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Optimization of vegetarian burger patties through mushroom substitution with peanut: Formulation, nutritional Profile, bioactivity, and consumer acceptability evaluation

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Abstract

The consumption of meat has been linked to an increasing prevalence of various diseases and negative environmental impacts, highlighting the necessity for alternative protein sources. Oyster mushrooms (*Pleurotus ostreatus*) and peanuts (*Arachis hypogaea*) offer promising alternatives for use in vegetarian burger patties. Achieving the optimal ratio between oyster mushrooms and peanuts is crucial to produce a vegetarian burger that meets the desired standards of consumer acceptability, nutritional value, and bioactivity. Four different formulations of vegetarian burgers were developed, each varying in the level of mushroom-peanut substitution (0%, 5%, 10%, and 15%). Each formula was evaluated based on its nutritional value (moisture, ash, protein, fat, carbohydrate, and dietary fiber), bioactive compounds (antioxidant activity, total phenolic compounds, and β-glucan), and sensory attributes (color, aroma, flavor, texture, and overall acceptability). The selected formula was characterized for its amino acid profile. The results revealed that substituting mushrooms with peanuts led to an increase in protein content, lipid content, and total phenolic compounds, significantly impacting consumer acceptance in all vegetarian burger patty attributes. On the contrary, the addition of peanuts resulted in a reduction in moisture content, ash content, dietary fiber content, antioxidant activity, and β-glucan content. Meanwhile, it did not significantly impact consumer acceptance in all vegetarian burger patty attributes. After a thorough assessment, the formulation featuring a 10% peanut substitution was identified as the most optimal, striking a balance between sensory evaluation, nutritional value, and bioactivity of the vegetarian burger patty. Afterward, the selected formula was characterized for its free amino acids profile.

Keywords: Oyster mushroom, Peanut, Non-meat product, Optimization formula

1. Introduction

Meat has long been referred as the primary source of protein with its consumption reaching 34.1 kg/year per capita [1]. However, its consumption has been associated with an increased risk of several health problems, such as cardiovascular disease, colon cancer, and type-2 diabetes [1]. Moreover, meat production is also considered to impact the environment due to greenhouse gas (GHG) emissions and inefficient energy usage, as it consumes a large amount of land and water, contributing to deforestation and biodiversity loss [1]. Consequently, campaigns promoting reduced meat consumption have become more popular, alongside the increasing trend of vegan, vegetarian, and flexitarian lifestyles. While vegans abstain from all animal-based products, vegetarians include milk and eggs in their diet, and flexitarians limit their meat intake. These lifestyles have increased the demand for alternative sources of protein. It is expected that this demand will increase by 200% in Asia-Pacific countries such as China and Thailand [2].

Burger patty is traditionally a product derived from minced beef. Several plant-based proteins can be an alternative for making burger patties. One of the most popular plant-based proteins used is soybean, whether in the form of isolated protein, by-product, or fermented (tempeh) [3–6]. However, amidst the rising demand for plant-based meat alternatives, oyster mushrooms (*P. ostreatus*) have emerged as a viable option. Oyster mushrooms

are packed with nutrients, rich in protein, dietary fiber, and minerals. They also contain health-promoting bioactive substances such as β-glucan, polyphenols, and terpenoids. In terms of maintenance, they require relatively small amounts of land for its cultivation, making them an excellent choice for creating meat substitutes [7]. In addition, adding mushrooms to meat-based dishes could enhance the taste and attractiveness of plant-based foods that promote health, all without compromising nutritional quality [8].

Peanuts may also be added to vegetarian meat-based products since they offer another avenue for enhancing these products by providing texture, flavor, and nutritional value, which may be lacking in other plant protein products [9]. Peanuts are rich in protein, monounsaturated fatty acids, fiber, vitamins, and minerals, making them valuable ingredients in plant-based diets. Additionally, nuts or peanut protein may add a distinct flavor and richness to the patties, enhancing their taste and overall eating experience, and leading to increased sensory acceptance of the burger [10]. Therefore, this study aims to formulate a vegetarian burger patty using an optimized ratio of oyster mushrooms and peanuts, and subsequently characterize the quality of the selected formula in terms of its nutritional value, bioactivity, and sensory acceptance.

2. Materials and methods

2.1. Material

The defatted peanut flour was provided by CV Saffa Tjahya Lestari, while the rest of the vegetarian burger patty ingredients were purchased from the local market in Bogor, West Java. The chemical reagents were purchased from Merck® as analytical grade unless otherwise stated.

2.2. Method

The preparation of the vegetarian burger patty and bioactivity analysis was conducted in the Edible Mushroom Laboratory, Research Center for Applied Microbiology, Bogor. Subsequently, the sensory evaluation, proximate, and quality analysis were conducted at PT Saraswanti Indo Genetech, Bogor.

2.2.1. Vegetarian burger patty preparation

The process and formula of the vegetarian burger patty have been registered as intellectual property under patent registration number P00202203713. İn the preparation process, the oyster mushrooms were cleaned, diced, and boiled for 10 minutes before being mixed with the remaining ingredients. The mixture was then cooked for 15 minutes until the patty dough was formed. Subsequently, the vegetarian patty dough was portioned into 75-gram portion, molded into patty shapes, and steamed for 15 minutes. After steaming, the patties were cooled to room temperature, packed in sealable plastic bags, and frozen until further analysis. A detailed breakdown of the ingredients for each formula is provided in Table 1.

Table 1 Ingredients of vegetarian burger patty.

Ingredient	Weight (%)			
	Formula I	Formula II	Formula III	Formula IV
Oyster mushroom	50	45	40	35
Defatted peanut flour	0	5	10	15
Water	18	18	18	18
Mixed Flour	10	10	10	10
Onion and garlic	9	9	9	9
Carrageenan	3.5	3.5	3.5	3.5
Egg white	3	3	3	3
Flavoring	2.5	2.5	2.5	2.5
Soy sauce (sweet and salty variants)	2	2	2	2
Vegetable oil	1.8	1.8	1.8	1.8
Spices	0.2	0.2	0.2	0.2

2.2.2. Nutritional value analysis

The proximate analysis, including moisture, ash, lipid, protein, carbohydrate content, and dietary fiber, was conducted following the methodology outlined by Setyawan et. al [11].

2.2.3. Bioactive compound analysis

The antioxidant activity was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method, and the total phenolic compound (TPC) was analyzed using the Folin-Ciocalteu method [12]. Additionally, β-glucan analysis was conducted using the β-Glucan Assay Kit (Yeast & Mushroom) from Megazyme®, Ireland.

2.2.4. Sensory evaluation and analysis

The sensory analysis of the vegetarian burger patty was conducted through a hedonic test, assessing color, aroma, taste, texture and overall attributes using a 5-point scale ranging from 1 (dislike), 2 (rather dislike), 3 (neutral), 4 (rather like), and 5 (really like). This test involved 20 subjects aged from 20 – 40 years, encompassing both male and female employees of PT Saraswanti Indo Genetech. The patties were prepared by thawing the frozen product and cooking them on a non-stick pan for 3-5 minutes each on each side over medium heat before serving them to the subjects.

2.2.5. Free amino acid profile analysis

The quality assessment of the selected formula for the vegetarian burger patty included characterizing its amino acid composition. Tryptophan amino acid content was determined using High Performance Liquid Chromatography (HPLC), while methionine and cysteine were detected Liquid Chromatography with Tandem Mass Spectrometry (LCMS/MS). The remaining amino acids were identified using Ultra Performance Liquid Chromatography (UPLC), following the methodology outlined by Setyawan et. al [11].

2.2.6. Statistical analysis

All results were underwent One-Way ANOVA analysis, followed by post hoc multiple comparisons using The Tukey test. Statistically significance was established at an alpha level of 0.05.

3. Results and discussion

3.1. Nutritional value of the vegetarian burger patty

The supplementation of different levels of peanuts in each formula significantly impacted the nutritional value of the vegetarian burger patty (Table 2). Peanut supplementation led to a reduction in moisture content. This can be attributed to the strong-water binding ability of peanut protein within the food product, potentially retaining more water within its structure. However, during processing, such as exposure to intense heat, protein denaturation and crosslinking can occur. Additionally, the heating process may increase pressure and the movement of water molecules, causing some bound water to evaporate due to the heat [13, 14]. Consequently, the final vegetarian burger patty exhibited lower moisture content peanut supplementation increased. Higher moisture levels in food products also can accelerate deterioration processes, resulting in a shorter shelf life due to increased microbial activity and enzymatic degradation. Conversely, the patties' ability to retain moisture impacts their texture characteristics, especially hardness, springiness, gumminess, and chewiness [6]. Thus, achieving the optimal balance of peanut supplementation in the vegetarian patty is necessary.

Substituting oyster mushrooms with peanuts in the vegetarian burger patty resulted in increased levels of protein and fat, while decreasing the levels of ash, carbohydrates, and dietary fiber due to the differing nutritional compositions of oyster mushrooms and peanuts [7, 13]. Peanuts offer a rich source of protein and fat, particularly high in unsaturated fatty acid, making them a viable option as vegetarian alternatives to meat-based products. Conversely, oyster mushrooms are abundant in minerals such as iron, zinc, calcium, and selenium, as well as vitamin D, addressing potential deficiencies in vegetarian diets [9]. Dietary fiber consumption has been associated with reduced risks of cardiovascular disease, type 2 diabetes, and cancer, particularly pancreatic cancer, by lowering cholesterol concentrations in the body, slowing glucose absorption, and improving insulin sensitivity. Conversely, high-fat diets have been linked to adverse effects, emphasizing the need for moderation in fat consumption [1].

According to the National Standard for Meat Burger Patty in Indonesia (SNI 8503-2018), burger patty must contain more than 13% protein and not exceed 20% fat content. Among the formulation tested, only Formula II and Formula III met this regulation criteria as oyster mushroom-peanut burger patties. Comparatively, when compared to five other plant-based burger products available in the USA market from three different producers, the formulated vegetarian burger patties made from oyster mushrooms and peanuts exhibited similar moisture content, ranging from 49.7% to 63.3%. Notably, Formulas II and III demonstrated fat content comparable to the commercial products, ranging from 10.8% to 13.1%. Furthermore, the oyster mushroom-peanut burger patties

showcased higher ash content compared to the marketed products, which ranged from 1.5% to 3%, indicating their potential as a superior micronutrient source. However, this study lacks an analysis of mineral composition, which could offer valuable insights into its micronutrient profile, warranting further research and development opportunities. A notable weakness identified is the lower protein content of the formulated burger patty compared to the commercial product, which ranged from 17.2% to 24%. Consequently, further development is necessary to enhance the protein content of the formulated oyster-peanut burger patty.

Table 2 Proximate analysis of vegetarian burger patty.

Formula	Proximate					
	Water (%)	Ash (%db)	Protein (%db)	Fat (%db)	Carbohydrate (%db)	Dietary fiber (%db)
I	60.78 ± 0.26[a]	14.71 ± 0.28[a]	11.12 ± 0.17[c]	5.55 ± 0.35[a]	68.62 ± 0.03[a]	27.54 ± 0.33[a]
II	61.59 ± 0.16[a]	11.16 ± 0.18[b]	15.57 ± 0.37[b]	12.53 ± 0.04[b]	60.74 ± 0.30[b]	25.73 ± 0.06[b]
III	53.36 ± 0.22[b]	9.85 ± 0.05[c]	15.27 ± 0.19[b]	14.36 ± 0.03[c]	60.52 ± 0.28[b]	25.99 ± 0.08[b]
IV	52.56 ± 0.23[b]	8.14 ± 0.07[d]	16.64 ± 0.13[a]	20.51 ± 0.004[d]	54.71 ± 0.13[c]	24.11 ± 0.07[c]
CPA1*	63.3	1.5	20	10.8		
CPA2*	49.7	1.7	18.6	13.1		
CPB1*	55.0	1.5	22	12		
CPB2*	52.3	2.5	17.2	11.7		
CPC*	69.3	1.3	24	10.8		

The mean ± standard deviation of three replicates with different letters in a column were significantly different (P<0.05). CPA1= Commercial product A1; CPA2= Commercial product A2; CPB1= Commercial product B1; CPB2= Commercial product 2; CPC= Commercial product C. *Data collected from commercial vegetarian burger patty products in the USA market [15].

3.2. Bioactive compounds of the vegetarian burger patty

In vegetarian burger patties, the inclusion of peanuts led to a reduction in antioxidant activity and β-glucan content, while relatively not influencing their TPC (Table 3). Cultivated oyster mushrooms can contain soluble dietary fiber β-glucan of up to 25% of their dry matter, primarily found in their cell wall [16]. Hence, the reduced mushroom content resulted in lower β-glucan and dietary fiber in the vegetarian burger patty. β-glucan from mushrooms has been studied for its health benefits, including immunomodulatory, antitumor, anti-inflammatory, hypocholesterolemic, hypoglycemic, and prebiotic activity [16, 17]. Moreover, β-glucan may influence food mouthfeel and texture, as it has the potential to act as a fat replacer [18], which could be advantageous, especially in plant-based protein products like vegetarian burger patties, with potentially different rheological properties compared to conventional meat products [9].

Additionally, β-glucan in mushrooms possesses antioxidant activity, and the decline in β-glucan concentration was followed by a reduction in antioxidant activity in the patties. Aside from β-glucans, antioxidant activity in food products can be attributed to compounds such as phenolic and terpenoid compounds. These compounds not only offer health benefits but also help prolong the shelf-life of food products by preventing fat rancidity and protein oxidation [19].

Oyster mushroom possesses a TPC content of 325 μg/g GAE (Gallic Acid Equivalents) [20], while roasted peanut flour boasts a higher concentration at 1170 μg/g GAE [21]. Therefore, incorporating peanut supplementation may elevate the TPC levels in vegetarian burger patties. Phenolic compounds found in food play a role in influencing flavor, color, and aroma. They are often associated with perceptions of bitterness and astringency [22]. Additionally, these compounds may contribute to the browning reaction catalyzed by the enzyme polyphenol oxidase (PPO) [22], which can impact both the color and aroma of vegetarian burger patties.

Table 3 Bioactive compounds analysis of vegetarian burger patty.

Formula	Bioactive compound		
	Antioxidant activity (% inhibition)	Total phenolic compound (μg/g GAE)	β-glucan (%)
I	69.91 ± 0.70[a]	2.94 ± 0.09[b]	32.48 ± 0.04[a]
II	67.52 ± 0.84[b]	3.17 ± 0.13[ab]	33.10 ± 0.65[a]
III	57.78 ± 0.62[c]	3.45 ± 0.09[a]	29.44 ± 0.08[b]
IV	49.08 + 0.68[d]	3.34 ± 0.13[a]	25.78 ± 0.46[c]

The mean ± standard deviation of three replicates with the different letters in a column were significantly different (P <0.05).

3.3. Sensory property of the vegetarian burger patty

Formula III of the vegetarian burger patty exhibited the highest acceptance in every attributed tested (aroma, flavor, texture, color, and overall). However, upon conducting statistical analysis, the differences were not found to be significant (Table 4). The lack of significant results in the sensory evaluation of the oyster mushroom-peanut burger patty could be attributed to the limited number of panelists (20 subjects) and the scale used (5-point scale) to assess each formula's hedonic score. Increasing the number of panelists enhances reliability and dependability,

while employing a broader scale, for example a 9-point scale, might facilitate easier measurement of the acceptability of a food product by the panelists [23].

The aroma and flavor of mushrooms are primarily attributed to their amino acids and odor volatiles. Amino acids aspartic and glutamic acid, abundant in mushrooms, contribute to the umami flavor [24]. Additionally, histidine, isoleucine, leucine and phenylalanine impart a bitter flavor in mushrooms, while serine and alanine contribute to sweetness. Compounds like arginine and proline present both sweet and bitter flavors, whereas cysteine and methionine provide a sulfuric sensation [7]. Alcohol-rich and carbonyl compounds containing eight carbons are odor volatiles responsible for the mushroom aroma, with 1-octen-3-ol contributing to around 70% of the volatile substances in oyster mushrooms [7]. Meanwhile, various compounds in peanuts may also contribute to the aroma and flavor of vegetarian burger patties, including 2-isobutyl-3-methoxypyrazine (earthy note); 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like note); 3,5-dimethyl-2-ethylpyrazine (nutty note); 2-methyl-3-furanthiol (roasty or meaty note); 2-acetyl-1-pyrroline and 2-propionyl-1-pyrroline (popcorn-like note); and 2-methoxy-4-vinylphenol (spicy or burnt note) [25].

The increasing amount of peanut in the formula of patties was accompanied by an increase in color acceptance, likely due to an increase in lightness [26]. Additionally, oyster mushrooms and defatted peanut flour, as the main ingredients of the vegetarian burger patty, contribute to a beige to yellow-brown color, which influences the final product's color. Similarly, substituting oyster mushrooms with peanuts also enhances the texture of the vegetarian burger patty. Peanut proteins exhibit a strong water and oil binding capacity, leading to higher viscosity and gel-forming properties after heating [13], which positively impacts the patties' texture, making them more desirable.

Despite Formula III achieving the highest acceptance among all tested formulas, none of the oyster mushroom-peanut patty burger formulas exceeded a 3 out of 5 on the hedonic scale, indicating relatively low acceptance levels. Therefore, it is crucial to implement strategies aimed at enhancing the sensory evaluation of the oyster mushroom-peanut patty burger. Given that flavor and texture are crucial factors influencing the preference for plant-based alternative products [8], prioritizing improvements in these attributes could prove to be a more effective approach in boosting the acceptance rate of vegetarian burger patties. One potential strategy involves gaining a deeper understanding of the protein functionality inherent in each ingredient used in the vegetarian alternative product and its impact on the vegetarian burger patty's texture. By leveraging this knowledge, it may be possible to enhance both the texture and flavor of plant-based patties, ultimately leading to a more favorable sensory profile and increased acceptance among consumers [27].

Formula III not only achieved the highest hedonic score among the oyster mushroom-peanut vegetarian burger patties but also presented trade-offs in terms of nutritional and bioactivity values. Incorporating a 10% peanut supplementation to replace oyster mushrooms, Formula III demonstrated improvements in moisture content, protein, fat, carbohydrate, antioxidant activity, and TPC. However, at this level of peanut supplementation, the values for ash, dietary fiber, and beta-glucan content were suboptimal, and further increases in peanut supplementation could exacerbate these deficiencies. Consequently, Formula III was chosen as the most promising candidate for further analysis.

Table 4 Sensory property of vegetarian burger patty.

Formula	Attributes				
	Aroma	Flavor	Texture	Color	Overall
I	3.18 ± 0.60[a]	2.55 ± 0.82[a]	2.64 ± 0.82[a]	2.91 ± 0.70[a]	2.82 ± 0.62[a]
II	2.91 ± 0.70[a]	3.00 ± 1.00[a]	2.91 ± 0.70[a]	3.18 ± 0.75[a]	3.00 ± 0.52[a]
III	3.27 ± 0.79[a]	3.18 ± 0.98[a]	3.09 ± 0.94[a]	3.27 ± 0.90[a]	3.20 ± 0.78[a]
IV	3.09 ± 0.70[a]	2.90 ± 0.83[a]	3.18 ± 0.87[a]	3.18 ± 0.98[a]	3.09 ± 0.71[a]

The mean ± standard deviation with different letters in a column were significantly different (P<0.05).

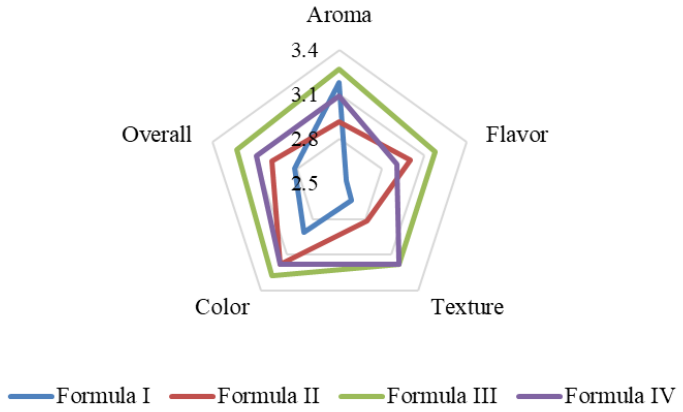

Figure 1 Sensory properties of vegetarian burger patty.

3.4. Free amino acid profile and contamination of selected vegetarian burger patty formula

The free amino acids profile of the selected formula of vegetarian burger patty was compared to several commercial plant-based burger patties from the USA (Table 4). In total, all the commercial products exhibited higher levels of amino acids compared to the selected formula. Hence, further development to enhance the amino acid quality in the selected patty is still necessary. The commercial burger patties utilized isolated protein from various sources, including peas, soy, and wheat as their main ingredients [15]. Consequently, their amino acid composition is more robust than that of the selected formula.

Essential amino acids are fundamental protein building blocks that cannot be synthesized in the body and must be obtained through diet. In contrast, conditional amino acids are typically nonessential under normal circumstances, as the body can synthesize them on its own. However, during conditions such as illness, stress, or specific metabolic disorders, the body's ability to produce these amino acids may be compromised. A deficiency of essential amino acids in the diet can hinder the utilization of other amino acids for protein synthesis and metabolism, potentially leading to malnutrition, emotional disorders, hormonal imbalance, growth and development delays, fertility issues, impaired immune responses, and worsened metabolic profile [28]. Therefore, the consumption of essential amino acids is critical for maintaining health [28] While the amino acid composition provides insight into the presence of individual amino acids in the formulated burger patty, it does not account for the protein's quality and digestibility. The Protein Digestibility Corrected Amino Acid Score (PDCAAS) assesses how effectively dietary protein can meet amino acid requirements and predict protein utilization levels. However, conducting PDCAAS analysis typically requires the involvement of human or animal subjects, presenting a limitation in this study [29]. The current Dietary Recommendation Intake (DRI) of essential amino acids (mg/kg body weight – day) includes phenylalanine + tyrosine: 33; leucine: 42, valine: 24, threonine: 20, isoleucine: 19, histidine: 14. lysine 38, tryptophan: 5, cysteine + methionine: 19 [28].

This study serves as a pivotal preliminary investigation into the development of non-animal-based protein products. Substituting meat with plant-based alternatives holds promise for enhancing health outcomes, potentially extending life expectancy by 7.6-8.7 months and reducing greenhouse gas emissions by 25% [30]. Additionally, this research maximizes the potential of oyster mushrooms and peanuts, contributing to the diversification of vegetarian products. Furthermore, it offers valuable insights into the formulation of vegetarian burger patties and related products, providing techniques for production and analysis.

Table 5 Free amino acid composition of vegetarian burger patty selected formula.

Amino Acid	Amount (mg/g)					
	Selected formula	CPA1*	CPA2*	CPB1*	CPB2*	CPC*
Glutamate	16.93	30.1	33.17	69.57	37.87	29.87
Arginine	8.20	15.32	16.5	9.17	11.4	8.16
Phenylalanine	5.37	9.87	11.04	11.82	8.65	7.15
Leucin	5.10	15.3	16.52	16.68	13	10.75
Aspartate	4.73	21.17	22.17	13.27	18.63	13.4
Serine	4.35	9.34	10.16	10.75	8.39	7.27
Proline	4.29	7.96	8.89	21.97	8.34	9.8
Glycine	4.19	7.36	8.06	8.85	7.09	4.7
Valine	3.49	9.12	10.4	10.52	8.17	6.86
Tyrosine	3.05	7.93	9.12	6.66	7.7	5.82
Threonine	2.98	6.67	7.3	6.98	6.57	5.03
Alanine	2.80	7.93	9.12	6.66	7.7	5.82
Isoleucine	2.78	8.74	9.41	9.19	7.82	6.08
Histidine	2.39	4.24	4.86	4.08	3.85	3.06
Lysine	2.21	13.28	12.82	7.63	10.29	6.82
Tryptophan	0.66	1.67	1.75	2.29	2.26	1.7
Cysteine	0.39	2.45	2.55	6.22	4.42	2.58
Methionine	0.04	1.61	2.53	3.28	2.01	2.79
Total essential amino acids**	25.03	70.5	76.63	72.47	62.62	50.24
Total conditionally essential amino acids***	20.1	70.24	77.42	124.62	75.61	60.42
Total non-essential amino acids****	28.80	38.44	41.45	30.68	34.72	26.49

CPA1= Commercial product A1; CPA2= Commercial product A2; CPB1= Commercial product B1; CPB2= Commercial product 2; CPC= Commercial product C. *Data collected from commercial vegetarian burger patty products in the USA market (15). **= phenylalanine, tyrosine, leucin, valine, threonine, isoleucine, histidine, lysine, tryptophan, cysteine, and methionine. ***= arginine, cysteine, glutamine, glycine, proline. ****= alanine, aspartic acid, glutamic acid, serine.

4. Conclusion

This study presents an innovative approach to crafting a vegetarian burger patty using oyster mushrooms and peanuts, offering a sustainable and nutritious option amidst the growing popularity of plant-based meat substitutes. It highlights the potential of these plant-based formulations, which feature elevated ash content and various bioactive compounds such as phenolic compounds, β-glucan, and antioxidant activity. While improvements in protein and essential amino acid content, as well as sensory evaluation, are necessary, the need for further formula refinement is emphasized. Additional analyses, including mineral composition, protein quality and functionality, and texture profiling could enhance the depth of the study.

5. Ethical approval

The study received approval from The Ethics Committee for Chemical Research, National Research and Innovation Agency, under number 005/KE.04/SK/12/2022.

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7. Conflict of interest

The authors declare that they have no conflict of interest.

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