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# FERMENTED CHICKPEA (*CICER ARIETINUM* L.) AS A FUNCTIONAL FOOD: MEATLESS "VEGAN" BURGERS

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*KEY WORDS:* *vegetarian products, fermentation, Aspergillus oryzae, umami taste, glutamic acid, protein content*

ABSTRACT

The global desire for ethical, sustainable, and nutritional food choices has intensified interest in plant-based meat alternatives. Researchers and food manufacturers have prioritized the development of superior alternatives to meat and dairy products due to the increasing popularity of vegetarian and vegan diets among consumers. Chickpeas, a leguminous source abundant in protein and fiber, and mushrooms are rich in umami chemicals and can potentially be essential ingredients in plant-based meat products. Plant-based foods' nutritional profile and sensory attributes can be improved through fermentation, a conventional method frequently employed in food production. This process can increase the allure of these foods to consumers. Therefore, this research aims to create a new product from plant sources that substitutes for meat products. The chickpea was fermented by *Aspergillus oryzae* (AUMC B2) for different fermentation periods (7, 10, and 14 days) to determine the optimum fermentation time to enhance the umami taste (meat flavor). Chickpeas and mushrooms were the primary raw materials for plant-based burgers. Fermented chickpeas were used to prepare vegan burgers at different fermentation times (7 days: FC7, 10 days: FC10, and 14 days: FC14). The sensory attributes of vegan burgers were compared to those of the non-fermented control sample. The results showed that the samples of FC10 meatless burgers recorded the highest score of taste and odor compared to the control. Based on these results, a chemical analysis was conducted for the meatless product FC10 and its control. The findings showed that the fermentation process increased the protein content and decreased the content of fats and carbohydrates in the fermented meatless burger.

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# ФЕРМЕНТИРОВАННЫЙ НУТ (*CICER ARIETINUM* L.) КАК ФУНКЦИОНАЛЬНЫЙ ПРОДУКТ: НЕ СОДЕРЖАЩИЕ МЯСО «ВЕГАНСКИЕ» БУРГЕРЫ

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*КЛЮЧЕВЫЕ СЛОВА:* *вегетарианские продукты, ферментация, Aspergillus oryzae, вкус умами, глутаминовая кислота, содержание белка*

АННОТАЦИЯ

Глобальный спрос на этичные, экологичные и питательные пищевые продукты усилил интерес к растительным альтернативам мясу. Ученые и производители пищевых продуктов уделяют первостепенное внимание разработке превосходных альтернатив мясным и молочным продуктам из-за увеличивающейся популярности вегетарианских и веганских диет среди потребителей. Нут — бобовый источник белка, содержащий большое количество белка и пищевых волокон, а также грибы богаты химическими веществами «умами» и потенциально могут быть исключительно важными ингредиентами в растительных альтернативах мясным продуктам. Пищевой профиль и сенсорные характеристики растительных продуктов могут быть улучшены в результате ферментации — традиционного метода, широко используемого при производстве пищевых продуктов. Этот процесс может повысить привлекательность этих продуктов для потребителей. В этой связи, целью данного исследования было создание нового продукта из растительных источников, который заменил бы мясные продукты. Нут был ферментирован *Aspergillus oryzae* (AUMC B2) в течение различных периодов ферментации (7, 10 и 14 дней) для определения оптимального времени ферментации для усиления вкуса умами (мясного вкуса). Нут и грибы были основным растительным сырьем для растительных бургеров. Для приготовления бургеров был использован ферментированный нут с разным периодом ферментации (7 дней: FC7, 10 дней: FC10, и 14 дней: FC14). Сенсорые показатели веганских бургеров сравнивали с таковыми неферментированного контрольного образца. Результаты показали, что образцы не содержащих мясо бургеров FC10 получили наивысший балл вкуса и запаха по сравнению с контролем. На основании этих результатов был проведен химический анализ для не содержащих мясо продукта FC10 и контроля. Полученные данные показали, что процесс ферментации повышал содержание белка и снижал содержание жиров и углеводов в ферментированном, не содержащем мясо бургере.

## 1. Introduction

In recent years, numerous challenges have been associated with producing animal-based products, such as meat and poultry. Among these are the increasing global population, the scarcity of animal sources, the substantial greenhouse gas emissions associated with the livestock supply chain, and health issues [1,2,3]. Therefore, the shortage of animal and poultry feed resources is one of the main obstacles to increasing livestock and poultry wealth. Lack of animal feed will make it more expensive to rear animals, raising the price of producing both the animals and the food required to feed society's members [4]. Additionally, cattle breeding leads to a substantial release of greenhouse gases into the environment. About 51% of all greenhouse gases, such as $NH_3$, $N_2O$, $CO_2$, and $CH_4$, are released by human activities. These gasses acidify ecosystems and produce acid rain [5].

The scarcity of animal feed resources is a significant impediment to the expansion of livestock and poultry prosperity. Insufficient nutrition increases animal production costs, elevating the overall cost of meat and poultry products [4]. Additionally, the environmental degradation that results from the rigorous agricultural methods necessary to meet the

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increasing food demand, which includes deforestation, water contamination, and biodiversity loss, is significant. Jiang et al. [6] have identified beef production as one of the most resource-intensive forms of animal agriculture, and research suggests that livestock farming is responsible for over 18% of global greenhouse gas emissions. Investigating alternative protein sources to mitigate environmental impacts and address food security concerns is becoming increasingly imperative.

Aside from environmental concerns, the health impacts of excessive meat consumption have been well-documented. Numerous studies have highlighted the link between high consumption of red and processed meats and the increased risk of chronic diseases, such as cardiovascular diseases, diabetes, and cancer [7]. The International Agency for Research on Cancer (IARC) has classified red and processed meats as carcinogenic, with evidence suggesting that the consumption of these meats, particularly in high quantities, may lead to an elevated risk of colorectal cancer [8]. In addition to the cancer risk, high meat consumption is also linked to metabolic disorders, including obesity, hypertension, and dyslipidemia, which can result in further health complications [9]. Consequently, there is a growing interest in reducing meat consumption and seeking healthier alternatives that provide the necessary nutrients without the associated health risks.

Plant-based meat alternatives are becoming progressively attractive substitutes for conventional meat products. These alternatives seek to emulate the sensory characteristics of meat, encompassing texture, flavor, and appearance while providing a more sustainable and health-oriented choice. Recent advancements in food technology have facilitated the creation of plant-based meats that closely replicate the sensory experience of traditional meat products. These plant-based substitutes encompass burgers, sausages, and nuggets, crafted to deliver equivalent sensory satisfaction as their animal-derived equivalents [10,11]. These products have been improved with innovative ingredients and processing methods that enhance their texture, flavor, and nutritional value, rendering them appealing to consumers aiming to minimize their environmental footprint while still enjoying meat-like foods.

Chickpea (*Cicer arietinum L.*), known as Garbanzo beans, has garnered considerable attention for its possible application in plant-based meat substitutes. Chickpeas are a rich source of protein, dietary fiber, and essential amino acids, especially lysine, frequently lacking in other plant protein sources. Consequently, chickpeas represent a top option for incorporating meat replacements, providing nutritional and functional advantages [12]. Chickpeas are not only a source of protein but also provide key micronutrients, including vitamins and minerals, vital for general health. Furthermore, chickpeas have demonstrated the ability to enhance digestive health and facilitate weight control owing to their substantial fiber content, rendering them a significant component of a plant-based diet [13]. Recent research has emphasized chickpeas' capacity to enhance blood sugar management, rendering them advantageous for those with or predisposed to diabetes [14].

While nutritionally beneficial, chickpeas possess antinutritional components such as trypsin inhibitors, phytic acid, and oligosaccharides that induce flatulence, potentially diminishing nutrient absorption and impairing protein digestion. Antinutritional factors can be mitigated through various processing techniques, such as fermentation. Fermentation enhances the nutritional quality of legumes by improving protein digestibility, diminishing antinutritional factors, and augmenting the availability of vitamins and minerals [15]. Fermented chickpeas may be optimal for plant-based meat alternatives, providing superior nutritional advantages and better sensory characteristics.

Protein-dense mushroom flour obtained from the mycelia of filamentous fungi represents a promising complete protein source for plant-based meat replacements. Mushrooms possess a beneficial amino acid composition, are low in fat, and have a fibrous texture analogous to meat, making them an appealing alternative for meat substitutes [16]. Mushrooms are known for their nutrition and health advantages, such as better cholesterol levels and increased muscle protein synthesis while exerting a considerably decreased environmental impact compared to animal proteins [16]. Incorporating mushrooms in plant-based meats has surged in popularity owing to its sustainability and the capacity of mushrooms to replicate the texture and mouthfeel of meat, thereby delivering a gratifying culinary experience for customers.

Fermentation enhances plant-based meat substitutes' flavor, texture, and nutritional quality. Recent studies indicate that fermenting plant-based components with fungi, such as *Aspergillus oryzae* and *Monascus purpureus*, can yield flavors and fragrances akin to conventional meats [17]. Fermentation enhances the sensory qualities of plant-based goods. It increases the bioavailability of essential nutrients, including a group of vitamin B complex and minerals, strengthening the nutritional value of a product [18].

This study examines the possibilities of producing meatless products using chickpeas fermented with *Aspergillus oryzae* and oyster mushrooms as nutritious and sustainable substitutes for meat. We focused on developing meat-free burgers, a popular and straightforward food choice that meets the growing consumer demand for healthy, convenient alternatives to meat and meat products. This study aims to enhance the nutritionally balanced and environmentally sustainable plant-based meat substitutes by utilizing the combined benefits of fermented chickpeas and mushrooms.

## 2. Materials and methods

### *2.1. Raw materials*

Oyster mushrooms, chickpeas (*Cicer arietinum*), curry powder, potato starch, flour, bread crumbs, vegetable shortening, salt, sugar, black pepper, onion powder, garlic powder, ground corn, beetroot, and spices were purchased from the local market of Giza, Egypt. Both fermented chickpea and oyster mushrooms were used as main ingredients for fermented meatless burgers, as shown in Table 1.

### *2.2. Chemicals*

Carboxymethyl cellulose (CMC), petroleum ether, sodium hydroxide, sulphuric acid, and Tween 80 were obtained from Sigma Chemical Company (St. Louis, Mo., USA). Potato dextrose agar and corn starch were from Fluka Chemie AG (Buchs, Switzerland).

### *2.3. Microbial strain*

*Aspergillus oryzae* (AUMC B2) was used in this work and was obtained from Assiut University Moubasher Mycological Centre (AUMMC, Assiut, Egypt). It was allowed to grow in potato dextrose agar slants (PDA) and kept at a temperature of 4 °C.

### *2.4. Spore suspension of the microbial strain*

*Aspergillus oryzae* (AUMC: B2) was introduced to malt extract agar slants and cultured at 30 °C for five days to create a spore suspension, following the procedure of Mohamed et al. [19]. After culturing, the spores were collected and added into a saline solution containing 0.1% Tween 80, with a volume of 50 ml. The gathered spores were used as a reservoir of inoculum after being quantified under a microscope (CARL ZEISS, Montagesatz T, West Germany) ($3.4 \times 10^5$ spores/ml).

### *2.5. Preparation of fermented chickpeas (FC)*

The chickpeas (CPs) were soaked in water (with a chickpeas-to-water ratio of 1:2, w/v) for six hours at room temperature (26 °C) and subsequently sterilized at a temperature of 121 °C in an autoclave for 15 minutes. Next, CPs were inoculated with *Aspergillus oryzae* (AUMC: B2) and incubated for 7, 10, and 14 days to finish the fermentation process. The samples were collected at three time points: 7 days (FC7), 10 days (FC10), and 14 days (FC14). After each collection, reducing the RNA concentration in the fermented sample was essential. Thus, thermal treatment of the fermented sample was applied at a temperature of 73 °C for 35 minutes using a shaking water bath (Blue M Magni Whirl Constant Temperature Shaking Heated Water Bath Model MSB-1122A-1, USA) with agitation. The treatment, as mentioned above, also deactivated the growth of mycelium.

### *2.6. Preparing mushrooms*

Potable water was used to clean every mushroom. A vegetable spinner was used to remove water from mushrooms. Slices of each mushroom, each 2.5 mm thick, were steam-blanched for 5 seconds at a temperature of 130 °C. The mushrooms were kept at a temperature of −20 °C until needed.

### *2.7. Meatless food production processes*

The prepared oyster mushrooms were combined with the fermented chickpeas and other ingredients, as mentioned in Table 1. Next, the whole mixture was formed into the shape of burger patties using a manual burger mold. Each piece weighed 50 grams. Then, they were packed into polypropylene packages and stored at −18 °C until further analysis.

### *2.8. Chemical analysis*

The moisture, protein, fat, crude fibers, total ash, and total carbs of raw materials (mushroom and chickpeas) and samples of meatless burgers were analyzed according to AOAC [20]. Moisture content was determined: 3 to 5 g of the sample was placed in a convection oven at 105 °C until the weight remained constant. Ash content was determined by burning a sample in a muffle furnace at 525 °C. Protein content was determined using the Kjeldahl technique. Fat content was determined using the Soxhlet extraction technique. The total carbohydrates were estimated using the phenol — sulfuric acid method. By the difference, the fiber's mathematics can be calculated. Free amino acids composition (FAA) was analyzed by HPLC (HPLC, Smart line, Knauer, German) [21]. Twenty microliters of the

Table 1. **Formulation of fermented meatless burger patties**

Таблица 1. **Рецептура ферментированных не содержащих мясо бургеров**

| Ingredients, % | | | |
|---|---|---|---|
| **Main formula** | | **Other additives** | |
| Oyster mushroom | 30 | Curry powder | 1 |
| Fermented chickpeas (FC7, FC10 and FC14) and unfermented chickpeas (control) | 20 | Potato starch | 12 |
| | | Salt | 1 |
| | | Sugar | 0.1 |
| | | Onion powder | 10 |
| | | Garlic powder | 2 |
| | | Vegetable shortening | 4 |
| | | Black pepper | 0.4 |
| | | CMC (Carboxymethyl cellulose) | 1 |
| | | Beetroot | 2.5 |
| | | Bread crumb | 6 |
| | | Corn flour | 5 |
| | | Wheat flour | 5 |

FC7: fermented chickpeas after 7 days, FC10: fermented chickpeas after 10 days, FC14: fermented chickpeas after 14 days of the fermentation process.

hydrolyzed sample were injected into HPLC, which was equipped with a C18 reverse phase (RP) column and a fluorescence detector. The amino acids were identified and quantified by comparing the retention periods and peak regions with that of the amino acid standard.

*2.9. Sensory evaluation*

The sensory evaluation of the burgers was conducted by 50 untrained panelists of the Food Science Department, Faculty of Agriculture, Cairo University, Egypt, who evaluated the meatless burgers for various sensory attributes. The quality of the samples was assessed based on multiple criteria, including color, taste, odor, texture, physical appearance, and general acceptability, using a 9-point hedonic scale. The scoring system utilized a Likert scale ranging from 1 (dislike very much) to 9 (strongly like) to assess the level of preference [22].

*2.10. Statistical analysis*

The CoStat Version 6.45 was used for all statistical analysis (CoHort Software Version 6.45, Monterey, CA, USA). The primary statistical analysis approach was a one-way variance analysis (ANOVA). The data was analyzed using the randomized complete block design with one factor. Each parameter was replicated three times, and the data were presented as the average of four experiments. Estimates of LSD were calculated to test the significance of differences among means with a 5% significant point.

## 3. Results and discussion

*3.1. Nutritional analysis of mushrooms and chickpeas*

The nutritional value of mushrooms and chickpeas was analyzed by estimating their protein, fiber, fat, ash, carbohydrates, and moisture content. The results (Figure 1) showed the samples' high protein, carbohydrate, and fiber content, making them good raw materials suitable for manufacturing plant-based meat products. These results are consistent with many researchers in this field [14,23].

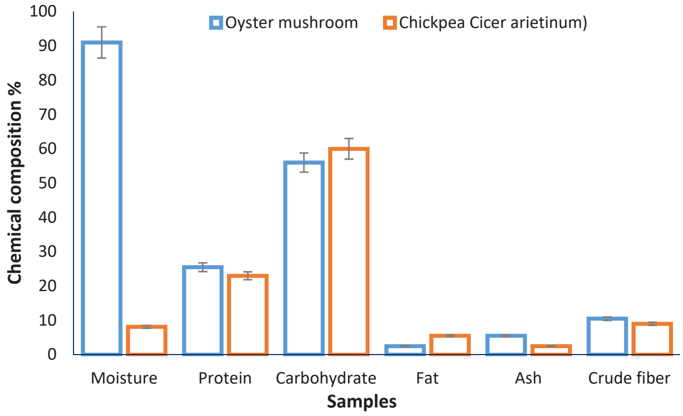


Figure 1. **Chemical composition (%) of oyster mushrooms and chickpeas**

Рисунок 1. **Химический состав (%) вешенок и нута**

Summo et al. [24] showed the highest lipid, dietary fiber, protein, and carbohydrate contents (4.1, 11.0, 20.5, and 61.0 g 100 $g^{-1}$, respectively) in beige chickpeas. Also, Ereifej et al. [25] found the highest protein and fat contents (21.2 and 7.09 g.100 $g^{-1}$, respectively) in Jubeiha-3 chickpea seed. Xiao et al. [26] examined the chemical composition of four chickpea species from Xinjiang, China: Muying-1, Keying-1, Desi-1, and Desi-2. The range of the moisture content was 7.64 to 7.89%. The total ash concentration varied between 2.59 and 2.69%. The range of the lipid content was 6.35 to 9.35%. The range of the protein level was 19.79–23.38%. According to Krüzselyi et al. [27], oyster mushrooms have a moderately high dry matter (DM) content (10.0%) for caps and a very high DM level (19.4%) for stipes. Oyster mushrooms have far more significant quantities of crude protein: 18.9% DM for caps and 11.3% DM for stipes. Compared to caps, stipes have a more substantial total carbohydrate content (63% for caps against 78% for stripes) [27]. The crude protein content of the cap, stalk, and combination (cap and stalk) is 34.19, 20.96, and 30.48%, respectively, according to Oluwafemi et al. [28]. The primary ingredient of edible mushrooms is carbohydrates, which comprise 52.9% of the cap, 61.8% of the stem, and 51.9% of the mixture. The crude fiber content of the edible mushroom varied by 3.1, 7.5, and 8.1% for the cap, stalk, and mixture, while the fat content was 1.60, 1.50, and 1.50% for the cap, stalk, and combination, respectively.

*3.2. Profile of free amino acids (FAAs) in fermented and unfermented chickpeas*

Cereals, nuts, and seeds have lower lysine contents than animal products but have comparable levels of sulfur amino acids (cysteine and methionine). Conversely, legumes often contain fewer sulfur amino acids and more lysine than other plant-based diets [29]. Therefore, a diet rich in different pulses can help you get the essential amino acids [30,31]. However, antinutritional factors (ANFs) in plant diets have been connected to the variation in protein bioavailability among dietary sources [32]. Meanwhile, the fermentation process can enhance the protein quality

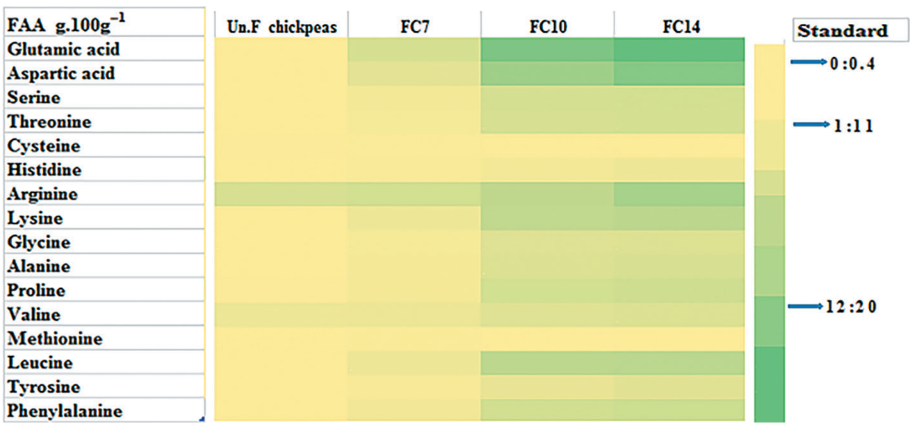


Figure 2. **Heat map of correlation between the profile of free amino acids (FAAs) and fermentation time. Un-FC: unfermented chickpeas, FC7: fermented chickpeas after 7 days, FC10: fermented chickpeas after 10 days, FC14: fermented chickpeas after 14 days of fermentation process**

Рисунок 2. **Тепловая карта корреляции между профилем свободных аминокислот (FAA) и временем ферментации. Un-FC: неферментированный нут, FC7: ферментированный нут после 7 дней, FC10: ферментированный нут после 10 дней, FC14: ферментированный нут после 14 дней процесса ферментации** of legumes by increasing essential amino acid concentrations in pigeon peas, chickpeas, and red beans and reducing ANFs. Their synergistic actions enhance the amino acid profile of fermented legume-based foods, resulting in more outstanding balance and nutritional value [33]. Free amino acids were estimated in fermented chickpeas at different fermentation periods (7, 10, and 14 days) and then compared to unfermented chickpeas (control). Table 2 and Figure 2 identified the change in the FAA content and its effect on sensory evaluation as meat-free products' flavor improvement (umami flavor). The results showed a significant increase in glutamic and aspartic acid during fermentation. The values recorded after 14 days of fermentation were 18 and 14 g/100 g, respectively. These acids are responsible for the appearance of the umami taste (meaty taste) and for improving the flavor of the final product. Also, it was observed that there was an increase in most free amino acids, with a decrease in both methionine and cysteine.

Xing et al. [34] reported that *Pediococcus* spp. fermentation may change the structure of proteins and make it easier for digestive enzymes to get to the substrate. Furthermore, bacteria can partially degrade intact proteins, increasing the concentration of free amino groups in fermented chickpeas. De Pasquale et al. [35] found that fermentation with LAB provided for a further rise in most FAA in black chickpeas. Aside from its nutritional value, the high concentration of FAA is linked to an enhancement in the sensory profile of the final product. Glutamic is the primary amino acid responsible for sapidity perception. Likewise, Sáez et al. [36] reported the same results for the amino acid composition. The findings of Liu et al. [37] indicate that *lactobacillus* fermentation modifies the multilevel structures of chickpea protein, resulting in a loose protein conformation that enhances hydrolysis during digestion. This improvement in protein digestibility underscores the capacity of fermentation to elevate the nutritional quality of chickpea-derived food products.

Table 2. **Profile of free amino acids (FAAs) in fermented and unfermented chickpeas**

Таблица 2. **Профиль свободных аминокислот (FAA) в ферментированном и неферментированном нуте**

| FAA g·100 g$^{-1}$ | Unfermented chickpeas | FC7 | FC10 | FC14 |
|---|---|---|---|---|
| Glutamic acid | 0.11 | 4.70 | 15 | 18 |
| Aspartic acid | – | 3.09 | 11 | 14 |
| Serine | – | 1.40 | 4.8 | 5.0 |
| Threonine | – | 1.05 | 4.5 | 4.7 |
| Cysteine | 0.25 | 0.37 | 0.15 | 0.08 |
| Histidine | 0.17 | 0.69 | 1.45 | 1.87 |
| Arginine | 4.50 | 5.08 | 7.13 | 10 |
| Lysine | 0.02 | 1.87 | 6.89 | 7.4 |
| Glycine | 0.05 | 1.07 | 3.73 | 3.9 |
| Alanine | - | 1.24 | 4.01 | 4.5 |
| Proline | 0.27 | 1.20 | 5.10 | 5.6 |
| Valine | 2.10 | 2.25 | 3.60 | 4.01 |
| Methionine | 0.12 | 0.36 | 0.21 | 0.11 |
| Leucine | – | 2.04 | 7.30 | 7.54 |
| Tyrosine | – | 0.75 | 2.60 | 3.5 |
| Phenylalanine | 0.02 | 1.57 | 5.18 | 5.8 |
| Tryptophan | ND | ND | ND | ND |

FC7: fermented chickpea, period 7 days; FC10: fermented chickpea, period 10 days; FC14: fermented chickpea, period 14 days.

### *3.3. Sensory evaluation of meatless products*

All samples of meatless burgers were prepared for sensory evaluation using fermented chickpeas (FC) at different fermentation periods, which were 7 days, 10 days, and 14 days. The sensory evaluation results are recorded in Table 3.

Results in Table 3 show that samples FC10 and FC14 had a clear difference in their superiority in terms of color, taste, odor, texture, and appearance compared to samples FC7 and C. Therefore, the best samples are samples FC10 and FC14. Still, the decision was made from an economic point of view to choose the sample FC10 as the best one to save energy and time spent when increasing the fermentation period. Much evidence states that the nutritional benefit of legumes can be improved by treatments such as fermentation before their incorporation into legume-supplemented products. Being relatively simple and economical, solid state fermentation (SSF) of chickpea flour (CF) can induce biochemical changes such as an increase in free limiting amino acids and available vitamins and a decrease in antinutritional factors, thus improving the functional and nutritional properties of the product.

Xiao et al. [38] demonstrated that solid-state fermentation of chickpea flour using the filamentous fungus *Cordyceps militaris* improved its crude protein content, essential amino acids, small-sized peptides and in vitro protein digestibility. Also, Liu et al. [39] reported that by changing the multilayer structures of chickpea protein, *Lactobacillus* fermentation made the protein more soluble and improved its breakdown during stomach and intestinal digestion.

Moreover, the phenolic content increased throughout fermentation, and the inhibitory activities of trypsin and chymotrypsin decreased. This happened due to fermentation producing hydrolytic enzymes, such as trypsin and chymotrypsin, which became hydrolyzed and inactivated) [40]. *Aspergillus oryzae* fermented four types of beans in the previous studies. The results showed a decrease in the carbohydrate content during fermentation, while an increase in the content of amino and fatty acids was observed [41]. The following are the processes by which microbes and their enzymes break down proteins during fermentation. Protein's envelope proteinase initially breaks down proteins into oligopeptides, which are then broken down by proline-specific peptidases, other intracellular peptidases, and exopeptidases into amino acids and shorter peptides, enhancing the flavor [42,43].

The study by Razavizadeh et al. [44] aimed to ascertain how fermentation affected okara in producing meat substitutes. The strains of *L. acidophilus* 308 and *L. plantarum* P1 were used to ferment the okara samples. The meat analogs were created by including 3% and 6% fermented okara into the matrices. The results showed that fermentation may reduce hardness and protein oxidation levels and improve the water-holding capacity of meat substitutes and sensory attributes. It has been demonstrated that okara provides enough fiber, plant-based proteins, and necessary amino acids.

High water absorption flours have more hydrophilic components, including polysaccharides, since WAC indicates a macromolecule's capacity to bind water [24]. Some scientists [45] say WAC may also be connected to protein composition and content. The texture, oil, water binding capacity, and gelling ability of chickpea protein are excellent. Another essential characteristic of chickpea protein is its capacity to stabilize emulsions and foam, making it equivalent to whey proteins and soy protein isolate. The beneficial impact of chickpeas on the color of the meat analog is one of its main benefits. Research has indicated that chickpea flour instead of some textured vegetable protein in vegetarian nuggets significantly improved color acceptability due to its carotenoid content [46,47]. Meat consumers frequently contrast meat substitutes with traditional beef, mutton, or pork. Customers have been advised to eat less meat to improve the environment and lead healthier lives. Although mushroom-based meat analogs are a viable alternative to animal meat, public acceptance of these products is still relatively low, possibly due to their flavor and taste [48]. Therefore, identifying the sensory characteristics that require optimization to enhance palatability is crucial [49]. Thus, in this study, the flavor was enhanced using fermented chickpeas to increase the sensory acceptance of meatless products.

### *3.4. Chemical analysis of meatless burgers*

Table 4 shows that burgers prepared from fermented chickpeas significantly increased protein values. The protein content reached 15.54%. Compared to the control, the protein value was 10.48% for unfermented chickpea burgers.

Table 3. **Sensory evaluation of meatless burgers**

Таблица 3. **Сенсорная оценка не содержащих мясо бургеров**

| Samples | Color | Taste | Odor | Texture | Appearance | Overall acceptability |
|---|---|---|---|---|---|---|
| C | $6.18 \pm 0.32^b$ | $5.56 \pm 0.34^b$ | $5.80 \pm 0.30^b$ | $5.73 \pm 0.34^b$ | $6.35 \pm 0.32^b$ | $6.13 \pm 0.30^b$ |
| FC7 | $5.55 \pm 0.33^b$ | $4.85 \pm 0.34^c$ | $5.20 \pm 0.29^b$ | $5.30 \pm 0.37^b$ | $5.58 \pm 0.33^c$ | $5.25 \pm 0.31^c$ |
| FC10 | $6.93 \pm 0.31^a$ | $7.13 \pm 0.29^a$ | $7.05 \pm 0.30^a$ | $7.03 \pm 0.30^a$ | $7.05 \pm 0.39^a$ | $7.03 \pm 0.30^a$ |
| FC14 | $6.95 \pm 0.35^a$ | $7.10 \pm 0.32^a$ | $6.90 \pm 0.34^a$ | $7.18 \pm 0.31^a$ | $7.10 \pm 0.32^a$ | $7.10 \pm 0.32^a$ |

Where C: unfermented chickpeas, FC7: fermented chickpeas, period 7 days; FC10: fermented chickpeas, period 10 days; FC14: fermented chickpeas, period 14 days. Mean values with different superscript letters in each row differ significantly ($p < 0.05$).

Table 4. **Chemical composition of meatless burgers**

Таблица 4. **Химический состав не содержащих мясо бургеров**

| Samples | Moisture | Ash | Carbohydrates | Fat | Protein | Crude fiber |
|---|---|---|---|---|---|---|
| C | 57.51±0.29$^a$ | 8.00±0.29$^a$ | 64.15±0.60$^a$ | 13.51±0.29$^a$ | 10.19±0.61$^b$ | 5.14±0.33$^a$ |
| FC10 | 56.17±0.44$^b$ | 7.42±0.52$^a$ | 63.74±0.41$^b$ | 9.28±0.43$^b$ | 15.75±0.39$^a$ | 3.81±0.36$^b$ |

C: unfermented chickpea, FC10: fermented chickpea, period 10 days.
Mean values with different superscript letters in each row differ significantly ($p < 0.05$).

Moreover, the results showed a significant difference in the fiber percentage in the fermented sample compared to the unfermented one. The fiber level was 3.81% in the fermented burger and 5.14% in the non-fermented control sample. This is due to the growth of the *Aspergillus oryzae* on the chickpeas and enzymatic degradation of the fiber. Likewise, the fat content decreased (9.28% in fermented burgers and 13.5% in the control. This is due to the enzymatic degradation of fats by *Aspergillus oryzae* during chickpea fermentation. Previous studies have documented that the fermentation process improves the sensory characteristics in terms of taste and smell to produce the umami taste responsible for the appearance of the meaty flavor of meat plant-based products.

In the future, mushrooms and fermented foods may represent a new class of plant proteins due to their meat-like flavor, extended shelf life, and high nutritional content.

## 4. Conclusions

Ensuring sustainable methods to fulfill the demands of a rising population while limiting environmental damage is a significant problem facing the global food business. Simultaneously, the need for high-quality products and customer awareness spur innovation and constructive changes in the food supply chain. This study aims to develop a more nutrient-rich and sustainable substitute by describing meatless burgers' chemical and sensory properties: an innovative approach to popular food products. We believe that the most superb method to increase pulse intake is to create enticing, nourishing, and easy ready-to-eat legume-based meals. The different fermentation periods of chickpeas (*Cicer arietinum*) mixed with oyster mushrooms have significantly affected the organoleptic properties of burger substitutes. The ten days as a fermentation period showed promising results, especially in protein. Burger substitutes with 10 days as the fermentation period exhibited better textural properties and sensory mean scores. However, samples with no fermentation and 7 days for the fermentation period were not the best options for producing burger substitutes, and they failed to satisfy the sensory panelists as the added ingredient affected the texture and taste. It can be concluded that meatless burgers, after 10 days of fermentation, represent acceptable plant-based products with good sensory acceptability. Due to the breakdown of non-nutritive components, which the microbes present during the fermentation may use as an energy source to grow, the fermented proteins have excellent protein quality. The solubility of plant-based proteins in water is impacted because, during fermentation, the hydrophobicity of the protein surface rises. Furthermore, some non-nutritive substances in plant-based proteins changed during fermentation.

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