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Article

Microbial and sensory evaluation of Bangladeshi frozen chicken sausage

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Abstract: The purpose of this study was to evaluate the microbiological safety and the effect of some synthetic chemicals with comparison to natural additives such as rosemary extract in preventing microbial growth and as a source of antioxidant by maintaining GHP as well as GMP of Bangladeshi processed chicken sausages with low fat content to correlate some scientific evidence in producing cancer and cardiovascular diseases by the consumption of meat product. Two experimental batches of chicken sausage were prepared according to Bangladeshi standard using different parameters of ingredients. Then centesimal parameter, P^H and instrumental color of the product were analyzed according to standard protocol. A sensory parameter was also checked by ranking the acceptance level of consumers about different batches of chicken sausage. The sensory properties and the instrumental color parameter of two experimental batches of chicken sausage 'A' and 'B' were found almost similar in comparison to the commercial cured chicken sausage 'C' but quite dissimilar in comparison to commercial uncured sausage 'D'. Total coliforms were observed 4.4 × 10 for sausage 'A' and 5.3 × 10 for sausage 'B', while the most probable number (MPN) for *E. coli* was 1.7 for both products. The counting of sulphite reducing *Clostridium* was 2 cfu/g and 3 cfu/g for both of the batches 'A' and 'B' consecutively. The coagulase-positive Staphylococci and lactose fermenting *Salmonella* was not found in any batches of chicken sausage.The results of this experiment clearly mentioned the possibility of producing safe and high quality chicken sausage with reduced fat content in Bangladesh by using natural pigments and antioxidants.

Keywords: coliforms; centesimal parameter; rosemary extract; sensory analysis; chicken sausage

1. Introduction

Now a day's diseases such as obesity, heart disease and diabetes are becoming most common diseases across Bangladesh as well as throughout the world, the food industries have come under mounting pressure to improve the nutritional quality of their products with low fat content. Particular attention has focused on the health problems associated with fat content in food and consumers are looking for no or low fat meat products (Ali *et al.*, 2011). A relationship between a diet containingan excess of energy-dense foods and the emergence ofa range of chronic diseases including colon cancer, obesity, cardiovascular diseases, and several other disorders has been proved by some recent studies (Bhat *et al.*, 2011; Zargar *et al.*, 2014). A positive association between meat consumption and colorectal cancer has also been stated by Sadri and Mahjub (Sadri *et al.*, 2006).Therefore, an increase in the level of dietary fiber in the daily diet can be beneficial for a healthy life

(Bhat *et al.,* 2011). Dietary fibers are the key ingredient lacking in the meat and meat products and regular consumption of latter is being associated with various health disorders. In our study, we used wheat and rice flours that are containing 14% and 15% dietary fiber consecutively. The addition of flours improved the composition of dietary fiber in chicken sausages. Various reports have revealed that intake of fiber reduces the risk of such diseases. Several studies have proven that dietary fibers have the potential to reduce blood low density lipoprotein cholesterol, risk of diabetes mellitus type 2, coronary heart disease, blood pressure and colorectal cancer (Zargar *et al.,* 2014).

Sausages are industrialized meat products that produced with or without the supplementation of fat tissue. Fresh sausages are produced without heat treatment to reduce the microbes. The microbiological stability of these types of sausages may be increased by the addition of curing ingredients. The curing process is occupied by the addition of salt and nitrate and other ingredients that significantly modify the physical, chemical and microbiological properties of meat products (Venturini *et al.,* 2011). Instead of using nitrate we used rosemary extract for cheaking the availability of producing antioxidant as well as to inhibit microbial activity. The stability of lipids against oxidation was supported by the rosemary extract; especially the sausage with 0.5 g/kg of the extract showed lower TBARS values than lycopene and its combination with the rosemary extract (Rohlik *et al.,* 2013; Szymanczuk *et al.,* 2011). A variety of beneficial effect of rosemary extract on human health has been observed by different studies (Aberle *et al.,* 2001). Some studies stated that it provides adequate antioxidant,protects brain cells from the normal effects of aging, may slow down the progression of Alzheimer's disease, protects cells from carcinogens, inhibits growth of cancer cells, helps in reducing allergy symptoms, especially to dust mites, increases potency of vitamin E and helps in reducing hypertension (Yu *et al.,* 2002). In this experiment, two Bangladeshi formulations of processed chicken sausage were studied with the supplementation of natural cochineal carmine dye and rosemary extracts against synthetic antioxidants for maintaining color, appearance and pleasant flavor to both of the batches. Considering the increasing demand of consumers for healthier and safe foods, the sausages were processed with bone less chicken flesh in an alternative system.

Therefore, the objectives of this study was to observe the microbial load, the centesimal composition, instrumental color in Bangladeshi processed fresh chicken sausages prepared with reduced fat content in different concentrations of carmine and rosemary extract and also to verify the preference of consumers in accepting different color, tenderness, flavor and texture between the experimental batches of chicken sausage and commercially available chicken sausage.

2. Materials and Methods

2.1. Selection of chicken

All the birds used in this experiment were broiler and within the age of 28 to 35 days, average weight was 1.5 kg. All the chickens were sacrificed by knife and thrown into boil water for 40 seconds to 1 minute, and then feathers were removed. Then the chickens were processed for manufacturing chicken sausages.

2.2. Composition of experimental batches of chicken sausages

Two experimental batches 'A' and 'B' were processed according to a selected parameter (Table 1) by following the codes of Good Manufacturing Practice (GMP). Manufacturing of the chicken sausage used skin and bone less chicken flesh, then the flesh and skin were grounded and transported to the mixture machine where flake ice and other ingredients were added to improve the color and texture. After preparing the mixture, it was stuffed in nojex casing (food grade) and tied with thread for making a desired size. Then it was exposed to steaming and smoking chamber. The sausage were then stored in a dark chamber at -18^{o}C for twenty days.

Table 1. Composition of ingredients used in manufacturing of uncured fresh Chicken Sausage 'A' and 'B'.

Components and additives	Batch 'A' (%)	Batch 'B' (%)
Bone less chicken flesh	79.5	79.5
Skin	6.2	6.2
Refined wheat flour	3	3
Refined rice flour	4.5	4.5
Flake ice	2.9	2.9
NaCl	0.4	0.4

Components and additives	Batch 'A' (%)	Batch 'B' (%)
Glucono-delta-lactones	0.5	0.5
Cochineal carmine	0.01	0.05
Red pepper	0.3	0.3
Seasoning	1.4	1.4
Malt dextrin	1.5	1.5
Garlic pest	0.15	0.15
Rosemary extract	0.4	-
Synthetic antioxidant	-	0.25

2.3. Processing and manipulation of chicken sausage

During the processing and manipulation room temperature was 17^{o}C and water used in the recipe was UV treated.

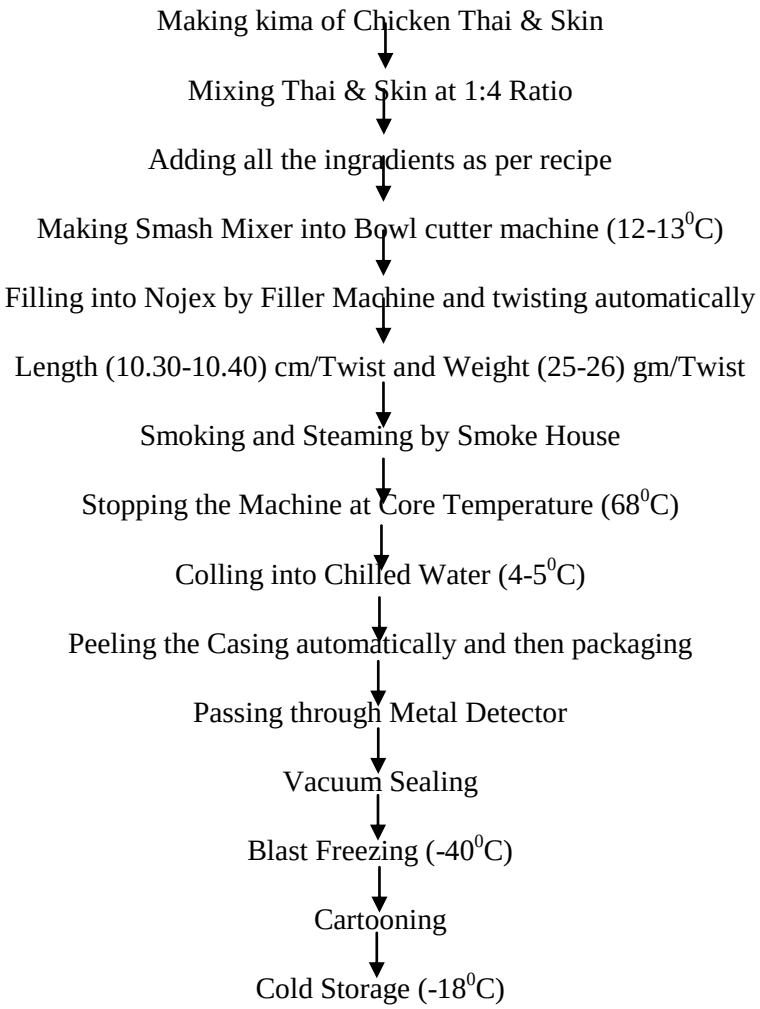

Figure 1. Flow diagram of the production process of experimental batches of chicken sausage.

2.4. P^{H}

All samples of chicken sausage were subjected for the measurement of P^{H} immediately before storage and at the end of storage. The P^{H} of two batches 'A' and 'B' were measured by glass electrodes which were placed directly into the mixed meat mass.

2.5. Centesimal parameter analysis

The total protein, fat and moisture content of both batch 'A' and 'B' were measured according to the methodology provided by AOAC International, USA.

2.6. Microbial analysis

To ensure the food safety by maintaining the proper food hygiene protocol the analysis of the microbial quality of both batches was determined before commencement of storage and at the end of storage at -18^{o}C temperature for twenty days through the determination of total and fecal coliform bacteria per gram of food using standard protocol (Ismail *et al.,* 2002; APHA 2002). The presence of *Staphylococcus* spp., *Clostridium* spp. and *Salmonella* spp. were also evaluated.

2.7. Determination of psychrophilic counts

The pour plate technique recommended by American Public Health Association was applied using Standard Plate Count Agar medium (Oxoid-CM463) and incubated at 7°C for 10 days at the end of the storage -18^{o}C for twenty days.

2.8. Determination of mesophilic counts

The pour plate technique recommended by American Public Health Association was applied using standard plate count agar medium (Oxoid-CM463) and incubated at 37°C for 24 hours at the end of the storage -18^{o}C for twenty days.

2.9. Sensory analysis

The both experimental batches of chicken sausages 'A' and 'B' were compared with another two commercial brands of sausage 'C' and 'D'. A total of 40 consumers were occupied in separate booths on the exposure of white light at 25^{o}C temperature. The both raw and fried samples were tested in three steps. In first step the sensory evaluation was done for checking proper color and appearance of raw sausages before frying, it was performed for ranking the preference of consumers when they were showed a product over another. The 'A' and 'B' batches of chicken sausages were thawed for balancing temperature in refrigerator for 24 hours. The samples were then served randomly in plastic cups. The results were statistically analyzed according to the method stated by Newell, which defines the value of critical differences between the total ordering at level of 5%. The fried sausages of both experimental batches were evaluated by nine point hedonic scale, where 1 was stands for disliked extremely, 2 was for disliked much,3 was for disliked moderately, 4 was for disliked slightly, 5 was for neither liked nor disliked, 6 was for liked slightly, 7 was for liked moderately, 8 was for liked very much and 9 was stands for liked extremely. The purchase tendency of processed products was measured by a five point scale where (1) I would certainly buy, (2) I would probably buy, (3) maybe I would buy / not buy, (4) I would probably not buy, and (5) I would certainly not buy. The sensory analysis was done according to Venturini *et al.* (2011).

3. Results and Discussion

3.1. Centesimal parameter analysis

The average of protein fraction, fat fraction and moisture contents of experimental chicken sausages were 18.88%, 7.6% and 72.56% respectively. Addition of wheat and rice flours increased a little bit protein concentration because the wheat flour contains 26% and the rice flour contains 19% protein. The standard parameter was fixed for chicken sausage in Brazil stated that fresh-type sausages should have 70% maximum moisture content, 30% maximum fat content and 12% minimum protein content (Demeyer *et al.*, 2008). The fat content of different batches of sausages was influenced by the addition of skin in the recipe. This results of this study is in consonance of Venturini *et al.,* 2011 but slightly varied from those that reported by Newell *et al.,* 1987. The protein content found in this study was significantly higher than the maximum limit, while the moisture content was slightly over the recommended percentage. The reduced fat content product should have a minimum fat reduction of 25% when compared to the regular conventional product but the low fat content product should have the fat content is lower than 3% in solid products (Lee *et al.*, 1999). In this work, chicken sausages made only with fat from the bone less chicken flesh (A and B) were considered as having reduced fat content, since they presented reduction of 41.95% (7.6% of fat), when compared to conventional products made with chicken meat, which have about 17.4% of fat before frying (Newell *et al.,* 1987).

3.2. P^{H}

The average pH of all batches was 6.50, 6.35, 6.25, and 6.20 respectively before storage and 5.95, 5.80, 5.75 and 5.70 consecutively after storage. During storage under freezing, the P^{H} of sausages A and B decreased significantly over time. The rapid decline in pH after 24 hours of storage was probably due to the conversion of

glucono-delta-lactone into gluconic acid (Venturini *et al.,* 2011). The pH may interact with many other factors such as water activity, salt, temperature, oxide reduction potential, and preservatives to inhibit growth of pathogenic bacteria (Kroger *et al.,* 2003). In addition to interfering in acidity, glucono-delta-lactone can also act as flavoring agent that helps in making the final product tastier.

3.3. Microbial analysis

Total coliforms were observed 0.8 × 10 before storage and 4.4 × 10 after storage for sausage 'A' and 1.3 × 10 before storage and 5.3 × 10 after storage for sausage 'B', while the MPN for *E. coli* was 0.8 before storage and 1.7 after storage for both products. The counting of sulphite-reducing *Clostridium* was 2 cfu/g and 3 cfu/g for both of the batches 'A' and 'B' consecutively. The coagulase-positive *Staphylococci* and lactose fermenting *Salmonella* was not found in any batches of chicken sausage. This finding is slightly variable with those that found by Venturini *et al.,* 2011, but quite disagreed with the findings stated by Chakraborty *et al.,* 2015.

3.4. An average of mesophilic and psychrophilic counts

The count of Mesophilic and Psychrophilic bacteria was obtained before and after storage of all batches of samples. Results of the counts are summarized in Table 2.

Table 2. An average of mesophilic and psychrophilic counts of all batches of sausages before and after storage.

Batches of chicken sausage	Mesophilic counts		Psychrophilic counts	
	Before storage	After storage	Before storage	After storage
'A' uncured	1.4×10^4	1.7×10^4	1.3×10^3	1.6×10^3
'B' uncured	0.8×10^5	1.3×10^5	0.7×10^4	1.1×10^4
'C' commercial cured	1.1×10^5	1.3×10^5	1.6×10^4	1.9×10^4
'D' commercial uncured	1.3×10^4	1.7×10^4	0.7×10^3	1.3×10^3

The total mesophilic and psychrophilic counts of all batches of sausages obtained in this study are almost in consonance of the findings that previously stated by Ismail *et al.,* 2002.

3.5. Instrumental evaluation

The averages of instrumental color of raw fresh chicken sausages were oriented in Table 3. In this study color saturation c* value of the processed sausage 'B' was significantly higher than that of the processed sausage 'A' but the color saturation of commercial cured sausage 'C' was found almost similar. This data is highly agreed with those findings that stated by Venturini *et al.,* 2011. The h* value which is stands for hue of processed sausage 'B' received a higher concentration of carmine extract was found lower than the other batches of sausage. The darkening of product 'D', in comparison to the other batches of sausage seems to be correlated both to the absence of nitrite and caramine as well as to the absence of antioxidant. Lee *et al.* 1999 observed that the reduction of antioxidants inhibits the formation of metamyoglobin which is responsible for improving the red color in flesh and flesh products. Meanwhile Yu *et al.,* 2002 stated that natural extracts used in meat product may have antioxidant effect comparable to synthetic antioxidants for maintaining the color and prevention of lipid oxidation during refrigeration at 2^oC temperature. The high cochineal carmine concentration effect in sample 'B' can be determined by the high values of red a* and chroma c* which were found higher than the other batch of sausage except for the batch'D'.

Table 3. Averages of instrumental color of raw chicken sausage for color, flavor, tenderness and appearance attributes after 20 days of storage at -18^{o}C temperature.

Batch of chicken sausage	Instrumental color[1]				
	L^*	a^*	b^*	c^*	h^*
'A' uncured	49.2 ± 0.9^b	9.1 ± 0.4^b	8.1 ± 0.9^b	12.56 ± 0.8^b	40.8 ± 3.1^c
'B' uncured	43.8 ± 2.1^a	17.9 ± 1.3^a	4.1 ± 0.4^a	19.22 ± 1.4^a	12.1 ± 1.9^b
'C' commercial cured	$49.8 \pm 1.7^{b,c}$	16.1 ± 1.9^c	10.3 ± 1.3^b	17.77 ± 2.2^a	32.23 ± 2.8^a
'D' commercial uncured	48.1 ± 2.5^c	3.8 ± 0.5^d	7.9 ± 3.1^b	10.1 ± 0.5^c	65.35 ± 9.8^d

Averages existed in the same column with different alphabet are found statistically variable.

3.6. Sensory evaluation

The preference ranking test of consumers are gained from individual booth are showed in Table 4. The descending order was obtained from the most preferred to the least preferred opinion of consumers is established for the color and texture attributes of raw chicken sausages were found as 'C' is greater than 'A' when 'A' is greater than 'B' and then 'B' is greater than 'D'.

Table 4. Cumulative values attained from the preference ranking test about raw chicken sausage before frying.

Batch of chicken sausage	Sensory attributes	
	Color	Texture
'A' uncured	52[a]	58[a]
'B' uncured	38[a]	47[a]
'C' commercial cured	49[a]	52[a]
'D' commercial uncured	41[a]	44[a]

Averages existed in the same column with different alphabet are found statistically variable.

However statistically significant but no variation were observed between the experimental batches of sausage. The color and texture of commercial cured sausage with a supplementation of nitrate/nitrite were the most preferred but the color and texture of commercial uncured sausages were the least preferred which indicates the main factor for purchasing the raw sausages was the notion of the color oxidation in the meat product which was more visible in sample 'D', as expressed in Table 1. On the other hand the significantly decreased saturation of red color and the improved tone of brown mentioned that these batches of sausage had more visible oxidized color. The scores of the preference test exhibited more informative answers about the differences between the batches of sausages. The average scores assigned by consumers for the color, flavor, tenderness and overall appearance attributes of fresh chicken sausages is shown in Table 5.

Table 5. Averages of the sensory scores of fried sausages for the color, flavor, tenderness and texture attributes twenty days of storage at -18^{o}C temperature.

Chicken sausage	Sensory attributes			
	Color	Tenderness	Flavor	Texture
'A' uncured	5.9±2.2[a]	7.6±2.0[a,b]	6.8±2.0[a,b]	7.3±1.1[a]
'B' uncured	6.4±1.9[a]	7.3±1.7[a]	7.6±1.7[a]	6.8±1.3[a]
'C' commercial cured	6.8±2.3[a]	6.8±2.2[a]	8.2±0.8[a]	7.7±1.4[a]
'D' commercial uncured	5.9±1.9[b]	7.1±0.8[b]	5.9±1.7[b]	6.2±0.8[b]

Averages existed in the same column with different alphabet are found statistically variable.

The scores obtained about the sensory color, tenderness, flavor and texture of uncured sausages 'A' and 'B' manufactured without supplementation of nitrate/nitrite was not varied significantly from the scores gained about the commercial sausages made with the addition of nitrite 'C'. But the sensory color and texture of the batch 'A' and 'B' were statistically higher than those batch of commercial uncured sausages 'D' which is also made without nitrite supplementation Roberts *et al.,* 1975. This variation may be correlated to the absence of carmine and antioxidant in producing the commercial uncured product which may have adverse effect on the color, flavor and texture of the products during refrigeration due to the oxidation of oxymyoglobin to metamyoglobin which converts the red color to brown (Shahidi *et al.,* 1992; Sindelar *et al.,* 2007). The scores of the preference test showed more definitive information about the variation between the batches of sausages. Table 4 exhibits the average scores assigned by consumers about the color, flavor, tenderness and texture attributes of fresh chicken sausages. The response of consumers reveled that they would certainly or probably buy the processed sausages 'A' and 'B' meanwhile only 32.1% responded they would buy the commercial uncured sausage 'D'.

Table 6. Consumer percentage of preference test about purchase tendency.

Parameter	'A' uncured	'B' uncured	'C' commercial cured	'D' commercial uncured
Would be certainly bought	25.8	26.2	33.02	19.1
Would be probably bought	41.7	31.8	16.98	13
May or may not be bought	20.8	27.7	27.2	36.8
Would not probably bought	11.7	10.3	18.1	31.1
Would not certainly bought	0.0	4.0	4.7	0.0

The supplementation of natural antioxidant in lower carmine concentration of sausage reduced darkening of the batch of sausage 'A'. Thus the batch of sausage 'C' has showed the same preference level in comparison to 'A". The batch of sausage 'B' consisted with highest concentration of carmine showed improved intensity of red color before frying, which was rejected by a certain amount of consumers (Vanderzant *et al.*, 1992).

4. Conclusions

The result of this study is clearly indicates the possibilities of producing high quality chicken sausage by using natural antioxidants in Bangladesh. Avoiding the use of nitrate is exhibiting more fruitful result to produce chicken sausage in a preferable quality without significant alteration of color, tenderness, flavor and texture. Maintenance of proper hygienic protocol is necessary to satisfy GHP as well as GMP. The microbial load of raw chicken sausage was observed in this study found within the acceptable range. Handling, processing and manipulation should be maintained in an aseptic manner thus the microbial load will be decreased. Practicing GHP will improve the quality of the product and reduce the probability of spoilage during storage. Maintenance of proper temperature during processing, storage, transportation, distribution and retailing is very much important. Improper maintenance of temperature will facilitate multiplication of spoilage causing bacteria thus the bacterial activity will alter the color, flavor, texture and nutritional facts of produced chicken sausage. Frozen meat product such as chicken sausage is becoming very popular in Bangladesh. Increasing production of such product in an industrial manner may be able to fulfill native demand as well as can initiate exporting opportunities across the world.

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Conflict of interest

None to declear

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