Nutritional Utilization of Pomegranate Pills Powder (Punica granatum) as Alternative Lipid in Beef Sausage and Testing Blood Lipid Parameters in Rats

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ABSTRACT

This investigation was carried out to utilize the pomegranate pills (Punica granatum) as alternative lipid to increase shelf life in beef sausage and lowering blood lipid parameters in rats. The pomegranate powder was prepared from pills to use as alternative lipid at 25, 50, 75 and 100% in beef sausage which had contained 20% sheep fat considerable as control. Chemical constituents, physical characteristics, cooking loss texture profile and organoleptic evaluation were determined in beef sausage and its formula. Biological experiment was used to measure biological evaluation and biochemical analysis. The results observed that the protein in formula did not change in different substituted sheep fat at 25, 50, 75 and 100% level, whereas, the total lipid and cholesterol content were decreased in the formula by increasing pomegranate powder. Moreover, they did not change in the ash and crude fiber for the formula at different levels. The average phenolic content was significantly higher with formula 100% of pomegranate powder. Plasticity, water holding capacity (WHC), cooking loss total volatile nitrogen (TVN) and thiobarbituric acid (TBA) were higher in control sausage than other formula. The texture properties analysis after replacing fat with pomegranate powder in sausage and their formula reported higher numbers value in formula than control. Organoleptic properties showed that low values nearly control sausage. Whereas, the sausage replacement fat with pomegranate powder at level 100% sheep fat was the lowest acceptability. Initial body weight showed non-significant increase, but body weight gain and feed efficiency ratio recorded significant increase compared to positive control, food intake found significant decrease compared to positive control hypercholesterolemia rats. Biochemical analysis illustrated that the alternative fat in the beef sausage formula had lowered the lipid parameter and fasting blood sugar in hypercholesterolemia rats. From the obvious results, it could be recommended that the replacement of fat with pomegranate powder was acceptable till 75% cause pomegranate powder is a good source for many nutrients such as protein and natural antioxidants.

Key words: pomegranate pills, sausage, phenolic content, organoleptic properties, blood lipid parameters

INTRODUCTION

In recent years, health concerns about fat consumption and changes in consumer’s preferences have led to extensive research on low-fat foods (1-9). Meat and meat products are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients. Microbial growth and oxidative rancidity are the major problems causing shelf life quality deterioration; therefore, preservation technologies must be applied in order to preserve its safety and quality (2, 10-14). Irradiation is known to be the best method for the control of both spoilage and potentially pathogenic microorganisms in meat without affecting its physical state (3, 15-17). Also, synthetic additives can reduce food spoilage, but consumers are concerned about chemical residues in food (4, 5, 18-21).
Meat products could be spoiled by two major causes: microbial growth and chemical deterioration. The most common form of chemical deterioration is oxidative rancidity (6, 22-24). The alterations of oxidative rancidity occurred in meat and meat products can vary greatly, ranging from extensive flavor changes, color losses and structural damage on proteins to a more subtle “loss of freshness” that discourages repeat purchases by consumers (7, 25-29).

Oxidation of meat lipids is a complex process and its dynamics depend on numerous factors, including chemical composition of meat, light and oxygen access and storage temperature6. The rate of the oxidation process is also affected by some technological procedures to which meat is subjected during its processing (8, 30-34). Because lipid oxidation leads to the formation of numerous other compounds which have adverse effects on the quality attributes and nutritive value of meat products (9, 35-41), this process frequently limits the shelf-life of processed meat.

Lipid oxidation can have negative effects on the quality of meat and meat products causing changes in sensory attributes (color, texture, odor, and flavor) and nutritional quality (9, 10, 42-45). One method to reduce lipid oxidation is the application of antioxidants. Antioxidants are the chemical substances that reduce or prevent oxidation and have ability to counteract damaging effects arteriosclerosis, heart disease and several other diseases of free radicals in tissues and thus are believed to protect against cancer (11, 46-49).

Pomegranate is one of the important and oldest edible fruits of tropical and subtropical regions, which originated in the Middle East. The plant is also cultivated in Iran, USA, Turkey, Egypt, Italy, India, Chile and Spain. The world pomegranate production amounts to approximately 1, 500,000 tons (12, 50-53).

Pomegranate is an important source of bioactive compounds and has been used in folk medicine for many centuries. Most pomegranate fruit parts are known to possess enormous antioxidant activity. In India, pomegranate pills are used and processed into juice. Pomegranate juice has been demonstrated to have high antioxidant activity and is effective in prevention of atherosclerosis, low-density lipoprotein oxidation, prostate cancer, platelet aggregation and various cardiovascular diseases (13, 54-58). The pomegranate juice or rind powder extract at a level of 10 mg equivalent phenolics/100 g meat would be sufficient to protect chicken patties against oxidative rancidity for periods longer than the most commonly used synthetic antioxidant like BHT (14, 59-63).

The aim of this study was to evaluate the effect of pomegranate aril powder as alternative fat in beef sausage and effect of natural antioxidant on serum total lipids parameters and blood sugar in hypercholesterolemia in rats.

MATERIALS AND METHODS

Materials:

Fresh pomegranate fruit and imported Brazil beef (frozen) and fat tissues (sheep tallow) were purchased from the private sector shop in the local market at Saudi Arabia. Cholesterol standard was purchased from Sigma Chemical Co., St. Louis, MO.

Kits of glucose and lipid parameters were obtained from Bicon Diagnosemittel GmbH and Co. KG Hecke 8 made in Germany.

Methods:

Preparation of pomegranate powder:

Mature pomegranate fruits were washed and cut manually to separate the rind and pills. Powder from pomegranate aril was prepared by drying in an air circulatory tray drier (WT- bimder Tuttlingen / Germany) at 60 °C for 48 h. The dried pomegranate pills were cooled and powdered in a kitchen grinder and sieved using a 60 mesh sieve, and packed into high density polyethylene bags and stored at – 4 °C in refrigerator until used.

Four formula beef sausage were manufactured in this study. The sausage control formula was prepared according to Rocco et al. (15, 64). The first, second, third and fourth formula had alternative fat at levels 25, 50, 75 and 100%
from aril powder as a natural antioxidant to increase shelf life and compared with beef sausage had contained 20% fat considerable as control.

**Chemical analysis of sausage formula:**

Chemical compositions (moisture, crude protein, total fat and total ash) were determined in fat replacers of the beef meat sausage and different formula according to AOAC (16, 65). Carbohydrate contents were calculated by difference.

Cholesterol content was determined in sausage and their formula by the procedure described with 17 by the following saponification with 8 ml of 15% KOH (in 90% ethanol) and 2 ml of 15% pirogallol for 20 min, in a shaking water bath at 80°C. When cooled, 5 ml of distilled water were added and the unsaponifiable material was extracted twice with hexane. A cholesterol standard curve was constructed using purified cholesterol for measuring the absorbance (17, 66-68).

The total amount of phenols was determined by using Folin-Ciocalteu’s phenol reagent and spectrophotometric determination. Samples (200 mL, three replicates) were mixed with 1.0 mL Folin-Ciocalteu’s phenol reagent (diluted 1:10 with water) and 0.8 mL of a 7.5% (w/v) sodium carbonate solution was added. The reaction mixture was stored for 30 min. at room temperature before measuring the absorbance at 765 nm on a Cintra 40, UV-Visible spectrophotometer (GBC scientific equipment, USA) according to Escarpa and Gonzalez (18, 69).

**Physical properties of sausage and their formula:**

Water Holding Capacity (WHC) and plasticity in the sausage and their formula were measured according to the filter press method of Soloviev (19, 70). The cooking loss of sausage and their formula were determined as the method described by AMSA (20, 70). Thiobarbituric acid (TBA) and total volatile nitrogen (TVN) were determined in the beef meat sausage and different formula according to the method described by Egan et al. (21) and Winton and Winton (22) respectively.

**Texture profile analysis**

The texture profile analysis (TPA) indices of the beef meat sausage and different formula were determined using a texture analyzer (Cometech, B type, Taiwan). The conditions of texture analyzer were provided with software, 35 mm diameter compression disc was used. Two cycles were applied at a constant crosshead velocity of 1 mm/s, to 30% of sample depth and then returned. From the resulting force-time curve the values for texture attributes, i.e. firmness, gumminess, chewiness, adhesiveness, cohesiveness, springiness and resilience were calculated from TPA graphic according to Bourne (23), gumminess and chewiness were determined from the following calculations: 

\[
\text{gumminess} = \text{hardness} \times \text{cohesiveness}; \\
\text{chewiness} = \text{hardness} \times \text{cohesiveness} \times \text{springiness}.
\]

**Organoleptic evaluation of different sausage treatments**

Organoleptic evaluation of the beef meat sausage and different formula were carried out according to Morr (24). Judging scale for each factor was as follows: excellent (8:9), Very good (7:<8), Good (6:<7), Fair (5:< 6), Poor (4:<5) and Rejected (<4).

**Biological experiment:**

Male albino rats Sprague Dawley strain (36 animals) weighing 170- 180 g were housed in individual cages with screen bottoms and fed on basal diet for one week. It consisted of casein 10%, corn oil 10%, cellulose 5%, salt mixture 4%, vitamin mixture 1% and corn starch 70%. The salt mixture used was that proposed by Hegested et al. (25). The vitamin mixture was that of Campbell (26).

After feeding on basal diet for eight days, rats were divided into two groups. The first group (6 rats) was fed on the basal diet for another four weeks and considered as negative control. The second main group (30 rats) was fasted overnight and injected with streptozootocin (dissolved in 0.1M citric acid buffer and adjusted at pH 4.5) into the leg muscle (5mg /100g body weight) to induce hypercholesterolemia rats according to Madar (27). After 48 hr. of
injection the second main group was divided into five sub groups (6 rats for each). The first one (6 rats) was continued to be fed on basal diet and considered as positive control. The second fifth subgroups (6 rats for each) were fed on basal diet fortified with 20% separately different sausage formula. Each rat was weighed every two days and the food consumption was calculated.

At the end of experimental period (four weeks), the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm to obtain blood serum and kept on a deep-freezer at -20°C until their analyses.

Biochemical analysis:

Serum fasting blood glucose, total lipids, total cholesterol and triglycerides were determined according to (28-31), respectively. High and low density lipoprotein- cholesterol in serum was determined according to (32, 33).

Statistical analysis:

The data obtained in the present study was analyzed by ANOVA. For all analyses, when a significant difference (p≤0.05) was detected in some variable, the data means test was applied to evaluate the difference between the samples. The results were analyzed with the aid of the software SAS System for Windows (34).

RESULTS AND DISCUSSION

Chemical compositions of sausage control and their formulae:

Chemical constituents were determined in sausage as control and their formulae made from pomegranate powder at different levels and the results are reported in Table (3). From the resultant, it could be noticed that the protein in formula did not change in different substituted sheep fat at 25, 50, 75 and 100% were 14.87, 14.94, 14.94 and 14.95%, respectively on wet weight basis compared with control sausage (14.81 %). Whereas, the total lipid was decreased in the formulae by increasing pomegranate powder were 15.23, 10.39, 5.43 and 2.31%, respectively on wet weight basis compared with control sausage 21.14%. These results indicated that the gradually lower total lipid in the sausage formulae may be caused the pomegranate powder was alternative the sheep fat in the formulae at different levels. Moreover, it did not change in the ash and crude fiber for the formulae at different levels, this may be that the pomegranate powder did not contain any fiber. Moreover, the determination of cholesterol content in sausage and their formulae showed that the control sausage was significantly higher (70.63mg/100g), than formulae sausages were 68.13, 57.81, 45.28 and 20.34 mg/100g, respectively and the results illustrated that the lower cholesterol content after reducing the fat content in sausage formulae. Whereas, the formula sausage 100% defatted was obtained the lowest cholesterol content may be cause the formulae did not content any fat except the fat in the minced meat.

The average phenolic content was significantly higher in formula (4) 116.9 mg GAE/100 g had contained 15g pomegranate powder substituted with fat followed by substituted fat with 10 and 5g pomegranate powder (101.5 and 89.3 mg GAE/100 g) than control free pomegranate powder was (52.6 mg GAE/100 g). Pomegranate fruit parts are known to contain phenolics compounds and antioxidant characteristics of pomegranate seeds and rind powder have also been reported (35).

Physical characteristics of sausage and their formulae:

Plasticity, water holding capacity (WHC), cooking loss total volatile nitrogen (TVN) and thiobarbituric acid (TBA) were determined in beef sausage as control and their formula made from pomegranate powder at different levels and the results are reported in tables (2). The resultant showed that the plasticity and WHC in sausage as control was the highest and increased in WHC. Water binding capacity of meat products with replaced fat is often poor. A few hydrocolloid systems are used to replace fat due to their high water binding capacity which promote the formation of gel, for example, alginate, carrageenan, xanthan gum, cellulose derivate, starch and pectin (36).
Table (1): Chemical compositions of beef sausage and their formulae substituted with pomegranate powder on wet weight:

<table>
<thead>
<tr>
<th>Formulae</th>
<th>Moisture</th>
<th>Protein</th>
<th>Lipids</th>
<th>Ash</th>
<th>Crude fiber</th>
<th>T.C.</th>
<th>Total phenolic</th>
<th>T. Cho. mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.15</td>
<td>14.81</td>
<td>21.41</td>
<td>2.24</td>
<td>1.24</td>
<td>6.35</td>
<td>52.6</td>
<td>70.36</td>
</tr>
<tr>
<td>Formula 1</td>
<td>54.61</td>
<td>14.87</td>
<td>15.23</td>
<td>2.29</td>
<td>1.29</td>
<td>11.01</td>
<td>79.2</td>
<td>68.13</td>
</tr>
<tr>
<td>Formula 2</td>
<td>54.76</td>
<td>14.94</td>
<td>10.39</td>
<td>2.31</td>
<td>1.25</td>
<td>14.05</td>
<td>89.3</td>
<td>57.81</td>
</tr>
<tr>
<td>Formula 3</td>
<td>54.75</td>
<td>14.94</td>
<td>5.43</td>
<td>2.33</td>
<td>1.27</td>
<td>18.08</td>
<td>101.5</td>
<td>45.28</td>
</tr>
<tr>
<td>Formula 4</td>
<td>55.05</td>
<td>14.95</td>
<td>2.31</td>
<td>2.25</td>
<td>1.25</td>
<td>19.59</td>
<td>116.8</td>
<td>20.34</td>
</tr>
</tbody>
</table>

Control made from sausage with 20% fat.
Formula 1 contained from sausage substitute 25% fat with 5g pomegranate powder.
Formula 2 prepared from sausage substitute 50% fat with 10g pomegranate powder.
Formula 3 consisted of sausages substitute 75% fat with 15g pomegranate powder.
Formula 4 consisted of sausages substitute 100% fat with 20g pomegranate powder.

The data presented in Table (2) shows that cooking loss were increased in sausage and it was decreased in their formulae by increasing the replacement fat using pomegranate powder reported with (37) who found that protein binds fat and reduces fat loss in cooking drip. The increases in cooking yield were improved by the texture of sausage protein for all formulae.

The mean values of total volatile nitrogen (TVN) are summarized in the same table and it was estimating the degree of meat deterioration during the storage period. The results showed that when storage period increased, the TVN values increased for all the sausage samples. This may be attributed to the breakdown of proteins as a result of activity of microbial strains and proteolytic enzymes. EOS (38) stated that 20 mg TVN/100 gm raw samples indicates the spoilage of minced meat. The highest rate of TVN values was recorded at the end of storage period in the sausage as control followed by formula low fat and formula replacement fat with 25, 50, 75 and 100% pomegranate powder. These results may be able the pomegranate powder is more effective in delaying increased rate of TVN.

The evaluation of thiobarbituric acid (TBA) mean values of sausage control and their formula were showed that highest incremental rate was recorded in the sausage as control followed by replacement fat with 25, 50, 75 and 100% pomegranate powder. The reduction of thiobarbituric acid reactive substances (TBARS) values recorded for sausage and their formula with increasing concentration of pomegranate powder could be due to the pomegranate powder had contained the highest natural antioxidant and delay the fat rancidity.

Table (3) showed that the results of texture properties analysis after replacing fat with pomegranate powder in sausage and their formulae. Firmness was between 4.29 and 9.86; cohesiveness between 0.51 and 1.12, gumminess between 2.12 to 10.68, chewiness between 1.51 and 2.32, springiness between from 0.60 to 0.71 and finely, resilience was between from 0.45 to 0.55. The addition of pomegranate powder id not influence the texture of sausages and its formula which may be caused by pomegranate powder being high contained in natural antioxidant.

Chambers and Bowers (39) suggested that of the characteristics of texture, hardness is the most significant factor in influencing consumer preference towards meat products, so this study primarily examined the hardness of Chinese-style sausages.

Table (2): Physical characteristics of sausage and their formulae substituted with pomegranate powder:

<table>
<thead>
<tr>
<th>Formulae</th>
<th>WHC cm²/0.3</th>
<th>Plasticity cm²/0.3</th>
<th>Cooking loss %</th>
<th>TVN mg %</th>
<th>TBA mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.70</td>
<td>3.20</td>
<td>23.43</td>
<td>9.79</td>
<td>0.47</td>
</tr>
<tr>
<td>Formula 1</td>
<td>0.99</td>
<td>3.19</td>
<td>19.59</td>
<td>9.46</td>
<td>0.36</td>
</tr>
<tr>
<td>Formula 2</td>
<td>0.97</td>
<td>3.15</td>
<td>19.88</td>
<td>9.49</td>
<td>0.35</td>
</tr>
<tr>
<td>Formula 3</td>
<td>0.95</td>
<td>3.12</td>
<td>19.86</td>
<td>9.51</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Viuda-Martos et al. (40) found that adding 1% orange dietary fiber to the Spanish emulsified meat mortadella significantly increased the hardness of the product, primarily because the bonding capacity of fiber particles and the emulsified protein system were strengthened through the heating process (41). Consequently, the resulting gumminess and chewiness of the sausages after the addition of different types of pomegranate powder as alternative fat followed the same general trend as the hardness of the sausage.

Table (3): Texture profile analysis of sausage and their formula substituted with pomegranate powder:

<table>
<thead>
<tr>
<th>Formulae</th>
<th>Firmness</th>
<th>Cohesiveness</th>
<th>Gumminess</th>
<th>Chewiness</th>
<th>Springiness</th>
<th>Resilience</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.29</td>
<td>0.51</td>
<td>2.12</td>
<td>1.51</td>
<td>0.60</td>
<td>0.45</td>
</tr>
<tr>
<td>Formula1</td>
<td>5.32</td>
<td>0.79</td>
<td>5.24</td>
<td>1.86</td>
<td>0.64</td>
<td>0.47</td>
</tr>
<tr>
<td>Formula2</td>
<td>7.24</td>
<td>0.88</td>
<td>7.36</td>
<td>2.01</td>
<td>0.67</td>
<td>0.49</td>
</tr>
<tr>
<td>Formula3</td>
<td>8.61</td>
<td>0.95</td>
<td>8.54</td>
<td>2.14</td>
<td>0.69</td>
<td>0.52</td>
</tr>
<tr>
<td>Formula4</td>
<td>9.13</td>
<td>1.12</td>
<td>10.24</td>
<td>2.32</td>
<td>0.71</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Organoleptic properties of sausage and their formulae substituted with pomegranate powder:

Sensory evaluation is used to measure human responses to foods with different techniques to get important and useful information (42). Sensory evaluation is defined as a scientific method for analyzing, evoking, measuring and interpreting responses to products under controlled conditions with the help of sight, smell, touch, taste and hearing. The tested samples are often labeled with random numbers and served in different orders for counterbalance of other judgments than the sensory experience.

Tables (4) showed that the organoleptic properties of sausage and their formula substituted with pomegranate powder. The resultant from Table (4), it could be noticed that the sausage replacement fat with pomegranate powder at level 25, 50 and 75%, respectively were acceptability (45.0, 40.0 and 37.5) and nearly control sausage (45.0%). Whereas, the sausage replacement fat with pomegranate powder at level 100% sheep fat was at the lowest acceptability (25.0 %). These results showed that the replacement fat with pomegranate powder was acceptable since 75% caused pomegranate powder is a good source for many nutrients such as natural antioxidants.

Color and flavor are the first stimuli for the consumers to purchase meat and meat products. However, lipid oxidation abbreviates the quality and acceptability of meat and meat products due to discoloration, drip losses, off-odor and off-flavor development, and the production of potentially toxic compounds (43).

Table (4): Organoleptic properties of sausage and their formula substituted with pomegranate powder:

<table>
<thead>
<tr>
<th>Formulae</th>
<th>Aroma (10)</th>
<th>Taste (10)</th>
<th>Color (10)</th>
<th>Texture (10)</th>
<th>Flavor (10)</th>
<th>Over all acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.00 ±0.12</td>
<td>9.00 ±0.10</td>
<td>9.00 ±0.11</td>
<td>9.00 ±0.11</td>
<td>9.00 ±0.12</td>
<td>9.0</td>
</tr>
<tr>
<td>Formula1</td>
<td>9.00 ±0.14</td>
<td>9.00 ±0.12</td>
<td>9.00 ±0.12</td>
<td>9.00 ±0.12</td>
<td>9.00 ±0.12</td>
<td>9.0</td>
</tr>
<tr>
<td>Formula2</td>
<td>8.00 ±0.15</td>
<td>8.00 ±0.16</td>
<td>8.00 ±0.15</td>
<td>8.00 ±0.14</td>
<td>8.00 ±0.14</td>
<td>8.0</td>
</tr>
<tr>
<td>Formula3</td>
<td>7.50 ±0.12</td>
<td>7.50 ±0.13</td>
<td>7.50 ±0.13</td>
<td>7.50 ±0.14</td>
<td>7.50 ±0.14</td>
<td>7.5</td>
</tr>
</tbody>
</table>
Biology evaluation:

Effect of feeding pomegranate powder on the initial body weight, gain body weight, total food intake and feed efficiency ratio in the experimental hypercholesterolemia rats:

Initial body weight, gain body weight, total food intake and feed efficiency ratio in the experimental hypercholesterolemia rats which were fed separately on 20% from different sausage formula substituted fat with 25, 50, 75 and 100% pomegranate powder and the results are reported in Table (5). The mean values of initial body weight of all groups after adaptation feeding on basal diet were ranged from 173.2 to 177.4 g.

At the end of experimental period (4 weeks), the final body weight of negative control hypercholesterolemia rats was higher than the positive control. The hypercholesterolemia rats were fed on sponge cake had lower final body weight than those of the hypercholesterolemia rats' positive control.

The obtained results illustrated that the gain in body weight at the end of experimental period for the negative control fed on basal diet was increased to 146.6 g, while the hypercholesterolemia positive control fed on hypercholesterolemia diet was 46.0 g. Feeding on basal diet separately on 20% from different sausage formula substituted fat with 25, 50, 75 and 100% pomegranate powder had lowered in body weight gain 90.3, 81.8, 70.5 and 55.3 g respectively than negative control (146.6 g).

Concerning food intake, the results indicated that rats fed on basal diet and different sausage formula are reported in the same table. The values of food intake for negative control were 560 g and 530 g for hypercholesterolemia rats as positive control. Whereas, the rats group 1, 2, 3 and 4 fed on different sausage formula, the food intake were nearly values 490, 470, 420 and 400 g, respectively for four weeks.

The calculate data of feed efficiency ratio (FER) for rats fed on basal diet and different sausage formula summarized in the same table. From the results, it can be observed that the value of feed efficiency ratio of basal diet was 26.17%, which was depressed to 8.68% for hypercholesterolemia control positive. The FER values of rats group 1, 2, 3 and 4 were 18.43, 17.40, 16.78 and 13.82%, respectively fed on different sausage formula substituted fat with 25, 50, 75 and 100% pomegranate powder.

The gain body weight, food intake and feed efficiency ratio were decreased in rats group 1, 2, 3 and 4 respectively, may be due to the groups fed on sausage formula substituted fat with 25, 50, 75 and 100% pomegranate powder had contained rich amounts from natural antioxidants, the results are significantly greater reduction of weigh, food intake and feed efficiency ratio.


<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial body weight (g)</th>
<th>Body weight gain (g)</th>
<th>Food intake (g)</th>
<th>Feed efficiency ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control negative</td>
<td>175.0 ± 2.70a</td>
<td>46.6 ± 2.70a</td>
<td>560.0 ±6.24a</td>
<td>26.17 ±0.05a</td>
</tr>
<tr>
<td>Control positive</td>
<td>173.2 ± 2.58a</td>
<td>46.0 ± 2.44a</td>
<td>530.0 ±6.25a</td>
<td>8.68 ±0.04c</td>
</tr>
<tr>
<td>Group 1</td>
<td>176.2 ±2.34a</td>
<td>90.3 ±1.95ab</td>
<td>490.0 ±5.43b</td>
<td>18.43 ±0.04ab</td>
</tr>
<tr>
<td>Group 2</td>
<td>173.8 ± 3.49a</td>
<td>81.8 ± 5.10b</td>
<td>470.0 ± 5.36b</td>
<td>17.40 ±0.08ab</td>
</tr>
</tbody>
</table>
Biochemical analysis:

Serum triglycerides, total lipids, cholesterol profile and blood sugar of the hypercholesterolemia rats:

At the end of biological experimental period the total lipid, triglyceride cholesterol profile and blood sugar were determined in all groups fed on basal diet substitute with 20% from sausage formula substituted fat with 25, 50, 75 and 100% pomegranate powder and the results are reported in Table (6). From the results in Table (6), it could be noticed that the total lipid and triglyceride were increased in control positive (1.42g/dl and 245.7 mg/dl) than control negative was 0.65g/dl and 112.3 mg/ dl, respectively. Moreover, the results illustrated that the hypercholesterolemia rats fed on sausage made from 100% pomegranate powder; the total lipid and triglyceride were the lowest 0.55 g/dl and 110.5 mg/dl followed by hypercholesterolemia rats fed on 75% pomegranate powder was amounted 0.68 g/dl and 115.1 mg/dl. The hypercholesterolemia rats fed on sausage made from 25% pomegranate powder was increased in total lipid and triglyceride 0.97g/dl and 170.5 mg/dl than other group fed on sausage different formula. These results showed that all the groups were fed on pomegranate powder during experimental period; the total lipid and triglyceride were decreased at the end of experimental due to the pomegranate powder having contained high natural antioxidants amount that increases degradation of cholesterol to fecal bile acids.

From the results in the same table, it could be observed that the total cholesterol in control positive was the highest amounted (297.3 mg/dl) than other group due to the positive control fed on basal diet during the experimental period. Moreover, the results illustrated that the hypercholesterolemia rats fed on sausage made from 75% pomegranate powder, the total cholesterol had the lowest (200.0 mg/dl) contained and nearly the negative healthy control 187.3 mg/dl fed on basal diet. These lowering results may be caused from the sausage made from 75% pomegranate powder which highly amounts from natural antioxidants. The hypercholesterolemia rats fed on sausage contained of 50% pomegranate powder had lowered cholesterol 227.0 mg/dl followed by hypercholesterolemia rats fed on sausage prepared from 25% pomegranate powder was 240.6 mg/dl. Moreover, the results illustrated that the LDL in positive control was the highest amounted 131.7 mg/dl and the control negative was the lowest amounted 25.0mg/dl as well as the rats group fed on 75% sausage pomegranate powder was 30.7 mg/dl followed by 50% was 40.6 mg/dl and 25% was 44.3 mg/dl, respectively. High density lipoprotein (HDL) was determined in all groups and the best group from the results was the rats fed on sausage made from 75% pomegranate powder was  80.0 mg/dl followed by 50% was 74.0 mg/dl and 25% was 67.5 mg/dl, respectively.

Whereas, according to the blood glucose from the obviously results, it could be noticed that the positive control was the highest amounted 169.3 mg/dl followed by rats fed on basal diet substitute 20% from sausage made from 25% pomegranate powder was 130.1mg/dl. Whilst, the groups fed on basal diet substitute with 20% from sausage made from50% and 75% pomegranate powder were decreased 125.3 and 110.7 mg/dl, respectively, than control positive and nearly to control negative 115.3 mg/dl.

The obtained results are in agreement with (44) who found that the mechanism of the observed hypotriglyceridemia effect may be due to decreased fatty acid synthesis, increased lipolytic activity by inhibition of hormone-sensitive tissue lipases or suppression of lipogenic enzymes, Activation of LCAT and tissues lipases.

Pomegranate fruit parts have bioactive compounds which prevent low-density lipoprotein oxidation, prostate cancer, platelet aggregation and various cardiovascular diseases 13. Ozkal and Dinc (45) reported the presence oftannins, anthocyanins and flavonoids in pomegranate rind. Pomegranate peel is a rich source of tannins and other phenolic compounds14. The meat industry can use these fruits or fruit byproducts as a potential source of phenolics as they have immense nutraceutical value and can be used to produce functional meat products of commercial interest.
Table (6): Serum triglycerides, total lipids, cholesterol profile and blood sugar (after 4 weeks) of the experimental hypercholesterolemia rats:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total lipids (g/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>Fasting blood sugar (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control negative</td>
<td>0.65 ±0.03 c</td>
<td>112.3 ±6.1 c</td>
<td>187.3 ±1.1 c</td>
<td>83.7 ±4.0 a</td>
<td>25.0 ±5.56 c</td>
<td>115.3 ±5.7 c</td>
</tr>
<tr>
<td>Control positive</td>
<td>1.42 ±0.17 a</td>
<td>245.7 ±27.9 a</td>
<td>297.3 ±6.5 a</td>
<td>47.3 ±7.24 d</td>
<td>131.7 ±20.2 a</td>
<td>169.3 ±3.8 a</td>
</tr>
<tr>
<td>Group 1</td>
<td>0.97 ±1.02 ab</td>
<td>170.5 ±10.5 ab</td>
<td>240.6 ±4.1 b</td>
<td>67.5 ±3.1 b</td>
<td>44.3 ±9.8 b</td>
<td>130.1 ±2.3 b</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.78 ±0.13 b</td>
<td>141.0 ±30.0 b</td>
<td>227.0 ±7.0 ab</td>
<td>74.0 ±5.3 b</td>
<td>40.6 ±10.0 ab</td>
<td>125.3 ±1.2 ab</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.68 ±0.19 c</td>
<td>115.1 ±7.5 c</td>
<td>200.0 ±6.3 c</td>
<td>80.0 ±4.2 a</td>
<td>30.7 ±6.34 c</td>
<td>110.7 ±2.8 c</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.55 ±0.12 d</td>
<td>110.5 ±5.23 c</td>
<td>190.0 ±4.2 d</td>
<td>82.3 ±4.2 a</td>
<td>28.3 ±3.52 d</td>
<td>110.7 ±2.8 c</td>
</tr>
</tbody>
</table>

CONCLUSION

Meat is still regarded as valuable protein source food in the world. However, consumer's preference on healthy meat product is also increasing due to various chronic diseases like cardiovascular diseases, cancer, cholesterolemia and diabetics. Reducing or replacing saturated animal fat could be the solution for producing nutritious product but there are challenges for preparing a healthy product and maintaining its sensory quality for the consumers. There are so many types of technological advancement have been developed to reduce or replace the animal fat, for example, incorporating the pomegranate powder as a fat mimetic. Incorporation of the pomegranate powder could be health beneficial, but, it may negatively affect the overall quality of the product. Therefore, research in advance is necessary to produce healthy and tasteful meat products. Meat content needs to be increased to compensate the reduction of fat in the meat products, and this will cause the increased redness, and firmness.

REFERENCES


