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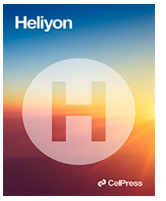

Research article

Effect of roasting temperature and soaking time on the nutritional, antinutrional and sensory properties of protein-based meat analog from lupine

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ABSTRACT

White lupine is a legume crop rich in adequate valuable nutrient profiles especially used as a possible source of proteins where animal-based proteins are scarce. However, there is little documented information about the effect of processing conditions to produce lupine protein-based meat analog. This study explores the impact of roasting temperature (raw, 130, 140, and 150 °C) and soaking time (raw, 2, 4, and 6 days) on the chemical compositions, physical quality, and sensory attributes of meat analog. The result showed that roasting at 140 °C and soaking for 4 days significantly increased ($p<0.05$) the proximate and mineral contents of the meat analog. The highest protein content (82.46 %) was obtained at T_2t_2 (roasted at 140 °C and soaked for 4 days). While the lowest protein content (62.47 %) was observed at T_3t_3 (roasted at 150 °C and soaked for 4 days). Similarly, the highest (93.17 %) and lowest (79.47 %) cooking yields were obtained at T_2t_2 and T_3t_3 respectively. Roasting and soaking conditions also showed a significant effect ($p<0.05$) on the anti-nutrient contents of meat analog. The highest overall sensory acceptability (6.40) of the meat analog was observed at T_2t_2. The research suggests that suitable processing conditions can enhance the nutritional profiles of lupine protein-based meat analog, potentially enabling its industrial production and global market entry.

1. Introduction

Animal proteins are more expensive than plant-based proteins, which lead to scarcity, and malnutrition, which is a serious problem in the world right now, especially in developing nations. There have been over 870 million people worldwide experience hunger every day, and 793 million people are still considered to be undernourished [1]. The prevalence number of protein energy malnutrition in 2019 was 147,672,757 and has been projected more than 160 million by 2044 in the world [2]. Meat is the top option for non-vegetarian consumers worldwide because it can fulfil all of their desired requirements i.e. meeting their nutritional needs [3]. However, it has different types of drawbacks such as type 2 diabetes, cardiovascular diseases and cancers [4].

Moreover, the increasing size of the human population is one of the most significant arguments against eating too much meat. In 2017, there were 7.5 billion people on the planet, but according to UN projections, that number will rise to 8.5 billion in 2030 and even 10 billion in 2050 [5]. A very serious issue arises when feeding such a big population due to the observed rapid population expansion

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and the inefficiency of present food habits. Instead of low-calorie intake, a protein shortage is the primary cause of malnutrition [5]. Presently, the production of cattle accounts for 14.5–18 % of human-caused greenhouse gas (GHG) emissions i.e. methane and nitrous oxide are gases that are released during the production of cattle and have a greater potential to cause global warming than carbon dioxide [6]. With the present and forecast consumption rates, meat production will be unsustainable by 2050 [7]. The world's animal protein demand and production have increased significantly and are projected from 229 billion kg for 6 billion people in 2000 to 465 billion kg for 10 billion people by 2050) [8]. Plants use about 18 times less land and 10 times less water to produce 1 kg of protein than beef does [9]. With the manufacture of tofu, tempeh, seitan, and other conventionally produced plant-based foods, the development of protein-rich plant-based foods with the potential to replace meat in a nutritional sense has already been investigated [10].

In recent years, there has been a growing interest in plant protein-based meat analogs [11]. These products offer several advantages over traditional meat, including a higher protein content, a lower glycemic index, and a more sustainable production process. Meat analogs are plant-based products that are designed to mimic the taste, texture, and appearance of meat [12]. In comparison to natural meat, plant-based meat analogs have demonstrated a number of benefits, including decreased obesity, blood pressure, and cholesterol, as well as favourable psychological effects on human health [6,13]. Additionally, since plant-based meat substitutes are meatless, they also solve concerns related to animal welfare [14]. In general, various factors, such as health issues like sensitivities to animal proteins, saturated fats, trans fats, and milk hormones, ethical and environmental concerns from some consumer groups, and positive health claims associated with plant-protein-based diets, have all contributed to the continued growth in demand and drive towards plant protein consumption [15].

Meat analogs are made from a variety of ingredients, including soy protein, wheat gluten, pea protein, textured vegetable protein (TVP) and mycoproteins (found in mushrooms), insects, and microalgae [16]. Plant-based meat analog is commonly made from soya proteins [17] which are relatively costive and more utilized than lupine. Soybean is used for different commercial products like edible oils, milk, cheese, and yogurt. So, the production of meat analog from soybeans may reduce utilization of it for other food products [18]. Even though, though lupine has the potential to provide significant nutrition for the global population due to its high protein content, it is an underutilized legume crop due to its antinutritional content and lack of awareness. Lupine protein-based meat analogs are a type of plant-based meat alternative that is made from lupine protein isolate, that is a good source of protein, fiber, iron, zinc, and magnesium [4,8]. It is also a good source of vitamins (B-vitamins), phenolic compounds and antioxidants [19]. The fiber from lupine can help to improve digestion and reduce the risk of chronic diseases such as heart disease and diabetes [20].

White lupine (*Lupinus albus* L.*)* is a legume that is native to the Mediterranean region and has been made available as a novel food source in 1996 [21]. White lupine is comparable even higher in protein content (40–45 %) [22] than soybean protein content (37–42 %) [18] and less expensive. White lupine is a good source of fiber (25–30 %), iron (essential for red blood cell production), zinc and magnesium (important for bone health and muscle function) [23]. The protein content of lupine is comparable to even higher than that of red meat, the amount of protein in 100 g of raw red muscular beef is about 20–25 g, while cooked red meat has a protein value of 28–36 g/100 g [24]. White lupine has little usage in commercial products since it is considered a food source of low income for people and underutilized due to its anti-nutrient contents (quinolizidine component of alkaloids especially lupanine and sparteine) which can be removed by soaking and roasting [25]. In 2018, there were 1,188,212 tons of lupines produced worldwide, with Australia accounting for 60.11 % of the entire supply [26]. In the 2017/2018 crop year, Ethiopia produced 246,294.20 quintals of lupine [26]. However, there is a limited trend to change white lupine as a commercial product due to bitter alkaloid components and other anti-nutrient factors [27]. Different processing methods can be applied to mitigate the aforementioned problem. Pretreatments such as roasting and soaking are known processing methods that can improve the quality of lupine protein by reducing its anti-nutrient contents, especially its bitter components [28]. Anti-nutrients can bind the protein and affect mineral absorption. There have been several kinds of literature that can elaborate on why we are using these processing methods of lupine mentioned above. Therefore, this study aimed to investigate the effects of roasting temperature, soaking time and their combined effect on the chemical compositions, physical quality and sensory attributes of lupin protein-based meat analog.

2. Materials and methods

2.1. Materials and chemicals collection

The white lupine sample was randomly purchased from the local market of Sekela district, Amhara, Ethiopia and packed in polyethylene bags. The other raw materials (extra virgin olive oil, cornstarch, salt, food colorant, and sodium mono-glutamic acid as a flavoring agent) were purchased from the supermarket available in Bahir Dar City. All reagents and laboratory chemicals used in this work were analytical grade purchased from (Sigma-Aldrich Chemie GmbH Eschenstr. 5, 82024 Taufkirchen, Germany).

2.2. Sample preparation

2.2.1. White lupine flour preparation

White lupine flour was prepared according to Ref. [29] with little modification. A white lupine seed sample was graded, sorted, and cleaned manually to remove foreign matter, and immature and damaged seeds. Then about 20 kg of cleaned raw white lupine bean was taken and 10 kg was roasted at 130, 140, and 150 °C for 12 min and the remaining was soaked for 2, 4, and 6 days. The roasted lupine was soaked for 2, 4, and 6 days in a bucket with tap water (1:10, lupine: tap water) and the water was changed within 8 h intervals. The roasted and soaked lupine was de-hulled, washed with tap water, and dried at 60 °C using oven drying (PHG-9140, China). The dried beans were milled with the laboratory grinder (Coffee Grinder-120 g, China) and sieved to pass a 60 μm sieve, packed in sealable

polyethylene bags, and stored at refrigerated temperature (4 °C) until protein isolation and analysis were carried out.

2.2.2. Lupine protein isolation

Lupine protein isolate was prepared by wet fractionation process based on the method of Ferawati [30] shown in Fig. 1. Roasting and soaking conditions of lupine have both negative and positive effects on the resulting lupine protein isolate. Roasting and soaking of lupine at optimum conditions can improve the quality of lupine protein isolate while at unoptimal conditions can affect negatively the quality of lupine protein such as sensory, functional quality attributes and its yield as well.

2.3. Development of white lupine protein-based meat analog

Lupine meat analog was developed according to the method of Bakhsh, Lee, Sabikun et al. [31], (Fig. 2). A preliminary experiment was done to estimate the desired pasting temperature, the proportion of minor ingredients, and water. The pasting temperature of the protein isolate was good at 70 °C. The obtained meat analog was oven-dried (PHG-9140, China) at 50 °C according to Seleem & Omran [32] and then ground in the laboratory grinder (Coffee Grinder-120 g, China) to produce flour which passes through a 60-mesh sieve size. The meat analog flours were then packed in a polyethylene bag and stored at 4 °C -refrigerated temperature-for further analysis.

2.4. Physical and chemical quality analysis of meat analog

2.4.1. Physical quality analysis

The physical quality of the meat analog (diameter, thickness reduction and cooking yield) was determined according to the method of Mishal et al., [33]. The pH was determined according to AOAC [34] with the official method number 942.15. The texture was determined by the method of Anton & Luciano [35].

The diameter and thickness reduction were calculated using equations (1) and (2) respectively.

$$\text{Diameter reduction } (\%) = \frac{D_1 - D_2}{D_1} \times 100 \quad [1]$$

where: D_1 = diameter of raw meat analog patties, D_2 = diameter of cooked analog patties

$$\textit{Thickness reduction } (\%) = \frac{r_1 - r_2}{r_1} \times 100 \quad [2]$$

where: r_1 = thickness of raw analog patties, r_2 = thickness of cooked analog patties.

The cooking yield was calculated from the following equation (3):

$$\textit{Cooking yield } (\%) = \frac{\textit{weight of cooked patty}}{\textit{weight of raw patty}} \times 100 \quad [3]$$

The texture of the meat anaolog was determined according to Ref. [35]. The texture analyzer (TA. XT plus, Stable Micro Systems, UK) equipped with a 2 kg load cell was used. The texture analyzer was calibrated at trigger force = 5 N, distance = 35 mm, speed = 4 mm/s, and loading weight = 2 kg. The meat analog of 40 mm long was compressed with a probe 35 mm long at a crosshead speed of 3 mm/s to 4 mm/s of the original diameter of the meat analog. The compression generated a curve with the force over a distance. The distance, force, and time were recorded at peak value. The highest values of force (peak value) were taken as a measurement for hardness.

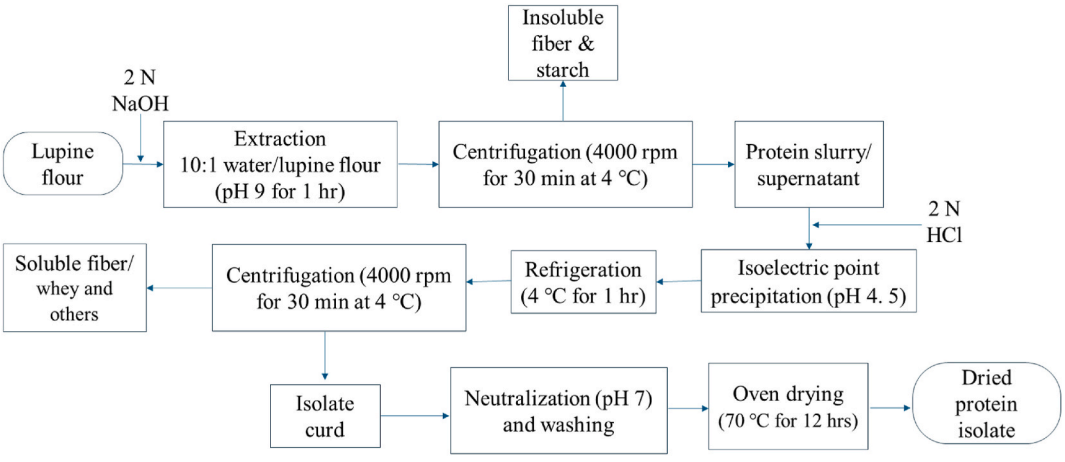

Fig. 1. Lupine protein isolation process flowchart.

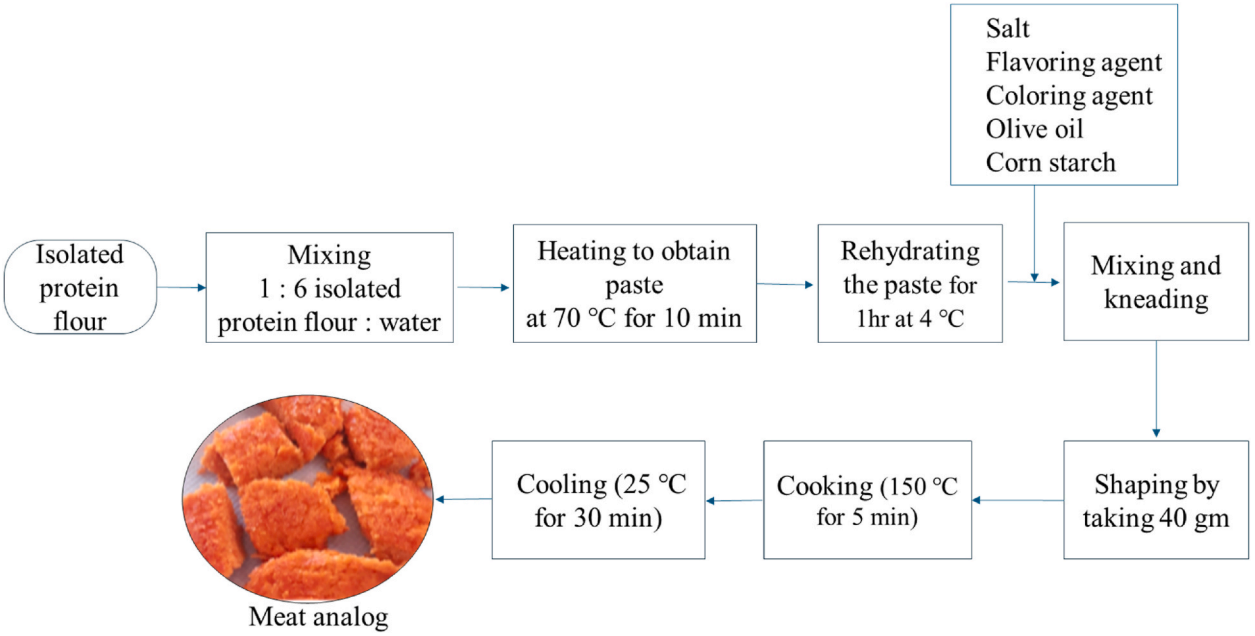

Fig. 2. Lupine protein-based meat analog development process.

An electronic benchtop pH meter (PHS–3C pH meter, China) was used to measure the pH. The standard buffer of pH 4 and pH 7 were used to calibrate the pH meter before the reading. Around 4 g of meat analog samples were homogenized with 25 mL of distilled water and used to measure pH.

2.4.2. *Proximate analysis*

The moisture content of meat analog samples was determined following AOAC [34] using the official method number 925.09 which was an oven-drying method (PHG-9140, China). Crude protein content was analyzed by the Kjeldahl method (DK6, VELP Scientific, Italy) according to AOAC [34] using the official method number 979.09. The crude fat content of meat analog was determined according to AOAC [34] official method number 4.5.01 using the soxhlet extraction method (heater model-98-I-BN, China). The total ash content of meat analog was determined using the official method number 923.03 of AOAC [34] using a muffle furnace (FHX-05, Korea). The crude fiber content of meat analog was determined according to AOAC [34] official methods number 962.09 by using base and acid digestion methods. The total carbohydrate content of meat analog was determined by a difference method described by Anberbir et al., [36]. The total energy of the meat analog sample was determined according to AOAC [34] by multiplying the value of total carbohydrate, crude protein and crude fat by the factors 4, 4 and 9 respectively. The results are reported on dry base value.

2.4.3. *Mineral analysis*

The iron (Fe) content of meat analog was determined according to AOAC [34] the recommended procedures of a photo calorimeter (Model number 1312, India) at a wavelength of 470 nm.

The zinc content was determined according to AOAC [36] official method No.985.35 using atomic fluorescence spectrophotometry (AF420, USA) with a hollow cathode lamp (213.9 nm wavelength).

The metal contents of Fe and Zn were calculated using equation (4):

$$\text{Metal content}\left(\frac{\text{mg}}{100\ \text{g}}\right) = \frac{(\text{a} - \text{b})}{\text{W} \times 10} \times \text{V} \quad [4]$$

where: W = weight in gm of sample, V = volume in ml of the extract, a = concentration in ppm of sample solution and b = concentration in ppm of blank solution.

2.4.4. *Anti-nutrient contents analysis*

The alkaloid content of meat analog was determined gravimetrically by the method of Abeshu & Kefale [37]. Total alkaloid content was calculated from equation (5):

$$\text{Alkaloid content}\ (\%) = \frac{(W_{fb} - W_{fa})}{W_1} \times 100 \quad [5]$$

where: W_{fb} = Weight of filtrate before drying (g), W_{fa} = Weight of filtrate after drying (g) and W_1= Weight of initial sample (g).

The phytate content of white lupine protein meat analog was determined based on the method described by Embaby [38]. The phytate content was calculated using equation (6) as follows:

$$Phytic\ acid\left(\frac{mg}{100\ g}\right)=\frac{[(A_s{-}A_b)-intercept)\times 10]}{(Slope\times W\times 3)} \quad [6]$$

where: A_b = absorbance of blank sample, A_s = absorbance of sample, intercept = the value found from linear equation (3) = the volume extracted from the sample (supernatant), W = the weight of the sample in grams and 10 = the aliquots.

The tannin content of the lupine protein meat analog was determined using a UV spectrophotometer (PerkinElmer Lambda 950, UK) and vanillin-HCl assay method [38]. The tannin content was calculated using equation (7):

$$\text{Tannin content}\left(\frac{\text{mg}}{100\ \text{g}}\right)=\frac{[(\text{A}_\text{s}-\text{A}_\text{b})-\text{intercept}]\times 10)}{\text{Slope}\times \text{d}\times \text{W}} \quad [7]$$

where: A_s = the sample absorbance, A_b = the blank absorbance, intercept = the value found from the linear equation, d = the density of the solution (0.791 g/ml), W = the weight of the sample in grams, and 10 = the aliquots.

2.4.5. Sensory analysis

The sensory evaluation (color, aroma, taste, tenderness, mouthfeel, and overall acceptability) of lupine-based meat analogs was conducted using the seven-point hedonic scale (1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly 4 = neither like nor dislike, 5 = like slightly 6 = like moderately and 7 = like very much) was used. Thirty untrained panellists (staff and postgraduate students) were invited from Bahir Dar Institute of Technology, Food Engineering Department. Each meat analog sample was coded and given to each panellist. The panellists were oriented on the objective of the study, testing procedures, and how they could compare and complete the scorecard regarding their evaluation.

2.5. Data analysis

The data was analyzed by two-way analysis of variance (ANOVA) by the Minitab software version 19.1.0.1 using a General Linear Model. The significance of variations between treatments was done using the Fisher LSD comparison Method, with a 95 % confidence level and a p-value of $p < 0.05$ significance level. The results were reported in terms of mean ± standard deviation. The model significance ($p < 0.05$) served as the basis for evaluating the model's suitability and the results were plotted using Sigma Plot Software Version 15.0. The radar chart for sensory quality attributes was plotted by Excell Software 2016.

3. Results and discussion

3.1. Effect of roasting temperature on the physical quality of the meat analog

Roasting treatment did not show any significant effect on the pH value of the meat analog. However, roasting had a significant effect (P = 0.019) on the diameter reduction value of the meat analog (Table 1). The highest diameter reduction value (2.81 %) was observed in the control sample. This might be due to the incomplete removal of anti-nutrient contents of lupine seeds, while the lowest value (2.03 %) was observed at T_2 (roasted at 140 °C). This might be due to the removal of undesired compounds especially anti-nutrients in lupine seeds during roasting. Gujral et al. [39], described that the addition of fiber-rich and non-meat protein raw materials may minimize the degree of shrinkage and weight loss of animal protein-based meat analogs like pork patty.

In addition, roasting had no significant effect (p = 0.993) on the texture value but had a significant effect (P = 0.026) on the thickness reduction value of the meat analog meat analog sample. The highest thickness reduction value (13.09 %) was observed in the control sample. This may be due to the presence of nitrogenous compounds that can hinder the desired thickness, while the lowest reduction value (11.59 %) was observed at T_2 (roasted at 140 °C) (Table 1). This is because of the removal of nitrogenous compounds during roasting at optimum temperature. Similarly, roasting had a significant effect ($p < 0.001$) on the cooking yield meat analog. The highest cooking yield (91.44 %) of the meat analog was observed at T_2 (roasted at 140 °C). This might be due to the removal of compounds that can affect the cooking yield of meat analog samples, while the lowest cooking yield (80.15 %) was observed in the control sample (Table 1). This may be because of incomplete removal of anti-nutrients such as phytic acids. As Hyun Jung Ko and Yaxin Wen [40] stated, moisture libration during heating is the noted reason for cooking loss that could reduce the yield after cooking.

Table 1
Effect of roasting temperature on the physical quality of lupine protein-based meat analog.

Roasting	Texture (N)	pH	Diameter reduction (%)	Thickness reduction (%)	Cooking yield (%)
Raw	0.17 ± 0.07a	5.74 ± 0.23a	2.81 ± 0.17a	13.09 ± 0.43a	80.15 ± 1.98b
T1	0.19 ± 0.12a	5.53 ± 0.29a	2.30 ± 0.18b	12.70 ± 0.44a	80.19 ± 1.10b
T2	0.19 ± 0.06a	5.93 ± 0.20a	2.03 ± 0.07b	11.59 ± 0.52b	91.44 ± 1.28a
T3	0.18 ± 0.06a	5.95 ± 0.18a	2.34 ± 0.40b	12.53 ± 0.51a	89.57 ± 1.29a
P-value	0.993	0.160	0.019	0.026	<0.001

Soaking had a significant effect (p<0.001) on the cooking yield of meat analog.

3.2. Effect of soaking time on the physical quality of the meat analog

Soaking treatment did not show a significant effect ($p < 0.001$) on the texture of lupine protein meat analog sample but there was a little significant effect ($p < 0.01$) on the pH value of the soaked lupine flour sample (Table 2). The highest value (6.27) of pH was observed at t_2 (soaked for 4 days), while the lowest value (5.74) was observed in the control sample. The lowest value of pH was observed in the control sample might be due to the presence of acidic nature compounds in lupine seeds. As Eun Yeong Lee et al. [41], mentioned, the qualitative and functional properties of muscle proteins are significantly influenced by the pH of the meat; higher pH values raise the water-holding capacity of meat because they change the electrical charge levels in the muscle proteins.

Soaking had also shown a significant effect (p<0.001) on the diameter reduction value of the meat analog. The highest diameter reduction value (3.03 %) of meat analog was observed at t_3 (soaked for 6 days) (Table 2). This may be because of the degradation of valuable compounds such as water-soluble proteins and fats. Soaking had also shown a significant effect (p<0.01) on the diameter reduction value of the meat analog sample. The highest thickness reduction (13.09 %) of meat analog was observed in the control sample while the lowest value (11.09 %) was observed at t_2 (soaked for 4 days). This may be due to the complete removal of undesired compounds that cause desired thickness during soaking. The lowest cooking yield (78.07 %) of the meat analog sample was observed at t_1 (soaked for 2 days) (Table 2). This might be due to the presence of larger compounds which have a great potential for cooking yields. The highest cooking yield (91.50 %) of the meat analog sample was observed at t_2 (soaked for 4 days). This may be due to the removal of anti-nutrients through leaching during soaking.

3.3. Effect of combined treatment (soaking after roasting) on the physical quality of the meat analog

The combined effect showed a significant effect (p<0.001) on the texture of meat analog. The highest hardness value (1.45 N) was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days) (Table 3). This is due to the breakdown of polysaccharide compounds and the removal of anti-nutrient contents that could hinder the functional properties of proteins. The lowest hardness value (0.13 N) was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days). This is because of the denaturation of proteins that can contribute to a good texture profile of the product by overheating and leaching of proteins by oversoaking conditions. Danowska-Oziewicz [42] reported that the lowest value of shear force (degree of shrinkage) for soy isolate protein as compared to pork patty (due to denaturation of myofibrillar proteins which causes meat toughening) was obtained. This revealed that the texture value of plant protein-based meat analogs is comparable with/even higher than animal proteins. Palanisamy et al. [43], also reported that the increase in temperature from 145 to 160 °C had no significant effect on the cutting force meat analogs; however, there was a significant increment in the cutting force value at 175 °C. The combined effect had shown a highly significant effect (p<0.01) on the pH value of meat analog. The lowest pH value (4.95) of the meat analog sample was observed at T_2t_1 (roasted at 140 °C and soaked for 2 days), while the highest pH value (5. 74) of the meat analog sample was observed in the control sample (Table 3). The pH value was high in the control sample due to the partial basicity nature of lupine beans. The pH increases, the acidity decreases and vice versa. As it has been reported by Bakhsh, Lee, & Hwang [44] in a comparison study of meat analog and beef and pork meat, the high pH (7.42–7.43) in the meat analog patties was due to the partial alkalinity of TVP as compared to beef and pork meat. The authors also explained that the lower pH value of beef and pork was due to the glycolytic changes that occur in traditional/conventional meat. There was a highly significant difference (p<0.001) in the interaction (soaked after roasted) meat analog sample on diameter reduction. The lowest value (2.03 %) of diameter reduction was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days), while the highest value (3.14 %) was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days) (Table 3). According to the report of Bakhsh, Lee, Sabikun et al. [45], the diameter reduction after cooking the pork patty ranged from 17.46 to 22.68 %, while the diameter reduction of TVP ranged from 4.23 to 12.28 %. As it has been described by the authors, the degree of shrinkage for TVP after cooking was lower than that of the degree of shrinkage of pork patty due to the connective tissue denaturation and fluid (moisture and fat loss). There was a highly significant difference (p<0.001) in the interaction (soaked after roasted) meat analog sample on thickness reduction. The lowest thickness reduction (10.29 %) was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days), while the highest value (13.09 %) was observed in the control sample (Table 3). The highest thickness reduction was observed in the control sample, this might be due to the presence of anti-nutrients that hinder functional properties by binding proteins. As reported by Byarugaba et al. [46], roasting temperature and soaking time increased the functional properties of food products decreased.

Combined effect had shown a significant effect (p<0.001) on the cooking yield of the meat analog sample. The highest cooking yield (93.17 %) was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days) (Table 3). This may be due to the removal of heat and water-

Table 2
Effect of soaking time on the physical quality of lupine protein meat analog.

Soaking	Texture (N)	pH	Diameter reduction (%)	Thickness reduction (%)	Cooking yield (%)
Raw	0.17 ± 0.07a	5.74 ± 0.23b	2.81 ± 0.17a	13.09 ± 0.43a	80.15 ± 1.98bc
t_1	0.14 ± 0.03a	6.13 ± 0.11 ab	2.13 ± 0.22b	12.05 ± 0.46b	78.07 ± 0.99c
t_2	0.21 ± 0.06a	6.27 ± 0.44a	2.13 ± 0.18b	11.09 ± 0.42c	91.50 ± 0.44a
t_3	0.16 ± 0.02a	6.05 ± 0.15 ab	3.03 ± 0.08a	12.30 ± 0.42 ab	81.86 ± 0.15b
P-Value	<0.001	<0.01	<0.001	<0.001	<0.001

Table 3
Effect of combined treatment on the physical quality of lupine protein meat analog.

Soaking after roasting	Texture (N)	pH	Diameter Reduction (%)	Thickness reduction (%)	Cooking yield (%)
Raw	0.17 ± 0.07^{d}	5.74 ± 0.23^{a}	2.81 ± 0.17^{b}	13.09 ± 0.43^{a}	80.15 ± 1.98^{cde}
T_1t_1	0.88 ± 0.06^{c}	5.78 ± 0.25^{a}	2.88 ± 0.22^{ab}	12.11 ± 0.30^{bc}	81.39 ± 1.20^{c}
T_1t_2	0.87 ± 0.04^{c}	5.46 ± 0.26^{ab}	2.46 ± 0.36^{cd}	10.88 ± 0.47^{de}	84.70 ± 0.26^{b}
T_1t_3	1.06 ± 0.12^{b}	5.06 ± 0.10^{bcd}	2.92 ± 0.08^{ab}	11.31 ± 0.49^{cd}	85.44 ± 0.49^{b}
T_2t_1	1.34 ± 0.11^{a}	4.95 ± 0.20^{d}	2.42 ± 0.10^{cd}	11.67 ± 0.52^{bcd}	85.38 ± 0.70^{b}
T_2t_2	1.45 ± 0.08^{a}	5.03 ± 0.27^{cd}	2.03 ± 0.06^{e}	10.29 ± 0.42^{e}	93.17 ± 0.90^{a}
T_2t_3	1.33 ± 0.06^{a}	5.24 ± 0.10^{bcd}	2.18 ± 0.17^{de}	11.68 ± 0.57^{bcd}	81.69 ± 1.10^{c}
T_3t_1	1.12 ± 0.10^{b}	5.09 ± 0.21^{bcd}	2.69 ± 0.20^{bc}	11.98 ± 0.75^{bc}	80.86 ± 0.64^{cd}
T_3t_2	0.81 ± 0.05^{c}	5.37 ± 0.27^{abc}	2.85 ± 0.23^{ab}	12.06 ± 0.17^{bc}	78.39 ± 1.51^{e}
T_3t_3	0.13 ± 0.03^{d}	5.26 ± 0.41^{bcd}	3.14 ± 0.14^{a}	12.22 ± 0.64^{b}	79.47 ± 0.64^{de}
P-value	<0.001	<0.01	<0.001	<0.001	<0.001

Values are reported as means of triplicate samples ± standard deviation. Values with the same letters in a column are not significantly different while values with different letters are significantly different ($p<0.05$).

sensitive components at different processing operations. The lowest cooking yield (79.47 %) of the meat analog sample was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days). This is because of excessive fat loss and water liberation during soaking and roasting respectively. According to Godschalk-Broers et al. [47], significant differences in cooking loss of meat analog burgers (4–23 %) and chicken meat analogs (7–19 %) were reported.

3.4. Effect of roasting temperature on proximate compositions of meat analog

The roasting temperature showed a significant effect ($p<0.001$) on the moisture content of the meat analog samples. The moisture content of the meat analog samples in roasting treatment ranged from 4.22 to 5.80 % (Table 4). The highest moisture content was observed at T_3 (roasted at 150 °C). This may be due to the migration of moisture from the environment to the sample. Byarugaba et al. [46], reported that the moisture content of common bean flour ranged from 6.72 to 4.58 %, roasted at 140 °C for 5 min and 200 °C for 15 min respectively. Roasting had a significant effect ($p<0.001$) on the total ash content of meat analog. There was a decrement effect of roasting on the crude ash content of meat analog (Table 4). The highest crude ash content (2.74 %) was observed in the control sample. This may be due to the presence of some roasting-sensitive compounds in lupine seeds, while the lowest value (2.17 %) was observed at T_3 (roasted at 150 °C). This may be due to the removal of heat-sensitive compounds by roasting. Roy et al. [48], reported that the ash contents of the raw, boiled, and roasted samples of kidney beans were 4.82 %, 3.35 %, and 4.40 % respectively.

Roasting had shown a significant effect ($p < 0.05$) on the fat content of meat analog samples. The lowest fat content (2.51 %) of the meat analog sample was observed at T_3 (roasted at 150 °C) (Table 4). This is due to the formation of oxidation reactions by high temperature, while the highest value (4.46 %) was observed in the control sample. This can be supported by Durmaz & Gökmen, 2010 [49] described that high-temperature roasting significantly affects fatty acid content, particularly polyunsaturated fatty acids, through oxidative reactions and triglyceride breakdown, and may also form trans fatty acids through isomerization. Similarly, Belcadi-Haloui et al., 2010 [50] mild roasting can inhibit enzymes like lipases and lipoxygenases that can cause fatty acid oxidation, thereby enhancing the stability of oils against oxidation. There was a significant difference ($p<0.001$) in the crude protein content of the meat analog sample in roasting treatment. The highest protein content (75.28 %) of the meat analog sample was observed at T_2 (roasted at 140 °C). This is due to the removal of compounds (anti-nutrients) that hinder proteins by roasting, while the lowest value (68.42 %) was observed at T_3 (roasted at 150 °C) (Table 4). This is because of the denaturation of protein at high temperatures. According to the report of Wang & Guo [51], Legumes' phytic acid can hinder mineral bioavailability and protein utilization, unless thermal and no-thermal processing methods like roasting and soaking are used. Roasting treatment had a significant effect ($p < 0.05$) on the digestible CHO content of the meat analog sample. The highest CHO content (18.60 %) of the meat analog sample was observed at T_3 (roasted at 150 °C). This may be due to the removal of undesired compounds that possibly affect the CHO content during roasting, while the lowest value (11.19 %) was observed at T_2 (roasted at 140 °C) (Table 4). This is agreed in line with Navicha, Willard Burton et al. [52], who reported soymilk processed at 120 °C and 130 °C for 100–120 min showed an increased trend in the amount of carbohydrates. Roasting had a significant effect ($p < 0.001$) on the crude fiber content of lupine protein meat analog sample. The highest crude fiber content (3.78 %) of the meat analog sample was observed at T_2 (roasted at 140 °C). This may be due to the increment of valuable compounds during the processing of lupine seeds at optimum temperature, while the lowest value (1.74 %) was

Table 4
Effect of roasting temperature on proximate contents of lupine protein meat analog.

Roasting	Mc (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	CHO (%)	GE (kcal/100 g)
Raw	4.22 ± 0.07^{c}	2.74 ± 0.08^{a}	4.46 ± 0.10^{a}	73.02 ± 0.17^{b}	3.19 ± 0.09^{b}	12.38 ± 0.14^{c}	381.72 ± 0.38^{a}
T_1	4.93 ± 0.37^{b}	2.29 ± 0.05^{b}	3.73 ± 0.05^{b}	71.12 ± 0.11^{c}	1.74 ± 0.07^{d}	17.16 ± 0.63^{b}	379.46 ± 1.66^{ab}
T_2	3.74 ± 0.17^{d}	2.20 ± 0.01^{bc}	2.85 ± 0.04^{c}	75.28 ± 0.07^{a}	3.78 ± 0.23^{a}	11.19 ± 0.43^{d}	378.82 ± 1.09^{b}
T_3	5.80 ± 0.20^{a}	2.17 ± 0.03^{c}	2.51 ± 0.09^{d}	68.42 ± 0.38^{d}	2.50 ± 0.15^{c}	18.60 ± 0.57^{a}	370.68 ± 1.50^{c}
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

observed at T_1 (roasted at 130 °C) (Table 4). This agreed in line with Dhingra et al. [53], who reviewed the study reveals that heating kidney beans leads to a decrease in fiber content due to the solubilization of polysaccharides while soaking increases crude fiber by removing water-soluble oligosaccharide compounds. Roasting treatment significantly affects the gross energy content of the meat analog, with T_3 having the lowest (370.68 Kcal/100 g) due to protein, fat, and CHO reduction, while the control sample had the highest (381.72 Kcal/100 g) (Table 4).

3.5. Effect of soaking time on proximate compositions of meat analog

There was a significant effect ($p<0.001$) on the moisture content of the lupine protein meat analog sample in the soaking treatment. The highest moisture content (6.69 %) of the meat analog sample was observed at t_3 (soaked for 6 days). This is due to the absorption of moisture content during soaking, while the least moisture content (4.16 %) of the meat analog sample was observed at t_1 (soaked for 2 days) (Table 5). Soaking had a significant effect on the crude ash content of meat analog. The lowest crude ash content (1.41 %) of meat analog was observed at t_3 (soaked for 6 days) (Table 5). This may be due to the excessive loss of valuable compounds that contribute to the increment of ash content by over-soaking of lupine seeds, while the highest value (2.80 %) was observed at t_2 (soaking for 4 days). This may be due to the reduction of anti-nutrients that can hinder the availability of possible minerals associated with crude ash. There was a significant effect ($p<0.001$) on the fat content of meat analog in the soaking treatment. The highest fat content (4.16 %) of the meat analog sample was observed at t_2 (soaked for 4 days), while the lowest value (2.90 %) was observed at t_3 (soaked for 6 days) (Table 5). The possible reason that fat content decreased at t_3 could be due to the reduction of water-soluble fats by over-soaking. Moreover, the decrement in fat content is because of the production of free fatty acids and the activity of lipase [54]. Soaking had a significant effect ($p < 0.001$) on the CHO content of meat analog. The highest CHO content (22.14 %) of the meat analog sample was observed at t_2 (soaked for 4 days). This may be due to the removal of anti-nutrients by soaking, while the lowest value (10.51 %) was observed at t_3 (soaked for 6 days) (Table 5). This may be due to the removal of water-soluble CHO by over-soaking lupine seeds. Soaking had shown a significant effect on the protein content of meat analog samples. The highest protein content (74.90 %) of the meat analog sample was observed at t_2 (soaked for 4 days). This is because of the removal of anti-nutrients by soaking, while the lowest value (65.33 %) was observed at t_3 (soaked for 6 days) (Table 5). This is due to the removal of water-soluble proteins by over-soaking.

The highest crude fiber content (4.07 %) of the meat analog sample was observed at t_2 (soaked for 4 days). This might be due to the removal of compounds that can reduce the availability of crude fiber by soaking, while the lowest value (1.53 %) was observed at t_3 (soaked for 6 days) (Table 5). There was a significant difference ($P<0.01$) on the gross energy content of the meat analog samples in the soaking treatment. The highest gross energy content (380.88 Kcal/100 g) of the meat anlog sample was observed at t_1 (soaked for 2 days), while the lowest value (375.997 kcal/100 g) was observed at t_3 (soaked for 6 days) (Table 5). This can be supported by the report of P. Thakur et al. [55], who reported a decrement effect of the calorific value of amaranths as soaking time increased from 12 to 24 h i. e. decreased from 377.93 ± 0.47 to 368.75 ± 2.01 Kcal/100 g respectively.

3.6. Effect of combined treatment on proximate compositions of meat analog

The combined effect had a highly significant effect ($p<0.001$) on the moisture content of the meat analog samples. The highest moisture content (7.03 %) was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days), while the lowest moisture content (3.11 %) was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days) (Table 6). This is due to methylcellulose content variation because of the treatment effect i.e. as temperature increases, its content decreases and vice versa can be a barrier to avoiding moisture loss [44]. As reported by Agume et al. [56], roasting led to a significant decrement in moisture content from 7.2 to 5.7 g/100 g of soybean. The combined effect had shown a significant effect ($p<0.001$) on the crude ash content of the meat analog samples. The highest ash content (3.13 %) of the meat analog sample was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days). This is because of the removal of nitrogenous compounds at appropriate processing conditions, while the lowest value (1.05 %) was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days) (Table 6). This might be the removal of heat-sensitive inorganic compounds by over-roasting lupine seeds.

According to the report of Simachew & Fikre [57], the total ash content of lupine protein (isolated from lupine soaked with water) was changed from 3.54 to 2.96 %). The highest fat content (5.33 %) was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days), while the lowest value (2.18 %) was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days) (Table 6). The combined effect showed a highly significant effect ($p<0.001$) on the crude fat content of the meat analog sample. The fat content was high at T_2t_2 because of the inactivation of lipolytic enzyme activity [58]. The lowest value (62.47 %) of the crude protein content of the meat analog sample was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days) (Table 6). This might be due to protein denaturation at high temperatures. In addition to this, soaking for a longer time could be the main cause of crude protein decrement due to the loss of water-soluble proteins

Table 5
Effect of soaking time on proximate contents of lupine protein meat analog.

Soaking	Mc (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	CHO (%)	GE (kcal/100 g)
Raw	4.22 ± 0.07^{b}	2.74 ± 0.08^{a}	4.46 ± 0.10^{a}	73.02 ± 0.17^{b}	3.19 ± 0.09^{b}	12.38 ± 0.14^{c}	381.72 ± 0.38^{a}
t_1	4.16 ± 0.05^{b}	2.05 ± 0.05^{b}	3.55 ± 0.06^{c}	67.14 ± 0.23^{c}	3.02 ± 0.14^{b}	20.09 ± 0.08^{b}	380.88 ± 1.22^{a}
t_2	3.57 ± 0.40^{c}	2.80 ± 0.01^{a}	4.16 ± 0.08^{b}	74.90 ± 0.11^{a}	4.07 ± 0.10^{a}	10.51 ± 0.48^{d}	379.05 ± 2.19^{a}
t_3	6.69 ± 0.43^{a}	1.41 ± 0.03^{c}	2.90 ± 0.10^{d}	65.33 ± 0.06^{d}	1.53 ± 0.08^{c}	22.14 ± 0.64^{a}	375.997 ± 1.50^{b}
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01

Table 6
Effect of soaking after roasting conditions on proximate contents of lupine protein meat analog.

Soaking after roasting	Mc (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	CHO (%)	GE (kcal/100 g)
Raw	4.22 ± 0.07b	2.74 ± 0.08b	4.46 ± 0.10c	73.02 ± 0.17cd	3.19 ± 0.09d	12.38 ± 0.14f	381.72 ± 0.38abc
T1t1	4.71 ± 0.19b	2.51 ± 0.03d	3.19 ± 0.22 fg	72.44 ± 0.56d	2.69 ± 0.10e	14.47 ± 0.70e	376.31 ± 1.43ef
T1t2	4.17 ± 0.21b	2.12 ± 0.03f	3.91 ± 0.09d	73.72 ± 0.84c	3.89 ± 0.22c	12.20 ± 0.86f	378.83 ± 1.04cde
T1t3	4.18 ± 0.22b	1.64 ± 0.05h	3.03 ± 0.07g	70.80 ± 0.10e	2.06 ± 0.06f	18.29 ± 0.26b	383.61 ± 0.92a
T2t1	3.42 ± 0.50c	2.74 ± 0.04b	4.95 ± 0.06b	77.22 ± 0.23b	4.22 ± 0.08b	7.45 ± 0.39g	383.22 ± 1.75a
T2t2	3.11 ± 0.17c	3.13 ± 0.06a	5.33 ± 0.16a	82.46 ± 0.56a	4.83 ± 0.41a	1.12 ± 0.57h	382.35 ± 1.51 ab
T2t3	3.33 ± 0.40c	2.64 ± 0.058c	3.45 ± 0.07e	72.59 ± 0.13d	3.32 ± 0.18d	14.67 ± 0.23e	380.05 ± 0.94bcd
T3t1	4.81 ± 0.30b	1.84 ± 0.057g	3.29 ± 0.01ef	70.07 ± 0.07f	2.82 ± 0.08e	17.16 ± 0.36c	378.53 ± 0.12de
T3t2	4.70 ± 0.96b	2.41 ± 0.01e	4.04 ± 0.06d	69.40 ± 0.10f	3.64 ± 0.25c	15.81 ± 1.00d	377.22 ± 3.84de
T3t3	7.03 ± 0.16a	1.05 ± 0.014i	2.18 ± 0.18h	62.47 ± 0.55g	1.27 ± 0.11g	26.00 ± 0.63a	373.46 ± 1.89f
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001 <0.001	

Values are reported as means of triplicate samples ± standard deviation. Values with the same letters in a column are not significantly different while values with different letters are significantly different (p<0.05).

[56]. Combined treatment had shown a highly significant effect (p<0.001) on the crude protein content of the meat analog sample. The highest crude protein content (82.46 %) was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days). The increment in protein content might be due to the removal of nitrogenous compounds and non-protein nitrogen compounds that bind the protein contents of meat analog samples at different processing operations. The lowest value (62.47 %) of the crude protein content of the meat analog sample was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days) (Table 6). This might be due to protein denaturation at high temperatures. In addition to this, soaking for a longer time could be the main cause of crude protein decrement due to the loss of water-soluble proteins [56].

The combined treatment had shown a significant effect ($p < 0.001$) on the fiber content of meat analog samples. The highest crude fiber content value (4.83 %) was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days), while the lowest value (1.27 %) was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days) (Table 6). The possible reason that crude fiber content increased at T_3t_3 may be due to the excessive removal of water-soluble and heat-sensitive fibers by soaking and roasting respectively. Similar trends have been reported by Liao et al. [59], who stated that there was an increased trend in fiber content related to roasting in high temperatures. Interaction (soaking after roasting) had shown a highly significant effect ($p < 0.05$) on the CHO content of meat analog samples. The

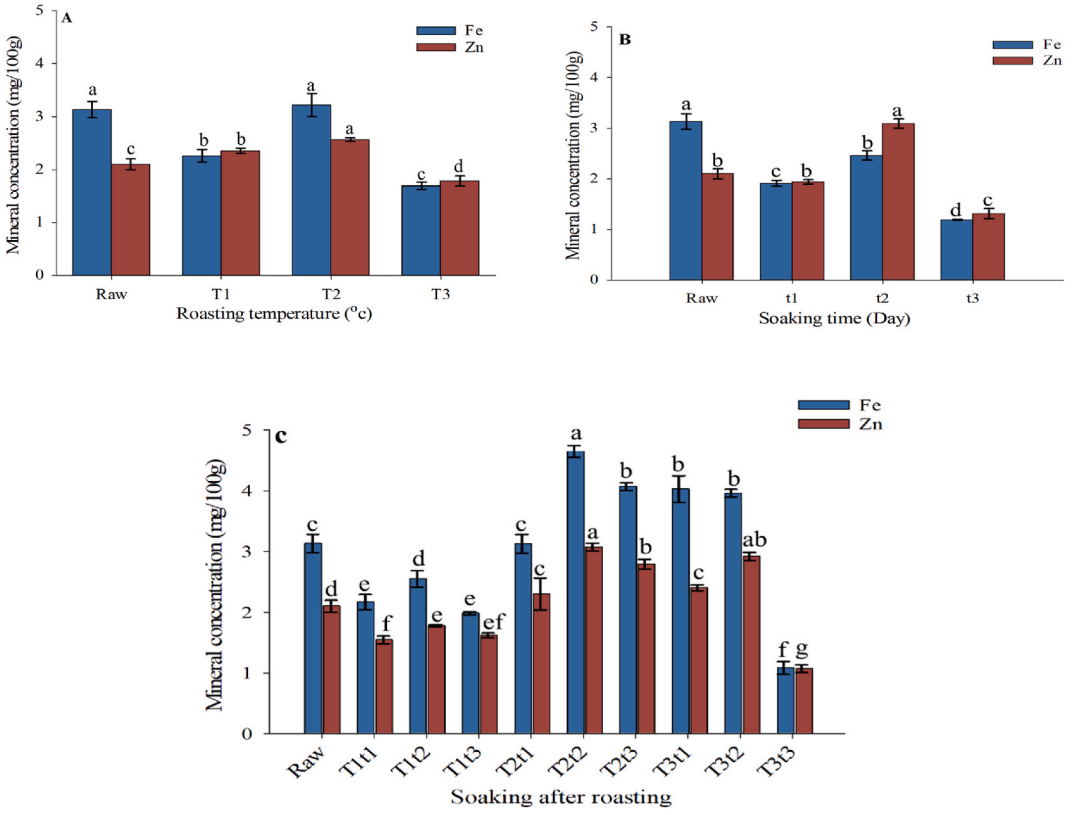

Fig. 3. Effect of A) roasting temperature B) Soaking time and C) combined effect on Fe and Zn content of meat analog.

highest CHO content (26.0 %) of the meat analog sample was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days). This is because of protein denaturation and excessive loss of water-soluble storage proteins during lupine processing conditions, while the lowest value (1.12 %) was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days) (Table 6). This might be due to the removal of large compounds such as polysaccharides by leaching during soaking and by heating. As Agume et al. [56], reported, the carbohydrate content of roasted and soaked soybean flour ranged from 22.8 to 27.9 % on a dry weight basis. The combined effect showed a highly significant effect ($p<0.001$) gross energy content of the meat analog sample. The highest gross energy content (382.35 Kcal/100 g) of the meat analog sample was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days), while the lowest value (373.46 Kcal/100 g) was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days) (Table 6). The gross energy content increased by roasting and soaking conditions might be due to the increment of fat and protein contents of meat analog samples at the recommended processing conditions of lupine seeds.

3.7. Effect of roasting temperature on the mineral contents of meat analog

Roasting treatment had a significant effect ($p<0.05$) on the Fe content of lupine protein meat analog sample. The highest Fe content (3.22 mg/g) was observed at T_2 (roasted at 140 °C), while the lowest value (1.70 mg/100 g) was observed at T_3 (roasted at 150 °C) (Fig. 3A). This may be due to the formation of oxidation reaction during roasting at high temperatures. As Miraji et al. [60], studied, the Fe content increased as the roasting temperature increased compared to unroasted rice-based products due to inward diffusion into endosperm. Roasting had a significant effect ($p<0.05$) on the Zn content of lupine protein meat analog sample. The highest Zn content (2.57 mg/100 g) was observed at T_2 (roasted at 140 °C), while the lowest value (1.78 mg/100 g) was observed at T_3 (roasted at 150 °C) (Fig. 3A). The possible reason that the Zn content decreased at T_3 may be due to the formation of an oxidation reaction during roasting at high temperatures. As Miraji et al. [60], reported, roasting slightly reduced the zinc content of rice -based products.

3.8. Effect of soaking time on the mineral contents of meat analog

There was a significant difference ($p<0.05$) on the Fe content of the meat analog sample in the soaking treatment. The highest Fe

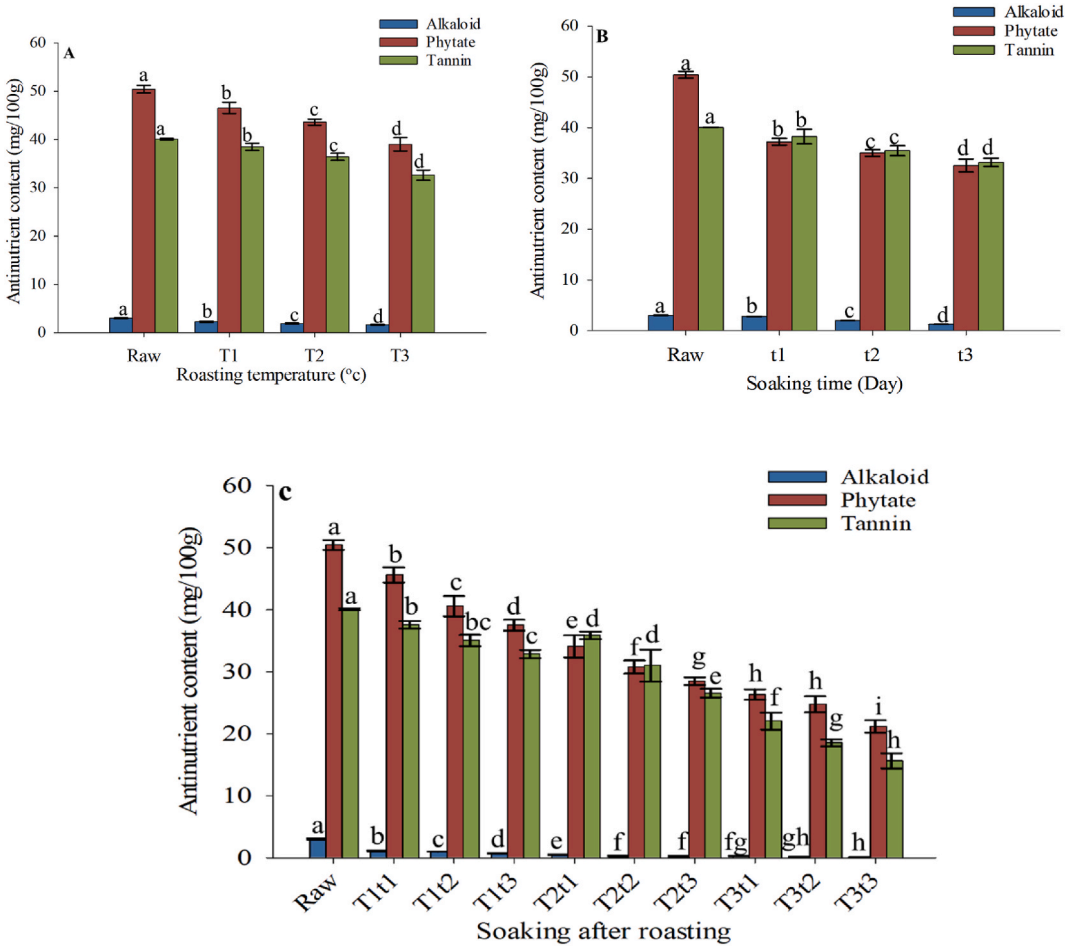

Fig. 4. Effect of A) roasting temperature B) Soaking time and C) combined effect on anti-nutrient contents of meat analog.

content (3.333 mg/100 g) was observed in the control sample, while the lowest value (1.192 mg/100 g) was observed at t_3 (soaked for 6 days) (Fig. 3B). Most probably, the possible reason that the Fe content of the meat analog sample increased at t_3 might be due to the formation of oxidation reaction and leaching by soaking lupine seeds for a long time. Soaking had shown a significant effect on the Zn content of meat analog samples. The highest Zn content (3.09 mg/100 g) was observed at t_2 (soaked for 4 days). This is because of the lowest leaching effect by soaking lupine seeds at an optimum soaking time, while the lowest value (1.31 mg/100 g) was observed at t_3 (soaked for 6 days) (Fig. 3B). This may be due to the formation of leaching and oxidation reactions during the soaking of lupine seeds for a long time.

3.9. Effect of combined treatment on the mineral contents of meat analog

The combined treatment had shown a highly significant effect ($p<0.05$) on the Fe content of the meat analog sample. The highest Fe content (4.65 mg/g) was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days). This is because of the reduction of anti-nutrient contents especially phytic acids which hinders mineral availability, while the lowest value (1.08 mg/100 g) was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days) (Fig. 3C). This is due to the occurrence of oxidation reaction and leaching during over-roasting and soaking respectively. According to the report of Alemu [29], the Fe content of bread made from wheat and lupine was increased from 0.37 mg/100 g (soaked for 10 days and roasted at 130 °C) to 0.61 mg/100 g (soaked for 10 days and roasted at 140 °C). There was a highly significant difference ($p<0.05$) in the Zn content of the lupine protein meat analog sample in combined effect. The highest value (3.07 mg/100 g) of Zn content was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days). The lowest Zn content (1.07 mg/100 g) was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days) (Fig. 3C). This may be due to the removal of water-soluble and heat-sensitive anti-nutritional components like phytates, tannins and alkaloids during soaking and roasting.

3.10. Effect of roasting temperature on anti-nutrient contents of meat analog

Roasting had a significant effect ($p<0.05$) on the anti-nutrient content of meat analog. The highest alkaloid reduction content (1.65 %) of the meat analog sample was observed at T_3 (roasted at 150 °C). This may be due to the heat sensitivity property of the alkaloid, while the lowest value (3.023 %) was observed in the control sample (Fig. 4A). As it has been studied by Alemu [29], the contents of alkaloids in lupine were significantly reduced by thermal and steeping conditions like roasting and soaking ranging from 2.591 to 0.064 %. More specifically, roasting at 140 °C and immersing for 15 days nearly eliminated the alkaloid contents. The percentage of alkaloids found in both the raw and processed white lupine beans.

There was a significant difference ($p<0.05$) in the phytate content of the meat analogs in roasting treatment. The highest phytate reduction content (39 mg/100 g) of the meat analog sample was observed at T_3 (roasted at 150 °C). This is due to the complete removal of its content by roasting, while the lowest value (50.43 mg/100 g) was observed in the control sample (Fig. 4A). Roasting had a significant effect ($p<0.05$) on the tannin content of lupine protein meat analog sample. The highest tannin reduction content (32.6 mg/100 g) of the meat analog sample was observed at T_3 (roasted at 150 °C). This may be due to the heat-labile property of tannin, while the lowest value of (40.067 mg/100 g) was observed in the control sample (Fig. 4A). This is agreed in line with Al-Amrousi et al. [61], reviewed that roasting has the potential to degrade and oxidize substances like phenolic compounds, especially tannins. There was a significant difference ($p<0.05$) in the alkaloid content of the meat analog in the soaking treatment. The highest alkaloid reduction content (1.25 %) of meat analog was observed at t_3 (soaked for 6 days). This is due to the water solubility nature of alkaloids that can be reduced by soaking, while the lowest reduction value (3.023 %) was observed in the control sample (Fig. 4B). Soaking had shown a significant effect on the phytate content of meat analog samples. The highest phytate reduction content (32.53 mg/100 g) of meat analog was observed at t_3 (soaked for 6 days). This is due to leaching by soaking lupine seeds, while the lowest value (50.433 mg/100 g) was observed in the control sample (Fig. 4B). Alkaline treatment and cooking process may also affect the anti-nutrient contents of lupine proteins. Rahate et al. [62],reviewed that the decrease in phytic acid content during soaking was mostly caused by the leaching out action; there was a 32.7 % decrease in phytic acid following the 12 h soaking period of fava beans. Soaking had shown a significant effect on the tannin content of meat analog samples.

3.11. Effect of soaking time on anti-nutrient contents of meat analog

The highest tannin reduction content (33.167 mg/100 g) of the meat analog sample was observed at t_3 (soaked for 6 days). This is due to the wash-away of its content by water, while the lowest value (40.067 mg/100 g) was observed in the control sample (Fig. 4B). As it has been described by Ramli et al. [54], the tannin content reduced with the increment of soaking time of crops. There was a significant difference ($p<0.05$) on the alkaloid content of the meat analog sample in combined treatment. The highest alkaloid reduction content (0.1033 %) of the meat analog sample was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days). This may be due to the heat and water sensitivity properties of alkaloids, while the lowest value (3.0233 %) was observed in the control sample (Fig. 4C). As it has been reported by Ezegbe et al. [63], the reduction of the anti-nutritional factors got greater with an increment of roasting temperature and soaking time.

3.12. Effect of combined treatment on anti-nutrient contents of meat analog

Interaction (soaking after roasting) treatment had a significant effect on the phytate content of the meat analog sample ($p<0.05$). The highest phytate reduction content (21.167 mg/100 g) of the meat analog sample was observed at T_3t_3 (roasted at 150 °C and

soaked for 6 days), while the lowest value (50.433 mg/100g) was observed in the control sample (Fig. 4C). As it has been stated by Ezegbe et al. [63], the highest phytate reduction content by soaking was reported which reduced from 1.50 % (the raw) to 0.42 % (soaked for 3 days) in mucuna pruriens seed. There was a significant difference ($p<0.05$) in the tannin content of the meat analog sample in interaction (soaking after roasting) treatment. The highest tannin reduction content (15.633 mg/100 g) was observed at T_3t_3 (roasted at 150 °C and soaked for 6 days), while the lowest value (40.067 mg/100 g) was observed in the control sample (Fig. 4C). It can be further described as the roasting temperature and soaking time of crops especially legume seeds increased the tannin content.

3.13. Effect of processing conditions on sensory quality attributes of lupine protein meat analog

There was a significant effect ($p<0.05$) on the sensory quality attributes of meat analog samples (Fig. 5). The interaction treatment showed a highly significant effect ($p<0.05$) on the sensory quality attributes of lupine protein meat analog samples. From this, one can understand that roasting and soaking conditions of lupine are commonly practiced inseparately. Soaking potentially reduce anti-nutrient contents of lupine and roasting improve the flavor and finally the combined effect can improve overall sensory quality attributes of lupine based meat analog. The highest-scored value (6.40) and the lowest-scored value (2.85) of all the sensory attributes (color, aroma, taste, tenderness, mouthfeel, and overall acceptability) of meat analog samples were observed at T_2t_2 (roasted at 140 °C and soaked for 4 days) and raw meat analog samples respectively. The reason that the sensory quality attributes of meat analog samples had shown a better sensory profiles at T_2t_2 is due to the reduction of undesired compounds at different processing operations (roasting, soaking, alkaline extraction and cooking). According to Wang et al. [11], off-flavor chemicals are formed when hydro-peroxides (ROOHs), the primary products of lipid oxidation, react further to generate a variety of volatile and nonvolatile secondary products roasting lupine seeds at high temperatures. Roasting and soaking treatments had also a significant effect ($p<0.05$) on the sensory quality attributes of lupine protein meat analog samples. According to the report of Eun Yeong Lee et al. [41], meat color may be adversely affected by heat denaturation and oxidation of the color pigments as compared to the fresh meat color sensory evaluation.

The highest scored value (4.75) of the overall sensory acceptability of the meat analog sample was observed at T_2 (roasted at 140 °C), while the lowest scored value (2.85) was observed in the control meat analog sample. The highest scored value (4.40) of the overall sensory acceptability of the meat analog sample was observed at t_2 (soaked for 4 days), this may be due to the removal of anti-nutrients by soaking, while the lowest scored value (2.85) was observed in the control sample. This indicates that if we isolate lupine protein from properly processed lupine flour, we can develop the most likely and delicious meat analog product, which is comparable with the sensory profiles of beef meat. However, if there are over or under-processing conditions, the acceptance of meat analog by the panelists decreases due to the formation of undesirable components and the presence of anti-nutrients, especially quinolizidine alkaloids which contribute bitterness of the product. According to the report of Bakhsh, Lee, & Hwang [44], plant protein-based meat analogs had a higher firmness compared to pork and beef meat. The authors mentioned that the overall acceptability of plant protein meat analog was comparable to pork and beef without significant differences; it was more chewy sensations, elastic, and rubbery. This could be due to the agglomeration properties of the plant-derived proteins.

This research concluded that the recommended processing conditions of white lupine beans were roasting at 140 °C and soaking for 4 days. In general, the panellists preferred lupine protein-based meat analog. This was a good indicator of the consumers currently

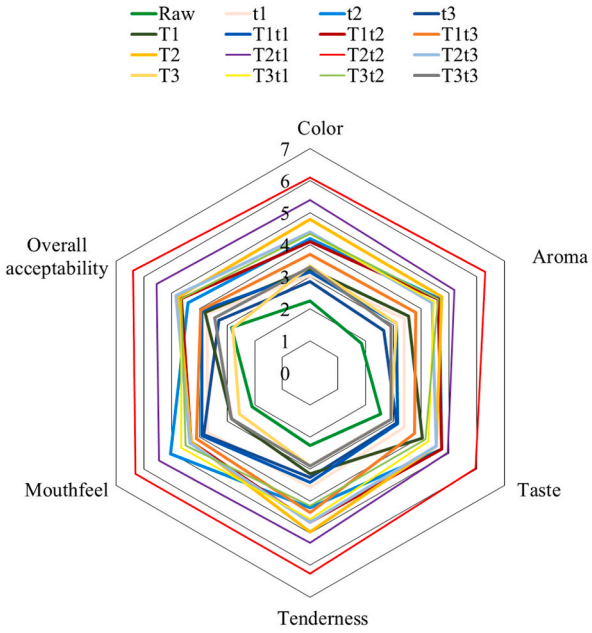

Fig. 5. Evaluation of the sensory quality attributes of lupine protein-based meat analog.

choosing plant protein-based food products.

4. Conclusion

Plant-based meat analog have become the best alternatives to animal proteins. White lupine is rich in adequate valuable nutrient profiles especially proteins when there are proper processing conditions to reduce its anti-nutrient contents. Processing conditions such as roasting and soaking show a significant effect on the chemical compositions and sensory quality attributes of lupine protein meat analog samples. Interaction treatment was the best to produce a comparable/even higher texture profile of meat analog. The recommended processing conditions to develop meat analog were roasting at 140 °C, soaking for 4 days and roasting at 140 °C and then soaking for 4 days. In addition, the highest scored value (6.40) of the overall sensory acceptability of lupine protein-based meat analog samples was observed at T_2t_2 (roasted at 140 °C and soaked for 4 days). This indicated that selecting proper processing conditions will enhance the quality of lupine protein-based meat analog. Recently, plant protein-based meat analogs have been attracting the scientific communities due to their highest protein source and eco-friendly to the environment. Further research is better to conducted on the nutritional profile and acceptance level of plant-based meat analogs comparing with conventional meat.

Ethics approval

The study complies with all regulations of the country for sensory evaluation and the informed written consent was obtained from the panelists.

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Consent for publication

All the authors have given approval for the publication of this manuscript.

Data availability

All the data used in this research are included in the article.

CRediT authorship contribution statement

Dessalew Birlew Ayalew: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Biresaw Demelash Abera:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Yemenue Lake Adiss:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis.

Declaration of competing interest

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33122.

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