milk and milk

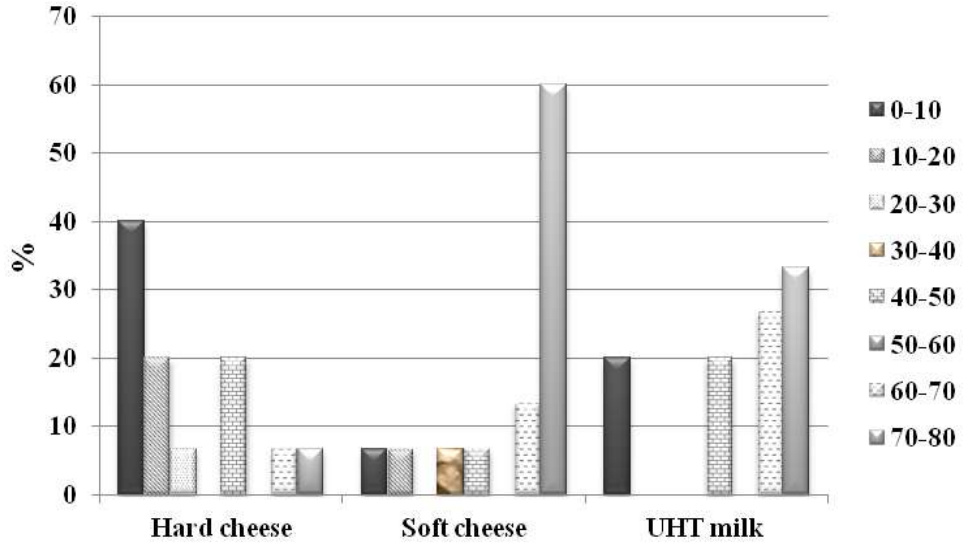


**Figure 2.** Frequency distribution of the examined samples based on their aflatoxin M1 concentration (ppt).

products may be attributed to greater amount of grass consumed by dairy cattle and lower amount of concentrates (Brown, 1982; Egmond, 1991; Dragacci and Fremy, 1996).

## Conclusion

The results obtained from this work allow us to concluded that the majority of yogurt, hard, soft and processed cheese exposed for sale in Giza governorate are considered being hazardous to the consumers.

Tetracyclines were present in samples of UHT milk with variable percentages, while aflatoxin M1 was detected in all examined samples of UHT milk and cheese, sorbic acid, benzoic acid, sulphur dioxide and nitrite were detected in some samples of cheese. Therefore, in order to produce safe milk products, contamination should be avoided in order to reduce economic losses and expenses. To ensure safety in dairy industries the following suggestions should always be observed:

1) Strict hygienic measures should be adopted in dairy farms to ensure production of high quality milk.
2) Good sanitary conditions should be applied during production and processing of milk. Educational programs should be imposed to producers, processors and and handlers to improve the quality of the product and to ensure the maximum safety to the consumers.
3) Application of effective technological measures (pasteurization, sterilization, acidification in technological processes to prolong the product shelf life and decreases or eliminations of pathogens in milk and milk products.
4) Application of adequate control measures through periodical examination of market milk and milk products by specialists to ensure maximum safety to the consumers.
5) Good manufacturing practices should be maintained throughout and HACCP should be applied to ensure safety of the finished products.

## Conflict of interests

The authors did not declare any conflict of interest.

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academicJournals

Vol. 9(4), pp. 194-199, April, 2015
DOI: 10.5897/AJFS2014.1209
Article Number: 9CD387751591
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Effect of concentration of debittering agent on the mineral, vitamin and phytochemical contents of *Lasianthera africana* leafy vegetable

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Received 11 September, 2014; Accepted 26 February, 2015

**White variety of *Lasianthera africana* leaves were blanched in hot water (control) and in different concentrations (0.25, 0.50, 0,75, 1.00 and 1.25%) of unripe plantain peel ash solution for 3 min at 100°C, cooled, drained, oven dried (50°C) for 36 h. Then, raw leaf and all oven dried samples were analyzed for minerals, vitamins and phytochemicals. The raw leaf contained potassium (78.98±0.78 mg/100 g), calcium (190.25±0.44 mg/100 g), sodium (75.69±0.95 mg/100 g), magnesium (14.68±0.74 mg/100 g), iron (3.96±0.55 mg/100 g), zinc (5.95±0.52 mg/100 g), phosphorus (17.79±0.81 mg/100 g), ascorbic acid (109.64±0.08 mg/100 g), beta-carotene (2.86±0.04 mg/100 g), riboflavin (0.22±0.03 mg/100 g), thiamine (1.01±0.06 mg/100 g), alkaloids (2.67±0.33 g/100 g), flavonoids (0.32±0.03 g/100 g), saponins (3.09±0.04 g/100 g) and tannins (0.28±0.01 g/100 g). Blanching the leaves either in hot water or in different concentrations of unripe plantain peel ash solution led to varying losses of the minerals, vitamins and phytochemicals. Samples blanched in different levels of ash solution retained higher mineral content than hot water blanched samples. Percentage minerals retained increase with increased levels of ash in the blanching solution. Conversely, percentage retention of vitamins and phytochemicals decreased with increase in the levels of ash in the blanching solution. Ash concentration had no significant ($p>0.05$) effect on the levels of magnesium, zinc, beta-carotene and tannins retained in the blanched samples. For higher retention of vitamins and health benefiting phytochemicals, lower concentration of unripe plantain peel ash solution (0.50% to 0.75%) should be used to debitter *L. africana* leaf.**

**Keywords:** *Lasianthera africana* leaf, debittering agent, minerals, vitamins, phytochemicals.

## INTRODUCTION

Green leafy vegetables (wild or cultivated) are important items of diets in many Nigerian home as valuable sources of nutrients especially in rural areas where they contribute substantially to micronutrients which are usually in short supply in most diets. Leaves in general are important sources of iron, potassium, calcium, magnesium, zinc, provitamin A, thiamine, riboflavin, ascorbic acid and folic acid (Uwaegbute, 1989; Fasuyi,

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2006). The dietary fibre in vegetables increases bulk and reduces the incidence of constipation and other related diseases (Jalili et al., 2000). Green leafy vegetables also contain bioactive compounds (phytochemicals) that have potentials in helping to reduce the risk of several deadly diseases in man (Sofowora, 1989; Williamson et al., 1997; Chung et al., 1998; Agte et al., 2000). Epidemiological studies have shown that consumption of vegetables can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Ames et al., 1981). High consumption of green leafy vegetables therefore plays vital role in human nutrition and health (Odukoya et al., 2007). Various traditional post harvest processing treatments such as soaking in hot water, boiling, squeeze washing with water, and hot water blanching may however lead to losses of some of the characteristics which initially made green leafy vegetables consumers delight (Adeboye and Babajide, 2007).

*Lasianthera africana* is one of the top six commonly consumed green leafy vegetables by Efik and Ibibio ethnic groups of Nigeria (Williams *et al.,* 2009). It belongs to the family *Icacinaceae*. It is called “editan” in Efik and Ibibio local dialects of Nigeria. It is a perennial, glabrous, shrub that reaches a height of 61-136 cm (Hutchinson and Dalziel, 1973). Among the Ibibios, four local varieties distinguished by their taste, leaf colour and ecological distribution are known (Bassey *et al*., 2006). The varieties are “afia” (white variety), “obubit” (black variety), “idim” (riverine variety) and “akai” (forest variety). The leaf has been used since pre-historic time for preparing soup and in many traditional concoctions for the treatment of various ailments (Sofowora, 1989). Ebana et al. (1996) reported that the leaves of *L. africana* are rich in chemical compounds of nutritional and medicinal importance. Preliminary screening of the leaves for phytochemicals indicated the presence of alkaloids, flavonoids, saponins, anthraquinones, glycosides and tannins in all the four ethnovarieties (Bassey et al., 2006).

One unique characteristic of *L. africana* leaf is that it has bitter taste that requires debittering prior to culinary use. Debittering helps to enhance palatability and acceptability of the soup prepared with the leaf. Traditionally, the leaf is usually debittered by squeeze washing with water or treatment with aqueous extract from unripe plantain peel ash. The use of higher concentration of unripe plantain peel ash usually gives objectionable flavour to the soup prepared with the debittered leaf and may have negative effect on other beneficial constituents in the leaf. Presently, nothing is known about the effect of debittering treatments on the nutrients, especially the water soluble and heat sensitive nutrients as well as constituents of medicinal value. This study was therefore conducted to assess the effects of debittering *L. africana* leaves in different concentrations of unripe plantain peel ash solution on the nutrients and phytochemical content in the leaves.

## MATERIALS AND METHODS

### Sample collection and preparation

Twigs of *L. africana* (white variety) were harvested from a garden at Aka Offot in Uyo Local Government Area of Akwa Ibom State and authenticated at the taxonomy unit of the Department of Botany and Ecological Sciences, University of Uyo. The harvested twigs were destalked, washed in potable water, spread out under a shade to air dry. The leaves were cut (2 mm width), shared into six portions (1 kg each) and blanched in 0.00, 0.25, 0.50, 0.75, 1.00 and 1.25% solution of unripe plantain peel ash (1:3 w/v) at 100°C for 3 min. The blanched samples were drained immediately, cooled, dried (50°C) for 36 h in a conventional air oven (Model P.P. 22 US, Genlab, England), milled, packaged in plastic containers, labeled and stored in a refrigerator (4°C) for analyses. The contents of minerals, vitamins and phytochemicals in the cut raw leaf were also determined.

### Mineral analysis

The mineral elements (K, Na, Ca, Mg, Zn, Fe and P) were determined using atomic absorption spectrophotometer (UNICAM, Model, 939, UK) as described by AOAC (2000).

### Vitamin analysis

Ascorbic acid, beta-carotene, thiamine and riboflavin were determined using the method described by AOAC (2000).

### Phytochemical analysis

Alkaloid and flavonoid were determined using the method of Harborne (1973). Saponin and tannin were determined by AOAC (2000) method.

### Statistical analysis

Data obtained were subjected to one way analysis of variance (ANOVA) using SPSS version 18 statistical package (SPSS, Inc., USA) to determine variation between treatments. Means of data generated were separated using Duncan multiple range test (DMRT). Results were expressed as mean ± SD (standard deviation) of triplicate determinations. Significant variation was accepted at $p<0.05$.

## RESULTS AND DISCUSSION

Table 1 shows the mineral content of *L. africana* leaves blanched in hot water (control) and in different concentrations of unripe plantain peel ash solution. The raw leaves had higher mineral content than the blanched and dried samples. Hot water blanched and dried samples exhibited higher losses of minerals than ash solution blanched and dried samples.

The potassium content in the raw (unblanched) leaf was 78.98±0.78 mg/100 g. The level of potassium in the hot water blanched and dried samples was 70.43±0.10 mg/100 g, whereas the values for samples blanched in different concentrations of unripe plantain peel ash

**Table 1.** Effect of concentration (%) of unripe plantain peel ash blanching solution on the mineral content of *L. africana* leaf (mg/100 g).

| Mineral | Ash concentration (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0.00 | 0.25 | 0.50 | 0.75 | 1.00 | 1.25 |
| K | $70.43^{d}$±0.14 | $72.20^{c}$±0.33 | $72.95^{c}$±0.31 | $73.92^{b}$±0.09 | $74.31^{a}$±0.14 | $74.88^{a}$±0.27 |
| Na | $61.09^{c}$±0.13 | $61.47^{c}$±0.21 | $62.35^{b}$±0.28 | $62.79^{ab}$±0.29 | $63.04^{ab}$±0.04 | $63.57^{a}$±0.06 |
| Ca | $177.91^{d}$±0.16 | $178.62^{c}$±0.16 | $179.00^{c}$±0.04 | $179.53^{b}$±0.16 | $179.82^{ab}$±0.08 | $180.06^{a}$±0.08 |
| Mg | $12.23^{a}$±0.17 | $12.24^{a}$±0.19 | $12.30^{a}$±0.14 | $12.35^{a}$±0.14 | $12.38^{a}$±0.07 | $12.42^{a}$±0.16 |
| Zn | $5.010^{a}$±0.13 | $5.14^{a}$±0.04 | $5.17^{a}$±0.03 | $5.18^{a}$±0.06 | $5.20^{a}$±0.03 | $5.21^{a}$±0.01 |
| Fe | $3.45^{a}$±0.44 | $3.49^{a}$±0.21 | $3.53^{a}$±0.08 | $3.56^{a}$±0.00 | $3.57^{a}$±0.13 | $3.61^{a}$±0.29 |
| P | $15.34^{b}$±0.19 | $15.93^{b}$±0.41 | $16.22^{b}$±0.39 | $16.79^{a}$±0.45 | $16.86^{a}$±0.23 | $17.01^{a}$±0.41 |

Values are means ± SD of triplicate determinations. Means on the same row with different superscripts are significantly different at $p<0.05$.

solution ranged from 72.20±0.33 mg/100 g in 0.25% ash solution blanched and dried samples to 74.88±0.27 mg/100 g in 1.25% ash solution blanched and dried samples (Table 1). Significantly, $p<0.05$ higher retention of potassium in ash solution blanched and dried samples relative to hot water blanched and dried samples could be due to differences in the soluble solute concentration of the blanching medium.

Ejoh et al. (2007) similarly reported that leafy vegetables blanched in 2.5 and 5.0% "kanwa" solution exhibited lower mineral losses than hot water blanched samples.

The sodium content in the raw leaf was 75.69±0.95 mg/100 g. The hot water blanched and dried samples had lower sodium content (61.09±0.14 mg/100 g). The ash solution blanched and dried samples had higher sodium content than hot water blanched and dried samples. The values for ash solutions blanched and dried samples ranged from 61.47±0.21 mg/100 g for sample blanched in 0.25% ash solution to 63.57±0.66 mg/100 g for samples blanched in 1.25% ash solution. This shows that sodium retention (%) increased with increase in ash concentration (Table 1).

The result suggests that the use of high concentration of unripe plantain peel ash to debitter *L. africana* leaves should not be encouraged as high sodium intake is an identified risk factor for the development of hypertension in susceptible individuals (Tobian, 1997; Campbell-Platt, 2009). Salt restriction and low salt diets have been shown to lower blood pressure in both normotensive and hypertensive subjects (Ebuehi et al., 2003).

Calcium was the most predominant mineral element in raw *L. africana* leaf (190.25±0.44 mg/100 g). The high calcium content in the leaf is significant because the cells need calcium and more than 99% of calcium in the body is used as a structural component of bones and teeth. This represents about 40% of all minerals present in the body (Grosvenor and Smolin, 2002). The blanched and dried samples had lower calcium content than the raw leaf. This could be due to leaching into the blanching medium. Samples blanched in ash solutions had significantly ($p<0.05$) higher calcium content than hot water blanched samples (Table 1). This could be due to differences in soluble solute concentration of the blanching medium. Percentage calcium retention increased with increase in the ash concentration (%) in the blanching medium ranging from 93.89% for samples blanched in 0.25% ash solution to 94.64% for samples blanched in 1.25% ash solution. Similar observation was made by Ejoh et al. (2007) who reported calcium content of 0.83±0.02 g/100 g, 1.04±0.11 g/100 g and 1.28±0.01 g/100 g for *Vernonia calvoana* blanched in hot water, 2.5% "kanwa" and 5.0% "kanwa" solutions, respectively.

The contents of magnesium, zinc, iron and phosphorus in the raw leaf were 14.68±0.74 mg/100 g, 5.95±0.52 mg/100 g, 3.96±0.55 mg/100 g and 17.79±0.81 mg/100 g, respectively. Their values in the blanched and dried samples were lower. This could be due to leaching of these minerals into the blanching medium. The hot water blanched and dried samples had lower content of these minerals than ash solutions blanched and dried samples. There were systematic increases in these minerals with increases in the concentration of ash in the blanching solutions. This could be due to possible contributions from the unripe plantain peel ash in the solutions used for blanching the leaves. Similar observation was reported by Ejoh et al. (2007) for *Vernonia calvoana* leaf blanched in hot water and in "kanwa" solutions.

Data on vitamin analysis (Table 2) indicate that there was inverse relationship between the increase in the concentrations of unripe plantain peel ash in the blanching solution and the level of vitamins retained in the blanched and dried samples. There were consistent reductions in the vitamins analyzed with increase in the concentration of ash in the solutions used for blanching the leaves.

The ascorbic acid content in the raw leaf was 109.64±0.08 mg/100 g. The human body cannot produce ascorbic acid, so it must be obtained entirely through one's diet. Hot water blanched and dried samples had the ascorbic acid content reduced to 65.12±0.14 mg/100 g.

**Table 2.** Effect of concentration (%) of blanching ash solutions on some vitamins in *L. africana* leaf (mg/100 g).

| Ash concentration (%) | Ascorbic acid | Beta-carotene | Riboflavin | Thiamine |
|---|---|---|---|---|
| 0.00 | 65.12$^{a}$ ±0.14 (59.39) | 1.89$^{a}$ ±0.04 (66.08) | 0.19$^{a}$ ±0.03 (86.36) | 0.61$^{a}$ ±0.01 (60.40) |
| 0.25 | 61.43$^{b}$±0.04 (56.03) | 1.87$^{a}$±0.06 (65.39) | 0.17$^{ab}$±0.01 (77.27) | 0.57$^{ab}$±0.03 (56.44) |
| 0.50 | 59.72$^{c}$±0.17 (54.45) | 1.86$^{a}$±0.04 (65.04) | 0.16$^{ab}$±0.00 (72.73) | 0.54$^{ab}$±0.03 (53.47) |
| 0.75 | 55.90$^{d}$±0.09 (51.90) | 1.86±0.02 (65.04) | 0.14$^{ab}$±0.03 (63.64) | 0.52$^{ab}$±0.05 (51.49) |
| 1.00 | 55.03$^{e}$±0.04 (50.19) | 1.85±0.07 (64.69) | 0.13$^{ab}$±0.01 (59.09) | 0.51$^{ab}$±0.01 (50.50) |
| 1.25 | 53.79$^{f}$±0.04 (49.06) | 1.85±0.00 (64.69) | 0.11$^{b}$±0.01 (50.50) | 0.49$^{b}$±0.04 (48.51) |

Values are means ± SD of triplicate determinations. Mean on the same column with different superscripts are significantly different at p<0.05. Values in parenthesis indicate percentage retention of vitamins.

This could be attributed to leaching, thermal decomposition and to a lesser extents, oxidation (Fellows, 2000).

Ascorbic acid contents of unripe plantain peel ash solution blanched and dried samples were significantly (p<0.05) lower than the content in hot water blanched and dries samples (Table 2). The values for samples blanched in different concentrations of ash solution ranged from 53.79±0.04 mg/100 g for samples blanched in 1.25% ash solution to 61.43±0.94 mg/100 g for samples blanched in 0.25% ash solution. Ascorbic acid is not only soluble in water but is also the most heat labile of the vitamins (Grosvenor and Smolin, 2002; Bolarin, 2006).

The lower levels of ascorbic acid in samples blanched in solutions of unripe plantain peel ash relative to hot water blanched samples could be due to the fact that the alkaline media predisposed the vitamin to decomposition which enhanced the level of losses. Ihekoronye and Ngoddy (1985) noted that when leafy vegetables are cooked with baking soda to retain the greenness, they lose much of their ascorbic acid. Grosvenor and Smolin (2002) also stated that the loss of ascorbic acid is accelerated by low acid conditions.

The beta-carotene content in the raw leaf was 2.86±0.04 mg/100 g. Hot water blanched and dried samples had lower content of beta-carotene (1.89±0.06 mg/100 g) than the value in the raw leaf. This reduction in beta-carotene could be attributed to possible destruction by heat and oxidation reaction. According to Meyer (1978), McDowell (1989) and Roche (1990), beta-carotene is especially prone to oxidative destruction in the presence of heat, light, oxygen and metal ions. Blanching the leaves in various concentrations of ash solution did not have any significant (p>0.05) effect on the beta-carotene content of the blanched samples (Table 2). There were however, slight reductions in beta-carotene of the blanched leaves with increase in the concentration of ash in the solution ranging from 1.87±0.06 mg/100 g for samples blanched in 0.25% ash solution to 1.85±0.00 mg/100 g for samples blanched in 1.25% ash solution.

The non-significant difference (p>0.05) in the beta-carotene content of samples blanched in different concentrations of ash solution and hot water confirms the fact that beta-carotene is not affected by changes in pH (Meyer, 1978). Ihekoronye and Ngoddy (1985) also stated that dilute acid, bases and water have little effect on carotenoids.

The riboflavin content in the raw leaf was 0.22±0.03 mg/100 g. Samples blanched in either hot water or various concentrations of unripe plantain peel ash solution and dried had lower riboflavin contents than the value in raw leaf. Sample blanched in hot water had higher riboflavin content (0.19±0.03 mg/100 g) than samples that were blanched in different concentrations of ash solution whose values ranged from 0.17±0.01 mg/100 g for samples blanched in 0.25% ash solution to 0.11±0.01 mg/100 g for samples blanched in 1.25% ash solution.

The observed higher retention of riboflavin in hot water blanched and dried samples (86.36%) when compared with ash solutions blanched and dried samples (77.22 – 59.00%) could be due to the fact that riboflavin is more soluble in alkaline solution than in water (Jacob, 1999), and is destroyed under alkaline condition, light and excessive heat (Fellows, 2000).

The thiamine content in the raw leaf was 1.01±0.06 mg/100 g. Hot water blanched and dried samples and ash solutions blanched and dried samples had lower thiamine content than the raw leaf. Thiamine is known to

**Table 3.** Effect of concentration (%) of blanching ash solutions on the phytochemical content in *L. africana* leaf (g/100 g).

| Ash concentration (%) | Alkaloid | Flavonoid | Saponins | Tannins |
|---|---|---|---|---|
| 0.00 | $2.14^{a}$ ±0.06 (19.85) | $0.21^{a}$ ±0.01 (34.37) | $2.98^{a}$ ±0.06 (3.56) | $0.24^{a}$ ±0.04 (14.29) |
| 0.25 | $1.96^{ab}$±0.04 (26.59) | $0.16^{ab}$±0.03 (50.00) | $2.33^{b}$±0.04 (24.60) | $0.19^{a}$±0.00 (32.14) |
| 0.50 | $1.84^{bc}$±0.06 (31.08) | $0.15^{bc}$±0.00 (53.12) | $2.31^{b}$±0.03 (25.24) | $0.19^{a}$±0.01 (32.14) |
| 0.75 | $1.76^{bc}$±0.07 (34.09) | $0.13^{bc}$±0.01 (59.37) | $2.27^{b}$±0.04 (26.54) | $0.16^{a}$±0.04 (42.86) |
| 1.00 | $1.69^{cd}$±0.56 (36.70) | $0.10^{cd}$±0.00 (68.75) | $2.25^{b}$±0.07 (27.18) | $0.15^{a}$±0.01 (46.43) |
| 1.25 | $1.60^{cd}$±0.00 (40.07) | $0.09^{cd}$±0.01 (71.87) | $2.20^{b}$±0.00 (28.80) | $0.14^{a}$±0.03 (50.00) |

Values are means ± SD of triplicate determinations. Mean on the same column with different superscripts are significantly different at $p<0.05$ values in parenthesis indicate percentage reduction of phytochemicals.

decompose readily in alkaline and neutral solutions if heated (Jacob, 1999; Fellows, 2000; Grosvenor and Smolin, 2002). Hot water blanched and dried samples had higher thiamine content (0.61±0.03 mg/100 g) than ash solutions blanched and dried samples whose values ranged from 0.57±0.03 mg/100 g for samples blanched in 0.25% ash solution to 0.49±0.04 mg/100 g for samples blanched in 1.25% ash solution (Table 2). The value for hot water blanched sample was only significantly ($p<0.05$) higher than that of 1.25% ash solution blanched sample, whereas the rest of the values were not significantly ($p>0.05$) different from each other.

The contents of alkaloid, flavonoid, saponin and tannins in the raw *L. africana* leaf were 2.67±0.33 g/100 g, 0.32±0.03 g/100 g, 3.09±0.04 g/100 g and 0.28±0.01 g/100 g, respectively. The presence of these phytochemicals in the leaf is significant because of their health protecting and promotion potentials. Blanching the leaves in either hot water or different concentrations of unripe plantain peel ash solution had negative effect on these phytochemicals as their values in the blanched and dried samples were lower than those in the raw leaf (Table 3). This could be due to leaching of these phytochemicals into the blanching media. Similar reduction of phytochemical constituents in vegetables as a result of blanching had been reported by Adeboye and Babajide (2007), Onyeka et al. (2010) and Nkafamiya et al. (2010).

The unripe plantain peel ash solutions blanched and dried samples had lower alkaloids, flavonoids, saponins and tannins than hot water blanched and dried samples. This result corresponded with the report of Adeboye and Babajide (2007) who reported that leafy vegetables blanched in hot water contained higher phytochemicals than those blanched in 1% potash solution for five minutes. The reduction of alkaloids, flavonoids, saponins and tannins consistently increased with increase in the level of unripe plantain peel ash in the blanching solution. Percentage reduction of alkaloids, flavonoids, saponins and tannins ranged from 40.07, 71.87, 28.80 and 50.00% for 1.25% ash solution blanched and dried samples to 26.59, 50.00, 24.60 and 32.14% for 0.25% ash solution blanched and dried samples.

It is important to minimize losses of these phytochemicals as they are implicated with various health benefits. Alkaloids and flavonoids for instance are listed among the plant chemical constituents responsible for hypoglycemic effect in humans (Akah et al., 2002, Li et al., 2004). Flavonoids have strong anti-cancer properties and protection against cardiovascular disease by inhibiting the oxidation of low density lipoprotein (Grosvenor and Smolin, 2002, Okwu, 2004).

Saponin in food decreases the cholesterol absorption from the gastrointestinal tract and therefore lower blood cholesterol, a major risk factor for cardiovascular disease (Topping et al., 1980; Grosvenor and Smolin, 2002). Tannins on the other hand can form complexes with protein and iron thereby inhibiting their bioavailability (Grosvenor and Smolin, 2002). Besides this deleterious effect of tannins, they exhibit antioxidant properties and may inhibit activation of carcinogens and cancer promotion (Grosvenor and Smolin, 2002). This implies that tannin can serve dual purpose of reducing some essential nutrients and protecting the body against cancer.

## Conclusion

In conclusion, debittering *L. africana* leaf in hot water and different concentrations of unripe plantain peel ash solution led to varying losses of minerals, vitamins and phytoche-

micals in the leaf. Mineral losses decreased with increase in the levels of ash concentration in the blanching solution. Conversely, losses of vitamins and phytochemicals increased with increase in the level of ash in the blanching solution. For higher retention of vitamins and health benefiting phytochemicals, lower ash concentration (0.50% to 0.75%) should be used for debittering the leaf.

## Conflict of interests

The authors did not declare any conflict of interest.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge Mr. E. Umoh of the Biochemistry Department, University of Uyo, Akwa Ibom State, Nigeria for the technical assistance.

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academicJournals

Vol. 9(4), pp. 200-207, April, 2015
DOI: 10.5897/AJFS2014.1250
Article Number: DB84B6E51598
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Influence of smoking method on quality of traditional smoked Bonga shad (*Ethmalosa frimbriata*) fish from Lagos State, Nigeria

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Received 12 December 2014; Accepted 18 March 2015

**This study was carried out to assess the influence of smoking method on quality attributes of traditional smoked bonga shad fish. Fresh bonga shad fish (100 samples) and smoked bonga shad fish (100 samples) were collected from 20 different processing centres and the fresh samples were smoked with convectional smoking kiln as the control. Laboratory analyses were conducted and each batch was assessed for; proximate and quality analyses. The results obtained show significant variations ($p<0.05$) for all the proximate composition and quality indices of the smoked bonga shad fish samples. The mean moisture content of fresh bonga shad ranged from 72.96 - 76.89% and that of the smoked bonga shad and control ranged from 10.89 - 14.38% and 8.56 - 10.12%, respectively. The mean protein content of 15.18 - 16.95% was recorded for fresh bonga shad samples and 51.86 - 60.24% and 58.86 - 64.84% for samples of smoked bonga shad. The fat content was determined at 6.46 - 8.84%, while the smoked bonga shad and the control samples were found to have fat content at 16.13 - 20.84% and 12.87 - 17.34%, respectively. The mean pH, thiobarbituric acid value (TBA), total volatile base- nitrogen (TVB-N), TMA, trimethylamine value (TMA) and Free fatty acids values (FFA) values of the smoked bonga shad were lower than the control and the fresh samples. However, all values are within the range of legislative standard.**

**Key words:** Bonga shad, smoking, traditional, proximate, quality indices.

## INTRODUCTION

In most sub-saharan Africa countries, traditional fishing is practiced in almost all rivers, lakes, ponds, and seas. It represents an important part of total fish captures and is an important sector in the national strategies of fight against poverty, food security and food safety (Ahmed et al., 2011). Fish is an important dietary component of people all around the world and represents a relatively cheap and accessible source of high quality protein for poorer households (Ikutegbe and Sikoki, 2014). In West Africa, fish has been reported to provide 40–70% of the protein intake of the population (Béné and Heck, 2005; Ikutegbe and Sikoki, 2014) and it is a critical source of

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dietary protein that is not readily available in the carbohydrate-based staple foods of the populations. In Nigeria, fish has an edge over meat because it is cheaper and relatively more abundant (Eyo, 2001) and constitutes about 40% of the animal protein intake (Eyo, 2001; Abolagba and Melle, 2008). Fish can be consumed in several forms; fresh, dried, frozen, fermented, and brined depending on the consumer preference. In a study by Mafimisebi (2012), it was discovered that majority of the Nigerian people had a preference for fresh fish; however limitations such as the low keeping quality of fish after harvested and the tendency of fish to spoil quickly make this very difficult. This results in a higher reported consumption of smoke-dried fish, which has relatively longer shelf life (Mafimisebi, 2012).

Fish is highly perishable and a considerable effort has been directed to extend the shelf-life of fish using presservation and processing techniques, such as refrigeration, freezing, canning, smoking, salting, and drying (Nwachukwu and Madubuko, 2013). Besides, some of these techniques can also be used to enhance the value of fish, such as smoked fish.

The smoking of fish smoldering dates back to civilization (Eyo, 2001). Smoking method mostly imparts a desirable flavour and inhibits the growth of micro-organisms (Swastawati, 2008). It has been used for centuries in food preservation, and is still widely used for this purpose among several communities in the third world where up to 70% of the catch is smoked for preservation (Omojowo and Raji, 2010). Consumers are rediscovering the good taste of smoked seafood, including smoked catfish. To satisfy the consumer demand, it is necessary to produce good quality and safe smoked seafood products (Omojowo and Raji, 2010).

There is insufficient data on the quality characteristics of traditional smoked wild fish from Nigeria despite the fact that it constitutes a substantial portion of fish available for Nigerians. This study is carried out to investigate the influence of smoking method on quality of traditional smoked bonga shad from Lagos State.

## MATERIALS AND METHODS

### Fish used

Fresh bonga shad fish (100 samples) and smoked bonga shad fish (100 samples) were collected from 20 different processing centres from fishing communities of Badagry and Epe Local Government Areas of Lagos State, Nigeria. The fresh bonga shad fish samples were freshly harvested. The fresh samples were taken to the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR) laboratory, Federal University of Agriculture, Abeokuta for smoking.

### Area of study

Using a current geopolitical map of Nigeria, Lagos State (Figure 1) lies to the south-western part of Nigeria and has boundaries with Ogun State both in the north and east. It is bordered on the west by the Republic of Benin and in the south, stretches for 180 km along the coast of the Atlantic Ocean. It therefore has 22.5% of Nigeria's coastline and occupies an area of 3,577 sq km land mass with about 786.94 sq km (22%) of it being lagoons and creeks. The state is endowed with marine, brackish and fresh water ecological zones with varying fish species that provide productive fishing opportunity for fishermen. Two local government areas (Badagry and Epe Local Government) were covered because they are highly densed fish processing centers. They were selected for the study and hazard analyses of the products.

### Sampling procedure

Fresh samples (100) and smoked bonga shad samples (100) were collected from each of the 10 processing centres from each of the two local government areas (Badagry and Epe Local Government) by purposive sampling in sterile containers (Ziploc).

All freshly harvested bonga shad samples were kept on ice during transportation to the laboratory and smoked on the same day. Smoked bonga shad fish samples were analyzed immediately.

### Fish smoking process

Smoked fish was prepared following the method as described by Crapo (2011) with modifications. Bonga shad fish samples were carefully cleaned to remove slime, blood and harmful bacteria. The fish were eviscerated, leaving the skin on the fish. The fish were cut into uniform pieces (fillet) so that no parts will get overheated.

The bonga shad fish were smoked to 80°C internal temperature for 18 h. The kiln temperature was adjusted as needed throughout this smoking period to maintain the 80°C internal temperature. Hands, utensils and work surfaces were cleaned when transferring fish from smoker to oven to cool down to avoid cross-contamination. Smoking was done for 24 h until the fish is fully dried.

### Proximate analysis

The following proximate analyses were carried out on fresh bonga shad and the smoked bonga shad samples collected from the processing centres. The moisture content of the fresh bonga shad and smoked bonga shad were determined by the oven-drying method. Protein contents of the bonga shad were extracted and fractionated by the method of AOAC (2000) method. The crude fat, crude fibre and ash content of the fresh bonga shad and smoked bonga shad were determined by AOAC (2000) method.

### Physico-chemical analysis

Kent pH meter (Kent Ind. Measurement Ltd., survey) model 7020 equipped with a glass electrode was used to measure the pH of the flesh, employing 10 g of fish homogenized in 10 ml of distilled water. Triplicate determinations were made in all cases. The pH meter was calibrated using pH 4.0 and pH 7.0 buffers. The total volatile base- nitrogen, trimethylamine value (TMA), thio-barbituric acid value, peroxide value and free fatty acid value of the fresh fish and smoked fish were determined by AOAC (2000) method. All chemicals used in this study were of the analytical grade unless stated otherwise.

### Data analysis

All data analyses were done in triplicates. The data obtained were


**Figure 1.** Map of Lagos State showing the 20 Local Government Areas

subjected to descriptive statistics using IBM SPSS version 21.0 software. One way analysis of variance (ANOVA) was done using Duncan's Multiple Range Test ($p<0.05$) to study the difference between means.

## RESULTS AND DISCUSION

Moisture content of fresh bonga shad samples ranged from 72.96 - 76.89% (Table 1) and that of smoked bonga shad samples obtained using local drum kiln and conventional smoke kiln ranged from 11.22 - 14.64% and 8.64 - 10.32% (Table 2). In contrary to protein, fat, and ash, the moisture content of fresh bonga shad samples decreased sharply after the smoking process. This decrease was due to loss of water during smoking. Moisture content of fish is of great importance as it promotes microbial growth. In the present study, the protein content of the fresh bonga shad samples ranged from 15.18 - 16.95% and that of the smoked bonga shad samples ranged from 51.86 - 60.24% and 58.86 - 64.84%. There was an inverse relationship between the moisture and protein content in the smoked bonga shad. Fat content of fresh bonga shad samples ranged from 6.46% - 8.84% and that of smoked bonga shad samples ranged from 16.13 - 20.84% and 12.87 - 17.34%. Crude fibre content of fresh bonga shad samples ranged from0.21 - 0.37% and crude fibre content of smoked bonga shad samples ranged from 2.14 - 4.32% and 2.26 - 4.56%. Ash content of fresh bonga shad samples ranged from 0.12 - 0.16% and ash content of smoked bonga shad samples ranged from 1.18 - 1.46% and 1.24 - 1.79% . The increase in mineral content, ash and crude fibre can be attributed to an increase in the dry matter content per unit weight following sample dehydration

**Table 1.** Proximate composition of fresh Bonga shad (*Ethmalosa frimbriata*) samples from 20 different processing centres.

| Processing centres | Moisture % | Protein % | Fat % | Crude fibre % | Ash % | Carbohydrate % |
|---|---|---|---|---|---|---|
| Agbalata | $74.81_{a}$ | $15.18_{a}$ | $7.81_{cdefg}$ | $0.29_{abc}$ | $0.15_{a}$ | $0.92_{ab}$ |
| Ajido | $75.68_{a}$ | $15.96_{a}$ | $5.73_{a}$ | $0.26_{abc}$ | $0.12_{a}$ | $1.04_{ab}$ |
| Asakpo | $76.54_{a}$ | $15.79_{a}$ | $7.86_{defg}$ | $0.3_{4bc}$ | $0.14_{a}$ | $1.01_{ab}$ |
| Boguru | $73.86_{a}$ | $16.21_{a}$ | $7.90_{fg}$ | $0.21_{a}$ | $0.12_{a}$ | $1.05_{ab}$ |
| Fvanoveh | $75.87_{a}$ | $16.95_{a}$ | $8.84_{fg}$ | $0.26_{abc}$ | $0.12_{a}$ | $0.94_{ab}$ |
| Gberefun | $74.69_{a}$ | $15.72_{a}$ | $8.45_{efg}$ | $0.23_{ab}$ | $0.13_{a}$ | $0.99_{ab}$ |
| Gbetrome | $74.75_{a}$ | $16.78_{a}$ | $8.87_{g}$ | $0.33_{bc}$ | $0.12_{a}$ | $1.01_{ab}$ |
| Ilaje | 75.91a | $16.26_{a}$ | $7.49_{bcde}$ | $0.31_{abc}$ | $0.14_{a}$ | $1.16_{ab}$ |
| Kofegameh | $74.62_{a}$ | $15.93_{a}$ | $6.73_{abc}$ | $0.24_{ab}$ | $0.14_{a}$ | $1.08_{ab}$ |
| Pako | $76.72_{a}$ | $16.11_{a}$ | $7.94_{defg}$ | $0.27_{abc}$ | $0.16_{a}$ | $0.95_{ab}$ |
| Afuye | $74.95_{a}$ | $16._{89a}$ | $8.84_{fg}$ | $0.25_{abc}$ | $0.14_{a}$ | $0.92_{ab}$ |
| BodinYawa | $73.89_{a}$ | $16.61_{a}$ | $6.93_{bcd}$ | $0.31_{abc}$ | $0.12_{a}$ | $1.06_{ab}$ |
| Idale | $74.63_{a}$ | $16.96_{a}$ | $7.89_{bcd}$ | $0.21_{a}$ | $0.13_{a}$ | $0.95_{ab}$ |
| Igbodun | $75.36_{a}$ | $16.83_{a}$ | $8.43_{efg}$ | $0.23_{ab}$ | $0.14_{a}$ | $1.04_{ab}$ |
| Ilogun | $76.52_{a}$ | $15.68_{a}$ | $7.68_{cdef}$ | $0.30_{abc}$ | $0.12_{a}$ | $1.24_{b}$ |
| Mejona | $74.93_{a}$ | $16.89_{a}$ | $7.74_{cdef}$ | $0.24_{ab}$ | $0.15_{a}$ | $1.12_{ab}$ |
| Oluwo | $75.84_{a}$ | $16.48_{a}$ | $6.46_{ab}$ | $0.37_{c}$ | $0.14_{a}$ | $1.01_{ab}$ |
| Okorisan | $77.69_{a}$ | $16.91_{a}$ | $7.90_{defg}$ | $0.34_{bc}$ | $0.12_{a}$ | $0.9_{a}$ |
| Orita | $76.92_{a}$ | $16.86_{a}$ | $7.82_{defg}$ | $0.31_{abc}$ | $0.14_{a}$ | $1.23_{b}$ |
| Orogoro | $75.45_{a}$ | $16.62_{a}$ | $7.96_{defg}$ | $0.28_{abc}$ | $0.13_{a}$ | $1.09_{ab}$ |

Data are means of 3 replicates. Data with the same subscript are not significantly different at $p<0.05$.

**Table 2.** Proximate composition of smoked Bonga shad (*Ethmalosa frimbriata*) from 20 different processing centres using local drum kiln and convective smoking kiln.

| Processing centres | Moisture % | | Protein % | | Fat % | | Crude fibre % | | Ash % | | Carbohydrate % | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Local | Convect | Local | Convect | Local | Convect | Local | Convect | Local | Convect | Local | Convect |
| Agbalata | 13.37gh | 9.23e | 56.18h | 63.18m | 18.09g | 13.31c | 2.91e | 2.98f | 1.46hi | 1.79j | 7.99g | 9.51hi |
| Ajido | 13.84i | 9.56f | 59.06p | 64.06p | 16.61c | 12.98b | 2.63c | 2.87e | 1.18a | 1.33ab | 6.68b | 9.20f |
| Asakpo | 14.31k | 10.12h | 54.39e | 61.39h | 18.15gh | 14.43f | 3.24g | 3.56i | 1.36efgh | 1.54efg | 8.55h | 8.96e |
| Boguru | 12.59e | 8.78bc | 57.21k | 63.21n | 18.20gh | 14.60g | 2.14a | 2.43b | 1.24abcd | 1.61ghi | 8.62h | 9.37g |
| Fvanoveh | 12.11d | 8.64a | 60.24q | 64.84q | 17.13e | 12.87a | 2.65c | 2.78d | 1.19a | 1.24a | 6.68b | 9.63jk |
| Gberefun | 14.26jk | 10.06h | 53.72c | 60.72d | 20.58k | 14.15d | 2.38b | 2.59c | 1.32cdef | 1.68i | 7.74e | 10.80n |

**Table 2.** Contd

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gbetrome | 11.83c | 8.89c | 55.48g | 60.48c | 19.27j | 15.82l | 3.32gh | 3.46h | 1.21ab | 1.43bcde | 8.89j | 9.59ij |
| Ilaje | 12.67e | 8.68ab | 58.26n | 63.86o | 18.15gh | 13.36c | 3.09f | 3.32g | 1.40efgh | 1.62ghi | 6.43a | 9.16f |
| Kofegameh | 13.28g | 9.09d | 57.63l | 62.63l | 16.43b | 14.53g | 2.43b | 2.57c | 1.44ghi | 1.73i | 8.79i | 9.45gh |
| Pako | 11.54b | 8.83c | 54.11d | 61.11g | 18.72i | 16.87o | 2.72cd | 2.91ef | 1.61j | 1.47cde | 11.30m | 8.81d |
| Afuye | 12.04d | 8.78bc | 51.86a | 58.86a | 20.84l | 17.34p | 4.32j | 4.56l | 1.42fghi | 1.73i | 9.52k | 8.73d |
| Bodin Yawa | 14.16j | 10.32i | 58.21mn | 61.61i | 16.13a | 14.28e | 3.10f | 3.34g | 1.18a | 1.65hi | 7.22c | 8.80d |
| Idale | 12.10d | 8.65a | 52.96b | 60.96f | 16.84d | 16.76n | 2.18a | 2.26a | 1.25abcd | 1.54efg | 14.67n | 9.83l |
| Igbodun | 11.33a | 9.68g | 58.13m | 62.13k | 18.25h | 16.35m | 2.25a | 2.41b | 1.42fghi | 1.38bcd | 8.62h | 8.05a |
| Ilogun | 13.28g | 9.08d | 57.18k | 60.18b | 16.46b | 17.82h | 3.04f | 3.32g | 1.16a | 1.51def | 8.62h | 8.01a |
| Mejona | 13.41h | 9.23e | 54.89f | 62.89m | 17.73f | 15.13j | 2.46b | 2.64c | 1.51i | 1.69i | 10.00l | 8.42c |
| Oluwo | 12.94l | 8.88c | 58.42o | 61.72j | 16.12a | 14.82h | 3.79j | 3.91j | 1.30bcde | 1.43bcde | 7.43d | 10.24m |
| Okorisan | 11.22i | 9.56f | 56.94j | 60.94f | 19.44j | 15.58k | 3.41h | 3.57j | 1.23abc | 1.36abc | 7.76f | 8.99e |
| Orita | 14.64 | 10.23i | 53.83c | 60.83e | 18.64i | 13.31c | 4.11i | 4.23k | 1.41fghi | 1.71i | 7.37d | 9.69k |
| Orogoro | 13.89 | 9.71g | 56.18h | 63.18m | 18.15gh | 15.89l | 2.82de | 2.96ef | 1.34defg | 1.63ghi | 7.46d | 8.17b |

Local = Local drum kiln; Convect = Convective smoking kiln. Data are means of 3 replicates. Data with the same subscript are not significantly different at $p<0.05$.

and during the smoking process. In this study carbohydrate content is given by difference that is the percentage of water, protein, fat and ash subtracted from 100. The carbohydrate content of fresh unsmoked bonga shad samples ranged from 0.92 - 1.24% and carbohydrate content of smoked bonga shad and control samples ranged from 6.43 - 10.00% and 8.01 - 10.24%. Carbohydrate content of smoked bonga shad samples is low because it is a proteinous food.

The quality indices of the fresh and smoked bonga shad were studied (Tables 3 and 4). Fats undergo changes during storage which result in production of an unpleasant taste and odour which is commonly referred to as rancidity. The peroxide value (PV) results are similar in pattern to TBA. In this study PV of fresh bonga shad samples was 6.11 – 8.59 mg Eqperoxide/kg and 6.22 – 10.41 mg Eqperoxide/kg and 7.12 – 9.86 mgEq.peroxide/kg for smoked bonga shad and control samples. These values are below the recommended value of between 20 and 40 mgEq.peroxide/kg for rancid taste to begin. Free fatty acids values (FFA) of fresh unsmoked bonga shad samples was 0.86 - 1.13% while that of smoked bonga shad and control samples ranged from 1.05 - 1.26% and 1.00% - 1.19%. These values are very low and below the threshold for rancidity detection in smoked fish. The thiobar-bituric acid value (TBA) is used to assess the degree of fish spoilage especially in fatty fish. The TBA test measures a secondary product of lipid oxidation, malonaldehyde. The TBA values of fresh bonga shad samples ranged from 0.84 – 1.11 mgMol/kg and thio-barbituric acid value (TBA) of smoked bonga shad samples and control samples ranged from 1.01 – 1.23 mgMol/kg and 1.01 – 1.15 mgMol/kg. The TBA (1.00 to 1.15 mg TBA/kg) did not exceed 1 to 2 mg TBA/kg which was well within acceptable limits. The sensory threshold level for detecting rancidity in fresh meat was reported to be between 1 and 2 TBA (Calhoun et al., 1999). The increased TBA values in the smoked fish probably originated from the breakdown of oxidation products, mainly malonaldehyde, during smoking due to the high temperature (Goktepe and Moody, 1998). Beltran and Moral (1991) reported that high TBA values are correlated with the degree of oxidation of fats in hot smoked sardines.

The legislative standard for TVB-N include: 20 mgN/100 g for fresh fish, 30 mgN/100 g stale fish and 40 mgN/100 g for fish that is unfit for human consumption but can be used for animal feed (da Silva, 2002). In this study, the total volatile base-nitrogen (TVB-N) of fresh bonga shad ranged from 13.38 – 15.57 mgN/kg and TVB-N of smoked bonga shad and control samples ranged from 16.43 – 19.36 mgN/kg and 15.49 – 18.83mgN/kg. These values are within the range of legislative

**Table 3.** Quality indices of fresh Bonga shad (*Ethmalosa frimbriata*) samples from 20 different processing centres.

| Processing centres | Peroxide value (PV)(mEq. peroxide/kg) | Free fatty acid (FFA) % | Thiobarbituric acid (TBA) (mg Mol/kg) | Total volatile base-nitrogen (TVB-N) (mgN/kg) | Trimethyl amine value (TMA) (mgN/kg) | pH |
|---|---|---|---|---|---|---|
| Agbalata | $6.11_{a}$ | $1.03_{bcdefg}$ | $0.96_{abcde}$ | $13.89_{e}$ | $2.42_{ghij}$ | $6.96_{abcd}$ |
| Ajido | $6.57_{ab}$ | $1.08_{efgh}$ | $1.00_{bcde}$ | $13.56_{bc}$ | $1.98_{abc}$ | $7.03_{bcdef}$ |
| Asakpo | $8.04_{def}$ | $1.00_{bcdef}$ | $1.19_{g}$ | $15.38_{k}$ | $2.31_{efghi}$ | $7.18_{fgh}$ |
| Boguru | $6.38_{a}$ | $0.95_{abc}$ | $1.07_{efg}$ | $13.49_{ab}$ | $2.49_{ijk}$ | $6.91_{ab}$ |
| Fvanuveh | $7.20_{bc}$ | $1.06_{defg}$ | $1.11_{fg}$ | $15.73_{l}$ | $2.26_{efgh}$ | $6.93_{abc}$ |
| Gberefun | $7.63_{cde}$ | $1.01_{bcdef}$ | $0.89_{abc}$ | $14.63_{gh}$ | $2.03_{abcd}$ | $7.11_{defgh}$ |
| Gbetrome | $8.13_{def}$ | $1.09_{efgh}$ | $0.94_{abcde}$ | $14.92_{i}$ | $2.51_{jk}$ | $6.93_{abc}$ |
| Ilaje | $8.00_{def}$ | $1.11_{fgh}$ | $1.13_{efg}$ | $13.71_{cd}$ | $2.43_{hijk}$ | $6.82_{a}$ |
| Kofegameh | $6.75_{ab}$ | $0.93_{abc}$ | $0.86_{ab}$ | $13.47_{ab}$ | $2.56_{k}$ | $6.86_{a}$ |
| Pako | $8.27_{def}$ | $0.98_{bcde}$ | $1.04_{def}$ | $14.78_{hi}$ | $2.12_{abcde}$ | $7.07_{bcdef}$ |
| Afuye | $7.18_{bc}$ | $1.04_{cdefg}$ | $0.95_{abcde}$ | $15.17_{j}$ | $1.94_{a}$ | $7.10_{defgh}$ |
| BodinYawa | $6.46_{a}$ | $1.07_{efgh}$ | $1.02_{cdef}$ | $13.79_{de}$ | $2.23_{defg}$ | $7.13_{efgh}$ |
| Idale | $6.31_{a}$ | $1.05_{defg}$ | $1.00_{bcdef}$ | $13.73_{de}$ | $2.16_{bcde}$ | $7.24_{h}$ |
| Igbodun | $8.35_{ef}$ | $1.18_{h}$ | $0.85_{a}$ | $15.57_{l}$ | $2.11_{abcde}$ | $7.00_{bcde}$ |
| Ilogun | $8.49_{ef}$ | $0.87_{a}$ | $0.97_{abcdef}$ | $14.45_{f}$ | $1.96_{ab}$ | $7.14_{efgh}$ |
| Mejona | $7.59_{cd}$ | $0.92_{ab}$ | $0.84_{a}$ | $15.63_{l}$ | $1.93_{a}$ | $6.81_{a}$ |
| Oluwo | $8.06_{def}$ | $0.86_{a}$ | $1.08_{efg}$ | $13.85_{de}$ | 1.99abc | $7.20_{gh}$ |
| Okorisan | $7.61_{cd}$ | $1.06_{defg}$ | $1.05_{defg}$ | $14.91_{i}$ | $2.17_{cdef}$ | $7.09_{cdefg}$ |
| Orita | $6.38_{a}$ | $1.13_{gh}$ | $0.91_{abcd}$ | $14.57_{fg}$ | $2.36_{fghi}$ | $7.01_{bcdef}$ |
| Orogoro | $8.59_{cd}$ | $1.02_{bcdef}$ | $0.94_{abcdef}$ | $13.38_{a}$ | $2.13_{abcde}$ | $7.13_{efgh}$ |

Data are means of 3 replicates. Data with the same subscript are not significantly different at $p<0.05$.

**Table 4.** Quality indices of smoked Bonga shad (*Ethmalosa frimbriata*) from 20 different processing centres using local drum kiln and convective smoking kiln.

| Processing centres | Peroxide value (PV)(mEq,peroxide/kg) | | Free fatty acid(FFA) % | | Thiobarbituric acid (TBA) (mg Mol/kg) | | Total volatile base-Nitrogen (TVB-N) (mgN/100g) | | Trimethyl amine value (TMA)(mgN/kg) | pH | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Local | Convect | Local | Convect | Local | Local | Local | Convect | Local | Convect | Local | Convect |
| Agbalata | $9.05_{ghi}$ | $8.92_{n}$ | $1.13_{abc}$ | $1.10_{b}$ | $1.07_{ab}$ | $1.02_{a}$ | $18.33_{g}$ | $16.62_{c}$ | $2.61_{i}$ | $2.49_{kl}$ | $6.41_{defg}$ | $6.56_{a}$ |
| Ajido | $8.96_{gh}$ | $7.78_{g}$ | $1.18_{cd}$ | $1.12_{b}$ | $1.12_{ab}$ | $1.09_{ab}$ | $19.06_{i}$ | $18.31_{k}$ | $2.36_{def}$ | $2.18_{cde}$ | $6.38_{cdef}$ | $6.72_{bcde}$ |
| Asakpo | $9.01_{ghi}$ | $8.21_{hi}$ | $1.26_{d}$ | $1.19_{b}$ | $1.18_{ab}$ | $1.15_{bc}$ | $17.54_{c}$ | $16.96_{d}$ | $2.56_{ij}$ | $2.37_{ij}$ | $6.62_{ij}$ | $6.93_{i}$ |
| Boguru | $9.13_{i}$ | $8.46_{l}$ | $1.05_{ab}$ | $1.03_{b}$ | $1.04_{ab}$ | $1.02_{a}$ | $17.31_{b}$ | $16.63_{c}$ | $2.62_{jk}$ | $2.51_{lm}$ | $6.29_{abcd}$ | $6.65_{abc}$ |
| Fvanuveh | $9.94_{k}$ | $8.63_{m}$ | $1.09_{abc}$ | $1.04_{b}$ | $1.06_{ab}$ | $1.04_{ab}$ | $19.11_{i}$ | $17.23_{e}$ | $2.49_{hi}$ | $2.30_{ghi}$ | $6.58_{hij}$ | $6.90_{hi}$ |

**Table 4.** Contd

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gberefun | $9.09_{hi}$ | $8.32_{k}$ | $1.06_{ab}$ | $1.03_{b}$ | $1.02_{ab}$ | $1.00_{a}$ | $17.29_{b}$ | $16.36_{b}$ | $2.27_{abcd}$ | $2.10_{abc}$ | $6.53_{fghi}$ | $6.61_{ab}$ |
| Gbetrome | $8.77_{f}$ | $7.61_{e}$ | $1.04_{a}$ | $1.01_{b}$ | $1.01_{a}$ | $1.00_{a}$ | $19.36_{j}$ | $17.62_{hi}$ | $2.73_{jk}$ | $2.59_{mn}$ | $6.37_{bcde}$ | $6.72_{def}$ |
| Ilaje | $8.92_{fg}$ | $7.68_{ef}$ | $1.19_{cd}$ | $1.15_{b}$ | $1.11_{ab}$ | $1.10_{abc}$ | $19.18_{i}$ | $18.23_{k}$ | $2.64_{i}$ | $2.52_{lm}$ | $6.43_{defgh}$ | $6.84_{ghi}$ |
| Kofegameh | $9.11_{hi}$ | $8.31_{jk}$ | $1.16_{bcd}$ | $1.11_{b}$ | $1.13_{ab}$ | $1.11_{abc}$ | $18.24_{fg}$ | $17.67_{i}$ | $2.81_{l}$ | $2.61_{n}$ | $6.71_{j}$ | $6.63_{abc}$ |
| Pako | $8.98_{ghi}$ | $8.95_{n}$ | $1.10_{abc}$ | $1.07_{b}$ | $1.08_{ab}$ | $1.06_{ab}$ | $17.96_{d}$ | $16.82_{c}$ | $2.35_{cdef}$ | $2.19_{cde}$ | $6.24_{abc}$ | $6.91_{i}$ |
| Afuye | $7.99_{d}$ | $7.73_{fg}$ | $1.08_{abc}$ | $1.06_{b}$ | $1.02_{a}$ | $1.01_{a}$ | $17.28_{b}$ | $16.58_{c}$ | $2.32_{cde}$ | $2.14_{bcd}$ | $6.32_{abcde}$ | $6.65_{abc}$ |
| BodinYawa | $10.41_{l}$ | $9.86_{o}$ | $1.12_{abc}$ | $1.08_{b}$ | $1.05_{ab}$ | $1.03_{ab}$ | $16.43_{a}$ | $15.49_{a}$ | $2.47_{ghi}$ | $2.34_{hij}$ | $6.42_{defgh}$ | $6.71_{bcde}$ |
| Idale | $6.22_{a}$ | $6.09_{a}$ | $1.06_{ab}$ | $1.02_{b}$ | $1.01_{a}$ | $1.01_{a}$ | $17.91_{d}$ | $16.68_{c}$ | $2.38_{defg}$ | $2.18_{cde}$ | $6.21_{a}$ | $6.64_{abc}$ |
| Igbodun | $8.43_{e}$ | $8.23_{ij}$ | $1.13_{abc}$ | $1.12_{b}$ | $1.07_{ab}$ | $1.04_{ab}$ | $19.46_{j}$ | $18.34_{k}$ | $2.31_{bcde}$ | $2.22_{def}$ | $6.58_{hij}$ | $6.77_{def}$ |
| Ilogun | $9.61_{j}$ | $8.47_{l}$ | $1.08_{abc}$ | $1.05_{b}$ | $1.03_{ab}$ | $1.01_{a}$ | $18.21_{efg}$ | $17.53_{gh}$ | $2.17_{a}$ | $2.0_{4a}$ | $6.41_{defg}$ | $6.93_{i}$ |
| Mejona | $9.59_{j}$ | $8.14_{h}$ | $1.12_{abc}$ | $1.10_{a}$ | $1.08_{ab}$ | $1.06_{ab}$ | $19.06_{i}$ | $18.12_{j}$ | $2.20_{ab}$ | $2.08_{ab}$ | $6.22_{ab}$ | $6.72_{bcde}$ |
| Oluwo | $8.01_{d}$ | $8.41_{l}$ | $1.10_{abc}$ | $1.05_{b}$ | $1.23_{b}$ | $1.21_{c}$ | $18.19_{ef}$ | $17.32_{f}$ | $2.23_{abc}$ | $2.05_{ab}$ | $6.56_{ghij}$ | $6.80_{efg}$ |
| Okorisan | $7.69_{c}$ | $7.32_{c}$ | $1.02_{a}$ | $1.00_{b}$ | $1.14_{ab}$ | $1.13_{abc}$ | $18.09_{e}$ | $17.46_{g}$ | $2.42_{efgh}$ | $2.24_{ef}$ | $6.23_{abc}$ | $6.68_{bcd}$ |
| Orita | $6.66_{b}$ | $7.12_{b}$ | $1.08_{abc}$ | $1.03_{b}$ | $1.01_{a}$ | $1.01_{a}$ | $18.79_{h}$ | $16.31_{b}$ | $2.57_{ij}$ | $2.41_{jk}$ | $6.20_{a}$ | $6.74_{cdef}$ |
| Orogoro | $7.51_{c}$ | $7.45_{d}$ | $1.11_{abc}$ | $1.10_{b}$ | $1.06_{ab}$ | $1.05_{ab}$ | $19.14_{i}$ | $18.83_{l}$ | $2.35_{cdef}$ | $2.26_{efg}$ | $6.46_{efgh}$ | $6.92_{i}$ |

Local = Local drum kiln; Convect = Convective smoking kiln. Data are means of 3 replicates. Data with the same subscript are not significantly different at $p<0.05$.

standard for TVB-N which is 20 mgN/100 g for fresh fish. This suggests that the level of protein decomposition or breakdown in all the samples is low.

Trimethylamine (TMA) is a reduction product of trimethylamine oxide during spoilage and ammonia is mainly formed as a product of protein break-down. Trimethylamine (TMA) is one of the volatile amines plus ammonia which can be used as an index of spoilage (da Silva, 2002). In this study, the trimethylamine value (TMA) for fresh unsmoked bonga shad samples ranged from 1.93 –2.56 mgN/kg and 2.17 – 2.81mgN/kg and 2.05 – 2.61 mgN/kg for smoked bonga shad and control samples. The trimethylamine value (TMA) of 1.93 –2.56mgN/kg for fresh bonga shad samples and 2.17 – 2.81 mgN/kg and 2.05 – 2.61 mgN/kg for smoked bonga shad samples are within the range of < 3 mgN/100g for fresh fish, >8 mgN/100g for spoiled fish and $\underline{>}$ 5 mgN/100g for doubtful quality specified U.S.F.D.A (da Silva, et al., 2008). pH is the most critical factors affecting microbial growth and spoilage of foods. The pH value of fresh unsmoked bonga shad samples ranged from 6.81 – 7.24 and pH value of smoked bonga shad samples ranged from 6.20 – 6.71 and 6.56 – 6.93. The pH values of the fresh bonga shad samples was high compared to smoked bonga and control samples; this may be due to biochemical reac-tions and enzyme action as a result of delay in reaching the shore from the sea because most of the fishermen had no cooling system in their boats or canoes. However, the pH in fish tissues drops due to smoking.

## Conclusion

This research work revealed that in contrary to protein, fat, and ash, the moisture content of fresh bonga shad samples decreased sharply after the smoking process.

This decrease had been found to be due to loss of water during smoking. Protein content in smoked bonga shad fish and control samples has been found to increase due to an increase in the dry matter content per unit weight following sample dehydration during smoking compared to fresh samples this is important- as so many people in West Africa eat smoked dried fish and are involved in producing smoked dried fish- has a high protein content lends support to national strategies of fight against poverty, food shortage and malnutrition. There was an inverse relationship between the moisture and protein content in the smoked bonga shad fish. There were increases in mineral content, ash, crude fibre, TBA and pH values. Convective smoking kiln did not significantly affect pH and proximate composition of smoked fish. It, however, significantly

($p<0.05$) reduced the moisture content of smoked fish and the quality indices such as FFA, TBA and PV.

## Conflict of interests

The authors did not declare any conflict of interest.

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Vol. 9(4), pp. 208-222, April, 2015
DOI: 10.5897/AJFS2015.1269
Article Number: 0C7677651601
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Antioxidant-mediated protective effect of hawthorn (*Crataegus mexicana*) peel extract in erythrocytes against oxidative damage

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Received 20 January 2015; Accepted 16 March 2015

**Hawthorn (*Crataegus Mexicana*) is a traditional fruit in Mexican gastronomy and is used to treat many ailments. Previous studies have shown that acetone extracts derived from hawthorn peel (HPE) possess a strong antioxidant activity in chemical and biological model systems *in vitro*, attributable to their polyphenolic content. The main objective of this study was to investigate the ability of HPE to protect erythrocytes against oxidative damage, *in vitro*. The protection rendered by the HPE in erythrocytes was studied in terms of protection to oxidative damage by the inhibition of thiobarbituric acid reactive substances (TBARS) assays, morphological changes by light microscopy, and electrophoretic analysis of banding pattern of the red blood cells (RBCs). $FeSO_4$ was chosen to induce lipid peroxidation in human RBCs membranes and cytoskeleton proteins. The total polyphenol content in HPE was found to be 0.68 mg/g (SD 0.001) as the equivalent of gallic acid per gram. Trapping of DPPH was calculated by $IC_{50}$ in 15.26 mg/L (SD 0.20). Better inhibition of TBARS formation by HPE was 16.78 mg/L (SD 0.33). HPE retards the morphological alteration in the erythrocytes-eryptosis. The electrophoretic pattern showed that some protein bands were not altered during a long period of incubation in HPE. Furthermore, it was found that HPE offers significant protection to human membrane erythrocyte up to for 28 days from the oxidative damage. In conclusion, our results indicate that HPE is capable of protecting erythrocytes against oxidative damage and morphologic changes by acting as a strong antioxidant.**

**Key words:** Antioxidants, *Crataegus mexicana*, free radicals, Hawthorn, red blood cells, thiobarbituric acid reactive substances (TBARS).

## INTRODUCTION

The pathophysiology of many blood diseases is associated with an increase of free radicals derived from reactive oxygen species (ROS) in the blood cells. Oxidative stress in red blood cells (RBCs) induces damage by injuring the

protein cytoskeleton (Berlett and Stadtman, 1997; Dean et al., 1997), and the phospholipid cell membrane (Kowalczyk et al., 2012) causing directly and indirectly morphologic and microrheologic changes (Hebbel et al., 1990), thus premature eryptosis (Nagababu et al, 2008; Kempe et al., 2006; Lang et al., 2006). These changes and the reduced life span of the cells are involved in the pathogenesis of a number of blood diseases, including different types of anemia, altered vascular disorders, coagulopathies or clog veins alterations, also it is important in the life span of RBCs' bags (Baek et al., 2012). ROS are also involved in a number of other chronic human diseases (Kowalczyk et al., 2012).

In the bi-lipid cell membrane of the erythrocytes oxidation occurs by ROS, and malondialdehyde (MDA) formation can be measured and determined due the altered redox status of the cell (Singh and Rajini, 2008). The higher production of ROS is capable of causing oxidative damage in proteins by oxidation of amino acid residue side chain, formation of protein-protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation (Berlett and Stadtman, 1997). Because of RBCs do not have nucleus or mitochondria organelles, their defense and reparation mechanisms against ROS became vulnerable (Pandey and Rizvi, 2011). However, RBCs have an effective anti-oxidant system that includes high levels of the enzyme catalase (CAT) (Cañas et al., 1988; Aebi et al., 1968), lower levels of superoxide dismutase (SOD) (Lynch et al., 1976), and the reduced glutathione peroxidase (GSH) (Van Asbeck et al., 1985), that make this cells mobile free radical scavengers.

A large number of fruits containing high amounts of nutrients are capable of inhibiting the oxidative stress and ROS formation; this is possible mainly by the action of antioxidant molecules, such as vitamin C, polyphenols, flavonoids and other antioxidant enzyme systems (Edwards et al., 2012; Cai et al., 2004). The clinical benefits after the intake of fruits have shown that they can protect and prevent the complications of chronic degenerative diseases, such as atherosclerosis, chronic inflammation, diabetes mellitus, cataract, coronary diseases and some types of cancer (Edwards et al., 2012; Willis and Wians, 2003; Middleton et al, 2000). Antioxidant activity of ethanolic plant extracts on cells has been demonstrated (Salawu et al., 2011; Nugraheni et al., 2011), including the inhibitory effects in RBCs hemolysis by the action of antioxidant extracts of cocoa plant (Zhu et al., 2005, 2002), potato peel (Singh and Rajini, 2008), and flaxseed oil (Liu et al., 2012). The activity has been related to flavonoids (Lam et al., 2007; Asgary et al., 2005; Sorata et al., 1984) to protect the erythrocytes membrane against deformation.

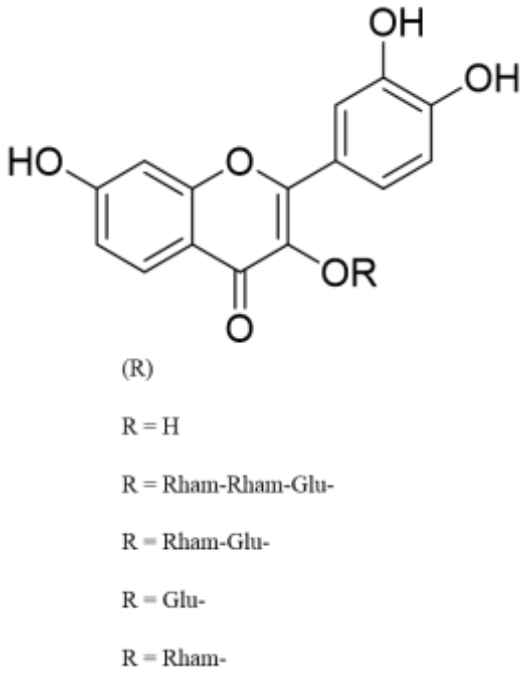


**Figure 1.** Structure of the quercetin glucosides in hawthorn.

The hawthorn *Crataegus mexicana*, is a traditional fruit which is a medicinal herb and in the Mexican gastronomy since ancient times. The wild type also named "tejocote" is widespread in almost all around Mexico. This variety of hawthorn has been the subject of little clinical studies for its medicinal properties and benefits (Arrieta et al., 2010; Andrade-Cetto and Heinrich, 2005) and only one study has been conducted to determine its phytochemical composition in their flowers (García-Mateos et al., 2012) showing the presence of polyphenols such as quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-rhamnosyl-(1→2)-[rhamnosyl-(1→6)]-glucoside, and quercetin 3-*O*-rhamnosyl-(1→6)-glucoside (rutin) (Figure 1).

In a previous study, we have demonstrated the antioxidant capacity of the hawthorn through lipid peroxidation inhibition in *in vitro* rat brain cells (Méndez-Iturbide et al., 2013). In this context to gain insight into the potential interaction of erythrocytes with the antioxidants found in Mexican fruits the present work aimed to assess the antioxidant activity of the acetone extract derived from the hawthorn peel, to inhibit lipid peroxidation and morphology changes in the red blood cell membrane during the life span of the erythrocyte *in vitro* experiments. The purpose of the study was to test the hypothesis that lipid peroxidation on the erythrocyt cell membrane is inhibited by HPE from *C. mexicana*.

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## MATERIALS AND METHODS

For TBARS analysis, the following reagents were purchased from Sigma-Aldrich Co (St Louis, MO, USA): Polyphenol (quercetin), ferrous sulphate ($FeSO_4$), NaCl, NaOH, KCl, $NaHPO_4$, $KH_2PO_4$, trichloroacetic acid, ethylenediaminetetraacetic acid (EDTA), and 2-thiobarbituric acid (TBA).

For Lowry protein concentration, the following reagents were acquired: Bovine Serum Albumin (BSA) [A-7906], Folin–Ciocalteu reagent, phosphate buffer saline (PBS) (9.5 mM, pH 7.4), $Na_2CO_3$, 2%; NaOH, 0.1 N; tetrahydrate potassium sodium tartrate 2% [$KNaC_4H_4O_6{\cdot}4H_2O$] and $CuSO_4$, 1% (Sigma-Aldrich Co. St Louis, MO, USA).

For scavenging effect on free radicals, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Co. (St Louis, MO, USA).

To measure total phenolic compounds, Folin-Ciocalteu reagent, 0.3% HCl, sodium carbonate (2% $Na_2CO_3$), and gallic acid, were purchased from Sigma-Aldrich Co. (St Louis, MO, USA).

Cell staining was achieved by preparing 10% neutral buffered formalin, and Haematoxylin-Eosin (HE) stain solutions (Sigma-Aldrich Co. St. Louis, MO, USA), ethanol, xylene, and mounting resin were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA).

For SDS-PAGE, the following reagents were purchased: Sodium chloride, isobutyl alcohol, methanol, and acetic acid were acquired from J.T. Baker Chemical Company (Phillipsburg, NJ, USA). SDS, Tris, glycine, coomassie brilliant blue R-250, silver stain kit, TEMED, ammonium persulfate, N,N-1-methyl-bis-acrylamide, acrylamide and low molecular weight markers were from Bio-Rad (Richmond, CA, USA).

All other reagents including 2*d*-deionized water (18.3 MΩ, System Milli Q-II. Millipore, USA) were high quality analytical grade and pyrogenous free.

### Source of samples

Four kilograms of hawthorn fruits were gathered in the State of Tlaxcala, México, in the month of December 2013. All samples had the same morphological characteristics in size, color, and damage free of the surface. Only skin samples of hawthorn were studied. The fruits were hand washed and the skin was manually peeled off, frozen and lyophilized. A total of 36.0 g of peel was obtained and then mashed (mesh size 20). Samples were maintained frozen at -20°C until use.

### Preparation of hawthorn fruits peels extracts

Thirty grams of the resulting powder were homogenized at room temperature. Then, the homogenate was sequentially extracted for 72 h using 300 mL of the following solvents: *n*-hexane, dichloromethane, ethyl acetate, acetone, ethanol and finally methanol to obtain from the same homogenate one extract per solvent. All the extracts were decanted and evaporated until dry and kept inside a closed amber flask at 5°C until use. Only the acetone HPE was used for this study.

### Scavenging effect on DPPH

The scavenging effect of antioxidants in HPE on free radicals was determined by using DPPH (Kim et al., 2005). Briefly, 100 µL of HPE were prepared at a concentration of 20 mg/L in DMSO. Then, 4000, 400, and 40 mg/L final dilutions in cool ethanol were prepared. DPPH was dissolved in ethanol (1 mM). A total of 50 µL of HPE and 150 µL of DPPH (final concentration of 1000, 100, and 10 mg/L of HPE) were mixed and incubated for 30 min at 37°C in an orbital shaker. The absorbance at 517 nm was recorded using an Elisa microplate reader (Bio-Tek EL800, USA). The percent-reduced activity of DPPH was calculated and expressed as the $IC_{50}$ (Khattak et al., 2008) using six different concentrations of HPE (5.6 - 100 mg/L). Quercetin was used as standard.

### Determination of total phenolic compounds

The total phenol content of the acetone HPE was determined using the Folin-Ciocalteu reagent method (Folin and Ciocalteu, 1927) with minor modifications as described previously (Méndez-Iturbide et al., 2013). Briefly, 3 mg of HPE were added to 40 mL of methanol/water (50:50, v/v). The mixture was left to stand for 1 h at 25°C, and the supernatant was then collected and stored (aqueous methanol extract). The residual sample was added to 40 mL of acetone/water (70/30, v/v) and treated as described before. The aqueous methanol and acetone extracts were mixed, evaporated and re-dissolved in 10 mL of ethanol (aqueous ethanol extract). Finally, the residual sample was dissolved in 10 mL of $H_2O$ (aqueous extract). One mL of each aqueous and ethanol extract was brought to a volume of 2.5 mL using 0.3% HCl. Subsequently, 50 µL of either this sample or the standard quercetin was added to 1 mL of 2% $Na_2CO_3$, followed by the addition of 50 µL of Folin-Ciocalteu reagent diluted in water (1:1). After 30 min incubation at 25 °C, the absorbance at 750 nm was recorded using a spectrophotometer (Genesys 10 UV, Thermo Electron Co. USA). The total phenol content was expressed as milligrams of gallic acid equivalent per gram of HPE (GAE/g) using a standard curve (concentrations 0.2 - 1.0 mg/mL) for a freshly prepared solution of gallic acid.

### Subjects selection and clinical examination

This study included eight healthy males. Their average age was 32 years old (SD 11.1). Exclusion criteria included that none of them had any chronic disease such as diabetes mellitus, cardiovascular disease, anemia; they were not under drug treatment or having history of alcoholism and/or smoking, and neither excessive exercise, which induce oxidative stress. The study was explained to all subjects, and their consent signature was taken on a voluntary bases. Also, this study was approved by the Institutional Ethical Committee for Human Research, Faculty of Health Sciences and Nutrition in the State University of Tlaxcala. Body mass index (BMI) was obtained by accurately measuring height and weight of each individual (BMI = weight [kg] / height [$m^2$]).

### Blood sample collection and preparation

Seven mL of fasting venous blood sample was collected at 10:00 am from each subject under aseptic conditions, in sterile EDTA-vacutainer tubes (Becton Dickinson Co. NJ, USA). RBCs and serum were separated immediately by centrifugation at 3000 *g* for 10 min at 25 °C. Hematocrit and total hemoglobin were determined in each individual blood sample. After centrifugation, RBCs were washed and centrifuged 3 times in PBS [0.1 M (0.2 g KCl, 0.2 g $KH_2PO_4$, 8 g NaCl, and 2.16 g $NaHPO_4{\cdot}7H_2O$)/L), pH 7.4 (with 10% NaOH] and white cells were pipetted out and discharged. RBCs samples were stored in PBS as the initial hematocrit conditions (1:1) and used for all experiments. All samples were prepared and analyzed the same day of collection, unless otherwise specified.

### Ghost cells (GC) preparation

Four mL of separate RBCs in PBS were used to prepare hemoglobin-free erythrocyte membranes according to a hypotonic-

hypertonic lysis procedure (Matteucci et al., 1995; Krishnaveni et al., 2013; Jangde, 2011).

**Lipid peroxidation induced by $FeSO_4$ in RBCs and GC (TBARS)**

Antioxidant activity from the HPE was estimated by using the spectrophotometric method described by Ohkawa et al. (1979). Briefly, extract samples at 10, 30, 50, 100, and 1000 mg/L concentration were prepared in order to inhibit lipid peroxidation induced by $Fe^{2+}$ in RBCs and GC.

Ghost cells and RBCs were washed and homogenized separately in 10 mL of PBS (pH 7.4) at 5°C (1:10 w/v) (Dominguez et al., 2005). The homogenate was centrifuged for 10 min at 3000 g, and the supernatant was adjusted at 2.66 mg of protein/mL in PBS to be used for total protein determination according to the method of Lowry et al. (1951). BSA was used to elaborate the standard curve. Then 375 µL (1 mg protein content) of RBCs or GCs were previously incubated in an orbital shaker with 50 µL of EDTA 10 µM, and 50 µL of each one of the different extract concentrations of HPE at 37 °C for 30 min for a working dilution concentrations of 3-50 mg/L. Lipid peroxidation was initiated by the addition of 50 µL of $FeSO_4$ (final concentration = 25 µM) (Ng et al., 2000). After 1 h, samples were cooled on ice, and 0.5 mL of TBA (1% 2-thiobarbituric acid in 0.05 N NaOH and 30% trichloroacetic acid, 1:1) was added. Samples were centrifuged 10 min at 3000 g. Lipid peroxidation and their final products in RBCs and ghost cells, were assayed by malondialdehyde (MDA) determination using TBARS assay method (Kibanova et al., 2009). Samples were heated at 94 °C for 30 min in a boiling water bath. After cooling on ice, 200 µL of each sample and a blank sample, were placed in an Elisa microplate reader and the absorbance was recorded at 540 nm. TBARS results were expressed as the equivalent of MDA, compared with 1,1,3,3-tetramethoxipropane as the standard. The percentage of inhibition is defined as the decrease of TBARS formation, due to the extract quelation (Esterbauer and Cheeseman, 1990). HPE was evaluated to determine the minimal concentration (µg/mL) to inhibit lipid peroxidation up to 50% ($IC_{50}$ µg/mL).

**Lipid peroxidation induced by $FeSO_4$ in RBCs to morphologic analysis**

HPE was diluted at 5, 10, 100, and 1000 mg/L in PBS with the pro-oxidant $FeSO_4$ (100 µM). Then, 25 µL (~ 125 x $10^6$ cells) of RBCs were added in each dilution and incubated at 25 °C during 5, 30, 120, 180 min, 1, 2, 15, 22, 24 and 28 days. The samples without $FeSO_4$ or HPE were prepared as control blanks. Experiments were carried out in triplicate in RBCs.

**Cytological analysis**

RBCs samples (2 □L = ~10 x $10^6$ cells) were taken from different incubation times, and they were extended on a glass slide and immediately fixed with 10% formaldehyde buffer. Cells were stained with HE and a glass cover was mounted for microscope analysis at a magnification of 40x, and 100x using an oil immersion objective. An Olympus CH30RF100 microscope (Olympus Optical Co. LTD, Japan) with a digital camera connected at one PC and an image analysis software system (Motic Images Plus 2.0, Motic China Group Co. LTD) was used to quantify static and kinetic RCBs and the morphology changes.

**Histometrical measurements**

Histometrical measurements were performed at a magnification of 40x and 100x. The examiner was blinded to which group each specimen belonged to. RCBs were counted in three standard areas of each slide (0.1 mm x 0.1 mm each). In each area three different squares of 70 µm were selected to cell counting. Intensity of colour stain (Anisochromy), size (Anisocytic), shape (Poikilocytic), inclusions and other morphological changes in RBCs were determined as described by Mackenzie (2000) and Brecher and Bessis (1972) as follow:

| | |
|---|---|
| HE Stain: | Normochromic |
| | Hypochromic |
| | Hyperchromic |
| Size: | Normocytic (7 – 8 µm) |
| | Microcytic (less than 7 µm) |
| | Macrocytic (more than 8 µm) |
| Shape: | Acanthocyte (crenated) |
| | Echinocyte |
| Inclusions: | Heinz bodies |
| Others: | Hemolysis |
| | Schistocyte |

**Dynamic flow RBCs assay**

The dynamic flow of RBCs was assayed using a flow channel (20 µm wide, 10 µm high, 5 mm long) cut on a glass microscope slide made in the laboratory and covered with a glass-cover slide to permit direct visualization during the entire flow study (Hillery et al., 1996). The experiments were performed at 25°C. A clean glass slide was put with a $5^o$ inclination on the microscope slide to permit continuous and free flow of RBCs and to avoid shear strength. Two microliters of the above mentioned 2 h-incubated RBCs in PBS or HPE, were dropped on one extreme of the chamber and let to flow freely for 120 min. The number of adherent cells, crenation (acanthocytes and echinocytes), and deformability against time, were counted by direct microscopic visualization and a video image system (20 frames per second) providing multidirectional pictures of the erythrocytes. In order to avoid glass near deformation of RBCs or echinocytes formation (Brecher and Bessis, 1972; Bronkhorst et al., 1995), only cells flowing in the middle of the channel were analyzed.

**Polyacrylamide gel electrophoresis**

Protein determination and standardization of RBCs concentration was determined as described above by the method of Lowry. After washing with PBS, 25 µL of each incubated sample of RBCs were reconstituted under non-denaturing conditions in 20 µL of sample solubilizing buffer (0.125 M Tris-HCl, 4% SDS, 20% glycerol, pH 6.8, 0.002% bromophenol blue) and sonicated for 3 min prior to running the gels. 1 µL of each individual RBC sample (considering that in 1 µL of blood exist ~ 5 x $10^6$ red blood cells) were subjected to SDS-PAGE electrophoresis at room temperature in a discontinuous gel system according to the method described by Laemmli (1970), using a 3% stacking gel and a 10% separating gel. Molecular-weight standards were used in all gels. Electrophoresis was carried out at 100V constant current until the dye front of the gel reached the bottom of the chamber. A minigel apparatus (Mighty Small II vertical slab unit; Hoefer Scientific Ins., San Francisco CA., USA) was used for this purpose.

***Gel staining***

Gels were fixed and stained successively with 0.1% Coomassie brilliant blue R250 in methanol-water-acetic acid (1:8:1), and silver stain. To enhance and obtain a good pattern of staining bands, gels were initially stained with the solution of Coomassie blue for 1 h and distained gradually with several changes of distain solution

(methanol-water-acetic acid, 1:8:1) for approximately 60 min until the bands showed the appropriate stain.

***Identification of membrane proteins***

Proteins were identified according to their relative mobility in the gel and stain patterns following the criteria described by Ballas (1977), Mackenzie (2000), and Singh and Rajini (2008). Membrane and cytoskeletal molecules were scored according to the size and stain intensity of the bands as: absent (-), present (+), low intensity and size (-+), and high intensity and size (++). However, because of the subjectivity inherent in these parameters, protein bands were also scored only as present or absent.

**Statistical analysis**

***TBARS analysis***

Data in the text, tables and figures were expressed as means with standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to compare the antioxidant capacity of HPE in RBCs, GC, and quercetin/GCs.

***RBCs counting***

All data to count the RBCs were presented as mean and the standard error of the mean (SEM). The significance between RBCs control group and RBCs experimental group with HPE, was determined using two-way ANOVA repeated measures analysis. HPE was considered as the first variable, and the time as the second variable. A subsequent Bonferroni correction test was performed to determine difference between groups and baseline. A $P<0•05$ was considered to be statistically significant. In all cases, each experiment was run in triplicate within two control samples. The GraphPad Prism version program (*v*.5.01 for Windows) was used for this purpose.

## RESULTS

### Total phenolic compounds, free radical capture of DPPH and $IC_{50}$

A total of 2.2 g of HPE was obtained from 30 g of hawthorn dried skin. The total polyphenol content in HPE was found to be 0.68 (SD 0.001) mg of gallic acid/g. The amount of DPPH reduced was quantified measuring the decrease in absorbance of DPPH by the HPE. The $IC_{50}$ of the HPE to scavenging free radicals was 15.26 ± 0.20 mg/L as compared with the $IC_{50}$ of the pure antioxidant quercetin [5.37 (SD 0.14) mg/L]. It was shown that acetone extract is three times less active than quercetin in scavenging DPPH radical.

### MDA determination in RBCs and ghost cells by TBARS

Red blood cells have both saturated and unsaturated lipids and fatty acids on its membrane, which are susceptible to be peroxidized by the ROS. Malondialdehyde (MDA) is one of the final products of cell membrane lipoperoxidation. In order to determine the antioxidant capacity of hawthorn fruit peel extracts, $FeSO_4$ was used to induce the lipoperoxidation in RBCs and erythrocyte ghost. The ferrous ion ($Fe^{2+}$) is capable of forming lipid peroxides that induce the formation of highly reactive substances as hydroxyl radicals, perferryl and ferryl species.

To evaluate the effectiveness of hawthorn extracts to protect the RBCs and ghost cells from the lipoperoxidation, different concentrations of extracts were used as described in material and methods. The acetone HPE had a better performance to inhibit lipid peroxidation in RBCs (46.84%) and in ghost cells (47.91%) in a concentration of 50 mg/L, as compared with GC/quercetin (41.58%) (Figure 2).

### RBCs quantitation and morphological changes

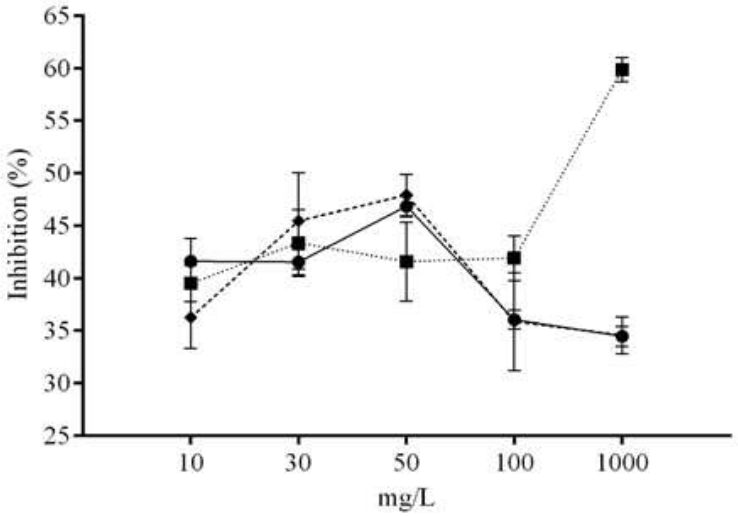
The body mass index and RBCs samples from 8 subjects were analyzed to determine their good health status to standardize experimental conditions. Means of BMI was 21.4 kg/m$^2$ (SD 5.2), hematocrit 0.49% (SD 0.05), and hemoglobin 12.5 mg/dL (SD 1.8). All experiments were run immediately after each blood collection.

RBCs were incubated in HPE with the prooxidant $FeSO_4$ at different concentrations and analyzed for morphological changes, eryptosis and hemolysis. The extent of hemolysis of RBCs in the different HPE concentrations, showed a time dependent concentration in the erythrocyte damages during the 28 days of incubation. RBCs with higher HPE concentration were less damaged as compared with a lower concentration of the extract as visually demonstrated by the presence of less hemoglobin accumulation in the incubation media (no quantified). This suggests a low-level of hemolysis and damage on the membranes of the erythrocytes, keeping the colour, and less cell adhesion in the erythrocyte-clot formation (Figure 3). These observations suggest an antioxidant effect of HPE in RBCs, and are similar to those found by Baek et al. (2012) that described the attenuation of cell lesion using haptoglobin therapy in guinea pigs red blood cells.

RBCs exposed to the different concentrations of HPE retained in greater or lesser extent their normal morphology and phenotypic characteristics during all the time of the experimental conditions. A concentration of 100 mg/L showed a better and repeatability pattern under the microscopic analysis. The hemolysis process was faster in untreated RBCs (Figure 4A to F) and started at day 7 of incubation, as compared with the RBCs treated with HPE (Figure 4G to L), where the hemolysis process started later. All morphological cell changes are described independently and also the sum of these changes.

***Normocytic and normochromic erythrocytes***

Microscopic counting of RBCs was similar in both cases, showing normocytic cells at the beginning of the




| | 10 mg/L | | 30 mg/L | | 50 mg/L | | 100 mg/L | | 1000 mg/L | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Inhibition % | SME | Inhibition % | SME | Inhibition % | SME | Inhibition % | SME | Inhibition % | SME |
| Total RBCs –●– | 41.59 | 2.15 | 41.52 | 1.22 | 46.84 | 1.01 | 36.03 | 0.91 | 34.45 | 0.95 |
| Ghost cells –♦– | 36.25 | 2.94 | 44.45 | 4.59 | 47.91 | 1.99 | 35.86 | 4.66 | 34.56 | 1.75 |
| Quercetin –■– | 39.50 | 1.74 | 43.35 | 3.20 | 41.58 | 3.75 | 41.91 | 2.13 | 59.84 | 1.16 |

**Figure 2.** Increasing concentrations of HPE from *Crataegus mexicana* on the production of TBARS in RBCs, and ghost cells. Data is the percent of inhibition and the SME from nine different experiments; each one by triplicate. Statistical differences among groups were at *P*<0•0001.

experiment incubated a non-incubated with the HPE. The counting of cells was easier in treated RBCs than in untreated RBCs, because the cytological smear in untreated RBCs showed large amounts of aggregated cells that was made difficult to separate each cell from the other.

Smear from treated RBCs with HPE was homogeneous and the cells showed a better separation among them; this could be due to the antioxidant activity since the first moment that the cells were incubated in the extract. Treated RBCs were longer hydrated, that suggests no loss of water inside cells by keeping the cell membrane intact for longer periods of time. Evident morphologic changes and hemolysis of RBCs began at 180 min either treated and untreated erythrocytes with HPE, and hemolysis started at 48 h in untreated RBCs (Figure 5a). By contrast, the beginning of hemolysis in RBCs treated with HPE started later than in untreated cells, maintaining the morphology for 28 days (Figure 4K to L). Total RBCs quantitation showed the effect of HPE (*P*<0•01), during time of incubation in min and days (*P*<0•001). A comparable dose-response relationship was observed in RBCs at different time points of incubation.

### *Anisocytic RBCs*

An increased number of microcytic erythrocytes was observed during the first 120 min in both incubated PBS/$FeSO_4$ cells and in PBS/$FeSO_4$/HPE cells. Following this microcytic formation, a decrease in these cell number was observed until they became lysed. However, the cells treated with the HPE remains up to for 28 days. This suggests that the crenated cell formation and the eryptosis process is retarded with the HPE extract (Figure 5b). No effect in the formation of microcytic cells was observed by the treatment (*P*<0•05), however the time of incubation (*P*<0•0001) and interaction (*P*<0•005) induce microcytic formation.

### *Crenated, acanthocytes*

The oxidation of the main structural proteins spectrin and actin that form the submembrane cytoskeletal meshwork of RBCs, induced morphological and viscoelastic changes in the cell membrane that were observed as blebs formation on the surface of the cells (Figure 4B to C, and 5c). RBCs with the HPE showed an early inhibition in the formation of these continuous bebbling oxidative phenomenon, thus also inhibited the eryptosis and hemolysis process in the life span of the erythrocyte (Figure 4I to L, and 5c) as compared with the RBCs from the control group. These data suggest that the acetone extract is acting to reduce membrane cytoskeletal deformation caused by the $FeSO_4$ oxidative effect on the lipoperoxidation of phospholipid on the membrane bilayer, and besides inhibit the oxidation of amino acid

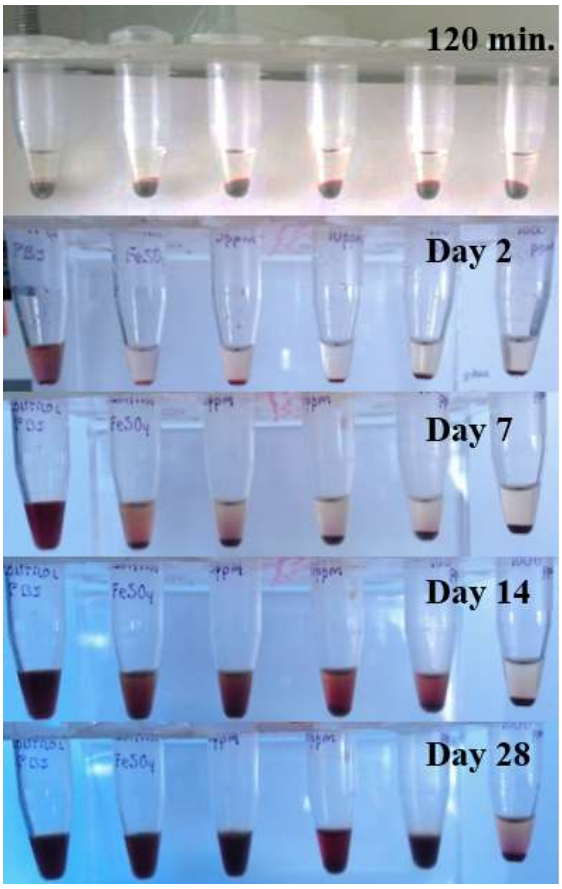



**Figure 3.** Effect by increasing concentrations of HPE from *Crataegus mexicana* in the lesion of RBCs at different times of incubation (left to right): RBCs/PBS, RBCs/PBS/$FeSO_4$, RBCs/PBS//$FeSO_4$/ with 5, 10, 100, and 1000 mg/L HPE respectively. Samples showed less hemoglobin accumulation in the incubation media at ongoing time of incubation and increase HPE concentration.

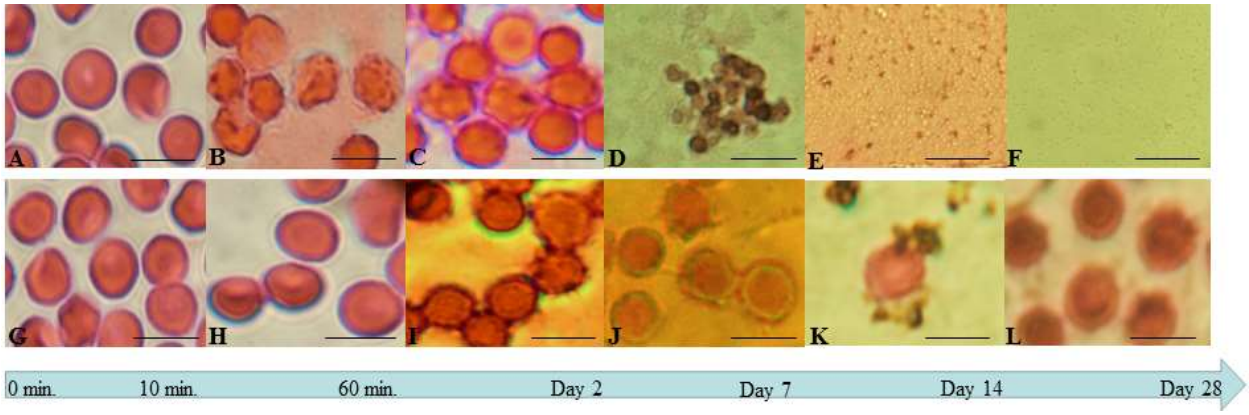



**Figure 4.** Effects of HPE on the contour and morphology of erythrocytes. RBCs incubated only with PBS (upper) and RBCs incubated in 100 mg/L of HPE (lower) at different time, both containing $FeSO_4$ 100 μM. Cell morphology was examined by light microscopy. Panel A-F RBCs incubated in PBS/$FeSO_4$; Panel G-L RBCs incubated in PBS/$FeSO_4$/100 mg/L of HPE. Incubation time is indicated. Further experimental details are given in material and methods. HE 100x, using different microscopy filters, Bar = 10 μm.

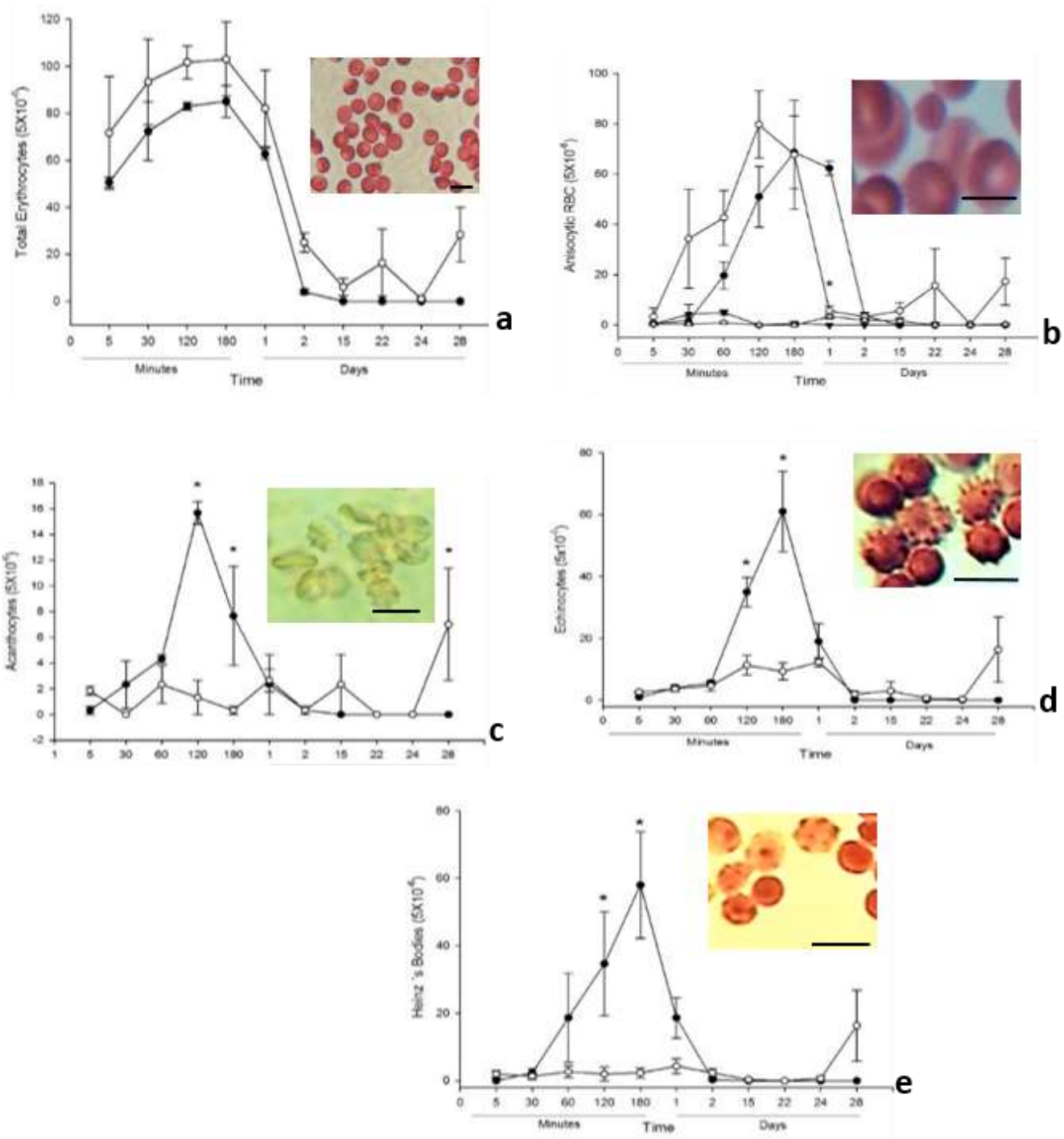



**Figure 5** (**a**) Comparative quantitation between total RBCs in PBS/$FeSO_4$ (•), and RBC in PBS/$FeSO_4$/HPE (o), and time. Insert: Light microscopy of normocytic and normochromic RBCs at 5 min. HE 40x, bar = 10 µm. (**b**) Interaction between microcytic RBCs in PBS/$FeSO_4$ (•), and microcytic RBCs in PBS/$FeSO_4$/HPE (o), and time. Also macrocytic RBCs in PBS/$FeSO_4$ (▼), and macrocytic RBCs in PBS/$FeSO_4$/HPE (⌂). Insert: Light microscopy of a microcyte at 180 min. HE 100x, bar = 10 µm. (**c**) Interaction between RBCs in PBS/$FeSO_4$ (•), and RBC in PBS/$FeSO_4$/HPE (o), and time. Comparing the presence and absence of acanthocytes. Insert: Light microscopy of acanthocytes. Bebbling formation is observed at 120 min. *In vitro* 100x, bar = 10 µm. (**d**) Interaction between RBC in PBS/$FeSO_4$ (•), and RBC in PBS/$FeSO_4$/HPE (o), and time. Comparing the presence and absence of echinocytes. Insert: Light microscopy of echinocytes. Spiny form structures are observed on the surface of the cell membrane at 180 min. HE 100x, bar = 10 µm. (**e**) Interaction between RBC in PBS/FeSO4 (o), and RBC in PBS/FeSO4/HPE (•), and time. Comparing the presence and absence of Heinz bodies at different time points. Insert: Light microscopy of RBCs. Heinz bodies are observed in the cells at 180 min. HE 40x, bar = 10 µm. values are Means and SEM of three different experiments; each one by triplicate. Statistical differences was with Bonferroni correction test*

residue side chains, formation of protein-protein cross-link, and oxidation of the cytoskeletal backbone of proteins in the erythrocyte. These morphological changes observed during the first 24 h reached the maximun peak at 120 min of the untreated red blood cells. A less formation of crenated cells is related to the HPE ($P$<0•01), time ($P$<0•0001), and interaction factors ($P$<0•0001). These morphological changes have been described as Type I and II (Brecher and Bessis, 1972) in the eryptosis process of the RBCs. As we already mentioned, HPE-treated RBCs did not increase these changes in a time dependent manner.

### *Crenated, echinocytes*

As the time increased the morphology changes in RBCs became more evident. The oxidative process and the crenation in RBCs induced the formation of cytoplasmic prolongations due the membrane deformation by cytoskeletal protein and lipid oxidation. These changes became non-reversible and were determinant in the eryptosis process of RBCs (Figure 4C, and 5d). The HPE from *C. mexicana* inhibited echinocytes formation following the time point in the acanthocytes formation, retarding cell deformation and the hemolysis process (Figure 4I – K, and 5d). A time dependent action in echinocyte formation ($P$<0•0001) and its interaction with the HPE ($P$<0•01) was observed. This morphology change in the cell membrane of the erythrocyte has been denominated as Type III (Brecher and Bessis, 1972) with many different forms in membrane deformation, and is considered as a non-reversible morphological alteration during the life span of the erythrocyte and therefore in the eryptosis process. The biological process in acanthocytes formation by radical-mediated damage in proteins and membrane, is similar in echynocites when they became $FeSO_4$ oxidized.

### *Heinz bodies*

The oxidation of hemoglobin produced in their primary structure disulphide cross-links between adjacent globin chains which become distorted and eventually lead to visible precipitates in the inner membrane of RBCs. HPE was able to inhibit the formation of these bodies from the beginning of *in vitro* experiments up to the day 24. RBCs without the acetone extract initiated the formation of Heinz bodies at min 30, increasing the formation in the next min and days. The increase in hemoglobin precipitation was associated by the distortion of the cell at the final phase of acanthocytes formation during the eryptosis process. This data suggest that HPE inhibited the oxidative process of hemoglobin for a longer period of time ($P$<0•0001), this interaction permits the extension of the life span of the cell ($P$<0•0001). Afterwards, the hemolysis process continued and began the formation of schistocytes. This changes last longer than the control took less time (Figure 4B, 4I, and 5e). group in which the Heinz body formation and hemolysis.

### *Schistocyte*

The schistocytes formation was observed on the first day of incubation, this is due to the deformation, bebbling formation and separation of liposomes from the surface of the cell membrane to be completely degraded (Figure 4D to E). However, schistocytes were observed in the RBCs incubated in HPE up to for 28 days, probably because the cells keeps their membrane for longer time as is deduced observing all the graphics in the different experiments (Figure 4K).

### Dynamic flow of RBCs assay

To critically evaluate the potential contribution of HPE to inhibit any cellular morphologic change by protein and lipid membrane oxidation, RBCs flow rate, adhesion, and morphologic changes were quantified from video recording of nine individual cells in the flow channel over a period of 180 min. RBCs incubated in HPE demonstrated a longer period of time of flowing through the flow channel up to for 180 min as compared with RBCs in PBS whose cells were dehydrated in less than 6 min. Morphologic changes such as acanthocytes and echinocytes formation were observed, and an increase of adhesion among cells was demonstrated in RBCs incubated only in PBS during the first 60 min as compared with RBCs incubated with the HPE (Figure 6A to J) without changes in the morphology up to 120 min. It is particularly notable in these experiments and suggest that RBCs maintain their morphologic and the micro-rheologic characteristics in the micromolar concentrations of HPE. This could be because there is not lipid or protein oxidation in the cell membrane, thus no dehydration and crenation, and therefore inhibition of cell deformity and viscosity and better flow of RBCs. However, life span of RBCs with or without the extract is going to continue its eryptosis process, thus the cell will lose their biological properties and finally will be cell lysed.

### Polyacrylamide gel electrophoresis

The banding pattern of some cytoskeletal proteins of RBCs were stained and identified in SDS-PAGE. Band staining intensity was observed during almost all time points of incubation of RBCs with the different concentrations of the hawthorn extract.

The qualitative analysis showed that some bands keep the stain intensity in relation to a major concentration of the HPE. In these experiment seven main bands were observed at day 1 of the experiments (Figure 7). The colour intensity and size of some bands were maintained over a longer period of time with increasing the extract concentration and suggest an antioxidant protective effect of the HPE on the cytoskeletal proteins of the RBCs. This data is related to what we observed in the

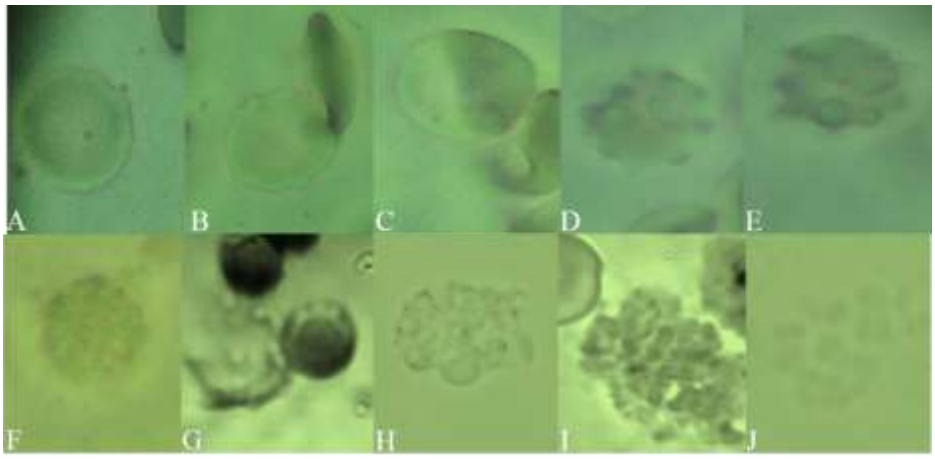



**Figure 6.** Morphologic changes of a representative RBC *in vitro* after exposure in HPE at 100 mg/L in a time lapse of 180 min in the flow channel. Eryptosis was retarded as compared with control RBCs. (A) normocyte; (B) beginning of Phase I; (C) beginning of crenation in phase II; (D) achantocyte and bebbling formation in phase II; (E) echinocyte in the beginning of phase III; (F, G, and H) beginning of vesicles formation and cell membrane disruption; (I) schistocytes formation, and (J) schistocytes and cell lysis.

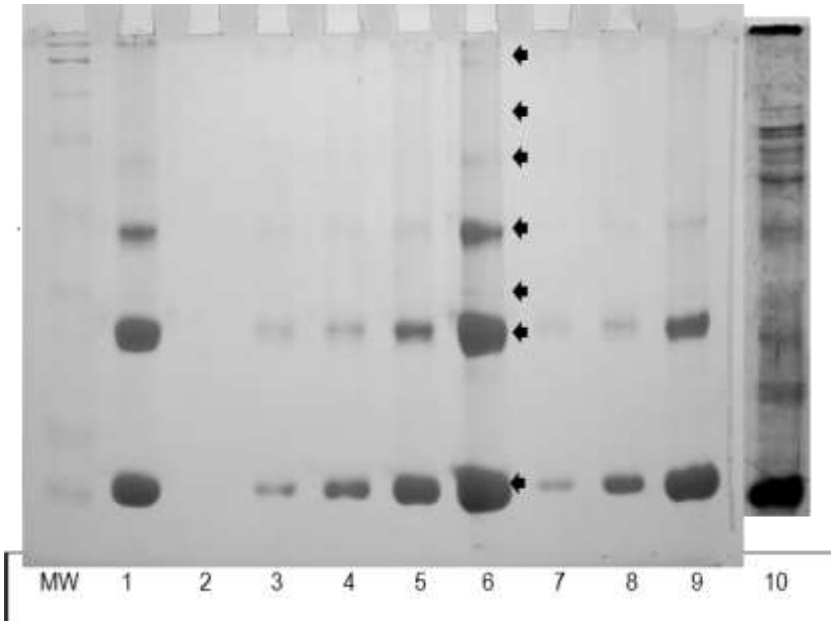



**Figure 7.** Determination of membrane proteins in RBCs in a representative SDS-PAGE stained with coomassie brilliant blue R250. Differences and variability in the molecular pattern (arrows) between different concentrations of HPE at day 7 (channels 1- control RBC, channel 2- PBS/$FeSO_4$, channel 3- RBC/HPE 10 mg/L, channel 4- RBC/HPE 30 mg/L, channel 5- RBC/HPE 50 mg/L, channel 6- RBC/HPE 100 mg/L), and day 14 (channel 7- RBC/HPE 10 mg/L, channel 8- RBC/HPE 30 mg/L, channel 9- (RBC/HSE 50 mg/L), channel 10- RBCs at day 1. One µL per channel according to material and methods. MW= molecular weight standards.

course of incubation, where the higher is the concentration, the lesser is the lesion in RBCs. This supports the fact that the cytoskeleton is protected against the oxidative process induced by the $FeSO_4$, therefore the cells will keep their morphology and rheology characteristics over a longer period of time.

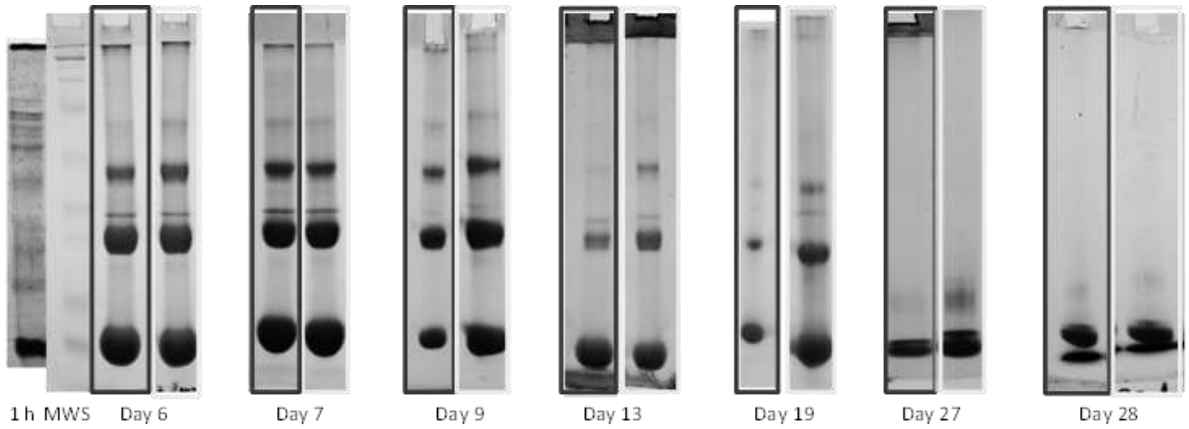



**Figure 8**. Band pattern of cytoskeletal proteins in RBCs at different days of incubation in the HPE from *Crataegus mexicana*, all lines at 100 mg/L. The protective effect of the extract on $FeSO_4$-induced protein damage is observed at different time periods of incubation. RBCs incubated in PBS (black margin) shown a decrease in size and band intensity as the incubation time is longer. RBCs incubated in HPE (white margin) shown that some bands are kept for a longer period of time. 10% SDS-PAGE. Experiments were run as triplicate: 1 µL per channel according to material and methods. MW= molecular weight standards.

**Table 1.** Scored distribution of RBCs protein bands with and without HPE at different times.

| Molecule scored* | Time of incubation | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 h | | day 6 | | day 7 | | day 9 | | day 13 | | day 19 | | day 27 | | Day28 | |
| | C | E | C | E | C | E | C | E | C | E | C | E | C | E | C | E |
| band 1 | +++ | +++ | +++ | +++ | +++ | +++ | + | + | +- | +- | + | + | + | - | - | - |
| New band | | | | | | | | + | - | + | - | +- | - | - | - | - |
| band 2 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +- | + | - | +- | - | - | - | - |
| band 3 | +++ | +++ | +++ | +++ | +++ | +++ | + | +++ | +- | ++ | - | +- | - | - | - | - |
| band 4 | +++ | +++ | +++ | +++ | +++ | +++ | + | ++ | ++ | ++ | - | - | - | - | - | - |
| band 5 | +++ | +++ | +++ | +++ | +++ | +++ | ++ | +++ | +- | ++ | + | +++ | - | - | - | - |
| New band | | | | | | | | | + | + | - | +- | - | - | - | - |
| New band | | | | | | | | | | | | +- | - | - | - | - |
| band 6 | + | + | + | + | + | + | - | + | - | -+ | - | -+ | - | +- | - | - |
| New band | | | | | | | | | | | | | +- | + | +- | + |
| band 7 | +++ | +++ | +++ | +++ | +++ | +++ | ++ | +++ | +++ | +++ | ++ | +++ | ++ | ++ | ++ | ++ |
| New band | | | | | | | | | | | | | ++ | ++ | ++ | ++ |

Values are the number of bands in RBCs indicated in each case. * Molecule scored according to the size and stain intensity as described in material and methods. C = control RBCs/PBS; E = RBCs/HPE. n = 3.

The banding pattern was compared between RBCs in PBS, and RBCs in HPE in an attempt to determine time dependent antioxidant protective effect on the cytoskeleton proteins. Meanwhile the banding pattern of some protein bands were observed in some cases up to the day 28 when the RBCs are incubated with HPE, the proteins in the control group were degraded, and some of them in a time point of incubation were absent (Figure 8). Because the band pattern was constant in each different concentration, it has been only shown the band pattern with HPE in a concentration at 100 mg/L. These observations were scored (Table 1).

Singh and Rajini (2008), in a similar experiment showed the antioxidant protective activity against ferrous- ascorbate using potato peel extract in different concentrations, but they did not determine time dependent changes during long periods of time. This makes clear that incubating RBCs in extracts containing polyphenols improve the antioxidant effect on the cytoskeletal proteins. Many pro-oxidants such as $H_2O_2$, $Fe^{2+}$-ascorbate and AAPH are capable to induce alterations in the erythrocyte membrane and a decrease in cytoskeletal proteins that lead a series of changes in the morphology and rheology properties of the cells (Singh and Rajini, 2008; Battistelli

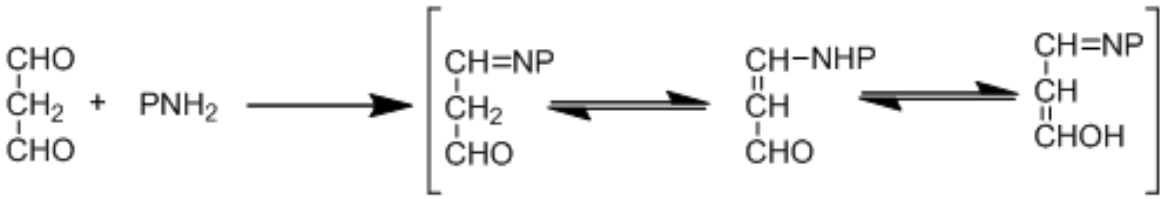



**Figure 9.** Formation of protein carbonyls by reaction of protein amino groups ($PNH_2$) with peroxidation products (MDA) of polyunsaturated fatty acids (PUFA).

et al., 2005; Srour et al., 2000). In these experiments, the use of the pro-oxidant $FeSO_4$ is responsible of the oxidative attack inducing this changes and subsequent non-enzymatic protein fragmentation and membrane deformation, but the HPE of *C. mexicana* is capable of protecting the RBCs of the oxidative process reducing the morphologic changes and retarding the hemolysis in the eryptosis process.

## DISCUSSION

The most abundant cells in the blood are the RBCs with a 45% of its total volume, with an average life span of 120 days, and upon which main function is transportation of $O_2$ and $CO_2$. During their life span RBCs are exposed to many stressful situations through the capillaries, and suffering osmotic, energetic, and oxidative stress. Therefore, the high concentration of oxygen and haemoglobin promote the oxidative process and impaired RBCs functions and damage in the cell. The oxidative stress process is catalysed by $Fe^{2+}$, via the Fenton reaction to produce hydroxyl-radical; a process that has been well documented (Cheng and Li, 2007). The oxidation of the polypeptide backbone is $O_2^{\cdot-}$ dependent; is caused by the $^{\cdot}OH$-dependent abstraction of the α-hydrogen atom of an amino acid residue, this results in the formation of a carbon-centered radical that react with $O_2$ in a series of molecular reactions, forming alkoxyl radicals and aldehydes, the last one may participate in Schiff-base formation and Michael addition (Dean et al., 1997). Many of the steps in this pathway that are mediated by interactions with $HO_2^{\cdot}$ can also be catalysed also by $Fe^{2+}$. The intermediate radicals may undergo side chain reactions with other amino acid residues in the same or a different protein molecule to generate a new carbon-centered radical and the cleavage of the peptide bond of proteins. In addition, carbonyl groups may be introduced into proteins by reactions with aldehydes (MDA) produced during lipid peroxidation (Figure 9).

This carbonyl groups and MDA are associated with aging, oxidative stress, many chronic diseases, and human red blood cells (Berlett and Stadtman, 1997). Therefore, these oxidized products in the cell proteins of RBCs, can contribute in many morphologic changes and reduced life span of the erythrocyte. An increase in oxidation raises hydrophobicity, while a limited oxidation increases hydrophilicity. This change in turn produces inner and outer rheology alterations on the cell bilayer membrane, and such responses will further heighten the eryptosis process in RBCs.

Hawthorn is a source of natural polyphenol antioxidants (García-Mateos et al., 2012). Acetone extract from hawthorn *C. mexicana* inhibited oxidative stress induced by the pro-oxidant $FeSO_4$ in RBCs. It was demonstrated and confirmed by TBARS analysis, evidences in cell morphology, and protein electrophoretic pattern using different concentrations. Our results by TBARS analysis showed a relationship between low doses of HPE to inhibit MDA formation induced by $FeSO_4$ in the RBCs. These results are similar to those demonstrated in previous studies using homogenate from rat brain cells.

$FeSO_4$-induced lesion of the cell membrane; hemolysis and morphologic changes in the RBCs were inhibited in a concentration-dependent manner in the presence of HPE, and the life span of the erythrocytes were extended in the RBCs samples that contained the acetone extract. The morphologic changes associated to the eryptosis process were retarded during the time of the experiments. It was observed that the crenated cells, acanthocytes, and echinocyte were present for longer periods of time, before the hemolysis process and/or schistocyte formation took place. This protection supplied by the hawthorn extract was observed during the first 24 h of the experiment, as compared with the control group, where the eryptosis and life span of RBCs are reduced in a significant period of time. Zhu et al. (2002), determined the antioxidant protective effect of the cocoa feeding in rats, to inhibit erythrocyte hemolysis up to for 240 min in a dose dependent manner, suggesting that some polyphenols in HPE have the capacity of quenching free radicals in the cell membrane of the erythrocyte. Our study also showed a longer period of time on the inhibition of $FeSO_4$-induced hemolysis and morphologic changes in the erythrocytes, in a concentration-dependent manner up to for 28 days. This results support the fact that polyphenols in hawthorn fruits improve the antioxidant protective effects in RBCs, and may be working in an interactive way on their lipid bilayer of the cells.

Changes in the number, relative mobility and staining intensity of the protein bands observed in the electrophoretic gels, indicate that cytoskeletal proteins are susceptible to oxidation due the pro-oxidant $FeSO_4$ and during life span of the erythrocyte under the experimental condition. Furthermore, RBCs samples with the different hawthorn extract concentrations showed that many proteins bands are maintained during different times of the experiment and, also with a higher concentration of the extract the band pattern staining of some proteins was more intense. This may indicate that the extract contribute in some way in the inhibition of the protein oxidation for a longer and extended period of time, probably by protecting the –SH protein groups against oxidation. Also, it can inhibit the ability of proteases to degrade the oxidized forms of other proteins, and therefore to increase cellular resistance to oxidative stress (Asgary, et al., 2005). The delay time for protein oxidation and polymerization has a relationship on the stability and morphology of the erythrocytes. The lipid bilayer membrane and protein stability should be related to antioxidants concentration present in the HPE of *C. mexicana*, reducing cell dehydration, K loss and other osmotic cell changes (Wandersee et al., 2005). This protection in the cytoskeletal proteins is related to better form and shape of the erythrocyte as compared with those RBCs without the extract of *C. mexicana*. The observed reduction in the number and intensity of the band pattern could be related to increased proteolysis or instability of the absent bands, but is also unlikely in view of the finding that results were similar in all the experiments in RBCs comparing with or without the acetone extract. The pathophysiology of this changes, remains to be determined using specific and purified antioxidants.

The deformability of the red blood cells is an important factor in the rheology of the blood (Bronkhorst et al., 1995; Dupire et al., 2012), and it is related to many important cell properties, flow rate and blood diseases. The deformability is the result of several mechanical and geometrical properties of the RBCs, and is determinant in the stability of the membrane lipids and cytoskeletal proteins of the cell. Consequent disruption of the membrane-cytoskeleton by any oxidative process may be responsible for the bebbling formation in RBCs (Spangler, 2011; Liu et al., 2012). Thus, hydrodynamic stress and oxidation of proteins and phospholipids can also induce morphological and rheological changes of the cell. HPE of *C. mexicana*, was able to inhibit oxidative stress maintaining the structural and morphologic integrity of the cell, with less changes and damage in the morphology of the erythrocyte under the experimental conditions. HPE reduce the eryptosis process, retarding the formation of crenated cells and increasing the flow rate of the erythrocyte, contributing to the cell motion and avoid energetically a costly oxidation and deformation, to keep the cell elasticity and shape. This could be possible by the presence of antioxidants metabolites in the acetone HPE. This capacity of the hawthorn may be used to avoid erythrocyte adhesion and vasoocclusion in the blood stream in many blood diseases, and to inhibit peroxidation of biomembranes that can induce damage during ischemia/reperfusion, inflammation and aging.

In spite of this, is well known that echinocytes are formed after 3 to 4 days in whole blood kept at $4^o$ C and within 24 h in blood incubated at $37^o$ C (Brecher and Bessis, 1972), but with the acetone extract of *C. mexicana* the crenated cells, acanthocytes and echinocytes, were formed later compared with the control group and the shape of the cell membrane was kept for 28 days, showing inhibition during the time of the experiment of these morphological changes. Thus, may be, the oxidative process in the cells is inhibited by the antioxidants in the extracts, that maintain the morphology of the cell and its life span for a longer time, reducing the eryptosis process.

The attenuation of RBCs lesion was demonstrated in our study, and this is similar to those reported by Baek et al. (2012) in guinea pigs by haptoglobin therapy. However, the hawthorn extract is a natural product than can be consumed regularly by the human being in healthy and disease conditions. Wautier et al. (1981) mentioned the increased adhesion of erythrocytes to endothelial cells in diabetes mellitus and its relation to vascular complications, therefore, the nutraceutical application of hawthorn could inhibit the adhesion by keeping the RBC membrane from being oxidized by free radicals.

These results are in broad agreement with the published reports regarding anti-oxidant capacity of other fruits and vegetables extracts, such as potato peel extracts, cocoa extracts, and flaxseed oil. A large body of evidence implicates oxidative stress in the pathogenesis of red blood cell diseases, affecting their life span, shortening the eryptosis process, and increasing the hemolysis process. This process might also result in a systemic inflammatory response. Therefore, we favor the hypothesis that the observed protective effects on the morphology and life span of RBCs is due to the presence and collective action of the antioxidant molecules present in the acetone HPE. These results are related to the use of hawthorn in Mexico and other countries since ancient times in order to treat blood and other related systemic diseases. However many of this complex dynamics and interactions remain unexplored experimentally.

There is a need to identify more effective and novel compounds of fruits according to food consumption in traditional diets like the Mexican one, as well as how theirs constituents are acting against oxidative stress in cells, tissues and, in human diseases. The broad range of fruits and theirs parts to inhibit and remove ROS and intermediate radicals in many forms encourage continuing the purification of antioxidants in Mexican fruits and determining their mechanisms of action in lipids and proteins in red blood cells.

## Conclusions

This study provides the first *in vitro* evidence for an active role of *C. mexicana* as antioxidant on erythrocytes. Hawthorn is a fruit with anti-oxidant potential, reduces morphological changes and extends life span of the red blood cells *in vitro*. Such protection could be due to the inhibition of membrane lipoperoxidation and oxidative damage in cytoskeletal proteins. The conjunction of antioxidants in this extract, and not just specific purified antioxidants, could be helpful to keep normal the rheology properties of the erythrocyte apparently without any pro-oxidative damage.

The antioxidants present in the acetone extract may contribute to protect the erythrocyte cell membrane against lipid peroxidation induced by oxidative stress, thereby participating in diminishing the morphological and many rheology changes that may happen in the pathogenesis of vasoocclusive diseases, such diabetes mellitus, metabolic syndrome, different types of anemia, and the inhibition of cell storage lesion in RBCs.

More studies are required to correctly identify the bioactive compounds of *C. mexicana* and how it contribute to the erythrocyte protection, and their mechanisms of action in the protection of the RBCs. This study give the scientific biochemical bases to use hawthorn extracts in a clinical setting, to prevent or treat many diseases and conditions associated to oxidative stress.

## Conflict of interests

The authors did not declare any conflict of interest.

## ACKNOWLEDGMENTS

This study was supported by grant 105663 from the Mexican Council of Science and Technology (CONACYT). We also acknowledge CONACYT for providing the financial support (54150) to José Antonio Banderas Tarabay during his Ph.D. studies.

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academicJournals

Vol. 9(4), pp. 223-229, April, 2015
DOI: 10.5897/AJFS2014.1220
Article Number: DA2726C51603
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Particulating broiler feeds into forms and sizes for nutritional and economic benefits (part 1)

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Received 20 October, 2014; Accepted 26 February, 2015

**A study was conducted to examine the nutritional and economic effects of using mash, crumbs and pellet diets for broiler starter birds using Arbor Acre broilers chickens. The study examines the starter phase (0-28 days) of 288 Arbor Acre broiler chickens fed mash, 2 mm pellet size diet and feed crumbs. All diets had identical nutrients composition and the study was conducted under similar environmental conditions and management practices. Data on growth performance, nitrogen utilization, carcass and visceral organs characteristics, hematology and serum biochemistry were collected and evaluated. Economic analyses were also conducted. Early growth performances were more evidently manifested (P<0.05) in chicks fed 2 mm pellet diets where the weight gain was highest at 46.93±4.19 g/bird/day and the feed conversion ratio (FCR) was also lowest for the same set of birds at 1.85±0.14. Most other examined organs and carcasses indicated better and significant values for chicks on 2 mm pellet diet. These diets also enhanced most carcass and organs characteristics. Hematological and blood biochemistry indices were not adversely affected. There was an overall better net return per bird for birds on 2 mm diets in broiler starter.**

**Key words:** Pellet feeds, Arbor Acre, performance, biometry.

## INTRODUCTION

Successful broiler development is dependent on optimal feed intake throughout the growing period. Optimal feed intake is dependent on a number of factors such as environmental temperature and diet nutrient density. The physical feed quality is considered to have a very significant impact on broiler growth (Jafarnejad et al., 2010). Growth is emphatically dependent upon feed intake, which in turn is influenced by feed form. The most logical reasons for pelleting are that the heat generated in conditioning and pelleting makes the feedstuffs more digestible by breaking down the starches (Ghazi et al., 2012). By combining moisture, heat and pressure on feed ingredients, a degree of gelatinization is produced which allows animals and poultry to better utilize the nutrients in these ingredients. Reasons for the enhanced performance may be due to increased digestibility, decreased ingredient segregation, reduction of energy during prehension, and increased palatability (McKinney and

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Teeter, 2004). Pelleting of feed also provides the benefits of increasing the bulk density of feed; improving feed flow ability; and providing opportunities to reduce feed formula costs through the use of alternative feed ingredients (Ghazi et al., 2012).

However, disadvantages associated with pelleting feeds include the increase in the cost of the feed due to the pelleting process; the excessive heat generated during the pelleting process may decrease the availability of the amino acids such as lysine and destroy some vitamins.

Although several studies have investigated and reported the effect of feeding different forms of feed (mash, pellet and crumble) on the productive performance of broiler, there has been a dearth of reports on the actual comparison of broiler performances on three diets of equal nutrient compositions but with varying physical types and forms. The present study has been carried out for comparison among broiler birds raised on the three feed types of equal nutrient compositions (mash, pellet and crumble feeds) using their growth rate, feed efficiency and other productive characters and to determine the most economic and suitable forms of feed in view of the productive performance of broilers.

## MATERIALS AND METHODS

### Experimental site and preparation

The study was carried out at the Poultry Research Unit of the Teaching and Research Farm of Ekiti State University Ado-Ekiti. The experimental site was properly cleaned and disinfected.

### Experimental design

The experiment was carried out using the completely randomized design. The experiment had 3 dietary treatments with 4 replicates consisting of 20 birds each making a total of 288 birds in this study. There were three experimental diets in this experiment. Diet 1 (mash feed) was the control diet. Diet 2 consisted of 2 mm pellet feed while diet 3 consisted of crumbled feed.

### Experimental animals and management

A total of 288 Arbor Acre day old chicks were sexed using the method described by Laseinde and Oluyemi (1997) for the first experiment. The chicks were randomly selected into each treatment such that equal numbers of male and female (equal sex ratio) of uniform batch weights make up each of the three treatments. The birds were raised on conventional deep litter system, where they were supplied with feed and clean water *ad-libitum*. The following veterinary routines were observed from day old:

i. Intraocular vaccination against Newcastle disease at day one.
ii. Neoceryl (Antibiotics) for a period of 4 days from 3 days of age.
iii. Coccidiostat for the treatment/control of coccidiosis and chronic respiratory diseases.
iv. Gumboro vaccine at two weeks of age.
v. Lasota vaccine (New castle booster) administered in a day at the age of about three weeks.

### Experimental diets

Three forms of broiler starter diets were used at this phase of the experiment (mash, 2 mm pellet and crumbs). All experimental diets were isocaloric and isonitrogenous (Table 1) compounded with the same quantities of ingredients. Particle sized screen was achieved by using a hammer mill with small sizes of screen openings. The fine mash was prepared by mixing corn and soybean meal that was grounded through a screen, and then blended with all other ingredients to yield a final mash diet. Manufactured experimental feeds were made into pellets using diesel flat die feed pellet mill with customized sizes of 2.0/3.0/4.0/6.0/8.0 mm. Power rating is 8-55 HP and has a capacity of 70-1000 kg/h. Crumbling of pellet feed was achieved by a further coarse milling of the already pellet diet into crumbs.

### Data collection

The weights of the experimental birds were taken every 3 days to record the weight gain. Feed consumption was recorded on daily basis and these data were used to generate other data such as feed conversion ratio and protein efficiency ratio. Nitrogen digestibility trial was carried out for each study to determine the nitrogen retention and utilization. Carcass and relative organs characteristics were recorded after slaughter at the termination of the experiment. Haematological parameters such as haemoglobin concentration, HBC; packed cell volume, PCV; red blood cell, RBC; erythrocyte sedimentation rates, ESR; mean corpuscular volume, MCV; mean corpuscular haemoglobin, MCH and mean corpuscular haemoglobin concentration, MCHC were determined for the two studies. Biochemical components such as total serum protein, albumin, globulin and albumin/globulin ratio were also determined for the two studies. Cost benefit analysis was done using simple economic indices such as feed cost, cost of feed/intake of bird, total revenue and total net returns.

### Carcass, muscle and organ measurements

The measurement of the carcass traits (dressed weight %, eviscerated weight %, thigh, drumstick, shank, chest, back, neck, wing, belly fat and head) were taken before dissecting out the organs. The organs measured were the liver, kidneys, lungs, pancreas, heart, spleen, bursa of fabricus and gizzard. The following muscles: inner chest muscle (*Supracoracoideus*) outer chest muscle (*Pectoralis major*) and thigh (*Iliotibialis*) were carefully dissected out from their points of origin and insertion. All the carcass traits, except the dressed and eviscerated weights, were expressed as percentages of the live weight while the organs and muscles were expressed in g $kg^{-1}$ body weight, while the length and breadth of the muscles were expressed in cm $kg^{-1}$ body weight.

### Estimation of nitrogen retention, nitrogen digestibility and protein efficiency ratio

Total faeces voided during the last 5 days were collected, weighed, dried at 65-70°C in an air circulating oven for 72 h and preserved while the corresponding feed consumed was also recorded for nitrogen studies. The nitrogen contents of the samples were determined by the method of AOAC (2010) and nitrogen retained was calculated as the algebraic difference between feed nitrogen and fecal nitrogen (on dry matter basis) for the period.

### Blood sampling

Blood from two slaughtered birds from each treatment were allowed

**Table 1.** Composition of the experimental diets (0-28 days).

| Ingredient | Dietary treatments | | |
|---|---|---|---|
| | 1 (Mash) | 2 (Pellet) | 3 (Crumble) |
| Maize (9% CP) | 50.2 | 50.2 | 50.2 |
| Soya bean meal (45% CP) | 33.5 | 33.5 | 33.5 |
| Palm kernel cake (18.8% CP) | 10.0 | 10.0 | 10.0 |
| Fish meal (68% CP) | 2.0 | 2.0 | 2.0 |
| Bone meal | 2.50 | 2.50 | 2.50 |
| Oyster shell | 0.50 | 0.50 | 0.50 |
| Salt | 0.50 | 0.50 | 0.50 |
| DL-methionine | 0.15 | 0.15 | 0.15 |
| L-lysine | 0.15 | 0.15 | 0.15 |
| Premix | 0.50 | 0.50 | 0.50 |
| Total calculated: | 100 | 100 | 100 |
| Crude protein,% | 22.9 | 22.9 | 22.9 |
| Me (kcal/kg) | 2980.5 | 2980.5 | 2980.5 |
| Crude fibre,% | 3.3 | 3.3 | 3.3 |
| Ether extract,% Analysed: | 8.1 | 8.1 | 8.1 |
| Ash,% | 8.79 | 10.33 | 8.30 |
| Moisture content,% | 14.77 | 16.15 | 16.24 |
| Crude protein,% | 21.64 | 21.87 | 20.78 |
| Ether extract,% | 3.68 | 3.71 | 3.64 |
| Crude fibre,% | 3.61 | 3.59 | 3.66 |

% CP, percentage crude protein; broilers vitamin premix supplied the following vitamins and trace elements per kg of diet: vit A 7812.50IU; vit D 1562.50 IU; vit E 25 mg; vit K 1.25 mg; vit B1 1.88 mg; vit B2 3.44 mg; niacin 34.38 mg; calcium pantothenate 7.19 mg; vit B 36 .13 mg; vit B 102 0.016 mg; choline chloride 312.50 mg: folic acid 0.62 mg; biotin 0.05; Mn 75 mg; Fe 62.5 mg: Zn 50 mg; Cu 5.31 mg; iodine 0.94 mg; Co 0.19 mg; Se 0.07 mg and antioxidant 75 mg.

to flow freely into labeled bottles one of which contained a speck of EDTA while the other without EDTA was processed for serum. The serum was kept deep frozen prior to analysis. The packed cell volume (PCV%) was estimated in heparinized capillary tubes in an haematocrit micro centrifuge for 5 min while the total red blood cell (RBC) count was determined using normal saline as the diluting fluid. The haemoglobin concentration (Hbc) was estimated using cyanomethaemoglobin method while the mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and the mean corpuscular volume (MCV) were calculated.

#### Economic analysis

The cost of birds, feed and medication incurred during the two phases of the experiment were collected from the income and expenditure statements. Revenue generated from the sale of the birds was recorded. The cost per kilogram gain, total profit and returns to naira invested were also calculated.

#### Statistical analysis

All recorded and calculated data were statistically analyzed with the standard procedures of analysis of variance (One Way ANOVA) technique by a computer using Minitab statistical computer software package (2005 version). Results were expressed as mean ± standard deviation of two measurements.

## RESULTS

### Average growth performance

Performance characteristics of broiler chicks fed mash, 2 mm pellet diet and crumble diet are presented in Table 2. The feed intake (FI) value obtained for birds on mash diet (diet 1) had the highest value of 81.4 ± 0.29 g/bird/day and was significantly different ($P<0.05$) from the lowest value obtained for birds fed 2 mm pellet diet (diet 2) at 79.4± 0.92 g/bird/day. The highest weight gain value was recorded for birds placed on pellets (diet 2) at 46.9±4.17 g/bird/day although similar ($P>0.05$) to those on crumbles (diet 3).

The least and optimum feed conversion ratio was obtained for birds placed on pellets (diet 2) at 1.85±0.14 g/day and this valu-e was significantly different ($P<0.05$) from those placed on mash and crumbles. The highest and significantly different ($P<0.05$) protein efficiency ratio value of 2.69±0.2 was obtained for birds placed on pellets (diet 2).

**Table 2.** Average growth performance of broilers fed various feed forms (0-28 days).

| Parameter | Dietary treatment | | |
|---|---|---|---|
| | 1 (Control/mash) | 2 (Pellet) | 3 (Crumble) |
| Average daily feed intake (g/bird/day) | 81.4±0.29[a] | 79.4±0.92[b] | 80.8±0.76[ab] |
| Average daily weight gain (g/bird/day) | 37.5±3.44[a] | 46.9±4.19[b] | 40.8±0.99[ab] |
| Feed conversion ratio (FCR) | 2.35±0.08[a] | 1.85±0.14[b] | 2.15±0.08[a] |
| Protein efficiency ratio (PER) | 2.10±0.2[a] | 2.69±0.2[b] | 2.44±0.08[c] |

Means within a row with different superscript are significantly different (P<0.05).

**Table 3.** Nitrogen utilization of broilers fed various feed forms (0-28 days).

| Parameter | Dietary treatment | | |
|---|---|---|---|
| | 1 (Control/mash) | 2 (Pellet) | 3 (Crumble) |
| Nitrogen intake (g) | 3.43±0.04[a] | 3.46±0.3[b] | 3.28±0.34[a] |
| Faecal nitrogen (g) | 0.92±0.01[a] | 1.39±0.1[a] | 1.14±0.2[a] |
| Nitrogen retention (g) | 0.62±0.34[a] | 1.31±0.49[a] | 0.61±0.20[a] |
| Apparent nitrogen digestibility (%) | 17.82±9.84[a] | 37.44±14.19[a] | 18.27±6.03[a] |

Means within a row with different superscript are significantly different (P<0.05).

**Table 4.** Carcass characteristics of broilers fed various feed forms (0-28 days).

| Parameter | Dietary treatment | | |
|---|---|---|---|
| | 1 (Control/mash) | 2 (Pellet) | 3 (Crumble) |
| Live weight, g | 1059.0±50.0[a] | 1453.0±30.5[b] | 1401.0±42.1[b] |
| Carcass weight, g | 960.0±45.2[a] | 1282.0±50.0[b] | 1275.0±35.2[b] |
| Dressing Percentage, % | 90.7±0.5[a] | 88.2±0.15[b] | 91.0±0.10[a] |
| Head, g | 39.0±9.5[a] | 39.0±0.5[a] | 37.0±0.6[b] |
| Wings, g | 78.0±0.5[a] | 124.0±0.5[b] | 104.0±0.5[c] |
| Neck, g | 51.0±0.5[a] | 71.0±0.5[b] | 69.0±0.5[c] |
| Thigh, g | 102.0±0.5[a] | 145.0±0.5[b] | 132.0±0.5[c] |
| Drumstick, g | 89.0±0.5[a] | 135.0±0.5[b] | 127.0±0.5[c] |
| Breast Muscle, g | 238.0±0.5[a] | 354±0.5[b] | 286±0.5[c] |
| Back, g | 88.00±0.5[a] | 135.0±0.5[b] | 110.0±0.5[c] |
| Shank, g | 49.0± 0.15[a] | 59.0±0.5[a] | 66.0±0.2[b] |

Means within a row with different superscript are significantly different (P<0.05).

## Nitrogen utilization

Nitrogen utilization for birds at the starter phase is shown in Table 3. All the values obtained for nitrogen utilization (nitrogen intake, faecal nitrogen, nitrogen retention and apparent nitrogen digestibility) were similar (P>0.05) in all experimental diets. However, birds on the pellets (diet 2) had the highest numerical value for nitrogen retention of 1.31 ± 0.49.

## Carcass characteristics

Carcass characteristics of broiler chicks fed mash, 2 mm pellet and crumble diets are presented in Table 4. Live weight value was highest for birds placed on diet 2 (pellet) at 1453.0±30.5 g which was similar to those placed on crumbles (diet 3) at 1401.0±42.1 g. These values were significantly higher (P<0.05) than those placed on the diet 1 (mash) with the lowest value of

**Table 5.** Relative organs weights broiler chicks fed various feed forms (0-28 days).

| Parameter | Dietary treatment | | |
|---|---|---|---|
| | 1 (Control/mash) | 2 (Pellet) | 3 (Crumble) |
| Liver | 29 ±0.5$^{a}$ | 21 ± 0.5$^{b}$ | 34 ± 0.5$^{c}$ |
| Kidney | 6.0 ± 0.5$^{ab}$ | 5.0 ± 0.5$^{a}$ | 7.0 ± 0.5$^{b}$ |
| Heart | 4.0 ± 0.5$^{ab}$ | 7.0 ± 0.5$^{b}$ | 6.0 ± 0.5$^{b}$ |
| Spleen | 1.0 ± 0.5 | 1.0 ± 0.5 | 2.0 ± 0.5 |
| Gizzard | 25 ± 0.5 | 26.0 ± 0.5 | 26.0 ± 0.5 |
| Lungs | 7.0 ±0.5$^{a}$ | 7.0 ± 0.5$^{a}$ | 9.0 ± 0.5$^{b}$ |
| Intestine | 84 ± 0.5$^{a}$ | 79 ± 0.5$^{b}$ | 133 ± 0.5$^{c}$ |
| Bursa of fabricius | 7.0±0.5$^{a}$ | 10.0± 0.5$^{b}$ | 8.0± 0.5$^{c}$ |

Means within a row with different superscript are significantly different (P<0.05).

1059±50.0 g.

Highest carcass weight was also obtained for birds on the pellet diet (diet 2) at 1282±50.0 g which was similar (P>0.05) to those on crumble diet (diet 3) at 1275±35.1 g and significantly different (P<0.05) from the lowest value obtained for birds on mash diets (diet 1) at 960±45.2 g.

There were similarities (P>0.05) in dressing percentage values obtained for birds placed on mash diet (diet 1) and crumble diets (diet 3) at 90.7±0.5 and 91.0±0.15 g, respectively and were significantly different (P<0.05) from those placed on pellet diets (diet 2) with the lowest value of 88.23±0.10 g. Carcass characteristics value of birds significantly varied (P<0.05) across the experimental diets. However, the highest values were obtained mostly for birds on pellet diets (diet 2).

### Visceral organs characteristics

Relative organs of broiler chicks fed mash, 2 mm pellet and crumble diets are presented in Table 5. Except for spleen and gizzard, all other visceral organs were significantly lower (P<0.05) in measured weights for birds on mash (diet 1).

Other carcass characteristics such as head, wings, neck, thigh, drumstick, breast muscle, back and shank had significantly higher or similar values for broiler starter birds on 2 mm pellet diets and broiler finisher birds at 4 mm pellet diets. There is no doubt that these particulate feed form and sizes augur well for the two phases of broiler production in terms of growth and muscle development.

Most visceral organs such as liver, heart, kidney, lungs, proventriculus, crop, intestine and pancreas showed similarity of growth at broiler starter and finisher phases, indicating similar organ development.

### Haematological and serum biochemical parameters

Table 6 shows the haematological and serum biochemistry of broiler birds at starter phase placed on the different feed forms. There were no significant differences (P<0.05) among the haematological para-meters examined for birds at this phase.

### Economic analysis (cost benefit analysis)

The cost implication indices for the broiler starting phase are shown in Table 7. The costs of diets were calculated using the prevailing current prices of the feed ingredients. The cost of the diets were calculated with the basic assumption that labour and other overhead costs were similar for all diets prepared and did not need to be considered in the calculation.

The cost of finished feed (diets) expectedly revealed that the pelleted feed (diet 2) was the most expensive at N68.5 per kg of feed while the mash had the lowest cost of N58.0. Even though the highest cost of production of N470.42 was recorded for birds on the pellet diets, the total net return per bird was also highest at N470.1 for birds on the pellet diet.

It is evidently clear that 2 mm pellet diets at broiler starter phase of broiler production generated more total net returns per bird and would be more profitable for the purpose of commercial broiler production.

## DISCUSSION

### Average growth performance

Generally, growth performance results for this experiment showed that optimal performances were achieved in the pellet diets. It is commonly accepted that pelleting poultry rations increases weight gain and improves feed efficiency. These improvements have been attributed to the higher density, improved starch digestibility resulting from chemical changes during pelleting, increased

**Table 6.** Haematological and biochemical profile of broilers fed various feed forms (0-28 days).

| Parameter | Dietary treatment | | |
|---|---|---|---|
| | 1 (Control/mash) | 2 (Pellet) | 3 (Crumble) |
| Hbc (g/dl) | 5.45±0.34 | 6.01±0.42 | 6.01±0.45 |
| PCV (%) | 23 .00±0.45 | 24.00±0.65 | 24.00±0.52 |
| RBC x $10^6$ ($mm^3$) | 1.42±0.42 | 1.54±0.43 | 1.63±0.54 |
| ESRs ($mm^{3/}$l) | 4.00±0.43 | 3.14±0.50 | 4.01±0.43 |
| MCV x $10^{-6}$ (µl) | 0.10±0.71 | 0.12±0.43 | 0.11±0.32 |
| MCH x $10^{-6}$ (µg) | 4.45±0.22 | 4.34±0.45 | 4.29±0.54 |
| MCHC (g/dl) | 41.50±0.40 | 40.16±0.41 | 36.94±0.63 |
| **Serum biochemical parameters (g/100 ml)** | | | |
| Total serum protein | 16.42±0.43 | 17.01±0.78 | 17.51±0.43 |
| Albumin | 15.67±0.45 | 15.06±0.56 | 15.56±0.56 |
| Globulin | 8.72±0.42 | 8.41±0.41 | 8.56±0.32 |
| Albumin/globulin ratio | 1.80±0.30 | 1.79±0.31 | 1.81±0.51 |

Means within a row with different superscript are significantly different (P<0.05).

**Table 7.** Economic analysis of the broilers fed various feed forms (0-28 day of age).

| Parameter | Dietary treatment | | |
|---|---|---|---|
| | 1 (Control/mash) | 2 (Pellet) | 3 (Crumble) |
| Total feed intake (kg/bird) | 1.53 | 1.61 | 1.57 |
| Feed cost per Kg of diet (N/kg) | 58 | 68.5 | 63.25 |
| Cost of feed intake / bird (N/bird) | 177.5 | 220.42 | 198.96 |
| Cost of Day old chicks (N/bird) | 250 | 250 | 250 |
| Total Cost of production/bird (N/bird) | 427.5 | 470.42 | 448.96 |
| Av. body wt. at $28^{th}$ day old (kg/bird) | 1.19 | 1.27 | 1.21 |
| Cost of 1kg of poultry meat (N/kg) | 750 | 750 | 750 |
| Total Revenue/bird (N/bird) | 892.5 | 952.5 | 906.5 |
| Total net returns/bird (N/bird) | 465 | 470.08 | 457.54 |

nutrient intake, changes in physical form, reduced feed wastage and decreased energy spent for eating (Jensen, 2000; Ghazi et al., 2012). The present study is consistent with previous findings that pelleting complete diets improved weight gain, feed conversion (Douglas et al., 1990) and feed intake (Nir et al., 1995) in broilers. Protein efficiency ratio (PER) is an important protein quality index (Fasuyi, 2006). The high PER value of birds on pellets indicated that protein was most efficiently utilized in birds on the pelleted diets in spite of the fact that all diets were formulated isonitrogenous with the same quantity and quality of ingredients.

## Nitrogen utilization

The fact that nitrogen retention values were similar for experimental birds on all feed types and sizes indicated that feed forms and sizes in broiler starter may have little or no remarkable effect as long as adequate intake of the feed and nitrogen is ensured.

## Carcass characteristics

Most carcass characteristics examined had significantly higher values for birds on the pellet diets indicating that there was ample benefit in the consumption of pellet diets over the other diets which translated into better carcass formation and muscle development. This is supported by previous studies (Oluyemi and Roberts, 1979; Rodehutscord et al., 2004).

## Visceral organs characteristics

The uniformity in the growth rate and muscle development

compared favourably with previous standard growth pattern and muscle development of birds of the same age and strain (Oluyemi and Roberts, 1979; Rodehutscord et al., 2004).

### Haematological and serum biochemical parameters

The similarity in all blood parameters examined for broiler starter birds indicated that feed forms had no marked effect on the birds at that stage of production and may not be predisposed to any health hazards as a result of the feed forms. The erythrocyte sedimentation rates (ESRs) of broiler starter birds were similar and compared with ESRs obtained for healthy birds in literature (Oluyemi and Roberts, 1979; Rodehutscord et al., 2004).

The variations in total serum protein (TSP), albumin, globulin and albumin/globulin ratio could be have been influenced by the dietary treatments. Total serum protein (TSP) is indirect indices for measuring the nutritional protein adequacy.

### Economic analysis (cost benefit analysis)

It is obvious that birds on the pellet diets had the highest and most profitable commercial value as the total net return per bird was most favourable for commercial broiler starter production. About 7% increase in revenue generated from the production of broilers on pellet feeds compared to mash have been previously reported (Banerjee, 1987).

### Conclusion

The present study reveals that pelleting broiler poultry at the starter phase of broiler production had nutritional advantage as it attracted better feed intake, increased growth rate and better feed efficiency. Pellet feeds also enhanced carcass and other organs characteristics of broiler starter. Hematological and blood biochemistry indices were not adversely affected. Expectedly, the cost of pelleting broiler feeds initially attracted extra cost of broiler production but eventually culminated into a better total net return per bird which translated into higher profitability for birds reared on pellet diets at broiler starter phase of production.

### Conflict of interests

The authors did not declare any conflict of interest.

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academicJournals

Vol. 9(4), pp. 230-236, April, 2015
DOI: 10.5897/AJFS2014.1221
Article Number: 62868E051608
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Effects of coffee processing technologies on physico-chemical properties and sensory qualities of coffee

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Received 21 October 2014; Accepted 16 March 2015

**The study aimed at comparing the effects of three coffee pulping methods on the physico-chemical properties and sensory qualities of coffee. The coffee cherries were processed by disc pulper, drum pulper and eco-pulper methods which varied on mode of operations and mucilage removal methods. The coffee parchment were dried to moisture content of 10 ± 1% and green coffee beans were evaluated for parameters including moisture content, mass, volume, density, pH, titratable acidity, protein, sucrose and lipids content of green coffee beans. The parameters such as moisture, mass, volume and density were determined by actual measurements. Protein was determined by Kjeldhal method, lipids were extracted by Soxhlet method and sucrose extracted and determined by HPLC. The pH showed some significant difference ($p<0.05$) between the treatments. There was no significant difference on other parameters such as mass, volume, density and titratable acidity, protein, lipids and sucrose. The processing methods showed similar levels on the scores of sensory attributes analyzed by qualified panelist and the scores varied between 7.0-10. The final quality was not significantly different between the processing methods and no defects and faults noted in the samples. It was concluded that the three processing methods do not vary on the levels of physico-chemical components of coffee and gives similar characteristics on sensory attributes and final quality of coffee beverage.**

**Key words:** Coffee processing, pulping methods, fermentation, physico-chemical composition, sensory quality.

## INTRODUCTION

Coffee quality is associated to a set of factors that involve physico-chemical and sensorial aspects which, in turn, depend on the post-harvest handling and processing (Coradi et al., 2007; Afonso Júnior, 2001, Lima et al., 2008). Wet processing is more commonly preferred than the dry method due to its production of good quality coffee. Wet coffee processing consists in removing pulp and skin from fresh berries using a pulping machine (Murthy and Naidu, 2011). These machines remove the skin and pulp from coffee cherries leaving viscous

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mucilage adhering to the parchment. Mucilage is traditionally removed by use of natural fermentation process where the natural micro flora degrade it hence facilitating its elimination by washing (Murthy and Naidu, 2011, Avallone, 2000).The whole process entails use of large quantities of water during pulping, washing and grading stages which contributes to the pollution of water in countries where it is commonly used (Gonzalez-Rios et al., 2007, Bailly et al., 1992). A new technology called ecological processing was developed in Colombia and Brazil, utilizes little water, pulp coffee and removes mucilage by mechanical processes without the fermentation stage (Roa et al, 2012, Gonzalez-Rios et al, 2007, Puerta-Quintero, 1999). Fermentation is a crucial stage in coffee wet processing because apart from enhancing the removal of mucilage from parchments it is reported to improve the quality of coffee (Nigam and Singh, 2014). Whereas the disc and drum pulpers utilizes fermentation during mucilage removal, the eco-pulper method uses mechanical process, hence the coffee produce may vary in terms of the physico-chemical properties which may leads to variations in the sensory quality of coffee. There is limited information on the effects of the new technology on the physico-chemical composition of coffee and the sensory characteristics of coffee brew. This study was undertaken to investigate the effects of the disc, drum and eco-pulper methods on the physic-chemical composition and sensory qualities of coffee.

## METHODOLOGY

### Site and sample preparation

Coffee cherries of variety commonly referred as SL 28 (Arabica coffee) were harvested during the short and peak harvesting seasons between the year 2012 and 2013. The coffee cherries were harvested from Dedan Kimathi University farm located in Nyeri County Kenya (0° 25' 0" S / 36° 57' 0" E). Ripe cherries were obtained from coffee plants grown on the same field, by selective picking method, sorted and processed by three different pulping methods which include the disc pulper, drum pulper and an eco-pulper method. Disc pulper method was done by continuous pulping operation with water involve in pulping, transport and grading of parchment. The parchments obtained were put in plastic containers with perforation at the bottom and allowed to ferment by dry method for 16 to 18 h. Once fermentation was complete, the parchments were washed to remove the mucilage and graded to have heavier and the lighter parchment. The heavier parchments were selected for research and transferred to the drying tables for drying in the sun to attain moisture content of 10 ±1%.

Drum pulper method was done by use of a motorized manual pulping machine and little water used during pulping. The parchment obtained were subjected to fermentation in plastic containers with perforation at the bottom to allow dry fermentation for 16 to 18 h. Once fermentation was complete, the parchment were washed and graded to separate lighter and heavier parchment. The heavier parchments were selected and transferred to the drying tables for drying in the sun to the required moisture content of 10 ± 1%. The eco-pulper method was done by first sub-Merging coffee cherries with water in a tank to separate the heavier and lighter cherries which were then pulped separately using an eco-pulper.

Heavier cherries were selected for research and after pulping the parchment were passed through a demucilager unit which removes the mucilage mechanically with some water poured continuously to clean the parchment. Clean coffee parchments were put in trays and transferred to drying tables for drying in the sun to the required moisture content of 10 ± 1%. The dry parchments from the three processing methods were package in sealed polythene bags and stored in a freezer at -18°C until time for analysis.

### Analysis of composition

#### *Chemicals*

The chemical used in the analysis of parameters such as proteins, lipids and sucrose included; concentrated sulphuric acid, absolute ethanol, concentrated hydrochloric acid, hexane, sodium hydroxide, methanol, acetonitrile and were all obtained from Sigma Aldrich (Germany).

#### *Mass, volume and density*

Volume of coffee beans were determined according to the method of Dutra et al. (2001) and described by Ismail et al (2013).

#### *Moisture content*

The moisture content was determined by use of the methods of Reh et al. (2006) and described by Ismail et al. (2013).

#### *Lipids*

The lipids were determined by the Soxhlet method. Some 5 g of powdered samples were refluxed with hexane for 8 h in round bottom flasks. The extracts were then evaporated by a rotary evaporator at 60°C until all the hexane was evaporated. The flasks containing the lipid were dried in an oven until a constant weight was achieved (Ismail et al., 2013).

#### *Crude protein content*

Crude protein was determined by Kjeldahl method after digesting samples (1 g) with sulphuric acid and the crude protein calculated as nitrogen × 6.25 according to the method described by AOAC (1995).

#### *Total titratable acidity and pH*

This was done according to the method described by Tawfik and EL Bader (2005). The pH was determined by taking 2.25 g of ground samples and mixing with 10 ml of hot water at 80°C, cooled to room temperature and the pH determined. Total titratable acidity was determined by taking 10 g of ground coffee, mixed with 75ml of 80% ethanol and kept under gentle agitation for 16 h. A portion of 25 ml of the extract was diluted to 100 ml with distilled water. Titratable acidity was determined by titrating with 0.1 N sodium hydroxide and three drops of phenolphthalein indicator added. The

results were expressed in percentage.

***Determination of sugars***

Approximately 1 g of sample was weighed into 250 ml round bottomed flask and 50 ml 95% ethanol added. The mixture was reflux for 1 h, cooled and filtered using filter paper (Whatman No. 42). It was then evaporated to dryness using rotor vapor and reconstituted with 10 ml mobile phase containing acetonitrile: water (75:25) and filtered using micro filter 0.45 and 20 µm injected to HPLC (Knauer, Germany) fitted with a refractive Index detector.

**Sensory analysis**

The sensorial analysis of coffee samples were carried out to determine the quality of coffee and was done by six qualified cuppers according to the method described by Kathurima et al. (2009). Scores were awarded to each sensorial attribute depending on their intensity in the samples. For each sensorial attribute, the samples received a score on a scale of 0 to 10. The attributes for aroma, aftertaste, acidity, body, flavor, clean, sweet, balance and overall score were evaluated, resulting in a final score count that indicated the quality of coffees.

**Statistical analysis**

All treatments were done in triplicates and analysis of data evaluated using the statistical package for social scientist (SPSS version 18). Experimental design was performed using complete randomized design (CRD). Analysis of variance (ANOVA) was conducted, and the differences between group means analyzed using the least significant difference (LSD). Statistical significance was established at $p \leq 0.05$.

## RESULTS AND DISCUSSION

The data used in this study was for coffee samples obtained during the short and main harvesting seasons and categorized as seasons 1 and 2, respectively. The results for the physical and chemical properties of coffee processed by the three processing methods studied for two harvesting seasons are shown in Table 1. The moisture content for the samples was within the recommended levels of 10-12% (Patui et al., 2014). The eco-pulper method showed slightly lower moisture content than the disc and drum pulper methods, and this could be attributed to faster rate of drying since parchment are exposed to drying immediately after processing. Therefore methods which use fermentation takes long time between 16 and 20 h before coffee parchment is subjected to drying. The level of the moisture content is important for preservation of coffee quality because higher moisture levels than 12% will favour the growth of moulds and cause off flavours affecting the taste qualities of coffee (Bucheli et al., 1998; Ismail et al., 2013). Regarding the mass of coffee samples, disc pulper method showed a significantly lower value than the eco-pulper method during season 1 harvest (Table 1). The same trend was observed in the second season but the values were not significantly different. The reduction in mass in the processes using fermentation may occur mainly due to the effect of microbial activities and leaching of components to the washing water (Roa et al., 2012). However the eco-pulper method, though it uses little water could also lead to losses of components due to the squeezing of coffee parchment through narrow space and high speed rotation which occur in the demucilager. Regarding the volume of coffee samples the results showed no significant differences between the treatments as observed for the two seasons (Table 1). The volume depends on the size of the coffee beans which is mainly influenced more by factors such as climatic and agronomic practices than the processing methods. The values for season 2 was slightly higher than season 1 indicating the effects of the climatic conditions since season 2 occur under the peak period of coffee harvesting with more favorable climatic conditions. In regard to the density, the results were only significant during season 1 with the eco-pulper samples showing higher values compared to the disc pulper samples. This could be attributed to the effect of mass which was higher in the eco-pulper samples than those of the disc pulper samples. This is consistent with the report of Ismail et al. (2013) who also noted that the density of coffee beans are influenced by the mass and not the volume. From this study the values of density varied from 895.81 to 1227.0 kg/cm$^3$ which was similar to the range reported by other authors such as Ismail et al. (2013) as 992.68 to 1138.25 kg/m$^3$ and Franca et al. (2005) as 1200 to 1300 kg/m$^3$ for crude coffee beans.

Considering the pH the results indicate that the disc and drum pulper methods showed significantly lower pH than the eco-pulper samples (Table 1). This is expected because the disc and drum pulper samples were processed through fermentation to remove the mucilage while the eco-pulper samples were processed by mechanical method. Similar observation were reported by other authors such as Ferreira et al. (2013) who explains that pH is reduced in washed coffee due to fermentation. The acids produced during fermentation such as acetic acid may penetrate the husks of the coffee bean influencing the changes observed for pH. This is in agreement with the report of Nigam and Singh (2014). From the study, the pH values range from 5.91 to 6.11 which was within the range reported in the literature such as Franca et al. (2005) reporting pH range of 5.3 to 6.52 and Butt et al. (2011) reporting pH range of 4.89 to 5.98 for green coffee samples. For total titratable acidity, there was no significant difference between the different processing methods studied. The change in titratable acidity could occur due to the levels of acid present in the coffee beans. The common acids present in the coffee beans include; citric, acetic, malic, chlorogenic and quinic acids (Butt et al.,

**Table 1.** Physico-chemical properties of green coffee from different processing methods for two harvesting seasons.

| Parameter | Treatment | | | |
|---|---|---|---|---|
| | Seasons | Disc pulper | Drum pulper | Eco-pulper |
| Moisture % | 1 | 10.84 ± 0.29a | 10.33 ± 0.15ab | 10.17 ± 0.29b |
| | 2 | 10.66 ± 0.05a | 10.55 ± 0.16a | 10.27 ± 0.04a |
| Mass (g) | 1 | 12.67 ± 0.57b | 14.67 ± 0.58ab | 16.67 ± 1.15a |
| | 2 | 16.33 ± 0.58a | 17.33 ± 0.58a | 18.67 ± 3.06a |
| Volume ($cm^3$) | 1 | 14.26 ± 1.4a | 14.11 ± 3.43a | 13.95 ± 3.21a |
| | 2 | 17.02 ± 1.97a | 17.55 ± 4.06a | 15.56 ± 4.48a |
| Density ($kg/m^3$) | 1 | 891.82 ± 53.4b | 1063.13 ± 146a | 1222.62 ± 184a |
| | 2 | 967.46 ± 102a | 972.54 ± 148a | 1226.2 ± 152a |
| pH | 1 | 5.95 ± 0.03b | 5.94 ± 0.03b | 6.04 ± 0.02a |
| | 2 | 5.92 ± 0.04b | 5.91 ± 0.06b | 6.03 ± 0.01a |
| Titratable acidity (%) | 1 | 1.37 ± 0.22a | 1.50 ± 0.13a | 1.62 ± 0.13a |
| | 2 | 1.54 ± 0.08a | 1.54 ± 0.34a | 2.0 ± 0.25a |
| Protein % | 1 | 13.17 ± 1.7a | 13.83 ± 1.9a | 15.57 ± 1.6a |
| | 2 | 14.26 ± 0.8aA | 13.62 ± 1.9a | 13.40 ± 1.0a |
| Sucrose % | 1 | 8.03 ± 0.20a | 7.38 ± 0.57a | 7.78 ± 0.54a |
| | 2 | 8.58 ± 0.56a | 8.51 ± 0.8a | 9.02 ± 0.71a |
| Fat % | 1 | 17.39 ± 0.64a | 17.08 ± 0.39a | 17.54 ± 0.29a |
| | 2 | 17.01 ± 0.01a | 16.82 ± 0.5a | 16.75 ± 0.14a |

[1]Values are means (± SD) of triplicate determinations; [2]Means designated by different letters are significantly different at ($P < 0.05$).

2011). Therefore the processing methods could have similar levels of this organic acids hence no variations in their titratable acidity levels. Ferreira et al. (2013) studying the effects of washed and dry processes did not find any significant differences in total acidity of coffee beans. However Tawfik and EL Bader (2005) reported significant variations between green coffee beans from different varieties. The total acidity and pH are important parameters influencing the quality of coffee beverage.

Protein, fats and sucrose are important components of green coffee which are known to influence the aroma characteristics of roasted coffee. The level of protein content varied from 13.17 to 15.57%. This was within the range reported by Rodrigues et al. (2010) as 9.3 to 20.8% in Arabica coffee. The results indicate that there were no significant differences between the processing methods. The literature report that the enzymes present in green coffee could degrade protein to polypeptides and free amino acids during fermentation for mucilage removal (Nigam and Singh, 2014). From our study, no indication of loss of protein or peptides by the processing methods especially those using fermentation processes. The proteins and peptides may complex with polyphenols (Nigam and Singh, 2014) and hence remain intact in the cell structure.

A study by Arnold and Ludwig (1996) noted that the total concentration of free amino acids and protein content does not change significantly with the chemical reaction occurring during the harvest season and the postharvest processing steps, such as fermentation, drying and storage. Regarding the sucrose content, there was no significant difference between treatments with the values ranging from 7.38 to 9.02%. Similar observation was reported by Knopp et al. (2006) who noted that sucrose and other low molecular weight sugars are not affected by the wet and dry processing of coffee cherries. It could be expected that the processes which use fermentation and excess water during washing loss the sucrose through leaching but no significant loss was observed in the methods studied. Some literature indicates that only soluble sugars such as glucose and fructose may be loss to the processing water and not the

non-reducing sugars such as sucrose (Knopp et al., 2006). This is also supported by the report that metabolic processes occurring at the start of drying takes a short period and mainly affects the reducing sugars such as glucose and fructose and sucrose remains intact as a storage compound (Kleinwachter and Selmar, 2010). Lipids represent a significant part of dry matter in green coffee consisting mainly of triacylglycerol with a range of 13 to 17% (Patui et al., 2014; Joët et al., 2010). The level of lipids in our analysis was slightly higher at a range of 16.75 to 19.60%. Butt et al (2011) reported a range of 9.3 to 12.3%.

The variations could be attributed to the difference in extraction methods, variety or geographical factors (Hurtado and Dorado, 2013). The results also indicate no significant differences between the processing methods on the level of lipid contents of coffee samples. Joët et al. (2010) noted that significant metabolism occurs during wet processing which could lead to increase of chemical substances such as lipids. In our study there was no indication of increase of lipids by the processing methods but the processing methods used may induce similar metabolic processes which result at similar levels of the lipid content. The lipase activity is also reported to be present in the coffee seed but it is mainly induced by germination processes while the presence of parchment prevent oxidation of liphophilic fractions (Patui et al., 2014).

**Effects of coffee processing methods on sensory attributes and quality of coffee**

The results for analysis of sensory attributes and total quality of coffee are presented in Figures 1 and 2 respectively. The green coffee bean were roasted, ground, brewed and evaluated for cup profiles according to the method described by Kathurima et al. (2009). Ten sensory attributes were evaluated in coffee samples which include aroma, flavor, aftertaste, acidity, body, balance, clean cup, uniformity, sweetness and overall quality. The results of evaluation of the attributes for the different processing methods presented close values (Figure 1). Sensory parameters such as clean cup, uniformity and sweetness were rated with the highest score of 10 for all the treatments. There were no defects noted in all the samples such as stinkers, bitterness and sourness. The cup parameters such as aroma, flavor, aftertaste, acidity, body, balance and overall acceptability were rated as 7.6, 7.6, 7.7, 7.8, 7.6, 7.6 and 7.7 respectively for the eco-pulper samples. For the disc pulper samples, the aroma, flavor, aftertaste, acidity, body, balance and overall acceptability were rated as 7.5, 7.6, 7.6, 7.7, 7.6, 7.6, and 7.6 respectively. Similar levels were observed for the drum pulper samples with the aroma, flavor, aftertaste, acidity, body, balance and over-all being rated as 7.6, 7.7, 7.7, 7.7, 7.6, 7.6 and 7.7 respectively (Figure 1). Generally, the study did not find much variations in the levels of the sensory attributes from coffee brew processed by the disc, drum and eco-pulper methods. Considering each of the sensory attributes it was observed that the eco-pulper showed slightly higher value for the aroma and was at par with the drum pulper for this attributes. For the flavor, the drum pulper showed slightly higher value than the other processing methods. The acidity was slightly higher for the eco-pulper samples than the drum and disc pulper samples.

The processing methods were all at par for the body and balance attributes while the eco-pulper and drum pulper showed slightly higher levels for the overall acceptability of the coffee brew samples. The values for the sensory attributes were not statistically significant between the treatments. This confirms the similarity between the coffee brews processed by the eco-pulper, disc and drum pulper samples. The coffee samples were described to have attractive flavors such as fruity, floral, lemon like and caramel. The eco-pulper samples were described to have well balance body and acidity with floral flavor. The disc pulper samples were described with a winny, fruity and slightly floral flavor. The drum pulper samples also were described to have good body, winny and floral flavor. Hence the processing methods did not vary on the levels of sensory attributes. Due to lack of significant variations between the processing methods on the levels of the sensory attributes, this then confirms their similarity in the levels of the chemical components as observed earlier in green coffee. The results for the final quality of coffee samples are shown in Figure 2. The scores for all the sensory attributes were summed up to give a total score which represents the final quality of coffee. The results indicate that the eco-pulper, disc and drum pulper samples showed a final quality of 83.45, 83.11 and 83.58% respectively in season 1. In season 2 similar results were observed with the eco-pulper, disc and drum pulper giving final quality of 84.0, 83.77 and 83.98% respectively. From the results then it was observed that there were no significant variations in the final quality of coffee processed by the eco-pulper, drum pulper and the disc pulper. The characteristics of the sensory attributes and final quality of coffee mainly depends on the physico-chemical components of roasted coffee which relates to the chemical composition of green coffee (Ferreira et al., 2013; Pimenta, 2003). From our study there were no significant variations in the level of physico-chemical components of green coffee such as protein, sucrose and lipids as observed for the different processing methods. Season 2 samples were also observed to have a slightly higher final quality than season 1 samples. This could be attributed to the for the physical and chemical components between the two seasons. Hence season two which occurs under the peak

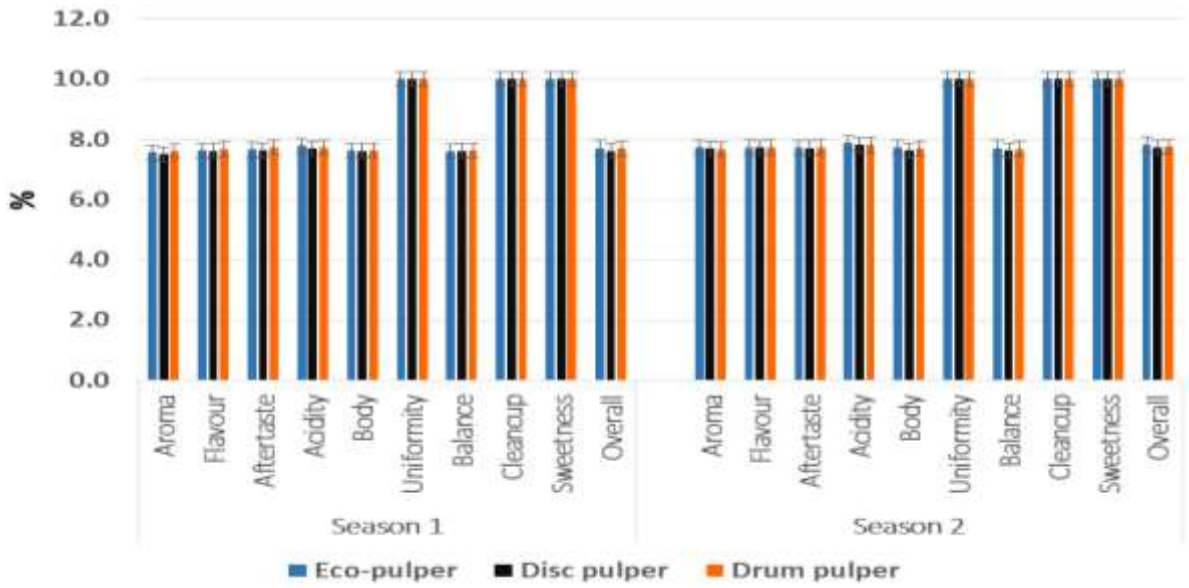


**Figure 1.** Sensory attributes of coffee beverage processed by three different pulping methods for two harvesting seasons.

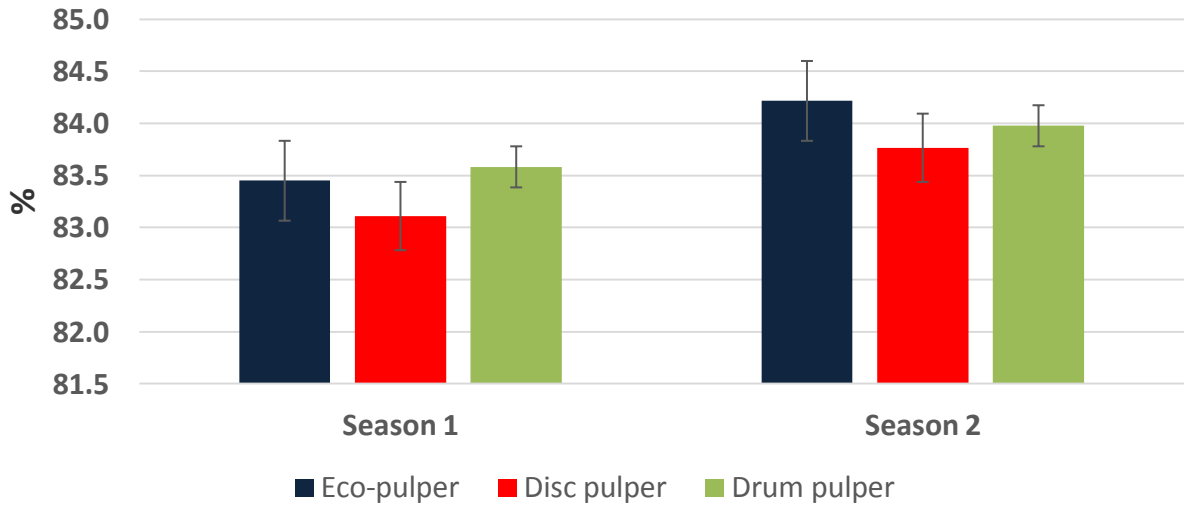


**Figure 2.** Total quality level of coffee brew processed by three pulping methods for two harvesting seasons.

season of coffee harvesting is observed to have better quality of coffee than the short season of coffee harvesting.

## Conclusion

From this study, we investigated the effects of the coffee processing methods, mainly the eco-pulper, the disc and drum pulper on the levels of physico-chemical parameters of coffee such as mass, volume, density, titratable acidity, colour, proteins, sucrose and lipids contents. There was no significant variation between the processing methods on the level of these parameters. The few variations observed for parameters such as density and pH did not have much influence on the sensory attributes and final quality of coffee. The scores for the sensory attributes were very close and final quality of coffee brew was also similar between the treatments.

Therefore, it was concluded that the coffee processing methods such as eco-pulper, disc and drum pulper do not vary on the levels of physico-chemical parameters and gives the same levels of the sensory attributes and final quality of coffee. The important consideration for the processors is the cost effectiveness of the processing methods since all processes gives similar levels of the sensory attributes and final quality of coffee.

## Conflict of interest

The authors would like to certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest as regards the subject matter or materials discussed in this manuscript.

## ACKNOWLEDGEMENTS

This work was sponsored by the National Council of Science and Technology (NACOSTI). We acknowledge the services of technical staff of Coffee Research Foundation and Jomo Kenyatta University of Agriculture and Technology.

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academicJournals

Vol. 9(4), pp. 237-242, April, 2015
DOI: 10.5897/AJFS2014.1188
Article Number: 24FCC1151612
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Preliminary studies on the effect of shea kernel size on shea butter quality

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Received 24 June, 2014; Accepted 6 March, 2015

**The study was carried out between March, 2012 and January, 2013 at the University for Development Studies, Nyankpala campus, Ghana. The objectives of the study were to characterize farmer shea nut varieties to determine the physical and chemical characteristics of the shea nut butter and determine appropriate shea nut quality for the domestic market. The shea nuts were characterized as T1 (small nuts), T2 (medium nuts), T3 (big nuts) and T4 (very big nuts). These nuts were arranged in a completely randomized design and replicated three times for shea nut butter extraction and assessed for free fatty acid (FFA), moisture content and sensory quality. The study reveals strong correlation between shea nut size and butter quality with bigger shea nuts recording lower FFA and moisture content hence higher butter quality. There were differences in colour and texture of the shea butter extracted from the different shea nut sizes. It is recommended that, further research be carried out at different geographical locations to assess the effect of shea nut size on butter quality.**

**Key words:** Shea kernel, free fatty acid, moisture content, sensory quality.

## INTRODUCTION

Shea nut is known as '*Kpihi*', '*Nyuuni*' and '*Kyuuma'* among the Dagomba, Frafra and Wala people of Ghana, respectively. It is obtained from the fruit of shea tree *(Vitellaria paradoxa),* which exists in the wild and grows in an uncultivated state in most parts of Africa. Shea fruit is made up of a green epicarp, a fleshy mesocarp (pulp) and a relatively hard shell (endocarp) which encloses the shea kernel (embryo). The kernel, according to Axtell et al. (1993) contains about 60% edible fat (shea butter) and the residual product, from which the butter is extracted (shea cake), is an excellent ingredient for livestock feed production. Shea butter is as good as table oil because of its high nutritive value. It is widely used locally for curing leprosy and other ailments. It also has various industrial uses that include edible oil, soap making, cosmetics, pharmaceuticals, lubricants and paints. According to Russo and Etherington (2001), shea products such as solid fat (butter or stearin) and the liquid oil (olein) are ideal for use as raw materials for cooking oils, margarines, cosmetics, soaps, detergents and candles. However, they have found their primary market as a substitute for cocoa butter in the chocolate and

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confectionary industry.

According to the American shea Butter Institute (ASBI, 2004), 100% pure natural shea butter is an all-natural vitamin A cream. Shea butter has been shown to be a superb moisturizer, with exceptional skin healing properties. ASBI (2004) compiled a list of skin conditions where 100% shea butter has been proved to be effective in healing. These skin conditions are dry skin, skin rash, skin peeling after tanning, blemishes and wrinkles, itching skin, sunburn, shaving cream for a smooth silky shave, small skin wounds, skin cracks and tough or rough skin (on feet). Others are cold weather, frost bites, stretch marks prevention during pregnancy, insect bites, health skin, muscle fatigue, aches and tension, skin allergies such as poison ivy or poison oaks, eczema, dermatitis and skin damage from heat. It is because of these unique healing properties that the shea tree is called karite tree which means tree of life (ASBI, 2004). It was reported by ASBI (2004) that shea butter has unparalleled moisturizing property and this is due to several natural moisturizers present in it. It was also discovered by ASBI (2004) that the moisturizers in shea butter are the same moisturizers produced by the sebaceous glands in the skin.

In the same development, ASBI (2004) reported that the positive biochemical and physiological effects that shea butter has on skin injuries make it ideal for wound healing. Reports from many users of shea butter have shown that this product promotes and accelerates wound healing. Furthermore, vitamin E in shea butter is helpful to the skin and such benefits could be accomplished by increasing the microcirculation to the skin. This eventually results in increased blood supply to and from the skin. Also, vitamin E in shea butter may serve as an anti-free radical agent thereby preventing the deleterious effects of sun and environmental exposure.

Determination of free fatty acid of shea nut butter is important especially for the screening purposes of large quantities of samples during processing and marketing (Quainoo et al., 2012).

## MATERIALS AND METHODS

The study was carried out from March, 2012 to January, 2013 at the Spanish Laboratory, University for Development Studies, Nyankpala. Nyanpkala is located between latitude 09° 25.925 N and longitude 01° 00,420 W. The area is in the Tolon District of the Northern Region of Ghana. The Agro-ecological zone of the area is Guinea Savanna. Shea nut samples were collected in the Zoolanyili community located in the Tolon district near Nyankpala. They were collected by farmers based on their own characterization into the various nut sizes from trees that bare fruits of different sizes. The farmer varieties were re-classified into four size groups for the purpose of this study. The four size groups are shown in Table 1 and Plates 1 to 4, respectively.

Treatments comprising four farmer varieties were used to carry out the study. The four treatments were arranged in a completely randomized design and replicated three times for laboratory analysis for FFA's and moisture content.

**Table 1.** Size ranges of nut varieties.

| Treatment no. | Treatment description | Kernel length (cm) |
|---|---|---|
| (T1) | Small nuts | 2.45 – 2.60 |
| (T2) | Medium sized nuts | 2.65 – 2.81 |
| (T3) | Big nuts | 2.95 – 3.00 |
| (T4) | Very big nuts | 3.25 – 3.45 |


**Plate 1.** Small nuts.


**Plate 2.** Medium sized nuts.

Snowball sampling was used to select respondents for sensory quality survey on butter colour and texture. Forty (40) women were selected from the Tamale Metropolis for the sensory quality survey on color, texture, taste and aroma.

Shea butter made from the different size categories (Plate 1a and b) were used for the study.


**Plate 3.** Big nuts.


**Plate 4.** Very big nuts.

#### Butter extraction procedure

Until recently, almost all shea butter was extracted by use of small-scale extraction methods. In Ghana, the method of extraction used locally is the same throughout the north. The collection and extraction of the shea nut were carried out based on the following procedure: collection of the shea nuts from the field, drying of the shea nuts, extraction of the kernels from the shells for further drying, washing of the dry kernels and crushing of the kernels into pieces to ensure roasting.

The roasted kernels were cooled and milled into paste using a grinding mill. The paste was then mixed thoroughly with cold and hot water until a cream coloured crude butter separated and floated on the surface of the water. The crude butter on top was collected, washed in clean water and then boiled to separate the oil from the impurities in the cream crude butter. The oil was put in clean containers and cooled whilst steering until it solidified into the familiar solid shea butter.

#### Laboratory determination of FFA

1% Phenolphthalein in 95% ethanol; 0.1 N potassium hydroxide; Standard solvent: Mix 25 mL ether, 25 mL 95% alcohol and 1 mL of 1% phenolphthalein solution and neutralize with N/10 alkali.

#### Procedure

1-10 g of oil or melted fat was dissolved in 50 ml of the standard solvent in a 250 mL conical flask. Few drops of phenolphthalein was added to the oil in the standard solvent and titrated against 0.1 N potassium hydroxide. The content was shaken until a pink colour persisted for fifteen seconds.

#### Calculation

$$\text{Acid Value (mg KOH/g)} = \frac{V_1 - V_0 \times N \times 56.1}{W}$$

$V_1$ = Titrate value of sample; $V_0$ = titrate value for blank solution; N = normality of titration; W = weight of sample in grams; the free fatty acid (as oleic) is estimated by dividing the acid value by 1.99.

#### Laboratory determination of moisture content

The digital moisture content analyzer (Sartorius, MA 45C-000230V1, June 2003) was used for the estimation of moisture content. Sample of the butter was placed on a sample holder and placed inside the analyzer. The machine was tarred and set to record the moisture content of the butter. The machine also recorded the weight of the sample, the dry matter content and the moisture dry matter ratio.

#### Analysis of data

Microsoft excel and genstat was used to analyze the data and standard error of difference (SED) to compare the means.

## RESULTS

### Effect of shea nut size on FFA content of shea butter

Figure 1 shows the levels of FFA (expressed as % oleic acid) in shea butter extracted from shea kernels. There were significant differences in the mean FFA values of butter extracted from the shea nuts of the various sizes used in this study. Shea butter extracted from big nuts recorded the lowest mean FFA value of 1.133 (Figure 1).

The Ghana Standards Authority criteria indicated that butter extracted from big, medium and small nuts with mean FFA values of 1.133, 2.227 and 2.420 respectively were grade two while very big nuts with mean FFA value of 3.637 was grade three (Table 2).

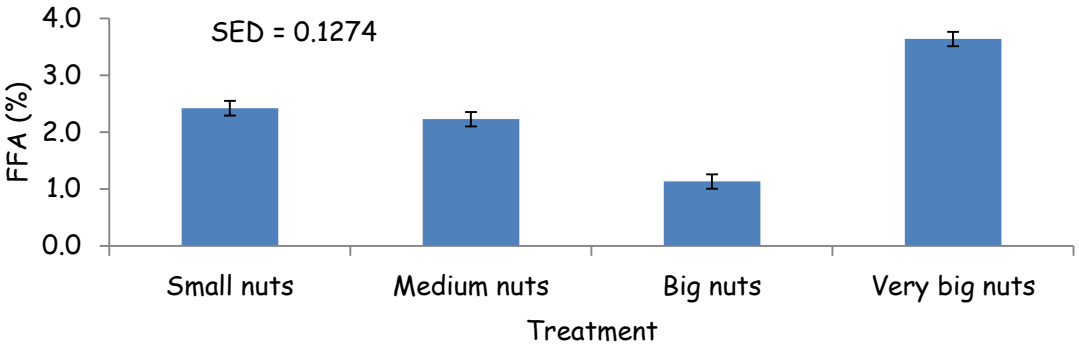


**Figure 1.** Effect of shea nut size on free fatty acid content of shea butter.

**Table 2.** Ghana Standards Authority grading of FFA values of shea butter.

| Treatment | Grade 1 (1) | Grade 2 (>1.0-3.0) | Grade 3 (>3.0-8.0) |
|---|---|---|---|
| Small nuts | - | 2.420 | - |
| Medium nuts | - | 2.227 | - |
| Big nuts | - | 1.133 | - |
| Very big nuts | - | - | 3.637 |

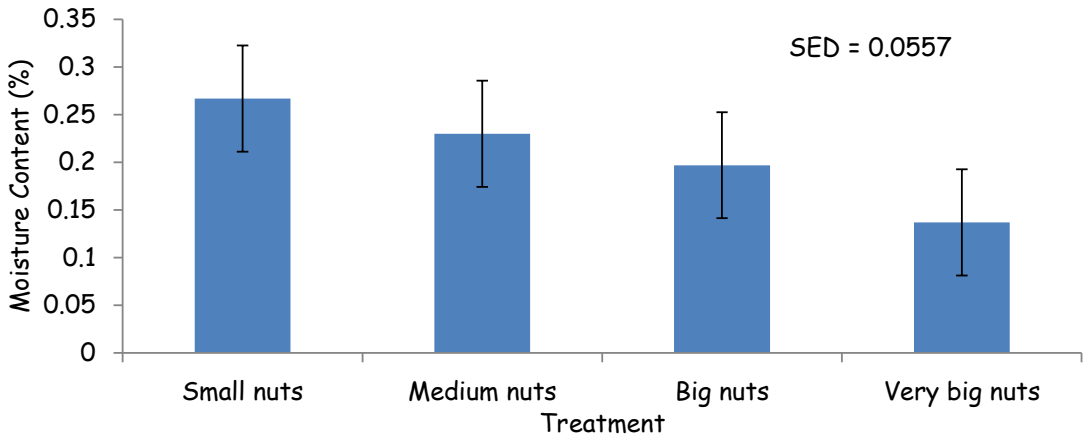


**Figure 2.** Effect of shea nut size on moisture contents of butter.

## Effect of nut size on moisture content of shea butter

The result of the mean moisture contents of shea butter extracted from the different shea nuts is presented in Figure 2.

The results indicate that there were no significant differences in the mean moisture content values of shea butter extracted from the shea nuts of different sizes. Butter extracted from very big shea nuts has the least amount of moisture with a mean moisture content of 0.137% with small shea nuts recording the highest moisture content of 0.267% (Figure 2).

The Ghana Standards Authority criteria indicated that shea butter extracted from very big and big shea nuts with mean moisture contents of 0.137 and 0.197% respectively, were grade 2 while butter from medium and small shea nuts with mean moisture contents of 0.230 and 0.267% respectively, were grade 3 (Table 3).

## Sensory qualities of shea nut butter

The sensory qualities of shea nut butter studied were color, texture, taste and aroma. These qualities were

**Table 3.** Ghana Standards Authority grading of moisture content of shea butter.

| Treatment | Grade 1 (0.05%) | Grade 2 (>0.05-0.2%) | Grade 3 (>0.2-2.0%) |
|---|---|---|---|
| Small nuts | - | - | 0.267 |
| Medium nuts | - | - | 0.230 |
| Big nuts | - | 0.197 | - |
| Very big nuts | - | 0.137 | - |

**Table 4.** Sensory qualities of shea nut butter as assessed by forty respondents.

| Treatment | No. of respondents who agreed on purity of butter colour | No. of respondents who agreed on the smoothness of butter texture |
|---|---|---|
| Small nuts | 16 | 18 |
| Medium nuts | 12 | 11 |
| Big nuts | 8 | 7 |
| Very big nuts | 4 | 4 |

observed based on indigenous knowledge. Individual participants did not find any distinction in the taste and aroma of the shea butter extracted from the different shea nut sizes. There were differences in colour and texture of the shea butter extracted from the different shea nut sizes (Table 4).

Table 4 indicated that for color, sixteen people observed shea butter extracted from small nuts to be the whitest, followed by butter from medium nuts with twelve people, then butter from big nuts with eight people and finally, butter from very big nuts with four people observing it to be the whitest.

For texture, 1 people observed shea butter extracted from small nuts to be the smoothest, followed by butter from medium nuts with eleven people, then followed by butter from big nuts with seven people and four people observing butter from very big nuts to be the smoothest.

## DISCUSSION

### Free fatty acid (FFA)

Shea nut butter extracted from the big nuts recorded the lowest mean FFA value with shea nuts from the very big nuts recording the highest mean FFA value. The lower mean FFA value for the shea nut butter extracted from big shea nuts may be attributed to their lower moisture contents.

The study revealed that, generally the bigger the shea nut kernels, the lower the FFA values and the higher the quality according to Ghana Standards Authority GSA (Figure 1). The only exception was the very big nuts which had very high FFA content. This indicates the need for further classification and categorization of nuts in repeated experiments to arrive at a conclusive relationship between the nut size and the FFA content

Shea nut butter quality is high when its free fatty acid content is low. Free fatty acid content is naturally low in fresh nuts, but it increases rapidly through hydrolysis under poor storage conditions. Hydrolysis occurs through the lipolytic activity of the fruit lipase and micro-organisms and halted by heating by reducing the moisture content to levels lower than 8%.

Results obtained by grading the four different shea nut sizes revealed that shea nut butter from big nuts, medium nuts, and small nuts were graded as high quality butter (grade 2) and can meet the requirement of the food industry (confectionary, chocolate, edible oil or as basis for margarines) based on the GSA grading criteria. However, shea nut butter from very big nuts was graded as low quality butter (grade 3) and may serve the needs of the soap making industries or can be refined for direct consumption (Table 2).

### Moisture content

Shea nut butter extracted from the very big nuts recorded the lowest moisture content with shea nut butter from the small nuts recording the highest moisture content. The results show that, the bigger the nuts, the lower the moisture content and this may be attributed to the fact that, bigger nuts have a larger surface area and are therefore subject to more rapid drying than smaller nuts (Figure 2).

Results obtained from grading the four different shea nut sizes based on GSA grading criteria on moisture content revealed that, shea nut butter extracted from very big nuts and big nuts were graded as high quality butter (grade 2), and may serve the needs of the food industry (confectionary, chocolate, edible oil or as basis for mar-

garines). Whiles butter from medium nuts and small nuts were graded as low quality butter (grade 3) and may serve the needs of the soap-making industry or may be refined for direct consumption (Table 3).

Furthermore, shea nut butter with low or no moisture content stores better than one with high moisture content. This is because shea nut butter with higher moisture content is prone to spoilage within one year of storage (Yidana, 2007).

Also, high moisture content in plant fats and oils usually leads to increased microbial load and lipid oxidation resulting in rancidity (Hee, 2011).

## Sensory qualities

The colour of the shea nut butter samples after extraction maintained a bright colour with slight differences among them. Sensory analysis conducted by Akingbala et al. (2006) found that unrefined shea nut butter gained lower scores than refined shea nut butter based on color. However, refining procedure also caused the loss of minor but valuable components such as unsaponifiable fraction with medicinal properties (Tasan and Demirci, 2005; Moharram et al., 2006; Van Hoed et al., 2006).

Texture of the extracted shea nut butter was similar to commercially available body butter or vaseline. Since the shea nut butter samples were all solid at room temperature, the first feeling was to take a small portion from the bulk shea nut butter using the fingertip but it was not as smooth as creams or lotion. However, once applied on the skin, all the samples were melted and showed nice creamy texture when spread over the back of the hand.

The results obtained from the study however indicated that for shea nut butter colour, butter extracted from small nuts was the brightest, followed by shea nut butter from medium, big nuts and very big nuts in that order respectively. The texture of the shea nut butter from the small nuts was the smoothest, followed by medium, big and very big nuts respectively in that order. The variations in colour and texture of the shea nut butter extracted from the different shea nut sizes may be probably due to some varying responses of the nut sizes to practices in the extraction process especially washing and roasting of the shea nut kernels.

The quality criteria usually used by buyers at the market level to assess shea nut butter for purchases are color and texture since the two characteristics of shea nut butter may be easily assessed visually and by feeling. Bright solidified shea nut butter with smooth texture is usually preferred by buyers.

## Conclusion and recommendation

The research was carried out to assess the chemical and physical characteristics of shea kernel samples obtained from the Zoolanyili community in the Tolon District of Northern Region. The usefulness of shea nut butter is based on its chemical composition. However, the market value of the shea nut butter is determined by both the physical appearance and the chemical composition. The results of the FFA and moisture content test carried out indicated that nut size have a significant effect on the quality of the extracted shea nut butter.

It is recommended that, further research be carried out at different geographical locations so as to provide more thorough understanding of the effects of shea nut kernel size on the quality of shea nut butter.

## Conflict of interests

The authors did not declare any conflict of interest.

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academicJournals

Vol. 9(4), pp. 243-251, April, 2015
DOI: 10.5897/AJFS2013.1118
Article Number: C6F504551617
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Development and evaluation of egg based ready-to-eat (RTE) products in flexible retort pouches

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Received 29 November 2013; Accepted 2 March 2015

**Effect of retort (thermal) processing on the shelf life and safety of ethnic Indian food products namely egg curry and egg burji were investigated. Ready-to-eat egg products were packed in four layer laminated retort pouches and processed in a steam-air retort with overriding pressure. Time-temperature profile of thermal processing was determined and the same was used for heat penetration characteristics. The thermal processing parameters like retort temperature, heating lag factor ($J_h$), heating rate index ($f_h$), process time (B), $F_0$ value and cook value ($C_g$) were determined. The retort processed egg products were analysed for microbiological sensory and chemical characteristics under ambient (27-30°C) and accelerated temperature (45°C) for a period of 12 months. Microbiological analysis indicated that retort processing has significantly reduced the microbial loads ($P < 0.05$). The changes in chemical characteristics and sensory quality on storage were insignificant. Microbiological analysis revealed that product was commercially sterile and fit for consumption. The samples were rated excellent by the taste panel and remained in good condition even after 12 months of storage under ambient conditions.**

**Key words:** Retort processing, egg curry, egg burji, free fatty acid, peroxide value and cook value.

## INTRODUCTION

There is an increasing consumer demand for high quality convenient ready-to-eat (RTE) food products and has led to an increase in the commercial production of ready-to-eat products (Kamatt, et al., 2005, Karadag and Gunes, 2008). Retort processing has evolved significantly since its incorporation into Department of Defence (DoD) and NASA food systems. While the technology still relies on aggressive application and penetration of heat throughout foods, recent advancements in process engineering coupled with evolution of packaging technologies have allowed for an overall improvement of the technology (Lopez, 1987; Goddard, 1994; Jun et al., 2006). The current state of the art retort pouch processing has increased commercial value and can offer to consumers a level of quality, safety and convenience not realized by other means (Brody, 2002). Recent work has also

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suggested that the unique properties of retort pouches allow maximum heat penetration and reduction of nutrient losses associated with standard processing of cans (Chia et al., 1983).

Packaging has a large influence on the shelf-life of food products (Rodriguez et al., 2002). Rigid metal containers are still the most common packaging type for thermally sterilized food, but there are other packaging types with many advantages. The retort pouch is a packaging type that allows faster heat transfer than the traditional metal or glass containers, owing to their slimmer profile, or more specifically, the higher surface area to volume ratio (Awuah et al., 2007; Rodriguez et al., 2002). The same microbial lethality can be achieved with a 30-50% shorter processing time, as compared to retorting of metal cans. The difference in processing time is largest for foods in which there is no natural convection in the case of metal cans (Snyder and Henderson, 2007). Most retort pouches are constructed as four-ply laminates of different packaging films that can withstand high process temperature and pressure (Jun et al., 2006). The typical retortable pouches consist of 12 µm polyester, 15 µm nylon, 9 µm aluminium foil, 80 µm cast-polypropylene. Outer polyester (polyethylene terephthalate) layer is for heat resistance and printability, an aluminium foil layer as a barrier for oxygen and light, biaxial oriented nylon for resilience, and an inner layer of cast polypropylene for pack sealing (Shihab et al., 2013; Holdsworth and Simpson, 2007). An adhesive between each layer bind the materials. Instead of using aluminium foil, some pouch materials contain silicon oxide (Holdsworth and Simpson, 2007). There is evidence that wild birds' eggs were included in the diets of primitive people long before the development of agriculture. Today, egg remain a staple food within the human diet, consumed by people throughout the world. They are recognised by consumers as versatile and wholesome and they have a natural balance of essential nutrients. However, eggs have to compete for sales with an increasing number of other products in the modern food industry and to compete successfully they have to overcome certain disad-vantages. For example, they are fragile and they deteriorate in quality with age. Furthermore, due to the natural variability in the major components of egg such as the albumen, the yolk and the shell, it poses a great challenge to meet the current demand and expectation of the consumer. Eggs consist of approximately 9.5% eggshell (including shell membrane), 63% albumen and 27.5% yolk (Cotterill and Geiger, 1977).

The main components are water (75%), proteins (12%) and lipids (12%), as well as carbohydrates and minerals (Li-Chan et al., 1995). The proteins are distributed throughout the egg, with the majority found in the egg yolk and egg white, and a small proportion in the eggshell and shell membrane (Mine, 2002). The lipids are found almost exclusively in the egg yolk, mainly in the form of lipoproteins. Several minerals have also been found in eggs, most of them in the eggshell. Carbohydrates are a minor egg component, present throughout the egg, as both free and conjugated forms, attached to proteins and lipids. The egg white or albumen makes up ~60% of the total egg weight of which water and protein are the major constituents. Egg white protein includes ovalbumin, which is the major protein followed by ovotransferrin and ovomucoid. The protein ovomucoid, is responsible for the viscosity of the albumen, lysozme, avidin, cystatin, ovoinhibitor and ovomacroglobulin (ovastatin).

Ready-to-Eat (RTE) foods are pre processed foods which are normally packed and served or consumed when required. Technological innovations, particularly in the field of food processing equipment, processing and packaging materials have brought about revolutions in the field of RTE. Indian RTE food scenario is exhibiting tremendous growth rate in the recent years and today it has become a multi billion industry with large number of firms involved. The changes in the socio-economic pattern of the society, like the changing life style, increasing number of working women, increase in the family income of people, awareness about healthy foods, changes in the meal pattern and existing food habits, desire to taste new food products have all contributed to the growth of RTE industry. Ready to eat thermally processed foods have the additional advantage that they can be stored for a period of more than one year without employing cold chain. Therefore, in this study, an attempt has been made to develop shelf-stable ready-to-eat egg curry and egg burji using retort pouch processing technique as well as to evaluate the changes in quality attributes under different storage.

## MATERIALS AND METHODS

### Egg products preparation

The ingredients used for the preparations of egg curry and egg burji are given in Table 1.

#### *Egg curry*

Eggs were boiled in water for 15-20 min, cooled and peeled off outer shell and cut into halves vertically. Gravy was prepared as per the standardised procedure. Gravy was filled in pouches followed by boiled egg pieces, by maintaining the ratio of drain weight.

#### *Egg Burji*

Egg bhurji was prepared as per the standardised procedure. The prepared egg bhurji was filled and sealed in retort pouch according to the required weight.

### Retort pouch processing of RTE products

Retort pouch processing of egg products were carried out in a steam-air retort, method followed by Kumar et al. (2013). The retort was equipped with facility for using compressed air for over-riding

**Table 1.** Ingredients used for the preparation of Egg curry and Egg burji

| Egg curry | | Egg burji | |
|---|---|---|---|
| **Ingredient** | **Weight** | **Ingredients** | **Weight** |
| Egg (Nos.) | 40 | Egg (Nos.) | 80 |
| Onion( kg) | 1.5 | Onion( kg) | 2 |
| Tomato (kg) | 1 | Tomato bit (g) | 500 |
| Ginger paste (g) | 100 | Garlic paste (g) | 50 |
| Dhania powder (g) | 70 | Dhania powder (g) | 70 |
| Chilli powder (gm) | 50 | Green chilli (gm) | 100 |
| Garam Masala (g) | 20 | Turmeric powder (g) | 5 |
| Turmeric powder (g) | 5 | White pepper powder (g) | 20 |
| Corn flour (g) | 30 | Oil (ml) | 40 |
| Oil (ml) | 200 | Salt (g) | 80 |
| Salt (g) | 150 | | |
| Water (L) | 3 | | |

pressure and a high-pressure water-circulating pump for pressurized cooling. The temperature of the product was continuously recorded during heat processing, through copper-constantan thermo couples, which were fixed at the geometric centres of the pouches. The pouches were placed at different locations in the retort. The temperature of the pouch and retort was calculated from the thermo-electro-motive-force at regular intervals of 1 min. The $F_0$ value was calculated from the temperature and time history. The pouches were initially heated till the inside temperature reached 100°C. Subsequently, the pressure of the steam was raised in stages; from 5 to 15 lbs gauge pressure with the increase of temperature progressively. The processing was carried out to achieve required $F_0$ value with maximum temperature of 121.1°C. After attaining the required $F_0$ value, the product temperature was brought down to 50-55°C by pressurized cooling (compressed air and water) in 4-5 min. The cooled pouches were wiped dry and examined for any visual defects.

#### Proximate and chemical analysis of the sample

Proximate analysis of the samples was estimated according to AOAC (1990). Percentage of free fatty acid (FFA) expressed as oleic acid and peroxide value (PV) were estimated according to the method of Ranganna (2000).

#### Microbiological analysis

The egg products were analysed for their commercial sterility. The pouches were incubated at 37 and 55°C for seven days. SPC was determined using dextrose tryptone agar (DTA) after incubation for 48 h at 30ºC. Yeast and moulds were estimated with the help of acidified potato dextrose agar (PDA), after incubation at 30°C for four to five days. Spore formers were determined after killing the vegetative cells by keeping the sample in boiling water bath for 10 to 20 min and subsequently incubated at 37 and 55°C for 48 h after incubation (Harrigan and McCance, 1976).

#### Storage

Egg products were stored under different temperatures, that is, cold storage (4-5°C), ambient temperature (27-30°C), and elevated temperature (45°C). The samples were analyzed periodically at 2 month interval for changes in peroxide value (PV) and free fatty acids (FFA).

#### Sensory evaluation

The egg products were evaluated at the interval of 4 months for quality and acceptability on a 9 point hedonic scale by semi-trained panellists with score 9 for samples excellent in all respects, while 1 for highly disliked ones (Ranganna, 2000).

#### Data analysis

All analysis were carried out in triplicate. The data were analysed statistically to find out standard deviations and significance (Snedecor and Cochran, 1988).

## RESULTS AND DISCUSSION

### Effect of retort processing on RTE egg products

The egg products were prepared as per the recipe standardized by this laboratory and retort processed as prescribed by Code of Federal Regulations. During retort processing, the core temperature of the products, which was measured by thermocouples, was found to increase gradually with the increase of processing time as shown in Figure 1. A reference temperature of 121.1°C was used to calculate the process lethality for *C. Botulinum*. A thermal resistance (z) value of 10 obtained for the similar products was used in this calculation. The come up time for the egg products to reach 100°C was 10-12 min. After attaining 100°C, the product was subjected to steam-air mixture (15 + 5 lbs) and the product temperature close to 118°C in 15-16 min. At 118°C, the product was held for 3 min. After achieving the desired $F_0$ value, the product temperature of 118°C was brought


**Figure 1.** Heat penetration characteristics and $F_0$ value for RTE Egg products.

**Table 2.** Thermal processing parameters for Egg based RTE products.

| Parameters | Egg curry | Egg burji |
|---|---|---|
| Retort Temperature (°C) | 121.1 | 121.1 |
| Heating lag factor $J_h$ | 0.38 | 0.38 |
| Heating rate index $f_h$ min | 17.3 | 16.0 |
| Process time (B) min | 21.0 | 20.1 |
| $F_0$ value | 7.77 | 7.46 |
| Cook value $C_g$ | 70.12 | 65.89 |

**Table 3.** Proximate composition for egg based RTE products.

| Parameters | Egg curry | Egg burji |
|---|---|---|
| Moisture (%) | 84 ± 1.05 | 64 ± 0.85 |
| Protein (%) | 4.5 ± 0.05 | 10.7 ± 0.01 |
| Fat (%) | 5.5 ± 0.02 | 16.5 ± 0.01 |
| Carbohydrate (%) | 4 ± 0.005 | 6.8 ± 0.003 |
| Energy Kcal/100g | 84 | 219 |

to 50-55°C by pressurized water cooling. The time-temperature history curves of the products are shown in Figure 1. The retort processed parameters like heating lag factor, heating rate index, total process time, $F_0$ values and cool values are reported in Table 2. The retort temperature was maintained at 121.1°C for both products. The $F_0$ values achieved was 7.77 and 7.46 for egg curry and egg burji, respectively. Our results are in accordance with other author like Frott and Lewis (1994) who studied retort processed meat products and recommended the $F_0$ value between 8 and 20 min for retort processed meat products. Ranganna (2000) also reported $F_0$ values between 8 and 12 for meat products. Gopal et al. (2001) also processed Kerala style fish curry at 121.1°C to $F_0$ values of 6.56 and 8.43 min. There are no literatures available regarding retort processed egg products like egg curry and egg burji in flexible laminated pouches. Whereas precooked specialty egg products such as egg patties, omelettes or cook-in-bag scrambled eggs are mainly commercialized in frozen form to fast food outlet chains in the food service industry (Baker and Bruce, 1995). However, precooked egg products, to be stored at room temperature, are not yet available in the market. In fact, only a few companies offer ready-to-eat scrambled eggs and whole hard-cooked/peeled eggs with longevity of 6 to 12 weeks at refrigerated conditions (AEB, 2003). The main challenge is to assure product safety during cooling, packaging and post packaging stages. This requires either additional thermal treatment, or an alternative treatment that has minimal effects on the product's final quality. Thermal processing is one of the important preservation technologies for RTE food products and can be stored at ambient temperature with minimum of one year shelf life.

The retort processed egg products were analyzed for their proximate composition as given in Table 3. Moisture (%), protein (%), fat (%), carbohydrate (%) and Energy (Kcal/100g) were 84 ± 1.05, 4.5 ± 0.05, 5.5 ± 0.02, 4 ± 0.005, 84 and 64 ± 0.85, 10.7 ± 0.01, 16.5 ± 0.01, 6.8 ± 0.003, 219 for egg curry and egg burji, respectively. The FFA percentage and PV were 0.15 ± 0.03, 0.16 ± 0.03

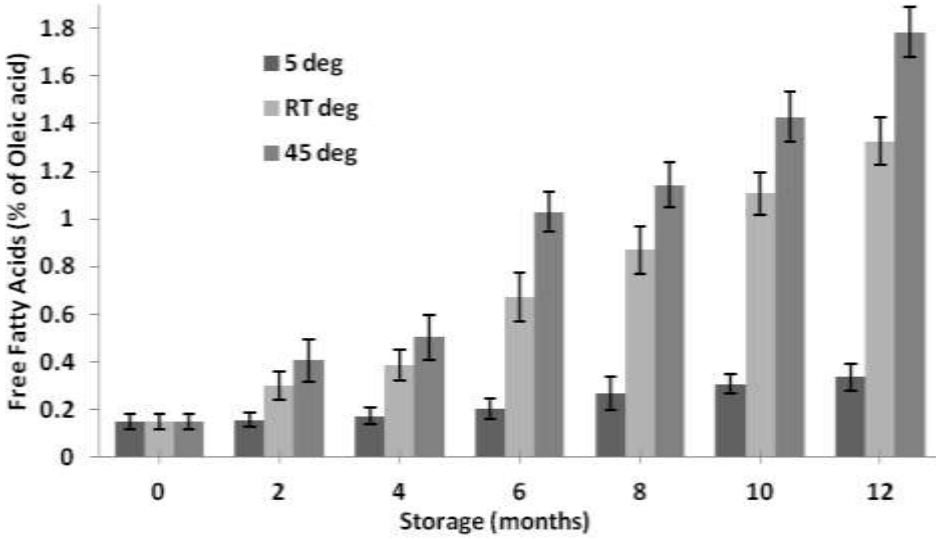


**Figure 2.** Changes in free fatty acid (FFA) content of retort processed egg curry under different temperature storage.

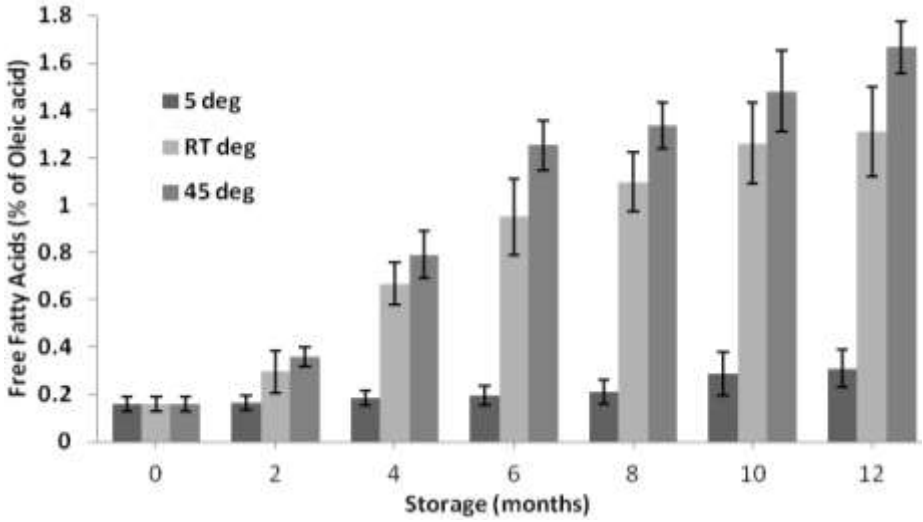


**Figure 3.** Changes in FFA content of retort processed egg burji under different temperature storage.

and 0.00 ± 0.00 for egg curry and egg burji, respectively, and the same is presented in Figures 2 to 5. The RTE egg products were stored under different temperatures: (4-5°C) ambient (27-30°C) and accelerated temperature (45°C) and analyzed for its quality deterioration.

## Effect of retort processing on micro flora in egg products

The microbiological analysis of the egg products were found to be nil and also the products remained commercially sterile during the entire period of the storage and confirmed the adequacy of the processing as well as it's safety for consumption (data not shown). The proper $F_0$ value of the products rendered the commercial sterility of egg curry and egg burji.

Our result were in accordance with authors (Kumar et al., 2007; Mohammedali et al., 2013; Agathian et al., 2009) who studied retort processed ready-to-eat food; they also found commercial sterility after retort process and the entire period of the storage under different temperature. The results were in agreement with the findings of other researchers. Rajkumar et al. (2010)

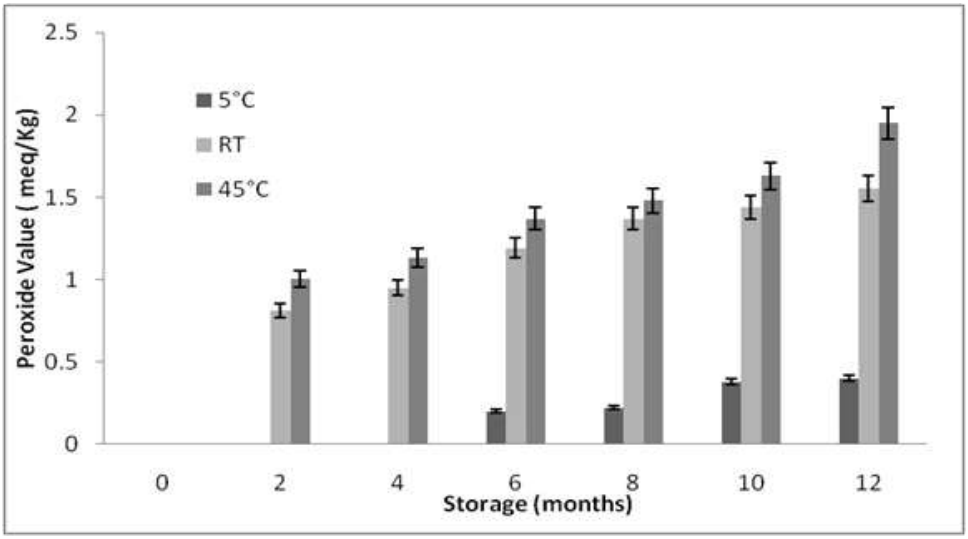


**Figure 4.** Changes in peroxide value (PV) content of Retort processed Egg Curry under different temperature storage.

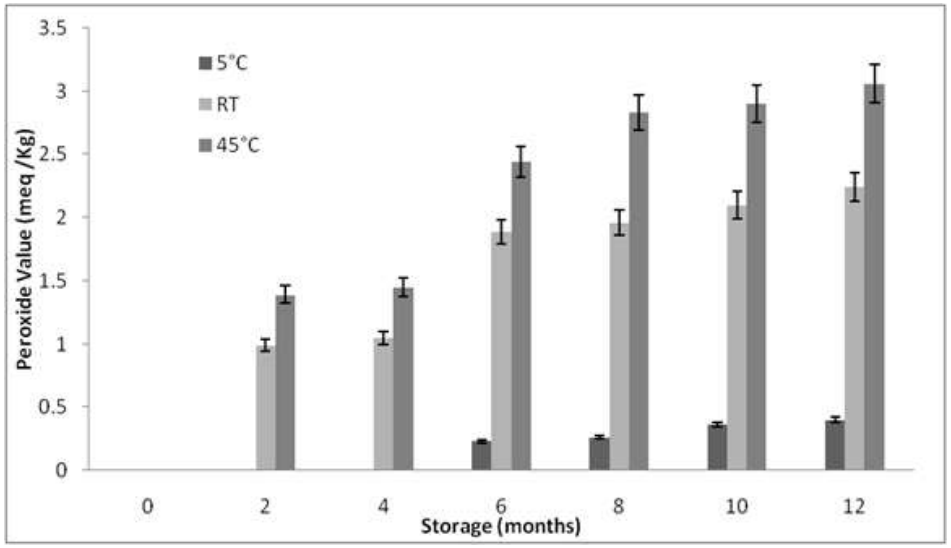


**Figure 5.** Changes in peroxide value (PV) content of Retort processed Egg Burji under different temperature storage.

determined total viable, anaerobic, coliform, staphylococcal, streptococcal, clostridial and yeast and mould counts of Chettinad goat meat curry retorted to an $F_0$ value of 12.1 min and showed that the product was commercially sterile.

## Effect of Retort processing on physico-mechanical and barrier properties of retort pouches

Physico-mechanical and barrier properties of the retort pouches were evaluated before and after processing (Table 4). The pouch material used is similar to or better as compared to those used in similar studies. For example it has a water transmission of <0.019 g/m$^2$. This can be compared with e.g. the 0.18 g/m$^2$ for the pouches used by Rajkumar et al. (2010) and 0.21 g/m$^2$ for the pouches used by Mohan et al. (2008). According to Rajan et al. (2011) a low water vapour transmission is an indicator of suitability of the pouches for retort processing. It is also positive for the shelf-life of the product. The strength of the pouch and seal is very critical and important for avoiding bursting during processing or handling. The seal strength is also an

**Table 4.** Physico-mechanical and barrier properties of multi layer laminate retort pouch.

| Parameter | [a]BP | [b]AP | Test Method |
|---|---|---|---|
| 12 µm Polyethylene terephthalate (PET) / 9 µm aluminium foil / 15 µm nylon / 70 µm cast polypropylene | | | |
| Total thickness µm | 110.00 | 110.00 | |
| Tensile strength (machine direction) kg/cm$^2$ | 446.58 | 437.49 | ASTM D 882 |
| Tensile strength (cross direction) kg/cm$^2$ | 358.00 | 349.00 | |
| Elongation at break (machine direction)% | 156.00 | 149.00 | |
| Elongation at break (cross direction) % | 124.00 | 112.00 | |
| Tearing strength (machine direction) g | 116.00 | 108.00 | ASTM D1004 |
| Tearing strength (cross direction) g | 102.00 | 96.00 | |
| Seal strength (top) kg/10 mm | 3.96 | 3.78 | ASTM F88/F88M |
| Seal strength (side) kg/10 mm | 4.86 | 4.26 | |
| Seal strength (bottom) kg/10 mm | 4.02 | 3.86 | |
| Gas transmission rate (ml/m$^2$/day) | <0.021 | <0.038 | ASTM F 2622-08 |
| Water vapour rate (g/m$^2$/day) | <0.019 | <0.032 | ASTM F 1249 |
| **Total migration (mg/kg)** | | | |
| Distilled water | 26.0 | 28.0 | |
| 3% Acetic acid | 38.0 | 42.0 | |
| 50% Ethyl alcohol | 26.0 | 29.0 | |
| n- Heptane | 24.0 | 26.0 | |

[a]Before retort processing; [b] after retort processing.

indicator of shelf-life (Rajan et al., 2011). The heat seal strength of the pouch used is 3.96 kg/10 mm if a sealing temperature of 220°C is used. This can be compared with the pouches used by Rajkumar et al. (2010) which showed a heat seal strength of 5.9-6.5 kg/15 mm. The processing effect on physico-mechanical and barrier properties of retort pouches were also evaluated and it was found that the effect of processing was insignificant. The packaging system based on aluminium foil has been reported to provide barrier against mass transfer, light and micro-organism and thus the moisture content of the product was almost retained till the completion of storage studies (Ghosh et al., 1980).

## Changes in FFA and PV during storage

Figures 2 and 3 shows changes in free fatty acid (FFA) content during storage at different temperature of retort processed egg curry and egg burji had no significant (P >0.05) effect on total acidity. The FFA content of egg curry and egg bhurji increased up to 1.32 ± 0.100%, 1.78 ± 0.105% and 1.31 ± 0.19%, 1.66 ± 0.11% under ambient temperature (27-30°C) and accelerated temperature (45°C) storage respectively. FFA correlates the possibility of breakage of long chain fatty acid chain into individual fatty acid moieties. Our results were in accordance with other authors (Aubourg et al., 1997; Aubourg et al., 1990) who studied the FFA changes in canned processed products. FFA content of the egg curry and egg burji was increased gradually, accelerated temperature (45°C) storage had a higher level when compared with other storage but the increase was within the acceptable level. Increase in FFA content was mainly due to the increased lipid hydrolysis at elevated temperature. Kumar et al. (2007), Mohammedali et al. (2013) and Agathian et al. (2009) also studied the retort processed ready-to-eat foods; and they also found a slight increase in FFA content was observed at the end of 12$^{th}$ month of storage period but the product was acceptable by the panellist.

The formation of peroxide during storage is slow at an induction period, the length of which will depend on the nature of fat and the presence of antioxidant. The observation also confirmed that the peroxide value of the product did not increased due to non-availability of oxygen, controlled by the packaging system (Figures 4 and 5). Peroxide values of egg curry and egg burji were increased upto 1.57 ± 0.09 meq/kg, 1.95 ± 0.17 meq/kg and 2.25 ± 0.135 meq/kg, 3.06 ± 0.135 meq/kg under ambient temperature (27-30°C) and accelerated temperature (45°C) storage, respectively. The increase in peroxide value has no significant (p > 0.05) changes as compared to samples stored under lower temperature (4-5°C). It is clearly indicating that the rancidity formation was temperature dependent. Other authors (Kumar et al., 2007; Agathian et al., 2009; Mohammedali et al., 2013) also found a slight increase in the peroxide value of retort processed samples when stored under different storage temperatures. The lipid oxidation was attributed to the

combination of free radicals with oxygen and to form hydroperoxides (Gracey et al., 1999). Hence, the free fatty acids of the egg products were less with the non availability of oxygen rendering less production of hydroperoxides in retort pouch processed egg products.

### Effect of retort processing on sensory analysis in egg products

The sensory analysis of egg curry and egg burji using a 9-point hedonic (data not shown) revealed that initially the product scored 8.6 ± 0.32, 8.5 ± 0.28 for colour, 8.5 ± 0.26, 8.4 ± 0.23 for flavour, 8.4 ± 0.15, 8.5 ± 0.25 for taste, 8.4 ± 0.28, 8.4 ± 0.21 for texture and 8.4 ± 0.22, 8.4 ± 0.23 for the overall acceptability respectively. On storage, the sensory scores of the product were decreased under ambient temperature (27-30°C) and accelerated temperature (45°C) storage conditions. Under ambient conditions, the sensory scores decreased to 8.1 ± 0.22, 8.1 ± 0.24 for colour, 8.1 ± 0.30, 8.1 ± 0.30 for flavour, 8.1 ± 0.25, 8.1 ± 0.25 for taste, 8.0 ± 0.24, 8.0 ± 0.14 for texture and 8.0 ± 0.25, 8.0 ± 0.25 for the overall acceptance. At 45°C, the decrease was 8.0 ± 0.10, 8.0 ± 0.19 for colour, 8.0 ± 0.12, 8.0 ± 0.20 for flavour, 8.0 ± 0.22, 8.0 ± 0.30 for taste, 8.0 ± 0.38, 8.0 ± 0.28 for texture and 8.0 ± 0.30, 8.0 ± 0.32 for overall acceptability for egg curry and egg burji, respectively. These results clearly indicate the effect of storage conditions on the quality attributes of the product. Our results were in accordance with that of Gopal et al. (2001) who evaluated Kerala style fish curry and showed an overall acceptance of 8.0 on a 9-point scale rating after heat treatment, which decreased to 7.5 after 12 months of storage. Rajkumar et al. (2010) also evaluated appearance, colour, flavour, juiciness, texture and overall acceptability for Chettinad goat meat curry, showing scores of 8.0-8.4 on a 9-point hedonic scale after heat treatment.

### Conclusion

Results from the temperature measurements and microbiological tests showed that the products were commercially sterile throughout the storage period. The changes in FFA and peroxide value of thermally processed egg curry and egg bhurji were insignificant, and it is possible to develop shelf stable egg products in retort pouches with good quality.

### Conflict of interests

The authors did not declare any conflict of interest.

### ACKNOWLEDGEMENT

The authors express deep gratitude to The Director, Defence Food Research Laboratory, Mysore, for his constant support and encouragement.

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academicJournals

Vol. 9(4), pp. 252-256, April, 2015
DOI: 10.5897/AJFS2014.1204
Article Number: B1EFD8451623
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

*Full Length Research Paper*

# Bacteriological quality of garri sold in Owerri open markets, Imo state, Nigeria

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Received 30 August, 2014; Accepted 16 March, 2015

**Garri as popularly known in Nigeria is a general food consumed by most people in Nigeria. It could be eaten by reconstituting it with hot water, stirred to form a thick paste and eaten with soup or stew. It could also be taken dried or mixed with cold water and sugar/milk, as a snack. As a part of check mating the public health risk associated with this general dependence of the population on garri, the bacteriological quality of garri sold in Owerri open market was examined with the aim of investigating the bacterial contamination of garri due to exposure in the market as well as establish the hygienic statue of garri taken as snacks by many Nigeria. To achieve this, a total of one hundred and ten (110) garri samples were collected from Eke-ukwu market, relief market, Eke-Mmegbu market, Orji market, IMSU gate market and some local garri processing factories within Oweri metropolis to serve as control. The samples were analyzed bacteriologically for viable heterotrophic bacteria and coliform bacteria counts on Nutrient and MacConkey agar respectively, using pour plate method. The mean value results from all the markets revealed high bacterial contamination, except from the factory. The resultant data were analyzed statistically using Chi-square to determine if there is a significant difference between the five markets. When calculated, the value of the five markets was 35.75 while the tabulated was 11.07. This implies that there was a significant difference between the five markets. Identified bacteria included: *Staphylococcus aureus, Staphylococcus epidenmidis, Bacilus cerus, Escherichia coli* and *Klebsielia aerogenes*. Isolation of these bacteria is a sign of danger, hence, Imo State government is advised to take measures such as making environmental sanitation a priority project in the state to save the lives of the citizens.**

**Key words:** Market, sample, snack, organism, contamination, Owerri.

## INTRODUCTION

"Garri" as popularly known in Nigeria is a staple food for most Nigerians. It is prepared by fermenting grated fresh cassava (*Manihot esculenta* (rantz) roots. This cassava originated from tropical America and is cultivated today in all tropical regions of the World (Scolt et al., 2000). It could be eaten, by reconstituting with hot water, stirred to form a thick paste and eaten with soup or stew and could be eaten dried or mixed with cold water and sugar-/milk, taken as a snack (Nweke, 1988).

In Nigeria, the sales and distribution of garri in local markets is associated with practice such as displaying of the products in open buckets, bowls and mats at points of

sales and the use of bare hands during handling and sales. These unhygienic practices, may lead to microbial contamination due to deposition of bioaerosols on exposed products (Amadi and Adebola, 2008).

There are many factors that could result to food contamination. Trickett (1992), listed his own sources of contamination. According to him, the larger the surface area of the food exposed, the higher the load of micro organism, as well as the greater availability of oxygen for the metabolic activities of aerosol organisms.

Microorganism, especially bacteria vary from species to species in nutritional requirement (Asegbeloyin and Onyimonyi, 2007). Their presence in food at any stage, depend on the nutritional status of the food at that stage, temperature, water content, pH as well as the nature of the organism. The bacteria that cause food poisoning have a similar nutritional requirement with that of human (Baine, 2000). He also states that food poisoning could have been minimized, if the food producers and processors are trained in safe – food – handling and consumers are better adviced in the choice of food.

Microorganism associated with food exposed to environment are *Salmonella* Typhi which was incriminated in *Salmonella* food poisoning outbreak in Germany and Great Britain in 1988 and 1971 respectively (Tietjen and Fung, 1980), *Shigella flaxner* associated with food contaminated with feacal materials. Others include: *Bacillus cerus*, *pseudomonas* spp.; *clostridium* spp.; *Klebsiella* spp.; and *S. aureus* (Nkanga and Nduka, 1980; Ijebadeniyi, 2007). In Nigeria, it has become a common practice to eat garri raw or as snacks especially among students who resort to it as faster alternative to preparation of cooked food, without considering the bacteriological implication. This work therefore is aimed at investigating the bacterial contamination of garri due to exposure in the market, as well as to establish the hygiene status of garri taken as snacks by many Nigerians.

## MATERIALS AND METHODS

### Sample collection

Garri samples used for this study were purchased randomly on two spaced time points between the month of January and November 2012 from sellers in five selective open markets: Eke-ukwu, Relief, Eke-Mmegbu, Orji and IMSU gate, as well as from different local garri processing factory within the locality to serve as control. A total of one hundred and ten (110) samples were obtained, twenty (20) samples from each market and ten from factories. The samples were collected into sterile polyethylene bags, using standard procedure and were transported to the microbiology laboratory of the Department of Medical Laboratory Science, Imo State University, Owerri for analysis within 3 h of collection.

### Preparation of samples

Ten (10) grams proportion of each sample was aseptically weighed after thorough mixing, transferred into a sterile 500 ml beaker containing 90 ml of peptone water and allowed to soak for 5 minwith occasional stirring using sterile glass rod. The supernatant was decanted into another sterile beaker and 10 fold serial dilutions of the sample supernatant were prepared by transferring successsively 1 ml aliquot of the supernatant into 9 ml of sterile distilled water up to $10^{-3}$ dilutions (Osoagbaka, 1996).

### Cultivation of samples

0.1 ml aliquot of each dilution was plated on nutrient agar (Biotec) for total viable heterotrophic bacterial counts and MacConkey agar (Biotec) for total coliform counts by pour plate method. The plates were incubated at 30°C for 24 h. At the end the incubation period, descrete colonies were enumerated and expressed as log of colony – forming units per gram (log CFU/g) of sample.

Representative bacterial colonies obtained after incubation were purified by subculturing on nutrient agar using the streak method. The purity of isolates was determined using the Gram's stain reaction. The purified isolates were then characterized and identified using their colonial morphologies and biochemical characteristics as described by Cheesbrough (2000) and with reference to the bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

### Statistical analysis

Chi-square statistical method was adopted to determine if there is a significant difference between the five markets.

## RESULTS

Table 1 shows the prevalence of Bacterial contamination distribution among the different market samples; relief market recorded 100% contamination, followed by Eke-Ukwu (90%) and IMSU gate with the least contamination of 75%.Table 2 shows specific sites of bacterial contaminants. This indicates that *Staphylococcus* was the predominate isolate, while *E. coli* was isolated from four out of the five markets. *B.cereus* and *Staphylococcus epidemidis* were isolated from three different market each and *Klebsiella aerogenes* was isolated from only one market.

Figure 1 shows the rate of occurrence of the different isolate. *S. saprophyticus* occurred in 35 representing 37% occurrence, *E. coli* in 24 (25.5%), *B. cereus* 18(19.2%), *K. aerogenes* 10 (10.6%) and *S. epidemidis* 7

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**Table 1.** Prevalence of Bacterial contamination of different market samples.

| Parameter | Eke ukwu | Relief | Eke mmegbu | Orji | Imsu gate | Control factory | Row total |
|---|---|---|---|---|---|---|---|
| No of Samples collected | 20(21.11) | 20 (22.22) | 20 (20.00) | 20 (20.56) | 20 (19.44) | 10 (6.67) | 110 |
| No of Samples that yielded growth | 18 (16.89) | 20 (17.78) | 16 (16.00) | 17 (16.44) | 15 (15.56) | 2 (5.33) | 88 |
| Coloumn total | 38 | 40 | 36 | 37 | 35 | 12 | 198 |

The value of the five markets =35.75. The value of tabulated =11.07.

**Table 2.** Specific sites of Bacterial contaminants.

| Bacterial isolates | Eke-Ukwu | Relief | Eke Mmegbu | Orji | IMSU Gate | Factory (control) |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | + | + | + | + | + | + |
| *Staphylococcus epidermdis* | + | + | - | - | - | - |
| *Bacillus cerus* | + | - | - | + | + | + |
| *Escherichia coli* | + | + | + | + | - | - |
| *Klebsiella aeroganes* | - | - | + | + | - | - |

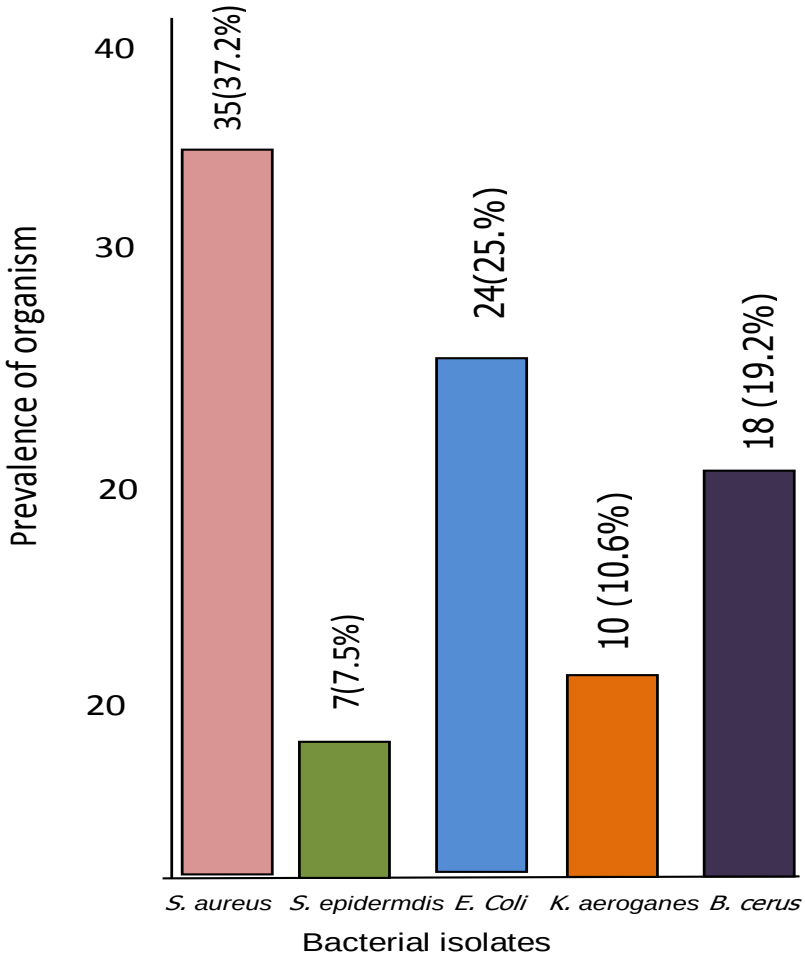


**Figure 1.** Chat representation of the occurrence of different isolates.

(97.5%).The heterotrophic count when compared with the control showed a statistical significant increase at P>0.05.

The coliform count of Eke- Ukwu, Relief, Eke-Mmegbu and Orji when compared with the control showed a statistical significant increase at P > 0.05 while that of IMSU gate showed a statistical decrease at P< 0.05.

## DISCUSSION

In addition to death and ill health caused by food poisoning, individuals, families, health care system and society, as well as commercial enterprises incure tremendous economic lost. These lost include, loss of income due to the cost of medical care, the cost of investigating food contamination outbreaks, loss of income due to closure of business, legal costs and fine (Baine, 2000). Hence taking "Garri" dry as snacks or with cold water is exposure to health risk due to the microbial status.

This study reveals high prevalence of bacterial contamination of samples from different markets, when compared with that of control. Relief market has the highest prevalence of 100%. This may be attributed to dirty environment associated with these markets. Indiscriminate dumping of refuse is a common practice around these markets. This is in agreement with the results of Trickett (1992) who listed his own sources of food contamination among other factors to include, dust, and waste products from the environment.

The 20% prevalence recorded against the control can be attributed to the unskilled nature of the garri producers who introduce contaminants to their products, especially during the cooling and packaging phase of their production. This corroborates the reports of Baine (2000) who states that food poisoning could have been reduced if the food producers and processors are educated and trained in safe food handling and consumers are better adviced in their choice of food.

The coliform count of IMSU gate when compared with that of control showed significant difference against other markets. This decrease can be attributed to the fact that IMSU gate market is not a large market, rather a mini market and so does not accommodate large population. Refuse disposal in this market is more properly managed than what is obtainable in other markets. The major source of contamination in this market is likely to be from the sellers and the aerosols. This supports the observation of Mankee et al. (2003) who states that the vendors can be carriers of pathrogens like *E. coli*, *Salmonella*, *Shigella*, *Campylobacter* and *S. aureus,* who eventually transfer these food borne harzards to consumers.

The distribution of the organism varied from the different markets. Eke – Ukwu market is located at the heart of the town with great number of people coming in for marketing purpose. Relief market though not completely at the heart of the town, harbours many refuse dumps, gutters and water logged areas containing stagnant water from sewages, hence, recorded more contamination than others. This corroborates Almeida, (1994) who attributes the variation in distribution of organisms to environmental condition and practice of the food handlers.

From the result of this work, *Staphylococcus* spp. *(S. epidermidis* and *S. aureus*) had the highest rate of occurrence with a total of 44.7% (37.2 and 7.5 respectively). They might have found their way into the garri through carriers, since the organisms are found around the nose, throats, hands and clottings of these carriers (Cheesbrough, 2000).

The isolation of *E. coli* and *K. aerogenes* from some of the markets, though, bellow infection causing value (*E.coli*: ≥1.0 x $10^6$ and *Klebsiella erogenous* ≥ 5.9x$10^3$) (Solberg et al., 1976) indicates recent feacal contamination which can be attributed to the refuse dumps as well as the stagnant waters from sewages seen around some of these markets. This is in agreement with the results of Dike- Ndudim et al. (2014) who attribute the isolation of the same organisms from smoked fish sold in Owerri to feacal contamination of water sources in Owerri.

The result of this study has proven that "Garri" which were sold in Owerri markets are highly contaminated with pathogenic organism. Although, the coliform (CFU/G) fall below the value that can result to infection, this implies that, increase intake of garri as snacks among people of Owerri may lead to a disease condition such as gastro enteritis.

We therefore suggest that the state government establishes standard garri processing industries to be managed by trained personnel in food handling, to reduce contamination due to poor handling. Also, environmental sanitation should be taken as a priority project to reduced food-borne disease. Finally, residents of the state should be educated on the dangers of food poisoning to enhance personal hygiene.

## Conflict of interest

Authors did not declare any conflict of interest.

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