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INVESTIGATION OF THE CHEMICAL COMPOSITION, PHYSICOCHEMICAL PROPERTIES, AND MICROSTRUCTURE OF MEAT PATTIES WITH AMARANTH FLOUR

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Abstract
This study aimed to investigate the effect of adding amaranth flour to meat patties on their chemical composition. Four different variations of meat patties were prepared, with amaranth flour added at concentrations of 5%, 10%, and 15% in place of beef. The control sample was prepared without any addition. The results of the study showed that the addition of amaranth flour led to a significant decrease in the moisture content of the meat patties, while the proportions of carbohydrates, fat, and ash increased. Specifically, the patties with the highest concentration of amaranth flour (15%) had the highest proportions of carbohydrates and fat with the lowest proportion of moisture. The control sample had the highest moisture content and the lowest proportion of carbohydrates, fat, and ash. The addition of amaranth flour increased the water-holding capacity of the meat patties, with the highest increase observed in the sample with 15% amaranth flour (82.21%). The overall score of sensory evaluation of the meat patties did not significantly decrease with the addition of up to 10% amaranth flour, according to the sensory evaluation. The study provides evidence that up to 10% amaranth flour can be used as a substitute for beef in meat patties, which can lead to an increase in the fat and carbohydrate content and mineral composition and improvement of the water-holding capacity of the final product.

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Introduction

In recent decades, there has been a growing trend in the meat product market toward the consumption of combined meat-plant products [1,2]. Meat products with plant ingredients are food products that contain both animal-based protein and plant-based ingredients. Adding plant ingredients to meat products can enhance their nutritional value by increasing the protein, fiber, vitamin, and mineral content [3–5]. One approach to enhance the textural characteristics of meat products is to incorporate plant-based ingredients. Plant-based ingredients can provide a range of functional benefits, such as enhancing water-binding capacity, emulsification, and gelling properties, which can improve the texture and mouthfeel of meat products [6,7].

Using plant ingredients in meat products can help to reduce the amount of meat that is consumed, which is beneficial for both health and environmental reasons [8–10]. Plant-based proteins are often less expensive and have a smaller environmental impact compared to animal-based proteins, so incorporating them into meat products can help to reduce the cost and environmental footprint of meat production [11,12].

Amaranth is a group of plants that belong to the genus Amaranthus [13]. Amaranth is grown and consumed in many parts of the world, but the largest producers of amaranth are mainly concentrated in Latin America (Peru, Mexico) and Asia (China, India, Nepal). Amaranth has been cultivated for thousands of years for its edible leaves, seeds, and stems, and is an important source of nutrition and income for many communities [14,15]. The consumption of amaranth is recommended for patients with ischemic heart disease and atherosclerosis, diabetes and obesity, cancer, and a weakened immune system. Amaranth flour and products made from it have a preventive effect on many body systems: they lower cholesterol levels, improve the condition of arteries, reduce the risk of cardiovascular and oncological diseases, promote the elimination of toxins [16,17].

The seeds of the amaranth plant are also highly nutritious and are often used as a gluten-free grain. Amaranth seeds are a good source of protein, fiber, iron, magnesium, and other essential nutrients. According to the chemical composition, amaranth contains 18.0–19.6% protein, 8.0–8.6% fat, 3.5–5.5% fiber, and 65.0–70.0% carbohydrates [18,19]. Amaranth is particularly high in essential amino acids, which are amino acids that the body cannot produce and must obtain from food. The amino acid profile of amaranth is notable for its high content of glutamic acid, aspartic acid, and arginine. These three amino acids alone make up over 45% of the total amino acid content of amaranth, with glutamic acid being the most abundant at 23.82 g/100g [20]. Other amino acids that are present in high amounts in amaranth include glycine, leucine, lysine, serine, phenylalanine, and valine. These amino acids are all important for various functions in the body, including protein synthesis, energy production, and the maintenance of healthy tissues [21,22].

In addition, amaranth seeds are rich in a range of B-group vitamins, tocopherol, provitamin A, ascorbic acid, and vitamin D. Amaranth flour is also a good source of various minerals, including iron, calcium, potassium, phosphorus, magnesium, copper, and others. Moreover, amaranth flour contains dietary fibers, which have an important advantage in reducing the calorie content of the diet and promoting a persistent sense of satiety [23]. Amaranth flour can be used in sausage production as a gluten-free and nutrient-dense ingredient that can improve the texture, flavor, and nutritional value of the final product [24,25].

The aim of this study is to investigate the influence of amaranth flour on the physicochemical properties, sensory characteristics, and nutritional value of meat patties.

Materials and Methods

Production of meat patties

Chicken meat and beef were deboned and trimmed of tendons, and connective tissues. The weighed meat was cut into pieces and ground using a meat grinder with a 3 mm plate. To prepare the forcemeat, the ground meat and non-meat ingredients, water, and spices were weighed and loaded into a meat mixer and mixed for 3–5 minutes.

Next, oval-shaped patties with a thickness of 2–2.5 cm were formed from the prepared forcemeat. The forming process was done using the IPCS-123M patty-making machine (Elf 4M Company, Russia). After forming, the semi-finished products were placed in a single row and sent to a freezing unit (at a temperature of –35 °C). The frozen semi-finished products were then packaged in a polymer, cardboard, or other packaging material and stored at a temperature of –10 °C to –30 °C.

According to the recipe (Table 1), four variations of meat patties were prepared, with the addition of 5%, 10%, and 15% amaranth flour instead of beef, along with a control sample without any addition.

Table 1. Recipe variants of meat patties, %

Ingredient	% of amaranth flour			
	1	2	3	4
Chicken meat	28	28	28	28
Beef	37	32	27	22
Amaranth flour	0	5	10	15
White bread	10	10	10	10
Milk	11	11	11	11
Egg	4.5	4.5	4.5	4.5
Creamy butter	4.5	4.5	4.5	4.5
Onion	4.5	4.5	4.5	4.5
Salt	0.4	0.4	0.4	0.4
Black pepper	0.1	0.1	0.1	0.1

Determination of chemical composition

The determination of the chemical composition (moisture, fat, ash, and protein) was based on the methods previously described in [26].

Determination of the mineral composition

A high-pressure Teflon container was used to hold between one and two grams of the sample. The sample was then burned in a muffle furnace for 4 hours at 400 °C, followed by an additional 2 hours at 600 °C. One gram of the resulting ash (measured by dry weight) was then digested by adding 3 mL of HNO_3 and 2 mL of HF. The mixture was then heated in a Berghof Speed Wave microwave system at 200 °C for 20 minutes. After this process, the samples were placed in a 10 mL container and diluted with 1% HNO_3. The content of elements in muscle samples was determined with an inductively coupled plasma mass spectrometric method (ICP-MS, Varian-820 MS, Varian Company, Canberra, Australia) [27].

pH determination

To determine the active acidity (pH), the potentiometric method was used. The sample was ground twice and mixed with distilled water in a ratio of 1:10. This mixture was then stirred on a magnetic stirrer for 30 minutes. The pH value was determined by HI 99163 instrument (Hanna Instruments Inc., USA) [28].

Determination of water-binding capacity

Water-binding capacity (WBC) was identified by the method proposed by Grau and Hamm using filter paper and a weighted press. The weight of the test sample was 0.3 g [29].

Statistical analysis

The experiments were carried out in triplicate. Standard deviation values are given for all measurements. Differences between the experimental and control groups were calculated using a one-way ANOVA with Tukey test. $p < 0.05$ was considered significant.

Results and discussion

Chemical composition of meat patties

The addition of amaranth flour significantly affected the moisture, fat, ash, and carbohydrate content of meat patties. For instance, replacing beef mince with amaranth flour at a concentration of 5% to 15% resulted in a decrease in moisture from 70.25% to 66.49%-58.91%, respectively (Table 2). This can be primarily attributed to the high content of dry matter in the flour, which, in turn, substantially increased the carbohydrate content from 5.86% to 16.36%. A slight increase in ash and fat was observed in the experimental samples of patties with amaranth flour. The protein content underwent little significant change, which characterizes the equivalent substitution of animal protein with plant protein.

The observed changes in the composition of meat patties resulting from the addition of amaranth flour have important implications for the nutritional and sensory quality of the final product. The reduction in the moisture content may contribute to a firmer texture and longer shelf life, as lower moisture levels limit microbial growth and spoilage. However, excessively low moisture levels can also result in a dry, unappetizing product [30]. The significant increase in the carbohydrate content resulting from the addition of amaranth flour is noteworthy, as it can provide an additional source of dietary fiber and nutrients for consumers [31,32].

Table 2. Chemical composition of meat patties, %

Indicator	% of amaranth flour			
	0	5	10	15
Moisture	70.25±0.93^{d}	66.49±0.91^{c}	62.69±0.68^{b}	58.91±0.76^{a}
Protein	14.83±0.34^{a}	14.74±0.30^{a}	14.66±0.29^{a}	14.56±0.22^{a}
Fat	7.88±0.17^{a}	8.16±0.14^{a}	8.47±0.16^{b}	8.78±0.13^{b}
Ash	1.16±0.02^{a}	1.26±0.02^{a}	1.30±0.02^{b}	1.37±0.03^{b}
Carbohydrate	5.86±0.13^{a}	9.33±0.18^{b}	12.86±0.12^{c}	16.36±0.20^{d}

$^{a, b, c, d}$ **means within the same row, with different letters meaning there is a significant difference among different samples of sausages ($p < 0.05$)**

Verma *et al.* [33] reported comparable findings, indicating that goat meat nuggets containing 3% amaranth flour and 1.5% quinoa had substantially lower moisture levels compared to the control group. Similar to our findings, Bağdatli [34] determined that the protein content of meatballs with quinoa flour did not change significantly, but had a significant effect on the fat and moisture contents of beef meatballs. The study showed that substituting wheat rolls with a combination of hemp seeds, amaranth, and golden flaxseed resulted in a positive impact on the protein content of poultry pates [35].

Mineral composition of meat patties

The mineral composition of meat patties underwent changes upon the addition of amaranth flour (Table 3). The calcium content increased to 48.91 mg/100g in variant 2, 55.46 mg/100g in variant 3, and up to 62.01 mg/100g in variant 4 in comparison to variant 1 without amaranth flour. A similar trend was observed for potassium, magnesium, phosphorus, iron, manganese, and copper (Table 3). Meanwhile, the sodium, sulfur, and zinc content were slightly reduced. The changes in the quantitative composition of the mineral substances in different variants of the meat patties can be explained by the content of these mineral substances in amaranth flour. Amaranth is rich in calcium (up to 150 mg/100g), magnesium (200–240 mg/100g), potassium (430–490 mg/100g), iron (up to 7.6 mg/100g), and other elements.

Table 3. Mineral composition of meat patties, mg/100g

Element	% of amaranth flour			
	0	5	10	15
Potassium	318.13±4.83^{a}	326.53±4.59^{a}	334.93±3.31^{b}	343.33±3.60^{b}
Calcium	42.36±0.68^{a}	48.91±0.91^{b}	55.46±0.58^{c}	62.01±0.89^{d}
Magnesium	21.07±0.16^{a}	32.32±0.42^{b}	43.57±0.59^{c}	54.82±0.96^{d}
Sodium	100.13±2.16^{b}	97.48±0.72^{b}	94.83±0.57^{a}	92.18±0.57^{a}
Sulfur	138.78±2.01^{d}	127.70±1.62^{c}	116.61±1.07^{b}	105.53±1.36^{a}
Phosphorus	160.49±2.51^{a}	178.14±2.39^{b}	195.79±2.66^{c}	213.44±2.99^{d}
Iron	1.21±0.02^{a}	1.61±0.03^{b}	1.93±0.04^{c}	2.17±0.04^{d}
Manganese	0.13±0.002^{a}	0.30±0.003^{b}	0.46±0.004^{c}	0.61±0.01^{d}
Copper	0.07±0.001^{a}	0.09±0.002^{b}	0.12±0.002^{c}	0.14±0.002^{d}
Zinc	1.75±0.03^{b}	1.69±0.02^{b}	1.62±0.03^{ab}	1.56±0.02^{a}

$^{a, b, c, d}$ **means within the same row, with different letters meaning there is a significant difference among different samples of sausages ($p < 0.05$)**

The findings revealed that the addition of amaranth flour to meatballs significantly increased their iron content. The control sample without the addition of amaranth flour had a mean iron content of 1.21 mg/100g. In contrast, the experimental samples with 5%, 10%, and 15% amaranth flour had mean iron contents of 1.61 mg/100g, 1.93 mg/100g, and 2.17 mg/100g, respectively. The highest iron content was observed in the experimental sample with 15% amaranth flour, indicating a dose-dependent response. The observed increase in the iron content may be attributed to the high iron concentration of amaranth flour. Amaranth is a good source of bioavailable iron, which is essential for human health [36].

The control sample without adding amaranth flour had a magnesium content of 21.07 mg/100g. This value served as a reference point for the other experimental samples. The results show that as the percentage of amaranth flour increased in the experimental samples, the magnesium content also increased. Specifically, the sample with 5% amaranth flour had a magnesium content of 32.32 mg/100g, while the sample with 10% of amaranth flour had a magnesium content of 43.57 mg/100g. The highest magnesium content was observed in the experimental sample with 15% of amaranth flour, which had a magnesium content of 54.82 mg/100g.

Magnesium is an essential mineral that plays a crucial role in many bodily functions, such as muscle and nerve function, blood pressure regulation, and bone health [37,38]. These findings suggest that the addition of amaranth flour to meat cutlets can increase their magnesium content. Amaranth flour is a good source of magnesium, and its incorporation into meat cutlets can be an effective way to increase the magnesium content of meat-based dishes. The observed increase in the magnesium content with increasing amounts of amaranth flour could be due to the higher magnesium content of amaranth flour, as well as the fact that the amaranth flour may have facilitated the absorption of magnesium in the meat patties.

The control sample without amaranth flour had the highest zinc content at 1.75 mg/100g. In contrast, the experimental samples with 5%, 10%, and 15% amaranth flour had progressively lower zinc content of 1.69 mg/100g, 1.62 mg/100g, and 1.56 mg/100g, respectively. The results show that as the proportion of amaranth flour increased, the zinc content decreased, indicating an inverse relationship between the two variables. The difference in the zinc content between the control and experimental samples was minimal, with a maximum reduction of 0.19 mg/100g.

Zinc is an essential nutrient that plays a critical role in various physiological functions, including immunity, wound healing, and growth and development. However, zinc overdose can cause gastrointestinal issues, copper deficiency, reduced immune function, anemia, headaches, fatigue, and reduced HDL cholesterol [39,40]. The decrease in zinc the content with the addition of amaranth flour could be due to various factors. Amaranth flour is rich in phytates, which are compounds that can bind to minerals like zinc, making them less available for absorption in the body. Additionally, the heat processing during cooking could have also contributed to the decrease in the zinc content [41].

The study conducted by Kobzhasarova *et al.* [42] found that the use of amaranth flour in cooked sausage increased the content of macroelements such as calcium by 69.7%, potassium by 6.4%, phosphorus by 39.96%, and magnesium by 59.5% compared to cooked sausage without amaranth flour. According to Behailu's research conducted in 2020, the amount of calcium present in beef sausage demonstrated a significant rise (at a significance level of 0.05) when the level of soybean and millet flours inclusion increased [43].

Microstructure of meat patties

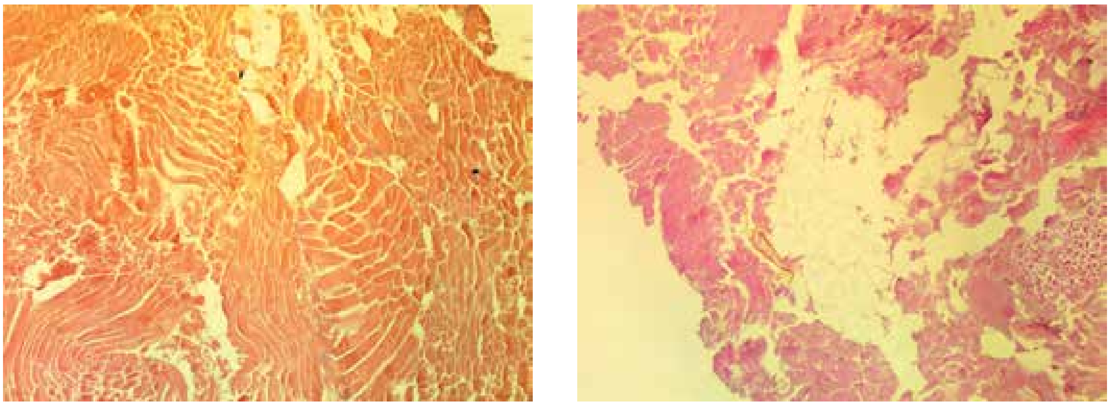
While examining the microstructure of meat patties, it was discovered that there were sections in the ground meat composition that contained plant ingredients. In the microstructure of the meat mixture that contained plant additives, muscle fibers that were cut both longitudinally and transversely were arranged with small gaps in between them (Figure 1). Furthermore, there was a finely structured pink-colored mass. The fatty tissue had a distinctive cellular structure with visible cell membranes and unpigmented content. Additionally, the presence of amaranth flour may increase the number of small spaces and gaps between muscle fibers, as the flour particles may interfere with the arrangement of the fibers during cooking. Microscopic analysis may reveal the presence of amaranth flour particles within the meat matrix, as well as changes in the organization and spacing of the muscle fibers. Furthermore, the addition of flour may alter the color and appearance of the meat, as the flour particles may absorb or reflect light differently than the meat tissue [44,45].

Overall, the microstructural changes that occur when mixing ground meat with amaranth flour may have implications for the texture, nutritional content, and sensory properties of the final product. Further research is needed to fully understand the impact of different types and levels of amaranth flour on the microstructure and quality of meat products.

In the histological preparation, fragments of connective tissue and large and small particles of amaranth flour were observed among loosely arranged muscle fibers. The nuclei of the plant additive cells were round and significantly larger than the nuclei of muscle fibers and connective tissue cells. The nuclei of muscle fibers and connective tissue cells were poorly visible compared to the nuclei of plant tissue cells when viewed under a x4 objective lens.

Sensory evaluation

The evaluation results of the sensory characteristics by the taste panel showed the highest score for variant 3 with the addition of 10% amaranth flour. In this variant, consistency, aroma, taste, and appearance of the meat patties were rated the highest compared to the other variants (Figure 2).



Control

Meat patties with 10% of amaranth flour

Figure 1. Microstructure of meat patties

Overall, variants 1, 2, and 3 were positively evaluated by the tasters, with minor differences. The lowest score was recorded for variant 4 (15% amaranth flour in the composition). This variant showed crumbliness, deterioration in taste, the appearance of a pronounced odor not typical for meat patties, and a disruption in consistency. The results of the sensory analysis indicate that adding up to 10% amaranth flour to the composition of meat patties does not lead to significant losses in quality and appearance.

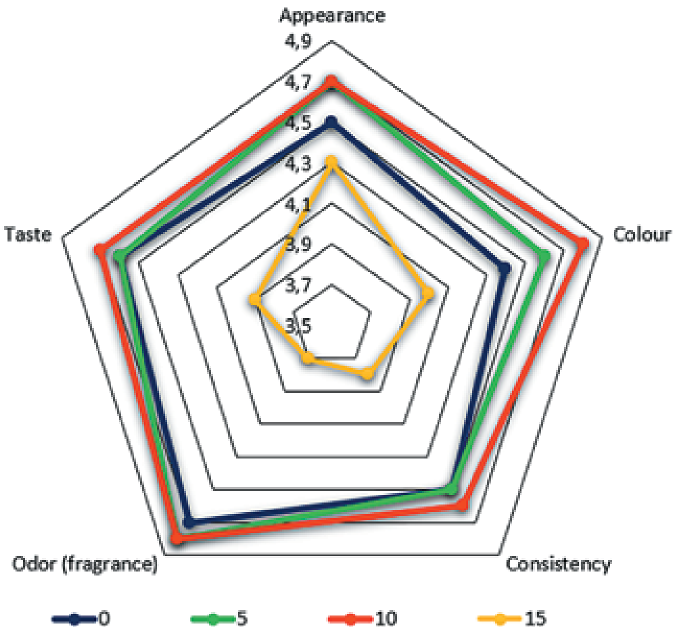


Figure 2. Sensory score diagram of meat patties

Behailu *et al.* [43] reported that the addition of the addition of soybean and finger millet flours to the beef sausage product enhanced the sensory quality of the sausage. The sausage products were generally accepted, and 20% soy and millet flour inclusion was "liked very much" [43]. In the study conducted by Muchekeza *et al.* [46] indicated no significant differences in the color of the sausages made with amaranth, quinoa, and corn-starch binder flours.

Determination of pH and water-holding capacity

The pH of meat is an important characteristic that influences product quality. In this study, pH values of meat patties were investigated after adding different concentrations of amaranth flour. The control sample without amaranth flour had a pH value of 6.14, which is in line with the normal pH range for meat products (Figure 3). The experimental samples with amaranth flour showed a slight decrease in pH as the concentration of amaranth flour increased. The sample with 5% amaranth flour had a pH of 6.10, while the samples with 10% amaranth flour had a pH of 6.10%.

The lowest pH value was observed in the sample with the highest concentration of amaranth flour (15%), which had a pH value of 6.0. This result suggests that the addition of amaranth flour to meat patties can lead to a slight decrease in pH, which may be attributed to the acidity of amaranth flour. It is important to note that the observed differences in pH values were relatively small and may not have a significant impact on the overall quality of the product.

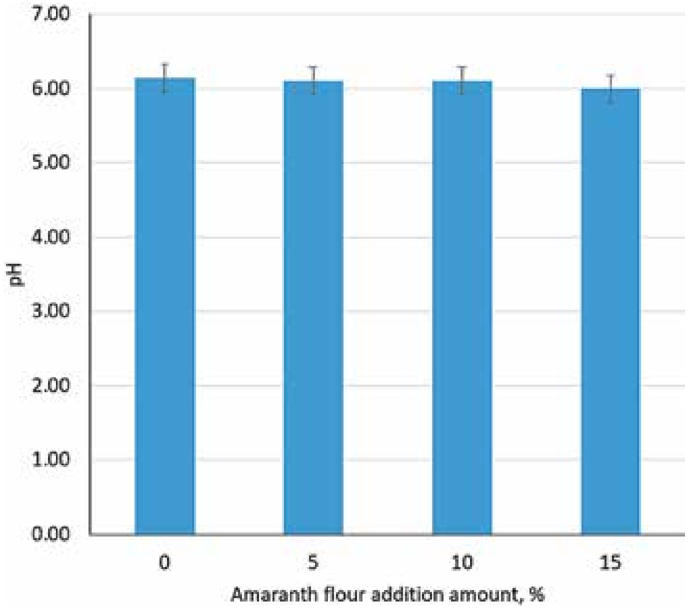


Figure 3. pH of meat patties

Water-holding capacity is a key characteristic of meat products, as it affects juiciness, texture, and overall quality. The results indicate that the addition of amaranth flour led to an increase in the water-holding capacity compared to the control sample. The water-binding capacity of the control sample and experimental samples with 5% and 10% of amaranth flour was 68.54%, 71.24%, and 74.58%, respectively (Figure 4). The maximum water-binding capacity was observed in the sample with 15% amaranth flour, which was 81.21%. These findings suggest that amaranth flour has the potential to improve the water-binding capacity of meat products. Ostoja *et al.* [47] found that the use of crude amaranth seed grit improved the ability of meat-fat batter to hold water, resulting in decreased cooking losses of canned meat after pasteurization or sterilization.

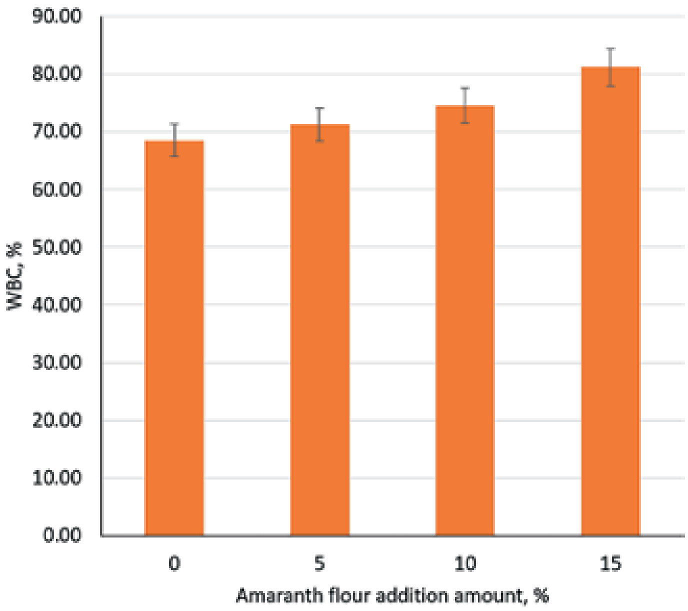


Figure 4. Water-holding capacity of meat patties

The mechanism behind this effect is likely due to the unique properties of amaranth flour, which contains high levels of soluble fiber and protein. Soluble fiber has been shown to increase water-binding capacity by forming gels that trap water molecules, while protein can contribute to the formation of a stable emulsion that helps retain water [48,49].

Conclusion

Based on the research findings, it can be concluded that the addition of amaranth flour to meat patties can lead to significant improvements in their nutritional and sensory properties. The chemical composition of the patties was affected, with a decrease in moisture and slight increases in ash and fat observed in the experimental samples. The addition of amaranth flour also led to a significant increase in the content of important minerals such as calcium, potassium, magnesium, phosphorus, iron, manganese, and copper. The sensory evaluation of the patties showed that the addition of 10% amaranth flour resulted in the highest score for overall sensory characteristics. Furthermore, the incorporation of amaranth flour resulted in an enhanced water-binding capacity of the meat patties compared to the control sample. Overall, these findings suggest that using amaranth flour in meat patties formulation could be a promising strategy for improving their nutritional value and sensory quality.

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