

Evaluation of Chemical Analyses of Experimentally Prepared Fermented and Heat-Treated Sausages

Eser İnce¹, Mukaddes Merve Efil², Nesrin Özfiliz^{1*}

Abstract: Sausages are very open to adulteration because of their preparation procedures. The purpose of this study was to examine the chemical composition of fermented and heat-treated sausages prepared experimentally with the addition of tissues and organs that are likely to be used in adulterated meat products and to determine whether the chemical analyses provided adequate information about sausage composition. The experimental fermented or heat-treated sausages were prepared with the addition of tissues and organs (seven tissue/organ combinations were studied; head meat-lung, tongue-liver, trachea-rumen, spleen-intestine, mammary gland-brain, heart-testis and kidney-oesophagus) that are likely to be used to adulterate sausage meat products. Appropriate control sausages not containing any organ additions were prepared according to the Turkish Food Codex. The most remarkable result is that contents of moisture, fat, ash and total protein are not sufficient criteria to determine the quality of the sausages. However, hydroxyproline content is an important criterion for the detection of collagen tissue, and this chemical analysis must be supplemented by histological analysis in future studies.

Keywords: chemical analysis, fermented sausages, heat-treated sausages, hydroxyproline.

Introduction

Meat is a nutrient source that is extremely rich in protein, vitamins and minerals, but is low in carbohydrates. At the same time, the nutrients in meat exist in vegetables at low levels, or their bioavailability is extremely poor. Meat has important nutrients for adequate and balanced nutrition and for the protection of human health and prevention of diseases (Muguerza *et al.*, 2004; Biesalski, 2005; Ekici and Ercoşkun, 2007). Meat, with its high amounts of water and a favourable pH, is a suitable medium for the development of microorganisms (Nychas and Arkoudelos, 1990; Serdaroglu and Sapanci Özsümer, 2000). Thus, various products have been manufactured in different societies throughout history to increase meat durability and to provide different flavours and aromas. The methods used in meat production are salting, drying, curing, cooking, cooling, freezing, fermentation, heat-treatment, irradiation and addition of chemicals to the meat (Çon *et al.*, 2002; Öztan, 2013; Anar, 2015). Fermentation is one of the oldest methods used for long-term preservation of foods (Caplice and Fitzgerald, 1999; Nassu *et al.*, 2003). When fermentation is applied

together with the methods of adding salt and reducing water, high quality products are made, and effective protection is achieved as well (Molly *et al.*, 1997; Çiçek *et al.*, 2015).

Fermented sausage is one of the oldest meat product types manufactured in Turkey, and its processing technology resembles that of fermented dry salami and sausages produced in Europe and America (Gökalp *et al.*, 2004). In addition to fermented sausages, heat-treated sausage production occurred in time, and recently, with developed technologies, these products have become common (Güner *et al.*, 2011; Pehlivanoglu *et al.*, 2015). Sausages are meat products that are open to adulteration due to their production methods (Güçer and Gövercin, 2010; İnce and Özfiliz, 2018). To prevent adulteration and provide appropriate standards in meat production, in 1860, legislative regulations were developed and implemented for the first time in England, and other countries later followed these regulations. To determine the adulteration of meat products, physical, chemical, microbiological and histological standards have been reported (Ekici and Ercoşkun, 2007). In countries where sausage products are widely consumed, a number of studies have examined wheth-

¹ University of Uludag, Faculty of Veterinary Medicine, Department of Histology and Embryology, 16059-Gorukle, Bursa, Turkey;

² University of Uludag, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, 16059-Gorukle, Bursa, Turkey.

*Corresponding author: Nesrin Özfiliz, nesrin@uludag.edu.tr

er products comply with these physical, chemical, microbiological standards (Salgado *et al.*, 2006; Marcinčák *et al.*, 2014).

The purposes of this study were to examine the chemical composition of fermented and heat-treated sausages prepared experimentally with the addition of tissues and organs that are likely to be used to produce adulterated sausage products and to determine whether the chemical analyses provide adequate information about sausage quality.

Materials and Methods

As controls, both fermented and heat-treated sausages were formulated with beef meat and fat without additives according to the Turkish Food Codex. In the experimental groups, sausages were formulated with the addition of two tissues and/or organs in equal proportions of 10% to make up a total of 20% (seven tissue/organ combinations were studied; head meat-lung, tongue-liver, trachea-rumen, spleen-intestine, mammary gland-brain, heart-testis and kidney-oesophagus). The fermented sausages were fermented for 8 days under air flow at 0.5–1 m/second, relative humidity of 75–90% and at 18–22°C until maturation. On the other hand, the heat-treated sausages were subjected to a heating process in a baking oven until a central temperature of 68°C was reached. Following these procedures, the sausages were vacuum-packed and stored at 4°C.

Chemical analyses

All chemical analyses were conducted according to standard methods (AOAC, 2003), as detailed below. Sausage material containing ca. 2 g dry matter was dried in mechanical convection oven at 105°C for 12 h. After drying, each sample was cooled in a desiccator and weighed. The loss in weight was recorded as the amount of moisture (AOAC 950.46). Approximately 2 g sausage sample was put into a porcelain crucible and burned in an ash oven at 550°C for 6 h. After burning, the sample was cooled in a desiccator and weighed. The weight lost was subtracted from the initial sample weight and the remaining weight was recorded as the amount of crude ash (AOAC 923.03). Fat content was measured by the Soxhlet system. Roughly 4 g of sausage sample was weighed onto filter paper and subjected to extraction in the Soxhlet extractor for 6 h. The solvent used was diethyl ether, and the extract was collected in a suitable container. When the extraction process ceased, the container was dried in an oven at

105°C for 1 hour and subsequently cooled in a desiccator. After cooling down, the remaining extract was recorded as the amount of fat (AOAC 960.39). Protein content was measured by the Kjeldahl (N \times 6.25) method. Approximately 1 g of sausage sample was digested at high temperature with 98% concentrated sulphuric acid to convert nitrogenous substances to ammonium salts. Then, ammonium salts were converted to free ammonia by applying 40% concentrated NaOH. Ammonia was titrated with hydrochloric acid to determine the N content. Since N forms 16% of protein molecule, the N content found was multiplied by 6.25 to calculate amount of total protein (AOAC 928.08). 4-Hydroxyproline was used as an indicator of collagen in the sausage samples. Therefore, 4 g of the homogenate obtained from a 200 g sausage sample was transferred to an Erlenmeyer flask and hydrolysed with sulphuric acid at 105°C for 16 h. After hydroxylation, the hydrolysate was diluted with distilled water and filtered. The concentration of 4-hydroxyproline was measured by spectrophotometer (AOAC 990.26).

Statistical analyses

Statistical analyses were conducted in the SPSS 23.0 package program (*International Business Machines*, 2015). The normal distribution of continuous variables was evaluated by Shapiro-Wilk test. A single sample *t*-test was used for comparisons of two groups of variables that were not normally distributed. The descriptive statistics of the continuous variables are shown by mean and standard deviation values and categorical variables by frequency and percentage. Comparisons below $p < 0.05$ were considered statistically significant in all statistical analyses in the study.

Results and Discussion

Fermented and heat-treated sausages were evaluated in terms of moisture, ash, fat, total protein and hydroxyproline contents for the purpose of determining quality. The results are shown in Tables 1 and 2.

Moisture levels of control sausages and the mean of the test groups were 54.72% and 55.50%, respectively, in fermented sausages and 54.46% and 56.37%, respectively, in heat-treated sausages. Our results are higher than the following results; 43.08% moisture levels for fermented sausages in the Istanbul markets, 44.60% in Spanish Androlla sausage, 47.58% in Afyon province sausages, 20.78% in fer-

mented sausages sold in Kahramanmaraş province, 38.75% in the Elazığ markets, 32.20% and 29.76% in Spain in homemade and industrial sausages, respectively, and 42.33% in Bosnian sausages (Pehlivanoglu et al., 2015; Lorenzo et al., 2000; Doğu et al., 2002; Erdoğan and Ergün, 2005; Öksüztepe et al., 2011; Salgado et al., 2006; Operata and Smajic, 2012). The average moisture content of Botillo, produced in Spain, was 55.90%, which is similar to our results (Lorenzo et al., 2000).

The amount of ash was 1.81% and 1.45% in fermented control and experimental sausages (means are presented), respectively, and 1.84% and 1.72% in heat-treated control and experimental sausages, respectively. In our study, no cartilage or bone tissue-containing pieces of meat were added to the con-

trol or to the experimental sausages, which resulted in no difference between the amounts of ash in the control and experimental sausages. In 1982–2016 in Turkey, the amount of ash in fermented sausages ranged from 3.85% to 5.88% (Ertaş, 1982; Ertaş and Kolsarıcı, 1983; Kolsarıcı et al., 1986; Sancak et al., 1996; Atasever et al., 1998; Erdoğan and Ergün, 2005; Öksüztepe et al., 2011). The lower ash contents obtained in our study may be due to the absence of cartilage and bone tissue, which are not allowed in sausages in Turkey, but which were identified in histological studies on Turkish sausages (İnal, 1992; Atasever et al., 1999; Erdoğan, 2002; Erdost et al., 2016; Ince and Özfiliz, 2016). The amounts of ash were 5.63% and 5.32% (Salgado et al., 2006) in Spanish homemade and industrial sau-

Table 1. Chemical composition of fermented sausages

Sausage type	Moisture %	Ash %	Fat %	Total Protein %	Hydroxyproline (mg/100g)
Cf	54.72	1.81	19.98	21.92	116
Ef1	55.41	1.43	16.93	23.24	389
Ef2	55.86	1.42	17.32	22.20	375
Ef3	54.93	1.51	18.10	21.30	379
Ef4	58.54	1.40	18.04	20.71	355
Ef5	57.68	1.46	19.33	21.82	367
Ef6	51.38	1.67	18.59	24.54	371
Ef7	54.72	1.29	18.50	23.30	358
MEf	55.50±2.31	1.45±0.12	18.12±0.81	22.44±1.32	370.57±11.86

Legend: Cf – Control Turkish type fermented sausage; Ef – Experimental Turkish type fermented sausages; MEf – Mean of experimental Turkish type fermented sausages

Table 2. Chemical composition of heat-treated sausages

Sausage type	Moisture %	Ash %	Fat %	Total Protein %	Hydroxyproline (mg/100g)
Ch	54.46	1.84	14.92	22.06	126
Eh1	56.90	1.56	13.88	22.27	399
Eh2	55.02	1.66	15.03	22.33	381
Eh3	55.70	1.45	15.97	21.53	402
Eh4	58.20	1.72	15.68	22.14	357
Eh5	58.03	1.83	12.63	21.69	358
Eh6	54.49	1.96	16.64	23.62	375
Eh7	56.26	1.85	11.77	23.46	384
MEh	56.37±1.43	1.72±0.18	14.51±1.81	22.43±0.81	379.43±17.79

Legend: Ch – Control Turkish type heat-treated sausage; Eh – Experimental Turkish type heat-treated sausage; MEh – Mean of experimental Turkish type heat-treated sausages

sages, respectively, and 4.95% in the study reported by Operta and Smajic (2012) in Bosnian sausages. This difference in ash content between our study and these other studies can be attributed to the use of MRM (mechanically recovered meat) and stripping meat, which are prohibited in Turkey, but are allowed in Europe (Tremlová *et al.*, 2006; Komrska *et al.*, 2011).

The amounts of fat in the control and test groups (means) were determined as 19.98% and 18.12%, respectively, in fermented sausages and 14.92% and 14.51% respectively, in heat-treated sausages. Studies in Turkey on the amount of fat in fermented sausages have found an average between 28.09% and 39.97% of fat (Ertaş, 1982; Ertaş and Kolsarıcı, 1983; Kolsarıcı *et al.*, 1986; Sancak *et al.*, 1996; Atasever *et al.*, 1998; Çon and Gökalp, 1998; Doğu *et al.*, 2002; Erdoğan and Ergün, 2005; Öksüztepe *et al.*, 2011). In homemade and industrial sausages and in Androlla and Botillo in Spain, the average fat content was 46.50%, 47.85%, 21.22%, and 14.82%, respectively (Lorenzo *et al.*, 2000; Salgado *et al.*, 2006). In a study of Bosnian sausages, the average amount of fat was 25.28% (Operta and Smajic, 2012). Animal fats are the source of essential fatty acids and fat soluble vitamins (Ekşi and Ertaş, 2011).

In the control and experimental groups, the total amounts of protein were determined as 21.92% and 22.44%, respectively, in fermented sausages and 22.06% and 22.43%, respectively, in heat-treated sausages. In 1982–2015, for fermented sausages produced in Turkey, the protein levels were between 17.16% and 27.3%, whereas the results in our current study are at the lower end of this spectrum (Ertaş, 1982; Ertaş and Kolsarıcı, 1983; Kolsarıcı *et al.*, 1986; Sancak *et al.*, 1996; Atasever *et al.*, 1998; Doğu *et al.*, 2002; Erdoğan and Ergün, 2005; Öksüztepe *et al.*, 2011; Pehlivanoglu *et al.*, 2015). Our results for total protein were similar to Spanish Androlla at 22.66%, lower than Bosnian sausages at 26.57%, higher than Botillo at 17.73%, homemade sausages at 13.69%, and Spanish industrial sausages at 15.16% (Lorenzo *et al.*, 2000; Salgado *et al.*, 2006; Operta and Smajic, 2012).

In terms of total protein content (%), there was no statistically significant difference between experimental sausages and control group (means) in both fermented ($p=0.335$) and heat-treated sausages ($p=0.268$), as shown in Figure 1. These results show that the control and experimental groups prepared by adding tissue and organs contained similar amounts of protein.

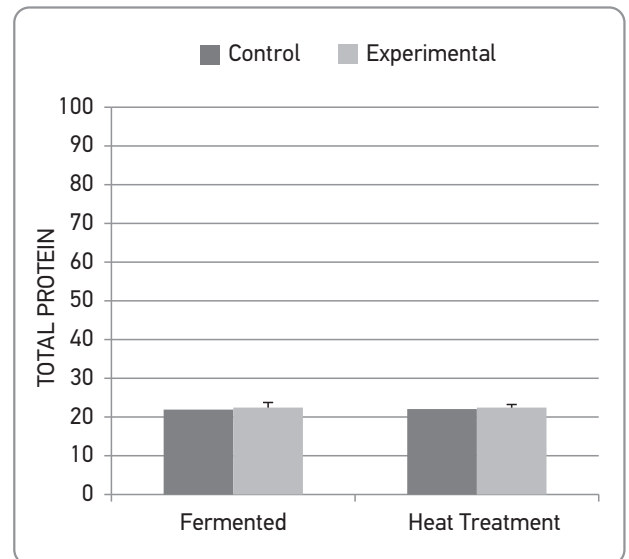


Figure 1. Comparison of total amounts of protein level (%).

The amounts of hydroxyproline in the control and experimental groups (means) were 116 mg/100 g and 355 to 389 mg/100 g, respectively, in fermented sausages, and 126 mg/100 g and 357 to 402 mg/100 g, respectively, in heat-treated sausages. In Turkey hydroxyproline levels in fermented sausages have reported in the ranged of 127mg-530mg/100g (Ertaş and Kolsarıcı, 1983; Kolsarıcı and Ertaş, 1986; İnce *et al.*, 2018), while in other countries, the levels ranged from 90mg/100g to 970mg/100g (Lorenzo *et al.*, 2000; Salgado *et al.*, 2006; González-Martín *et al.*, 2009; Mazorra-Manzano *et al.*, 2012).

In terms of the hydroxyproline levels, there was a statistically significant difference between

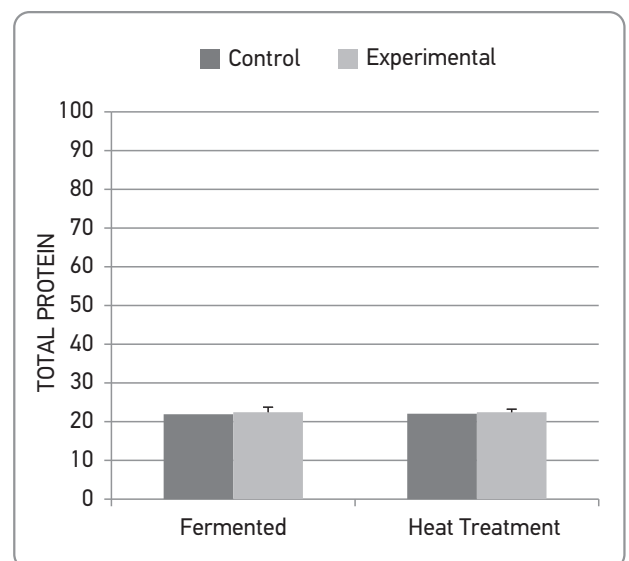


Figure 2. Comparison of hydroxyproline level (mg/100g).

the experimental and control groups (means) of fermented ($p < 0.001$) and heat-treated ($p < 0.001$) sausages, as shown in Figure 2. The results indicate that the addition of tissues and organs, except for muscle tissue, to sausages affects the level of hydroxyproline. We suggest that this result may be due to the amount of connective tissue-derived collagen.

However, our statistical results regarding total protein and hydroxyproline levels indicated that the total protein content of the fermented and heat-treated sausages is not sufficient to provide information on the quality and bioavailability of the protein (Kolsarıcı and Ertaş, 1986; González-Martín et al., 2009). Studies conducted by Brito et al. (2010) on traditional and industrial sausages showed that the total protein ratios and hydroxyproline levels of traditional sausages were high, while in sausage samples examined by Kurćubić et al. (2012), the total protein ratio was low and the hydroxyproline level was high. Thus, the hydroxyproline level is important. However, the hydroxyproline results do not address the issue of whether the products sold on markets are poor quality meat or whether different tissues and organs were used to adulterate the sausages. Although the

levels of hydroxyproline show the presence of connective tissue-derived collagen tissue, for indicating from which tissues or organs the collagen originates, histological examinations must be conducted (Atasever et al., 1999; Erdoğan, 2002; Sezer et al., 2013; Latorre et al., 2015; İnce and Özfiliz, 2016).

Conclusions

The results of our study show that the moisture, fat, ash and total protein analysis results do not differ between the control and organ-added experimental groups of sausages. However, there was a statistically significant difference in hydroxyproline levels between the control and organ-added sausages. The most remarkable result is that moisture, oil, ash, and total protein contents are not sufficient criteria to determine the quality of the sausage products.

It was also concluded that hydroxyproline analysis is an important criterion for the detection of collagen tissue from connective tissue proteins in sausages, while histological analysis must be conducted to determine the origin of the adulteration.

Ocena hemijskih analiza eksperimentalno pripremljenih fermentisanih i termički obrađenih kobasica

Eser İnce, Mukaddes Merve Efil, Nesrin Özfiliz

Apstrakt: Kobasice su veoma otvorene za patvorenje (oponašanje) zbog postupka njihove pripreme. Svrha ovog istraživanja je bila da se ispita hemijski sastav fermentisanih i termički obrađenih kobasica pripremljenih eksperimentalno uz dodatak tkiva i organa koji će se verovatno koristiti u patvorenim proizvodima od mesa i da se utvrdi da li su hemijske analize dale adekvatne informacije o sastavu kobasica. Eksperimentalne fermentisane ili termički obrađene kobasice pripremane su uz dodatak tkiva i organa (proučavano je sedam kombinacija tkivo/organ; meso glave -pluća, jezik-jetra, dušnik-burag, slezina-crevo, mlečna žlezda-mozak, srce-testis i bubreg-jednjak) koji će se verovatno koristiti za patvorenje mesnih proizvoda od kobasica. Odgovarajuće kontrolne kobasice bez dodatka organa pripremljene su prema turskom kodeksu za hranu. Najznačajniji rezultat je da sadržaj vlage, masti, pepela i ukupnih proteina nije dovoljan kriterijum za određivanje kvaliteta kobasica. Međutim, sadržaj hidroksiprolina je važan kriterijum za detekciju kolagenog tkiva, a ova hemijska analiza mora biti dopunjena histološkom analizom u budućim istraživanjima.

Cljučne reči: hemijska analiza, fermentisane kobasice, termički obrađene kobasice, hidroksiprolin

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