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Phytosterol supplementation improves antioxidant enzymes status and broiler meat quality

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ABSTRACT

The present study was aimed to assess the phytosterol supplementation on broiler meat quality and mechanism involved. One hundred and twelve days old Avian male Ross broiler chickens were offered commercial diet (control), second group fed with Castastesrone, a kind of Polyhydroxy phytosterol, (15, 20, and 25 g/kg diet) and third group fed with, β-sitosterol, a kind of hydroxyl phytosterol, (25, 50, and 75 g/kg diet) for 21 days. Phytosterol showed non-significant ($p<0.05$) effect on the feed efficiency and feed conversion ratio (FCR), however body weight momentously increased in phytosterol group. The body weight showed significant correlation with hydroxyl Phytosterol content. Fast growing chickens have larger diameter fibers than slow growing lines due to supplementation of β-sitosterol. Supplementation of 50g and 75g β-sitosterol resulted in the body weight of 656.07±12.52g and 647.13±7.45g, respectively. Moreover, antioxidant capability of broilers was improved significantly ($p<0.05$) by the addition of dietary phytosterol in feed. Finally, phytosterol addition did not affect the texture properties ($p<0.05$) of meat. The present results suggest that phytosterols improve meat quality by increasing the antioxidant enzyme levels of broiler chickens.

Keywords: Polyhydroxy Phytosterol; Hydroxy Phytosterol, Antioxidant status, Meat quality, Broiler chicken

INTRODUCTION

The consumption of poultry meat has become very popular due to their nutritional characteristics. In fact, chicken meat supplies high protein (around 20 g/100 g raw meat without skin) and low fat intakes (around 5 g/100 g raw meat without skin). It delivers essential vitamins and minerals and is among the most affordable meat sources (Buzby and Farah, 2006; Jinap *et al.*, 2013). Currently there is a focus on the development of functional poultry products capable of enrichment by phytosterol, iodine and unsaturated fatty acids. The oxidative stability of meat depends on anti and pro-oxidants balance, composition of oxidation substrates including polyunsaturated fatty acids (PUFAs), cholesterol, proteins and pigments (Yasin *et al.*, 2013). PUFAs are more susceptible to lipid oxidation and due to it chicken meat is more susceptible to oxidation (Yasin et al., 2013: Sohaib *et al.*, 2012). Consumption of poultry meat is constantly increasing and meat nutrition quality depends largely on composition of poultry feed (Schwartzkopf-Genswein *et al.*, 2012).

The broilers diet consists of cereals (wheat, corn, etc.) in general, but biological active additives are widely used in commercial broiler feed for enrichment of their nutritional value. In this regard, phytosterol has been regarded as an essential dietary supplement which is important for improving health and meat quality for human consumption that may boost immune function and reduce cancer risk as well (Bradford and Awad, 2007). Phytosterols are naturally occurring steroid alcohols and have a chemical structure which is similar to that of cholesterol. They are made up of tetracyclic cyclopenta phenanthrene ring and a long flexible side chain at C17 carbon atom (Moreau et al., 2002). More than 200 different types of phytosterols have been reported in plant species, the most abundant being-sitosterol (24- ethylcholesterol), campesterol (24-methylcholesterol) and stigmasterol (22, 24-ethylcholesterol). In general, vegetable oils and products derived from oils are regarded as richest natural sources of sterols, followed by cereal grains, nuts and vegetables. Metabolisation of phytosterols has been demonstrated in higher organisms (Moreau R.A. 2005) The physiological effects of phytosterols and mainly their cholesterol lowering properties have increased interest for their use in food products. Moreover, there is growing interest in quantification of phytosterols either intrinsic or added in foods. Phytosterols has attained remarkable attention to replace animal fat (cholesterol) in animal feed due to bovine spongiform encephalopathy (BSE) and dioxin crisis (Katan *et al.*, 2003). The aim of food researchers is to increase the nutritional value of food

without affecting sensory quality or consumer's acceptability. Human health may be improved with increased intake of biologically valuable ingredients. The food models gave simplified but multilevel insights into oxidative behaviour of phytosterols in common food lipids. In fact, combined feed with phytosterols and unsaturated fatty acids have been shown not only complementary but also synergistic effects on circulating lipid levels, without any adverse effects (Bradford and Awad, 2007; Moreau R.A. 2005). Therefore, combination of phytosterols and unsaturated fatty acids may offer greater cardiovascular benefits than alone (Nurulhuda *et al.*, 2013). The positive impact of food on human health becomes the basis for development of "functional foods" that displayed therapeutic effects. Functional food preparation resumed to use hypocholesterolemic properties of phytosterols (Moreau R.A. 2005; Nurulhuda *et al.*, 2013). Despite vast therapeutic benefits of phytosterols, most studies have neither eliminated nor quantified phytosterols in background diet and left many questions unanswered regarding the effective dose of phytosterols to derive health benefits and mechanisms by which such benefits occur. In order to enhance nutritional quality of poultry meat without alteration of sensory attributes, the present study was designed to assess the feeding regimens of variable phytosterol levels on broiler chickens quality.

MATERIALS AND METHODS

Experimental animals

A total of one hundred and twelve one day old avian broiler chickens were used in this experiment. Ross chicken strains were obtained from a commercial hatchery, Wuxi, China. The birds were housed in individual 40 x 45 x 50 cm cages in an environmentally controlled room at 24^{o}C and 60% relative humidity. All chickens kept in pens were given feed and water adds labium with 24 h continuous light and acquired immunity according to usual ways. The birds were randomly assigned for treatments after 3 days; this experiment used a completely randomized design with 7 treatments. Chicken (16 per diet) were systematically randomized to receive 7 treatment diets for 21 days, the birds were weighed, within ±20 g of the mean, and 4 replicates of 4 birds each, were prepared. Diets were formulated to meet or exceed nutrient requirements of broiler chicken consuming 120 g/d (Table 1). The experimental diet L1, L2 and L3 were prepared by the addition of 15, 20 and 25 g/kg, respectively, of polyhydroxy phytosterol (Castastesrone; purchased from Zhejiang Dawei pharmaceutical Co.,Ltd- China). Diet H1, H2 and H3 were prepared by the addition of 25, 50 and 75 g/kg of hydroxy phytosterol (β-sitosterol; obtained from zhejiang dawei pharmaceutical co., LTD - China). Mortalities and feed consumption per pen were recorded daily. Feed intake (FI), Body weights (BW) and feed conversion ratios (FCR) were determined weekly for each group. Twelve hours fasting birds (6 chickens per pen) of 21 days of age were weighed prior to slaughter while the 21 days experimental treatment birds were killed by cervical dislocation for meat analyses. Birds were slaughtered and dissected by a trained team. Muscles were collected and stored at −70^{o}C (ULT freezer: Thermo scientific Electronic LED-Gmbh -Germany) prior to analysis.

Determination of lipid peroxidation

The lipid peroxidation was expressed as malondialdehyde (MDA) in nanomoles per milligram protein. The method was used as described previously by James et al., (2002). MDA which is formed as an end product of lipid peroxidation was treated with thiobarbituric acid to generate a coloured product that was measured at 532 nm. MDA detecting kit was purchased from Jiancheng Bioengineering Institute, Nanjing, China.

Measurements of antioxidant status

Total antioxidant capacity (T-AOC), glutathione (GSH), oxidized glutathione (GSSG) and catalase (CAT) in tissue was assayed with the appropriate test kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, P.R. China).

Water-holding Capacity (WHC)

Pectoralis major muscles of broilers were taken from the carcass. Samples were trimmed to 5 × 2 ×1 cm size, blotted to remove the surface water, and the initial breast muscle weight was determined. Samples were placed in air-filled plastic bags and fastened to avoid evaporation and vertically hung in the refrigerator at 4^{o}C. The final breast muscle weight was determined after 24 and 48 h post mortem. The percentage of drip loss was calculated as followed:
Drip loss = 100× (initial breast muscle weight-final muscle fillet weight)/initial weight.

Mechanical properties measurement

Mechanical properties were analyzed according to TA.XT2i texture analyzer (Stable Microsystems, Godalming, U.K.). The force (N) was recorded continuously during compression in a texture profile

curve (Texture Profile Analysis (TPA60)) with the trigger force 5 g. The samples were compressed twice at a deformation rate of 1.0 mm/s to 60% of their original height (TPA60). The holding time between the compressions was 5 seconds. The maximum force of compression in the force time curve (hardness), as well as adhesiveness, cohesiveness, resilience, springiness, gumminess and chewiness were calculated through texture analyser machine. Measurements (n = 6) were run on each broiler breast samples.

Statistical analysis

The statistical analyses was done using the GLM procedures of SAS software, and Duncan's multiple range tests was used to compare treatment means. Differences at $p<0.05$ were considered to be significant. Replicate was considered as the experimental unit to determine the performance.

RESULTS AND DISCUSSION

Performance parameters

The results from the present investigation revealed in Table 2; final body weight (21 days) of birds significantly ($p<0.05$) increased by supplementation of H3, L3and H2. Feed consumption did not show any difference from each other ($p<0.05$). Treatment L3 achieved the highest value relative to feed efficiency compared to the rest of the treatments and control (Table 2). However leg meat, Breast meat, bursa, spleen, liver, heart, did not differ across treatment groups, but the net weight increased in phytosterol groups (Table 3). The body weight significantly correlated with diameter, area, and density of myofibers. We have already noted in another study that fast-growing chickens have larger diameter fibers than slow-growing lines (Taha et al., 2014) , which implies that the body weights increased in our present study was due to supplementation of HydroxyPhytosterol. This increase can also be associated with an increase in the number of giant fibers, which typically have cross-sectional areas three to five times larger than normal, although these may result from severe contraction (hypercontracted fibers). Smaller fiber diameters may allow a higher packing density and increase toughness of the meat (Chen and Opara, 2013; Phillips et al., 2010; Devatkal et al., 2011; Mckee L. H. 2012).

Reactive oxygen species and malondialdehyde levels

This would indicate that highly unsaturated oil is able to use more oxygen before the total destruction of the sterols through an oxidative pathway. This attack causes lipid peroxidation. Furthermore, the decomposition of peroxidized lipids yields a wide variety of end-products, including malondialdehyde (MDA), it is a major degradation product of lipid hydroperoxides has attracted much attention as a marker for assessing the extent of lipid peroxidation (Del Rio et al., 2005). This compound is of particular concern since it has been shown to be mutagenic, carcinogenic and implicated in other pathological process that is widely used in practice as an indicator of free radical damages (Del Rio et al., 2005; Sahin et al., 2010). The effects of phytosterol supplementations on malondialdehyde MDA concentrations are shown in (Table 4). Phytosterol addition in broiler feed significantly ($p<0.05$) decrease MDA levels compared to control group.

Antioxidant Enzyme Activity

Thus, when unsaturated oil such as soybean is subjected to the oxidative stresses, it is possible to recover more intact sterols and more sterol oxides. Data collected in this trial indicated that the antioxidant capability of broilers was greatly improved by dietary phytosterol. Total antioxidant capacity (TAOC) and concentration of a non-enzymatic substance (GSH) and catalase (CAT) were increased, while the metabolic product of lipid peroxides of malonaldehyde MDA and oxidized glutathione (GSSG) levels leads to which may cause oxidative modifications to lipids, nucleic acids and proteins content was decreased (Table 4). These trends corroborate with the work reported by (Grune et al., 2012; Wang et al., 2012). Plasma oxygen radical scavenging capacity values significantly ($P < 0.05$) decreased when rats were fed with atherogenic diets that contained the combination of phytosterol. . The activities of GSH, CAT, and T-AOC in broilers were increased by adding phytosterol. Significant ($p<0.05$) increase was observed by supplementation of hydroxy phytosterol at 75g level particularly. GSH, CAT, and T-AOC increased with the increasing level of phytosterol. However, the addition of 75g of hydroxy phytosterol to diet significantly decreased oxidized glutathione (GSSG) of broilers.

Water loss and Texture profile analysis (TPA)

Texture is one of the principal factors in determining the quality and acceptability of foods. TPA is an objective test commonly used in other industries for texture assessment of foods (Simonin et al., 2012). Texture profile analysis uses a double compression cycle to simulate the first and second bites, similar to

Table 1: Composition of the experimental diet (g/100 g of diet)

Ingredient (%)	Starter (1-15 days)	Grower (16-35 days)	Finisher (36-45days)
Corn	45.20	40.20	45.20
Wheat	8.00	8.30	8.50
Soybean meal	38.00	36.00	31.00
Calcium phosphate	1.90	1.50	1.20
Limestone	1.00	1.10	1.10
Salt	0.34	0.34	0.38
Vitamin-mineral premix*	0.30	0.30	0.30
Santoquin	0.04	0.04	0.04
Soybean oil	5.00	12.00	12.00
Polyhydroxy phytosterol	0, 1.5, 2.0, 2.5	0, 1.5, 2.0, 2.5	0,1.5,2.0,2.5
Hydroxy Phytosterol	0,2.5,5.0,7.5	0,2.5,5.0,7.5	0,2.5,5.0,7.5

*Supplied per kilogram of diet: riboflavin, 8.0 mg; niacin, 48 mg; pantothenic acid, 16 mg; 50% cholinechloride, 800 mg; cobalamin, 15 g; cholecalciferol, 18.5 g; vitamin E (DL-α-tocopherol acetate), 20 IU; vitamin A (trans-retinyl acetate), 10,000 IU; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4$ $7H_2O$, 300 mg; MnO, 100 mg; $CuSO_4$ $5H_2O$, 20 mg; $ZnSO_4$ H_2O, 150 mg; $NaSeO_3$, 0.15 mg; KI, 0.5 mg; ethoxyquin, 100 mg; and avoparcin, 15 mg. The carrier was zeolite.

Table 2: Effect of phytosterol levels on growth performance, feed consumption and feed efficiency of broiler chickens after 21 days

Feed		Growth consumption (g)	Feed efficiency (g)	Feed performance(g:g)
Control		634.00±9.77	133.33±10.53ab	1.79±0.18c
Polyhydroxy phytosterol	L1	631.73±26.50	149.17±1.03a	1.67±0.07c
	L2	642.00±3.33	147.63±2.67a	1.61±0.12bc
	L3	640.27±2.75	124.70±2.59b	1.46±0.08a
HydroxyPhytosterol	H1	624.27±12.01	124.50±5.25b	1.50±0.09bc
	H2	656.07±12.52	138.01±6.54ab	1.58±0.01bc
	H3	647.13±7.45	129.17±2.60b	1.53±0.17bc

Values shown are means± SD (g). a,b,c Values in column with different superscripts are significantly different ($P<0.05$) from each other.

Table 3: Effect of phytosterol levels on relative weight of organs (g) of broiler chickens after 21 days

		leg muscle	Breast meat	Liver	Kidney	bursa	spleen	heart
Control		75.50±2.47[b]	106.61±3.29[a]	40.34±3.13[ab]	11.11±1.13ab	3.20±0.31[a]	1.12±0.06[a]	7.85±0.472[a]
Polyhydroxy phytosterol	L1	86.81±2.37[ab]	120.39±5.34[a]	40.34±3.13[ab]	10.01±0.65b	3.49±0.56[a]	1.29±0.19[a]	8.05±0.489[a]
	L2	85.69±3.07[a]	112.54±3.96[a]	39.02±1.54[ab]	11.94±1.20ab	3.24±0.48[a]	1.30±0.13[a]	8.04±0.457[a]
	L3	82.30±5.02[ab]	114.25±6.57[a]	40.73±1.64[ab]	12.57±0.86ab	3.32±0.22[a]	1.36±0.15[a]	8.25±0.218[a]
Hydroxy phytosterol	H1	88.21±3.13a	113.31±4.41[a]	42.87±1.15[a]	12.16±1.27ab	2.81±0.41[a]	1.14±0.09[a]	8.35±0.198[a]
	H2	85.10±2.95[ab]	112.53±5.67[a]	35.74±3.53[b]	12.15±1.37ab	3.35±0.32[a]	1.21±0.13[a]	8.20±0.574[a]
	H3	85.49±4.66[ab]	117.87±3.42[a]	38.09±2.03[ab]	13.40±0.66a	3.65±0.36[a]	1.24±0.09[a]	7.76±0.171[a]

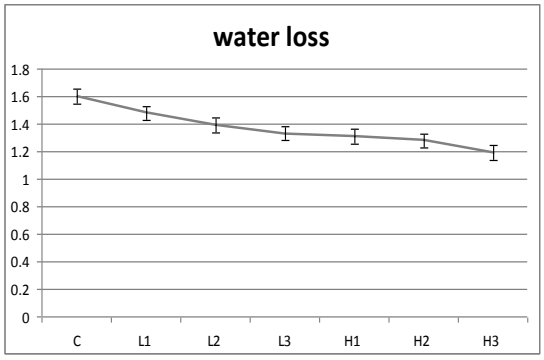
Values shown are means±SD for 16 birds. a,b,c Values with different superscripts are significantly different ($P < 0.05$) from each other

Table 4: Effect phytosterol levels on oxidative stability of broiler chickens meat after 21 days

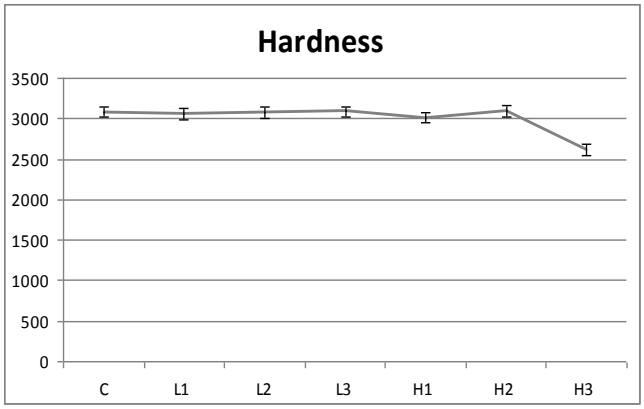
		MDA (U/mg of protein)	CAT (U/mg of protein)	TAC (U/mg of protein)	GSH (U/mg of protein)	GSSG (U/mg of protein)	GSH/GSSG (U/mg of protein)
Control		4.71±0.64a	2.889±0.47	0.21±0.04b	1.62±0.58c	14.77±2.10a	0.12±0.04d
Polyhydroxy phytosterol	L1	1.96±0.31b	5.028±1.03	0.63±0.06ab	1.78±0.38bc	12.71±2.12ab	0.14±0.04cd
	L2	1.56±0.18b	5.403±0.91	0.56±0.08ab	2.55±1.09abc	10.66±0.59bc	0.21±0.10bcd
	L3	1.68±0.23b	4.537±1.02	0.97±0.31a	3.24±1.01ab	9.89±2.14bc	0.29±0.13ab
Hydroxy Phytosterol	H1	2.65±0.43b	4.397±1.01	0.44±0.13ab	2.33±1.04abc	10.23±1.22bc	0.18±0.01bcd
	H2	2.50±0.24b	4.614±1.23	0.63±0.25ab	3.04±0.32abc	9.75±2.16bc	0.26±0.03abc
	H3	1.92±0.34b	6.045±1.71	0.92±0.30a	3.59±0.84a	9.110±0.87c	0.37±0.03a

.Values shown are means ± SEM for 16 birds. a,b,c,d Values with different superscripts are significantly different

Figure 1: Effects of phytosterol on texture profile analysis: Water-holding capacity (a), hardness (b), adhesiveness (c), , cohesiveness (d), gumminess (e), chewiness (f), springiness (g)and resilience (h) properties of broiler semitendinosus muscle after 21 days of age.



a



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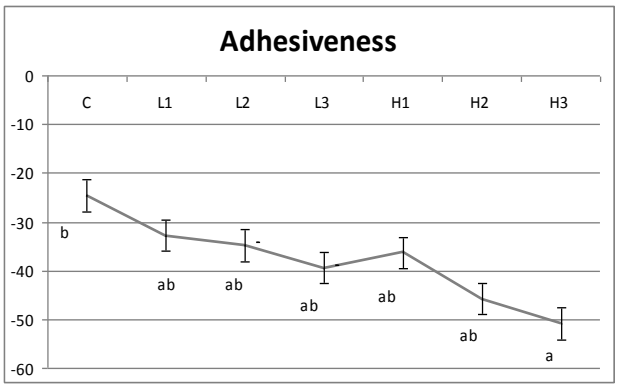

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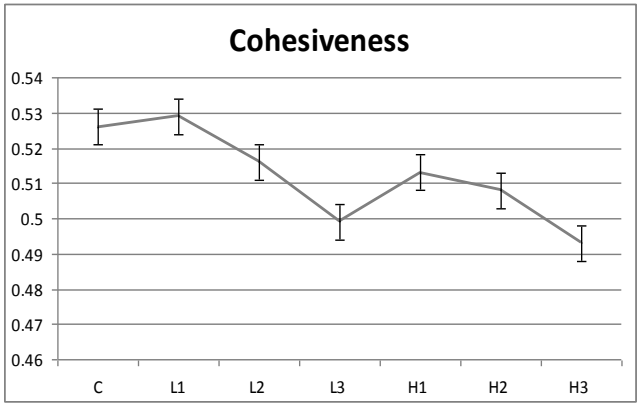

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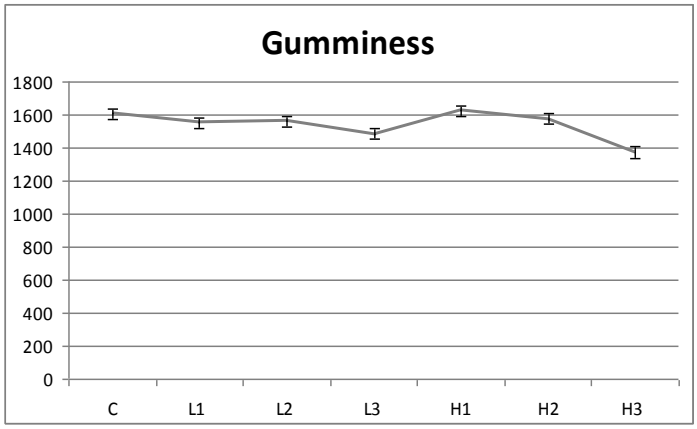
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L1
L2
L3
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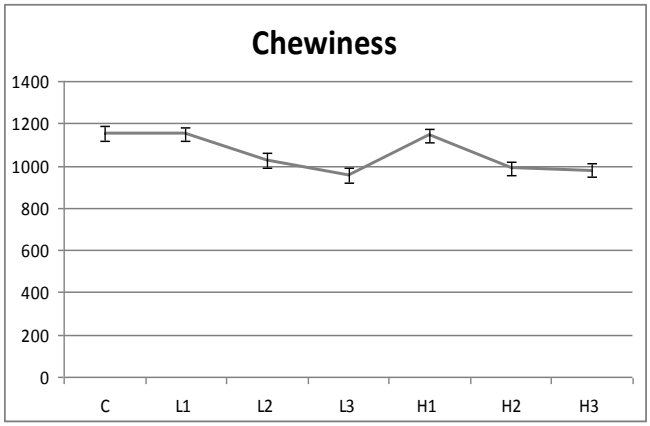
H2
H3

e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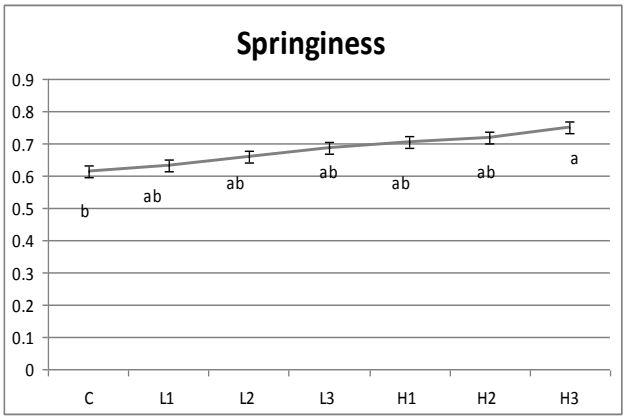
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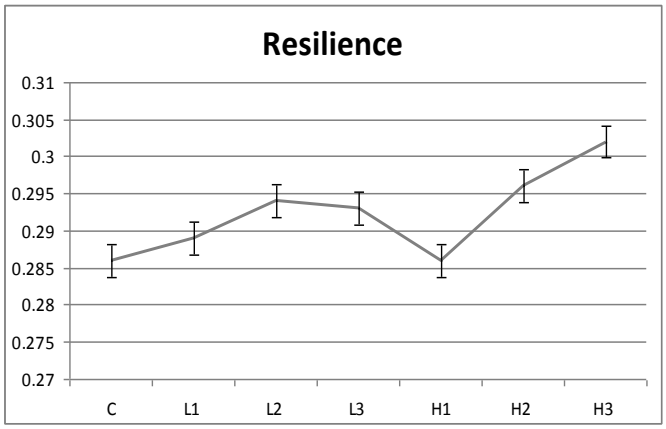
H3

g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0.29
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0.275
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C
L1
L2
L3
H1
H2
H3

h

a human subject. Texture parameters that can be assessed using TPA are: hardness, cohesiveness, springiness, gumminess, and chewiness. Utilization of these texture parameters has been proven to be a useful method in determining textural properties of food borne (Chen and Opara, 2013) it was found that water holding capacity values proportional with early content of products, where water holding capacity values were low in products high in fat. The water holding capacity functionality nature was influenced by how far effective protein matrix binding scattered

Texture profile analysis uses a double compression cycle to simulate the first and second bites, similar to a human subject. Texture parameters that can be assessed using TPA are: hardness, cohesiveness, springiness, gumminess, and chewiness. Utilization of these texture parameters has been proven to be a useful method in determining textural properties of food borne (Chen and Opara, 2013) it was found that water holding capacity values proportional with early content of products, where water holding capacity values were low in products high in fat. The water holding capacity functionality nature was influenced by how far effective protein matrix binding scattered excess fat and water in products (Katan et al., 2003). While most of the data spread by the companies producing high value broilers refers to some technological and economical features of their products (microclimate, nutrition requirements, weight gains, FCR, slaughtering efficiency), this paper brings some partial results from a study onto the textural quality of the broiler meat. Results of this study have shown that the increase in amount of phytosterol had only minor effects on the chicken meat properties (Phillips et al., 2010). Diet with the different amount of phytosterol had no significant effect on the water holding capacity, hardness, adhesiveness, springiness, cohesiveness, gumminess, resilience, and chewiness. However, in 21 days the meat of broilers groups L3, H3 had higher parameters compared to broilers from the other feeding and control groups ($p<0.05$)**.**There was no significant effect ($p<0.05$) observed on water loss (a) hardness (b), adhesiveness (c), cohesiveness (d), gumminess (e), chewiness (f), resilience (g), springiness (c), (Fig. 1). The results showed similar texture characteristics to that of control.

CONCLUSIONS

The diet containing phytosterol revealed increased antioxidant status and improved growth performance of the chickens. Consequently, varying the amount of phytosterol in feed had no negative effect on broiler meat and produced more healthy meat for human consumption. We conclude that addition of 25 g and 50.75 g of Castastesrone and β-sitosterol, respectively, per kg of diet and increased the level of antioxidant enzymes, and improved the broiler meat quality.

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Effects of calcium chloride on pH, peroxide value, oil stability index and fatty acid composition of Buffaloe's and Cow's butter-based low-fat spreads

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ABSTRACT

This work involved effects of $CaCl_2$ (0 (control), 0.02, 0.04, 0.06 and 0.08%) on properties of Buffaloe's butter-based low-fat spread (B-LFS) and Cow's butter-based low-fat spread (C-LFS) (fat 40%). The differences in pH values of $CaCl_2$ treatments (B-LFS and C-LFS) separately compared control samples were negligible, while all pH values significantly decreased during the storage periods (3 to 90 days) at 4 °C. The changes in peroxide values (PVs) of $CaCl_2$ treatments separately and control samples were fluctuating, however all PVs significantly increased during storage periods. The effects in oil stability index (OSI) of $CaCl_2$ treatments separately were without significant compared controls samples, exception levels of $CaCl_2$ 0.08% with B-LFS, $CaCl_2$ 0.06% (at 3 days) and $CaCl_2$ 0.08% (at 3, 30 and 90 days) with C-LFS were decreased significantly. In this study, we noticed changes in fatty acids composition (FAC) between $CaCl_2$ treatments compared control samples and during the storage periods.

Keywords: Buffaloe's butter; Cow's butter; Low fat spread; $CaCl_2$; Oil stability index; Fatty acid composition.

INTRODUCTION

Obviously there are problems associated with both Buffalo's and Cow's butter such as high cost and poor spreadability at refrigerator temperatures. Therefore, by reducing the fat phase and addition of water, biopolymer, emulsifier, protein and using of the homogenization may produce low fat butter spreads characterized with spreadability at refrigerator temperature. This product is economical, functional and low calorie alternative of butter. The large fat phase in low fat butter spreads determines the sensory evaluation, physical properties, rheological characteristics, chemical reactions and microbiological stability attributes.

In many developed countries the intake of calcium with the average diet is not adequate (Nelson 1996; Gennari 2001). That result in high risk of osteoporosis and other diseases related to deficiency of calcium and bone demineralization (Bryant *et al*. 1999; Flynn 2003). Calcium is nutritionally valuable in food products with a typical concentration ranging from 10 to 30 mM (Keowmaneechai and McClements 2006). Furthermore, the encapsulation technique used alginate as matrix has potential application for the fortification of emulsion-based foods with various minerals (i.e., Ca^{+2} and Cu^{+2}) simultaneously. This is essential for the industrial food applications as it may reduce costs by minimizing the steps required for the mineral supplementation of foods (Borgogna *et al*. 2010).

However, the addition of calcium may stabilize or destabilize oil-in-water emulsions depending on the concentration of added calcium and the protein (Dickinson and Golding 1998). Moreover, Sun (2006) found that an addition of 10 mM $CaCl_2$ and 200 μM EDTA before heating protected oil droplets against oxidation and stabilized the emulsions via ionic bonding and electrostatic screening.

Low fat butter spreads require four types of structuring agents that have been identified (Moran 1991) to provide the required structure alongside a 'plastic' flow (Evageliou *et al*. 1997). These include viscous (milk proteins or high-molecular weight polysaccharides), gelling (hydrocolloids used to gel the aqueous phase), phase-separating (with thermodynamically incompatible hydrocolloids) and synergistic (exploiting known synergistic interactions between hydrocolloids) agents. Commonly used ingredients, include proteins such as casein, whey, egg, soy or gelatin, and/or polysaccharides such as starch, carrageenan, alginate, pectin. These

ingredients result in a thickening effect on the aqueous phase and the resulting viscous water droplets have better stability against coalescence, which improves the overall stability of the spread against the changing in temperature (Bot and Vervoort 2006).

The objectives of this work were to study effects of $CaCl_2$ on pH, PVs, OSI and FAC of B-LFS and C-LFS.

MATERIALS AND METHODS

Materials

Buffaloe's butter (83.48% fat, solid not fat 2.91%, moisture 13.61% and peroxide value 0.145) was obtained from Department of Dairy Science, Faculty of Agriculture, Suez Canal University (Ismailia, Egypt). Cow's butter (82.68% fat, solid not fat 1.75%, moisture 15.57% and peroxide value 0.135), skim milk powder and sodium chloride (table salt) were purchased from a local market at Wuxi (Jiangsu, China). Halal gelatin (80-280 BLOOM) was purchased from Gelatin & Protein Co., Ltd. (Hangzhou, China). DIMODAN®HP-C distilled monogelyceride was obtained from Danisco Co. (Shanghai, China). Citric acid anhydrous, k-sorbate and calcium chloride were purchased from Shanghai Honghao Chemical Co., Ltd. (Shanghai, China). All other reagents and solvents were of analytical or chromatographic grade to suit analytical requirements.

Preparation of Buffaloe's and Cow's butter oil

According to Fatouh *et al*. (2003) both of Buffaloe's and Cow's butter were melted at 50 °C instead of 60 °C, and the top oil layer was decanted and filtered through glass wool. The oil was then refiltered under vacuum to obtain clear Buffaloe's and Cow's butter oil.

Preparation of B-LFS and C-LFS with different $CaCl_2$ concentrations

The recipe of making $CaCl_2$ treatments (B-LFS and C-LFS separately), was according to Madsen (2000) with some modifications. The treatments contained the following ingredients in percentage (w/w): Buffaloe's and Cow's butter oil 40%, DIMODAN®HP-C distilled monogelyceride 0.5%, halal gelatin 2%, skim milk powder 1%, NaCl 1%, $CaCl_2$ (control (0), 0.02, 0.04, 0.06 and 0.08%), k-sorbate 0.1% and distilled water (100-ingredients together). The steps of preparation $CaCl_2$ treatments were as a following:

1) The ingredients of water phase (halal gelatin, skim milk powder, NaCl and k-sorbate) were blended together into the distilled water at 70 °C for 10 min by JJ-1B Electric Blender (Changzhou Runhua Electric Appliance Co., Ltd, China). 2) The temperature of water phase reduced from 70 to 40 °C, and the pH adjusted [with citric acid 20% (w/w)] to 5.5 with the stirring by JJ-1B Electric Blender. 3) With regard to fat phase, a portion from melted Buffaloe's and Cow's butter oil (~5 × the weight of the emulsifier) was removed and heated to 70 °C with the stirring until dissolving of the emulsifier, and then added back to the melted Buffaloe's and Cow's butter oil at 40 °C. 4) The water phase was then slowly added to the fat phase and mixed using a homogenizer (IKA® T18 Basic ULTRA-TURRAX®, Germany) for 5 min at a speed No. 2. 5) Pasteurization at 75°C for 10 min in water bath with the stirring by JJ-1B Electric Blender. 6) The temperature of the mixture decreased from 75 to 60 °C, and calcium chloride (20% w/w) blended with the mixture using a homogenizer (IKA® T18 Basic ULTRA-TURRAX®, Germany) for 3 min at a speed No. 2. 7) The homogenization by laboratory Homogenizer (Model: GYB, Donghua High Pressure Homogenizer Factory, Shanghai, China) at a pressure 17 MPa and 60 °C (one step) has been done. 9) Calcium chloride treatments were kept in sterilized plastic cups (30 g) at room temperature for 15 h and moved to the refrigerator (4 °C).

pH values

The pH of $CaCl_2$ treatments were measured by pH meter (Mettler Toledo FE20, China). The samples moved out from the refrigerator and kept at room temperature for 2 h followed by the pH measurement. All of experiments were carried out in triplicate and mean results are reported.

Peroxide values

The PV was modified from International Dairy Federation (IDF) Standard 74:1974 (Alexa *et al*. 2010). Briefly, samples of $CaCl_2$ treatments (B-LFS and C-LFS separately) (40 g each) were placed into 50 mL conical centrifuge tubes and placed in a 50 °C water bath for 20 min, followed by centrifugation (RJ-TDL-50A, Low-speed desktop centrifuge, China) for 20 min at 5000 rpm. Then, the top fat layers in the conical centrifuge tubes were decanted into a beaker and then dried over excess anhydrous sodium sulfate to remove residual water. The fat was separated from the anhydrous sodium sulfate by vacuum filtration through Whatman No. 4 filter paper to obtain clear fat. A 0.1 mL of melted fat was dissolved into 10 mL of chloroform/methanol (70:30) mixture, followed by

addition of ammonium thiocyanate (0.05 mL) and ferrous chloride (0.05 mL), respectively. Using glass stoppers, the tubes were inverted and placed in dark cupboard for 10 min. Meanwhile, a blank test with only reagents (without sample) was carried out. The absorbance of the samples was read at 505 nm on a Spectrophotometer (Alpha-1500, China). After calibration, the blank value was subtracted from the sample values (1) and the PVs were calculated. All of experiments were carried out in triplicate and mean results are reported.

$$OD = Abs_{sample} - Abs_{standard} \quad (1)$$

where, OD is the optical density.

Oil stability index

The oxidation induction time (OIT) of fat (to extraction of fat see PV) from $CaCl_2$ treatments was determined by the AOCS method Cd 12b-92 (Firestone 2004) with the Rancimat 743 apparatus (Metrohm AG, Herison, Switzerland). Samples were prepared in duplicate by weighing 3 g of fat in the reaction vessels. The distilled water (50 mL) was added to the measuring vessels, which were maintained at room temperature. Electrodes were attached for measuring changes in conductivity. The samples were heated at 120 °C under a purified air flow rate of 20 L/h. The oxidation induction times (OIT) is defined as the time necessary to reach the inflection point of the conductivity curve.

Fatty acid composition

The fat phase was extracted (see PV) from $CaCl_2$ treatments, and weighed 60 mg fat into a 10 ml screw-capped test tube. Then, 5 ml of n-hexane to dissolve the sample, and 250 µL of 2 M potassium hydroxide in MeOH were added into the test tube. The mixtures were vigorously shaken for 2 min, and then 1 g $NaHSO_4$ added into the tube and the mixtures also were vigorously shaken for 2 min. After vortexing, 2 mL from the separated upper layer (hexane) was added into the screw-capped test tube, and then centrifuged in a high speed centrifuge (TGL-16B, Shanghai Anting scientific factory, China) for 10 min at 10,000 rpm.

A 1 µL of purified hexane extract was injected into a GC-14B gas chromatograph (GC) equipped with a fused-silica capillary column (CP-Sil88, 100 m × 0.25 mm × 0.2 mm) and a flame ionization detector (Shimadzu, Tokyo, Japan). Both of injector and detector temperatures were set at 250 °C. The column oven temperature were as follows: 45 °C for 4 min, raised at 13 °C/min to 175 °C, held for 27 min, raised at 4 °C /min to 215 °C, held for 20 min. Nitrogen was the carrier gas. The identification of the peaks was achieved by retention times and by comparing them with authentic standards analyzed under the same conditions. Results were expressed as w/w (%) total fatty acid.

Statistical analysis

Both of B-LFS and C-LFS with different $CaCl_2$ concentrations were analyzed separately, and values of different tests were expressed as mean ± standard deviation. One way analysis of variance using SPSS 16 for windows (SPSS Inc., Chicago, USA) was performed on all experimental data sets. Duncan analysis was applied to evaluate the significance of differences between means at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of $CaCl_2$ concentrations on pH of B-LFS and C-LFS

Effects of $CaCl_2$ concentrations on pH of B-LFS and C-LFS are shown in Table 1. In general, the differences in the pH values between $CaCl_2$ treatments (B-LFS and C-LFS separately and together) were negligible. Furthermore, within all $CaCl_2$ treatments, the pH values decreased significantly ($P < 0.05$) during the storage, and there was slight increase in pH values from 3 to 30 days, but after 30 to 90 days, the pH values decreased.

The declining in pH values of $CaCl_2$ treatments was lower in the beginning and end of storage periods, due to the preservative (k-sorbate) and the pasteurization. Our findings are in agreement with the results obtained by Samet-Bali *et al*. (2009); Glibowski *et al*. (2011). In addition, our results were consistent with that observed for pH values of NaCl treatments (B-LFS and C-LFS) during the storage (data not shown). In contrary Balasubramanyam and Kulkarni (1999); Patange *et al*. (2013) found that pH values of stored spread increased.

It could be noticed that, the declining in pH values of calcium chloride treatments, due to the microbiological growth during the storage.

Effect of $CaCl_2$ concentrations on PV of B-LFS and C-LFS

Effects of $CaCl_2$ concentrations on oxidation of B-LFS and C-LFS are presented in Table 2. The differences in PVs among $CaCl_2$ treatments (B-LFS and C-LFS separately) and control samples were fluctuating. In addition, all of PVs within $CaCl_2$ treatments significantly increased ($P < 0.05$) during the storage at 4 °C. The oxidation of B-LFS with different levels of $CaCl_2$ was higher than C-LFS,

because the coloring agent (β-carotene) was added to Cow's butter, which used with C-LFS, while B-LFS was without β-carotene. Therefore β-carotene has antioxidant activity, and lead to protect lipids from free radical autoxidation by reacting with peroxyl radicals, thereby inhibiting propagation and promoting termination of the oxidation chain reaction (Burton and Ingold 1984; Palozza and Krinsky 1991; Britton 1995; Mallia 2008). Samples are considered rancid and unacceptable when PV are over 5, while ideally PV should be below 1-1.5 (Stathopoulos et al. 2009). In our study, all of $CaCl_2$ treatments (B-LFS and C-LFS) were to be accepted in an industrial setting, as the highest PV found was 0.504 (B-LFS with $CaCl_2$ 0.08% at 90 days).

The oxidative stability in dairy products is influenced by oxygen, metals (Cu^{+2}, Fe^{+3}), antioxidants, water activity, etc., (O'Connor and O'Brien 1995). However, the oxidation promoted in our treatments, due to incorporation of air and commencement of oxidation during the preparation of butter oil (Alexa *et al*. 2010). Furthermore, the pasteurization, light exposure and moisture causes the oxidation in the treatments during storages (Mallia 2008). On the other hand, Sun *et al*. (2007); Sun and Gunasekaran (2010) revealed that the PVs of menhaden O/W emulsions were decreased with an increasing of heating time due to the higher denaturation rate for whey protein isolate (WPI) upon substantial thermal treatment (90 ◦C) leads to an increase in the adsorbed amount of WPI and facilitates WPI-WPI interactions at droplet surfaces, which results in the formation of viscoelastic WPI layers around the droplet. Therefore, increased amount of WPI is expected to be adsorbed at droplet surfaces, which renders better protection against oxidation since WPI can act as an effective antioxidant in the emulsions. Furthermore, Sun (2006) reported that an addition of 10 mM $CaCl_2$ and 200 μM EDTA before heating protected oil droplets against oxidation and stabilized the emulsions via ionic bonding and electrostatic screening.

It could be noticed that, $CaCl_2$ hasn't protective influence against oxidation among B-LFS and C-LFS separately and during the storage, however Kolanowski *et al*. (2007) found that PVs of spreadable fat which fortified with the natural milk minerals complex rich in calcium, phosphor and magnesium were lower than that of the control. Moreover, the viscosity of B-LFS and C-LFS decreased with increasing of $CaCl_2$ from 0 to 0.08% (data not shown), however an increase in viscosity did not delay the onset of oxidation during the storage (Basaran *et al*. 1999).

Effect of $CaCl_2$ concentrations on OSI values of B-LFS and C-LFS

Table 3 shows the values of OIT, which give an indication of the oxidative stability of milk fat. In $CaCl_2$ treatments (B-LFS), the differences among $CaCl_2$ 0.02, 0.04 and 0.06% and the control were small and not significant, while $CaCl_2$ 0.08% were decreased noticeably ($P > 0.05$) compared to the control samples. On the other hand, the OSI values for $CaCl_2$ treatments (C-LFS) were non significant compared control samples, except $CaCl_2$ 0.06% (at 3 days), $CaCl_2$ 0.08% (at 3, 30 and 90 days). During storing of all treatments, the OIT had decreased effects ($P < 0.05$), however our results were in agreement with the determinations of Krause *et al*. (2008) who found that, the OSI values for stick butter decreased (Cow's butter) with the storage at the refrigeration conditions. The correlation between OSI values and the PVs (Table 2) was reversible. Furthermore, all OSI values with $CaCl_2$ treatments (B-LFS) were lower than C-LFS (see PV), therefore β-carotene led to prolong of OIT for $CaCl_2$ treatments (C-LFS) than B-LFS. Krause *et al*. (2008) they found the OIT for Cow's butter at 110 °C and the air rate 0.05 mL/min was 2 times approximately during the storage from 0 to 3 months. Also, Fatouh *et al*. (2005) found the OIT for Buffaloe's butter oil at 110 °C was 8.2 h. Läubli and Bruttel (1986) reported that the OIT for cooking butter at 100, 110 and 120 °C were 20.88, 9.33 and 5.03 respectively. Furthermore, Mathäus (1996) determined the OIT for walnut oil at 110, 120 and 130 °C, the OSI values were 156, 84 and 45 min respectively.

Effect of $CaCl_2$ concentrations on FAC of B-LFS and C-LFS

The effects of different $CaCl_2$ concentrations on B-LFS and C-LFS are shown in Table 4. All of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and trans fatty acids (TFA) among $CaCl_2$ treatments (B-LS and C-LFS separately) were significantly different from the control samples, whereas saturated fatty acids (SFA) and total FA were without significant. Furthermore, we found $CaCl_2$ treatments (B-LFS) during the storage were only significant in PUFA (control) and TFA (NaCl 0 and 0.5%). On the other hand, the differences among $CaCl_2$ treatments (C-LFS) for PUFA and TFA were significantly compared with the control, while SFA, MUFA and total FA were without significant. In addition, during the storage,

C-LFS samples had only significant differences in PUFA ($CaCl_2$ 0.04 and 0.06%) and TFA ($CaCl_2$ 0, 0.04 and 0.06%). The changes in FAC of $CaCl_2$ treatments (B-LFS and C-LFS separately) during the

Table 1: Effects of different $CaCl_2$ concentrations on pH of B-LFS and C-LFS

Storage (Days)	pH values [a]				
	B-LFS				
	$CaCl_2$ 0% (Control)	$CaCl_2$ 0.02%	$CaCl_2$ 0.04%	$CaCl_2$ 0.06%	$CaCl_2$ 0.08%
3	5.56 ± 0.04^{bA}	5.50 ± 0.03^{bBC}	5.51 ± 0.03^{bAB}	5.47 ± 0.02^{bBC}	5.46 ± 0.02^{bC}
30	5.62 ± 0.03^{aA}	5.62 ± 0.02^{aA}	5.59 ± 0.02^{aAB}	5.56 ± 0.04^{aB}	5.60 ± 0.04^{aAB}
60	5.50 ± 0.02^{cC}	5.53 ± 0.02^{bAB}	5.55 ± 0.02^{abA}	5.50 ± 0.02^{bC}	5.50 ± 0.01^{bBC}
90	5.42 ± 0.04^{dA}	5.38 ± 0.04^{cA}	5.40 ± 0.04^{cA}	5.38 ± 0.04^{cA}	5.36 ± 0.03^{cA}
	C-LFS				
3	5.54 ± 0.02^{bA}	5.47 ± 0.01^{aB}	5.47 ± 0.03^{bB}	5.54 ± 0.03^{aA}	5.53 ± 0.03^{bA}
30	5.61 ± 0.04^{aA}	5.45 ± 0.04^{abC}	5.52 ± 0.02^{aB}	5.55 ± 0.03^{aB}	5.54 ± 0.03^{aA}
60	5.57 ± 0.03^{abA}	5.41 ± 0.01^{bC}	5.44 ± 0.03^{bC}	5.54 ± 0.03^{aAB}	5.53 ± 0.03^{bB}
90	5.47 ± 0.02^{cA}	5.34 ± 0.02^{cC}	5.29 ± 0.02^{cD}	5.42 ± 0.03^{bB}	5.44 ± 0.03^{cAB}

Capital letters: represent average values with different letters are statistically significant ($p < 0.05$) within each row. Small letters: represent average values with different letters are statistically significant ($p < 0.05$) within each column. [a,] mean±S.D, n = 3.

Table 2: Effects of different $CaCl_2$ concentrations on PVs of B-LFS and C-LFS

Storage (Days)	PVs values [a]				
	B-LFS				
	$CaCl_2$ 0% (Control)	$CaCl_2$ 0.02%	$CaCl_2$ 0.04%	$CaCl_2$ 0.06%	$CaCl_2$ 0.08%
3	0.219 ± 0.017^{cBC}	0.251 ± 0.024^{dAB}	0.261 ± 0.019^{cA}	0.217 ± 0.016^{dC}	0.276 ± 0.014^{dA}
30	0.307 ± 0.023^{bC}	0.323 ± 0.015^{cBC}	0.354 ± 0.026^{bAB}	0.347 ± 0.020^{cAB}	0.376 ± 0.014^{cA}
60	0.417 ± 0.015^{aB}	0.435 ± 0.013^{bB}	0.443 ± 0.013^{aAB}	0.437 ± 0.014^{bB}	0.471 ± 0.025^{bA}
90	0.444 ± 0.022^{aC}	0.467 ± 0.014^{aBC}	0.467 ± 0.011^{aBC}	0.492 ± 0.008^{aAB}	0.504 ± 0.015^{aA}
	C-LFS				
3	0.177 ± 0.022^{cAB}	0.195 ± 0.022^{cA}	0.154 ± 0.024^{cB}	0.195 ± 0.020^{cA}	0.167 ± 0.012^{dAB}
30	0.296 ± 0.010^{bA}	0.286 ± 0.024^{bAB}	0.255 ± 0.014^{bBC}	0.245 ± 0.024^{bC}	0.255 ± 0.018^{cBC}
60	0.322 ± 0.015^{bBC}	0.313 ± 0.014^{bBC}	0.293 ± 0.024^{bC}	0.362 ± 0.024^{aA}	0.345 ± 0.023^{bAB}
90	0.385 ± 0.018^{aA}	0.375 ± 0.022^{aA}	0.373 ± 0.023^{aA}	0.387 ± 0.018^{aA}	0.385 ± 0.028^{aA}

Capital letters: represent average values with different letters are statistically significant ($p < 0.05$) within each row. Small letters: represent average values with different letters are statistically significant ($p < 0.05$) within each column. [a,] mean±S.D, n = 3.

Table 3: Effects of $CaCl_2$ concentrations on OSI values of B-LFS and C-LFS

Storage (Days)	OSI values [a]				
	B-LFS				
	$CaCl_2$ 0% (Control)	$CaCl_2$ 0.02%	$CaCl_2$ 0.04%	$CaCl_2$ 0.06%	$CaCl_2$ 0.08%
3	4.56 ± 0.10^{aA}	4.54 ± 0.09^{aA}	4.39 ± 0.15^{aA}	4.54 ± 0.11^{aA}	4.06 ± 0.14^{aB}
30	4.45 ± 0.10^{abA}	4.39 ± 0.16^{abA}	4.24 ± 0.15^{abA}	4.36 ± 0.09^{abA}	3.79 ± 0.09^{bB}
60	4.25 ± 0.17^{bcA}	4.28 ± 0.08^{bA}	4.10 ± 0.14^{bA}	4.31 ± 0.11^{bA}	3.67 ± 0.15^{bB}
90	4.08 ± 0.10^{cAB}	4.00 ± 0.11^{cB}	4.02 ± 0.10^{bAB}	4.24 ± 0.17^{bA}	3.58 ± 0.14^{bC}
	C-LFS				
3	5.42 ± 0.08^{aA}	5.31 ± 0.08^{aAB}	5.24 ± 0.14^{aABC}	5.19 ± 0.17^{aBC}	5.04 ± 0.11^{aC}
30	5.16 ± 0.12^{abA}	5.15 ± 0.09^{aA}	5.04 ± 0.11^{abA}	5.04 ± 0.09^{abA}	4.81 ± 0.13^{abB}
60	4.96 ± 0.16^{bcA}	4.95 ± 0.11^{bA}	4.88 ± 0.11^{bA}	4.87 ± 0.13^{bcA}	4.74 ± 0.16^{bA}
90	4.77 ± 0.19^{cA}	4.69 ± 0.06^{cA}	4.63 ± 0.09^{cAB}	4.65 ± 0.11^{cAB}	4.43 ± 0.17^{cB}

Capital letters: represent average values with different letters are statistically significant ($p < 0.05$) within each row. Small letters: represent average values with different letters are statistically significant ($p < 0.05$) within each column. [a,] mean±S.D, n = 3.

storage were slightly, however our results were in agreement with Mallia (2008). Furthermore, our results were approximately resembled with that observed for FAC of pH and NaCl treatments (B-LFS and CLFS), which still under study in our lab.

Calcium chloride treatments (B-LFS) were lower than C-LFS in SFA, C6, C8, C10, C11, C12, C14, C18:3n3, TFA and C18:1T, while C4, C13, C15, C16, MUFA, C14:1, C15:1, C16:1, C17:1, C18:1 and C18:2T were higher in B-LFS samples than C-LFS, however Talpur *et al*. (2008) found that the mean concentrations of C-18:1T in Buffaloe's and Cow's milk fat were 2.00 g/100 g and 2.01 g/100 g respectively. On the other hand, Ahmad *et al*. (2013) reported that the proportions of C4, C16, C17, and C18 were higher, but C6, C8, C10, C12, C14, and C14:1 were lower in Buffaloe's than in Cow's milk fat. Patel *et al*. (2002) found the average of C4, C16, C17 and C18 in Buffaloe's milk fat was higher than Cow's milk fat, while C6, C8, C10, C10:1, C12, C14, C14:1 and C18:1 in Cow's milk fat was higher than Buffaloe's milk fat. Although, Talpur *et al.* (2008) noticed that the averages of four seasons as percentages of C4, C8 and C12 were higher in Buffaloe's than Cow's milk fat which reared under the traditional feeding system of Sindh, Pakistan. In contrast Varricchio *et al.* (2007); Ménard *et al*. (2010) reported that Buffaloe's milk fat contained a higher amounts of SFA and lower amounts of unsaturated fatty acids than Cow's milk fat, but the different with our study could be explained by results of the previous authors came from other breeds were different than breed of Egyptian Buffaloe's Animals. Furthermore, Samet-Bali *et al.* (2009) reported that milk FAC depends on several factors as animal species, nutrition, climate and environmental conditions. Additionally, the differences in FAC of Buffaloe's and Cow's milk fat due to the genetics of the two animal species considered **(**Ménard *et al*. 2010**)**. However, our results were in agreement with Haggag *et al*. (1987) who reported that an unsaturated fatty acids for Egyptian Buffaloe's milk were higher than Egyptian Cow's milk. We can say the differences in FAC during the storage periods related to the degradation by non-enzymatic, oxidative react ions occurring during the storage, by metabolic conversions mediated by enzymes produced by microorganisms introduced during the processing B-LFS and C-LFS.

Conclusions

Levels of calcium chloride do not affect pH values of treatments, and lacked protective influence against the oxidation of B-LFS and C-LFS separately and during the storage periods. On the other hand, an increase of $CaCl_2$ led to slight decrease in the OSI treatments. Furthermore, effects of $CaCl_2$ concentrations showed changes in FAC among B-LFS and C-LFS separately and during the storage period.

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Table 4: Effects of different $CaCl_2$concentrations on FAC of B-LFS and C-LFS

Fatty acids	Storage (Days)	B-LFS [a]					C-LFS [a]				
		$CaCl_2$ 0% (Control)	$CaCl_2$ 0.02%	$CaCl_2$ 0.04%	$CaCl_2$ 0.06%	$CaCl_2$ 0.08%	$CaCl_2$ 0% (Control)	$CaCl_2$ 0.02%	$CaCl_2$ 0.04%	$CaCl_2$ 0.06%	$CaCl_2$ 0.08%
S	3	65.53±2.46^{aA}	67.79±2.07^{aA}	66.47±2.29^{aA}	67.19±1.65^{aA}	68.25±2.55^{aA}	70.62±1.85^{aA}	70.42±2.27^{aA}	69.59±1.82^{aA}	71.78±2.10^{aA}	70.45±2.21^{aA}
	90	65.95±2.13^{aA}	64.20±2.13^{aA}	66.09±2.19^{aA}	67.00±2.36^{aA}	66.05±1.94^{aA}	68.86±2.29^{aA}	70.20±1.87^{aA}	68.48±1.89^{aA}	68.85±2.18^{aA}	68.92±2.24^{aA}
C4	3	1.96±0.13^{b}C	2.82±0.16^{a}B	1.86±0.17^{b}C	2.68±0.17^{aB}	3.53±0.17^{a}A	2.51±0.12^{a}A	2.03±0.15^{b}B	1.97±0.11^{aB}	2.66±0.17^{a}A	2.18±0.17^{aB}
	90	2.39±0.15^{a}B	2.46±0.16^{a}AB	2.52±0.12^{a}AB	2.55±0.15^{aA}B	2.70±0.11^{b}A	2.01±0.14^{b}C	2.98±0.15^{a}A	1.66±0.16^{b}D	2.48±0.17^{aB}	2.03±0.15^{aC}
C6	3	0.86±0.14^{a}B	1.66±0.12^{a}A	0.78±0.14^{b}B	0.78±0.13^{aB}	1.45±0.19^{a}A	1.59±0.08^{aB}	1.55±0.13^{aB}	1.33±0.03^{aC}	1.94±0.12^{a}A	1.56±0.12^{aB}
	90	0.93±0.17^{a}B	1.67±0.11^{a}A	1.15±0.08^{aB}	1.07±0.16^{aB}	1.01±0.08^{b}B	1.42±0.15^{a}AB	1.56±0.14^{a}A	1.28±0.15^{aB}	1.55±0.13^{b}A	1.57±0.03^{a}A
C8	3	0.58±0.08^{a}CD	1.22±0.09^{a}A	0.48±0.07^{a}D	0.62±0.04^{aC}	0.81±0.02^{aB}	1.19±0.06^{a}AB	1.12±0.09^{b}AB	1.02±0.01^{aB}	1.24±0.07^{b}A	1.25±0.06^{a}A
	90	0.49±0.07^{a}B	0.85±0.07^{b}A	0.54±0.07^{aB}	0.53±0.04^{aB}	0.54±0.06^{b}B	0.97±0.01^{b}C	1.34±0.01^{aB}	0.78±0.02^{b}D	1.47±0.08^{a}A	0.98±0.07^{b}C
C10	3	1.29±0.13^{a}C	2.45±0.11^{a}A	1.13±0.13^{aC}	1.71±0.15^{aB}	1.78±0.10^{aB}	2.65±0.14^{a}D	4.46±0.12^{a}A	2.60±0.03^{a}D	4.01±0.13^{aB}	3.76±0.14^{aC}
	90	1.23±0.10^{a}AB	1.36±0.14^{b}A	1.35±0.12^{a}A	1.14±0.10^{aB}	1.22±0.07^{b}AB	2.55±0.12^{aB}	3.21±0.12^{b}A	2.48±0.13^{aB}	3.20±0.12^{b}A	2.66±0.01^{b}B
C11	3	0.09±0.01^{a}A	0.08±0.02^{a}A	0.07±0.01^{a}A	0.08±0.03^{aA}	0.06±0.02^{a}A	0.36±0.03^{a}A	0.34±0.01^{a}A	0.29±0.00^{b}A	0.38±0.00^{a}A	0.36±0.01^{a}A
	90	0.09±0.02^{a}AB	0.11±0.02^{a}A	0.08±0.01^{a}AB	0.06±0.02^{aB}	0.08±0.03^{a}AB	0.25±0.02^{bE}	0.32±0.02^{aC}	0.55±0.03^{a}A	0.38±0.01^{aB}	0.29±0.04^{b}D
C12	3	1.73±0.15^{a}C	3.40±0.03^{a}A	1.67±0.16^{aC}	2.08±0.13^{aB}	2.16±0.16^{aB}	5.20±0.11^{a}A	5.09±0.09^{a}A	4.13±0.11^{a}D	4.85±0.10^{aB}	4.50±0.17^{aC}
	90	1.46±0.11^{a}BC	3.26±0.07^{b}A	1.56±0.14^{aB}	1.44±0.10^{bB}C	1.35±0.08^{b}C	3.93±0.13^{b}B	4.62±0.14^{b}A	4.03±0.12^{aB}	4.60±0.10^{a}A	4.07±0.15^{b}B
C13	3	0.29±0.05^{a}A	0.16±0.03^{a}C	0.27±0.06^{a}AB	0.31±0.08^{aA}	0.19±0.04^{b}BC	0.23±0.08^{a}A	0.17±0.01^{a}A	0.19±0.03^{a}A	0.21±0.02^{a}A	0.16±0.06^{a}A
	90	0.28±0.03^{a}A	0.15±0.06^{a}B	0.24±0.03^{a}A	0.24±0.05^{aA}	0.29±0.04^{a}A	0.35±0.01^{a}A	0.17±0.02^{aC}	0.18±0.03^{aB}C	0.18±0.02^{aB}C	0.21±0.02^{aB}
C14	3	9.99±0.63^{a}	10.88±0.5	9.82±0.55^{a}	9.95±0.59^{aA}	10.06±0.63	12.58±0.48	11.39±0.58	11.93±0.47	12.06±0.52	12.31±0.53

		A	4^{aA}	A		aA		aA	aB	aAB	aAB	aAB
	90	9.78±0.47^{a} A	10.28±0.50^{aA}	9.33±0.63^{a} A	9.64±0.56^{aA}	9.48±0.69^{a} A		11.60±0.55 aA	12.26±0.48 aA	11.41±0.53 aA	11.91±0.55 aA	11.48±0.59 aA
C15	3	1.62±0.16^{a} A	1.09±0.10^{b} B	1.63±0.11^{a} A	1.70±0.12^{b} A	1.70±0.11^{a} A		1.52±0.17^{a} A	1.31±0.13^{a} A	1.42±0.12^{a} A	1.33±0.12^{b} A	1.38±0.13^{a} A
	90	1.69±0.11^{a} B	1.61±0.14^{a} B	1.57±0.16^{aB}	2.85±0.16^{aA}	1.65±0.13^{aB}		1.22±0.14^{aB}	0.97±0.13^{b} C	1.29±0.12^{aB}	1.59±0.11^{a} A	1.28±0.14^{aB}
C16	3	35.55±0.77^{aB}	35.23±0.70^{aB}	36.97±0.74 aA	36.11±0.16 aAB	34.95±0.82 aB		31.21±0.64 bB	31.11±0.74 bB	33.06±0.63 aA	31.76±0.66 aB	31.20±0.68 aB
	90	36.68±0.51^{aA}	34.96±0.62^{aB}	36.99±0.91 aA	36.65±0.77 aA	36.12±0.86 aAB		33.03±0.65 aA	32.83±0.63 aA	32.01±0.34 aA	31.85±0.72 aA	32.73±0.83 aA
C17	3	0.80±0.07^{a} B	0.84±0.04^{a} AB	0.94±0.07^{a} A	0.87±0.08^{aA} B	0.91±0.03^{a} A		0.84±0.09^{a} A	0.88±0.06^{a} A	0.88±0.03^{a} A	0.90±0.08^{a} A	0.81±0.09^{a} A
	90	0.65±0.13^{a} B	0.59±0.12^{b} B	0.25±0.03^{b} C	0.55±0.06^{bB}	0.83±0.04^{b} A		0.60±0.10^{b} A	0.20±0.12^{b} B	0.71±0.11^{a} A	0.55±0.06^{b} A	0.71±0.03^{a} A
C18	3	10.75±0.46^{aA}	7.95±0.46^{b} B	10.85±0.37 aA	10.30±0.28 aA	10.65±0.45 aA		10.74±0.38 aA	10.97±0.46 aA	10.78±0.46 aA	10.44±0.44 aA	10.98±0.40 aA
	90	10.28±0.63^{aA}	10.17±0.43^{aA}	10.50±0.49 aA	10.28±0.44 aA	10.79±0.49 aA		10.93±0.54 aA	9.74±0.52^{b} B	11.20±0.45 aA	9.09±0.42^{b} B	10.90±0.43 aA
US												
Mono	3	28.05±1.04^{aA}	25.06±0.93^{aB}	28.24±0.87 aA	27.64±1.00 aA	27.10±0.97 aA		22.50±1.29 aA	23.65±0.99 aA	24.10±0.78 aA	22.59±0.91 aA	23.68±0.88 aA
	90	28.84±1.11^{aA}	26.07±0.89^{aB}	27.67±0.81 aAB	26.24±0.73 aB	28.72±1.05 aA		23.94±1.08 aA	24.19±1.00 aA	25.14±1.06 aA	24.55±0.90 aA	24.92±0.80 aA
C14:1	3	1.90±0.17^{a} A	1.28±0.10^{b} B	1.87±0.15^{a} A	1.78±0.08^{aA}	1.74±0.21^{a} A		0.60±0.09^{b} B	1.34±0.14^{a} A	1.40±0.09^{a} A	1.53±0.13^{a} A	1.43±0.16^{a} A
	90	1.54±0.12^{b} AB	1.76±0.16^{a} A	1.54±0.10^{b} AB	1.44±0.05^{bB}	1.60±0.15^{a} AB		1.33±0.15^{a} AB	1.09±0.14^{aC}	1.30±0.10^{aB} C	1.55±0.11^{a} A	1.28±0.13^{aB} C
C15:1	3	0.45±0.04^{a} BC	0.44±0.01^{a} C	0.43±0.05^{aC}	0.53±0.05^{aA} B	0.54±0.07^{a} A		0.28±0.06^{a} A	0.16±0.02^{aB}	0.28±0.05^{a} A	0.19±0.05^{a} AB	0.21±0.06^{a} AB
	90	0.40±0.05^{a} A	0.39±0.08^{a} A	0.43±0.06^{a} A	0.41±0.02^{b} A	0.41±0.03^{b} A		0.21±0.05^{aB} C	0.26±0.07^{a} AB	0.33±0.05^{a} A	0.20±0.03^{aB} C	0.13±0.05^{aC}
C16:1	3	3.18±0.17^{a} C	2.03±0.10^{b} D	3.82±0.20^{a} A	3.69±0.10^{aA} B	3.48±0.19^{aB}		2.40±0.20^{aB}	2.51±0.14^{a} AB	2.74±0.14^{a} A	2.54±0.12^{a} AB	2.47±0.17^{a} AB
	90	3.17±0.19^{a} AB	3.07±0.13^{a} B	3.34±0.14^{b} A	3.10±0.15^{b} AB	3.30±0.11^{a} AB		2.16±0.19^{aB}	1.70±0.16^{b} C	2.18±0.15^{b} B	2.80±0.15^{a} A	2.18±0.17^{aB}
C17:1	3	0.34±0.05^{a}	0.37±0.07^{a}	0.34±0.06^{a}	0.29±0.07^{b}	0.22±0.02^{b}		0.18±0.08^{aB}	0.16±0.06^{aB}	0.14±0.01^{b}	0.28±0.06^{a}	0.36±0.06^{a}

		A	A	A	AB	B				B	A	A
	90	0.45±0.06^{a} A	0.46±0.06^{a} A	0.19±0.05^{b} B	0.44±0.05^{aA}	0.45±0.10^{a} A		0.27±0.05^{a} A	0.14±0.06^{aB}	0.32±0.07^{a} A	0.33±0.06^{a} A	0.28±0.01^{a} A
C18:1	3	22.18±0.69^{aA}	20.94±0.68^{aB}	21.78±0.51 aAB	21.34±0.80 aAB	21.12±0.66 bAB		19.05±0.98 aAB	19.49±0.68 aA	19.54±0.60 aA	18.04±0.65 aB	19.21±0.56 bAB
	90	23.28±0.79^{aA}	20.39±0.62^{aC}	22.18±0.68 aAB	21.86±0.60 aB	22.97±0.72 aAB		19.98±0.74 aB	21.00±0.71 aAB	21.01±0.80 aAB	18.64±0.59 aC	21.56±0.60 aA
Poly	3	1.87±0.22^{a} B	2.12±0.06^{a} A	1.77±0.11^{b} B	2.00±0.14^{aA} B	1.88±0.08^{a} AB		2.46±0.17^{a} A	1.91±0.14^{aB}	2.44±0.02^{a} A	1.93±0.05^{b} B	1.97±0.04^{aB}
	90	1.84±0.02^{a} C	2.09±0.04^{a} A	1.95±0.02^{aB}	2.03±0.07^{aA}	1.86±0.02^{aC}		2.50±0.12^{a} A	1.94±0.03^{a} D	2.15±0.06^{b} C	2.35±0.02^{aB}	2.04±0.04^{aC} D
C18:2 C	3	1.15±0.10^{a} BC	1.51±0.10^{a} A	1.10±0.07^{aC}	1.29±0.09^{aB}	1.17±0.11^{aB} C		1.65±0.11^{a} A	1.14±0.10^{aC}	1.76±0.08^{a} A	1.14±0.10^{b} C	1.31±0.01^{b} B
	90	1.16±0.08^{a} B	1.45±0.09^{a} A	1.18±0.10^{aB}	1.22±0.08^{aB}	1.20±0.09^{aB}		1.58±0.09^{a} A	1.13±0.09^{aC}	1.38±0.01^{b} B	1.38±0.02^{aB}	1.37±0.02^{aB}
C18:3 n3	3	0.72±0.12^{a} A	0.61±0.04^{a} A	0.67±0.04^{a} A	0.71±0.05^{b} A	0.71±0.03^{a} A		0.81±0.06^{b} A	0.77±0.04^{a} AB	0.68±0.06a BC	0.79±0.05^{b} A	0.66±0.05^{aC}
	90	0.68±0.06^{a} BC	0.64±0.06^{a} C	0.77±0.08^{a} AB	0.81±0.02^{aA}	0.66±0.07^{aC}		0.92±0.03^{a} A	0.81±0.06^{aB}	0.77±0.06^{aB}	0.97±0.04^{a} A	0.67±0.06^{aC}
Trans	3	3.12±0.05^{b} AB	2.95±0.23^{b} BC	3.31±0.19^{a} A	3.18±0.16^{aA} B	2.66±0.12^{aC}		4.01±0.32^{b} A	3.71±0.09^{a} AB	3.68±0.05^{b} AB	3.63±0.21^{b} B	3.58±0.19^{aB}
	90	3.29±0.01^{a} B	3.51±0.19^{a} AB	3.54±0.11^{a} A	3.40±0.15^{aA} B	2.78±0.06^{aC}		4.63±0.04^{a} A	3.58±0.09^{aC}	3.97±0.08^{aB}	4.11±0.21^{aB}	3.67±0.15^{aC}
C18:1 T	3	2.51±0.10^{a} B	2.27±0.18^{b} BC	2.77±0.11^{a} A	2.45±0.10^{aB}	2.03±0.19^{aC}		3.26±0.27^{b} AB	3.38±0.05^{a} A	3.36±0.01^{b} A	3.20±0.17^{a} AB	3.05±0.14^{aB}
	90	2.66±0.10^{a} A	2.82±0.16^{a} A	2.81±0.08^{a} A	2.61±0.21^{aA}	2.13±0.09^{aB}		4.05±0.10^{a} A	3.44±0.03^{aB}	3.55±0.00^{aB}	3.45±0.15^{aB}	3.25±0.10^{aC}
C18:2 T	3	0.61±0.04^{a} BC	0.68±0.05^{a} AB	0.54±0.08^{b} C	0.73±0.06^{aA}	0.63±0.07^{a} ABC		0.76±0.05^{a} A	0.33±0.04^{a} D	0.32±0.05^{a} D	0.43±0.04^{b} C	0.53±0.06^{aB}
	90	0.63±0.09^{a} C	0.69±0.03^{a} BC	0.73±0.03^{a} AB	0.79±0.05^{aA}	0.65±0.03^{aB} C		0.58±0.06^{b} A	0.14±0.06^{b} C	0.43±0.08^{aB}	0.66±0.07^{a} A	0.42±0.06^{aB}
Total	3	98.57±3.65^{aA}	97.92±3.30^{aA}	99.79±3.25 aA	100.00±2.67^{aA}	99.88±3.71 aA		99.59±3.27 aA	99.67±3.48 aA	99.81±2.66 aA	99.92±3.27 aA	99.68±3.24 aA
	90	99.92±3.24^{aA}	95.86±3.24^{aA}	99.25±3.13 aA	98.67±3.31 aA	99.41±2.95 aA		99.94±3.45 aA	99.91±2.87 aA	99.73±2.96 aA	99.86±3.27 aA	99.55±2.65 aA

Capital letters: represent average values with different letters are statistically significant ($p < 0.05$) within each row. Small letters: represent average values with different letters are statistically significant ($p < 0.05$) within each column. [a,] mean±S.D, n = 3.

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Effect of different levels of pulp concentration on processing of guava drink

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ABSTRACT

Guava is known to be the cheapest and richest source of vitamin C and calcium in Pakistan. Guava fruit is also a good source of polyphenols and its extract has anti-microbial properties. Consumption of drink with guava pulp will aid in population health. Freshly harvested graded, ripened and fully matured guava were pulped and preserved chemically. The drinks with different concentration of pulp were made and preserved by chemicals. The samples were analyzed for physicochemical characteristics and sensory evaluation was carried out after suitable time intervals. The data obtained were subjected to statistical analysis. The analysis of variance and the significant of different were determined by Duncan's multiple range tests. The drink prepared with 8 percent pulp concentration (T2) was found to be the best so for as sensory evaluation and processing was concerned. Physicochemical analysis of drink showed that acidity and reducing sugar contents increased with storage, while pH and ascorbic acid of the drink decreased. Total soluble solids of the drink remained constant.

Key Words: Guava Drink, Pulp Levels, Storage Stability, Storage Effect on Nutrients

INTRODUCTION

Guava (Psidiumguajava L.) is a member of large Myrtaceac family. It is common in most tropical and subtropical region throughout the world. Guava is a good source of vitamin A (beta carotene) and a rich source of ascorbic acid (vitamin C). It is also high in dietary fiber and contains lycopene, a carotenoid with antioxidant properties (Celsoet el., 2008).

Guavas (Psidiumguajava) are cultivated in many tropical and subtropical countries for their edible fruit. Guavas are often considered as super fruits being rich in vitamins A and C in the pericarp, omega-3 and -6 polyunsaturated fatty acids in the seeds and especially have high levels of dietary fibre. A single guava fruit weighing 160-170 g contains over four times more of vitamin C compared to a single orange (220-230mg/100g) and also has adequate levels of dietary minerals, potassium, magnesium and an otherwise broad, low-calorie profile of essential nutrients (Mahendran, 2010).

The fruit, which has a size of 2-5cm, can be rounded or pear-shaped and grows on medium size trees. The tree matures after 6 years when grown from a seed. For the purpose of fruit juice industry, it is convenient to divide the different types broadly into white fleshed and pink-fleshed varieties of which the Beaumont is probably the best known (Hopper, 1996).

Single guava fruit without seed (90g) provides 46 kilocalories energy, contains 78% water, 5 gram dietary fiber, 1g fat, 11g carbohydrate, 1 g protein, 18mg calcium, 256 mg potassium, 9mg magnesium, 23 mg phosphorus, 71 RE vitamin A, 165 mg vitamin C and 1mg of niacin and vitamin E each (Murdock 2002). Guava seeds also contain protein which is used in guava seed meal. The essential amino acid profile of guava seed protein isolate, except for lysine contents, is above that proposed in the FAO/WHO pattern for adult human beings (Nicannor et al. 2001).

In Pakistan guava is grown on 58.5 thousand hectares of land with annual production of 468.3 thousand tons. Punjab has higher annual production among all the provinces of Pakistan about 406 thousand tons (Agri.Stat. Pakistan, 1999). The extract from Guava is found to possess strong antioxidant activity, the extract from leave found to be good in scavenging free radical and phenolic compounds from guava extract found to be good as antioxidant. (Chen and Gow 2006).

Four new atimicrobial components have been extracted from guava fruit and on the basis of chemical and spectrophotometrical basis there structures have been established. The components are glycosides, morin 3 O alfaLyxopyranoside, guaijavarin and quircetin. (Arima and Danno, 2002) Quick settling and separation is most common problem in guava drink. This is because the nature of puree of guava is different from other fruits. This gives undesirable visual separation which is unacceptable from commercial point of view so the acceptability and stability of guava drink depends upon certain chemical or ingredients which should be used in manufacturing (Hick, 1990).

Among all the commercial based products as guava jam, jellies, sweets, squashes, guava nectars and ready to serve

comminuted bottled guava drink are famous. In Pakistan the project as making guava drink is not famous and launched by few food processing companies only . Basically this project is designed to provide the guava drink is market throughout the year so that people of Pakistan can enjoy the guava drink as well as vitamin C and calcium.

Material and methods

Procurement of raw Material: Guava fruit was collected from the local farms of Faisalabad and other material for the preparation of the drink was purchased from the local market. The chemical required for shelf life extension of drink and analysis was collected from the laboratories of the University.

Preparation of guava drink: Sound healthy, optimized size and matured guavas were purchased from market. Sorting was done on the basis of overall appearance and free from diseases. Fruit was washed with tap water in order to remove dirt, dust, pesticide residues and microbial load. Coring was done with coring knife and size reduction with ordinary knife. Pulp was made by addition of 15% water in guavas and heating it for 4 minutes in boiling water in order to make them soft. Then this soft fruit was blended and homogenized. After that chemical preservatives (citric acid and sodium benzoate) were added and the pulp was stored in plastic cans. For making drink, guava pulp with different concentrations (Table I) was used and other ingredients were mixed with water so that the final product contains 13% sugar 0.035% preservatives (sodium benzoate) and 0.15% carboxymethyl cellulose. The drink was then pasteurized and 0.001% ascorbic acid (vitamin C) was added just before filling. Hot filling was made in clean and sterilized bottles and crown capped afterwards. The bottles were cooled at ambient temperature and then stored. Keeping the parameters same, the pulp concentrations in different drinks was changed (Table I). The drink sample was analyzed after a regular interval of 15 days i.e. 0, 15, 30 and 45 days for the following parameters, total soluble solids, acidity, reducing sugars, ascorbic acids and pH.

Analysis of guava drink:

Total soluble solids: The total soluble solids of guava were determined by hand refractometer according to the method of Ruck (1963) and expressed as brix.

Acidity and sugar of drink:

Acidity of the guava drink was measured following the method of Moing and Svanella (1998). Sugar of guava drink: Sugars were determined according to the method of Ruck (1963).

pH:

pH of the drink was recorded by using pH meter as describe in AOAC (1995).

Sensory Evaluation:

Sensory evaluation of the Guava drinks will be conducted through trained panel of judges by following the method as described by the Meilguard *et al*., (1997).

RESULTS AND DISCUSSION

The present study was undertaken to ascertain the suitability of pulp concentration for drink. The drinks were made with different levels of pulp concentration and product was evaluated for its storage (Table I).The physicochemical analysis of the drinks showed that acidity, reducing sugar and total soluble solids of the drink increased while ascorbic acid and pH of the drink decreased with the storage.

In our study TSS increased slightly with storage (Table II). Acidity of the drink (Table III) was increased with the storage time and maximum increase of the acidity was in the drink T1 that was 9.6%. Reducing sugars (Table IV) of the drink also increased with the storage, minimum increase was observed in the T1 that is 3.64% and maximum increased was observed in T2 that is 4.16%.There are different factors which can increase the reducing sugars like temperature, pH, acidity and time of storage. If we see the table IV, it is clear that the time of the storage is increased the reducing sugar in the drink is increased. Alaka at el. (2003) also observed similar results on storage of mango juice. They stored mango juice packed in different packages at different temperatures and observed that ascorbic acid decreased, non enzymatic browning and titrable acidity increased, with storage time and soluble solid remain constant.

Table V shows the decrease in % ascorbic acid in the guava drink during the storage of 45 days. The percentage loss of the ascorbic acid is in the range of 38-41.6%. Maximum loss was observed in the T3 drink and minimum loss was observed in T2 drink. Kabasakalis et al. (2000) observed similar results that ascorbic acid of fruit juices such as oranges, peaches, grape fruit, pineapple and mango fruit is readily oxidized and lost during storage of juice, with losses ranging from 21-41%, when stored at room temperature for four month.

The pH of the drinks decreased with the passage of time and the percentage decrease was in the range of 6.36-6.80 (Table VI). Similar trend of decrease of pH have been observed by Ahmad et al (2008) while preparing ready to serve mandarin drink.

Sensory evaluation of the guava drink showed that its quality deteriorates slightly up to the storage of 45 day, but it was still acceptable after the 45 days of storage.

Table 1: Treatment plan of Guava drink

Drinks	Pulp (%)
T_1	6%
T_2	8%
T_3	10%
T_4	12%

Table 2: Effects of pulp concentrations and storage on % total soluble solids of ready to serve guava drink

Drinks/ Days→ ↓	0	15	30	45	Means
T_1	13.0	13.10	13.12	13.15	13.09
T_2	13.0	13.12	13.13	13.16	13.10
T_3	13.0	13.09	13.12	13.13	13.08
T_4	13.0	13.13	13.13	13.15	13.10
Means	13.0	13.09	13.15	13.16	13.10

Table 3: Effects of pulp concentrations and storage on % acidity of ready to serve guava drink

Storage (Days)

Drinks/Days→ ↓	0	15	30	45	Means	%Increase
T_1	0.31	0.32	0.34	0.34	0.32	9.60
T_2	0.34	0.34	0.36	0.37	0.35	8.80
T_3	0.35	0.36	0.36	0.38	0.36	8.57
T_4	0.36	0.37	0.38	0.39	0.38	8.33
Means	0.341	0.350	0.363	0.383		

Table 4: Effects of pulp concentrations and storage on % reducing sugars of ready to serve guava drink

Storage (Days)

Drinks/Days→ ↓	0	15	30	45	Means	%Increase
T_1	3.57	3.60	3.67	3.70	3.63	3.64
T_2	3.60	3.62	3.68	3.75	3.66	4.16
T_3	3.61	3.63	3.69	3.75	3.67	3.87
T_4	3.66	3.68	3.71	3.80	3.71	3.82
Means	3.61	3.63	3.68	3.75		

Table 5: Effects of pulp concentration and storage on ascorbic acid (mg/100mL) of ready to serve guava drink at different Storage (Days)

Drinks/Days→ ↓	0	15	30	45	Means	% decrease
T_1	4.70	3.56	3.42	3.22	3.73	38.0
T_2	4.76	3.64	3.47	3.36	3.80	37.0
T_3	4.92	3.70	3.54	3.39	3.88	41.6
T_4	5.04	3.76	3.63	3.44	3.96	39.8
Means	4.85	3.66	3.51	3.35		

Table 6: Effects of pulp concentrations and storage on pH of ready to serve guava drink at different stored days

Drinks/Days→ ↓	0	15	30	45	Means	% decrease
T_1	3.91	3.83	3.75	3.65	3.78	6.58
T_2	3.82	3.77	3.69	3.56	3.71	6.80
T_3	3.77	3.73	3.64	3.53	3.67	6.36
T_4	3.70	3.61	3.55	3.45	3.57	6.75
Means	3.80	3.73	3.66	3.54		

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Effect of Thawing on Frozen Meat Quality: A comprehensive Review

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ABSTRACT

Meat and meat products provide essential nutrients such as protein, fat, vitamins and minerals by making an important role in dietary intake. The overall eating quality of meat and meat products is affected by characteristics like taste, texture, juiciness, appearance and odor. Texture is deemed to be most important characteristic of all. Nowadays in busy life meat in bulk quantity is purchased for further usage that required thawing i.e. a loss of nutrition as well. The quality of frozen foods is main concern in many cases due to less attention is paid towards thawing process. However, thawing is significant cause of quality damage in freezing process due to many reasons. Proper precautionary measures must be practiced during meat thawing process to avoid microbial spoilage, which includes temperature below danger zone and reduced thawing time. Improper thawing technique will lead to activation and multiplication of already residing dormant micro flora on meat surface. The purpose of this review is to describe the effects of thawing on the physicochemical quality parameters of meat.

Key words: Meat quality, Thawing methods, shelf stability, Texture

Background

The meat freezing is a practice to extend its shelf-life. Because of high quality product has been practiced for thousands of years, although most improvements in freezing technologies have occurred in the past century (Leygonie *et al*., 2012; Persson and Londahl, 1993).

In food processing thawing of frozen materials is important. Thawing time should be minimum to reduce microbial growth, chemical deterioration and excessive loss of water caused by dripping or dehydration (Taher and Farid, 2001). Generally speaking, the quality of frozen food is closely related to freezing and thawing processes. Freezing rate and the small ice crystals formation in freezing are critical for minimizing tissue damage and drip loss during thawing. Thawing process is slow than freezing. During thawing process foods are subject to damage by chemical and physical changes and microbial attack (Fennema *et al.,* 1973; Kalichevsky *et al.*, 1995). For assurance of food quality quick thawing at low temperature avoiding notable rise in temperature and increased dehydration of food is desirable. Longer the thawing treatment time, higher will be the microbial growth on product surface. Nutritional quality reduction due to leaching of soluble proteins, high energy consumption and large quantities of loaded waste-water are also other disadvantages of conventional thawing (Roberts *et al*., 1998). Freezing and thawing process mainly affect the water fraction of meat. Since the water is present intermuscular and intramuscular fibers of the meat, compartments are created in the tissue, which complicates the process. With the freezing of water, the concentration of the remaining solutes (proteins, carbohydrates, lipids, vitamins and minerals) increases, thereby upsetting the homeostasis of the complex meat system (Lawrie, 1998). The changes in the immediate

environment of the muscle fibers affect the cell membrane characteristics, which in turn affect the quality of the meat (Fellows, 2000). A perception of the changes that freezing and thawing bring about in different meat types and cuts is essential to the meat industry, as their main objective is to produce better-quality products with high resale values that are both attractive and pleasant to the consumer (Renerre, 1990). Research conducted on freezing and thawing of meat has main focus on the reduction of moisture loss. Anon and Calvelo were the leaders in researching the effects of freezing on meat quality from the 1970s to the 1990s. Their work was later expanded on by Farouk and Swan (1998) from the 1990s into the 2000s. The shelf-life of meat is generally determined by appearance, texture, flavor, color, microbial activity and nutritive value (McMillin, 2008). Of these characteristics, flavor measurement is the most difficult. Flavor compounds may originate from lipid and peptide components in the muscle or meat (Spanier, 1992). All of these parameters are affected by freezing, frozen storage and subsequent thawing.

How does freezing preserve foods?

Most pathogens don't replicate at freezer temperature and many of them die because their enzymes don't work properly to sustain normal activity of cell. Also, pathogens need water to grow and freezing changes the available water into solid ice crystals. Slower the freezing process larger the crystals become and the more cells they damage. Speed of freezing in food depends on the amount of solutes (soluble salts, proteins and carbohydrates) which affect the temperature at which ice crystals are formed. The higher the level of solutes the lower the temperature must be for the food to freeze (Gill, C.O. 2002).

Storage time for frozen food:

If the temperature in a freezer fluctuates, the length of time you can keep frozen foods is considerably reduced. Freezer doors should be kept closed as much as possible, and only a small amount of unfrozen food should be added at one time.

Thawing of meat

Thawing refers to the melting process of conversion from a frozen to a liquid state (melt) or to become free from the effect (as stiffness, numbness, or hardness) of cold as a result of exposure to warmth.

Thawing is a quite important process following the freezing in terms of meat quality. There are various ways of thawing meat such as slow thawing, ambient temperature (counter-top) thawing, water immersion thawing, and microwave thawing (Xia *et al*., 2009). Despite the fact that the counter-top or ambient thawing increases the drip loss and is not suggested by the food codes and regulation due to the risk of microbial spoilage, almost 50% of consumers are still favoring this thawing method due to simplicity. The increased drip loss of muscle can lead to less acceptability, due to the loss of tasteful constituents, such as some amino acids or nucleotides. Also, nutritional constituents such as heme iron and heme pigment must be preserved by these processes as these pigments are mostly found in beef (Met *et al*., 2013).

Methods of Thawing of meat:

Speeding up the process of thawing runs the risk of food spending long periods in the temperature danger zone. Options for thawing raw meats, fish or chicken, and the

limitations of each conventional and novel method are summarized below.

Conventional methods of thawing

- Refrigerator Thawing
- Cold Water Thawing
- Microwave Thawing
- Thawing at room temperature

Novel methods of thawing

- High pressure thawing
- Ohmic thawing
- Acoustic thawing

Refrigerator thawing

Use a refrigerator thermometer to be sure the refrigerator temperature is consistently 40 F° or below. Place frozen meats and poultry products on a plate or cookie sheet in your refrigerator to prevent juices from dripping onto other foods. Place thawing foods on the bottom shelf or below ready to eat foods. This way the raw juices do not drip onto ready to eat foods. Plan ahead when thawing in the refrigerator; it usually takes at least 24 hours to completely thaw.

Different sizes of meat or poultry packages will require different amounts of time to completely thaw. For example, one pound of hamburger will take less time than large cuts of meat, such as a pot roast. Cook meat and poultry products within the following time frame, once they have been completely thawed in the refrigerator. Thaw raw meats, poultry, and seafood on the bottom shelf of the refrigerator. Have a plate or a pan under them to catch any juices that may drip.

Cold water thawing

Using cold water for thawing is a fast way to thaw out frozen foods but some precautions are needed. Place the frozen food in a leak proof bag and submerge in cold tap water. Change the tap water every 30 minutes until the frozen food is thawed. As soon as the food is thawed either cook it immediately or put it in the refrigerator until ready to cook. Different amounts of time are needed for different types of meat and poultry

- A 1-2 pound package of beef or poultry takes approximately 1 hour to thaw
- A 3 or more pound package of beef or poultry takes approximately 2-3 hours to thaw

Microwave thawing

A microwave oven is another fast way to thaw out frozen foods. When the microwave is used to defrost, it will start cooking the frozen item. When using this method, the food needs to be cooked right after it is thawed to prevent bacterial growth on the food. Do not put foods thawed this way in the refrigerator unless they have been cooked.

Room temperature thawing

Rapid thawing technique but Potential growth of pathogens on the surface initially, then inside if food temperature rises into the danger zone. Monitor the temperature regularly to keep food below 4°C. Require protection against flies, pests and domestic pets. Cook food immediately after using this method (Gill, C.O. 2002).

High-pressure thawing

High-pressure thawing would be another new application of high pressure on food industry. Though less attention has been paid to high-pressure thawing in comparison with high-pressure freezing, recently some research

revealed that high-pressure thawing can preserve food quality and reduce the necessary thawing time (Makita, 1992; Zhao *et al.*, 1996; Zhao *et al*., 1998), suggesting its potential for the food industry. Makita (1992) found that high-pressure thawing at frozen meat required only one-third of the time necessary at atmospheric pressure but produced sensory qualities comparable to those of conventionally thawed products. High pressure thawing was more effective in texture improvement in frozen tofu than was atmospheric-pressure thawing. During high pressure thawing, the drip loss of beef was too small to detect and there were no negative effects ($p < 0:05$) on colour, penetration force or cooking loss of thawed beef (Zhao *et al*., 1998). The thawing rate depends only on the conduction of heat, as pressure is transmitted uniformly through the sample (Kalichevsky *et al*., 1995). Zhao *et al*. (1998) demonstrated that pressure level and treatment time affected thawing rate and product quality, while product characteristics, such as size and initial temperature, did not affect thawing rate, indicating that it is advantageous to thaw a larger amount of product at high pressure.

Limitations on the application of high-pressure thawing are mainly high cost, the same as high-pressure freezing encounters, and pressure-induced protein denaturation and meat discoloration (Kalichevsky *et al*., 1995; Mertens and Deplace, 1993). Therefore, studies on fundamental data influencing high-pressure thawing process and its optimization is important to its commercial application.

Ohmic thawing

When electric current passes through conducting food with high electrical resistance, heat is generated instantly inside the food, thus increasing the temperature of the food item (Fu and Hsieh, 1999). This heating technology is termed as ohmic heating or electro-heating. In the food industry, more attention has been paid to the application of ohmic heating on aseptic processing and pasteurisation of particulate foods. In comparison with microwave heating, ohmic heating is more efficient because nearly all of the energy enters the food as heat and ohmic heating has no limitation of penetration depth. Ohmic heating also has advantages over conventional heating such as high heating rate, high energy conversion efficiency, volumetric heating, etc. (Reznick, 1996; Fellows, 2000). Using ohmic heating to thaw frozen foods is an innovative method. Ohtsuki (1991, 1993) patented an ohmic thawing process where frozen foods positioned with negative electrons were introduced into a high voltage electrostatic field. Using this method, the thawing time for frozen tuna, beef and eggs was shortened to 1/4–1/3 of that under the same temperature condition. Yun, Lee, and Park (1998) examined ohmic thawing of frozen chucks of meat in combination with conventional water immersion thawing with 60–210V (A.C.) at frequencies of 60 Hz–60 kHz. It was found that frequency changes did not significantly affect thawing time and ohmically thawed samples showed reduced drip loss and improved water holding capacity when lower voltages were applied.

Acoustic thawing

The utilisation of acoustic energy to thaw frozen foodstuffs was investigated about 50 years ago; however, the negative aspects of poor penetration, localized heating and high power requirement hindered the development of this method (Brody and Antenevich, 1959).

Recently, work on relaxation mechanism showed more acoustic energy could be absorbed by frozen foods when a frequency in the relaxation frequency range of ice

crystals in the food was applied (Kissam, *et al*., 1981). Kissam *et al*. (1981) illustrated the thawing process under the relaxation frequency. Experiments showed that blocks of cod required 71% less time by using acoustically assisted water immersion thawing than that with water immersion only when 1500 Hz acoustic energy at 60 watts was applied. Miles *et al.* (1999) applied high power ultrasound to thaw meat and fish, their work indicated that acceptable ultrasonic thawing was achieved at frequencies around 500 kHz, which conformed to relaxation mechanism. Therefore, acoustic thawing still is a promising technology in the food industry if proper frequencies and acoustic power are chosen.

Meat quality attributes affected by freezing and thawing

- Moisture
- Protein denaturation
- Oxidation of lipids and proteins
- Color (myoglobin protiens)
- pH
- Tenderness (shear force)
- Microbial count
- Drip loss
- Meat texture
- Meat structure

Moisture content of meat

Freezing and thawing alters both the content and the supply of moisture in meat tissue. Moisture as a quality characteristic in meat can be evaluated in several ways, including drip loss; thaw loss; cooking loss; water binding capacity and total moisture content. Moisture loss in meat is inevitable post mortem due to the decrease in pH (closer to the isoelectric pH of proteins), the loss of adenosine triphosphate (ATP), and the steric effects due to shrinkage of the myofibrils as a result of rigor mortis and conditioning (Huff-Lonergan and Lonergan, 2005). These factors all act to release water that was previously immobilised and bound to proteins into the intrafibrillar spaces.

The released water is then redistributed into the sarcoplasmic and extracellular spaces. Freezing and thawing are known to affect the amount of exudate (thaw loss and/or drip loss). In terms of thawing, major differences in opinion exist regarding the correlation between the rate of thawing and the extent of exudates formation. Gonzalez-Sanguinetti, Anon, and Cavelo (1985) concluded that a decrease in thawing time (time elapsed from −5 °C to −1 °C) to below 50 min resulted in a decrease in exudate. This was attributed to the melting of ice in the extracellular spaces causing an increase in water activity, resulting in the net flow of water into the intracellular spaces and its subsequent re-absorption by the dehydrated fibres. Haugland (2002) also proposed that an increased rate (or decreas in time) of thawing caused less exudate to form. Ambrosiadis *et al.* (1994) reported that rapid thawing of meat by submergence in water decreased the drip loss. On the other hand, it was found in the latter study that microwave thawing (35 min to reach 0 °C) increased the drip loss to within the same range as ambient air thawing (5–7 h), but this drip loss was still less marked than in the case of refrigerated thawing (28 h), which resulted in the highest drip loss.

Protein Denaturation

It has been traditionally thought that protein denaturation could result during freezing due to an increased

intracellular ionic strength following the migration of water to the extracellular spaces. Nonetheless, this mechanism has been refuted by several authors. Anon and Cavelo (1980), Mietsch *et al.* (1994) and Ngapo *et al*. (1999) all suggested that protein denaturation does not contribute significantly to quality loss, as they found no significant differences in the amount and composition of proteins in the drip collected from fresh samples and those samples that had been frozen and immediately thawed. It was, however, noted by these authors that the time and temperature of the sample storage may have influenced the results obtained and no new explanations were offered with regard to the loss of meat quality during freezing. It would consequently be very beneficial to evaluate the drip composition of such samples using more modern techniques, such as proteomics.

Oxidation of Lipids and Protein

The final temperature to which meat is frozen and stored determines the amount of unfrozen water that remains available for chemical reactions to proceed. Petrovic (1982) showed that biochemical reactions could still take place in meat frozen and stored at temperatures higher than −20 °C, since sufficient unfrozen water remained available at these temperatures for such reactions to occur. The optimum temperature for the frozen storage of meat has been reported to be −40 °C, as only a very small percentage of water is unfrozen at this point (Estevez, 2011). This fraction of water is believed to be bound to other food constituents and thus is chemically inactive (Nesvadba, 2008; Singh and Heldman, 2001). The freezing of the water fraction also causes an increase in the solute concentration both intracellularly and extracellularly, which is thought to be the reason for the increased chemical reactivity during frozen storage (Fennema, 1975). The ice crystals, depending on their size and location, will disrupt the muscle cells, resulting in the release of mitochondrial and lysosomal enzymes into the sarcoplasm (Hamm, 1979).

The fraction of unfrozen water is also important in terms of oxidation, since chemical reactions can occur during frozen storage that initiate primary lipid oxidation (peroxidation) in the meat. This can lead to radical secondary lipid oxidation upon thawing (Owen and Lawrie, 1975) leading to adverse changes in colour, odour, flavor and healthfulness. This phenomenon has been demonstrated by Akamittath *et al.* (1990) and Hansen *et al*. (2004), who reported accelerated lipid oxidation in frozen–thawed meat that was subjected to a refrigerated shelf-life study. The quality of the secondary products of lipid oxidation is generally measured using the thiobarbituric acid reactive substances (TBARS) method. These secondary products cause rancid, fatty, pungent and other off-flavours. The development of these flavours was noted by Vieira *et al*. (2009), who stated that TBARS of fresh meat were significantly lower than meat stored for 90 days at −20 °C. Such observations indicate that frozen storage is not necessarily sufficient to prevent oxidation from occurring. Although peroxidation was not measured in the aforementioned study, it would be expected that primary lipid oxidation would cease at such low temperatures by 90 days and secondary lipid oxidation would commence, which should be detected by the TBARS method. Benjakul and Bauer (2001) also found that freezing and thawing of muscle tissue resulted in accelerated TBARS accumulation and attributed this finding to the damage of cell membranes by ice crystals and the subsequent release of pro-oxidants, especially the haem iron. There is also increasing evidence to indicate that lipid oxidation takes place primarily at the cellular

membrane level and not in the triglyceride fraction. Therefore, lipid oxidation has been reported in both lean and fatty meats (Thanonkaew *et al.*, 2006).

Protein oxidation can be linked to any of the pro-oxidative factors, such as oxidised lipids, free radicals, haem pigments and oxidative enzymes. Malonaldehyde is one of the substrates that react with protein derivatives to form carbonyls (ketones and aldehydes) (Xiong, 2000). Protein and lipid oxidation are, therefore, undoubtedly interlinked. Protein oxidation in meat may lead to decreased eating quality due to reduced tenderness and juiciness, flavour deterioration and

discolouration (Rowe, *et al.*, 2004). These changes are partially due to the formation of protein aggregates through both non-covalent and covalent intermolecular bonds as reactive oxygen species (ROS) attack the proteins. Other common changes in oxidised proteins include amino acid destruction; protein unfolding; increased surface hydrophobicity; fragmentation and protein crosslinking. These all lead to the formation of protein carbonyls (Benjakul *et al.*, 2003; Liu *et al.*, 2000; Xia *et al*., 2009). Freezing and thawing cause damage to the ultrastructure of the muscle cells with the ensuing release of mitochondrial and lysosomal enzymes, haem iron and other pro-oxidants. These increase the degree and rate of protein oxidation (Xiong, 2000). The amino acid residues that are mainly involved in these reactions are lysine, threonine and arginine, the oxidation of which leads to the polymerization of proteins as well as peptide scission (Liu *et al*., 2000; Xia *et al*., 2009; Xiong, 2000). These amino acids are mainly found in the myofibrillar proteins, which account for 55–65% of total muscle protein and are responsible for the majority of the physicochemical properties of muscle foods (Xia *et al*., 2009). Protein oxidation destabilises the protein matrix leading to increased toughness, loss of water-binding capacity and loss in protein solubility.

Color (Myoglobin Proteins)

Myoglobin has been identified in exudate by gel-electrophoresis, accounting in part for the change in the colour stability of meat after freezing and thawing (Anon and Cavelo, 1980). It has also been reported that denaturation of the globin moiety of the myoglobin molecule takes place at some stage during freezing, frozen storage and thawing (Calvelo, 1981). The denaturation leads to an increased susceptibility of myoglobin to autoxidation and subsequent loss of optimum colour presentation. This theory has been verified by many authors by comparing the degree of bloom and the ability of the meat to resist oxidation to metmyoglobin during refrigerated storage post freeze/thaw (Abdallah, Marchello, and Ahmad, 1999; Farouk and Swan, 1998; Lanari, *et al*, 1990; Lanari and Zaritzky, 1991; Leygonie, Britz, and Hoffman, 2011; Marriott, *et al.*, 1980; Otremba *et al.*, 1999). The existence of an enzyme system capable of reducing metmyoglobin back to myoglobin was proposed by Livingston and Brown (1981) and was termed the metmyoglobin reducing activity (MRA). The theory is that in fresh muscle the enzyme is very active and the metmyoglobin formed is quickly reduced to deoxymyoglobin and oxygenated back to oxymyoglobin, thereby retaining the bloomed colour. However, as the meat ages or is frozen, the activity of the MRA is decreased and metmyoglobin begins to accumulate on the surface of the meat at a rapid rate (Abdallah *et al*., 1999). Also, MRA and/or co-factors, such as NADH, could be ‘lost’ from the post mortem sarcoplasmic environment by leaching as exudate during thawing, and/or due to

oxidation, and/or be used by reactions unrelated to MRA, which will all contribute to accelerated oxidation and loss of bloom (Abdallah *et al.*, 1999).

pH

The pH of meat that has been frozen and thawed tends to be lower than prior to freezing (Leygonie *et al.*, 2011). As pH is a measure of the amount of free hydrogen ions (H+) in a solution, it is possible that freezing with subsequent exudate production could cause denaturation of buffer proteins, the release of hydrogen ions and a subsequent decrease in pH. Alternatively, the loss of fluid from the meat tissue may cause an increase in the concentration of the solutes, which results in a decrease in the pH. A further explanation for this finding may involve the deamination of proteins by microbial or enzymatic action, with the ensuing release of hydrogen atoms (Leygonie *et al*., 2011).

Tenderness (shear force)

There is general agreement in the literature that the tenderness of meat increases with freezing and thawing when measured with peak force (Farouke, *et al.*, 2003; Lagerstedt, *et al.*, 2008; Shanks, *et al.*, 2002; Wheeler, *et al.*, 1990). It has also been found that the increase in tenderness is correlated to the length of frozen storage and the degree to which the meat was aged prior to freezing. The tenderising effect of freezing seems to be negated when the meat was sufficiently aged prior to freezing (Vieira *et al.*, 2009). The mechanism involved in the tenderisation is thought to be a combination of the breakdown of the muscle fibres by enzymatic action during proteolysis, ageing, and the loss of structural integrity caused by ice crystal formation. The formation of large, extracellular ice crystals disrupts the physical structure, largely breaking myofibrils apart and resulting in tenderisation. However, the formation of small intracellular ice crystals increases the rate of ageing probably by the release of protease enzymes (Vieira *et al*., 2009), although many alternative postulations exist in the literature.

Contradictory results have been obtained from sensory evaluation of tenderness (Lagersted *et al*., 2008), where a lower peak forces was reported in freeze/thaw samples compared to chilled meat. In this case the trained sensory panel rated the freeze/thawed meat significantly less tender than the chilled meat. This sensory result was attributed to the loss of fluid during thawing that resulted in less water available to hydrate the muscle fibres; thus, a greater quantity of fibres per surface area seemed to increase the toughness as perceived by the sensory panel. The decrease in the shear force was attributed to the loss in membrane strength due to the ice crystal formation thereby reducing the force needed to shear the meat (Lui *et al.*, 2010).

Microbial count

Neither freezing nor thawing appears to decrease the number of viable microbes present in meat. During freezing, however, microbial spoilage is effectively terminated as the microbes become dormant. Unfortunately, they regain their activity during thawing (Londahl and Nilaaon, 1993). As thawing is a much slower process than freezing and is less uniform, certain areas of the meat will be exposed to more favourable temperature conditions for microbial growth. This is of particular concern when air thawing is employed. In addition to the risk of high temperature exposure, there is an increase in moisture and nutrients available to microbes post freeze/thaw due to exudates formation. The moisture lost during thawing is rich in proteins, vitamins

and minerals derived from the structural disarray caused by the freezing process, which consequently provides an excellent medium for microbial growth. For this reason, good hygiene and handling practices are even more important for meat that is to be frozen and thawed compared to that which is to be sold fresh (Pham, 2004). Vieira *et al.* (2009) found in their study that beef frozen for up to 90 days, previously aged for 3 and 10 days, did not spoil due to microbial growth. They did, however, report an increase in the levels of psychrotrophic bacteria during the 90-day frozen storage, which were probably favoured above the other bacteria by the thawing process (48 h at 4 °C in a cooler). Greer and Murray (1991) found that the lag phase of bacterial growth in frozen/thawed pork was shorter than for fresh meat, but that the time to develop spoilage

odours was not affected. Literature on the microbial quality and shelf-life post freeze/thaw is limited for all species of meat, but that which is available seems to indicate that the microbiological shelflife of fresh and frozen/thawed samples is similar.

Meat Structure

Most severe fibre deterioration during freezing and subsequent thawing was due to forming inter- and intracellular ice crystals, when the ice crystals formed between the fibres generate pressure which separates the fibres, while the ice crystals formed within the fibre generate pressure in the opposite direction. A less severe deterioration was noticed when only intra-cellular ice crystals were formed, when the pressure is generate in one direction only.

Moreover, the structure of the frozen meat was assessed according to the size of the cavities which become visible in the case of microscopy. In the case of frozen meat, these cavities can indicate the size of the ice crystals which appear during freezing, while for fresh meat they correspond to the space occupied by the extra-cellular fluid (Hansen, *et al.*, 2003). In this respect, studies on frozen meat at different rates showed the appearance of some big cavities which led to severe deterioration of the muscular cells structure (Ngapo, *et al.*, 1999). Nonetheless, it was noticed that after thawing, the ultra-structure of meat samples fully recovered from the totally damaged structure in the frozen samples (Ngapo, *et al.*, 1999). RMN studies were combined with microscopy by Mortensen *et al*. (2006) in order to highlight the influence of the freezing temperature and of the freezing rate on the ultra-structure of the thawed meat as well as on thawing loss. The samples were frozen at temperatures of -80°C (fast freezing) and -20°C (slow freezing) and stored at -20°C for 30 months.

After thawing it was noticed that in the case of the samples subjected to fast freezing the damages were more severe that in the case of the samples subjected to slow freezing, as well as the tendencies to have higher thawing loss. It is assumed that those small ice crystals turned into big ice crystals either as a consequence of re-crystallizing while storage or because of the slow thawing process.

Meat Texture

Meat texture is important sensory property for consumers and, implicitly, its juiciness. In this respect, many studies showed that the freezing process as well as the meat aging rate before and after freezing could contribute to changes in meat texture after thawing.

Thus, Shanks *et al.* (2002) showed differences between the shear force of aged meat chilled not frozen and the shear force of meat stored frozen for 2 months, the latter representing a lower shear force. It was presumed that

these results are a consequence of the fact that muscular cells were deteriorated because of intra-cellular ice forming during freezing which led to lowering the shear force in frozen and thawed samples. Similar results were obtained by Lagerstedt *et al.* (2008) who, comparing the values of the shear force in chilled meat samples as well as of the frozen samples after aging, obtained lower values of the shear force in the frozen samples. In this case, although the values of the shear force were lower for frozen meat than for chilled meat these were not consistent with the sensory evaluation, the thawed meat being significantly less tender than the chilled meat.

As regards the freezing rate, Dransfield, (1994) considered that it plays an important role in meat aging. Thus, unlike slow commercial freezing, fast freezing increased the rate of aging 3 times more than in chilled beef. In this situation, these results were explained by the appearance of cellular lesions which led to an increase of aging rate (Vieira *et al*., 2009).

Drip Loss

A very important aspect in meat industry – especially from a financial point of view – is the driploss after thawing. This is the reason why the factors which influence the drip loss after thawing should be identified. Previous studies have reported the link between drip loss, freezing speed and aging rate of the freezing meat. Ngapo *et al.*, (1999) showed the influence of freezing rate on dip loss. It was proven that in the case of fast frozen pork (for 12-120 minutes) the drip loss was the same as for refrigerated meat. Yet, in the case of slowly frozen meat (for 240-900 minutes) the drip loss was significantly higher than for refrigerated meat. Ngapo *et al.* (1999) suggested that protein concentration of drip obtained after different treatment (frozen and thawed and frozen, stored and thawed) and of drip of fresh samples showed that there are no significant differences between thawed samples and fresh ones. As regards the influence of freezing rate on drip loss for meat stored for 4 weeks, Ngapo *et al*. (1999) demonstrated that the freezing rate used before storage did not influence drip loss. Moreover, drip loss at samples stored for 4 weeks were significantly higher that drip loss at samples which were not stored. Concerning freezing temperature, Sakata *et al*. (1995) reported that no correlation between freezing temperature (-20°C and -80°C) and drip loss was found and no significant difference was noted. An analysis of the freezing speed was done by Petrovic *et al*. (1993) and it was demonstrated that in both cases (slow freezing and fast freezing) happened considerable deteriorations of fibres and micro-fibres, reduction of myofibrils proteins solubility, as well as great thawing loss. Hansen *et al*. (2003) found that using pressure while freezing affects the amount of exudate. Thus, drip loss from thawed, pressure shift- frozen was not different from drip loss from fresh meat samples, while cryogen-frozen and air frozen pork both had significantly higher drip loss.

In this case it was assumed that pressure causes protein denaturation and the insoluble proteins blocked the drainage of the muscular fluid leading to smaller quantities of thawing loss. It was tried to establish a relation between thawing rate and amount of exudate and it was noticed that meat exudate depends upon thawing time. Theories on the effect of thawing time on drip loss were contradictory, Gonzales-Sanguinetti *et al*. (1985) evidencing that, by lowering the thawing time the exudate is higher and Ngapo *et al*. (1999) demonstrating that drip loss is lower, proportionally to shorter thawing time period. Related to obtaining higher drip loss in the case of slow thawing, Linares *et al.* (2005) suggests that drip loss

could be linked with the speed of the thawing rate, and in the case of slow thawing the fluid released from fibres cannot be reabsorbed.

Similarly, in the case of slow thawing there is also the possibility of re-crystallising leading to high drip loss out of the fibres. A link between the optimum time post-mortem to freeze meat and drip loss was made by Yu *et al*. (2009). Thus, it was proven that freezing meat at 45 min instead of 24 h after slaughter resulted in lower drip loss after thawing. Consequently, it is recommended that the earlier the meat was frozen after slaughter, the lower drip loss at thawing, assuming that in this freezing phase more extra-cellular crystals than inter-cellular ones are formed. We know that storage while freezing is an important method of meat preservation.

Conclusion

As global trade increases and the distance between producer and consumer expand, there is great demand to freeze meat for transportation. Beef, lamb/mutton and chicken are the meat products that are produced world-wide in the greatest quantities majority of the research in the meat science discipline has main focus on these species. In this review effect of thawing on different parameters for assuring good quality meat are assessed. In recent years, the main focus of research into freezing and thawing mechanisms has been concentrated on the development of novel freezing and thawing methods. The commercial application of these processes is still disputed, however, even though scientific research indicates that they lead to an increase in the quality of meat.

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Health Benefits and Importance of Utilizing Wheat and Rye

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ABSTRACT

Cereals are being utilized as the staple food in most parts of the world. The food products prepared from the cereals are the essential part of the daily diet worldwide. Several studies indicate that the consumption of whole grain food products reduces the risk of wide spread chronic diseases. Wheat and rye are the members of *Gramineae* family and are used to cure some common ailments. These are rich in different nutrients and their bran is the excellent source of dietary fiber. They also provide substances such as the lignans, alkyresorcinol, phytosterols, phenolic acids, folates, tocopherols and tocotrienols. The biologically active components found in wheat and rye have several health benefits. These not only help to prevent digestive disorders and cancer but also provide protection against cardiovascular diseases and help in reduction of the different health problems such as constipation, obesity, diabetes and appendicitis.

Key words: Wheat, rye, fiber, cardiovascular diseases

INTRODUCTION:

Wheat is nutritious food worldwide and provides proteins, minerals, B-group vitamins and dietary fiber more in quantity than other cereal crops and help in preparation of different types of foods. The wheat grain consists of three distinct parts: bran (13-17%), germ (2-3%) and endosperm (80-85%) and contains all essential nutrients. In general, 70% carbohydrates, 12% water, 2% fat, 12% protein, 1.8% minerals, and 2.2% crude fiber are found in wheat grain kernel. It is also enriched with phosphorus, magnesium, manganese, zinc, selenium, iron, potassium and copper (Liu *et al.*, 2012). Wheat flour is the key ingredient in making the health beneficial foods. The dietary fibers in the wheat bran help to reduce colon cancer risk along with preventing and curing some digestive disorders (Qu *et al.*, 2005).

Rye is an important cereal crop, higher in fiber contents and has become the important ingredient of different foods considered beneficial from heath perspective. It contains biologically active substances showing antioxidant properties by acting as reducing agents, free radical-scavengers and thorugh formation of complexes with metals (Tanwir *et al.*, 2013). Bioactive compounds present in large quantity in the rye bran are lignans, alkyresorcinol, benzoxazinoids, phytosterols, phenolic acids, folates, tocopherols and tocotrienols. The mouth feel and taste of food is manipulated by the lignin and cellulose (Katina *et al*., 2007*).* Benzoxazinoids provide the health promoting effects such as weight reduction and appetite suppression effects, anti-allergic effect, anti-inflammatory effects and anti-carcinogenic effects (Hefni and Witthoft, 2012). Nutritionally optimized cereal foods are prepared by addition of rye flour. Dietary fiber present in rye may be soluble or insoluble. Mostly insoluble dietary fiber helps in enhancement of the digestibility and bioavailability of nutrients.

The cancer and chronic diseases risks factors can be minimized by the utilization of rye based products. It is thought that this reduction can take place by the activity of the antioxidants and through digestion of resistant carbohydrates and phytochemicals. The consumption of

rye based products is increasing these days as the consumers are well aware of health beneficial foods. Cardiovascular disease and type-2 diabetes can also be protected by the consumption of cereal based foods.

Cereal based industry is continuously growing and the production of rye based products is also increasing. Up to 80 % rue flour is used for bread baking while its utilization for the preparation of breakfast cereals and rye flakes is 55 % (Zeilinski *et al*., 2007). Baking quality of rye flour is positively influenced by water extractable arabinoxylan, which is the main component of rye. However, the baking quality is negatively affected by the non extractable arabinoxylan. Therefore both the amount and extractability of arabinoxylans are important in determining its role in bread making. Rye arabinoxylan has high water holding capacity which is responsible for the retrogradation of starch and results in staling of bread. Oat and barley also contain higher amount of β-Glucan than rye. According to the dose, molecular weight and viscosity, it also helps to lower the cholesterol and control the glucose level (Rakha *et al*., 2010).

Wheat and rye based food containing dietary fiber provide bulkiness to the foods when consumed (Korycinska *et al*., 2009). For the proper health of human eyes and skin, the effect of lutein along with zeaxanthin, is considered as substantial. Likewise, for intoxication and the treatment of biliousness, various cereal stems are used. Additionally, starches and fiber present in whole grains ferment and produce numerous substances in colon that may prevent the bile acid to promote cancer effect. These seed sprouts are also utilized to cure sore throat, thirst, spasmic pain, coldness, cough and constipation (Wang *et al.,* 2010).

Cereal grains have some specific constituents that have various health benefits for humans, such as anti-disease factors and antioxidants. In this context, Phytic acid was found to play a key role in the medication of hypercholesterolemia, cancer, kidney stones and hypercalcuria. Diets rich in dietary fiber and high in carbohydrates mostly originate from cereals that help in taking out of oral hypoglycemic agents or help in reduction of insulin in diabetic patients (Ragaee *et al.,* 2006). Immune functions are improved by the short chain fatty acid which help in the production of splenocyte cytokines, antibodies, T helper cells and leukocyte all of which have a vital role in immune protection. Lactic acid forming bacteria competitively hinder the growth of pathogenic bacteria which shows their positive control over immune functioning. This control over immune system helps in the reduction of insulinemia and glycemia. A diet low in fiber contents is responsible to the etiology of hemorrhoids. The Symptomatic hemorrhoids can be treated by increasing the fiber contents in the diet (Slavin *et al*., 2009).

Importance of health beneficial foods

In market, the fiber enriched healthy food with low calories and sugar free is in greater demand. Purposely, the researchers have developed the fiber enriched food products to cure the diabetes hypertension, colon cancer and many other health related problems. Quite a number of health benefits of using fibrous products have been reported especially that are rich in lignin, gums, cellulose and hemicelluloses. Similarly, a β-glucan rich fibrous food helps to reduce the absorption of glucose in diabetic patients (Sudha *et al*., 2007).

Importance of wheat

Wheat being used as staple food in Pakistan, fulfills the 60% of total calories and protein needed for the daily life. In the world about 65% of wheat grain is used by humans. In Pakistan, about 80% of total wheat is used for production of unleavened flat bread whereas about 20% is used for the production of bakery products (Khan *et al*., 2009). Nutritionally, wheat germ is rich in essential

vitamins, deficiency of which can lead to various cardiovascular diseases. Furthermore, the consumption of refined flour which is deficient in vitamins and minerals

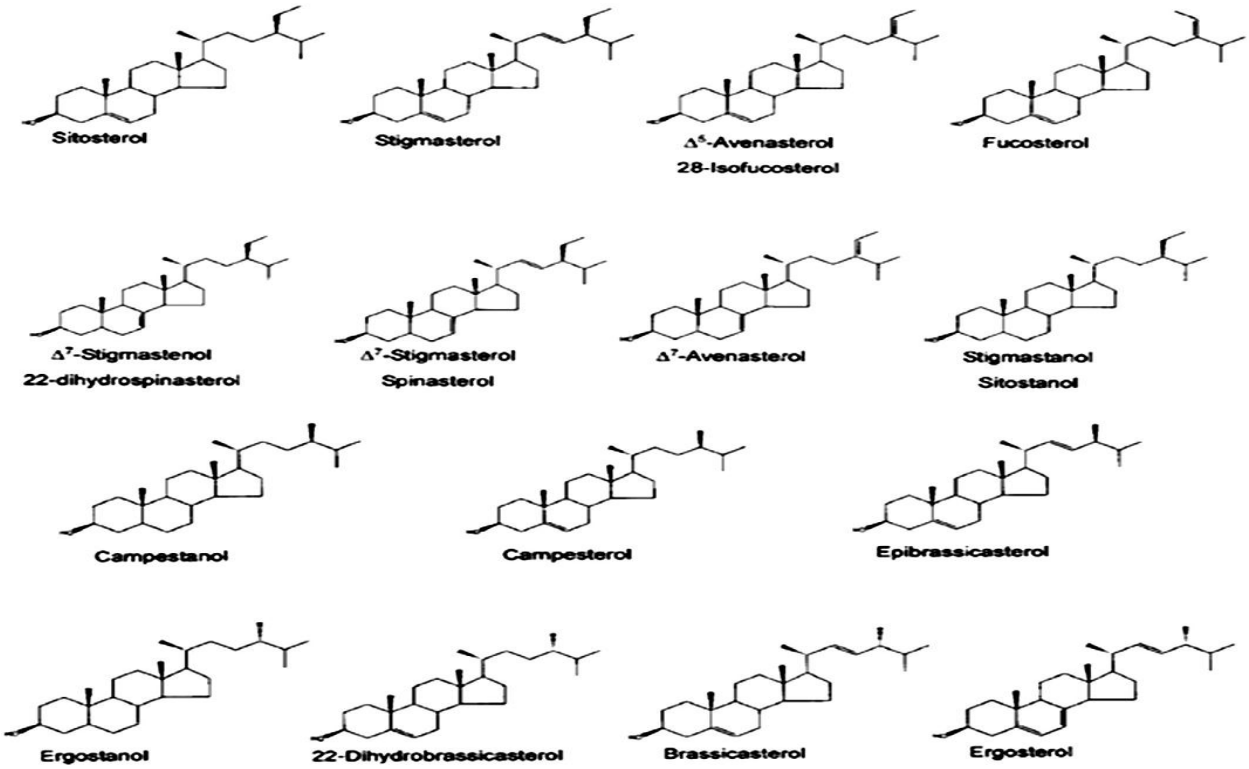

Figure 1:

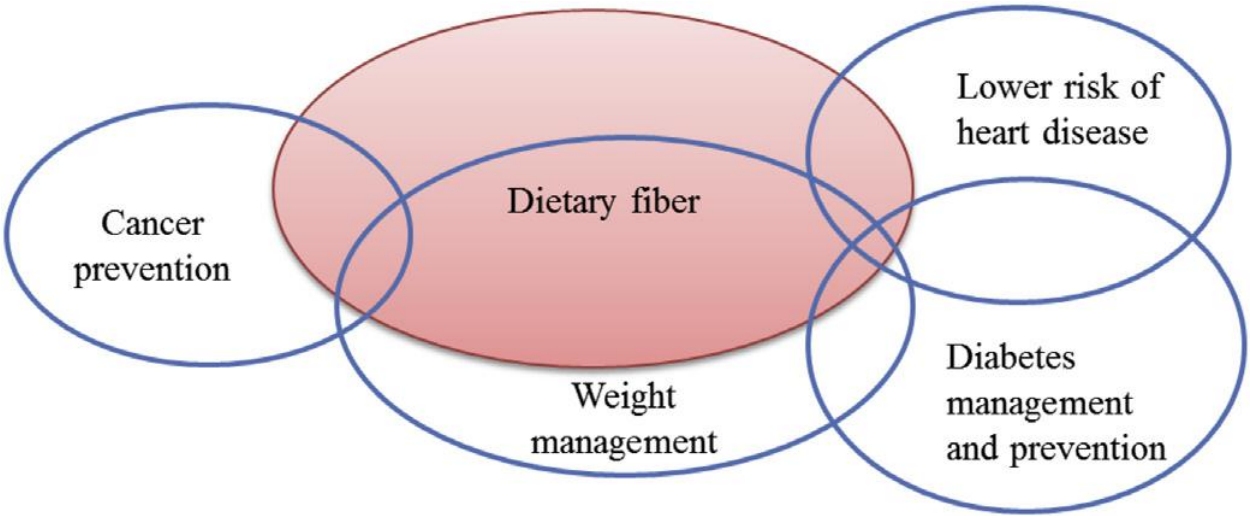

Figure 2:

lead to numerous digestive and nutritional disorders like constipation. However, in contrast to this, the usage of dietary fiber results in medication of various diseases like ischaemic heart disease, constipation, colon diseases such as appendicitis, diverticulum, diabetes and obesity (Kumar *et al*., 2011).

Importance of rye

Rye bran contain higher amount of dietary fiber and its complex comprehend the plant lignans along with various other bioactive compounds. Plant lignans e.g. enterolactone are being utilized as biomarkers for the intake of lignin rich food in blood concentration. However, numerous studies show that it really works, particularly in relation to upper digestive tract cancer. Quite a number of results have revealed that whole grain cereals such as rye are protective against myocardial infarctions. A consequent defensive effect has also been demonstrated against ischemic stroke and diabetes. It seems realistic to presume that the defensive effects are linked with many factors in dietary fiber complex (Hallmans *et al*., 2003).

Folates

Folate is a common term for various forms of folic acids including B vitamin. Folates are cofactors in many enzymic reactions, including the biosynthesis of amino acids and nucleotides. As folates are essential for proper body functioning, their deficiency may result in megaloblastic anemia and neural tube defects. Cereals, being a magnificent source of folate help to counteract with these ailments. In Finland, where the fortification of folic acid is not done, cereals supply 36-43 % of the folate (Kariluoto *et al.*, 2006).

Sterols

Plant stanols and sterols are the components that found naturally in cereal grains. Phytostanols occur in rye, corn, wheat, rice, fruits and vegetables. Different studies were performed to unveil the effects of food fortified with numerous plant sterols. Phytosterols which are structurally resembled with body's cholesterol, compete with cholesterol for absorption purpose, after consumption in digestive system. Resultantly, absorption of cholesterol is stopped and blood cholesterol level is decreased. Researchers have suggested that phytosterols provided in natural matrices are biologically active at various levels present in healthy diet and have enormous effects on whole body cholestrol metabolism.

Arbinooxylan

Arbinoxylan are the main non cellulosic polysaccharides in cereals. These are the polymer of xylose. These have several health benefits as a dietary fiber (Mendis and Semsick, 2013).

1. Immmuno modulatory activity
2. Exhibit cholesterol lowering activity
3. Prevention against alternate type 2 diabetes
4. Enhance the absorption of minerals
5. Act as feacal bulking agent
6. To have a prebiotic effect

Health benefits of wheat and rye

Cancer

The stomach, digestive tract, liver and colo-rectal cancer are the different forms of cancer whose prevalence varied among various countries. Different studies showed that Western lifestyle put an undesirable consequence on the incidence of various types of cancers. It is the localized and malignant cancer which usually spread and difficult to cure (Hallmans *et al*., 2003).

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Colon cancer

Colon cancer is one of the most widely spread diseases. In western world it is one of the major cause of death and mortality. Colon cancer occurred due to less consumption of dietary fibers. Various researches and studies have suggested an inverse association between high fiber consumption and colon cancer. The inhibitory effect of phytate against colon carcinogenesis has more importance. It has been explained by animal modeling that the inhibitory effects of dietary fiber on the development of colon cancer is influenced by its nature and source. In humans, oat and corn bran seems to be the less effective to overwhelm the incidence of cancerous growths in colon than that of wheat and rye bran (Rakha *et al*., 2010). During a study, the scientists exhibited that, colon cancer cells HT-29 in human were suppressed when subjected to phytate. A down parameter of tumors proliferation marker named PCNA was also noticed. Furthermore, the occurrence of aberrant crypts was reduced by the use of phytate as a biomarker for colon cancer. Different researchers have recommended that on large intestinal cancers there is the synergistic effect of inositol and phytate. Due to 1, 2dimethylhydrazine (DMH), a significant decline in the prevalence of large intestinal cancer was seen in mice. Likewise, the protective effect of phytate was also reported against the carcinogenic induction with azoxymethane. It was mentioned that to inhibit carcinogenesis phytate and lipid operate in an organized manner. Some metabolic studies have confirmed that the consumption of diet with high dietary fiber and low dietary fat by various populations, pose a low risk for colon cancer, by diminishing the excretion of colon tumors promoters like secondary bile acids as compared to the population those consume diet with high fat and low dietary fiber contents (Kumar *et al*., 2010).

Colorectal cancer

It is considered that wheat and rye has the greater dietary factors. Usually dietary fiber-rich food provides the protection against the colon and rectum cancer. It has been widely agreed that the people who consume less plant sterols as an important source of plasma but have dietary fiber enriched food in their diet have low risk of colorectal cancer. In another study, it is indicated that more than 60,000 individuals have increased risk of this cancer who utilize the less cereals, fruit and vegetables in their diet. Moreover, ample corroboration has been accumulated in favor of defending effect of high fiber diet against colorectal cancer risks. In an earlier study, the prospective information about colorectal cancers and the use of dietary fiber biomarkers complex such as avenantramides and alkyresorcinols have been revealed (Hallmans *et al*., 2003).

Stomach cancer

Different biological studies indicated that foods rich in fiber are considered as protective against stomach cancer. Mostly whole grain cereal fibers are at the top for its positive effect on health. Currently, a study was conducted on a population where the defensive organization was built for cardiac cancer and for adenocarcinomas of lower oesophagus. Usually there is no clue of defensive effect for oesophageal squamous cell carcinoma that sturdily aids the assumption. One stimulating probable mechanism for shielding effect of whole grain cereals are linked with their requisite properties in relation to N-nitroso compounds. Data was offered for associating the human health and whole grain

that leads toward a defensive influence of cereal grains in upper gastrointestinal track.

Hepatocellular carcinoma (HCC)

Hepatocellular carcinoma is found to be a fatal malignant disease due to minimal diagnosis of cancer cells in human liver. In humans, for the treatment of liver cancer line HepG2, phytate plays the potential role. It results in inhibition of growth of HepG2 cells and hampers the cells capability to form colonies. Furthermore, it also contributes the conversion of cancer cells to less destructive phenotypes production of alpha-fetoprotein and favored the variation of malignant cells. As a result of HepG2 cells treatment with phytate, an augmentation in the expression of p21WAF1protein and reduction in expression of mutant protein p53 were documented. This suggests that tumor suppressor gene activity enhanced by the phytates (Kumar *et al*., 2010).

Pancreatic cancer

Amongst various forms of cancer, pancreatic cancer proved to be the most resistant to a number of therapies. It was reported that about 31,270 numbers of mortalities were occurred in 2004 due to the effect of this cancer. Insensitivity of conventional therapies posed a conflict to apoptosis. It has been documented that the in vitro administration of phytate on human pancreatic adenocarcinoma cells (PANC) and MIAPACA, noticeably decrease their growth from 37.1 to 91.5%. This ultimately evinced the potential effectiveness of phytate for pancreatic cancer treatment. Moreover, a number of human studies are required to estimate safety and clinical utility of phytate to cure the pancreatic cancers suffering patients (Hallmans *et al*., 2003).

Blood/bone marrow cancer

The activity of phytate to reduce the growth of erythroleukaemia cells K-562c in humans has been tested. It has been observed that about 19-36% phytate causes the reduction of K-562 cell population that leads to increased demarcation, as evinced by ultra structural morphology and improved haemoglobin synthesis (Kumar *et al*., 2010).

Cancer preventive mechanism

The mechanism occupied by the anti neo-plastic potential of phytate is not completely explored. It was recommended that it offer some valuable effects via its chelating ability. Nevertheless, innumerable studies have been suggested the anti-cancer activity of inositol compounds which includes anti-oxidant functions, pH reduction and mineral chelating ability, cell cycle inhibition, interrupting cellular signal transduction and improving natural killer cells activity.

Cardiovascular disease, stroke and myocardial infarction

Some studies indicate a conspicuous relationship amongst a high utilization of fiber and reduced rate of cardiovascular heart diseases in people. Several epidemiological studies explained the remarkable protective effects of dietary fiber rich foods against the myocardial infractions. A study linking approximately 22000 specific age group people had an inverse relationship among the quantity of fiber in the diet and rate of CHD. So this group of people took under consideration for long period of time and it was seen that due to the consumption rye dietary fiber, these people were more negatively related with the danger of myocardial infarction than that of people consumed fruit and vegetable fiber. People with the highest intake of fiber have the risk of 0.45 times more as in contrast with people with low ingestion of fiber (Rakha *et al*., 2010).

A number of pathways are present for protection that includes the protective effects on insulin and lipid metabolism. The whole grains like wheat and rye are the rich source of Alkylresorcinols, phenols and lignans,

which possess strong antioxidants activity and anti-inflammatory properties that are linked to their disease preventing properties. There has been found a strong indication for protective consequences of whole grain products on diabetes, strokes and myocardial infarction. Conclusively, the involvement of whole grain based diet, e.g. wheat bran, rye bran etc, is very helpful in reducing the risk of different cardiac disorders.

Diabetes

Diabetes mellitus is an entrenched threat factor for cardiovascular diseases (CVD). In many trails it was observed that the intake of rye bran improves the metabolic status of human and animals. It was a comparative study in which the effect of rye bran incorporated products was checked on the patients with insulin-dependent diabetes and normal persons and found significant results. It was also depicted that the inclusions of high rye bran products in the diet lower the glucose profile and improve the insulin levels. Similar diabetes preventing effect was also observed in rats. The results shows that the rats fed on the bread with high-fiber significantly lower the body weight, blood glucose and urinary glucose excretion as compared to the animals fed bread with low-fiber content. Another scientist examined that the use of rye fiber can limit the weight gain in normal rats and prolonged the survival period of diabetic rats (Shewry, 2009). It has been recognized that the ingestion of high wheat bran or combination of wheat and rye bran improves glucose tolerance in human subjects without diabetes and in individuals with glucose intolerance. Currently, it was reported that high intake of whole grains also relates with improve in insulin sensitivity. In two large-scale studies on women's shows the clear association between high intakes of dietary fiber from cereal grain and a reduced risk of diabetes. One of the study shows a low glycemic index was concurrently related with a decreased risk in younger people. In the second study more recently on older people did not hold up this finding (Ragaee *et al.,* 2006).The conclusion of different experiments is that the fortification of refined grain products with whole grain products may lower the risk of diabetes and other linked complications. The use of high fiber products is also a sensible approach to retain good health. At present, here is need to explore the anti-diabetic potential of different high fiber cereal based foods and make them a safer and reliable remedies for metabolic disorders.

Whole Grains a remedy for Gallstones

The foods which have high indigestible fiber, like breads and other food products made with whole wheat can help to avoid gallstones. The researchers reported that the individuals consuming the more fiber (Soluble and Insoluble) are at 13% lower risk of developing gallstones as compared to women consuming the fewest fiber-rich foods and on the other hands which consume more insoluble fiber are at 17% lower risk as compared to women eating the least. The gallstones formations have the inverse relation with fiber consumption. A study shows that 5-gram hike in insoluble fiber intake can drop about 10% the risk of gallstone formation. Mostly the health benefits associated with insoluble fiber. Insoluble fiber not only speeds intestinal transit and movement of food in intestines, but also lowers the secretion of bile acids, excessive secretion leads to gallstone formation. It also improves insulin sensitivity and decreases triglycerides in the blood. Besides the cereal grains we can get handsome amount of insoluble fiber form nuts and the edible skin of fruits and vegetables including, berries, pear, apple, many squash, cucumbers, and tomatoes. The beans like kidney beans and sprouts are also provide a large amount of insoluble as well as soluble fiber for the healthy life (Shewry, 2009).

Treatments of Some Common Aliments

Internal Rejuvenation

Eight percent of the wheat grain consists of protein that has a particular benefit due to its eight essential amino acids occurs precisely in balanced proportions. The metabolism of wheat protein provide many health-building amino acids a complete internal rejuvenation takes place. These amino acids are involved in the construction of flexible muscle which has to come back to it's relax state after stretching and bending. The availability of these amino acid play a significant role in construction and working of skin, clear eyesight, hair growth, nourishment of heart and lungs, brain, nervous system and glandular network, tendons and ligaments (Vitaglione *et al.,* 2008).

The B-complex vitamins, specially thiamin, riboflavin and niacin offered by natural brown wheat endorse high energy and nourishment to blood vessels and skin. Natural brown wheat and rye help to nourish the hormonal system heal wounds and regulate blood pressure due to the presence of a large quantity of minerals. To maintain internal water balance wheat also offers iron to enrich the bloodstream and phosphorus and potassium along with other nutrients. For restoration of internal harmony wheat and rye found to be help full (Kumar *et al*., 2011). From the above discussion we can say that the proper inclusion of wheat and rye products in diet also retard the aging process and give a fresh look to human beings.

Tooth Disorders

Wheat takes more time to eat and compels the chewing of foods also than the other foods. It is beneficial for the teeth and gum exercise but also a great aid to digestion. For sore throats and pyorrhea wheat grass juice acts as an excellent mouth wash. It also prevents tooth aches and tooth decays. To chew wheat grasses consider beneficial which draws out toxins from the gums and inhibit the bacterial growth (Kumar *et al*., 2011).

Constipation

The addition of bran in wheat and the rye flour make it more wholesome and beneficial. It is considered as outstanding laxative. The laxative effects of cereals bran are much better than that of cellulose in fruits and green vegetables, because it is easily broken down by the intestinal micro flora. The high concentration of insoluble fiber (cellulose) it is highly beneficial in the prevention and in curing of constipation. Its consumption gives a bulk-mass in the intestines which play a smoothing effect and make easy movement due to increased peristalsis (Haripriya and Premakumari, 2010). Furthermore, it is also observed that by preventing we cannot only make easy of stool movement but also can be prevented from the associated issues like hemorrhoids.

Skin Diseases

Many researches explored that chlorophyll inhibit the proliferation of harmful bacteria. For skin diseases and ulcerated wounds wheat grass therapy can be effectively used. It promotes the cell activity and normal growth and retards bacterial action. Drinking of wheat grass juice creates unfavorable conditions for bacterial growth. Wheat grass juice poultice also have sterilization effects when applied on the infected area. Superficially over inflamed surface as in burns, scalds and various itching and burning eruptions wheat flour is useful as a dusting powder. To reduce freckles whole wheat flour, mixed with vinegar then boiled and applied outwardly (Shewry, 2009).

Digestive System Disorders

For the detoxification of digestive system wheat grass juices are best. It is very helpful in prevention of disorders of the ulcerative, mucous, and severe

constipation, colon colitis, and some other digestive ailments (Kumar *et al*., 2011).

Circulatory Disorders

In wheat and rye the chlorophyll content is present that enhances lung and heart functions. It helps to reduce the toxemia or blood poisoning and capillary activity also increases. The higher level of iron in blood improves the functioning of Lungs. The effect of carbon dioxide is minimized and oxygenation improves. So it is the reason that for circulatory disorders wheat grass juice or other cereal grass juices prescribed (Mulloy *et al*., 2009).

Ear diseases

It is used for resolving the problem of septic discharge from the ear and in relieving ear pain. So in this case the wheat and rye grass extract has revealed very excellent results. The extract of wheat and rye grass is obtained through different processes and it is used to treat the ear diseases. It is also suggested that it is more effects as compared to it is taken orally (Singhal *et al*., 2012).

Procurement of joint diseases

Different joint diseases found in people of different age groups such as bone rotting, pain in the joints, swelling on the joints, osteoarthritis, etc. The wheatgrass therapy has to be employed patiently for long time for treatment of joints pain. It has strong assurance that this therapy gives positive results (Mulloy *et al*., 2009).

Anti-asthematic and anti allergic agent

Wheat and some other cereals such as rye have the anti-allergic actions due to the presence of the rich vitamin and antioxidant content in them. It was seen that respiratory symptoms like wheeze, cough, and shortness of breath not linked with vitamin C intake. But has the inverse relation with cough. It was observed that due to high dosage of vitamin C, patients had a higher forced expiatory volume and higher forced vital capacity than those with a low vitamin C intake (Singhal *et al*., 2012).

Wheat for Treating Boils

If pus occurs in boils we can treat them at home with ease and without the help of surgeons knife. Prepare the fine powder of little Alse by grinding it. Fry the one table wheat flour in a little oil to a golden color. Then add tablespoon of water along with grounded Alse. Until the mixture turns thick then keep on stirring. Remove from fire and put on a clean piece of cloth, spread it over the cloth and bandage the boil when the mixture turns acceptably hot. Boil will burst giving instant relief within 2 to 3 days. A little boric has been added to a warm water and clean the boils and then apply sulphur ointment and bandage. Until the wound heal clean the wound and apply the ointment daily (Haripriya and Premakumari, 2010).

Wheat paste a remedy for Scars

Roasted wheat paste/oil is used to eliminate scars. Wheat grains are roasted until it turns black and then paste is formed after grinding. Squeeze out the oil after putting in cloth. The regular use of this paste or oil provides relief and itching disappears (Vitaglione *et al.*, 2008).

Wheat for Treating Acne or Pimples

After grinding the whole grains obtain the powder and then add the water in it and make the fine paste. This paste applies on pimples. For 1 hour keep it. After 1 hour wash it. Regularly do this so it found to be helpful for resolving the skin problems (Kumar *et al*., 2011).

Conclusion

The cereal grains and their products are consumed as the staple food since centuries. In Pakistan more than 80% of the energy requirements are fulfilled from the cereals products. Among which wheat and rye are most commonly used in Pakistan. They have the substantial contribution in provision of carbohydrates, proteins,

dietary fiber, vitamins and minerals. All the health benefits are linked with the provision of these nutrients and the bioactive components present in it. In this way, different illness like, diabetes, CVD, cancer, tooth decay, obesity, aging and many others disorders can be prevented through the utilization of wheat and rye. Due to lack of knowledge and the ineffective utilization of these grains we are lacking in getting desired benefits. In this review an attempt is made to educate the common peoples and to gain the attention of researches. A lot of area is yet needed to be explored like the identification of bioactive components, their mechanism, effectiveness, safety, and many other aspects. A new approach can also be made to investigate that how the complex antioxidants present in wheat, rye and other cereals can be liberated, how can they be metabolized and which metabolic pathways are affected by antioxidants. This will provide new information on the health benefits of whole-grain cereals.

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