



Research Article

## Nutritional, anti-nutritional, antioxidant, and sensory properties of meat alternatives derived from wheat gluten and pumpkin seeds

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### Abstract

This study evaluates the nutritional, anti-nutritional, antioxidant, and sensory properties of meat alternatives derived from wheat, gluten, soy, and pumpkin seeds. The raw materials included soybeans (*Glycine max*), pumpkin seeds (*Cucurbita maxima*), and wheat flour (*Triticum aestivum* L.), from which meat alternatives were prepared using soy curd, pumpkin seed curds (in ratios of 100%, 70:30, and 50:50 soy/pumpkin seeds) and wheat gluten. Moisture, ash, crude fiber, protein, fat, carbohydrate, and calorie contents were analyzed, along with screening for oxalate, phytate, and saponin contents. Additionally, antioxidant activities (DPPH and FRAP) and hedonic analysis were performed. The results showed varying contents of water (52.06% to 61.84%), protein (12.77% to 34.60%), fat (1.43% to 13.63%), and carbohydrates (50.11% to 76.31%) in the meat alternatives. Ash and calorie contents ranged between 1.85% and 5.83%, and 383.88 kcal and 450.24 kcal, respectively. No fiber was detected, while phytate and oxalate contents varied from 4.15% to 11.69% and 4.85% to 19.55%, respectively. Saponins were absent in the samples. The meat alternatives exhibited DPPH-scavenging activities with IC<sub>50</sub> values between 140.56 g/L and 183.80 g/L, and ferric-reducing antioxidant power with IC<sub>50</sub> values from 147.49 g/L to 166.21 g/L. Hedonic acceptability ranged from 56% to 98%, with taste being the most important descriptor, followed by aroma, texture, and appearance, as identified by perceptron artificial neural networks. Sensory discrimination analysis indicated that the meat alternatives shared the same sensory profile. Thus, the plant-based foods derived from wheat gluten and pumpkin seeds demonstrate their potential as viable meat alternatives.

## 1. Introduction

The current food system is grappling with multiple challenges, including a continuously growing demand for food due to the increasing global population, which is projected to surpass 10 billion by 2050 [1]. This growing demand is straining the limited available resources, such as land, water, and energy. The food production system is considered one of the

significant contributors to global environmental changes, including climate emergencies and biodiversity loss [2, 3]. Additionally, the current dietary patterns, which are high in meat and energy-dense foods and low in whole grains, fruits, and vegetables, are not sustainable and have severe consequences for human health [4].

To address these challenges and mitigate the environmental impact of the food sector on climate change, it is essential to reevaluate existing consumption practices, with a particular focus on meat consumption due to its substantial environmental burden [5]. Plant-based products have gained significant attention as a potential solution, with their demand increasing due to the growing popularity of veganism, vegetarianism, and the increased consumption of plant-based food in Western countries. Encouraging the consumption of plant-based foods is seen as a strategy to reduce the negative impacts associated with the modern food supply and improve human and global health [6, 7]. Plant-based meat alternatives (PBMA) are highly processed products designed to mimic the characteristics of animal meat products, such as the texture and appearance of a burger patty [8]. These alternatives aim to replace the meat component in various dishes, given their similarities in form, taste, and preparation method. However, despite the growing market for meat substitutes, many consumers remain unattracted to these products [9]. Incorporating PBMA into a "flexitarian/reducitarian" diet, which includes occasional consumption of meat and fish within a primarily vegetarian approach, holds significant potential for preventing premature deaths and mitigating greenhouse gas emissions [10]. Studies have shown that adopting plant-based diets can be cost-effective, low-risk interventions associated with various health benefits, including potential reductions in body mass index, blood pressure, HbA1C, and cholesterol levels, as well as decreased medication requirements for managing chronic diseases and lower mortality rates related to ischemic heart disease [11-13].

To facilitate the shift towards a plant-based eating pattern, researchers are exploring alternative protein sources that could serve as viable replacements for traditional animal-based proteins. While vegetarian and vegan diets have been prevalent for decades, and plant-based items like tofu and textured soy protein have been present in the Western world since the 1960s, there has been a growing variety of PBMA in recent years [14]. Initiating changes in meat consumption habits requires acknowledging the numerous socio-behavioral factors associated with

meat consumption [15,16], with sensory dimensions being crucial for consumer acceptance of dietary substitutes [17].

Among the various plant-based meat substitutes, tofu and seitan are notable examples. Tofu, made similarly to cheese from soymilk, possesses unique nutritional qualities, including dietary fibers and the absence of cholesterol [18,19]. Seitan, or "wheat meat," is derived from wheat flour and is known for its chewy texture and rich flavor, making it a suitable choice for individuals without gluten sensitivity [20]. Food products incorporating wheat gluten offer a diverse range of textured vegetable protein ingredients, serving as meat extenders and analog products [21, 22].

The antioxidant properties of meat alternatives, especially those from plant sources, have been a focus of research, with studies showing that meat analog products exhibit better antioxidant properties than traditional meat products [23, 24]. However, anti-nutrients in meat alternatives, particularly those based on plants, have raised concerns due to their high phytate content, which can hinder the absorption of minerals like iron and zinc [25].

Given the current shift towards plant-based diets and the demand for meat alternatives, various non-animal protein sources have been developed. However, there is limited comprehensive research evaluating the nutritional, antinutritional, antioxidant, and sensory properties of these meat alternatives. Therefore, this study aims to assess the nutritional content, anti-nutritional factors, antioxidant properties, and sensory attributes of meat alternatives made from wheat gluten and pumpkin seeds. Understanding the characteristics of these PBMA will provide valuable insights for consumers, food manufacturers, and nutritionists, ultimately contributing to promoting sustainable and healthy dietary choices.

## 2. Materials and methods

### 2.1. Plant material and chemical acquisition

Soybeans (*Glycine max*), pumpkin seeds (*Cucurbita maxima*), and wheat flour (*Triticum aestivum* L.), were purchased from the Bamenda main market in the Northwest region of Cameroon. They were transported to the laboratory in airtight containers and stored at 4°C before undergoing processing. All

chemicals used for the study are of analytical grades and were procured from Sigma-Aldrich (United Kingdom).

### 2.2. Preparation of tofu

The soy tofu preparation was done following the procedure outlined by Oboh and Omotosho [26]. One kilogram of soybeans was soaked in 6 liters of water at a temperature range of 27 to 32°C for 9 hours. After soaking, the beans were drained and ground in a grinder with tap water, resulting in a water-to-raw-bean ratio of 6:1 for extracting solids from soybeans into raw milk, bringing the total solid content of the soymilk to approximately 11%. The soymilk was then heated to 98°C and maintained for 1 minute before being transferred to the mixing tank. Once cooled to 87°C, 3 l of soymilk was mixed at 420 rpm with 0.5 l of vinegar (coagulant). After mixing, the mixture was held for 5 seconds and then filled onto tofu trays, allowing it to coagulate for 10 minutes. The bean curd was pressed and seasoned with 1 g of salt per 100 g of tofu. After pressing, the weight of the tofu was recorded. The produced tofu was immersed in water at 4°C overnight before analysis.

### 2.3. Preparation of pumpkin seed-derived meat alternative

The process for preparing meat alternatives from pumpkin milk curds was identical to that used for soybeans, except that no coagulant was added, as the proteins in the pumpkin milk coagulated naturally upon boiling.

### 2.4. Preparation of meat alternatives combining soy curd and pumpkin seed curd

A 70:30 ratio was employed to create a composite product of pumpkin seed/soy curd, where 70% of the previously prepared pumpkin seed curd was combined with 30% of already prepared soy curd and molded to form the composite product. Additionally, a 50:50 ratio was utilized to produce another product consisting of 50% soy curd and 50% pumpkin seed curd. The choice of the 70:30 and 50:50 ratios for the composite products was based on preliminary experiments that suggested these combinations would provide optimal texture, flavor, and nutritional profile results.

### 2.5. Wheat gluten extraction and preparation of seitan

The standard method, as outlined in AACC [27], was employed to extract wheat gluten. One kilogram of

all-purpose wheat flour was used to form a dough by adding approximately 1 l of water. This dough was covered and allowed to rest for 30 minutes, after which it was kneaded again to separate the starch granules from the gluten. Subsequently, the dough was washed under water until all the starch was removed, resulting in a brown, stretchy, meaty mass of wheat gluten. This gluten mass was later boiled (90°C for 10 min) with a choice of seasoning (1 g of salt per 100 g of wheat gluten) to give seitan. Seitan was stored in a polyethylene container in the refrigerator at 4 °C for further analysis.

### 2.6. Determination of proximate composition

The moisture, crude fiber, and ash content of the samples were analyzed following the procedures outlined in the AOAC methods [28]. The determination of crude protein was performed using the Kjeldahl method, while the Soxhlet extraction method was employed for determining crude fat. The total carbohydrate was calculated as follows:

$$\% \text{ Total carbohydrate} = (100 - (\% \text{ Ash} + \% \text{ fibre} + \% \text{ fat} + \% \text{ protein}))$$

The calorie content was determined as shown below:

$$\text{Calory content (Kcal/100g)} = (\text{crude protein} \times 4) + (\text{Total carbohydrate} \times 4) + (\text{crude fat} \times 9)$$

### 2.7. Determination of oxalate content

The levels of oxalate in the samples were determined using the procedure detailed by Day and Underwood [29]. Each 1 g sample was immersed in 75 mL of 1.5 N sulfuric acid for 1 hour, then filtered using No. 1 Whatman filter paper. Subsequently, 25 mL of the filtrate was transferred to a conical flask and titrated hot at approximately 80–90 °C against 0.1 M potassium permanganate until a persistent pink coloration endpoint was achieved for 15 seconds. The value obtained from the titration was then used to calculate the oxalate content.

$$\text{Oxalate content} \left( \frac{\text{mg}}{100\text{g}} \right) = \frac{(\text{Titre value} \times \text{volume} - \text{mass equivalent of KMnO}_4 \times \text{dilution factor})}{(\text{Molar equivalent of KMnO}_4 \times \text{Weight of sample used}) \times 100}$$

### 2.8. Determination of phytate content.

The phytate content of the samples was evaluated

using the method outlined by Wheeler and Ferrel [30]. A 4 g portion of each sample was weighed and immersed in a beaker containing 100 mL of 2% hydrochloric acid for 180 minutes, then filtered using a No. 1 Whatman filter paper. Subsequently, 25 mL of the filtrate was transferred to a conical flask, followed by the addition of 5 mL of 0.3% ammonium thiocyanate as an indicator and 53.5 mL of distilled water to achieve the appropriate acidity of the solution. The resulting solution was titrated against a standard FeCl<sub>3</sub> solution with a concentration of 5.66 mg/mL, containing approximately 1.95 g of iron/mL until a brownish-yellow coloration persisted for 5 minutes. The phytate contents of the samples were calculated from the titer value as follows:

$$\text{Phytate content} \left( \frac{\text{mg}}{100\text{g}} \right) = \frac{(\text{Titre value} \times \text{Iron equivalent} \times \text{dilution factor})}{(\text{Weight of sample used}) \times 100}$$

### 2.9. Determination of total saponin content

The determination of total saponin content followed the procedure outlined by Obadoni and Ochuko [31]. Initially, 0.5 g of the powdered sample was blended with 200 mL of 20% ethanol and agitated on a shaker for 30 minutes. Subsequently, the plant sample underwent a 4-hour heating process in a water bath at 55°C. The resulting mixture was filtered, and the residue was subjected to another round of extraction using an additional 200 mL of 20% ethanol. The combined extracts were then concentrated to 40 mL over a water bath at 90°C. The concentrated solution was transferred to a 250 mL separating funnel, undergoing two extractions with 20 mL of diethyl ether. The ether layer was discarded, and the aqueous layer was retained, followed by the addition of 60 mL n-butanol. The n-butanol extracts underwent two washes with 10 mL of 5% sodium chloride. The remaining solution was heated in a water bath and subsequently oven-dried at 40°C until a constant weight was achieved. The percentage of saponin content was calculated using the following formula:

$$\% \text{ Saponin} = \frac{\text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

### 2.10. Determination of DPPH-scavenging activity

The capacity of meat alternatives to neutralize the

DPPH radical was determined following the procedure outlined by Braca et al. [32]. A total of 4.5 mL of a 0.002% alcoholic solution of DPPH was combined with 0.5 mL of various concentrations (250, 500, 1000, and 2000 µg/mL) of samples and standard solutions separately, resulting in final product concentrations of 25-200 µg/mL. The samples were then stored at room temperature in the dark, and after 30 minutes, the absorbance of the resulting solution was measured at 517 nm. The absorbance of the samples, control, and blank was measured in comparison with methanol. The scavenging activity was expressed as the percentage of DPPH radicals scavenged:

$$\text{DPPH radical scavenged (\%inhibition)} = \frac{\text{Absorbance}_{\text{reference}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{reference}}} \times 100$$

The IC<sub>50</sub> value (g/L) was determined by plotting the percentage inhibition against the inhibitor concentration and using logit regression to interpolate the concentration at which the inhibition is 50%.

### 2.11. Determination of ferric-reducing antioxidant power (FRAP)

The ferric-reducing antioxidant capacity of meat alternatives was evaluated using the method described by Yildirim [33]. The dried extract (125–1000 µg) in 1 mL of the corresponding solvent was combined with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (K<sub>3</sub>Fe(CN)<sub>6</sub>; 10 g/L). The mixture was then incubated at 50 °C for 30 minutes. After incubation, 2.5 mL of trichloroacetic acid (100 g/L) was added, and the mixture was centrifuged at 1650 g for 10 minutes. Following centrifugation, 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl<sub>3</sub> (1 g/L), and the absorbance was measured at 700 nm. A higher absorbance value indicates greater reducing power. TBHQ, a potent ferric reducer, was used as a positive control to compare the reducing power of the extracts.

$$\text{FRAP} \left( \text{mg} \frac{\text{AAE}}{\text{g}} \right) = \frac{\text{Absorbance}_{\text{sample}} \times \text{Slope}_{\text{standard curve}} \times \text{dilution factor}}{\text{Weight of sample used}}$$

The IC<sub>50</sub> value (g/L) was determined by plotting the

**Table 1.** Proximate values of different meat alternatives made with pumpkin seeds, wheat gluten, and soybeans.

Content	Meat alternatives				
	Soybean	Pumpkin seed (100%)	Pumpkin seed/soy (50:50)	Pumpkin seed/soy (70:30)	Wheat gluten
Water (%)	56.42 ± 0.02 <sup>b</sup>	58.12 ± 1.75 <sup>b</sup>	52.06 ± 0.04 <sup>a</sup>	58.34 ± 0.11 <sup>b</sup>	61.84 ± 0.01 <sup>c</sup>
Proteins (%)	34.60 ± 0.70 <sup>c</sup>	16.43 ± 0.85 <sup>a</sup>	12.77 ± 0.51 <sup>a</sup>	21.25 ± 0.54 <sup>b</sup>	73.31 ± 2.82 <sup>d</sup>
Fats (%)	12.37 ± 0.19 <sup>c</sup>	1.43 ± 0.14 <sup>a</sup>	10.36 ± 0.18 <sup>b</sup>	13.63 ± 0.30 <sup>d</sup>	1.88 ± 0.07 <sup>a</sup>
Carbohydrates (%)	50.11 ± 0.78 <sup>b</sup>	76.31 ± 0.84 <sup>d</sup>	73.18 ± 0.58 <sup>d</sup>	61.15 ± 0.84 <sup>c</sup>	22.96 ± 2.84 <sup>a</sup>
Crude fiber (%)	ND	ND	ND	ND	ND
Ash (%)	2.91 ± 0.02 <sup>b</sup>	5.83 ± 0.02 <sup>e</sup>	3.68 ± 0.04 <sup>c</sup>	3.97 ± 0.03 <sup>d</sup>	1.85 ± 0.02 <sup>a</sup>
Energy (kcal)	450.24 ± 0.91 <sup>d</sup>	383.88 ± 0.76 <sup>a</sup>	437.07 ± 1.02 <sup>c</sup>	452.25 ± 1.51 <sup>d</sup>	402.00 ± 0.43 <sup>b</sup>

The figures carrying the same superscripts on the same row were not statistically significant ( $p \geq 0.05$ ). ND: not detectable

percentage inhibition against the inhibitor concentration and using logit regression to interpolate the concentration at which the inhibition is 50%.

### 2.12. Sensory analysis

The panel comprised individuals of both genders who met specific criteria: (1) absence of allergies to soybeans, pumpkin, and wheat, (2) not pregnant or attempting conception, (3) refraining from operating heavy machinery or driving within 2 hours post-sensory test, and (4) being of legal age, 21 years or older (the legal age of majority in Cameroon). Approval for the study was obtained from the Department of Nutrition, Food, and Bioresource, College of Technology, University of Bamenda, Bamili, Cameroon. Participants were provided with an information sheet and required to provide written consent before the experiments commenced. The sensory evaluation of meat alternatives involved 50 untrained panelists who participated in hedonic tests. The panel members, consisting of both males and females, assessed the attributes such as appearance, aroma, taste, and sweetness. A 9-point rating scale was utilized, ranging from (1) extremely unpleasant to (9) extremely pleasant, to evaluate each descriptor and the overall acceptability. Each sample, amounting to 30 g, was presented on a plate. To mitigate any taste interference, panelists were instructed to take a three-minute break and drink water between tastings. The samples were anonymized with 3-digit codes.

### 2.13. Statistical analysis

The data collected in this study was input into data entry forms using Microsoft Excel 365 and then analyzed using the Statistical Package for Social Science (SPSS) version 27. Continuous data was

presented as mean ± standard deviation, while categorical data was expressed in terms of frequency and percentage. To compare different meat alternatives in terms of nutritional, antinutritional, antioxidant, and sensory properties, one-way ANOVA (Analysis of Variance) coupled with Tukey tests was employed. A linear discriminant analysis model was constructed employing a stepwise approach to differentiate between meat alternatives based on sensory acceptability. This method selected predictor variables that exhibited the greatest influence on group discrimination. Before analysis, the data underwent preprocessing to assess normality and variance homogeneity. Model validation was conducted through leave-one-out cross-validation, offering an estimation of classification accuracy. Multi-layer Perceptron neural network models were utilized to assess the sensory factors influencing the acceptability of the five meat alternatives. The training process involves feeding input data through the network, computing errors, and updating weights using gradient descent. Three performance indicators were used to validate the performance of the models namely: Training accuracy, testing accuracy, and Area under the Curve (AUC). Differences between comparable sets of results were considered significant at  $p < 0.05$ .

## 3. Results and discussion

### 3.1 Proximate composition of the meat alternatives

The water content of the five meat alternatives varied from 52.06% to 61.84% (Table 1). Water plays a pivotal role in determining the quality of food products, influencing their acceptability, freshness, and storability. In this study, seitan demonstrated the

highest moisture content at 61.84%, attributed to its ability to absorb 1.3-1.5 times its weight in water. Following closely was the moisture content of the pumpkin seed curd/soy curd blend (ratio 70:30) at 58.34%. The incorporation of pumpkins into meat products has been shown to affect their water content. Serdaroğlu et al. [34] reported a water content range of 55.83% to 58.69% in cooked beef patties formulated with pumpkin mix at concentrations of 2% to 5%. This finding may be attributed to the higher water retention capacity of pumpkins. Similarly, Zargar et al. [35] observed significant increases in moisture content (63.11% - 67.63%) in chicken sausages formulated with pumpkin pulp at concentrations of 6% to 18%. The higher moisture content of fresh pumpkins may account for this increase. These studies suggest that the addition of pumpkins to meat products can have a significant impact on their moisture content.

The protein content in various meat alternatives produced ranged from 16.43% to 34.60% (Table 1). Soybean curd exhibited the highest protein content, surpassing that of tofu coagulated with steep water (17.6%),  $\text{CaCl}_2$ ,  $\text{H}_2\text{O}$  (15.1%), and alum (13.3%) as reported by Oboh and Omotosho [26]. The enhanced protein content may be attributed to vinegar creating a more conducive coagulating environment for proteins present in soymilk, as hypothesized by Oboh and Omotosho [26]. Pumpkin seed/soy curd with a ratio of 70:30 showed a comparable protein content (21.25%) to that found in cooked beef patties formulated with pumpkin mix (2% to 5%), falling within the range of 20.26% - 21.33% [34]. Likewise, a pumpkin seed/soy curd blend with a ratio of 50:50 had a protein content of 12.77%, which is similar to that of chicken sausages formulated with pumpkin pulp at concentrations of 6% to 18% (15.81%-16.55%) [35]. This indicates that a mixture of pumpkin seed/soy curd can replicate a similar protein content to that of certain meat hybrids.

The fat content ranged from 1.43% to 13.63% (Table 1). The pumpkin seed curd/soy curd blend with a ratio of 70:30 demonstrated the highest fat content at 13.63%. Conversely, the lowest fat content was observed in pumpkin seed curd (100%) at 1.43%. These fat levels were lower than those found in beef patties formulated with pumpkin mix (18.18%-20.26%) [34].

However, they were higher than the fat content in hybrid sausages containing broccoli, upcycled brewer's spent grain, and insect flour (5.6% - 8.7%) [36]. It is noteworthy that the fat content of the wheat gluten-derived meat alternative (1.88%) in this study was slightly higher than the value reported by Schopf et al. [37] at 1.34%. This suggests that the fat content of meat hybrids and plant-based meat alternatives is contingent on the specific formulation employed.

The carbohydrate content, which surpassed other nutrients, ranged from 22.96% to 76.31% (Table 1). In the findings presented in Table 1, the meat alternative from pumpkin seed curd (100%) exhibited the highest carbohydrate level of 76.3%, followed by the meat alternative from pumpkin seed/soy curd blend with a ratio of 50:50 at 73.18%. Plant-based meat analogs are recognized for having elevated levels of complex carbohydrates [38]. The wheat gluten-derived meat alternative in this study was determined to contain a higher amount of carbohydrates compared to the figure reported by Schopf et al. [37], which was 5.5%. In this study, all samples exhibited undetectable levels of fibers (Table 1). Surprisingly, certain studies have indicated pumpkin seeds and soybeans as good sources of dietary fiber, ranging around 6% for pumpkin seeds and 9% to 16.5% for soybeans [39-41]. This suggests that the curd processing method may not have retained the fibers. Wheat gluten is widely recognized for being low in fibers ( $\leq 1.5\%$ ) [42]. Researchers have reported appreciable fiber content in some meat hybrids, ranging from 1% to 4% [36,38,43]. Therefore, the fiber content of plant-based meat analogs is influenced by the specific ingredients used in their production. High-fiber foods, such as fruits, vegetables, and whole grains, have consistently demonstrated significant health benefits and effectively reduced the risk of disease [44].

The ash content varied between 1.85% and 5.83% (Table 1). As shown in Table 1, the meat alternative from pumpkin seed curd (100%) displayed the highest ash content at 5.83%, whereas the lowest ash content was observed in the wheat gluten-derived meat alternative at 1.85%. Notably, the ash content in the meat alternative pumpkin seed curd (100%) (5.83%) was slightly higher than that reported for pumpkin seed alone (4.77%) [44]. Similarly, the ash content in

**Table 2.** Phytochemical values of different meat alternatives made from pumpkin seeds, soya beans, and wheat gluten

Content (for 100g of DW)	Meat alternatives				
	Soybean	Pumpkin seed (100%)	Pumpkin seed/soy (50:50)	Pumpkin seed/soy (70:30)	Wheat gluten
Phytates (mg)	8.97 ± 0.13 <sup>c</sup>	5.52 ± 0.09 <sup>b</sup>	10.44 ± 0.57 <sup>d</sup>	11.69 ± 0.63 <sup>d</sup>	4.15 ± 0.64 <sup>a</sup>
Oxalates (mg)	7.10 ± 0.35 <sup>c</sup>	4.85 ± 0.20 <sup>a</sup>	6.14 ± 0.11 <sup>b</sup>	6.41 ± 0.07 <sup>b</sup>	19.55 ± 0.35 <sup>d</sup>
Saponins (mg)	ND	ND	ND	ND	ND

The figures carrying the same superscripts on the same row were not statistically significant ( $p \geq 0.05$ ). ND: not detectable

the wheat gluten-derived meat alternative exceeded the value reported by Schopf et al. [37], which was 0.93%. This observation may indicate a concentration of minerals during the preparation process.

The caloric content of samples ranged from 383.88 kcal to 450.24 kcal (Table 1). Within the samples, the highest caloric content was observed in the meat alternative from pumpkin seed curd/soy curd blend (70:30) at 452.25 kcal, while the lowest was recorded in the meat alternative derived from pumpkin seed curd (100%) with a caloric value of 383.88 kcal. Despite the common perception among consumers that plant-based meat analogs have lower caloric content, it was demonstrated that some exhibit comparable caloric values to animal products [38].

### 3.2. Phytochemical factors of produced meat alternatives.

The results show that the phytate content in the various meat alternatives varied from 5.52 to 11.69 mg/100g, with the highest level found in the meat alternatives derived from pumpkin seed curd/soy curd blend (70:30) (Table 2). These phytate levels are higher than the 0.35 mg/100g reported for raw seeds of pumpkin (*C. maxima*) used to prepare the meat alternatives [45]. However, the phytate levels in the curds are still lower than the value reported for raw soy, which was also used to prepare the meat alternatives, and are 11.73 mg/g (1173 mg/100g) [46]. This suggests that while the meat alternatives have higher phytate levels compared to raw pumpkin seeds, they are still lower than what would be expected based on the phytate content of raw soy. It is important to note that phytate, which inhibits mineral absorption, is known to accumulate in the protein fraction during the extraction of plant proteins, suggesting lower mineral bioavailability in meat alternatives [47]. According to Thompson [48], a diet with 10–60 mg/g phytate over an extended period can lead to reduced mineral bioavailability in monogastric

animals. In this study, the phytate content in meat alternatives derived from soybean, pumpkin seed, and wheat gluten fell below this specified range. Similarly, phytates have been shown to inhibit the bioavailability of iron and calcium in the diets of pregnant women in rural Bangladesh [49], highlighting the potential impact of phytates on human nutrition.

The highest concentration of oxalate was found in the wheat gluten-derived meat alternative (19.55 mg/100g), while the lowest was found in meat alternatives from pumpkin seed curd (4.85 mg/100g) (Table 2). Plant-based meat products often have protein sources that, in comparison to animal protein sources, are higher in oxalate. For instance, soy-based beef has the highest average oxalate content (18 mg per serving) [50]. Raw legumes also exhibit a wide range of oxalate content. Soybeans have the highest amount (370 mg/100 g DW), followed by lentils and peas (168–293 mg/100 g DW), chickpeas (192 mg/100 g DW), and common beans (98–117 mg/100 g DW) [51]. When comparing the oxalate content of raw legumes with that of the meat alternatives produced in this study, it suggests that curd preparation has led to a reduction in oxalate content in the samples. It is noteworthy that a diet high in oxalate may increase the risk of renal calcium absorption and has been associated with kidney stone formation [52–54].

No saponins were detected in any of the samples in this study (Table 2), even though saponins are known to be present in some of the ingredients used to prepare the meat alternatives. Saponins are present in pumpkin seeds (*C. maxima*) at a level of 3.42 mg/100g, soybeans at a level of 2.80 g/100g, and wheat flour at a level of 0.26% [45, 55, 56]. This suggests that the processing involved in making the meat alternatives may have reduced or eliminated the saponins. It is worth noting that plant-based meat analogs,

primarily composed of soy and legume proteins, often have flavors that tend to be somewhat astringent and bitter due to the presence of compounds such as saponins, among others [57, 58]. Thus, the absence of saponins in the samples from this research likely contributed to the enhanced sensory properties of the meat alternatives.

3.3. Antioxidant activities of produced meat alternatives.

Table 3 displays the IC50 values of various meat alternatives. These values were calculated to determine the amount of sample required to take up 50% of the radical or yield 50% of ferric reduction. The highest antioxidant activity is the one with the lowest IC50 value.

**Table 3.** Inhibitory concentration 50 (IC50) of meat alternatives using DPPH and FRAP tests

Meat alternative	IC50 (g/L)	
	DPPH	FRAP
Wheat gluten	178.24 ± 0.40 <sup>cd</sup>	165.30 ± 0.21 <sup>d</sup>
Soy curd	150.03 ± 12.01 <sup>bc</sup>	152.30 ± 0.18 <sup>c</sup>
Pumpkin seed curd (100%)	140.56 ± 12.98 <sup>b</sup>	147.73 ± 0.00 <sup>b</sup>
Pumpkin seed/soy curd 50:50	186.68 ± 1.84 <sup>d</sup>	147.49 ± 0.00 <sup>b</sup>
Pumpkin seed/soy curd 70:30	183.80 ± 1.57 <sup>d</sup>	166.21 ± 0.21 <sup>e</sup>
Butylhydroxytoluène (BHT)	49.49 ± 0.25 <sup>a</sup>	15.03 ± 0.05 <sup>a</sup>

The figures carrying the same superscripts on the same column were not statistically significant (p ≥ 0.05)

The results of this study revealed that the DPPH-scavenging activities of the meat alternatives varied, with values ranging from 140.56 g/L to 186.68 g/L (Table 3).

Among the different formulations tested, the meat alternative from pumpkin seed/soy curd blend at a ratio of 50:50 exhibited the highest IC50 value of 186.68 g/L, while the meat alternative from pure pumpkin seed curd had the lowest IC50 value of 140.56 g/L. These findings suggest that pumpkin seeds significantly contributed to the antioxidant properties of these meat alternatives, which is consistent with previous studies that have reported the DPPH-scavenging activities of pumpkin seeds (IC50 = 1337.87 ppm) [59].

Furthermore, our findings are in line with those of

other researchers who have reported the antioxidant activities of meat alternatives made from various plant-based ingredients. For instance, Song et al. [60] reported an IC50 value of 62 g/L for DPPH-scavenging in meat alternatives made from cashew nuts, walnut, soybean, black sesame, sesame, beet, onion, and gluten. Similarly, plant-based meat (PBM) incorporating novel plant-based ingredients, such as spirulina and yellow Chlorella, exhibited detectable levels of diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, with spirulina demonstrating the highest level of antioxidant activity [61]. Taken together, these results suggest that plant-based meat alternatives can provide a valuable source of antioxidants and may offer a healthier and more sustainable alternative to traditional meat products.

The ferric-reducing antioxidant activities of various meat alternatives ranged between 147.49 g/L and 166.21 g/L (Table 3). The IC50 values for FRAP revealed that the meat alternative from pumpkin seed/soy curd blend at a ratio of 70:30 exhibited the least antioxidant activity (166.21 g/L), while the highest antioxidant activity was observed in the meat alternative derived from pure pumpkin seed curd (147.13 g/L). When compared to BHT, all meat alternatives displayed relatively low ferric-reducing antioxidant activities. Meat analogs are known to possess iron-reducing antioxidant power varying between 3.46 and 7.51 mmol/g [23]. Additionally, researchers confirmed the ability to reduce iron (III) ions for pumpkin seeds, with the reducing power determined at the level of 8.67 mg AAE/g [62]. Soybeans are also known to possess iron-reducing antioxidant power varying between 8.18 and 13.75 mol TE/g [57].

The ferric-reducing antioxidant power (FRAP) of the meat alternatives was evaluated in this study, with values ranging from 147.49 g/L to 166.21 g/L (Table 3). The meat alternative from pumpkin seed/soy curd blend at a ratio of 70:30 exhibited the lowest antioxidant activity (166.21 g/L), while the highest antioxidant activity was observed in the meat alternative from pure pumpkin seed curd (147.13 g/L). These findings suggest that pumpkin seeds may be a significant contributor to the antioxidant properties of these meat alternatives, which is consistent with

previous studies that have reported the iron-reducing antioxidant power of pumpkin seeds (8.67 mg AAE/g) [60].

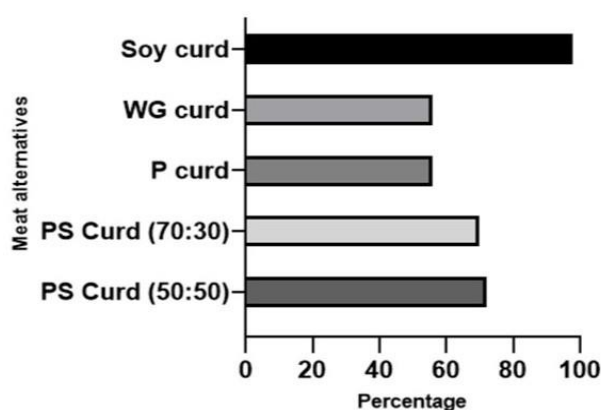
Previous studies have reported varying levels of iron-reducing antioxidant power in meat analogs, ranging from 3.46 to 7.51 mmol/g [23]. Soybeans, another ingredient used in some of the meat alternatives, have also been reported to possess iron-reducing antioxidant power varying between 8.18 and 13.75 mol TE/g [63]. Therefore, the FRAP values obtained in this study may be influenced by the specific ingredients and formulations used in each meat alternative.

The findings of this study indicated that the meat alternatives exhibited lower DPPH-scavenging and ferric-reducing activities compared to BHT, a synthetic antioxidant that is frequently utilized in the food industry. However, there is growing interest in the development of natural antioxidants from plant-based sources as alternatives to synthetic antioxidants due to concerns about the safety and potential adverse effects of synthetic antioxidants [64].

Overall, these findings suggest that the antioxidant activity of meat alternatives may vary depending on their composition and the assay used to evaluate their activity.

### 3.4. Sensory attributes of produced meat alternatives.

The sensory acceptability of the five meat alternatives is depicted in Fig. 1, showing an overall appreciation ranging from 56% to 98%.

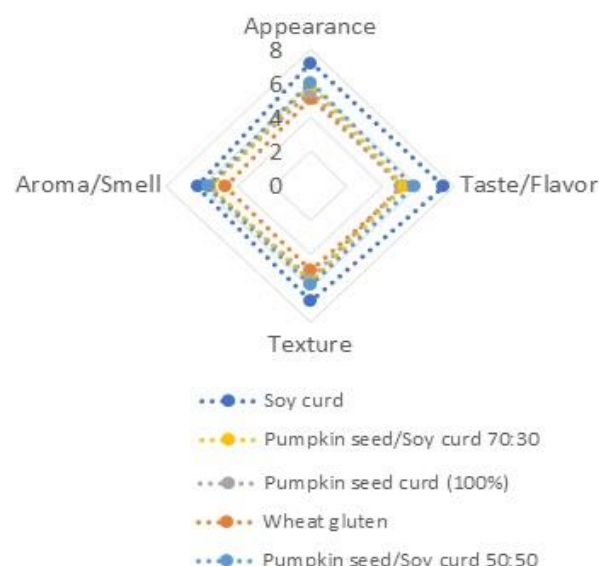


**Figure 1.** Liking rating of various meat alternatives  
WG curd: wheat gluten, P curd: pumpkin seed-based curd, PS curd (70:30): pumpkin seed/soy curd (70:30), and PS curd (50:50): pumpkin seed/soy curd (50:50).

Meat alternatives derived from wheat gluten and pumpkin seed curds (100%) received the lowest appreciation scores at 56%, while soy tofu had the highest at 98%. This suggests that soy tofu was the most preferred meat alternative among the options presented.

The combination of soy and pumpkin seeds led to an increased acceptability of meat alternatives derived from pumpkin seed curds, varying between 70% and 72%. This suggests that combining pumpkin seed curd with soy may improve its sensory properties and make it more appealing to consumers. It is important to note that crafting plant-based alternatives (PBAs) that are visually appealing, texturally satisfying, and flavorful, while preserving their nutritional value and functionality, remains a significant hurdle [65]. In this study, meat alternatives from the combination of soy and pumpkin seeds showed promising sensory acceptability and nutritional values.

The findings also suggested that the sensory properties of the meat alternatives varied depending on the type of samples (Fig. 2). The meat alternative derived from wheat gluten received neutral ratings for all sensory descriptors, indicating that it was neither liked nor disliked by consumers.



**Figure 2.** Hedonic evaluation of the different meat alternatives based on appearance, taste, texture, and aroma

**Table 4.** Validation of the various Perceptron Artificial Neural Network models

Variables	Soy curd	Wheat gluten	Pumpkin seed-based curd (100%)	Pumpkin seed/Soy curd (70:30)	Pumpkin seed/Soy curd (50:50)
Training accuracy	100.0%	93.3%	89.3%	88.2%	100.0%
Testing accuracy	100.0%	93.7%	90.0%	93.3%	86.7%
Area under the Curve (AUC)	1.000	0.976	0.939	0.948	0.992

**Table 5.** Importance of independent variables in determining overall sensory acceptability for meat alternatives produced from pumpkin seeds, soy, and wheat gluten.

Variables	Soy curd	Wheat-gluten	pumpkin seed-based curd (100%)	pumpkin seed/Soy 70:30 curd	pumpkin seed/Soy 50:50 curd
Appearance	0.128	0.159	0.124	0.249	0.129
Taste	0.361	0.501	0.461	0.356	0.450
Texture	0.178	0.105	0.210	0.230	0.115
Aroma	0.333	0.235	0.205	0.165	0.306

The soy-derived meat alternative, on the other hand, received moderately likable ratings for appearance, taste, and texture, and slightly likable ratings for aroma. The meat alternative derived from pumpkin seed curd (100%) received neutral ratings for taste and texture, and slightly likable ratings for appearance and aroma. The combination of pumpkin seed and soy curd in a 50:50 ratio resulted in slightly likable ratings for all sensory descriptors, while the 70:30 ratio received slightly likable ratings for all descriptors except for taste, which was rated as neutral.

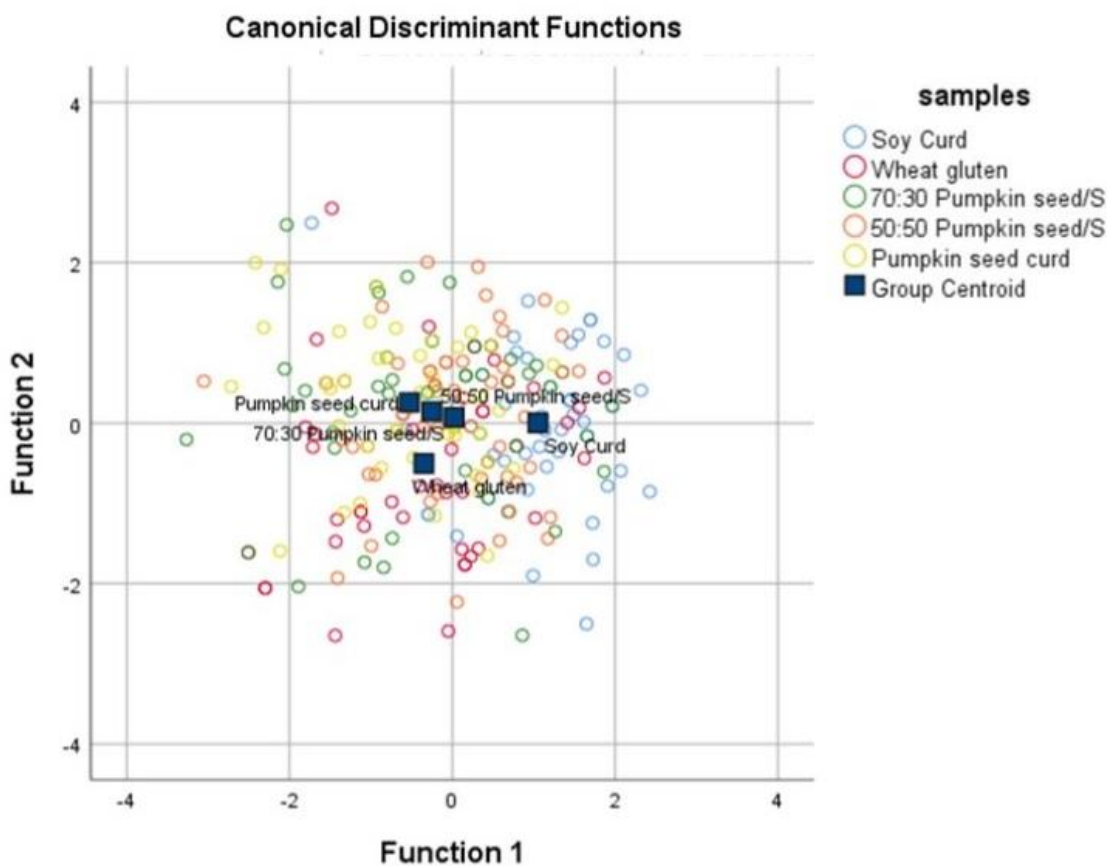
Overall, these findings suggested that the sensory properties of meat alternatives can be influenced by the type of foodstuff used and that combinations of different curds may lead to more desirable sensory characteristics. The results also indicate that while some meat alternatives may not be strongly liked or disliked, they may still be acceptable to consumers.

The results presented in Table 4 indicate that five artificial neural network models based on perceptron architecture were developed and validated to analyze the determinants of sensory acceptability for the five meat alternatives. The performance indicators suggest that all five machine learning algorithm models were effective in predicting factors associated with sensory acceptability, with both training and testing accuracies exceeding 70% and the area under the curve (AUC) approaching 1.

Moreover, the results presented in Table 5 indicate that taste is the most important factor to consider when developing the five meat alternatives, followed by aroma, texture, and appearance. However, research has shown that the primary challenge for product developers in creating plant-based dairy and meat alternatives that resemble conventional products is accurately replicating the textural properties [66]. This indicates that while taste is a critical factor in consumer acceptance, texture is also an essential component in creating a successful meat alternative. Therefore, product developers must prioritize both taste and texture to create plant-based meat alternatives that closely resemble conventional meat products.

Overall, these findings confirm the fact that artificial neural networks can be useful tools in predicting the sensory acceptability of meat alternatives and identifying the key sensory attributes that drive consumer acceptance as demonstrated by previous studies [67, 68]. This information can be valuable for food manufacturers aiming to develop plant-based meat alternatives that meet consumer expectations.

The factor analysis conducted to assess sensory discrimination of the five meat alternatives, including tofu, revealed that all five meat alternatives have a similar sensory profile in terms of appearance, taste, texture, and aroma (Fig. 3). This suggests that consumers may not be able to easily distinguish between the different meat alternatives based on



**Figure 3.** Analysis of sensory discrimination for meat alternatives derived from pumpkin seeds, soy, and wheat gluten across appearance, taste, texture, and aroma.

sensory characteristics alone. In this study, the meat alternatives developed using pumpkin seeds as the main ingredient were therefore found to have a sensory profile similar to soy tofu. Thus, it can be concluded that the developed pumpkin seed-based meat alternatives mimic the sensory characteristics of soy tofu that are generally well appreciated by consumers. The findings also imply that the five meat alternatives might be used interchangeably in recipes concerning their sensory properties.

#### 4. Conclusions

Several conclusions can be drawn based on the findings related to the nutritional, anti-nutritional, antioxidant, and sensory characteristics of the five meat alternatives. Firstly, meat alternatives derived from wheat gluten and pumpkin seed/soy curd at a ratio of 70:30 exhibited the highest protein content (21.25%-22.96%). The lowest fat content was observed in meat alternatives from wheat gluten and pumpkin seed-based curd (1.43%-1.88%). Moreover, meat

alternatives derived from pumpkin seed/soy curds at the ratio of 70:30 and 50:50 had the highest phytate content (10.44-11.69 mg/100g), while the one derived from wheat gluten contained the highest oxalate content (19.55 mg/100g). Additionally, the meat alternative derived from pumpkin seed curd blends at a ratio of 50:50 displayed the highest DPPH-scavenging activity (186.68 g/l). In contrast, the highest ferric-reducing antioxidant activity was observed in the meat alternative derived from pumpkin seed curd at the ratio of 50:50 (147.49 g/L) and pumpkin seed curd (100%) (147.73 g/L). Overall, the meat alternative derived from soy curd was the most highly appreciated (98%) though meat alternatives derived from pumpkin seed/soy curds at 70:30 and 50:50 showed acceptable appreciation, 70% and 72% respectively. Although meat alternatives shared a similar hedonic profile based on descriptors, taste, and aroma emerged as the primary hedonic promoters. Therefore, each of the meat alternatives exhibited interesting traits related to at least one of the

properties examined, underscoring their usefulness in the diet.

While the current study provides valuable insights into the characteristics of various meat alternatives, several areas warrant further investigation. Future research should focus on the long-term health effects of consuming these meat alternatives, including their impact on nutrient absorption and overall health outcomes. Exploring different processing techniques and their impact on the nutritional and sensory qualities of meat alternatives could lead to further optimization of these products. More extensive consumer studies are needed to understand the acceptance and preferences for these meat alternatives across different demographic groups and cultural contexts. Assessing the environmental impact and sustainability of producing these meat alternatives compared to traditional meat products is crucial for promoting their adoption.

The findings of this study have significant implications for the food industry. The high protein content and low fat content of certain meat alternatives make them attractive to health-conscious consumers, opening up new market opportunities. The diverse nutritional and sensory profiles of these meat alternatives can be leveraged to create a range of products tailored to different consumer preferences and dietary needs. While the nutritional benefits are clear, the cost of production and market pricing of these meat alternatives need to be carefully considered. Ensuring that these products are affordable will be key to their widespread adoption. The food industry can invest in research and development to improve the taste, texture, and overall quality of these meat alternatives, making them more competitive with traditional meat products.

By addressing these research gaps and capitalizing on the industrial prospects, the development and adoption of meat alternatives can be significantly advanced, contributing to a more sustainable and healthier food system.

### Authors' contributions

Conceived and designed the study, performed the data analysis, and wrote the original draft of the manuscript, B.U.F.S.; Conducted laboratory

experiments, including the preparation of meat alternatives and the analysis of nutritional, anti-nutritional, and antioxidant properties, F.N.; Helped in the design of the study and contributed to the interpretation of the results, I.T.; Supervised the research and provided critical feedback on the manuscript, R.A.E.

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### Availability of data and materials

The data used to support the findings of this study can be obtained from the corresponding author upon request.

### Conflicts of interest

The authors declare that they have no conflicts of interest.

### References

1. United Nations Department of Economic and Social Affairs, and Population Division. World Population Prospects 2022: Summary of Results. UN DESA/POP/2022/TR/NO. 3. New York, NY, 2022.
2. Willett, W.; Rockström, J.; Loken, B.; Springmann, M.; Lang, T.; Vermeulen, S.; Garnett, T.; Tilman, D.; DeClerck, F.; Wood, A.; Jonell, M.; Clark, M.; Gordon, L.J.; et al. Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. *Lancet*. 2019, 393, 447-492. [https://doi.org/10.1016/S0140-6736\(18\)31788-4](https://doi.org/10.1016/S0140-6736(18)31788-4).
3. Gibbs, J.; Cappuccino, F.P. Plant-based dietary patterns for human and planetary health. *Nutrients*. 2022, 14, 1614. <https://doi.org/10.3390/nu14081614>.
4. FAO. The Future of Food and Agriculture - Trends and Challenges, Rome: Food and Agriculture Organization of the United Nations, 2017.
5. Hoek, A.C.; Malekpour, S.; Raven, R.; Court, E.; Byrne, E. Towards environmentally sustainable food systems: Decision-making factors in sustainable food production and consumption. *Sustain. Prod. Consum.* 2021, 26, 610-626. <https://doi.org/10.1016/j.spc.2020.12.009>.

6. Alcorta, A.; Porta, A.; Tárrega, A.; Alvarez, M.D.; Vaquero, M.P. Foods for Plant-Based Diets: Challenges and Innovations. *Foods*. 2021, 10, 293. <https://doi.org/10.3390/foods10020293>.
7. McClements, D.J.; Grossmann, L. A Brief Review of the Science behind the Design of Healthy and Sustainable Plant-Based Foods. *NPJ Sci. Food*. 2021, 5, 17. <https://doi.org/10.1038/s41538-021-00099-y>.
8. Hu, F. B.; Otis, B. O.; McCarthy, G. Can Plant-Based Meat Alternatives Be Part of a Healthy and Sustainable Diet? *JAMA*. 2019, 322, 1547–1548. <https://doi.org/10.1001/jama.2019.13187>.
9. Gebhardt, B. Plant-Based for the Future. In *Insights on European Consumer and Expert Opinions*. University of Hohenheim, Germany, 2021.
10. Ritchie, H.; Reay, D. S.; Higgins, P. Potential of meat substitutes for climate change mitigation and improved human health in high-income markets. *Front. Sustain. Food Syst.* 2018, 2, 16. <https://doi.org/10.3389/fsufs.2018.00016>.
11. Tonstad, S.; Butler, T.; Yan, R.; Fraser, G. E. Type of vegetarian diet, body weight, and prevalence of type 2 diabetes. *Diabetes Care* 2009, 32, 791–796. <https://doi.org/10.2337/dc08-1886>.
12. Tusso, P. J.; Ismail, M. H.; Ha, B. P.; Bartolotto, C. Nutritional update for physicians: plant-based diets. *Perm. J.* 2013, 17, 61–66. <https://doi.org/10.7812/TPP/12-085>.
13. McMacken, M.; Shah, S. A plant-based diet for the prevention and treatment of type 2 diabetes. *J. Geriatr. Cardiol.* 2017, 14, 342–354. <https://doi.org/10.11909/j.issn.1671-5411.2017.05.009>.
14. Tilman, D.; Clark, M. Global diets link environmental sustainability and human health. *Nature* 2014, 515, 518–522. <https://doi.org/10.1038/nature13959>.
15. Dinnella, C.; Napolitano, F.; Spinelli, S.; Monteleone, E.; Pacelli, C.; Braghieri, A. Factors affecting stated liking for meat products: Focus on demographics, oral responsiveness, personality, and psycho-attitudinal traits. *Meat Sci.* 2023, 195, 109004. <https://doi.org/10.1016/j.meatsci.2022.109004>.
16. Godfray, H.C.J.; Aveyard, P.; Garnett, T.; Hall, J. W.; Key, T.J.; Lorimer, J.; Pierrehumbert, R.T.; Scarborough, P.; Springmann, M.; Jebb, S.A. Meat consumption, health, and the environment. *Science*. 2018, 361, eaam5324. <https://doi.org/10.1126/science.aam5324>.
17. Al-Thawadi, S. Public perception of algal consumption as an alternative food in the Kingdom of Bahrain. *Arab J. Basic Appl. Sci.* 2018, 25, 1–12. <https://doi.org/10.1080/25765299.2018.1449344>.
18. Mistry, M.; George, A.; Thomas, S. Alternatives to meat for halting the stable to table continuum – an update. *Arab J. Basic Appl. Sci.* 2020, 27, 324–334. <https://doi.org/10.1080/25765299.2020.1807084>.
19. Shuhong, L.; Dan, Z.; Kejuan, L.; Yinngnan, Y.; Zhongfang, L.; Zhenya, Z. Soy bean curd residue: composition, Utilization, and related limiting factors. *SRN Ind. Eng.* 2013, 41, 77–86. <https://doi.org/10.1155/2013/423590>.
20. Schepker, K. *Meet the Meatles(s), A Guide to Vegetarian Meat Substitutes*. Spring 2012, 13, 1.
21. Orcutt, M. W.; McMIndes, M. K.; Chu, H.; Mueller, I. N.; Bater, B.; Orcutt, A. L. Textured soy protein utilization in meat and meat analog products. In *Soy applications in food*; CRC Press: New York, 2006; pp. 155–184.
22. Riaz, M. N. Texturized soy protein as an ingredient. In *Proteins in food processing*; Yada, R. Y., Ed.; Woodhead Publishing Limited: England, 2004.
23. Abdullah, F.A.A.; Dordevic, D.; Kabourkova, E.; Zemancová, J.; Dordevic, S. Antioxidant and Sensorial Properties: Meat Analogues versus Conventional Meat Products. *Processes* 2022, 10, 1864. <https://doi.org/10.3390/pr10091864>.
24. Kołodziejczak, K.; Onopiuk, A.; Szpicer, A.; Poltorak, A. The effect of type of vegetable fat and addition of antioxidant components on the physicochemical properties of a pea-based meat analogue. *Foods*. 2023, 13, 71. <https://doi.org/10.3390/foods13010071>.
25. De Angelis, D.; Pasqualone, A.; Allegretta, I.; Porfido, C.; Terzano, R.; Squeo, G.; Summo, C. Antinutritional factors, mineral composition and functional properties of dry fractionated flours as influenced by the type of pulse. *Heliyon*. 2021, 7, e06177. <https://doi.org/10.1016/j.heliyon.2021.e06177>.
26. Oboh, G.; Omotosho, O.E. Effect of types of coagulant on the nutritive value and In vitro Multienzyme Protein Digestibility of Tofu. *J. Food Technol.* 2005, 3, 182–187.
27. A.A.C.C. *Approved methods of the American Association of Cereal Chemists*, St. Paul, Minnesota, U.S.A, 1998.
28. AOAC. *Association of Official Analytical Chemists. Official Methods of Analysis*, 15th Ed., Washington DC, 1990.
29. Day, R. A.; Underwood, A. L. *Quantitative Analysis*, Prentice Hall NJ, 1991.
30. Wheeler, E.L.; Ferrel, R.E.A method for phytic acid determination in wheat and wheat fractions. *Cereal Chem.* 1971, 48, 312–320.
31. Obadoni, B. O.; Ochuko, P. O. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci.* 2002, 8, 203–208. <https://doi.org/10.4314/gjpas.v8i2.16033>.

32. Braca, A.; Sortino, C.; Politi, M.; Morelli, I.; Mendez, J. Antioxidant activity of flavonoids from *Licania licaniaeflora*. J. Ethnopharmacol. 2002, 79, 379–381. [https://doi.org/10.1016/s0378-8741\(01\)00413-5](https://doi.org/10.1016/s0378-8741(01)00413-5).
33. Yildirim, A.; Mavi, A.; Oktay, A.A.; Algur, O.F.; Bilaloglu, V. Comparison of antioxidant and antimicrobial activity of tilia (*Tilia argenta* Desf. Ex. D.C.), sage (*Salvia triloba* L.), and black tea (*Camellia sinensis* L.) extracts. J. Agric. Food Chem. 2000, 48, 5030–5034. <https://doi.org/10.1021/jf000590k>.
34. Serdaroğlu, M.; Kavuşan, H.S.; İpek, G.; Öztürk, B. Evaluation of the Quality of Beef Patties Formulated with Dried Pumpkin Pulp and Seed. Korean J. Food Sci. Anim. Resour. 2018, 38, 1–13. <https://doi.org/10.5851/kosfa.2018.38.1.001>.
35. Zargar, F.A.; Kumar, S.; Bhat, Z.F.; Kumar, P. Effect of pumpkin on the quality characteristics and storage quality of aerobically packaged chicken sausages. Springerplus. 2014, 3, 39. <https://doi.org/10.1186/2193-1801-3-39>.
36. Talens, C.; Llorente, R.; Simó-Boyle, L.; Odriozola-Serrano, I.; Tueros, I.; Ibarra, M. Hybrid sausages: modelling the effect of partial meat replacement with broccoli, upcycled brewer's spent grain and insect flours. Foods. 2022, 11, 3396. <https://doi.org/10.3390/foods11213396>.
37. Schopf, M.; Wehrli, M.C.; Becker, T.; Jekle, M.; Scherf, K.A. Fundamental characterization of wheat gluten. Eur. Food Res. Technol. 2021, 247, 985–997. <https://doi.org/10.1007/s00217-020-03680-z>.
38. Costa-Catala, J.; Toro-Funes, N.; Comas-Basté, O.; Hernández-Macias, S.; Sánchez-Pérez, S.; Latorre-Moratalla, M.L.; Veciana-Nogués, M.T.; Castell-Garralda, V.; Vidal-Carou, M.C. Comparative assessment of the nutritional profile of meat products and their plant-based analogues. Nutrients. 2023, 15, 2807. <https://doi.org/10.3390/nu15122807>.
39. Redondo-Cuenca, A.; Villanueva-Suárez, M. J.; Rodríguez-Sevilla, M.D.; Mateos-Aparicio, I. Chemical composition and dietary fibre of yellow and green commercial soybeans (*Glycine max*). Food Chem. 2007, 101, 1216–1222. <https://doi.org/10.1016/j.foodchem.2006.03.025>.
40. Batool, M.; Ranjha M.M.A.N.; Roobab, U.; Manzoor, M.F.; Farooq, U.; Nadeem, H.R.; Nadeem, M.; Kanwal, R.; AbdElgawad, H.; Al Jaouni, S.K.; Selim, S.; Ibrahim, S.A. Nutritional value, phytochemical potential, and therapeutic benefits of Pumpkin (*Cucurbita* sp.). Plants. 2022, 24, 11(11), 1394. <https://doi.org/10.3390/plants11111394>.
41. Patel, K.; Soni, A.; Tripathi, R. Pumpkin seed: Nutritional composition, health benefits. Int. J. Home Sci. 2023, 9, 93–98.
42. Van Der Borgh, A.; Goesaert, H.; Veraverbeke, W.S.; Delcour, J.A. Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. J. Cereal Sci. 2005, 41, 221–237. <https://doi.org/10.1016/j.jcs.2004.09.008>.
43. Rizzolo-Brime, L.; Orta-Ramirez, A.; Puyol Martin, Y.; Jakszyn, P. Nutritional assessment of plant-based meat alternatives: A comparison of nutritional information of plant-based meat alternatives in spanish supermarkets. Nutrients. 2023, 15, 1325. <https://doi.org/10.3390/nu15061325>.
44. Ioniță-Mîndrican, C.B.; Ziani, K.; Mititelu, M.; Oprea, E.; Neacșu, S.M.; Moroșan, E.; Dumitrescu, D.E.; Roșca, A.C.; Drăgănescu, D.; Negrei, C. Therapeutic Benefits and Dietary Restrictions of Fiber Intake: A State-of-the-Art Review. Nutrients, 2022, 14, 2641. <https://doi.org/10.3390/nu14132641>.
45. Mohaammed, S.S.; Paiko, Y.B.; Mann, A.; Ndamitso, M.M.; Mathew, J.T.; Maaji, S. Proximate, mineral and anti-nutritional composition of *Cucurbita maxima* fruits parts. Nigerian J. Chem. Res. 2014, 19.
46. Redekar, N.R.; Glover, N.M.; Biyashev, R.M.; Ha, B.K.; Raboy, V.; Maroof, M.A.S. Genetic interactions regulating seed phytate and oligosaccharides in soybean (*Glycine max* L.). PLoS One 2020, 15, e0235120. <https://doi.org/10.1371/journal.pone.0235120>.
47. Labba, H.M.; Steinhausen, L.; Almius, K.E.; Bach Knudsen, A.S.; Sandberg, A. Nutritional composition and estimated iron and zinc bioavailability of meat substitutes available on the Swedish market. Nutrients. 2022, 14, 3903. <https://doi.org/10.3390/nu14193903>.
48. Thompson, L.U. Potential health benefits and problems associated with anti nutrients in foods. Int. J. Food Res. 1993, 26, 131–149. [https://doi.org/10.1016/0963-9969\(93\)90069-U](https://doi.org/10.1016/0963-9969(93)90069-U).
49. Al Hasan, S.M.; Hassan, M.; Saha, S.; Islam, M.; Billah, M.; Islam, S. Dietary phytate intake inhibits the bioavailability of iron and calcium in the diets of pregnant women in rural Bangladesh: a cross-sectional study. BMC Nutr. 2016, 2. <https://doi.org/10.1186/s40795-016-0064-8>.
50. Liaw, C.W.; Potretzke, A.M.; Winoker, J.S.; Matlaga, B.R.; Lieske, J.C.; Koo, K. Dietary assessment of lithogenic factors in plant-based meat products. J. Endourol. 2023, 37, 119–122. <https://doi.org/10.1089/end.2022.0189>.
51. Shi, L.; Arntfield, S.D.; Nickerson, M. Changes in levels of phytic acid, lectins and oxalates during soaking and cooking of Canadian pulses. Food Res. Int. 2018, 107, 660–668. <https://doi.org/10.1016/j.foodres.2018.02.056>.
52. Chai, W.; Liebman, M. Assessment of oxalate

- absorption from almonds and black beans with and without the use of an extrinsic label. *J. Urol.* 2004, 172, 953–957. <https://doi.org/10.1097/01.ju.0000135918.00761.8a>.
53. Mitchell, T.; Kumar, P.; Reddy, T.; Wood, K.D.; Knight, J.; Assimos, D.G.; Holmes, R.P. Dietary oxalate and kidney stone formation. *Am. J. Physiol. Renal. Physiol.* 2019, 316, F409-F413. <https://doi.org/10.1152/ajprenal.00373.2018>.
54. Chen, T.; Qian, B.; Zou, J.; Luo, P.; Zou, J.; Li, W.; Chen, Q.; Zheng, L. Oxalate as a potent promoter of kidney stone formation. *Front. Med.* 2023, 10, 1159616. <https://doi.org/10.3389/fmed.2023.1159616>.
55. Sun, H.; Meng, X.; Han, Y.; Li, X.; Li, X.; Li, Y. Soybean saponin content detection based on spectral and image information combination. *J. Spectrosc.* 2024, 2024, 12 pages. <https://doi.org/10.1155/2024/7599132>.
56. Pi, A.; Do, N. Phytochemical composition, physical and sensory properties of bread supplemented with fermented sweet orange peel flour. *Innovare J. Food Sci.* 2019, 7, 1–6.
57. Joshi, A.U.; Liu, C.; Sathe, S.K. Functional properties of select seed flours. *LWT - Food Sci. Technol.* 2015, 60, 325–331. <https://doi.org/10.1016/j.lwt.2014.08.038>.
58. Khan, M.I.; Jo, C.; Tariq, M.R. Meat flavor precursors and factors influencing flavor precursors—A systematic review. *Meat Sci.* 2015, 110, 278–284. <https://doi.org/10.1016/j.meatsci.2015.08.002>.
59. Jahan, F.; Islam, M. B.; Moulick, S.P.; Al Bashera, M.; Hasan, M.S.; Tasnim, N.; Saha, T.; Boby, F.; Waliullah, M.; Saha, A. K.; Hossain, A.; Ferdousi, L.; Rahman, M.M.; Saha, B.K.; Bhuiyan, M.N.H. Nutritional characterization and antioxidant properties of various edible portions of *Cucurbita maxima*: A potential source of nutraceuticals. *Heliyon.* 2023, 9, e16628. <https://doi.org/10.1016/j.heliyon.2023.e16628>.
60. Song, H.S.; Bae, J.K.; Park, I. Effect of heating on DPPH radical scavenging activity of meat substitute. *Prev. Nutr. Food Sci.* 2013, 18, 80–84. <https://doi.org/10.3746/pnf.2013.18.1.080>.
61. Bakhsh, A.; Park, J.; Baritugo, K.A.; Kim, B.; Sil Moon, S.; Rahman, A.; Park, S. A holistic approach toward development of plant-based meat alternatives through incorporation of novel microalgae-based ingredients. *Front. Nutr.* 2023, 10, 1110613. <https://doi.org/10.3389/fnut.2023.1110613>.
62. Akomolafe, S.F.; Oboh, G.; Oyeleye, S.I.; Molehin, O.R.; Ogunsuyi, O. Phenolic composition and inhibitory ability of methanolic extract from pumpkin (*Cucurbita pepo* L) seeds on Fe-induced thio-barbituric acid reactive species in albino rat's testicular tissue in-vitro. *J. Appl. Pharm. Sci.* 2016, 6, 115–120. <https://doi.org/10.7324/JAPS.2016.60917>.
63. Dajanta, K.; Janpum, P.; Leksing, W. Antioxidant capacities, total phenolics and flavonoids in black and yellow soybeans fermented by *Bacillus subtilis*: A comparative study of Thai fermented soybeans (Thua nao). *Int. Food Res. J.* 2013, 20, 3125–3132. <https://doi.org/10.4172/1948-5948.1000052>.
64. Lourenço, S.C.; Moldão-Martins, M.; Alves, V.D. Antioxidants of natural plant origins: from sources to food industry applications. *Molecules.* 2019, 24, 4132. <https://doi.org/10.3390/molecules24224132>.
65. Xing, Z.; Li, J.; Zhang, Y.; Gao, A.; Xie, H.; Gao, Z.; Chu, X.; Cai, Y.; Gu, C. Peptidomics comparison of plant-based meat alternatives and processed meat after in vitro digestion. *Food Res. Int.* 2022, 158, 111462. <https://doi.org/10.1016/j.foodres.2022.111462>.
66. Moss, R.; LeBlanc, J.; Gorman, M.; Ritchie, C.; Duizer, L.; McSweeney, M.B. A Prospective review of the sensory properties of plant-based dairy and meat alternatives with a focus on texture. *Foods* 2023, 12, 1709. <https://doi.org/10.3390/foods12081709>.
67. Pallavi, J.K.; Sukumar, M. Categorizing functional yoghurt using artificial neural network. *Asian J. Biol. Life Sci.* 2020, 9, 129–138. <https://doi.org/10.5530/ajbls.2020.9.20>.
68. Moss, R.; Barker, S.; Falkeisen, A.; Gorman, M.; Knowles, S.; McSweeney, M.B. An investigation into consumer perception and attitudes towards plant-based alternatives to milk. *Food Res. Int.* 2022, 159, 111648. <https://doi.org/10.1016/j.foodres.2022.111648>.