

QUALITY ASSESSMENT OF FORMULATED FISH SILVER CARP BURGERS DURING FROZEN STORAGE

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ABSTRACT

Quality attributes, sensory evaluation and microbial examination of fish burger prepared from silver carp (*Hypophthalmichthys molitrix*) supplemented with oat, soybean, chickpea flours and maltodextrin were determined during frozen storage at -18 °C for 6 months. The results showed that, cooking loss of the formulated burgers were significantly lower than that of control burger and the decrease is significant as a result of increasing the supplementation ratios. Shrinking percentage of unsupplemented fish burger was high comparing with supplemented samples. Samples containing soybean flour had the highest crude fibers; while those containing maltodextrin had the highest available carbohydrates. Burgers prepared from fish only had the highest percentage of moisture, crude protein, ether extract and ash. Palmitic and stearic acids were predominant saturated fatty acids in oil extracted from all samples. All samples had omega-3 fatty acids around 1%. Linoleic acid was the highest unsaturated fatty acid followed by oleic acid in all samples. Moisture contents of all fish burger samples decreased significantly during frozen storage. A significant decrease in the protein content was observed as a result of storage period. A decrease of non-protein nitrogen (NPN) was observed for all samples during frozen storage; but the decrement for control was the highest. The pH values increased significantly during the first 2 months; then slowly decreased during storage. PVs showed remarkable increase during frozen storage. TBA values of all samples increased (within acceptable limits) as frozen storage time continued. Free fatty acids (FFA) significantly increased through frozen storage. Control sample had higher FFA reaching about 18.75% than those of supplemented samples. All supplemented samples at 15% level had the lowest FFA. TVB-N of both supplemented and control samples significantly increased as a result of increasing storage period; but control sample had the highest TVB-N. Also, TVB-N decreased as a function of increasing supplemented ratios from 5 to 15%. Generally, from the organoleptic evaluation, fish burger supplemented with 15% chickpea had the highest taste and odor comparing with other burgers. While, the best color associated with addition of maltodextrin. Total plate count (TPC) of all samples decreased during frozen storage. Supplemented samples had TPC lower than that of control. Total coliform was not detected at all samples during frozen storage.

Keywords: Silver carp, burger, frozen storage, chemical composition, quality parameters and sensory evaluation.

INTRODUCTION

Fish is considered to provide important constituents for the human diets such as high biologically and readily digestive proteins, lipid soluble vitamins, micronutrients and polyunsaturated fatty acids (Simopoulos, 1997). Seafoods have high protein content, low saturated fat and also contain

vitamins and minerals. Minerals such as magnesium, calcium, zinc, iron and phosphorus are essential for human nutrition (Erkan and Özden, 2007). Carp fish, as one of the freshwater fish species, has been one of the most widely cultured species all over the world due to its fast growth rate, easy cultivation, high feed efficiency ratio as well as high nutritional value (Tokure *et al.*, 2006a). However, freshwater fish, including silver carp, often have a strong earthy/musty taste and odor and poor gel-forming ability, which largely limits the consumption of this kind of fish (Luo *et al.*, 2008). Silver carp (*Hypophthalmichthys molitrix*) is a freshwater species living in temperate conditions and its natural distribution is in Asia. As silver carps have no such demand in fresh condition, it has not received much attention from these researchers to explore its technological feasibility. Thus, silver carps have a good prospect for becoming one of the suitable species for value-added products. Aquaculture production of silver carp is the highest of any finfish species in the world; especially important in the Asia-Pacific region that has an annual global production of nearly 4.2 million metric tons (Gheyas *et al.*, 2009). Fish burgers are one of the value added and acceptable fast food products in the world (Taskaya *et al.*, 2003; Chomnawan *et al.*, 2007). These products are stored and sold in the frozen status (Tokure *et al.*, 2006a). During the frozen storage, deterioration is due to lipid oxidation and limit protein denaturation (Aubourg and Medina, 1999; Tokure *et al.*, 2006b). Thus, the objective of this research was to evaluate the prepared fish burger from silver carp supplemented with oat, soybean, chickpea flours and maltodextrin at different ratios. The quality attributes, microbial examination and sensory properties were studied during frozen storage at -18 °C for 6 months.

MATERIALS AND METHODS

MATERIALS:

Fresh silver carp (*Hypophthalmichthys molitrix*) female at age 18 months were purchased from a private fish farm in Baltem, Kafrelsheikh Governorate, Egypt. Fresh fish weight ranged between 1.850 and 2.653 kg. The flesh weight ranged between 0.935 to 1.225 kg. Whole flaked oat (*Avena sativa*) seeds of Australian white oat (Uncle Tobys) and chickpea seeds were obtained from the local market at Kafrelsheikh city, Egypt. Soybean was obtained from the soy processing unit, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. Maltodextrin was purchased from El-Gomhoria Company for Chemicals and Drugs at Tanta city, Egypt.

METHODS:

Sample preparation:

Fresh silver carp were washed in chilled water and transported to the processing hall in insulated box containing ice in the ratio of 1:1 (fish:ice). The fish samples were thoroughly washed and dressed to remove scales, head and viscera. They were washed once again in chilled water and filleted. Fish fillets were deboned manually. Fish mince was prepared by mechanical mincer initially with 5 mm holes mincer plate (15 cm diameter) and later with 2 mm holes mincer plate.

Oat, chickpea and soybean were ground into fine powder using a laboratory electronic mill (Braun, Model 2001 DL, Germany), then passed through a 60 mesh sieve screen. The flour was stored in tightly polyethylene bags at -18 °C until used.

Fish burger preparation:

Formulation of fish burgers were composed of 80% silver carp flesh and 20% other ingredients including: 5% crumble powder, 3.45% onion powder, 3% starch, 3% gluten, 1.2% salt, 1% garlic, 1% isolated soy protein, 1% sodium caseinate, 0.5% lime juice, 0.5% blend spices (red pepper, thyme and cumin), 0.3% sodium tripolyphosphate and 0.05% sodium ascorbate. Soybean, chickpea and maltodextrin at 5, 10 and 15% (from fish percentage) were replaced with fish meat. Produced fish burgers were packaged in polyethylene bags and then stored at -18 °C for 6 months (Mahmoudzadeh *et al.*, 2010).

Cooking loss and shrinkage:

Cooking loss values were determined by calculated the weight difference of three pieces before and after cooking divided into initial weight (Crehan *et al.*, 2000). Shrinkage was calculated as the diameter loss after frying divided into initial diameter according to the method of El-Akary (1986).

Gross chemical composition:

Moisture, crude protein, ash and crude fibers of fish burger samples were determined according to the methods of AOAC (2000). Total carbohydrates were determined by phenol-sulphuric acid according to the method outlined by Dubois *et al.* (1956). The available carbohydrates were calculated by subtracting the percentage of crude fibers from the percentage of total carbohydrates. Total lipid was extracted according to Bligh and Dyer (1959) method. Ten percent trichloroacetic acid extract was used to estimate non protein nitrogen (NPN) by using the micro-Kjeldahl method of AOAC (2000).

Fatty acids composition:

Fatty acids composition of fish burgers was extracted using n-hexane at room temperature according to the method described by AOCS (1998), and identified by gas liquid chromatography (GLC) technique according to the methods described by Radwan (1978) at Health Institute, Alexandria. HP (Hewlett Packard) 6890 GC. Detector: FID, Detector temperature: 250 °C, Injector temperature: 220 °C, injection volume 2 µl, splitless mode, Column: HP-5 (5% diphenyl, 95% dimethyl polysiloxane), 30m, 0.32mm ID, 0.25 µm film thickness, Carrier gas: Nitrogen, gas flow: 1ml/min

pH value was measured for the homogeneous blends of samples and distilled water (1:10, w:v) using a Metrohm model pH meter (Switzerland) according the AOAC (2000).

Total volatile base nitrogen (TVB-N, mgN/100g) was determined according to Safari and Yosefian (2006). About 10g of fish burgers was added to the Kjeldahl flask consisting of magnesium oxide and distilled water was added before connecting it to the Kjeldahl system. The transferred fluid was collected in an Erlenmeyer flask containing boric acid (2%) and methyl-red indicator

using 10N H₂SO₄ which changes the color from green to light red, TVB-N was calculated in 100g of fish burger as follow:

$TVB-N(\text{mg N}/100\text{ g}) = 1.4 \times \text{used H}_2\text{SO}_4 \times 100 \times \text{amount of sample}/1000\text{mg}$

Peroxide value (PV) was determined as described by Leonard *et al.*, (1987).

Free fatty acids were calculated as oleic acid from acid value/1.99.

Thiobarbituric acid value (TBA), mg malonaldehyde/kg) was colorimetrically determined according to the method described by Pearson (1981).

Sensory evaluation:

Organoleptic properties of cooked burger samples were performed by semi-trained panel of judges using nine-point hedonic-scale ratings for color, taste, odor and texture with 9 being the highest score, extremely liked, and 1 being the lowest score, extremely disliked (Watts *et al.*, 1989).

Microbial examination:

Total plate count and total coliform were determined using the recommended methods for the microbiological examination of foods published by APHA, (1971).

Statistical analysis:

The obtained data were statistically analyzed using General Linear Models Procedure Adapted by Statistical Package for the Social Sciences (SPSS, 1997) for user^s Guide Duncan Multiple Range Test was used to test the difference among means (Duncan, 1995).

RESULTS AND DISCUSSION

Gross chemical composition of fish burgers:

The prepared silver carp fish burger was supplemented with oat, soybean, chickpea flours and maltodextrin at different ratios (5, 10 and 15%). Gross chemical composition of processed burgers was determined and the results were presented in Table (1). Data reveal that, burgers prepared from fish only had the highest moisture, crude protein, ether extract and ash contents. Also, dry matters, available carbohydrates and crude fibers increased as a function of increasing supplementation ratios. Samples supplemented with soybean flour had the highest crude fibers; while supplemented with maltodextrin had the highest available carbohydrates. This may be due to the difference in chemical composition of raw materials. Siddaiah *et al.* (2001) found that moisture, total protein, total lipids and ash contents of minced silver carp fish were 80.97, 87.65, 7.46 and 6.36% on dry weight; respectively. Naseri *et al.* (2010) demonstrated that the moisture content of silver carp fish fillets was 74.15%. Crude protein, total lipids and ash recorded 66.5, 42.44 and 4.91%; respectively on dry weight. Also, Majumdare *et al.* (2012) reported that mince silver carp fish contained 78.68% moisture, crude protein 80.72% and total lipids 4.55% on dry weight.

Table (1):Gross chemical composition of supplemented silver carp fish burger before frying (dry weight basis).

Sample	Moisture	Crude protein	Ether extract	Ash	Total Carbohy.	Available carbohy.	Crude fibers
Control	77.50 ^a	74.89 ^a	16.53 ^a	9.47 ^a	0.00	0.00	0.00
Oat							
5%	76.40 ^b	65.25 ^b	9.45 ^c	7.92 ^b	18.56 ^j	5.17 ^f	13.39 ^h
10%	74.30 ^c	57.04 ^c	7.78 ^e	6.23 ^c	31.99 ^f	10.82 ^c	21.17 ^f
15%	73.10 ^d	52.08 ^g	6.54 ^f	6.58 ^c	39.89 ^b	10.67 ^c	29.22 ^b
Soybean							
5%	72.10 ^e	57.60 ^c	10.75 ^b	7.42 ^b	27.82 ^j	7.10 ^e	20.72 ^g
10%	71.20 ^f	53.70 ^g	9.03 ^c	6.64 ^c	34.81 ^e	7.33 ^e	27.48 ^c
15%	70.71 ^f	50.69 ⁱ	6.11 ^f	5.33 ^d	40.40 ^a	9.42 ^d	30.98 ^a
Chickpea							
5%	72.60 ^e	55.95 ^e	10.51 ^b	7.55 ^b	28.80 ^h	7.30 ^e	21.50 ^f
10%	72.31 ^e	57.71 ^c	7.48 ^e	7.15 ^b	29.83 ^g	7.69 ^e	22.14 ^e
15%	70.84 ^f	54.87 ^f	6.69 ^f	6.45 ^c	36.21 ^d	10.22 ^c	25.99 ^d
Maltodextrin							
5%	73.40 ^d	56.62 ^d	10.53 ^b	7.44 ^b	27.97 ⁱ	23.76 ^b	4.21 ^j
10%	72.31 ^e	51.35 ^h	8.41 ^d	6.39 ^c	37.70 ^c	32.39 ^a	5.31 ⁱ
15%	71.80 ^f	47.41 ^j	8.26 ^d	6.28 ^c	37.02 ^c	31.81 ^a	5.21 ⁱ

Means of treatments having the same case letter(s) within a column are not significantly different (at $p \leq 0.05$). N.S= not significant

Effect of frying on cooking loss and shrinking of silver carp burgers:

Table (2) shows cooking loss and shrinking of silver carp burgers after cooking. The results indicate that, cooking loss of supplemented burgers were significantly lower than that of control. Also, cooking loss of supplemented samples decreased significantly as a resulting of increasing supplementation ratio. Burgers supplemented with chickpea had the lowest cooking loss. Cooking loss may be due to moisture loss during cooking. This may be due to the addition of supplementation materials which is able to bind water and fat, consequently improved the cooking loss (Rinaudo, 2006). As for shrinking during cooking, it could be observed that, shrinking percentage of control fish burger was higher compared with supplemented samples. It may be due to the high content of protein in control samples. The results are in line with those reported by Ali (1995) and Metwalli (2005), who found that shrinkage of beef burger decreased with adding soy bean hull fibers. Also, the results are in agreement with those of Bessar (2008), who reported that, the reduction of shrinkage of beef burgers increased as a result of increasing orange and apple peels levels.

Table (2): Effect of frying on cooking loss and shrinking of silver carp burgers.

Sample	Cooking loss %	Shrinking %
Control	30.36 ^a	28.46 ^a
5%	26.28 ^c	23.38 ^c
Oat10%	24.06 ^d	22.80 ^d
15%	21.15 ^f	20.19 ^e
5%	27.82 ^b	19.83 ^e
Soybean10%	20.48 ^g	15.00 ^g
15%	16.37 ^j	15.65 ^g
5%	22.69 ^e	26.18 ^b
Chickpea10%	19.84 ^h	13.67 ^h
15%	18.44 ⁱ	10.04 ⁱ
5%	24.56 ^d	19.70 ^e
Maltodextrin10%	22.11 ^e	17.87 ^f
15%	18.44 ⁱ	17.14 ^f

Means of treatments having the same case letter(s) within a column are not significantly different (at $p \leq 0.05$). N.S= not significant

Data in Table (3) shows fatty acids composition of oil extracted from supplemented silver carp fish burgers. The results reflected that, control burger had 24.77% saturated and 59.45% unsaturated fatty acids. All supplemented samples had saturated fatty acids higher than that of control except for sample supplemented with 15% soybean which recorded 21.19%. Palmitic and stearic acids were the predominant saturated acids.

Table (3): Fatty acids composition (% of total fatty acids) of oil extracted from supplemented silver carp fish burgers.

Sample	Control	Oat			Soybean			Chickpea			Maltodextrin		
	0	5	10	15	5	10	15	5	10	15	5	10	15
Fatty acids													
C _{4:0}	0.53	2.52	0.14	2.95	0.15	0.19	0.38	0.30	0.46	0.26	ND	ND	0.10
C _{6:0}	3.00	16.05	1.08	17.37	2.03	2.08	2.93	1.70	3.83	2.72	3.01	2.03	1.02
C _{8:0}	3.29	1.32	2.05	1.29	1.81	1.56	1.37	1.99	1.52	1.87	2.03	2.07	1.57
C _{10:0}	0.22	0.14	0.18	0.12	0.16	0.14	0.11	0.19	0.18	0.17	0.23	0.17	0.17
C _{11:0}	ND	0.04	ND	0.02	ND	ND	0.01	ND	0.05	0.03	ND	ND	0.04
C _{12:0}	0.11	0.10	0.13	0.07	0.09	0.09	0.08	0.14	0.10	0.12	0.12	0.13	0.12
C _{13:0}	0.06	0.05	0.09	0.04	0.06	0.06	0.05	0.08	0.06	0.07	0.06	0.08	0.08
C _{14:0}	1.08	0.85	1.25	0.61	1.04	1.14	0.76	1.37	1.06	1.08	1.35	1.38	1.47
C _{15:0}	0.25	0.21	0.50	0.14	0.25	0.28	0.19	0.31	0.25	0.26	0.60	0.33	0.35
C _{16:0}	8.90	7.72	10.13	6.95	9.93	10.47	8.08	11.02	10.11	9.96	13.22	11.72	12.10
C _{17:0}	0.23	0.18	0.24	0.14	0.21	0.24	0.16	0.25	0.21	0.23	0.33	0.29	0.29
C _{18:0}	6.80	7.68	8.93	5.66	10.14	9.38	6.79	9.43	8.94	9.60	11.56	8.70	8.45
C _{20:0}	0.30	0.26	0.19	0.17	0.36	0.35	0.19	0.31	0.34	0.35	0.38	0.35	0.33
C _{24:0}	ND	0.07	ND	0.07	ND	ND	0.09	0.09	0.09	ND	ND	ND	ND
Total SFA	24.77	37.19	24.91	35.60	26.23	25.98	21.19	27.18	27.50	26.72	32.89	27.25	26.09
C _{14:1}	0.11	0.15	0.15	0.10	0.14	0.14	0.09	0.19	0.27	0.14	0.25	0.17	0.14
C _{15:1}	0.10	0.13	0.13	0.09	0.13	0.14	0.10	0.15	0.25	0.13	0.13	0.15	0.12
C _{16:1}	3.22	2.60	2.90	1.73	3.45	3.72	2.24	3.85	3.22	3.45	4.65	4.14	4.85
C _{17:1}	0.30	0.24	0.36	0.16	0.29	0.32	0.21	0.33	0.29	0.30	0.64	0.39	0.42
C _{18:3}	0.01	0.02	0.06	0.01	0.03	0.02	0.01	0.05	0.04	0.02	0.11	0.04	0.05
C _{18:2}	37.90	29.33	28.69	24.18	39.98	33.14	26.93	24.28	39.98	43.42	26.79	35.94	33.52
C _{18:1}	11.20	14.27	30.37	23.36	15.77	23.28	26.02	28.74	14.81	11.90	20.51	17.27	19.31
C _{20:5}	0.41	0.03	0.37	0.22	0.35	0.40	0.28	0.39	0.38	0.38	0.40	0.47	0.48
C _{20:4}	1.23	1.28	1.37	0.67	1.64	1.34	0.86	1.19	1.09	1.21	1.39	1.35	1.68
C _{20:3ω3}	0.37	ND	ND	0.18	ND	0.33	0.17	0.31	0.26	0.34	0.52	0.64	0.39
C _{20:3ω6}	0.90	0.72	0.82	0.51	0.89	0.96	0.59	0.87	0.76	0.83	0.98	1.05	1.12
C _{20:2}	1.04	0.86	1.06	0.64	1.05	1.05	0.85	1.08	0.98	1.07	1.19	1.27	1.20
C _{20:1}	0.94	0.69	0.70	0.55	0.98	0.91	0.39	0.82	0.80	0.90	0.98	1.04	1.20
C _{22:2}	1.40	1.13	1.39	0.80	1.29	1.40	0.91	1.33	1.15	1.26	1.59	1.59	1.59
C _{22:1}	0.06	0.06	ND	0.03	0.05	0.05	0.03	0.07	0.05	0.05	0.07	ND	0.06
C _{22:6}	0.26	0.22	0.32	0.24	0.27	0.27	0.29	0.26	0.29	0.28	0.20	0.27	0.23
Total UFA	59.45	51.73	68.69	53.47	66.31	67.47	59.97	63.91	64.62	65.68	58.23	65.78	66.36
U/S	2.40	1.39	2.76	1.50	2.53	2.60	2.83	2.35	2.35	2.46	1.77	2.41	2.54
Total F.A	84.22	88.92	93.60	89.07	92.54	93.45	81.16	91.09	92.12	92.40	91.12	93.25	92.45

Fatty acids composition of oil extracted from supplemented silver carp fish burgers:

Regarding to unsaturated fatty acids, all supplemented fish burgers had unsaturated acids higher than that of control except for supplemented with 5 and 15% oat. Also, it was observed from the same Table that, all samples had Omega 3 fatty acids around 1%. Linoleic was the highest unsaturated acids followed by oleic acid. Farmed carp flesh generally contains a high level of oleic acid and a low level of favorable n-3 HUFA (Csengeri, 1996). Carp have the ability to biosynthesis n-3 HUFA from its precursor 18:3n-3, α-

linolenic acid (Tocher, 2003; Zhenget al., 2004). Thus, a feed supplement rich in α -linolenic acid could be an alternative means to increase n-3 HUFA content in the carp flesh. The obtained results are in agreement with those reported by Naseriet al. (2013) who found that monounsaturated fatty acids of raw silver carp oil recorded about 52.14%, polyunsaturated fatty acids were 15.09% and saturated fatty acids were 26.18%. ω_3 fatty acids were 8.90% and ω_6 were 6.19%.

Changes in proximate chemical composition during frozen storage:

The changes in the moisture, protein, fat and NPN are presented in Table (4). Moisture contents of all samples decreased significantly as a function of increasing storage time at $-18\text{ }^{\circ}\text{C}$; but the decrement of supplemented burgers was lower than that of control. Also, supplemented samples at 15% level were the best in maintenance moisture loss during storage. Moisture content is a determinative indicator of surimi quality (Lanier and Lee 1992). Further, fish burgers showed a gradual decrease of moisture content during the period of storage. This may be due to the gradual denaturation and subsequent loss of the water-holding capacity of protein. Also, the lowest decrement of moisture in supplemented burgers may be due to the lowest moisture content of the added materials.

A significant decrease in the protein contents were observed as a result of storage time. The decrement of protein content in control was higher than those of supplemented samples. This may be due to further loss of protein with loss of moisture as drip. The observed decrease could be due to the loss of water extractable proteins in the thaw drip (Reddy et al. 1995). However, Boerrenson et al. (1985) attributed the decrease in water soluble protein to the denaturation of sarcoplasmic proteins. Decrease in the protein of the minced silver carp fish indicates progressive denaturation of myofibrillar proteins during frozen storage. Many factors, viz. hydrophobic effect of FFA on proteins (Sikorski et al., 1976), interaction of oxidized lipids with cystine-SH, the epsilon-NH₃ group of lysine and N-terminus group of aspartic acid, tyrosine, methionine and arginine (Kussiet al., 1975) of fish proteins are known to influence the solubility of both myofibrillar, and sarcoplasmic proteins. Srikanth et al. (1989) found that the oxidized products of lipid and FFA formed during storage influence the solubility of sarcoplasmic proteins.

A decreasing of non-protein nitrogen (NPN) was observed for all samples during frozen storage; but the decrement for control was the highest compared to supplemented samples. The reduction of NPN content may be related with their loss with drip. Majumdar et al. (2012) reported that the NPN content of mince silver carp decreased from 0.32% at day zero to 0.26% at day 135 of frozen storage at $-20\text{ }^{\circ}\text{C}$.

Changes in different quality parameters:

Although pH value is not a suitable index on its own to determine quality of fish, however, it can be useful as a guideline for quality control of fish when used with other quality parameters (Ruiz-Capillas and Moral, 2001). Initial post mortem pH can vary from 5.4 to 7.2 depending on fish species and other factors (Grigorakis et al., 2003). It could be noted from Table (5) that pH values of both supplemented and control fish burgers increased significantly during the first 2 months; but slowly decreased during 4 and 6

months of frozen storage. Increasing the pH value at the end of the second month could be caused by products such as ammonia and trimethylamine that these compounds were produced by the endogenous enzymes of fish and bacterial spoilage (Chomnawanget *al.*, 2007; Ozyurtet *al.*, 2007). Decrease in pH value at the fourth and six months of present study may be caused due to decrease in oxygen content since it is possible that aerobic microflora growth may have increased CO₂ content. At lower temperatures increased solubility of CO₂ in unbuffered solution can produce a drop in pH (Adams and Moss, 2000).

Rancidity development was measured by means of primary (PV) and secondary (TBA) lipid oxidation compounds formation. Apparent also, from the same Table (5) that PVs showed remarkable increase during frozen storage in both supplemented and control samples. This could be explained as a result of the presence of pro-oxidant enzymes (lipoxygenases and peroxidases) and chemical pro-oxidant molecules (namely, heme protein and metal ions) (Erickson 1997; Sikorski and Kolakowski 2000). Also, mechanical deboning and mincing accelerates the oxidative changes due to the separation of fat from tissue and skin during deboning (Webb *et al.*, 1976) and through physical surface effects while mincing (Mai and Kinsella, 1980). The PV (milliequivalent O₂/kg fat) was found to increase in control from initial value of 0.87 to 5.82 after 6 months of frozen storage. While, the increments were 3.00, 3.94, 3.09 and 3.80 for samples supplemented with oat, soybean, chickpea and maltodextrin at 15% level. The increment of PVs of supplemented samples was lower than that of control. This may be due to the presence of phenolic compounds of oat, soybean or chickpea flour that delay peroxide and hydroperoxide formation.

Thiobarbituric acid (TBA) test was based on appearance red color between malondialdehyde and TBA indicator. Malondialdehyde is one of the ingredients that is produced from fatty acid oxidation (Orak and Kayisoglu, 2008). TBA index is used as indicator for evaluation lipid oxidation levels and rancidity in the products and values above 3–4 mg malondialdehyde/kg show loss in quality. Results indicate that both supplemented and control samples were at the acceptance limit of quality. The results in Table (5) showed that TBA values of all samples increased (within acceptable limits) as frozen storage time continued, this may be result of interaction of decomposition products of proteins with malondialdehyde and formation of tertiary products (Hernandez-Herrero *et al.*, 1999).

The results cleared also that, free fatty acids (FFA) of supplemented and control samples significantly increased as a function of increasing frozen storage. Control sample had higher FFA reaching about 18.75% than those of supplemented samples. All supplemented samples at 15% levels had the lowest FFA that recorded 12.71, 13.17, 10.31 and 11.08% for oat, soybean, chickpea and maltodextrin; respectively. While the formation of FFA itself does not lead to nutritional losses, its assessment is deemed important when considering the development of rancidity. Thus, a pro-oxidant effect of FFA on lipid matter has been proposed and explained on the basis of a catalytic effect of the carboxyl group on the formation of free radicals by the decomposition of hydroperoxides (Yoshida *et al.*, 1992; Aubourg 2001). In addition, FFA has shown to interact with proteins leading to fish texture deterioration during frozen storage (Mackie, 1993).

As for TVB-N (mg/100g meat) that produced by microbial degradation of nitrogenous tissue compounds, the results were presented in Table (5). The data referred that, TVB-N of both supplemented and control samples significantly increased as a result of increasing storage time; but control sample had TVB-N higher than those of supplemented samples. Also, TVB-N decreased as a function of increasing supplemented ratio from 5 to 15%. Varelziset *al.* (1997) and Chomnawangetal. (2007) reported that an increase in TVB-N for fillets and minces of horse mackerel and Mediterranean hake during frozen storage. This increasing could be attributed to bacterial activity and endogenous enzymes of fish. As Özogul and Özogul (2000) reported spoilage bacteria that are mainly responsible for volatile base production are more active in aerobic than in anaerobic conditions. According to Connell (1975), 35-45 mg/100 g meat of TVB-N content is the limit of acceptability for chilled cod, frozen tuna and sword fish. On the other hand, Kimura and Kiamakura (1934) recommended 20 mg/100 g meat of TVB-N as the beginning of spoilage and 30 mg/100 g meat of TVB-N as spoiled, while the acceptability limit was between 18 and 24 mg/100 g meat for frozen stored pink perch mince (Vidya Sagar Reddy *et al.*, 1995)

Sensory evaluation:

Sensorial parameters of both control and supplemented fish burgers decreased significantly during frozen storage (Table 6). While, samples supplemented with ratio of 15% had taste, odor and texture better than those of others. On the other hand, burgers supplemented with maltodextrin had the highest color among all of the others samples. Because of pre-frying and high amount of lipid in control, losing of sensory attributes in this group was higher than those of supplemented samples. Oxidation of unsaturated fatty acids could produce ketones, aldehydes, alcohols, hydrocarbons, acids, and epoxides that interact with proteins thereby forming off-color during frozen storage. (Thanonkaew *et al.*, 2006) Also the formation of aldehydes and ketones can cause denaturation of myofibrillar proteins and rancid off-flavors that affect the sensory attributes even in a little amount (Tokure *et al.*, 2006a). Generally, from the organoleptic evaluation burger supplemented with 15% chickpea had the highest taste and odor compared with others. While, the best color associated with addition of maltodextrin.

Microbial examination:

Total count analysis of fish burgers did not exceed the maximum limits (7-10 cfu/g) of microbiological criteria for fresh and frozen fish given by The International Commission on Microbiological Specifications for Food (ICMSF, 1978). Total counts are considered a quality indicator for food samples; there is not direct correlation between this and the presence of pathogenic microorganisms (Arvanitoyannis *et al.*, 2005). Coliform counts should be present in numbers less than 100/g meat (Nollet, 2007), and maximum level of total coliform in fish products was given as 400 cfu/g by ICMSF (1978). It could be noted from Table (7) that, total plate count (TPC) of all samples decreased during frozen storage.

Table (7): Microbial counts (cfu/g)* of supplemented fish burgers at the end of frozen storage at -18°C for 6 months.

Samples	Total plate count		Total coliform	
	0 month	6 month	0 month	6 month
Control	4.8×10^3	2.6×10^3	0.00	0.00
5%	4.8×10^3	2.0×10^3	0.00	0.00
Oat 10%	4.8×10^3	1.8×10^3	0.00	0.00
15%	4.5×10^3	1.5×10^3	0.00	0.00
5% Soybean 10%	4.5×10^3	2.0×10^3	0.00	0.00
15%	4.3×10^3	1.8×10^3	0.00	0.00
5%	4.8×10^3	2.4×10^3	0.00	0.00
Chickpea 10%	4.8×10^3	1.9×10^3	0.00	0.00
15%	4.3×10^3	1.4×10^3	0.00	0.00
5%	4.8×10^3	2.6×10^3	0.00	0.00
Maltodextrin 10%	4.8×10^3	2.4×10^3	0.00	0.00
15%	4.8×10^3	2.4×10^3	0.00	0.00

Colony forming units.

Supplemented samples had TPC lower than that of control; it may be due to its contents of antimicrobial agent in phenolic compounds form. Total coliform was not detected at all samples during storage. Total bacterial count was found to be 2×10^5 CFU/g for unwashed mince mirror carp and 8×10^4 CFU/g for washed mince mirror carp, while *E. coli*, total coliforms and *staphylococcus* could not be found (Tokure *et al.*, 2006b).

CONCLUSION

In the present study, it could be concluded that, the fish burgers prepared from silver carp fish supplemented with oat, soybean, chickpea flour and maltodextrins an alternative products, can be stored for six months in a frozen state at -18 °C for 6 months without undesirable changes of chemical quality and microbial deterioration. Also, addition of these additives enhanced sensory parameters and become accepted.

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تقييم جودة خلطات برجر سمك المبروك الفضى خلال التخزين بالتجميد

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أجرى هذا البحث بهدف الاستفادة من المحتوى العالى لبروتين سمك المبروك الفضى فى تصنيع برجر السمك حيث أنه هنا كإقبال محدود على هذا النوع من الاسماك نظرا لخواصه العضوية الحسية الغير مرغوبه. لذلك تم عمل البرجر وخلطة بنسب استبدال ٥ ، ١٠ ، ١٥ % من مطحون كل من الشوفان وفول الصويا والحمص والمالتودكسترين بهدف رفع قيمته الغذائية وتخزينه على درجة حرارة -١٨٠ م لمدة ٦ شهور. تم تقدير بعض الخواص مثل الفقد فى الوزن ونسبة الانكماش بالطهى. كما تم تقدير التغير فى التركيب الكيماوى خلال التخزين والتغير فى بعض صفات الجودة مثل pH- رقم الثيوباربيتوريكو والقواعد النيتروجينية الكلية المتطايرة ورقم البيروكسيد والاحماض الدهنية الحرة. ايضا تم اجراء التقييم الحسى خلال مراحل التخزين واخيرا تقدير العد الميكروبي الكلى وعد مجموعة الكوليفورم بعد فترة التخزين حيث اظهرت النتائج أن:

- ١- يقل الفقد فى الوزن والانكماش أثناء الطهى فى العينات المدعمة مع زيادة نسبة الاستبدال.
- ٢- كما وجد أن العينات المدعمة بفول الصويا احتوت على أعلى نسبة من الألياف خام، المدعمة بالمالتودكسترين احتوت على أعلى نسبة من الكربوهيدرات.
- ٣- حامضى البالمتيك والاستياريك كانا الحامضان السائدان فى زيوت كل العينات، احتوت كل العينات على الاحماض الدهنيه أوميغا-٣ حول نسبة ١% . كما وجد أن حامض اللينوليك الاعلى بين الاحماض الدهنيهالغير مشبعة يلية حمض الاوليك.
- ٤- لوحظ أيضا انخفاض نسبة البروتين والنيتروجين غير البروتينى(NPN) فى كل العينات خلال فترة التخزين.
- ٥- درجة الحموضه(pH) ارتفعت فى كل العينات خلال الشهرين الاول والثانى من التخزين لكن انخفضت ببطء خلال باقى فترة التخزين.
- ٦- رقم البيروكسيد(PV) ارتفع تدريجيا خلال فترة التخزين فى كل العينات وايضا رقم حمض الثيوباربيتوريك(TBA) ازداد تدريجيا فى كل العينات خلال فترة التخزين وكلاهما كان فى الحدود المسموح بهما.
- ٧- الاحماض الدهنية الحرة(FFA) ارتفعت خلال فترة التخزين. وكانت العينات المدعمة بنسبة ١٥% هى التى احتوت على أقل نسبة منالأحماض الدهنية الحرة.
- ٨- القواعد النيتروجينية الكلية المتطايرة ازدادت تدريجيا خلال مراحل التخزين لكل العينات ووجد أن نسبة الزيادة فى الكنترول أعلى منها فى العينات المدعمة، كما وجد أن هذه النسبة انخفضت بزيادة نسب الاستبدال.
- ٩- من الخواص الحسية المقدرة وجد أن برجر السمك المدعم بنسبة ١٥% حمص كان له أحسن طعم ورائحة مقارنة بباقى العينات بينما المدعم بالمالتودكسترين كان أفضل لونا".
- ١٠- أظهر العد الكلى الميكروبي فى بدايه ونهاية فترة التخزين أن الحمولة الميكروبيه لعينات البرجر المدعم كانت أقل من البرجر الكنترول، وأن بكتريا الكوليفورم لا توجد فى كل عينات البرجر المدعمة والكنترول خلال التخزين بالتجميد.

Table (4): Changes in proximate chemical composition during frozen storage (at -18 °C for 6 months) of supplemented silver carp fish burgers.

Storage time (month)	Sample	Control	Oat			Soybean			Chickpea			Maltodextrin		
		0	5	10	15	5	10	15	5	10	15	5	10	15
0	Moisture	^a 77.50 ^a	^a 76.40 ^b	^a 74.30 ^c	^a 73.10 ^d	^a 72.10 ^f	^a 71.20 ^{gh}	^a 70.71 ⁱ	^a 72.60 ^e	^a 72.31 ^f	^a 70.84 ^j	^a 73.40 ^d	^a 72.31 ^f	^a 71.80 ^h
2		^a 77.05 ^a	^a 76.00 ^b	^a 74.22 ^c	^a 73.00 ^d	^a 71.56 ^g	^a 70.43 ⁱ	^a 70.11 ^h	^a 72.23 ^e	^a 72.00 ^{ef}	^a 70.08 ^j	^a 72.84 ^d	^a 71.86 ^g	^a 71.07 ^h
4		^b 74.19 ^a	^b 73.88 ^a	^b 72.19 ^b	^b 71.06 ^c	^b 69.14 ^e	^b 70.20 ^d	^b 69.27 ^e	^b 70.97 ^c	^b 70.13 ^d	^b 69.06 ^f	^b 71.13 ^c	^b 70.05 ^d	^b 70.13 ^d
6		^c 72.11 ^a	^c 71.58 ^b	^c 70.82 ^c	^c 70.11 ^d	^c 68.47 ^f	^c 69.17 ^e	^c 67.13 ^g	^c 68.10 ^f	^c 69.17 ^e	^c 68.04 ^f	^c 70.00 ^d	^c 69.11 ^e	^c 70.07 ^d
0	Protein	^a 74.89 ^a	^a 65.25 ^b	^a 57.04 ^d	^a 52.08 ^h	^a 57.60 ^c	^a 51.70 ⁱ	^a 48.69 ^j	^a 55.95 ^f	^a 57.71 ^c	^a 54.87 ^g	^a 56.62 ^e	^a 51.35 ⁱ	^a 47.41 ^h
2		^b 73.16 ^a	^b 64.00 ^b	^b 55.10 ^e	^b 51.12 ^h	^b 56.08 ^d	^b 51.00 ^h	^b 47.63 ^j	^b 54.88 ^f	^b 57.00 ^c	^b 53.15 ^g	^b 55.41 ^e	^b 50.13 ⁱ	^b 46.29 ^h
4		^c 71.82 ^a	^c 63.14 ^b	^c 54.09 ^d	^c 50.04 ^g	^c 55.37 ^c	^c 49.00 ^g	^c 46.09 ^j	^c 52.13 ^f	^c 55.22 ^c	^c 52.25 ^f	^c 53.00 ^e	^c 49.17 ^h	^c 45.17 ^j
6		^d 69.24 ^a	^d 61.11 ^b	^d 53.00 ^d	^d 49.00 ^g	^d 53.16 ^d	^d 48.50 ^h	^d 45.11 ⁱ	^d 51.63 ^f	^d 54.25 ^c	^d 51.41 ^f	^d 52.37 ^e	^d 48.28 ^h	^d 44.23 ^j
0	Fat	^{N.S.} 16.53 ^a	^{N.S.} 9.45 ^c	^{N.S.} 7.78 ^{de}	^{N.S.} 6.54 ^g	^{N.S.} 10.75 ^b	^{N.S.} 9.03 ^c	^{N.S.} 6.11 ^g	^{N.S.} 10.51 ^b	^{N.S.} 7.48 ^e	^{N.S.} 6.69 ^f	^{N.S.} 10.53 ^b	^{N.S.} 8.41 ^d	^{N.S.} 8.26 ^d
2		^{16.27^a}	^{9.30^c}	^{7.62^e}	^{6.43^f}	^{10.09^b}	^{9.30^c}	^{6.00^f}	^{10.53^b}	^{7.53^e}	^{6.24^f}	^{10.40^b}	^{8.12^d}	^{8.48^d}
4		^{16.42^a}	^{9.25^c}	^{7.84^e}	^{6.70^f}	^{10.69^b}	^{9.65^c}	^{6.37^f}	^{10.62^b}	^{7.89^e}	^{6.78^f}	^{10.57^b}	^{8.78^{cd}}	^{8.50^d}
6		^{16.65^a}	^{9.68^c}	^{7.93^e}	^{6.91^f}	^{10.84^b}	^{9.74^c}	^{6.53^f}	^{10.81^b}	^{7.91^e}	^{6.82^f}	^{10.87^b}	^{8.81^d}	^{8.90^d}
0	NPN	^a 0.38 ^{N.S.}	^a 0.36 ^a	^a 0.36 ^a	^a 0.35 ^a	^a 0.37 ^a	^a 0.37 ^a	^a 0.35 ^a	^a 0.36 ^a	^a 0.35 ^a	^a 0.36 ^a	^a 0.36 ^a	^a 0.34 ^a	^a 0.34 ^a
2		^b 0.33 ^{N.S.}	^a 0.35 ^a	^{ab} 0.35 ^a	^a 0.35 ^a	^a 0.35 ^a	^b 0.33 ^a	^a 0.35 ^a	^{ab} 0.33 ^a	^{ab} 0.33 ^a	^{ab} 0.33 ^a	^{ab} 0.33 ^a	^{ab} 0.34 ^a	^{ab} 0.33 ^a
4		^c 0.29 ^{N.S.}	^b 0.31 ^a	^b 0.32 ^a	^{ab} 0.33 ^a	^b 0.31 ^a	^b 0.33 ^a	^{ab} 0.33 ^a	^{bc} 0.30 ^a	^{ab} 0.33 ^a	^{ab} 0.33 ^a	^b 0.30 ^a	^b 0.30 ^a	^b 0.30 ^a
6		^d 0.24 ^b	^c 0.26 ^{ab}	^c 0.26 ^{ab}	^c 0.30 ^a	^c 0.27 ^{ab}	^c 0.29 ^{ab}	^c 0.31 ^a	^c 0.28 ^{ab}	^b 0.30 ^a	^b 0.30 ^a	^b 0.30 ^a	^b 0.25 ^{ab}	^b 0.27 ^{ab}

Means of treatments having the same right case letter(s) within a row are not significantly different (at p ≤ 0.05).

Means of storage periods having the same left case letter(s) within a column are not significantly different (at p ≤ 0.05).

N.S= Not significant. NPN (mg/100g burger).

Table (5): Changes in different quality parameters during frozen storage (at -18 °C for 6 months) of supplemented silver carp fish burgers.

Storage time (months)	Sample	Control	Oat			Soybean			Chickpea			Maltodextrin		
		0	5	10	15	5	10	15	5	10	15	5	10	15
0	pH	^a 6.65 ^{N.S.}	^a 6.52 ^a	^a 6.57 ^a	^a 6.62 ^a	^a 6.57 ^a	^a 6.60 ^a	^a 6.63 ^a	^a 6.50 ^a	^a 6.52 ^a	^a 6.59 ^a	^a 6.55 ^a	^a 6.61 ^a	^a 6.63 ^a
2		^a 7.60 ^a	^a 7.53 ^a	^a 7.50 ^a	^a 7.44 ^b	^a 7.60 ^a	^a 7.45 ^b	^a 7.43 ^b	^a 7.35 ^{bc}	^a 7.47 ^{ab}	^a 7.22 ^c	^b 7.12 ^{cd}	^b 7.00 ^d	^a 7.00 ^d
4		^b 7.37 ^a	^b 7.32 ^a	^b 7.28 ^{ab}	^b 7.25 ^b	^c 7.11 ^{cd}	^b 7.00 ^d	^b 7.22 ^{bc}	^b 7.30 ^a	^b 7.15 ^c	^b 7.08 ^d	^b 7.27 ^b	^b 7.15 ^c	^a 7.00 ^d
6		^c 7.25 ^a	^c 7.23 ^a	^b 7.20 ^{ab}	^b 7.20 ^{ab}	^b 7.23 ^a	^b 7.00 ^c	^c 7.10 ^b	^b 7.05 ^{bc}	^c 7.00 ^c	^b 7.03 ^{bc}	^c 6.98 ^{cd}	^b 7.00 ^c	^b 6.90 ^d
0	PV	^a 0.87 ^{N.S.}	^a 0.87 ^a	^a 0.87 ^a	^a 0.87 ^a	^a 0.87 ^a	^a 0.87 ^a	^a 0.87 ^a	^a 0.87 ^a	^a 0.87 ^a	^a 0.87 ^a	^a 0.87 ^a	^a 0.87 ^a	^a 0.87 ^a
2		^c 2.50 ^a	^c 2.00 ^b	^b 2.00 ^b	^c 1.90 ^c	^b 2.50 ^a	^c 2.35 ^a	^c 2.00 ^b	^c 2.44 ^a	^c 2.50 ^a	^b 2.13 ^{ab}	^b 1.97 ^{bc}	^b 1.53 ^d	^c 1.53 ^d
4		^b 4.11 ^a	^b 2.95 ^e	^a 3.00 ^e	^b 2.53 ^c	^a 3.71 ^b	^b 3.60 ^{bc}	^b 3.11 ^e	^b 3.15 ^{de}	^b 3.00 ^e	^a 2.86 ^f	^b 2.29 ^g	^a 3.11 ^d	^b 2.06 ^g
6		^a 5.82 ^a	^a 3.90 ^{cd}	^a 3.22 ^{ef}	^a 3.00 ^f	^a 3.44 ^e	^a 4.13 ^b	^a 3.94 ^{bc}	^a 4.27 ^b	^a 3.66 ^{de}	^a 3.09 ^f	^a 3.77 ^d	^a 3.17 ^d	^a 3.80 ^d
0	TBA	^a 0.54 ^{N.S.}	^a 0.54 ^a	^a 0.54 ^a	^a 0.54 ^a	^a 0.54 ^a	^a 0.54 ^a	^a 0.54 ^a	^a 0.54 ^a	^a 0.54 ^a	^a 0.54 ^a	^a 0.54 ^a	^a 0.54 ^a	^a 0.54 ^a
2		^c 1.00 ^a	^b 1.00 ^a	^b 0.95 ^a	^b 0.92 ^{ab}	^c 1.00 ^a	^b 1.00 ^a	^b 0.95 ^a	^c 1.00 ^a	^b 0.87 ^b	^c 0.85 ^b	^b 0.87 ^b	^c 0.85 ^b	^b 0.55 ^c
4		^b 1.22 ^a	^a 1.22 ^a	^{ab} 1.07 ^b	^b 1.07 ^b	^b 1.12 ^b	^a 1.21 ^a	^a 1.15 ^{ab}	^b 1.11 ^b	^a 1.05 ^{bc}	^b 1.00 ^c	^b 0.88 ^{cd}	^c 0.73 ^d	^a 0.72 ^d
6		^a 1.43 ^a	^a 1.27 ^b	^a 1.13 ^{cd}	^a 1.15 ^c	^a 1.25 ^b	^{ab} 1.13 ^{cd}	^a 1.20 ^c	^a 1.27 ^b	^a 1.09 ^d	^a 1.22 ^{bc}	^a 1.00 ^d	^a 1.00 ^d	^a 0.83 ^e
0	FFA	^a 5.52 ^{N.S.}	^a 5.50 ^a	^a 5.50 ^a	^a 5.45 ^a	^a 5.51 ^a	^a 5.45 ^a	^a 5.42 ^a	^a 5.50 ^a	^a 5.45 ^a	^a 5.45 ^a	^a 5.52 ^a	^a 5.50 ^a	^a 5.45 ^a
2		^c 8.37 ^a	^c 7.42 ^b	^c 7.11 ^{cd}	^c 7.11 ^{cd}	^c 7.24 ^c	^c 7.11 ^{cd}	^c 6.91 ^d	^c 7.19 ^c	^c 5.35 ^e	^c 5.00 ^f	^c 5.78 ^b	^b 7.42 ^b	^b 7.16 ^c
4		^b 14.53 ^a	^b 12.75 ^c	^b 11.81 ^d	^b 8.95 ^h	^b 13.07 ^{bc}	^b 11.73 ^d	^b 11.00 ^f	^b 8.63 ^h	^b 7.82 ⁱ	^b 7.08 ^j	^b 11.37 ^{df}	^a 10.25 ^g	^a 10.00 ^g
6		^a 18.75 ^a	^a 15.41 ^b	^a 15.16 ^b	^a 12.71 ^d	^a 15.09 ^b	^a 13.22 ^c	^a 13.17 ^c	^a 13.56 ^c	^a 11.80 ^e	^a 10.31 ^f	^a 13.26 ^c	^a 11.08 ^e	^a 11.08 ^e
0	TVB-N	^a 12.63 ^{N.S.}	^a 12.63 ^a	^a 12.50 ^a	^a 12.60 ^a	^a 12.63 ^a	^a 12.60 ^a	^a 12.53 ^a	^a 12.50 ^a	^a 12.50 ^a	^a 12.60 ^a	^a 12.60 ^a	^a 12.60 ^a	^a 12.60 ^a
2		^c 14.71 ^a	^c 14.35 ^a	^c 13.00 ^c	^b 13.00 ^c	^c 13.25 ^b	^c 13.00 ^c	^c 13.00 ^c	^c 14.00 ^{ab}	^b 14.20 ^a	^b 13.27 ^{cd}	^c 13.54 ^b	^b 12.91 ^d	^b 13.07 ^{cd}
4		^b 16.22 ^a	^b 15.80 ^{ab}	^b 14.09 ^c	^b 13.11 ^e	^b 14.73 ^b	^b 14.00 ^c	^b 13.12 ^e	^b 15.08 ^{ab}	^a 13.57 ^b	^a 14.29 ^c	^b 14.45 ^b	^b 13.66 ^d	^a 13.92 ^{cd}
6		^a 18.53 ^a	^a 16.95 ^b	^a 16.07 ^c	^a 15.00 ^e	^a 16.13 ^c	^a 15.07 ^e	^a 14.36 ^f	^a 16.08 ^c	^a 14.13 ^f	^a 14.86 ^{ef}	^a 15.70 ^d	^a 14.08 ^f	^a 14.11 ^f

Means of treatments having the same right case letter(s) within a row are not significantly different (at p ≤ 0.05).

Means of storage periods having the same left case letter(s) within a column are not significantly different (at p ≤ 0.05).

N.S= Not significant

Table (6): Sensory evaluation of supplemented silver carp fish burgers during frozen storage (at -18 °C for 6 months).

Storage time (months)	Samples	Control	Oat				Soybean			Chickpea			Maltodextrin		
		0	5	10	15	5	10	15	5	10	15	5	10	15	
0	Color	^a 8.50 ^b	^a 8.50 ^b	^a 8.50 ^b	^a 8.50 ^b	^a 8.50 ^b	^a 8.50 ^b	^a 9.00 ^a	^a 8.50 ^b	^a 8.50 ^b	^a 9.00 ^a	^a 8.50 ^b	^a 9.00 ^a	^a 9.00 ^a	
2		^a 8.50 ^b	^a 8.50 ^b	^a 8.50 ^b	^a 8.50 ^b	^a 8.50 ^b	^a 8.50 ^b	^a 9.00 ^a	^a 8.50 ^b	^a 8.50 ^b	^a 9.00 ^a	^a 8.50 ^b	^a 9.00 ^a	^a 9.00 ^a	
4		^b 7.50 ^c	^b 7.50 ^c	^b 8.00 ^b	^b 8.00 ^b	^b 7.50 ^c	^b 8.00 ^b	^b 8.50 ^a	^b 8.00 ^b	^b 8.00 ^b	^b 8.50 ^a	^b 7.00 ^d	^b 8.50 ^a	^b 8.50 ^a	
6		^c 7.00 ^d	^c 7.00 ^d	^c 7.50 ^c	^b 8.00 ^b	^c 7.00 ^d	^b 8.00 ^b	^c 8.00 ^b	^c 7.50 ^c	^c 7.50 ^c	^c 8.00 ^b	^b 7.00 ^d	^b 8.50 ^a	^b 8.50 ^a	
0	Taste	^a 7.50 ^b	^a 7.50 ^b	^a 7.50 ^b	^a 7.50 ^b	^a 7.50 ^b	^a 7.50 ^b	^a 8.00 ^a	^a 7.50 ^b	^a 7.50 ^b	^a 8.00 ^a	^a 7.50 ^b	^a 8.00 ^a	^a 8.00 ^a	
2		^b 7.00 ^c	^b 7.00 ^c	^b 7.00 ^c	^a 7.50 ^b	^b 7.00 ^c	^b 7.00 ^c	^b 7.50 ^b	^b 7.00 ^c	^a 7.50 ^b	^a 8.00 ^a	^b 7.00 ^c	^b 7.50 ^b	^a 8.00 ^a	
4		^c 6.00 ^d	^c 6.50 ^c	^c 6.50 ^c	^b 7.00 ^b	^c 6.00 ^d	^c 6.50 ^c	^c 7.00 ^b	^c 6.50 ^c	^b 6.50 ^c	^b 7.50 ^a	^c 6.50 ^c	^b 7.50 ^a	^b 7.00 ^b	
6		^d 5.50 ^d	^d 6.00 ^c	^c 6.50 ^b	^c 6.50 ^b	^c 6.00 ^d	^c 6.50 ^b	^d 6.50 ^b	^d 6.00 ^c	^b 6.50 ^c	^c 7.00 ^a	^d 6.00 ^c	^c 6.00 ^c	^c 6.00 ^c	
0	Odor	^a 8.00 ^b	^a 8.00 ^b	^a 8.50 ^a	^a 8.50 ^a	^a 8.00 ^b	^a 8.00 ^b	^a 8.00 ^b	^a 8.00 ^b	^a 8.50 ^a	^a 8.50 ^a	^a 8.00 ^b	^a 8.00 ^b	^a 8.50 ^a	
2		^a 8.00 ^b	^a 8.00 ^b	^b 8.00 ^b	^a 8.50 ^a	^a 8.00 ^b	^a 8.00 ^b	^a 8.00 ^b	^a 8.00 ^b	^a 8.50 ^a	^a 8.50 ^a	^b 8.00 ^b	^a 8.00 ^b	^a 8.50 ^a	
4		^b 7.00 ^c	^b 7.00 ^c	^c 7.50 ^b	^b 8.00 ^a	^b 7.00 ^c	^b 7.50 ^b	^a 8.00 ^a	^b 7.00 ^c	^b 7.50 ^b	^b 8.00 ^a	^c 7.50 ^b	^b 7.50 ^b	^b 7.50 ^b	
6		^c 5.00 ^f	^c 6.00 ^e	^d 7.00 ^c	^c 7.50 ^b	^c 6.00 ^e	^c 7.00 ^c	^b 7.50 ^b	^c 6.50 ^d	^b 7.50 ^b	^b 8.00 ^a	^d 6.00 ^e	^c 7.50 ^b	^b 7.50 ^b	
0	Texture	^a 7.50 ^{N.S}	^a 7.50	^a 7.50	^a 7.50	^a 7.50	^a 7.50	^a 7.50	^a 7.50	^a 7.50	^a 7.50	^a 7.50	^a 7.50		
2		^b 7.00 ^b	^b 7.00 ^b	^b 7.00 ^b	^a 7.50 ^a	^b 7.00 ^b	^a 7.50 ^a	^a 7.50 ^a	^b 7.00 ^b	^a 7.50 ^a	^a 7.50 ^a	^b 7.00 ^b	^b 7.00 ^b	^a 7.50 ^a	
4		^c 5.50 ^d	^c 6.00 ^c	^c 6.50 ^b	^b 7.00 ^a	^c 6.00 ^c	^b 7.00 ^a	^b 7.00 ^a	^c 6.50 ^b	^b 7.00 ^a	^b 7.00 ^a	^c 6.00 ^c	^c 6.50 ^b	^b 6.50 ^b	
6		^d 5.00 ^c	^d 5.00 ^c	^d 6.00 ^b	^c 6.50 ^a	^d 5.00 ^c	^c 6.50 ^a	^c 6.50 ^a	^d 6.00 ^b	^c 6.00 ^b	^c 6.50 ^a	^d 5.00 ^c	^d 6.00 ^b	^c 6.00 ^b	

Means of treatments having the same right case letter(s) within a row are not significantly different (at $p \leq 0.05$).

Means of storage periods having the same left case letter(s) within a column are not significantly different (at $p \leq 0.05$).

N.S= Not significant

