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Evaluation of Sausage Products Properties by Chemical, Microbial, and Histological Techniques in Qom Province, Iran

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ABSTRACT

Introduction: Sausages consist of principal processed meat products, including meat and other additives, making special texture and flavor. Consumption of these products has increased nowadays. This study aimed to evaluate the chemical, microbial, and histological properties of sausage products manufactured and distributed in Qom province, Iran.

Methods: In this study, 100 samples of varied types of sausage products were randomly collected from Oct-2021 to Jun-2022. Chemical, microbial, and histological techniques were applied to analyze samples. All measurements were implemented in triplicate, and the data were analyzed by SPSS software version 25.

Results: The highest amounts of total fat, sodium nitrite, and starch were observed in 40-50% of meat sausages and total protein and phosphate in 60-70%. *Salmonella spp. and Escherichia coli*, as dangerous pathogens, were not detected in collected samples. Total Bacterial Count, Coliform, *Clostridium perfringens*, Yeast and Mold were lower than the acceptable limit of the Iranian National Standard Organization (INSO 2303: 2021) ($p<0.05$). The histological techniques showed that lymphatic, skin, peritoneal fat and plant tissue, trachea, bone, and hyaline cartilage were used to produce sausage samples with no significant statistical differences ($p<0.05$).

Conclusion: The microbial properties of samples indicated suitable hygienic conditions of production and distribution. There was a strong consequence of dramatic variation in the processing and manufacturing conditions of collected sausage samples, due to the high standard deviation value in the chemical, microbial, and histological analysis results. However, the overall quality of production and distribution of analyzed sausage products were suitable.

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Abbreviations

AOAC=Association of Official Analytical Chemists
BPA=Baird-Parker Agar
MUG=MacConkey sorbitol agar-MU
PCA=Plate Count Agar
RVB=Rappaport-Vassiliadis Broth
SPS=Sulfite Polymyxin Sulfadiazine
TBARS=Thiobarbituric Acid Reactive Substances
TSB=Trypticase Soy Broth
TVBN=Total Volatile Basic Nitrogen
XLD=Xylose Lysine Deoxycholate

Introduction

Meat and meat products contain many minerals and nutrients recommended as daily intakes, but consuming these products should be associated with some nutritional problems. The nutritional value of sausages has changed due to the variation in meat composition, ingredients, and processing characteristics (1). Generally, meat products are manufactured from different raw materials, producing varied chemical, microbial, and sensory properties. The main composition of meat products associated with used raw materials includes protein, pigment, fat, and water contents (2). Sausage products are a varied group of meat products made from various meat species, including beef, buffalo, chicken, pork, and fish, with different spice formulations.

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Protein functionality is a critical characteristic in meat processing, directly related to the meat composition of raw material (3). In addition to safety and nutritional benefits, other characteristics of meat products consumers consider are sensory, price, and convenience properties (4). There should be more about appropriate safe and standard manufacturing strategies to produce healthier meat products such as sausages. Suitable quality properties of final sausage products, including chemical, microbial, texture, and sensory properties, are evidence of good manufacturing process, shelf life, and safety characteristics (5).

Many studies have demonstrated the hazards of sausage products for the consumer. The most important pathogens that have been detected in sausage products were *Clostridium botulinum, Clostridium perfringens, Listeria monocytogenes, Escherichia coli,* and *Salmonella typhimurium.* However, the products' salt, nitrite, thermal processing, and dryness significantly lead to antibacterial effects. In addition to foodborne pathogens, spoilage micro-organisms cause problems in consuming these products. Raw material properties, processing characteristics, and hygienic conditions of manufacturing are the key factors for sausage production with high-level safety. Furthermore, sensory attributes are essential for consumer acceptability (6).

Various factors affect the sensory properties of sausage products. Fat, water contents, and raw meat quality directly affect these products' texture and rheological properties (7). Since sausage products primarily comprise raw meat, high-quality meat without unauthorized tissue is essential. Because of the high price of meat in sausage production, manufacturers tend to use unauthorized tissue instead of meat in the formulation of these products.Animals' skin, fat, bone, and hyaline cartilage usually are used as unauthorized tissue in sausage manufacturing. Histological methods have been employed by researchers to detect unauthorized tissues in sausage products (8). Previous studies have investigated the histological methods to detect unauthorized tissues in the texture of sausage manufactured by Iranian producers (9), but their findings are controversial. This study aimed to evaluate the chemical, microbial, and histological findings of unauthorized tissue of sausage products manufactured and distributed in Qom province, Iran.

Material and Methods

Sampling Procedure

In this study, 100 samples of varied types of manufactured sausage were collected from markets located in different parts of Qom province, Iran. Samples were immediately transported by icebag at 4˚C to the laboratory for chemical, microbial, and histological analysis after coding (9).

Chemical Analysis

Chemical characteristics, including moisture, fat, protein, starch, ash, phosphate, and nitrite contents, were determined through the Gravimetric, Soxhlet, Kejldahl, Fehling, Dry, and Spectrophotometric methods, respectively, by AOAC (2005). All measurements were implemented in triplicate. Chemical characteristics including moisture, fat, protein, starch, ash, phosphate, and nitrite contents were determined by the methods suggested by AOAC (2005), (10). All measurements were implemented in triplicate.

Microbial analysis

About 10g of each sample was subjected to a homogenizer (IUL Instruments, Barcelona, Spain) and diluted with 90ml of a sterile solution of 0.1% (w/v) peptone water, 0.85% NaCl and 1% tween 80 as an emulsifier (Merck, Germany). Thus, ten-fold serial dilution was obtained by mixing 1m1 of a homogenized sample with a 9ml sterile solution of 0.1% peptone water. *Total Bacterial Count*, *Molds and yeasts*, and *Staphylococcus aureus* were determined using plate count agar: 37˚C/24-48h (PCA, United Kingdom), Baird-parker agar; 37˚C/24h (BPA, Merck, Germany) and Malt Extract Agar (Merck, Germany); 25˚C/5 days, respectively. Salmonella was detected using the method described below. Pre-enrichment media was done by Lactose Broth (25g of each sample added to LB)incubated at 37˚C/20h. Then, selective enrichment was performed by transferring 0.1ml of pre-enriched incubated media into the Rappaport-Vassiliadis broth (RVB, Oxoid) incubated at 42˚C/24h. Finally, enriched samples were streaked on Xylose Lysine Deoxycholate agar (XLD agar) (Merck, Germany) and incubated at 37˚C/48h. Lysine agar, Triple Sugar Iron agar as differential media, and Urea broth were used as complement media (37˚C/24h). Clostridia colonies were detected by inoculating 10ml of the sample dilution into 20ml SPS Agar (Sulfite Polymyxin Sulfadiazine) (Merck, Germany) tubes and

overlaying with 2ml of paraffin oil to provide anaerobic conditions. Colonies were grown in violet-red bile Agar (Merck, Germany), which was used to enumerate Coliforms via incubation at 37˚C/24h. Each sample was first enriched in Trypticase Soy Broth (TSB, Merck, Germany) in shaker incubator at 37˚C/18h to detect Escherichia coli. Then, the samples were linearly cultured on MacConkey sorbitol agar-MUG (MUG, Sigma-Chemical, USA) at 37˚C/24h. All experiments were implemented in triplicate (11).

Histological properties

Three similar parts were separated from each sample and then divided into four pieces for tissue analysis. The pieces were fixed in a 10% buffered formalin solution for light microscopy processing and then embedded in paraffin blocks. The blocks embedded with paraffin and processed samples were cut into pieces with a diameter of 6µm and stained using Hematoxylin and eosin (Vector, USA). An optic microscope (N-180 M, Novel, China) (MD-130, OME-TOP, Taiwan) was used to examine slides and detect unauthorized tissue in sausage samples. Then, the images were processed using Photoshop software CS (Adobe system, CA, USA). All measurements were carried out in triplicate (9).

Statistical Analysis

All analyses were performed in triplicate. One-way Analysis of Variance (ANOVA) was used to determine the difference ($P<0.05$) between treatments, and the contrast between means (Duncan`s multiple range test for chemical and microbial analysis) was used to assess the differences between the variables. Statistical analyses were conducted using SPSS software version 22 for Windows (Chicago, USA).

Table 1. Chemical properties of sausage samples manufactured and collected from Qom province, Iran

Meat content (%)	Chemical Properties						
	Total Moisture (%)	Total Fat (%)	Total Protein (%)	Starch (%)	Total Ash (%)^{NS}	Phosphate (%)	Sodium Nitrite (mg/kg)
40 – 50	54.31 ± 1.33^{a}	21.97 ± 1.54^{c}	11.24 ± 1.00^{a}	8.60 ± 0.65^{b}	1.97 ± 0.45^{b}	0.28 ± 0.12^{b}	44.75 ± 22.48^{b}
50 – 60	59.08 ± 4.21^{a}	16.96 ± 4.16^{b}	14.35 ± 2.47^{a}	5.30 ± 0.56^{a}	2.01 ± 0.46^{b}	0.29 ± 0.15^{b}	37.84 ± 26.58^{a}
60 – 70	61.68 ± 4.07^{a}	14.60 ± 2.71^{b}	18.30 ± 1.27^{b}	3.75 ± 1.76^{a}	1.83 ± 0.47^{a}	0.41 ± 0.13^{a}	32.87 ± 09.23^{a}
80 – 90	68.34 ± 4.24^{b}	9.17 ± 3.37^{a}	17.36 ± 1.04^{b}	3.17 ± 1.51^{a}	1.42 ± 0.28^{a}	0.37 ± 0.18^{a}	34.18 ± 15.68^{a}

Different letters between groups in each column denote significant differences ($p<0.05$).
^{NS} Not Significant

Results

Chemical Analysis of Sausage Samples

The chemical properties of sausage products with different meat content collected from different areas of Qom province are demonstrated in Table 1. These results showed that the total moisture content in group 89–90% meat content was higher than other groups ($p<0.05$). In addition, the nitrite and starch content of sausage samples included in the 40–50% meat content group was higher ($p<0.05$). The maximum fat content included 21.97% for the 40-50% meat content group ($p<0.05$). Further, the percent of total ash, phosphate, and protein were higher in the (50–60 and 60–70%) meat content group, respectively.

Table 2. Microbiological quality of sausage products produced and collected from Qom province, Iran

Microorganism	Mean	SD[a]
Total bacterial count (*Log CFU/g*)	+3.701	+2.621
***Coliform* (*Log CFU/g*)**	+2.155	+1.025
***E. coli* (*Log CFU/g*)**	0	0
***Salmonella* (*Log CFU/25 g*)**	0	0
***S. aureus* (*Log CFU/g*)**	+0.696	+0.060
***Clostridium perfringenes* (*Log CFU/g*)**	+0.954	+0.146
Yeast and Molds (*Log CFU/g*)	+1.568	+0.318

[a] SD = Standard Deviation

Microbial Analysis of Sausage Samples

The microbial properties of sausage product samples manufactured and collected from different areas in Qom province, Iran, are summarized in Table 2. *Salmonella* and *Escherichia coli* (E. coli) were not detected, but (*Log CFU/g*) (Mean±SD) of *Total Bacterial Count*, *Coliform*, S. aureus, *Clostridium perfringenes*, *Yeast, and Molds* were 3.701±2.621, 2.155±1.025, 0.696±0.060, 0.954±0.146 and 1.568±0.318, respectively.

Table 3. Histological properties of sausage samples collected from Qom province, Iran

Histological parameter	Mean (%)	SD[a]
Abdominal Cavity Tissue	0	0
Genitourinary Tissue	0	0
Nervous Tissue	0	0
Lymphatic Tissue	0.01	0.103
Skin Tissue	0.19	0.396
Peritoneal Fat Tissue	0	0
Bone and Hyaline Cartilage	0.16	0.368
Plant Tissue	0.02	0.145
Trachea	0.03	0.177
Breast tissue	0	0
Chicken Gizzard Tissue	0	0
Face Meat	0	0
Spleen Tissue	0	0
Lung Tissue	0	0
Liver Tissue	0	0

[a] SD = Standard Deviation

Histological Study for Detecting Unauthorized Tissue in Sausage Samples

Histological parameters of sausage samples are demonstrated in Table 3, revealing that collected samples detected lymphatic, skin, peritoneal fat, plant tissue, trachea, bone, and hyaline cartilage. The non-standard raw materials as unpermitted tissue, such as skin tissue (A), plant tissue (B), adipose tissue (C), connective tissue (D), and soy protein, are shown in Figure 1.

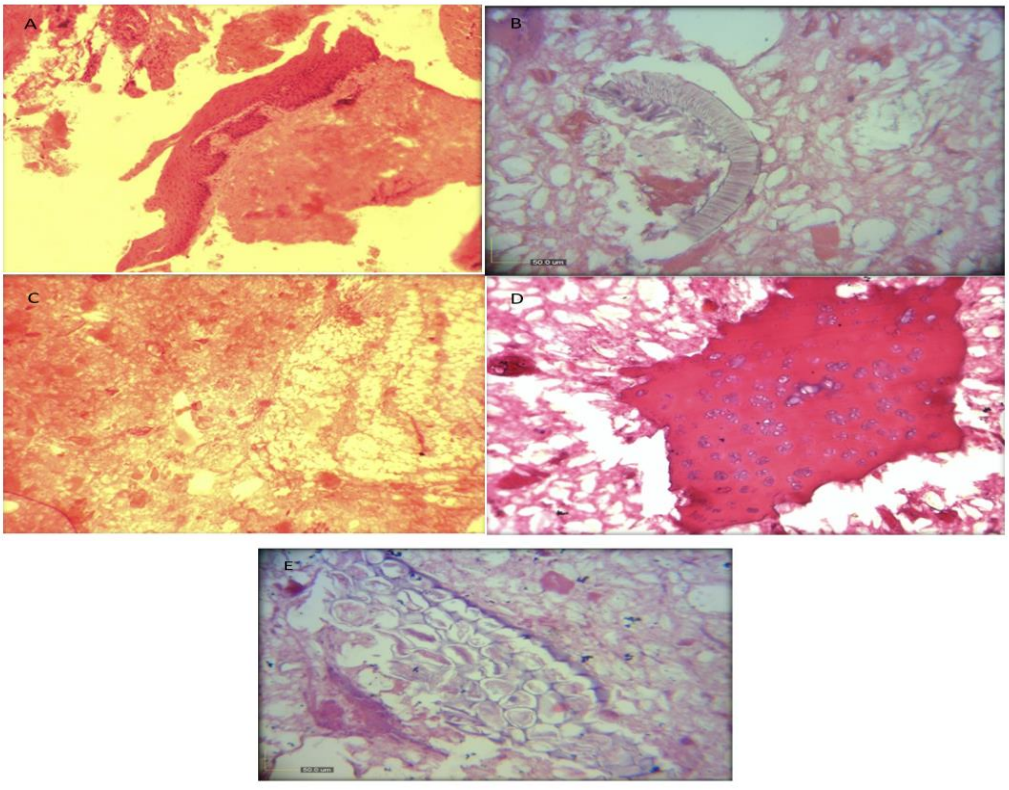

Figure 1. Microscopic image of skin tissue (A), plant tissue (B), adipose tissue (C), connective tissue (D) and soy protein (E) in sausage sample detected by histological method.

Discussion

Chemical Properties

Table 1 shows the chemical properties of sausage products collected from different regions of Qom province, Iran, with varying meat contents. The results showed that the total moisture in the 80-90% meat content group increased, and the amount of nitrite and starch decreased. In the 40-50% meat group, the amount of nitrite and starch was higher than in other groups, and the moisture content was also reduced ($p<0.05$). The results obtained for total protein indicate that the total moisture of sausage products increased as meat content increased and starch and nitrite contents decreased. Alamin et al. (2015) reported more than 70% moisture content in sausage samples manufactured by goat, camel, and beef meat (12). Moreover, there was a significant positive correlation between the meat and moisture contents of the samples. A maximum fat content of 21.97% was detected for the 40-50% meat content group, but Alamin et al. (2015) reported 2.31–3.45% fat content for sausage samples, which was lower than those of the present study (12). A higher fat content was observed in the present study, which decreases the nutritional value of meat products. Mallika and Prabhakar (2011) investigated the chemical characteristics of low-fat pork sausage. A lower fat content was found in produced samples in comparison with controls. In addition, the moisture content reported for low-fat pork sausage was significantly higher than our findings. Lower fat content promotes consumers' health and decreases cardiovascular diseases. The type of meat used to manufacture sausage products gives rise to variations in the fat content of final products that must be balanced before the formulation and processing. This high variation was observed due to different processing conditions and properties of employed meat in the formulation (13).

Zohdi et al. (2021) compared the safety and quality of high-priced (premium) and low-priced (economic) meat products. A total of 200 samples collected from the various grades of Egyptian beef luncheon sausage and burger patties commonly distributed in Egyptian markets were tested. All examined beef samples included the fatty acids profiles of chicken and buffalo meat. Moreover, the results revealed that the samples that exceeded the permissible limits of TBARS and TVBN values were more premium than those of economic products for luncheon and burger patties. Conversely, the economic luncheon indicated a higher percentage of samples that exceeded the permissible limit of residual nitrite compared to the premium one (14).

Microbial Properties

The microbial properties of sausage product samples manufactured and collected from different areas in Qom province, Iran, are summarized in Table 2. As shown in Table 2, yeast, moulds, and coliform counts were higher than in other studies. In addition, *Salmonella* and *E. coli* as dangerous pathogens were not detected in collected samples, indicating the suitable safe condition for consumption of these products. In the present study, *Staphylococcus aureus* (*S.aureus*) 0.696 *Log CFU/g* was lower than Drosinos et al. (2005). This study focuses on 2 *Log CFU/g* of *S.aureus* in traditional Greek fermented sausage products. In addition, 3.79 *Log CFU/g E. coli* were observed in sausage samples, indicating the low hygienic condition of the manufacturing process. The growth of other bacteria and lactic acid bacteria prevents increasing coliform and *E. coli* in meat products (15). Sohrabi et al. (2020) checked 101 samples of bovine minced meat (Group 1) and ready-to-cook meat products (Group 2) were collected from supermarkets in Turin, Italy. A higher presence of bacteria and inflammatory cells was detected in Group 1. Bacterial strains associated with inflammatory cells were detected with a higher score in Group 2. *Sarcocystis spp.* was present in 83.3% of Group 1 samples and 49.1% of Group 2 (16). In another study, more than 1.5 *Log CFU/g S.aureus* was found in processed buffalo sausage samples by Sachindra et al. (2005), indicating lower hygienic conditions of processing. In addition, 4–5 Log *CFU/g* coliforms were found in samples, while 2-3 *Log CFU/g Coliforms* should be attributed to employing higher microbial quality raw meat for the product's formulation. There is no considerable difference between yeast and mold counting of samples in this and the present study. The lower quality of raw materials should be attributed to increased *yeast, moulds*, and *Coliform* counting in meat-processed products such as sausages (17). Findings demonstrated that raw materials and the processing conditions of sausage products manufactured and

contributed to in Qom province, Iran, were at a suitable and standard level, considering the lack of salmonella detection in any collected sample.

Histological Findings

Histological techniques can be a simple, fast, economical, decisive, and conclusive tool for the quality control of some foodstuffs (18). Histologically, the meat comprises skeletal striated muscle fibers, adipose, water, and connective tissue. The histological examination allows the identification of tissue structures in meat products and, to a certain extent, the presence of unauthorized plant and animal ingredients. For this reason, the histological examination was adopted in some developed countries as a complementary method of assessing the integrity of the products. The histological examination provides concrete images regarding the tissues' morphological integrity in the meat's composition (19). The histological parameters of sausage samples are presented in Table 3. The collected samples detected Lymphatic, skin, peritoneal fat, plant tissue, trachea, bone, and hyaline cartilage. Izadi et al. (2016) investigated fraud in minced and processed meat by histological analysis, and they successfully implemented this method to detect unauthorized tissues (20). Latorre et al. (2015) analyzed unauthorized tissue in Kabab loghmeh, Kabab koobideh, handmade hamburgers, and sausage samples. In addition, plant tissue (soya), hyaline cartilage, and lymph nodes were also found in sausage product samples collected from different areas in Yazd province, Iran (9). Unauthorized tissue in formulating sausage and meat products indicated a lack of standard and hygienic conditions in food manufacturing regulation, leading to lower quality for consumers (21). The non-standard raw materials as unpermitted tissue are shown in Figure 1. Latorre et al. reported that histological methods and microscopic examination are valuable procedures for detecting unpermitted tissue in meat products (9). Malakauskiene et al. (2016) reported blood vessels, adipose, and connective tissues in sausage samples collected from Kaunas, Lithuania, by histological analysis. In addition, histological methods were introduced to detect unauthorized tissue in processed meat products (22).

Moghtaderi et al. (2019) applied histological methods to detect unauthorized tissues in the Iranian sausage samples, such as skeletal muscle fiber (100%), fat tissue (100%), and plant material (97.70%). A wide range of unauthorized tissues was detected, including dense connective tissue (6.66%), cartilage (28.30%), bone (8.30%), skin (51.60%), smooth muscle (1.66%), and blood vessels (11.66%). The results confirmed that the histological methods with Masson's trichrome staining are practical techniques for routine assessment of the authenticity and quality of sausage to protect consumers from adulteration (23).

In another study by Maghami et al. (2022), 34 samples of minced meat, hamburger, and sausage were randomly collected from the markets in northeast Iran. The results showed a distinctive difference in meat percentage compared to the labeled rate. Skeletal and smooth muscles, blood vessels, nerves, gizzard, adipose tissue, glandular tissue, cartilage, bone, tendon, skin, lymphatic tissues, and plant materials were observed (24).

This study showed that histological methods are helpful for the detection of unauthorized tissue in sausage products. However, the hygienic and safe sausage products consumed in Qom province, Iran, at an appropriate level, but the variation in production and distribution of these products were observed in higher values by other researchers.

Conclusion

The dramatic variation observed in results obtained for chemical, microbial, and histological analysis indicated various manufacturing and distribution conditions for these products in Qom province, Iran. The microbial characteristics of the samples can be due to the lower quality of raw materials and improper time-temperature conditions during distribution and storage in supermarkets. Histological parameters of sausage samples showed that applying unauthorized tissue in the formulation of sausage products can cause food loss of nutritional value. The chemical properties did not show a specific change in the amount of protein, fat, moisture, and carbohydrates, and other chemical tests, such as TVN, TBARS, and peroxide, are recommended to show spoilage. Therefore, health surveillance organizations should perform more accurate monitoring based on histological methods and chemical and microbial properties to prevent fraud, and new methods such as PCR should be recommended.

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