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A study on pork sausage incorporated with olive oil, dried apple pulp powder and pomegranate seed powder

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Abstract
The purpose of this study is to evaluate the effect of olive oil, dried apple pulp powder and pomegranate seed power in traditional pork sausage in refrigeration storage. Pork sausage were prepared, namely, T1 (traditional pork sausage batter was manually mixed) and T2 (low fat pork sausage batter was manually mixed). Pork sausage is 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 and pomegranate seed powder. The products were compared on the basis of physicochemical and sensory qualities at different interval under refrigerated (4±1 °C) conditions. Among the treatments, pH was recorded lower for T2 compared to T1 treatment with the advancement of the storage periods. The mean tyrosine value and thio-barbituric acid number of all the treatment showed increasing trend with the advancement of the storage period. The sensory evaluation revealed highest scores for colour and appearance, flavour, texture, and overall acceptability for T2 than T1 but juiciness score was more in T1. The manuscript has stated that adding of dried apple pulp powder and pomegranate seed powder improved all the attributes (physicochemical and sensory) and also ensured a longer shelf-life of the product.

Keywords: Low fat, pork sausage, shelf–life, apple pulp, pomegranate seed

Introduction
Ready to eat value added meat products are capturing the market due to the convenience as well as day-to-day fast lifestyle of today's world. At the same time consumers are equally aware of the food quality and safety related to human health. Hence, the meat industry is in continuous process of developing better meat products enriched with fibre and anti-oxidants along with a better shelf-life of the products. As we know, meat is zero fibre product, adding of fibres obviously add on to the quality of meat products.

The process of preserving meats by stuffing salted, chopped meats flavoured with spices into animal casings dates back to thousands of years, to the ancient Greeks and Romans, and earlier. Sausage is considered to be one of the oldest processed meat products. The word "sausage" is originated from the Latin word, "*salsus*", which means preserved or salted [19]. Pork sausage is a fresh, all pork product, and grayish-pink to grayish light red in colour. The meat is chopped or ground to a moderately coarse texture, and thoroughly blended and mixed with spices and salt [12]. Pork is a nutritious as well as a major animal originated food products that is rich in thiamin, phosphorus etc. Pork may be considered as healthy diet in moderate quantity. Proteins present in pork may contribute to a feeling of fullness, thus helping in prevention of over eating and pork is also devoid of trans fatty acids which are associated with high blood cholesterol levels. Convenient, value added and low fat meat product demand have increased resulting in the development of variety of fat substitutes for meat products. Objectives of fat substitutes are to contribute minimum calories without affecting palatability and processing characteristics [16]. Fat substitutes used in meat products are recognized as leaner meats, added water, protein based substitutes, carbohydrate based substitutes and synthetic components. Several studies have been conducted to evaluate the substitution of fat with water [5], carbohydrates [30], non-meat proteins [25] and formulated compounds [9]. Fruits and vegetables are generally recognized as important sources for a wide array of non-digestible components and phytochemicals, which act synergistically to contribute to the nutritional and health benefits if mixed with other food commodities [24].

Addition of dietary fibre from different sources in meat products would help to enhance their nutritional composition and desirability as well. Many traditional fruits have been recognized for many health benefits and especially apple pulp which is a rich source of dietary fibre [32]. Apples are rich sources of antioxidants. These compounds protect the body from losses suffered from free radical [1]. By-products from fruit processing plant can offer a practical and economic way of properly utilizing natural anti-oxidants that may replace synthetic anti-oxidants [7]. It further helps in production of economically sustainable product by reducing the cost of production. Adding olive oil could dramatically increase the percentage of MUFA, decreasing amounts of PUFA and saturated fatty acid SFA, increased tenderness, juiciness and cured red colour in final fat replaced meat products [11] and [20].

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 local market (Sihphir, Aizawl). 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 [10] 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 (Soant)	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

Control and treatment 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. Lean meat, 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	Treatment-1	Treatment-2
Pork lean meat	80%	80%
Pork Fat	10%	0
Olive oil	0	6%
Dried apple pulp powder	0	2%
Dried pomegranate Seed powder	0	2%
Condiments (Onion and Garlic, 3:1)	2.5%	2.5%
Dried spice mix	1%	1%
Common Salt	1.5%	1.5%
Ice water	5%	5%

Two treatments were prepared, namely, Treatment-1: Smoked pork sausage incorporated with 10% pork fat. Sausage batter was manually mixed. Treatment-2: Smoked pork sausage incorporated with 6% olive oil, 2% each of dried apple pulp powder and pomegranate seed powder. Sausage batter was manually mixed. 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 and sensory parameters at periodic intervals.

Physico-chemical parameters

pH. The pH of sample was determined using a pH Meter (Cyberscan 1,000 Euteoh instruments) and by following the methods as described by [3].

Tyrosine value and thiobarbituric acid number. Tyrosine value (TV) and thiobarbituric acid number (TBA No.) were determined by methods described by [28] and [34] in milligram tyrosine/100 g and milligram malonaldehyde/kg, respectively.

Organoleptic evaluation

Sensory evaluation. Standard sensory evaluation method using an 8 point descriptive scale [15] was followed with slight modification (where 8 stands for excellent; 1 for extremely poor). Pork sausages were preheated in microwave and then served to the panelists along with a glass of water to rinse their mouth on tasting the product samples. The sensory evaluation room was conducted in air-conditioned at 20±2°C, with R.H 45-55% and shadow-free illumination at 70-100 foot candles. The sensory evaluation was conducted late afternoon [21]. During evaluation, there was strict prohibition in interaction among panelists. The test samples were presented to the panelists after assigning the suitable codes.

Statistical analysis: The data collected from these experiments were analysed statistically as per method outlined by [27] using the SPSS version 20. Firstly, to normalize the data arcsine transformations have been used for proportional data. General Linear Model of two way ANOVA based on Fisher's Least Significant Difference method was

used to check the significant difference. The significant values in the ANOVA were further tested through Duncan multiple range test. Results are depicted as Mean±S.E and when $p< 0.05$, $p< 0.01$ and p=0.00 then the differences were considered significant.

Results and Discussion

Physico-chemical quality. Results in Table 3 showed that the pH value of the pork sausage during different storage periods under different treatment groups were significantly ($p<0.05$) higher in T1 than T2 during the storage period. It might be due to the use of apple pulp and pomegranate seed powder that inhibit the growth of the microbes more in T2 than T1. There was a significantly ($p<0.05$) increasing trend for T1, decreasing trend for T2, irrespective of the used of apple pulp and pomegranate seed powder. The increase in the pH of T1 on progressive storage period was in agreement with the findings of [14] who reported that an increase in pH is directly related to the degree of spoilage of the meat products. This might be due to protein breakdown, this leading to the production of free amino acids. As a result, formation of NH3 and amines takes place. Both of the compounds being alkaline in nature, results in increased pH values of meat products. The pH was lowest in T2, whereas more in T1 on the 12th day storage. Malic acid in apples and punicic acid, ellagic acid in pomegranate seed which significantly reduce the pH of T2 [17] and [8]. The variation in pH with ingredients might have been occurred due to multiple factors like formation of bacterial metabolites, deamination of proteins, growth of facultative anaerobes, lactic acid bacteria, antimicrobial substances [13].

The TV of pork sausage followed an increasing trend throughout the storage period irrespective of treatments. The TV was significantly ($p<0.05$) higher in T1 than T2 throughout the storage period; This might be due to increased proteolysis and denaturation of the proteins during storage period. In the smoked buffalo tripe rolls, the Tyrosine value non-significantly increased with the advancement of storage period up to 14th d of storage and significantly increased after 21st d of storage as per the report of [2]. It was seen that T1, even with similar low pork sausage (T2). This phenomenon of rising of TV also holds good for T2.

The TBA number of pork sausage increased gradually from the initial values with the advancement of the storage periods, all the treatments. The results of lipid oxidation evaluation were expressed by the TBA number under different treatment groups at different stages of storage periods. In the present study, even though there was an increase in the TBA number during storage, they were well within the threshold limit of 1-2 mg malonaldehyde/kg meat [33]. TBA numbers were significantly ($p<0.05$) higher in T1 than T2 throughout the storage period. [23] also reported that the high oxygen atmosphere packaging is connected with the increased TBA numbers during the advanced storage period and leads to the disruption of the integrity of muscle membranes, facilitates the interactions of pro-oxidants with unsaturated fatty acids and results in the propagation of oxidative reactions leading to the production of free radicals [18]. In the meat packaging system under aerobic condition, the lipid oxidation occur by several ways but the principally might be due to myoglobin, that is protein hemes, or other simple forms of Fe reacting as pro-oxidants as reported by [4]. On day 12th day storage, highest concentration of TBA number was recorded in T1 followed by T2. The increase in the TBA number during storage might be attributed to oxidation of meat lipid during the storage periods of respective treatments at refrigeration temperature. These results were in agreement with the findings of [7] in cooked goat meat patties and [22] in cooked chicken patties.

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

Treatments/days	1st	5th	7th	10th	12th
pH					
T1	5.56±0.006^{cA}	5.66±0.004^{cC}	5.68±0.004^{cD}	5.71±0.006^{cE}	5.63±0.008^{dB}
T2	5.28±0.008^{aA}	5.27±0.008^{aA}	5.26±0.010^{aA}	5.25±0.010^{aA}	5.27±0.010^{bA}
TV(mg tyrosine/100g)					
T1	3.25±0.006^{bA}	3.25±0.006^{aA}	3.42±0.004^{aB}	3.52±0.006^{aC}	4.35±0.002^{dD}
T2	3.22±0.006^{aA}	3.80±0.008^{cB}	3.82±0.006^{cC}	3.91±0.008^{cD}	4.15±0.006^{bE}
TBA (mg malonaldehyde/kg)					
T1	0.20±0.006^{bA}	0.20±0.006^{cA}	0.26±0.006^{cB}	0.37±0.006^{dC}	0.48±0.006^{dD}
T2	0.17±0.006^{aA}	0.18±0.006^{abA}	0.22±0.006^{bB}	0.25±0.004^{bC}	0.28±0.002^{bD}

Notes: *Mean ± S.E with different superscripts row wise (a-c) and column wise (A-D) differ significantly ($p<0.05$), ($n=4$); T1: traditional pork sausage batter was manually mixed, T2: low fat pork sausage batter was manually mixed). TV: Tyrosine value; TBA: Thiobarbituric acid number

Organoleptic evaluation

Sensory evaluation. Sensory panelists awarded higher ($p<0.05$) appearance scores to low fat pork sausage (T2) than traditional pork sausage (T1). Low fat pork sausage (T2) had attractive pinkish tinge colour, higher moisture content retention capacity. Apple pulp and pomegranate seed provided mild sweet fruity flavor, sour taste and musty/earthy and fruity odour. Overall aroma of pomegranate fruit was derived from a mixture of alcohols, aldehydes, terpenes and hexanal derivates and terpenes altogether provide green, woody, earthy, fruity, floral, sweet and musty flavor [26] and [31] as compared to traditional pork sausage (T1) showed in Table 4.

Table 4: Sensory quality of pork sausage stored at refrigeration temperature (4±1°C)

Treatments/days	1st	5th	7th	10th	12th
Colour and appearance					
T1	6.73±0.006^{aA}	6.70±0.006^{aAB}	6.68±0.005^{abAB}	6.65±0.006^{abB}	6.62±0.006^{abC}
T2	7.08±0.006^{bA}	6.91±0.006^{bB}	6.81±0.006^{cAB}	6.75±0.006^{abD}	6.70±0.004^{abC}
Flavour					
T1	6.30±0.006^{aA}	6.30±0.006^{aB}	6.20±0.006^{aAB}	6.02±0.011^{aAB}	5.80±0.006^{aC}

T2	6.81±0.008^{abA}	6.73±0.008^{abAB}	6.70±0.006^{abAB}	6.65±0.006^{abB}	6.60±0.006^{abC}
Texture					
T1	6.65±0.006^{abA}	6.63±0.006^{abAB}	6.66±0.006^{abB}	6.36±0.004^{aAB}	6.31±0.002^{abC}
T2	6.93±0.008^{bA}	6.88±0.004^{bB}	6.86±0.004^{bAB}	6.60±0.008^{abC}	6.50±0.009^{abD}
Juiciness					
T1	7.06±0.005^{abA}	6.80±0.008^{abB}	6.70±0.008^{bAB}	6.65±0.006^{bD}	6.60±0.008^{abC}
T2	6.96±0.008^{abA}	6.60±0.008^{bAB}	6.55±0.008^{aAB}	6.50±0.008^{aB}	6.48±0.006^{abC}
Overall acceptability					
T1	6.63±0.006^{aA}	6.63±0.006^{aB}	6.50±0.008^{abAB}	6.06±0.023^{aAB}	5.52±0.022^{aC}
T2	7.16±0.006^{abA}	7.06±0.023^{abB}	6.98±0.007^{abAB}	6.95±0.006^{abC}	6.80±0.011^{abD}

Notes: *Mean ± S.E with different superscripts row-wise (a-d) and column wise (A-D) differ significantly (p±0.05), (n=4); T1: traditional pork sausage batter was manually mixed, T2: low fat pork sausage batter was manually mixed).

Colour and appearance scores followed a decreasing trend throughout storage. Mean flavour scores were higher (p<0.05) in T2 than T1. However, mean flavour scores for all the treatments were reduced with the advancement in the storage period. It decreased significantly (p<0.05) due to increase in lipid oxidation, which can be correlated with the increase in TBA no. (Table 3). As per the report of the [6], there might be flavour deterioration in restructured pork rolls due to microbial growth and oxidative rancidity of meat products. Texture and juiciness scores followed declining trend in all the treatments during storage; this resulted in higher moisture loss occurring in low pork sausage (T1) than in traditional fat pork sausage (T2), which lead to harder texture and lower juiciness. Juiciness scores were lower in T2 compared to T1 on day 12th day of storage study. Mean texture scores were significantly higher (p<0.05) in T2 than T1, but mean juiciness were significantly higher (p<0.05) in T1 than T2. Decrease in texture and juiciness scores in meat products at end of storage might be due to moisture loss as reported by [29] in pork nuggets treated with bamboo shoot extract and it is also agreement with the results of proximate composition. The overall acceptability of all the treatments decreased with the advancement of storage period. Overall acceptability scores were also significantly (p<0.05) higher in T2 than T1. Reduction in overall acceptability scores with an increase in storage days was reported by [32] in low fat chicken nuggets and by [2] in smoked buffalo tripe rolls.

Conclusion

Low fat pork sausage was found to be relatively much superior in terms of physicochemical and sensory properties with a commendable shelf-life. 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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