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Nutritional characterisation of homemade beef sausage based on amino acid, biogenic amines, and fatty acid composition

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ABSTRACT

The aim of this study was to assess the nutritional quality of homemade beef sausages by examining the amino acid, biogenic amine, and fatty acid composition. The most abundant amino acids were Ala (15.56%), Leu (13.28%), Gly (8.64%), Pro (8.41%), Ser (8.26%), and Val (7.65%). The essential amino acids accounted for 44.30% of total amino acids. Apart from the protein building amino acids, the free amino acid content was relatively high, accounting for 10% of total amino acid content. The average biogenic amine concentration in the sausage samples was low (1.69 mg kg^{-1}). Saturated fatty acids accounted for 59.10% of total fatty acids, followed by monounsaturated (38.63%) and polyunsaturated fatty acids (2.27%). The fatty acid profile was dominated by oleic (C18:1, 34.37%) and palmitic (C16:0, 30.24%) acids, and short-chain fatty acids were also present, which may have a positive impact on gut health. The results show that beef sausages have a high nutritional value and are a good source of essential amino acids, free amino acids, and fatty acids that are important for human health.

KEYWORDS

biogenic amines, beef meat, essential amino acids, lipid, protein

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1. INTRODUCTION

The majority of the world's population regularly consumes meat, mainly pork, beef, sheep, goat, chicken, and other poultry, but primarily red meat. Because of its unique flavour and nutritional value, beef is one of the most popular red meats. The global beef production is estimated to be around 60 million tons per year, with the largest producers being the USA, Brazil, China, and Argentina (FAO, 2022). Beef is valued for its high protein content, essential fatty acids and micronutrients, such as iron, zinc, magnesium, and others (Cobos and Diaz, 2015).

Protein is one of the most important macronutrients, and its quality is determined by the amount of essential amino acids present (Simon Sarkadi, 2019). Approximately half of the 20 amino acids that make up proteins are considered essential, which means that our bodies cannot produce them and must be obtained through diet. Beef contains a high concentration of amino acids such as methionine, cysteine, lysine, and tyrosine (Ahmad et al., 2018).

Due to its high protein content, beef meat contains biogenic amines, which are the decarboxylated product of the corresponding amino acids. As some might have intolerance to high levels of biogenic amines such as histamine and tyramine, these compounds are important in terms of human health. Although many people are sensitive to biogenic amines, there is no general recommendation for their upper limit. However, there are certain food categories, including meat and meat products, have high levels of histamine. The European Union (EU) Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs establishes a maximum limit for histamine in meat products (excluding minced meat) of 200 mg kg^{-1}. The maximum limit for minced meat is 100 mg kg^{-1}. This is due to the larger surface area of minced meat, which allows for faster bacterial growth and the formation of biogenic amines such as histamine (European Commission, 2005). The European Food Safety Authority (EFSA) has also determined that a daily maximum of 50 mg of histamine and 600 mg of tyramine is acceptable for healthy individuals (EFSA Panel on Biological Hazards (BIOHAZ), 2011).

The nutritional value of beef is determined not only by its protein content, but also by its fatty acid profile. Beef contains a variety of essential fatty acids, including omega-3 and omega-6 fatty acids. Omega-3 fatty acids are important for regulating the body's inflammatory response as well as proper blood clotting and blood pressure regulation. Omega-6 fatty acids are important for the human body because they regulate the production of prostaglandins, which play an important role in hormone regulation and blood pressure control (Shen et al., 2022; Belury, 2023).

Beef meat is commonly processed into a variety of food products. Among these products, sausages have become a popular everyday food in many countries. Beef sausage is typically made by mixing ground beef meat with salt, spices, and other seasonings before encased in a skin made from animal intestines or synthetic materials. Given the popularity of beef sausage, it is important to investigate its nutritional value.

The aim of the research was to assess the nutritional quality of homemade beef sausages based on amino acid, biogenic amine, and fatty acid composition.

2. MATERIALS AND METHODS

2.1. Manufacturing of sausages

Beef sausages were provided by a local sausage manufacturer in Kosovo. Three samples of homemade beef sausage (2 kg) were made using a recipe that included 70% beef meat, 30% fat (from cattle), spices, salt, onion, and garlic. The ingredients were ground with an Alexanderwerk cutter (Alexanderwerk, Germany) and then placed in round beef casings of 3 cm in diameter and 45 cm in length (34/36 AB 15m"). The sausages were dried for 12 h at 80 °C in an air master chamber. This sausage contains no starter culture or other preservatives and can be stored in the refrigerator at 4–7 °C for 3 months or frozen (−20 °C) for up to a year. Following production, homemade beef sausage samples were transported to the laboratory and analysed.

2.2. Moisture content and protein determination

The ISO 1442:1973 method was used to determine the moisture content. The protein content was determined based on the Kjeldahl method (Kirk, 1950). Three parallel samples were measured.

2.3. Amino acid determination

2.3.1. Protein building amino acids. To determine protein building amino acids, 500 mg samples of homemade beef sausage were hydrolysed in a closed hydrolysing vessel (KUTESZ, Budapest, Hungary) with 10 mL 6 mol L^{-1} HCl under nitrogen atmosphere at 110 °C, for 24 h in a block thermostat (FALC Instruments, Treviglio, Italy). In a 25 ml volumetric flask, neutralisation was accomplished by adding 10 ml of 4 M L^{-1} NaOH to the hydrolysed sample and then filling the flask with buffer (pH 2.2). The neutralised samples were filtered through 0.25 µm membrane filter (Nalgene, Rochester, USA). Amino acids were determined using an Automatic Amino Acid Analyser AAA400 (Ingos Ltd., Prague, Czech Republic) equipped with a cation-exchange column (Ionex Ostion LCP5020 22 × 0.37 cm). Stepwise gradient elution with sodium buffer systems was used for separation. After post-column derivatisation with a ninhydrin reagent, colorimetric detection was carried out at 570 and 440 nm (for Pro). Three parallel samples were prepared for all analyses.

2.3.2. Free amino acids. For the determination of free amino acids (FAA), 1 g of homemade beef sausage was extracted with 10 mL 10% trichloroacetic acid for one hour at room temperature at 100 r.p.m. using a Laborshake (Gerhardt GmbH, Königswinter, Germany). Following extraction, samples were filtered twice, first through filter paper and then through 0.22 µm membrane filter (Nalgene, Rochester, USA). FAA analysis was carried out using the same equipment as for protein building amino acids, including the same detection procedure, but lithium buffer systems were used for the stepwise gradient separation.

2.4. Biogenic amine determination

The sample preparation for biogenic analysis was identical to that of free amino acids. Biogenic amines were determined using the same equipment and a different cation-exchange column

(Ostion LG ANB ion-exchange resin; 7.0 × 0.37 cm). Separation was carried out with Na+/K+ buffer system. After the ninhydrin reaction, detection was done at 570 nm.

2.5. Fatty acid determination

Sample preparation was made according to method of Bligh and Dyer (1959). Petroleum ether was used to extract the fat from the samples using a Soxhlet extractor.

2.5.1. Preparation of fatty acid esters. The extracted fat was re-dissolved in 10 mL of n-hexane using a Vortex. Then, 4 mL of methanol containing 4% w/v KOH was pipetted into the solution and stirred vigorously with a Vortex for 5 min. Following separation of the phases, the entire amount of the upper iso-octane phase was transferred to a clean test tube using a Pasteur pipette, 4 mL of distilled water was added and vortexed vigorously for 2 min. After the separation of the phases, a sufficient amount (100–500 µL) was transferred from the upper iso-octane phase for gas chromatography measurement in such a way that no aqueous phase was present, and the sample vessel was sealed. All samples were stored frozen at −18 °C until use.

Gas chromatography. Gas chromatography with flame ionisation detection (GC-FID, Thermo Finnigan Trace GC, AS 2000 sampler, San Jose, CA, USA) was used for the qualitative and quantitative analysis of fatty acid methyl esters (FAMEs). The FAMEs were separated using an SP2450 column (30 m × 0.32 mm × 0.2 µm); N_2 was used as a carrier gas at a flow rate of 0.5 mL min^{-1}, with a split ratio of 1:50. The injector was set to 180 °C. The heating program was maintained for 2 min at 70 °C, then increased to 140 °C at a rate of 4 °C min^{-1} and held for 1 min, then increased to 180 °C at a rate of 1 °C min^{-1} for 1 min, and finally increased to 250 °C at a rate of 40 °C min^{-1} and held for 1 min. The detector was operated at 280 °C.

To confirm component identification, gas chromatography coupled with mass spectrometry (GC-MS, Hewlett Packard 5890/II GC – 5971A MSD, Palo Alto, CA, USA) was applied. For the separation, an Ultra2 column (50 m × 0.2 mm × 0.33 µm) was used. Helium was applied as a carrier gas at a flow rate of 0.8 mL min^{-1} and a split ratio of 20:1. The sample was injected into a 250 °C injector. The oven temperature program was as follows: the column was held at 70 °C for 2 min, then heated to 280 °C at a rate of 4 °C·min^{-1} for 10 min. The detector's ion source was set to 280 °C, the ionisation method was EI, the electron energy was 70 eV, and the mass scan range was 40–350 amu. The results were evaluated using the HP MS Chemstation program.

2.6. Statistical analysis

Descriptive statistics were used to evaluate the data. The statistical analysis was performed by IBM SPSS ver. 25 software.

3. RESULTS AND DISCUSSIONS

3.1. Moisture, protein, and fat contents of beef sausage

The moisture, protein, and fat contents of homemade beef sausage are shown in Table 1. The moisture content of homemade beef sausage (31.28%) was lower compared to Australian retail raw beef sausage samples (58.8–70.2%) (Cunningham et al., 2015). In terms of protein content, the homemade beef sausage had a higher protein content (36.74%) than retail samples of

Table 1. Moisture, protein, and fat contents of homemade beef sausage

Composition	Mean ± sd
Moisture content (%)	31.28 ± 2.50
Protein (%)	36.74 ± 1.95
Fat (%)	26.25 ± 0.85

sd: standard deviation (n = 9)

Australian beef sausages (11.60–17.00%) and dry fermented beef sausages (19.33–26.51%) analysed by Olivares et al. (2011). Furthermore, the homemade beef sausage contained more fat (26.25%) than the Australian beef sausages (7.3–22.6%). These results show that the nutritional composition of beef sausages can vary greatly depending on factors such as the type of beef meat, the method of preparation, and the additives.

3.2. Amino acid composition of homemade beef sausage

The total amino acid (TAA) content of homemade beef sausage was 344.92 mg kg^{-1} (Table 2). The major amino acids were Ala (15.56%), Leu (13.28%), Gly (8.64%), Pro (8.41%), Ser (8.26%), and Val (7.65%) accounted for 61.80% of the TAA content. Cysteine (0.81%) was the minor amino acid. The amino acid profile of beef sausages is important in determining nutritional value and potential health effects. According to the results, homemade beef sausages are an excellent source of essential amino acids, accounting for 44.30% of the TAA composition (Table 2). In general, amino acid concentrations in beef sausage are lower than in beef meat

Table 2. Amino acid composition of homemade beef sausage

Amino acids	Mean ± sd (mg kg^{-1})	Mean (%)
Alanine	53.66 ± 0.02	15.56
Leucine*	45.80 ± 0.09	13.28
Glycine	29.79 ± 0.26	8.64
Proline	29.00 ± 0.01	8.41
Serine	28.50 ± 0.11	8.26
Valine*	26.39 ± 0.21	7.65
Lysine*	19.92 ± 0.53	5.78
Tyrosine	17.62 ± 0.32	5.11
Aspartic acid	16.90 ± 0.46	4.90
Histidine*	14.53 ± 0.03	4.21
Phenylalanine*	14.29 ± 0.01	4.14
Glutamic acid	13.83 ± 0.08	4.01
Arginine*	13.33 ± 0.13	3.86
Threonine*	9.63 ± 0.20	2.79
Methionine*	4.54 ± 0.04	1.32
Isoleucine*	4.40 ± 0.17	1.28
Cysteine	2.79 ± 0.35	0.81
Total amino acid content	344.92 ± 13.99	100
Total essential amino acids	152.83 ± 12.85	44.30

*:Essential amino acids; sd: standard deviation (n = 9)

(Jorfi et al., 2015). The amino acid profile of sausages can vary depending on factors such as manufacturing method and other ingredients used (Aro et al., 2010; Domínguez et al., 2016; Bär et al., 2020). Our results indicate that beef sausage has a high nutritional value and should be included in a healthy diet.

In addition to the protein-building amino acids, the free amino acid (FAA) composition of a food product is also an important aspect that determines its quality. Various factors such as temperature, ripening time, sausage making technology, and the starter culture used, all have been reported to influence the content and composition of FAA (Aro et al., 2010; Bär et al., 2020). In homemade beef sausage, 20 free amino acids were identified with 40.12 mg kg^{-1} accounting for approximately 10% of the TAA content (Table 3). The most abundant FAAs were Ala (22.81%), Glu (11.62%), Leu (9.20%), and Lys (7.68%) accounting for more than 50% of the total FAA. This is similar to the findings of Gallego et al. (2018), who investigated the free amino acid profile of European dry-fermented pork sausages and identified Ala, Glu, Leu, and Lys as the most abundant amino acids. Kononiuk and Karwowska (2020a) found that fermented sausages made from beef and fallow deer meat with sour whey contained higher concentrations of certain amino acids including Gly, Pro, and Val, than homemade beef sausages. Domínguez et al. (2016) investigated the effect of starter cultures on the free amino acid content of dry-cured foal sausage and found that different starter cultures cause large differences in amino acid composition. FAAs also play an important role in sensory qualities e.g., glutamic acid and aspartic acid contribute to the fresh flavour, glycine and alanine contribute to the sweet taste,

Table 3. Free amino acid composition of homemade beef sausage

Free amino acids	Mean ± sd (mg kg^{-1})	Mean (%)
Alanine	9.15 ± 0.02	22.81
Glutamine	4.66 ± 0.09	11.62
Leucine	3.69 ± 0.26	9.20
Lysine	3.08 ± 0.01	7.68
Glycine	2.25 ± 0.11	5.61
Ornithine	1.79 ± 0.25	4.46
Valine	1.73 ± 0.21	4.31
Threonine	1.72 ± 0.53	4.29
Histidine	1.72 ± 0.32	4.29
Isoleucine	1.56 ± 0.46	3.89
Serine	1.52 ± 0.03	3.79
Tyrosine	1.42 ± 0.01	3.54
Methionine	1.38 ± 0.08	3.44
Arginine	1.29 ± 0.13	3.22
Glutamic acid	0.84 ± 0.20	2.09
Phenylalanine	0.70 ± 0.10	1.74
Aspartic acid	0.66 ± 0.04	1.65
Proline	0.60 ± 0.17	1.50
Cystathionine	0.27 ± 0.35	0.67
Cysteine	0.09 ± 0.02	0.22
Total Free Amino Acids	40.12 ± 2.02	100

sd: standard deviation (n = 9)

arginine, leucine, valine, and phenylalanine contribute to bitter taste, and lysine contributes to both sweet and bitter tastes (Mau and Tseng, 1998).

3.3. Biogenic amine composition of homemade beef sausage

Table 4 shows the biogenic amine (BA) content of homemade beef sausage. The total concentration of biogenic amines was 1.69 mg kg^{-1}. Histamine, agmatine, and tyramine were the most abundant biogenic amines with concentrations of 1.03, 0.28 and 0.16 mg kg^{-1}, respectively. Spermine (Spm) and spermidine (Spd) concentrations of 0.04 and 0.03 mg kg^{-1} were also detected. The BA contents of homemade beef sausages were within the range reported by Kononiuk and Karwowska (2020b) in beef dry fermented sausages (1.7–2.1 mg kg^{-1}). However, the amine profile found in homemade beef sausage differs from what was reported in that study, with histamine being the most abundant rather than putrescine. BA contents in homemade beef sausages were significantly lower than those reported in traditional European pork sausages, where tyramine, putrescine, and cadaverine were the most commonly detected (Latorre-Moratalla et al., 2008). Other studies found significantly higher levels of Spm and Spd in foal sausage made with various starter cultures (Domínguez et al., 2016). Several factors influence the formation of biogenic amines in food, including microbial population growth and activity, amino acid decarboxylase activity, starter culture, and fermentation process (Roseiro et al., 2010; Schirone et al., 2022). The low concentration of biogenic amines in homemade beef sausages could be explained by a lack of starter culture or fermentation.

3.4. Fatty acid composition of homemade beef sausage

The fatty acid profile of the homemade beef sausages is shown in Table 5. The homemade beef sausages contained a total of 13 different fatty acids, with oleic acid (C18:1) accounting for 34.37%, followed by palmitic acid (C16:0; 30.24%) and stearic acid (C18:0) accounting for 18.78%. Short chain saturated fatty acids (SCFA) such as butyric acid (C4:0; 1.16%), caproic acid (C6:0; 0.20%), and capric acid (C8:0; 0.18%) were also present in lower concentrations in the sausages. The SCFA are well-known for their role in colon physiology, acting as energy sources for host cells and the intestinal microbiota, and taking part in various host-signalling mechanisms (Ríos-Covián et al., 2016).

Table 4. Biogenic amine composition of homemade beef sausage

Biogenic amines	Mean ± sd (mg kg^{-1})
Histamine	1.03 ± 1.79
Agmatine	0.28 ± 0.28
Tyramine	0.16 ± 2.25
Putrescine	0.09 ± 0.23
Cadaverine	0.06 ± 0.11
Spermine	0.04 ± 0.06
Spermidine	0.03 ± 0.05
Total biogenic amine content	1.69 ± 0.36

sd: standard deviation (n = 9)

Table 5. Fatty acid composition of homemade beef sausage

Fatty acids	%
Butyric acid C4:0	1.16
Caproic acid C6:0	0.20
Capric acid C8:0	0.18
Caprylic acid C10:0	0.56
Myristic acid C14:0	5.45
Pentadecanoic acid C15:0	0.80
Palmitic acid C16:0	30.24
Heptadecanoic acid C17:0	1.73
Stearic acid C18:0	18.78
Palmitoleic acid C16:1	4.26
Oleic acid C18:1	34.37
Linoleic acid C18:2	2.27
Saturated fatty acids	59.10
Monounsaturated fatty acids	38.63
Polyunsaturated fatty acids	2.27
Unsaturated fatty acids/saturated fatty acids ratio	0.69

The unsaturated fatty acids found in the homemade beef sausage included 34.37% oleic acid (C18:1) and 2.27% linoleic acid (C18:2). According to emerging scientific evidence, the type of fatty acids in the diet plays a crucial role in determining our health outcomes. Oleic acid has been shown to have a variety of health benefits, including lowering blood pressure and oxidative stress (Shen et al., 2022). On the other hand, linoleic acid, a polyunsaturated fatty acid, is considered an essential fatty acid because it cannot be synthesised by the body and must be obtained through diet. Recent research suggests that linoleic acid may protect against heart disease and improve cognitive function by acting as a precursor to important compounds such as prostaglandins and leukotrienes that are involved in inflammation and immune function regulation (Belury, 2023).

The homemade beef sausages contained high amounts of saturated fatty acids (59.10%) and monounsaturated fatty acids (38.63%), but only a low amount of polyunsaturated fatty acids (2.27%). The unsaturated to saturated fatty acid ratio was 0.69, indicating that the homemade beef sausages were high in saturated fatty acids and low in monounsaturated and polyunsaturated fatty acids.

4. CONCLUSIONS

Homemade beef sausage was investigated for amino acid, biogenic amine, and fatty acid contents. Given the limited information on beef sausage, this study provides useful nutritional data. The homemade beef sausage contains high-quality proteins with a variety of essential amino acids that are important for human health. We have found low level of biogenic amines, which also indicates good quality product. In terms of fatty acid composition, it is recommended to improve the technological process in order to produce a more nutritious homemade beef sausage.

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