Waste utilization of apple pomace as a source of functional ingredient in buffalo meat sausage

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Abstract: Apple pomace was analyzed for its proximate analysis which showed protein content of 4.50% and total dietary fiber of 62.67%. Its functional properties were also carried out which showed high water- and oil-holding capacity of 3.89 gH₂O g⁻¹ Solid and 1.92 ml g⁻¹, respectively. Its antimicrobial activity was also found against S. aureus, P. aeruginosa, and L. monocytogenes. Further, it has shown lower in vitro glucose retardation index. Buffalo meat sausages incorporated with apple pomace powder showed high cooking yield and emulsion stability of 94.46% and 74.70, respectively, as compared to control sausage. Additionally, the dietary fiber content got enhanced with the incorporation of apple pomace powder as expected.

Subjects: Food Science & Technology; Meat & Poultry; Waste & Recycling

Keywords: buffalo meat; apple pomace powder; sausages; in vitro glucose retardation index; dietary fiber

1. Introduction

Buffalo meat due to its unique flavor, low intramuscular fat, presence of essential amino acids, biological value, and iron content is getting recognition worldwide (Anjaneyulu, Lakshamanan, Sharma, & Kondiah, 1990). Buffalo meat can be processed into variety of products like sausages (Sachindra, Sakhare, Yashoda, & Narasimha Rao, 2005), patties (Suman & Sharma, 2003), and burgers (Modi, Mahendraker, Narasimha Rao, & Sachindra, 2003) which attributes its good functional properties. Due to low price of buffalo meat, there is an increasing demand in both domestic and international markets for its consumption as compared to mutton and other meat sources.

Nowadays, increased concerns about the health risks linked with the utilization of foods with low fiber content and with high fat percentage have pressurized food industry to produce healthier foods. Different fiber sources have been tried as partial fat replacer like inulin, cereal, and fruit fibers (García, Domínguez, Galvez, Casas, & Selgas, 2002; Mendoza, García, Casas, & Selgas, 2001).
Apple consumption is considered to prevent various diseases like coronary disease, cerebrovascular disease, lung cancer, and obstructive pulmonary disease (Knekt et al., 2002). It has been estimated that annual waste from apple processing was 3.0–4.2 million metric tons (Oreopoulou & Tzia, 2007). It is a good source of fiber and contains cellulose, hemicelluloses, lignin, and pectin (Nawirska & Kwaśniewska, 2005). Dietary fiber slows the absorption of glucose in the intestines, lowers the cholesterol and low density lipid levels, and enhances the intestinal health (Kosmala, Kozodziejczyk, Zdunczyk, Juskiewicz, & Boros, 2011). Fiber sources are not only used to improve nutritional value but also for their functional and technological properties, for example improving cooking yield, fat binding property and improving texture (Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997). Apples processing generates skin, stems, and residual flesh which are considered as a potential value added food ingredient (Wolfe, Wu, & Liu, 2003). Enrichment of apple pomace in foods with low dietary fiber, for example, meat could be an effective way to enhance nutritional value of product. No reports have been found on the properties of buffalo sausage containing different levels of apple pomace powder.

This study describes research carried out to examine the effect of incorporation of apple pomace powder in buffalo meat sausages in relation to enhance its physicochemical and nutritional value.

2. Materials and methods

2.1. Procurement of apple pomace
Apple pomace was collected from the juice vendors of Aligarh. The pomace was washed with clean water to remove the remained juice and any dirt present. After washing, the pomace was pressed in muslin cloth to express the water from it and was dried at a temperature of 58°C for 24 h in hot air drier. The dried pomace was then grinded in a laboratory grinder (Panasonic Super Mixer Grinder, Model No. MX AC 300 S) and was sieved through No. 12 (1.68 mm). The powder was then packed air tight in polythene bags and stored at −18°C for the further use.

2.2. Procurement of buffalo meat and fat
Boneless buffalo meat and fat was purchased from the local meat shop of Aligarh. Male buffalo with approximately 2 year age was slaughtered by halal method at slaughterhouse of municipal corporation, Aligarh. Deboned meat was frozen for 24 h in a deep freezer (−18°C).

2.3. Preparation of buffalo meat sausage
Frozen meat was grinded in an electrical mincer (Sirman TC 12 E) by passing it through 6-mm plate. The control sausage was prepared by adding, sodium chloride (1.6 g), sodium nitrite (0.015 g), spice mix (1.9 g), condiments paste (3 g), ice (8 g), and minced fat (10 g) to grinded meat (75.49 g). Other sausage samples were incorporated with apple pomace at four different levels viz. 2, 4, 6, and 8% by replacing lean meat, while the remaining ingredients were used in similar concentration as in control. All the ingredients were mixed in a bowl chopper for 4–6 min to prepare a stable emulsion. The emulsion was stuffed in artificial cellulose casings by hand operated sausage filling machine and cooked in a steam cooker for 30 min. After cooking, sausages were delinked, cooled to room temperature, packaged in low-density polythene bags and stored at refrigerated temperature for further study.

2.4. Physicochemical analysis
Moisture, ash, protein, and fat contents were analyzed as per the standard AACC methods (2000). Total dietary fiber, insoluble dietary fiber and soluble dietary fiber were estimated as per AOAC (1997)). pH measurement was done using pH meter (EUTECH Instruments CyberScan pH 1,500), following a method described by Troutt et al. (1992). Emulsion stability of sausage (Baliga & Madaiah, 1970) and emulsifying activity and emulsion stability of apple pomace powder was determined using method described by Chau and Huang (2003), water-holding capacity (Robetafika, Bchir, Blecker, & Richel, 2014), oil-holding capacity (Femenia, Lefebvre, Thebaudin, Robertson, & Bourgeois, 1997), swelling power, and solubility index of apple pomace (Abirami, Nagarani, & Siddhuraju, 2014).
Cooking yield of sausage was recorded before and after cooking and is expressed as percentage of weight of cooked product divided by weight of raw product (Gadekar, Sharma, Shinde, Verma, & Mendiratta, 2014). Shrinkage in diameter of sausage was measured as decrease in diameter after cooking. Water activity was measured with a AQUA LAB (Dew Point Water Activity Meter 4TE, USA). Sensory analysis of sausage was done using nine-point hedonic scale as described by Ranganna (2008).

2.5. Antimicrobial activity by well diffusion method

The antimicrobial activity of prepared extracts was determined using agar well diffusion method against S. aureus, L. monocytogenes, P. aeruginosa, and B. subtilis (Bayanova et al., 2005). Nutrient agar plate was prepared and 100 μL of overnight grown culture was spread over the media, wells were bored and sealed with soft agar. 100, 200, 300, and 400 μL of prepared extracts were added to respective wells. The antimicrobials present in the extracts were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms by incubating the plates overnight at 37°C. The resulting diameter of zone of inhibition was measured in millimeters using zone measuring scale.

2.6. Swelling power and solubility index of apple pomace powder

Swelling powder and solubility index was done as given by Ikegwu, Okechukwu, and Ekumankana (2010). A total of 1 g of sample was taken in a test tube and weighed (W₁). Distilled water 50 cm³ was added to it and was mixed. The slurry was heated at 85°C for 30 min in water bath. After cooling, the sample was centrifuged at 2,200 rpm for 15 min. The supernatant was collected in a dish and 5 ml of it was poured on tarred evaporating dish (A₁) and was dried at 100°C for 4 h and weighed again (A₂). Then, the weight of sediment was taken as (W₂).

\[
\text{Swelling power of sample(\%) = } \frac{W_2 - W_1}{\text{Weight of sample} \times 100}
\]

\[
\text{Solubility index(\%) = } \frac{A_2 - B_1}{\text{Weight of sample} \times 100}
\]

2.7. Determination of glucose diffusion retardation index of apple pomace powder

Glucose diffusion retardation index was carried out according to a method described by Chau, Huang, and Lee (2003) with slight modifications. Apple pomace powder (0.25 g) was mixed with 12.5 mL of glucose solution (50 mmol L⁻¹) in a dialysis membrane with a cut-off molecular weight of 12,000. The mixture solution was dialyzed against 100 mL of distilled water. After 20, 30, 60, and 120 min, the glucose content in the dialysate was measured using the glucose assay kit (BeneSphera Glucose reagent) to determine the glucose diffusion rate. A control test was carried out without the addition of apple pomace powder. It is calculated as below:

\[
\text{Glucose dialysis retardation index} = 100 - \left[\frac{(\text{glucose content with the addition of fiber})}{(\text{glucose content of control})}\right] \times 100.
\]

2.8. Glucose adsorption capacity of apple pomace powder

Glucose adsorption of apple pomace powder was measured as per the method described by Ou, Kwok, Li, and Fu (2001), in which 1 g of sample was mixed with 100 mL of glucose solution (10–200 mmol L⁻¹) and kept for 6 h. The whole mixture was centrifugated at 3,500 g for 15 min. The final glucose content in the decanting solution was determined to estimate the glucose adsorption capacity (millimoles per gram) of the fibers.

2.9. Instrumental texture analysis of sausage

Texture profile analysis was done according a procedure given by Bourne (1978). Samples with 50 mm diameter and 15 mm height were taken for texture analysis using TAHD Plus Texture Analyser (Stable Micro Systems, England). Samples were compressed to 50% of their original weight at a time.
interval of 5 s between two compression cycles. A 50-kg load cell was used at a speed of 2 mm s⁻¹ which gives the force time deformation curves.

2.10. Color analysis
Instrumental color was measured as L* (lightness), a* (redness), and b* (yellowness) using a MiniScan XE Plus. The instrument was calibrated with black and white standard plates.

2.11. Differential scanning calorimetry of sausage
DSC of fresh sausage was done according to Morin, Temelli, and McMullen (2004) with a slight modification. A DSC 200 F3, NETZSCH, Germany was used. A sausage sample of 10–20 mg were taken in hermetically sealed aluminum pan and scanned from 30 to 100°C at a rate of 5°C min⁻¹. An empty aluminum pan was used as a reference. The endothermic peak was determined from DSC curve.

2.12. Statistical analysis
All the experiments were done in triplicates and their mean and standard deviation were taken with the help of Microsoft Excel 7. One-way ANOVA was also used to compare control sausage with apple pomace powder incorporated sausage by using SPSS 16.0.

3. Result and discussion

3.1. Proximate analysis
Table 1 shows composition of apple pomace powder used in the study. As illustrated, apple pomace has moisture content (10.04%), protein (4.50%), ash content (1.59%), and fat content (2.14%). Chemical composition of the dietary fiber of apple pomace was 62.67% total dietary fiber, 8.55% soluble dietary fiber and 54.12% insoluble dietary fiber.

| Components          | Percentage       |
|---------------------|------------------|
| Moisture            | 10.04 ± 0.52     |
| Fat                 | 2.14 ± 0.35      |
| Protein             | 4.50 ± 0.81      |
| Ash                 | 1.59 ± 0.11      |
| Total dietary fiber | 62.67 ± 0.54     |
| Insoluble dietary fiber | 54.12 ± 0.48   |
| Soluble dietary fiber | 8.55 ± 0.55     |

| Parameter                      | Value           |
|--------------------------------|-----------------|
| pH                             | 4.2 ± 0.02      |
| WHC (gH₂O/g Solid)             | 3.89 ± 0.83     |
| OHC (ml g⁻¹)                   | 1.92 ± 0.10     |
| Solubility index (%)           | 1.83 ± 0.63     |
| Swelling capacity (%)          | 909.17 ± 0.72   |
| Glucose absorption capacity (mmol L⁻¹) | 0.23 ± 0.14    |
| Emulsion activity (%)          | 47.22 ± 0.32    |
| Emulsion stability (%)         | 94.44 ± 0.5     |
| Color (L*)                     | 48.25 ± 1.27    |
| Color (a*)                     | 9.85 ± 0.18     |
| Color (b*)                     | 24.33 ± 0.79    |
3.2. Physicochemical properties of apple pomace powder

Table 2 shows the pH, water-holding capacity, oil-holding capacity, solubility index, swelling capacity, glucose absorption capacity, emulsion activity, emulsion stability, and color of apple pomace powder. The result suggested that the apple pomace powder is able to bind more water and oil than its weight. Water-binding capacity has been widely studied in food functionality, due to its importance in foods. Water plays an important role in the major changes that occur during cooking, which include protein denaturation, enzyme inactivation, flavor and color formation (Pomeranz, 1985). Swelling capacity of apple pomace powder was high which indicates its water absorption capacity during heating while as solubility of apple pomace powder was lower. Glucose absorption capacity of apple pomace powder was 0.23 mmol L⁻¹ which shows its ability to absorb glucose even at low glucose concentration. This could help in lowering postprandial serum glucose. A similar result was observed in the previously reported research reported by Chau and Huang (2003). The value of emulsion stability and emulsion activity of apple pomace was 94.44 and 47.22%, respectively. These moderate values are due to low protein content of apple pomace powder. The evidence of emulsion activity of fibrous materials is its ability to absorb biliar acids in the small intestines. Hence, helps in reducing blood cholesterol levels (Lopez et al., 1997). The L*, a*, b* values of apple pomace powder were determined for color estimation. The L* (lightness) values of the apple pomace powder were positive and higher than the b’ and a’ values.

3.3. Glucose retardation index

In vitro glucose retardation index indicates the glucose diffusion rate through a dialysis bag. It was found that the dialysis bags contained with apple pomace powder along with glucose solution diffuses significantly slowly at different time intervals as compared to the dialyses bags containing only glucose solution (Table 3). The reason may be the absorption of glucose molecules by apple pomace powder. This property of apple pomace powder may be useful for lowering the glycemic index by its incorporation in foods with high glycemic index. Same glucose retardation index was also reported in different peels and fibers (Abirami et al., 2014).

3.4. Antimicrobial activity of apple pomace powder

By using agar well diffusion technique, methanol extract of apple pomace powder was used to check the antimicrobial activity on four different pathogenic micro-organisms as shown in Table 4. It was seen that the apple pomace extract was effective in inhibiting the growth of *S. aureus*, *P. aeruginosa*, and *L. monocytogenes*, while in case of *B. subtills* it had no effect at all concentrations. The growth inhibition property of apple pomace is due to the presence of polyphenols (Agourram et al., 2013) and apple is considered as good source of polyphenols (Suárez et al., 2010).

3.5. Analysis of sausages

Preliminary experiments were done so as to determine the best combination of ingredients taken for processing of buffalo meat sausage Apple pomace used for experiments was varied as 0, 2, 4, 6, and 8% with meat. As per sensory analysis, 6% apple pomace incorporated buffalo sausage got higher sensory scores (color, flavor, texture, juiciness, and mouth coating) performed by 9-point Hedonic Scale, as compared to the other samples so this combination was selected and further analysis was carried out.

3.6. Physicochemical properties of sausages

The effects on physicochemical properties of sausages incorporated with apple pomace powder are shown in Table 5. Fat content, cooking yield, emulsion stability, and water activity are directly or
indirectly linked to the properties like oil-holding and water-holding capacity of material. From the data, it has been shown that the values of fat content, cooking yield, emulsion stability, and water activity of control sausage differ significantly as compared to sausage incorporated with apple pomace powder. This may be due to water- and oil-holding capacity of apple pomace powder as shown in Table 4. Antimicrobial activity of apple pomace powder

| Test organism       | Concentration (μl) | Zone of inhibition (mm) |
|---------------------|-------------------|-------------------------|
| S. aureus           |                   |                         |
| 100                 | 0 ± 0             |
| 200                 | 0 ± 0             |
| 300                 | 13 ± 1            |
| 400                 | 17 ± 2            |
| P. aeruginosa       |                   |                         |
| 100                 | 12 ± 1            |
| 200                 | 13 ± 1            |
| 300                 | 16 ± 1            |
| 400                 | 19 ± 2            |
| L. monocytogenes    |                   |                         |
| 100                 | 10 ± 1            |
| 200                 | 11 ± 2            |
| 300                 | 15 ± 1            |
| 400                 | 17 ± 2            |
| B. subtilis         |                   |                         |
| 100                 | 0 ± 0             |
| 200                 | 0 ± 0             |
| 300                 | 0 ± 0             |
| 400                 | 0 ± 0             |

Table 5. Effect of apple pomace powder on physicochemical properties of buffalo meat sausage

| Parameters                     | Control sausage | Sausage incorporated with 6% Apple pomace powder |
|-------------------------------|-----------------|-----------------------------------------------|
| Fat (%)                       | 5.27 ± 0.25a    | 7.51 ± 0.3b                                  |
| Cooking yield (%)             | 89.98 ± 0.99a   | 94.46 ± 1.04a                                |
| Emulsion stability (%)        | 62.69 ± 0.62a   | 74.70 ± 0.73a                                |
| Water activity (aW)           | 0.97 ± 0.01a    | 0.99 ± 0.02a                                 |
| pH                            | 6.17 ± 0.03a    | 6.03 ± 0.01a                                 |
| Total dietary fiber (%)       | 0.50 ± 0.20a    | 3.82 ± 0.04a                                 |
| Insoluble dietary fiber (%)   | 0.35 ± 0.18a    | 3.27 ± 0.03a                                 |
| Soluble dietary fiber (%)     | 0.15 ± 0.03a    | 0.55 ± 0.03a                                 |
| Shrinkage diameter (mm)       | 3.59 ± 0.08a    | 1.72 ± 0.04a                                 |
| Surface color (L*)            | 33.68 ± 0.10a   | 36.58 ± 0.03a                                |
| Surface color (a*)            | 12.28 ± 0.04a   | 13.09 ± 0.01a                                |
| Surface color (b*)            | 10.91 ± 0.04a   | 12.35 ± 0.25a                                |
| Firmness (N)                  | 15.60 ± 0.78a   | 18.74 ± 0.65a                                |
| Toughness (N Sec.)            | 176.02 ± 2.18a  | 192.5 ± 1.8a                                 |
| Hardness (N)                  | 48.91 ± 0.82a   | 64.10 ± 2.93a                                |
| Springiness (mm)              | 0.78 ± 0.02a    | 0.81 ± 0.01a                                 |
| Gumminess (N)                 | 29.92 ± 2.05a   | 31.94 ± 1.84a                                |
| Cohesiveness                  | 0.61 ± 0.05a    | 0.51 ± 0.03a                                 |
| Chewiness (Nmm)               | 30.29 ± 1.21a   | 25.98 ± 1.61a                                |

Notes: Mean ± SD with different superscripts in a row for a particular parameter differ significantly (p ≤ 0.05).
in Table 2. Hence, less fat loss, high water activity, high cooking yield, and emulsion stability of sausage was observed. A similar trend have been seen in which the addition of wheat fiber improved cooking yield due to its water- and fat-holding abilities (Choe, Kim, Lee, Kim, & Kim, 2013).

The pH values varied between 6.17 and 6.03 for control and apple pomace powder incorporated sausages. As expected, the lower pH values were observed for apple pomace powder incorporated sausages than control sausages. The decrease in pH may be due to mild sourness of apple pomace. The results are in agreement with Verma, Sharma, and Banerjee (2010) in which significant decrease in pH was observed in chicken nuggets incorporated with apple pomace.

As such meat is deficient of dietary fiber; however, buffalo meat sausage has a small amount of dietary fiber added up by the spices used. Sausages incorporated with apple pomace powder showed significantly higher values than control sausage. Further, the soluble and insoluble dietary fiber content was also higher than the control sausage. This is because of the apple pomace which is rich in dietary fiber as described by Shah and Masoodi (1994). Similar results have been found by Verma et al. (2010).

Shrinking of sausage during cooking is due to the contraction of proteins during heating. It has been seen that sausages incorporated with apple pomace powder cause significantly less shrinkage than that of control sausage. It may be due to the jelling property of dietary fiber contained in apple pomace. Controlled shrinkage was also seen in a research in which polysaccharides has been used to stabilize shrinkage (Ayadi, Kechaou, Makni, & Attia, 2009).

Buffalo meat has usually dark color, however, the surface color properties of sausages was significantly affected by the incorporation of apple pomace powder. The higher values in L*, a*, and b* of apple pomace incorporated sausages may be due to the coloring properties of apple pomace (Verma et al., 2010).

The detailed textural analysis of sausage like firmness, toughness, hardness, springiness, and gumminess significantly increased with the incorporation of apple pomace powder, while as the cohesiveness and chewiness decreased. The increase in hardness may be due to jell structure formed by the apple pomace during cooking. The high water-holding capacity of apple pomace can also be the reason for high hardness due to its turgor pressure. The increase in hardness of bologna sausage was also seen by Fernandez-Gines, Fernandez-Lopez, Sayas-Barbera, Sendra, and...
Perez-Alvarez (2003), in which citrus fiber at various concentrations made the bologna sausage harder and less chewy.

3.7. Differential scanning calorimetry

Both control and apple pomace incorporated sausages were scanned for endothermic peaks. Sausage incorporated with 6% apple pomace showed high endothermic transition enthalpy curve than that of control sausage as shown in Figure 1. The high endothermic transition enthalpy may be due to the gel type network formation by apple pomace powder with the meat proteins. The peak ranging from 34 to 39°C shows the melting of fat and from 48 to 82°C the different peaks show denaturation of different types of proteins present in meat.

4. Conclusion

From the above research, it has been concluded that apple pomace has versatile functional properties like glucose diffusion retardation index, emulsifying activity, water-oil-holding capacity, and antimicrobial activity. Further, its high dietary fiber content appeals its utilization in foods like meat which is deficient in dietary fiber. The results showed that apple pomace powder incorporated in buffalo meat sausage improved its physicochemical and sensory properties. This clearly suggests that apple pomace can be used in different meat products which could be beneficial to industry as well as customers in respect of getting functional foods. This practice can also eliminate the problems related to gastrointestinal tract.

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Competing interests

The authors declare no competing interest.

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