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A Comparative Study on Microbiological and Compositional Aspect of Economy Pork Sausages Incorporated with Olive Oil, Apple Pulp and Pomegranate Seed Powder

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Abstract

Pork sausage were prepared, namely, T1 (traditional pork sausage, batter was manually mixed), T2 (low fat pork sausage, batter was manually mixed), T3 (traditional sausage, batter was in the form of emulsion) and T4 (low fat pork sausage, batter was in the form of emulsion). Pork sausages traditionally produced by minced pork meat and pork fat. Low fat pork sausage was prepared by using minced pork meat, olive oil, dried apple pulp powder (2%) and pomegranate seed powder (2%). Among the treatments the moisture and total ash content significantly ($p<0.05$) more in T2 and T4, protein and fat content significantly ($p<0.05$) more in T1 and T3. The microbiological quality had better in T2 and T4 than other treatments with lower total plate count, no significant ($p>0.05$) difference of protein, fat and total ash content in 12th day storage. Total plate count was found to differ significantly ($p<0.05$) throughout the period of storage. E. coli, Salmonella and Listeria monocytogenes were not detected in any of the samples on entire storage period, but Staphylococcus aureus were detected on 1st and 12th day in T1 and T3, but absent in T2 and T4.

Keywords: Apple Pulp, Low Fat, Olive Oil, Pomegranate Seed, Pork Sausage, Shelf – Life

Introduction

Animal intestines or skin are used for preparation of natural casings and meat can be preserved by stuffing salted, chopped meats flavored with spices into it. The word "sausage" is originated from the Latin word, "*salsus*", meaning preserved or literally salted (Lonergan *et al.,* 2018). Meat and meat products are an essential part of the human diet. They provide easily available proteins, minerals and all the B-complex vitamins. The excellent digestibility and well-balanced composition of essential amino acids make the meat a highly demanded article of human nutrition. Pork and organ meats are good sources of linoleic and linolenic acid (Das *et al*., 2020). Apart from all these nutrients, recent studies indicate that pork contains several bio-active peptides and neutraceutical substances required for promotion of health. Pork and pork products are very popular in North Eastern Region of India. Many conventional pork products like ham, bacon, sausages and salami etc. are being imported to this part of the country from other states (Chavhan *et al*., 2015).

Now a days health concerns about fat consumption and changes in consumer preferences have led to extensive research on low fat foods. In restructured meat products fat plays an important role in stabilizing meat emulsions, reducing cooking loss, improving water holding capacity, providing juiciness and improving other quality (Warriss, 2000). Traditional meat products show some negative aspects from the nutritional point of view as a consequence, among other reasons, of their high animal fat content. However, high fat contents in particular animal fat provides high amounts of saturated fatty acids and cholesterol. High animal fat diets are associated with several types of ailments like obesity, hypertension, cardiovascular diseases and coronary heart diseases. Thus, changes in consumer demand as well as increased global competition, has led to development of novel restructured meat products, which are ''natural, functional and nutritional'' as well (Rather *et al*., 2015).

Vegetable oils are free of cholesterol and have a higher ratio of unsaturated to saturated fatty acids than animal fats. Thus, the incorporation of vegetable oils in frankfurters may have positive effects on consumer health (Choi *et al*., 2009). Vegetable oils have also been used as partial substitutes of pork back fat in low-fat frankfurters and other type of cooked products giving rise to products with fatty acid profiles and cholesterol levels more adequate than the traditional ones. Reducing the fat content in frankfurters has been reported to increase firmness, gumminess and chewiness, and springiness, and to decrease juiciness. No significant effects have been observed in textural parameters when vegetable oils have been used as partial substitutes of pork back fat. It was also showed that low-fat frankfurter produced with vegetable oils were more firm and less juicy than high fat controls, although no differences were found in overall acceptability (Muguerza *et al*., 2001). Pomegranate rind and seeds are by-products obtained during processing of pomegranate juice. Pomegranate rind, pomegranate juice, and pomegranate seeds are reported to possess significant antioxidant activity due to poly-phenolic compounds. Oxidation of lipid and auto-oxidation are one of the major causes of quality deterioration and reduced shelf life of meat products. Pork and pork products has relatively high levels of polyunsaturated fatty acids (PUFA), including the long chain (C20-22) PUFA compared to other meats (Ahmad *et al*., 2015 and Qin *et al*., 2013). Meat and meat products are very poor sources of dietary fibre and their regular consumption is being associated with various health disorders. Thus, incorporation of dietary fibre from different sources in meat products would help to enhance their nutritional composition (Das *et al*., 2020). Traditional fruits have many health benefits and especially apple pulp is a typical source of dietary fibre, but the use of apple pulp as a source of dietary fibre in meat products is yet to be reported (Verma *et al*., 2010). The present study was undertaken to develop low fat and high fibre functional pork sausage through replacement of pork fat with olive oil, apple pulp and pomegranate seed powder inclusion in the low-fat pork sausage and observe their effect on the proximate composition, microbiology and cost of production.

Materials and Methods

Preparation of Meat Products

Pork meat was purchased from the freshly slaughtered Yorkshire pig carcasses of about 10 months to 1 year of age from the Aizawl market. The meat cuts were brought into laboratory in polyethylene bags and were trimmed off the external fat. The deboning of the cuts was carried out and back fat and other fats were separated from the lean meat. Meat was cut into uniform size in order to mince it. Natural casings were used for product making and casings were prepared in the laboratory from goat intestine. Edible olive oil was purchased from Aizawl market. Apple and pomegranate were purchased from the local market. Apple were washed properly, cut into thin slices and then dried in hot air oven at 70°C for overnight. Likewise, pomegranate seeds were taken out and dried overnight in hot air

oven at 70°C. Both the dried products were ground properly in a mixer grinder, sieved and kept in air tight containers for further use. External coverings of the onion and garlic were peeled off, weighed and taken in the ratio of 3:1. They were cut into smaller bits and blended into a fine paste and were used in the formulations. The spice-mix formula suggested by Hazarika (2005) was followed (Table 1). Spices were oven-dried at 50°C for 3 hrs. and were ground in a grinder and sieved through a fine mesh. The fine powder was weighed and taken into the required ratio for the preparation of the spice mixture, which was store in airtight container for further use.

Table 1: Composition of spice mixture

Spice ingredients	Percent of mixture
Anise seed (Soanf)	10
Black pepper (Kali mirch)	5
Capsicum (Mirch)	10
Caraway (Ajwain)	10
Cardamom (Elaichi)	4
Cinnamon (Dalchini)	4
Cloves (Laung)	2
Corriander (Dhania)	15
Cumin (Zeera)	20
Dry ginger (Sont)	10
Turmeric (Haldi)	10
Total	100

Traditional and low-fat pork sausages were prepared by using ingredients as presented in Table 2. Meat chunks and fat was minced in meat mincer. During mincing/chopping, the temperature was maintained around refrigeration temperature. Pork Fat, olive oil, dried apple pulp powder, dried pomegranate seed powder was added at various levels for different treatments during chopping of meat in bowl chopper along with other ingredients. The batter was transferred to stuffer for filling into natural casing; the encased mass was twisted and manually drawn together to form links and to form cylindrical loops. Sausages were cooked at 80°C for 20 minutes in water and smoked in an artificial smoking unit (Kerres Showsmoker CS 350 EL) for 20 minutes.

Table 2: Ingredients % for the preparation of control and treatment pork sausages

Ingredients	T1	T2	T3	T4
Pork lean meat	80%	80%	80%	80%
Pork Fat	10%	0	10%	0
Olive oil	0	6%	0	6%
Dried apple pulp powder	0	2%	0	2%
Dried pomegranate Seed powder	0	2%	0	2%
Condiments (Onion and Garlic, 3:1)	2.50%	2.50%	2.50%	2.50%
Dried spice mix	1%	1%	1%	1%
Common Salt	1.50%	1.50%	1.50%	1.50%
Ice water	5%	5%	5%	5%

Four treatments were prepared, namely, T1: Smoked pork sausage incorporated with 10% pork fat. Sausage batter was manually mixed. T2: Smoked pork sausage incorporated with 6% olive oil, 2% each of dried apple pulp powder and pomegranate seed powder. Sausage batter was manually mixed. T3: Smoked pork sausage incorporated with 10% pork fat. Sausage batter was in the form of emulsion. T4: Smoked pork sausage incorporated with 6% olive oil, dried apple pulp powder and pomegranate seed powder. Sausage batter was in the form of emulsion. The samples were aerobically packed and sealed in LDPE and kept under domestic refrigerator at 4±1˚c for 12 days and were analyzed for different physico-chemical, microbiological and sensory parameters at periodic intervals.

Physico-chemical Parameters

Proximate Composition

Proximate composition, namely, moisture, protein, fat and total ash percent of samples were determined by standard methods described by AOAC (1995).

Assay for Microbiological Quality

Total plate counts (TPC), *E. coli*, *Salmonella, Staphyloccocus aureus* and *Listeria monocytogenes* count, of the samples were enumerated following the methods as described by American Public Health Association (APHA, 2015). The average number of colonies was multiplied by the reciprocal of the dilution and expressed as $\log_{10}$cfu/g.

Statistical Analysis

Data were analyzed statistically on "SPSS-16.0" (SPSS Inc., Chicago, II USA) software package as per standard methods (Snedecor and Cochran, 1995). Duplicate samples were drawn for each parameter and the whole set of experiment was repeated three times to have n=*4* observations for all parameters. The data were statistically analyzed by two-way ANOVA at the 5 per cent level (p<0.05) and evaluated with Duncan's Multiple Range Test.

Results and Discussion

Proximate Composition

Results in Table 3 showed that the moisture content of traditional pork sausage (T1 and T3) and the low-fat pork sausage (T2 and T4) were non-significant (p>0.05) difference during the storage period, but a significant (p<0.05) difference could be observed between different treatments. The higher moisture content of low-fat pork sausage (T2 and T4) may be due to presence of apple pulp and pomegranate seed powder rich in fiber. Similar finding also recorded by Kumar & Sharma (2004) that pork patties treated with fiber rich barley flour as a result increased moisture content.

Table 3: Proximate composition of pork sausage stored at refrigeration temperature (4±1°C)

Days	Moisture				Protein			
	T1	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**
1st	48.56±0.037^{b}	51.61±0.074^{d}	47.91±0.038^{a}	50.63±0.013^{c}	24.75±0.008^{b}	23.9±0.018^{a}	24.72±0.015^{b}	23.87±0.018^{a}
12th	48.51±0.035^{b}	51.54±0.062^{d}	47.51±0.054^{a}	50.11±0.020^{c}	22.56±0.006^{c}	22.34±0.017^{b}	23.39±0.017^{d}	22.19±0.006^{a}
Days	Fat				Total ash			
	T1	T2	T3	T4	T1	T2	T3	T4
1st	24.49±0.010^{c}	22.45±0.012^{a}	25.18±0.004^{d}	23.03±0.006^{b}	2.35±0.006^{a}	2.41±0.006^{b}	2.37±0.004^{a}	2.42±0.006^{b}
12th	23.84±0.008^{c}	22.43±0.013^{a}	25.15±0.002^{d}	22.54±0.008^{b}	2.34±0.006^{a}	2.42±0.004^{c}	2.36±0.006^{b}	2.43±0.004^{c}

Mean ± S.E with different superscripts row wise (a-c) differ significantly (p<0.05), (N=6); T1: traditional pork sausage batter was manually mixed, T2: low fat pork sausage batter was manually mixed), T3: traditional sausage batter was in the form of emulsion and T4: low fat pork sausage batter was in the form of emulsion.

Protein content of low-fat pork sausage (T2 and T4) was significantly (P<0.05) lower than the traditional pork sausage (T1 and T3), but a non-significant (P>0.05) change in protein (for both traditional and low-fat pork sausage) were found during the storage period. Lower protein value of low-fat pork sausage (T2 and T4) may be due to the presence of non-meat ingredients when compared with traditional pork sausage (T1 and T3). Similar finding were reported by Huang *et al.* (2011) incorporated with wheat fiber, oat fiber, and inulin in Chinese-style sausages. Fat content of low-fat pork sausage (T2 and T4) was significantly (P<0.05) lower than the traditional pork sausage (T1 and T3), but a non-significant (P>0.05) change in fat (for both traditional and low-fat pork sausage) were found during the storage. Lower fat value of low-fat pork sausage (T2 and T4) may be due to pork fat replaced with olive oil, apple pulp and pomegranate seed powder. Similar findings also report by Thomas *et al*. (2016), where pork nuggets incorporating with kordoi (*Averrho acarambola*) fruit juice and bamboo (*Bambusa polymorpha*) shoot

extract decrease the fat content. A significantly higher (P<0.05) total ash content was observed for low fat pork sausage (T2 and T4) as compared to traditional pork sausage (T1 and T3), it can be due to the use of apple pulp and pomegranate seed powder for preparation of pork sausage, but a non-significant (P>0.05) change in total ash (for both traditional and low-fat pork sausage) were found during the storage. The findings of the present study that is increased total ash content is similar with the findings of Yadav *et al.* (2018) chicken sausages incorporated with wheat bran and dried carrot pomace resulted in the increase of total ash content.

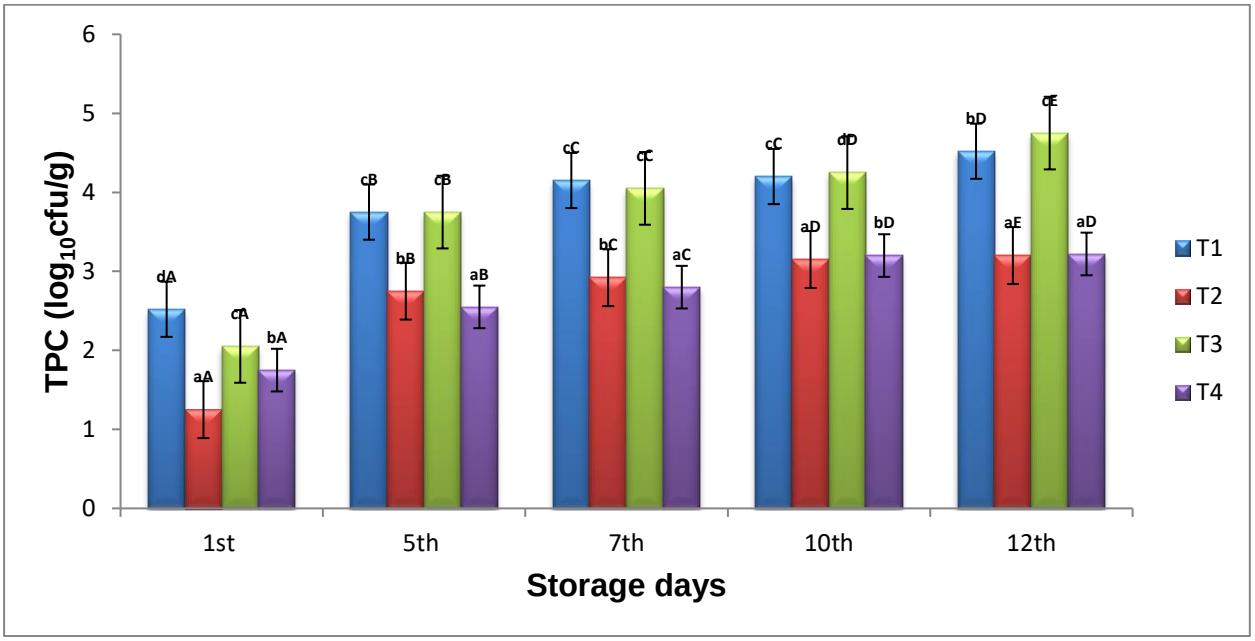
Microbiological Quality

TPC (Table 4 and Figure 1) was significantly (p<0.05) lower in T2 and T4 as compared to T1 and T3; this can be attributed to manifold inhibition of microbial cells due to the antimicrobial effect of apple pulp and pomegranate seed powder, cooking and smoking. Shahamirian *et al*. (2019) also reported that pomegranate rind powder extract and pomegranate juice has inhibitory effect on common spoilage micro-organisms in burgers. Shanmugam *et al*. (2017) also found same in mutton patties added apple and strawberries. TPC was lower than the threshold limit (5.33 $\log_{10}$cfu/g) in the treated product in all the treatments throughout storage period (Frazier and Westhoff, 2008). It was evident that smoking caused a decrease in the number of the viable aerobic organisms in all the treatments on storage. There was a gradual increase in the mean TPC in all the treatments throughout the storage period. Statistical analysis revealed highly significant (p<0.05) differences amongst the treatment throughout storage period. The counts of *E. coli* organisms were nil during the storage periods in all the treatments. Similar findings were reported by Shahamirian *et al*. (2019) pomegranate rind powder extract and pomegranate juice added in burgers (Shanmugam *et al*., 2017), apple and strawberries added in mutton patties. It could be due to destruction of this organism during cooking at 82°C and smoking at 75°C, above their thermal point of 57°C (Bhat & Pathak, 2012; Singh *et al*., 2015). *Salmonella* was not detected in all the treatments throughout the storage period. Ahmad and Amer, (2013) reported the absence of *Salmonella* in all the semi-dry fermented buffalo sausages at all stages during refrigerated storage (20°C) for 120 days. Gullon *et al*. (2016) also reported that pomegranate peel flour had strong bactericidal action against *Salmonella sp*. *Staphylococcus aureus* were found on 1st day and 12th day in treatment T1 and T3, but absent in T2 and T4. Meat samples smoked and packaged could not demonstrate any inhibitory effect and that such sample were found to be positive for *Staphylococcus aureus* organism even up to 12th d storage under refrigeration temperature. Apple and pomegranate act against *S. aureus* (Shanmugam *et al*., 2017 and Shahamirian *et al*., 2019). Gullon *et al*., (2016) also found that bactericidal action against *S. aureus* in pomegranate peel flour in case of T2 and T4. *Listeria monocyte genes* were not detected in all the samples on 1st and 12th d of storage which might be due to inhibitory effect of apple pulp and pomegranate seed powder. Similar findings were also reported by Shahamirian *et al*. (2019) pomegranate rind powder extract and pomegranate juice added in burgers Shanmugam *et al*. (2017) apple and strawberries added in mutton patties.

Table 4: Changes in TPC values ($\log_{10}$cfu/g) of pork sausage during refrigerated storage (4±1°C)

	Period of storage (d)				
Treatments	**1st**	**5th**	**7th**	**10th**	**12th**
T1	2.52±0.047^{dA}	3.75±0.004^{cB}	4.15±0.004^{cC}	4.20±0.006^{cC}	4.52±0.047^{bD}
T2	1.25±0.006^{aA}	2.75±0.006^{bB}	2.92±0.025^{bC}	3.15±0.006^{aD}	3.20±0.004^{aE}
T3	2.05±0.050^{cA}	3.75±0.006^{cB}	4.05±0.064^{cC}	4.25±0.006^{dD}	4.75±0.004^{cE}
T4	1.75±0.006^{bA}	2.55±0.064^{aB}	2.80±0.006^{aC}	3.20±0.006^{bD}	3.22±0.002^{aD}

Mean ± S.E with different superscripts row wise (a-d) and column wise (A-D) differ significantly (p<0.05), (N=6); T1: traditional pork sausage batter was manually mixed, T2: low fat pork sausage batter was manually mixed), T3: traditional sausage batter was in the form of emulsion and T4: low fat pork sausage batter was in the form of emulsion.



Mean ± S.E, with different superscripts row wise (a-d) and column wise (A-D) differ significantly (p<0.05), (N=6)

Figure 1: Changes in TPC values (log_{10}cfu/g) of pork sausage during refrigerated storage (4±1°C)

Production Cost of Low-Fat vs Traditional Pork Sausage

The comparative cost for formulation of 1 kg traditional pork sausage (T1 and T3) and low-fat pork sausage (T2 and T4) is presented in Table 5. It includes the cost of raw materials required for preparation of low fat and traditional pork sausage, which includes cost of pork meat, pork fat for traditional pork sausage and olive oil, dried apple pulp and pomegranate seed powder for low fat pork sausage. Other charge like casings, spice mix, condiments, salt, ice water, electricity, packaging and sawdust was similar for both low fat and traditional pork sausage. The retail prices for these ingredients are relatively stable in our marketing system. The production cost of traditional and low-fat pork sausage was calculated as ` 549/kg and ` 543/kg.

Table 5: Cost of production of traditional and low-fat pork sausage (for 1 kg of product)

Commodities	Traditional pork sausage	Low fat pork sausage
Pork lean meat 800 g	` 400.00	` 400.00
Pork fat 100 g	` 90.00	-
Edible olive oil 60 g	-	` 40.00
Dried apple pulp powder 20 g	-	` 20.00
Dried pomegranate seed powder 20 g	-	` 24.00
Condiments (onion and garlic; 3:1) 25 g	` 4.00	` 4.00
Spice mix 20 g	` 6.00	` 6.00
Salt 15 g	` 1.00	` 1.00
Ice water 50 g	` 1.00	` 1.00
Casing	` 20.00	` 20.00
Packaging	` 2.00	` 2.00
Sawdust	` 5.00	` 5.00
Electricity	` 20.00	` 20.00
Total cost per kg	**` 549.00**	**` 543.00**

Conclusion

Low fat pork sausage is considered relatively superior to traditional pork sausage due to its better proximate composition and safer microbiological qualities. So, it was initially thought that the cost of low-fat pork sausage will be higher but after this study, the end product found was much economical compared to present marketed product with better food value. Considering the above beneficial aspects, low fat pork sausage would provide better

scope and wider opportunities for marketing, besides commanding higher market price in comparison to traditional pork sausage, as the consumers of the present-day society are more quality-conscious and ready to pay more for better products. Besides, upgradation of production technology of traditional meat products is the need of the hour, as these products suit to the local people's taste and flavour.

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Conflict of Interests

There is no conflict of interest.

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