

QUALITY CHANGES OCCURING DURING FROZEN STORAGE OF CHICKEN SAUSAGE CONTAINING BUFFALO SPLEEN

MOUSTAFA M.M. IBRAHIM

Meat and Fish Tech. Res. Dept., Food Tech. Res. Inst., Agric. Res. Center, Giza, Egypt.

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Abstract

Buffalo boiled spleen was incorporated in chicken sausage formulation in ratio 30 and 50% as replaced chicken meat percent in blends B and C, respectively, to improve color, iron content and texture of samples. The effect of prolonged storage (at- 18 °C for 6 months) on samples for quality, Hunter color, heme pigments, micro-elements and microbiological loads were studied. Moreover, consumer panel data of samples during frozen storage were analyzed.

Amino nitrogen, total volatile nitrogen and thiobarbituric acid (TBA-value) were slightly increased during frozen storage, but they were within the permissible limits.

A significant higher values in redness (a*), saturation index and redness / yellowness (a*/ b*) ratio were found in blends B and C of prolonged frozen storage, while the A control (0 % spleen) had the lowest value. The nitrosoheme pigment and total pigment concentration were increased with increasing level of spleen in sausage, but the pigments were gradually decreased during storage, of which the A (control) had the lowest values. The iron content was increased, but zinc and copper contents were slightly decreased in samples with replacement spleen in blends.

The total aerobic plate count was decreased, but total psychrophilic count was increased progressively by storage time. *Salmonella* and *shigella* were not detected in all samples. Incorporated spleen in sausage (blends B and C) did not adversely affect the bacterial quality or shorten their useful shelf-life, and remained darker on each surface and contained more pigment than control. Furthermore, blend B had received high level of overall acceptability in consumer panel.

INTRODUCTION

Spleen, is considered as a delicacy by gourmets, with soft tender and mild in flavor. It is one of the economical variety meats and often used roasted, oven roasted and fried or browned in butter. Meat patties, meat loaves and stews of other meats often include diced or ground spleen,(Ibrahim, 1997).

Slesinski *et al.* (2000) reported that poultry producers could reduce pink color development in further-processed products by selective addition of dairy proteins. Claus *et al.* (1994) studied the storage effects on color values for cooked turkey

and the results illustrated that the L* and b* values were slightly decreased during storage. Mendenhall (1989) suggested that myoglobin denaturation is related to pigment concentration of the raw products. Ibrahim (1997) found that beef spleen contained iron, zinc and copper with a level of 87.76, 27.13 and 1.50 mg/100g (dry weight), respectively.

Bittel, *et al.* (1981 b) stated that frankfurters made with different levels of mechanically separated spleen (MSS) did not adversely affect microbiological quality of the finished product or shorten their useful shelf-life, as well as these levels did not influenced bacterial numbers. Ibrahim and Abbas (2000) found that total aerobic plate count (TAPC) and *staphylococcus* were usually decreased, while total psychrophillic count was gradually increased during frozen storage of ground beef. On the other hand, *salmonella* and *shigella* were not detected in all samples.

The present investigation was carried out to study quality aspects, Hunter color, heme pigment, micro-elements and microbiological changes occurring during frozen storage of chicken sausage.

MATERIALS AND METHODS

Materials

Eight female chicken (more than one year old, with an average weight of 3.5 kg) were obtained from the Ministry of Agric., Cairo, Egypt. Samples were slaughtered, defeathered, eviscerated and the edible carcasses were cleaned , put into ice box and transported to the Laboratory of Meat and Fish Tech. Res. Dept., Food Tech. Res. Inst., Agric. Res. Center, Giza, Egypt. The samples were cleaned, the skin and fat were removed, deboned and the muscles (breast and legs) were cut in small pieces, packaged and stored at – 18 °C until using in sausage blends.

Five buffalo spleens were obtained from slaughterhouse, Helwan, Cairo, Egypt. The samples were veterinary examined and were found free from infection or diseases and then transported to the laboratory inside ice box. The samples were cleaned and precooked in boiling water containing 2% salt for 20 min, cooled, then cut in small pieces, packaged and stored at – 18 °C until using in sausage blends.

Fat tissue (sheep tail) was cleaned and cut in small pieces, packaged and stored at – 18 °C.

Fine rusk, spices and salt were bought from local market in Giza City.

Technological methods

Preparation of sausage formula

Three blends of sausage were prepared as given in Table (1). The ingredients were mixed for 8-10 min (emulsified) using laboratory emulsifier for sausage (Hobart, Model 84486, USA). Sodium nitrite (0.001%) was dissolved in small amount of water before being added to the formula.

Table1. Formulation of different sausage blends.

Ingredients %	Control (A)	Blend (B)	Blend (C)
Chicken meat	67.0	46.90	33.50
Buffalo spleen	—	20.10	33.50
Minced fat tissues	15.00	15.00	15.00
Ice water (flaskers)	10.00	10.00	10.00
Fine dry rusk	5.00	5.00	5.00
Sodium chloride	2.00	2.00	2.00
Spices	1.00	1.00	1.00

The obtained emulsion was stuffed in previously cleaned and prepared natural mutton casings, then packaged in polyethylene and stored at -18°C until analysis at 0,2,4 and 6 months.

Physical analysis

Hunter colour values

Hunter colour values (L^* , a^* and b^*) of raw sausages were measured using colourimeter (colour Tee PCM color Meter, colour Tec, NJ,USA). Thawed raw sausages were wrapped in clear. Polyethylene before reading. Small square slices were cut in parallel to the flat surface in the center of sausage. These later samples were wrapped in plastic food wrap, and the colour was measured.

The value L^* was the lightness ranged from 0 to 100, a^* was a chromatically index where positive value indicating redness and negative value indicating greenness, while positive value of b^* indicating yellowness and negative value indicating blueness. Four random spots on each sample were measured and the average data were taken.

Saturation index: $S = (a^2 + b^2)^{1/2}$, higher values indicate more vivid redness, hue angle $(b/a)^{-\tan^{-1}}$, higher values indicate less redness and a^*/b^* ratio higher values indicate more redness. The aforementioned parameters were calculated according to Ana and Joseph (1996).

Analytical methods

The pH value: was measured using a pH – meter (A512, USA).

Titrateable acidity: was determined and expressed as ml / NaoH 0.1 N /100g sample according to Keeton and Melton (1978).

Total volatile nitrogen (TVN): was determined according to the method described in AOAC (1980).

Amino nitrogen (Amino N): was estimated using the formal volumetric titration method as described by Kolochoy (1952).

Thiobarbituric acid value (TBA value): was determined as described by the method of Pearson (1970).

Pigment determination

Total and nitroso-heme pigments were determined by the method of Bittell *et al.* (1981b) after 0,2,4 and 6 months storage.

Micro-elements analysis

The micro-elements namely iron (Fe), zinc (Zn) and copper (Cu) of the tested samples were determined using a Perkin-Elmer 403 atomic absorption spectrophotometer by Perkin-Elmer (1976) at zero time and after 4 months of frozen storage.

Microbiological analysis

Samples of sausage blends (20 g) were placed in warring blender with 180 ml. of 0.1 sterile peptone and homogenized for 1-2 min, then the extension of viable cells. Bacteria counts were calculated as CFU/g (colony forming unit) for the following:

Total aerobic plate count (TAPC): was counted on agar media (Difco, 1970). Plates were incubated at 35 °C for 48 hrs.

***Salmonella* and *shigella*:** were counted according to the method described in Difco, (1970) incubated at 35 °C for 18-24 hrs.

Total psychrophillic plate count (TPPC): was determined according to APHA (1976) incubated at 7 °C for 10 days.

Consumer panel

Frozen samples were thawed overnight in a refrigerator at 5 ° C, then samples were evaluated by 10 persons (female) unfamiliar to purchase the different meat products from Meat and Fish Tech. Res. Dept. and not informed with the nature of study. They received samples, coded package of sausage at three separate times during the first 2 months of the study. Raw products were evaluated and scored for color, odor and overall acceptability on a 5- Point rating scale (5= very desirable, 1= very undesirable) , while texture was rated from 5 (very firm) to 1 (very soft) according to Bittel *et al.* (1981 b).

Statistical analysis

The analysis of data was carried out by ANOVA, while, Dumcan's multiple range test was used to test the differences among means (SAS, 1992).

RESULTS AND DISCUSSION

1- Quality aspects changes during frozen storage.

Data in Table (2) show that the pH value of control was higher than blend B and C at 0 time. The pH values were slightly decreased at the 2 month of storage, but gradually increased during prolonged frozen storage. Nassos *et al.* (1985) evaluated the relationship between lactic acid concentration and microbial spoilage in ground beef, they reported that the level of pH correlated inversely with the proportion of lactic acid producers. Also, results revealed that the changes in acidity took the inverse direction of pH values.

A remarkable gradual increase in AN and TVN were found in all samples during frozen storage which indicates a slight proteolysis during storage. The TVN values of all samples were within the permissible limit < 30mg /100g (EOS, 2000).

The TBA values of samples were slight increased during storage, but the values of blends B and C were higher than A (control) probably due to the incorporated spleen in blends which precooked before added in formula. The TBA values were decreased after 4 months. Bhattacharya *et al.* (1988) stated that TBA values for both cooked and uncooked patties increased with increasing time of frozen storage for a period of 12-16 weeks, after which they decreased.

Table 2. Quality aspects changes during frozen storage of raw sausage blends.

Storage period (months)	pH value			Titratable acidity (ml NaOH/0.1N/100g)			Amino nitrogen (mg/100g)			Total volatile nitrogen (mg/100g)			TBA value (mg malonaldehyde/kg sample)		
	Blend A*	Blend B	Blend C	Blend A*	Blend B	Blend C	Blend A*	Blend B	Blend C	Blend A*	Blend B	Blend C	Blend A*	Blend B	Blend C
0	5.75	5.60	5.65	0.35	0.42	0.39	8.83	10.02	10.42	5.75	5.35	5.16	0.28	0.31	0.19
2	5.50	5.50	5.55	0.46	0.38	0.38	12.15	13.31	13.79	10.95	9.47	7.65	0.41	0.51	0.55
4	5.65	5.65	5.70	0.45	0.43	0.41	13.91	15.05	15.65	14.11	12.65	11.71	0.72	0.86	0.89
6	5.75	5.70	5.75	0.33	0.31	0.31	15.45	18.44	19.05	16.00	16.55	16.29	0.55	0.72	0.75

A* control

On wet weight basis

Results are average of three replicates.

Table 3. Hunter color (L*, a*, b*) changes during frozen storage of raw sausage blends.

Storage period (month)	L* value			a* value			b* value			Saturation index			Hue angle			a*/b* ratio		
	Blends			Blends			Blends			Blends			Blends			Blends		
	A*	B	C	A*	B	C	A*	B	C	A*	B	C	A*	B	C	A*	B	C
0	52.47 ^a	43.25 ^b	35.30 ^c	5.07 ^b	11.10 ^{ab}	11.28 ^{ab}	9.74 ^{ab}	11.41 ^a	9.36 ^{ab}	10.98 ^b	15.92 ^{ab}	14.66 ^{ab}	62.50 ^a	45.79 ^b	39.69 ^c	0.52 ^c	0.97 ^{ab}	1.21 ^{ab}
2	54.09 ^a	43.69 ^b	36.24 ^c	6.17 ^b	12.83 ^{ab}	12.69 ^{ab}	8.16 ^b	11.10 ^a	10.05 ^{ab}	10.23 ^b	16.97 ^a	16.19 ^a	52.90 ^a	40.87 ^b	38.38 ^c	0.76 ^b	1.16 ^{ab}	1.26 ^{ab}
4	57.85 ^a	44.16 ^b	39.79 ^c	7.43 ^b	15.98 ^a	14.82 ^a	7.85 ^b	10.68 ^a	11.55 ^a	10.80 ^b	19.22 ^a	18.79 ^a	46.57 ^b	33.76 ^c	37.93 ^c	0.95 ^b	1.51 ^a	1.28 ^{ab}
6	59.16 ^a	45.09 ^b	40.63 ^c	8.74 ^b	17.46 ^a	15.49 ^a	6.86 ^c	9.37 ^{ab}	11.89 ^a	11.11 ^b	19.82 ^a	19.53 ^a	38.13 ^c	28.22 ^{cd}	37.51 ^c	1.27 ^{ab}	1.86 ^a	1.30 ^{ab}

A* = control

Results are average of three replicates.

abc Means in a column with different letters are different (P < 0.05).

2- Hunter color (L^* , a^* , b^*) values changes during frozen storage.

Table (3) demonstrates the differences in Hunter color values between samples. The lightness (L^*) of samples was significantly different ($P < 0.05$) between samples during frozen storage. The L^* values of A (control) was higher than blend B and C.

A significant higher values in redness (a^*), saturation index and a^*/b^* ratio were found in blends B and C after prolonged frozen storage, while the A (control) had the lowest values.

The yellowness (b^*) values of blends B and C were not varied during storage, but the values revealed that slight decrease was found in blends A and B during storage. Claus *et al.* (1994) illustrated that the L^* and b^* values were slightly decreased during storage. Moreover, Bittel *et al.* (1981 b) stated that each product showed a significant change of external surface color during storage. The rate of exterior surface of frankfurter indicated that lightening was decreased with increased level of mechanically separated spleen (MSS), and it was increased with storage time Table (3).

Results of hue angle values were significantly different ($P < 0.05$) between most treatments. The hue angle values of blends B and A went down during storage, but blend B had the lowest values.

These results revealed that frozen storage of samples was not failed effect on Hunter color values, but the a^* , saturation index and a^*/b^* ratio values of blend B were highest during frozen storage. These results are closed with that reported by Slesinski *et al.* (2000) and Mendenhall (1989).

The results in Table (3) demonstrated that incorporated spleen in sausage formula remained darker on each surface and contained more pigment than the control sample.

Table 4. Heme pigments changes during frozen storage of raw sausage blends. (ppm / hematin).

Storage Period (months)	Nitrosoheme pigment						Total heme pigment					
	Blend A (control)		Blend B		Blend C		Blend A (control)		Blend B		Blend C	
	Initial value	%*	Initial value	%*	Initial value	%*	Initial value	%*	Initial value	%*	Initial value	%*
0	8.70	100.00	16.82	100.00	21.17	100.00	183.60	100.00	231.20	100.00	427.72	100.00
2	9.31	107.01	17.98	106.91	24.65	116.44	142.37	77.34	178.16	77.06	285.60	66.77
4	7.83	90.00	15.60	92.75	19.23	90.84	126.86	69.11	162.36	70.23	269.77	63.07
6	6.15	70.69	13.37	79.49	12.79	60.42	112.97	61.53	149.89	64.83	246.93	57.73

Results are average of three replicates.

* = % of initial value.

3- Heme pigments changes during frozen storage.

The interaction effects for total and nitroso-heme pigments are presented in Table (4). It can be seen that increased level of spleen in blends B and C increased the concentration of nitrosoheme pigment, but the nitrosoheme pigment values (as % of initial value) were gradually decreased in all samples during prolonged storage. The blends B and C were higher values than A (control) during storage.

As the same trend, the initial total pigment concentration increased linearly with increased level of spleen in blends B and C. The blends B and C had higher values of total pigment than A (control), while the blend C had the highest value and the blend A had the lowest.

The total heme pigment (as % of initial value) was decreased in all samples during frozen storage, but the decrease in blend C was faster than blend B. The increased intensity of brown-red pigmentation of frankfurters with increased level of mechanically separated spleen (MSS) was most probably a results of the abundant quantity of hemoglobin in the MSS (Bittel *et al.* 1981 b).

It should be noted that even after the noticeable degree of fading , each of sausage containing spleen in the formulation (blends B and C) remained darker on each surface and contained more pigments than control sample.

4-Micro-elements changes during frozen storage

Table 5. Micro-elements changes during frozen storage (ppm).

Elements	At zero time			After 4 months		
	Control	Blend	Blend	Control	Blend	Blend
	(A)	(B)	(C)	(A)	(B)	(C)
Fe	17.68	91.26	125.85	16.27	90.43	124.32
Zn	21.89	20.71	19.45	20.46	19.86	18.86
Cu	1.12	1.05	0.92	1.01	0.95	0.89

On wet weight basis

Means of triplicate analyses

Data in Table (5) reveal that zinc and copper concentration were slightly decreased with increased level of spleen, but iron concentration in blends B and C increased. Bittel *et al.* (1981 b) found that increasing levels of mechanically separated spleen (MSS) resulted significantly in increasing iron concentration in the frankfurters. While Bittle *et al.* (1981 a) found that the zinc contents of whole spleen and MSS were 20.9 and 19.7 ppm, respectively. Moreover, Ibrahim (1997) found that beef spleen contained iron, zinc and copper as 87.76, 27.13 and 1.50 mg/100g (dry weight), respectively, which was higher than others edible meat by-products. In general, frozen storage appeared to have little impact on concentration of elements in samples. Results, however, suggest that freezing and thawing meat organs may cause some loss of fluid (driploss), and this fluid will contain a small portion of minerals (Price and Schweigert, 1970).

In addition, results showed that blends B and C may provide with considerable amounts of micro - elements for human nutrition when compared to the % recommended dietary allowance (% RDA) and levels in 100g serving for adult male (NAS/ NRC, 1980).

5- Microbiological loads changes during frozen storage.

Total aerobic plate count (TAPC) of all samples were gradually decreased during prolonged storage probably due to these organisms were not adequately surviving the environment Table (6). Kraft *et al.* (1981) found that storage for six months at (- 20 °C) reduced bacterial numbers. The TAPC of blend A (control) was higher than blend B and C during storage, this indicated that the incorporated spleen in sausage did not increase bacterial count in the samples. These results are closed with that reported by Bittel *et al.* (1981 b).

The total psychrophilic plate count (TPPC) was gradually increased during prolonged storage of samples, but blend A had highest value. In general, the *salmonella* and *shigella* were not detected in all samples. The numbers of bacteria studied were in permissible limit set by EOS (1991). Results in Table (6) illustrated that incorporated buffalo spleen in sausage (blends B and C) did not adversely affect the microbiological quality of the final products or shorten their useful shelf life.

At the end of storage the samples were microbiologically acceptable and showed no signs of spoilage i.e. slime formation, off-odor or discoloration. These results are in agreement with those obtained by Bittel *et al.* (1981 b) and Ibrahim and Abbas (2000).

Table 6. Microbiological loads changes during frozen storage of raw sausage blends (CFU/g).

Storage period (months)	Total aerobic plate count (TAPC)			Total psychrophilic plate count (TPPC)			<i>Salmonella</i> and <i>shigella</i>		
	Blend A (control)	Blend B	Blend C	Blend A (control)	Blend B	Blend C	A	B	C
0	1.6x10 ³	0.9x10 ³	0.7x10 ²	0.6x10 ²	0.4x10 ²	0.4x10 ²	Not detected		
2	3.6x10 ²	3.2x10 ²	3.2x10 ²	3.1x10 ²	0.6x10 ²	0.7x10 ²	Not detected		
4	2.5x10 ²	2.2x10 ²	2.1x10 ²	2.3x10 ³	1.9x10 ³	1.8x10 ³	Not detected		
6	2.2x10 ²	2.1x10 ²	2.1x10 ²	3.2x10 ³	2.1x10 ³	2.2x10 ³	Not detected		

Results are average of three replicates

6- Consumer panel evaluation during frozen storage of raw samples.

Results in Table (7) illustrate that increasing the level of spleen in sausage blends B and C did not significantly alter the color and overall acceptability, but high significant differences ($P < 0.05$) were found between them and A (control). The color and overall acceptability scores were higher in blend B than blends C and A, respectively.

Consumer panel texture scores reflected the trend toward softer sausages with increased the spleen level in blends B and C. The scores of odor were slightly different between treatments, the data revealed that blends B and C had higher odor scores than A (control).

Bittel *et al.* (1981 b), stated that several panelists commented that the high level of mechanically separated spleen (MSS) in frankfurters (10 and 15 %) revealed a spicier, more intense flavor and a softer, pasty-like texture product, somewhat similar to that of liver sausage. Therefore, Field and Riely (1974) reported that incorporation MSS (mechanically separated spleen) into finely comminuted products improved emulsion stability, texture score, and reduced shrink in bologna due to lower connective tissue content of machine deboned mutton.

In general, frozen storage appeared to have a little impact on consumer panel between samples. Overall, these results indicated that as much as 30 % spleen (in blend B) can be incorporated in sausages without seriously altering or decreasing consumer acceptability.

Table 7. Consumer panel evaluation during frozen storage of raw sausage blends.

Storage period (month)	Color scores			Odor scores			Texture scores			Overall acceptability scores		
	Blend A (control)	Blend B	Blend C	Blend A (control)	Blend B	Blend C	Blend A (control)	Blend B	Blend C	Blend A (control)	Blend B	Blend C
0	2.98 ^d	4.65 ^a	4.45 ^{ab}	3.56 ^c	4.46 ^a	4.48 ^a	3.60 ^b	2.55 ^c	2.40 ^d	3.55 ^c	4.80 ^a	4.70 ^a
2	3.05 ^c	4.55 ^a	4.43 ^{ab}	3.42 ^c	4.38 ^a	4.38 ^a	3.60 ^b	2.50 ^c	2.35 ^d	3.40 ^c	4.70 ^a	4.55 ^a
4	3.10 ^c	4.45 ^{ab}	4.30 ^b	3.33 ^c	4.35 ^a	4.33 ^a	3.65 ^b	2.55 ^c	2.30 ^d	3.40 ^c	4.65 ^a	4.45 ^{ab}
6	3.13 ^c	4.40 ^{ab}	4.23 ^b	3.23 ^c	4.25 ^{ab}	4.20 ^{ab}	3.70 ^b	2.60 ^c	2.30 ^d	3.33 ^c	4.60 ^a	4.35 ^{ab}

abc means in a raw with different letters are different ($P < 0.05$).

Color, odor and overall acceptability: 5= very desirable, 1= very undesirable.

Texture: 5= very firm, 1= very soft.

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تغيرات الجودة الحادثة أثناء التخزين بالتجميد لسجق الدجاج المحتوى

على الطحال الجاموسى

مصطفى محمد محمد إبراهيم

قسم بحوث تكنولوجيا اللحوم والأسماك معهد بحوث تكنولوجيا الأغذية مركز البحوث الزراعية
جيزة مصر.

تم إدخال الطحال الجاموسى المسلوق فى تركيبة سجق الدجاج بنسبة ٣٠ ، ٥٠ % كنسبة استبدال من لحم الدجاج فى الخلطة B ، C بالترتيب وذلك لتحسين اللون ومحتوى الحديد والبطراوة فى العينات. تم دراسة تأثير التخزين بالتجميد على درجة حرارة ١٨ م على الجودة و Hunter color وصبغات الهيم والعناصر الصغرى والمحتوى الميكروبيولوجى بالعينات. علاوة على ذلك تم تحليل تقييم المستهلكين للعينات أثناء التخزين بالتجميد.

حدثت زيادة طفيفة فى كل من النتروجين الأمنى والمركبات النتروجينية الطيارة وقيم حمض الثيوباربيتوريك (TBA) خلال التخزين بالتجميد ، ولكن الزيادة داخل الحدود المسموح بها. وجدت قيم عالية معنوية فى الاحمرار (a*) والـ Saturation index ونسبة الاحمرار / الاصفرار a*/b* ratio فى الخلطات B ، C خلال فترات التخزين بالتجميد ، بينما أقل القيم كانت فى الخلطة A (لا تحتوى على الطحال). إزداد تركيز صبغة النيتروزهيم وصبغة الهيم الكلية بزيادة مستوى الطحال فى السجق، ولكن هذه الصبغات يقل تركيزها وبالتدرج خلال فترات التخزين وكانت أقل القيم فى الخلطة (الكنترول). يزداد محتوى الحديد ولكن ينخفض محتوى الزنك والنحاس بدرجة خفيفة فى العينات بزيادة إحلال الطحال فى الخلطات.

تناقصت الأعداد الكلية للبكتريا الهوائية ، ولكن تزايدت بالتدرج أعداد البكتريا المحبة للبرودة خلال فترات التخزين. ولم يستدل على وجود أى أعداد للسالمونيلا والشيجيلا فى كل العينات. إدخال الطحال فى السجق (خلطات B ، C) لم يؤثر عكسياً فى الجودة البكتيرية أو يقلل من صلاحيته للاستهلاك وتظل العينات ذات لون داكن ومحتواها أكثر فى الصبغات عن الكنترول. علاوة على ذلك نالت الخلطة B درجات عالية فى القبول العام لتقييم المستهلكين.