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Physicochemical and microbial quality of veggie burgers with fermented rapeseed meal as a potential protein source

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Abstract

Introduction and Objective. Rapeseed meal (RSM), a by-product of oil extraction, is rich in protein and holds potential as a replacement for animal-derived ingredients. However, its direct use in human nutrition is limited due to the presence of antinutritional compounds such as glucosinolates and phytic acid. Fermentation is a promising method to improve RSM's nutritional and functional properties by reducing these compounds and enhancing protein digestibility. The aim of this study is to evaluate the physicochemical and microbial quality of fermented rapeseed meal and assess its suitability for use in novel food applications.

Materials and Method. Three variants of veggie burgers with varying amounts of fermented dried meal (10g – 20 g), underwent microbiological and physicochemical analyses according to ISO standards.

Results. The raw burgers had high counts of lactic acid bacteria and yeast (approx. 7 log CFU/g) with no pathogens. The burger with 20 g meal contained almost 8% protein. Fermented rapeseed meal integrated effectively with other ingredients (carrot and nutritional yeast flakes) improving texture and binding. The fermentation process applied reduced the content of antinutritional substances in rapeseed meal.

Conclusions. The study provides valuable insights into the physicochemical and microbial quality of veggie burgers enriched with fermented rapeseed meal, demonstrating its potential as a safe and functional alternative source of protein. The burgers developed comply with consumer health and safety requirements, further supporting the feasibility of incorporating fermented rapeseed meal into the human diet; nevertheless, additional investigations are warranted. These findings may facilitate the development of novel food products and contribute to considerations of food safety.

Key words

food safety, plant proteins, Fermented rapeseed meal, veggie burgers

INTRODUCTION

The increasing demand for plant-based foods is evident not only globally but also in Poland. The main drivers of this pro-ecological and ethical trend include the significant increase in the human population, concerns about the environment, health, and animal welfare [1]. Global trends that limit meat-based proteins in daily diets are encouraging researchers to seek alternative protein sources, often requiring the development of innovative technologies [1, 2]. Plant-based products offer a promising solution. Vegetables (beans, chickpeas, peas, lentils), grains, nuts, legumes, and oilseeds (rapeseed, sunflower, flax, soy) are gaining popularity due to their high nutritional value, including adequate protein, minerals, and vitamins [1, 3]. Human health is significantly influenced by the nutrients present in the daily diet. Consuming plant proteins not only provides essential amino acids but also offers numerous health benefits, including the positive effects on cholesterol and glucose metabolism [1, 2]. Plant protein sources are particularly important for individuals following vegetarian or vegan diets, not only due to their high protein content, but also amino acids, carbohydrates, fibre, vitamins, and minerals [4]. In contrast, animal-derived proteins are associated with high production costs and increased risks of cardiovascular diseases [4]. However, plant proteins differ in composition and structure from meat proteins, which can reduce their appeal to consumers. This has led researchers to explore ways to improve the texture and flavour of plant-based proteins sources to make them more comparable to meat-based proteins [2, 3]. To enhance the sensory quality of plant-based protein products, various food additives are used. However, excessive use of additives transforms these potentially natural, health-promoting foods into ultra-processed products, as defined by the NOVA classification system. These products, which often contain colourants, preservatives, and flavour enhancers, may lead to consumer health issues, such as cancer, obesity, or cardiovascular diseases [3].

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Many countries face limited access to high-protein plant-based raw materials. Given that Poland is one of the largest producers of rapeseed, products derived from rapeseed processing, such as rapeseed meal, they should be utilized more efficiently. Currently, rapeseed meal is primarily used in animal feed, with limited application in human nutrition. This meal contains up to 30% protein and approximately 2–4% fat [5]. From a dietary perspective, it is a good source of tryptophan, threonine, sulfur-containing amino acids, as well as calcium and phosphorus. However, its use in the human diet is restricted due to the presence of anti-nutritional factors (ANFs) [6, 7].

Rapeseed protein is approved for use in human food in countries such as Canada, Japan, and the United States (e.g., in processed meats, pizzas, and bagels). However, the European Union has not approved rapeseed protein as a food ingredient due to the lack of established threshold values for certain undesirable compounds [6]. High fibre content and ANFs, including glucosinolates and glycosides, limit its application in human nutrition [4, 8, 9]. Consequently, there is growing interest in advanced technologies to reduce ANF levels. Fermentation is one such technology that not only reduces anti-nutritional compounds but also enriches rapeseed meal with valuable postbiotic substances, enhancing flavour and texture [1, 3]. This process improves the sensory, nutritional, and functional profiles of the product. Properly selected starter cultures increase digestibility and the bioavailability of micronutrients [6, 7, 10]. Additionally, microbial metabolites enhance the nutritional value, microbiological safety, and organoleptic properties of the final product [1].

The aim of this study is to evaluate the physicochemical and microbial quality of an innovative plant-based veggie burger formulated with fermented rapeseed meal as an alternative protein source. The research assessed the suitability of fermented rapeseed meal for novel food applications by analyzing its macronutrient content, caloric value, presence of different microbial groups, and potential safety hazards.

MATERIALS AND METHOD

Materials. The research material consisted of rapeseed meal (*Brassica napus* L. var. *napus*) obtained from a local farmer in the Łódź province, Poland. The rapeseed processing product underwent a 72-hour microbiological fermentation process, at a temperature of 21–22 °C and humidity levels ranging from 20% – 28%. A selected microbial culture from the collection of the Department of Food Quality was used for the fermentation. The starter culture included the following environmental isolates: *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Leuconostoc mesenteroides*, and *Saccharomyces cerevisiae*. The starter culture used for rapeseed fermentation consisted of bacteria and yeast in a 1:1 ratio, with each component present at a minimum concentration of 10^5 CFU/g. Such a composition of the starter culture ensured the reproducibility of the fermentation process of rapeseed meal. Upon completion of the fermentation process, the rapeseed meal was dried at a temperature of 48±2 °C until the water content reached 10%. The fermentation process of rapeseed meal has been described in detail in an earlier publication by the the authors [11].

METHODS

Chemical safety hazard analyses of starting material. For the chemical risk assessment, the levels of heavy metals-lead (Pb), cadmium (Cd), and mercury (Hg), as well as glucosinolates (progoitrin, epiprogoitrin, and sinigrin), were analysed in both fermented rapeseed meal (FRSM) and unfermented rapeseed meal (RSM). Mercury analysis was performed using the AMA 254 mercury analyzer (LECO Korea Co., Ltd.), based on the mercury vapour generation technique. The procedure followed test method PS-02, edition 3 (6 July 2009), developed by the Food Quality Department of IBPRS-PIB. The detection limit for Hg using this method was 0.02 mg/kg. Cadmium and lead concentrations were determined using Flame Atomic Absorption Spectrometry (FAAS) in accordance with ISO standard PN-EN 14082:2004, with detection limits of 0.003 mg/kg for Cd and 0.001 mg/kg for Pb. Additionally, glucosinolate content was quantified using high-performance liquid chromatography (HPLC) with a Shimadzu liquid chromatograph equipped with a UV detector to assess its potential impact on the safety of FRSM.

Preparation of veggie burger. The preparation of veggie burger variants with the addition of fermented and dried rapeseed meal (FDRSM) was carried out in three formulations, as follows:
- Variant I (FDRSM I): 20 g of ground FDRSM and 80 g of cooked carrots (Daucus carota L. subsp. sativus).
- Variant II (FDRSM II): 10 g of ground FDRSM and 90 g of cooked carrots
- Variant III (FDRSM III): 20 g of ground FDRSM, 78 g of cooked carrots, and 2 g of yeast flakes (Planteon, Poland).

Control sample (FDRSM) 100 g of ground FDRSM without ingredients.

Microbiological and physicochemical quality assessments were conducted on raw patties before heat treatment. The base ingredient for the veggie burgers was cooked carrot, chosen for its health benefits and nutritional properties, including carotenoids, vitamins, and minerals. The cooking process was selected based on studies by Buratia et al., which confirm that cooking increases the carotenoid content in carrots [10]. Yeast flakes were added as a flavour enhancer and a source of iron and vitamin B12.

Microbiological analyses. The veggie burger variants were evaluated for presence of *Salmonella* spp., *Listeria monocytogenes*, number of *Enterobacteriaceae*, *Escherichia coli* in accordance with applicable ISO standards, coagulase-positive staphylococci, lactic acid bacteria, *Bacillus cereus*, *Clostridium perfringens*, yeasts and moulds. Ten grams of each sample were weighed into sterile Whirl Bag Pack (Interscience, France) and homogenized in 90 mL of buffered peptone water in a Stomacher Model 400 (Seward Laboratory System, Inc., USA) for 2 min. Decimal dilutions were prepared with a sterile diluent (BPW) and used for plating 1,000 µL onto selective media.

Mesophilic LAB were counted after incubating inoculated de Man, Rogosa and Sharpe agar (MRS) plates at 30 °C for 72 h, likewise PN-ISO 15214:2002. Moulds and yeasts were inoculated on chloramphenicol agar (Ch) and incubated at 25±1 °C for 5 days by means of PN-ISO 7954:1999. *E. coli* enumeration was performed on tryptone bile x-glucuronide

Table 1. Content of heavy metals and glucosinolates in rapeseed meal before and after fermentation

Starting material	GLS [mmol/g s.m.b.]			Heavy metals [mg/kg]		
	Progoitrin	Epiprogoitrin	Sinigrin	Cadmium	Lead	Mercury
RSM	7.50±0.19	0.13±0.02	0.07±0.01	< 0.003**	< 0.020**	< 0.001**
FRSM	0.09±0.01*	0.06±0.01*	< 0.05*	< 0.003**	< 0.020**	< 0.001**

[1]GLS – glucosinolate; RSM rapeseed meal FRSM fermented rapeseed meal, s.m.b. – fat free dry matter; < 0.05 – below the level of quantification; the values are expressed as means ±SD; * means represent significant differences (p < 0.05), ** limits of quantification methods

agar (TBX) after incubation for 20 h at 44±1 °C, according to PN-ISO 16649–2:2004. *Enterobacteriaceae* were cultured on Violet Red Bile Glucose Agar (VRBGA), incubated at 37±1 °C for 24 h following PN-ISO 21528–2:2017–08. For estimation of the number of coagulase- positive staphylococci, samples were streaked on Rabbit Plasma Fibrinogen agar with supplement (RPF), incubated at 37±1 °C for 48 h based on PN-EN ISO 6888–2:2022–03+ A1:2024–02.

Bacillus cereus number was carried out with mannitol-egg yolk-polymyxin (MYP) agar incubated at 30±1 °C for 48 h, correspondingly PN-ISO 7932:2005.

The total *Clostridium perfringens* count was detected on tryptose sulfite cycloserine (TSC) agar supplemented with egg yolk emulsion and incubated at 37±1 °C for 24 h through PN-ISO 7937: 2005. Analysis for the presence of *Salmonella* spp. was performed in accordance with PN-EN ISO 6579–1:2017–04+A1:2020–09. This includes pre-enrichment in BPW and selective enrichment in Rappaport Vassiliadis Salmonella broth (RVS) and Muller-Kauffmann Tetrathionate-Novobiocin broth (MKTTn). Streaking from both broths was performed on Xylose Lysine Deoxycholate agar (XLD) and Hektoen agar plates and incubated at 37±1 °C for 24 h. The detection of *Listeria monocytogenes* was performed in accordance with PN-EN ISO 11290–1:2017–07. For the analysis of *Listeria* presence samples were stomached in pre-enrichment broth (half Fraser broth) and incubated at 30±1 °C for 24 h. This was followed by a secondary selective enrichment broth (Fraser broth) incubated at 37±1 °C for 24 h. The enriched samples were cultured on Listeria agar by Ottaviani and Agosti (ALOA) and Palcam agar plates at 37±1 °C for 48 h and checked for signs of growth after 24 h. All media were purchased from Oxoid Ltd., Basingstoke, UK. Each experiment was performed in triplicate and each analysis was conducted in duplicate.

Physicochemical quality analyses

Energy value. The energy value of 3 variants of veggie burgers with fermented dried rapeseed meal was tested. All the values used in the calculations, except water, were converted to dry weight according to the formula:

$$\text{score} \times 100\% \ / \ \text{dry weight}$$

Water content. Water content of a veggie burger was determined using the gravimetric method in accordance with PN-A-82100–1985. Five grams of each sample were dried at 105 °C until a constant weight was achieved. The result were expressed as dry matter content correspondingly.

Protein content. Protein content was determined using the Kjeldahl method in accordance with PN-A-04018:1975/Az3:2002. The total nitrogen content obtained was multiplied by the Kjeldahl conversion factor 6.25 to arrive at the protein content.

Fat content. The fat content was determined using the gravimetric method (Soxhlet extraction) according to PN-A-82100–1985.

Dietary fibre content. The fibre content determined by using the enzymatic technique in accordance with the brochure for the method AOAC985.29 (1997). Following the procedure, 1 g of the sample was exposed to an enzyme set: α-amylase, protease, amyloglucosidase. The individual stages of the study were performed in accordance with the technical brochure of the enzymatic method used.

Ash content. The ash content was analysed using the gravimetric method in accordance with PN-ISO 936:2000. The method consisted in incinerating 5 g of the sample in a muffle furnace at a temperature of 900° C±25 °C to a constant mass.

Sugar content. The sugar content was tested according to PN-A-82100–1985. The method involved the estimation of reducing sugars based on the amount of sodium thiosulfate solution used for titration of iodine, corresponding to the copper reduced by sugars contained in the tested sample.

Carbohydrate content. The carbohydrate content was calculated based on the evaluation of water, protein, fat and ash content according to the formula:

$$Wog = 100 - (B + W + T + P)$$

Wog – total carbohydrate content [g/100g]
B – protein content [g/100g]
W – water content [g/100g]
T – fat content [g/100g]
P – ash content [g/100g]

Statistics. Microsoft Excel 2016 (Microsoft Corporation, USA) was used for the statistical analysis of the study results. A one-way analysis of variance (ANOVA) was performed with a significance level of $p < 0.05$. The significance of differences between the means was assessed using the Bonferroni correction, with an adjusted p-value threshold of 0.0167. All measurements were performed in triplicate. Microorganism counts were normalized by Log10 transformation.

RESULTS

Chemical safety hazard of starting material. Table 1 presents the glucosinolate and heavy metal levels in non-fermented and fermented rapeseed meal. Heavy metals present in polluted soil can be absorbed by plants through the conductive system and accumulate in seeds. Given this potential risk, the concentrations of lead (Pb), cadmium (Cd), and mercury (Hg) were analyzed and compared with legal contamination limits. In addition, anti-nutritional factors (ANFs), such as glucosinolates (GLS), were assessed [12].

No risk due to Cd, Pb, and Hg contamination was identified in this study, as the concentrations of these toxic elements in RSM and FRSM were below detectable levels. Fermentation significantly ($p \leq 0.05$) reduced glucosinolate content compared to non-fermented rapeseed meal, with sinigrin being completely degraded (up to 100%). Therefore, the absence of these chemical compounds in the starting material indicates no hazard for human consumption from these toxins.

Microbiological quality. After the preparation of the 3 varieties of veggie burgers, they were analysed for microbiological quality (Tab. 2). Each burger was analyzed qualitatively for the presence of *Salmonella* spp., *Listeria monocytogenes* and quantitatively for *Enterobacteriacea*, *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *E.coli,* lactic acid bacteria, yeast and moulds.

Table 2. Microbiological quality for the three variants of veggie burgers

PARAMETERS	FDRSM	FDRSM I	FDRSM II	FDRSM III
Presence of *Salmonella* spp. in 25g	nd	nd	nd	nd
Presence of *Listeria monocytogenes* in 25g	nd	nd	nd	nd
Number of *Escherichia coli* [log cfu/g]	< 1	< 1	< 1	< 1
Number of *Staphylococcus aureus* [log cfu/g]	< 1	< 1	< 1	< 1
Number of *Enterobacteriacea* [log cfu/g]	< 1	< 1	< 1	< 1
Number of *Bacillus cereus* [log cfu/g]	< 1	< 1	< 1	< 1
Number of *Clostridium perfringens* [log cfu/g]	< 1	< 1	< 1	< 1
Number of lactic acid bacteria [log cfu/g]	6.9±0.02 ^{A}	3.2±0.01^{B}	2.6±0.02^{C}	3.4±0.02^{D}
Number of moulds	< 1	< 1	< 1	< 1
Number of yeast [log cfu/g]	7.2±0.02^{A}	2.9±0.02^{B}	2.8±0.01^{B}	3.1±0.03^{C}

[1] FDRSM – fermented dried rapeseed meal; nd – not detected. FDRSM I – 80g cooked carrots: 20g FDRSM. FDRSM II – 90g cooked carrots: 10g FDRSM. FDRSM III – 78g cooked carrots: 20g FDRSM+ 2g yeast flakes; The values are expressed as means ±SD, means in the same row followed by different uppercase letters (A– D) represent significant differences ($p < 0.05$)

None of the tested veggie burger samples were positive for *Salmonella* spp. or *Listeria monocytogenes*. Although *Enterobacteriaceae* and fecal coliforms are commonly found in plant-based products, the *Enterobacteriaceae* number was below the detectable limit. Moreover, all burgers were free of *Staphylococcus aureus* and *Escherichia coli*. The highest lactic acid bacteria (LAB) count was observed in FDRSM (6.9 log CFU/g), while the lowest was in FDRSM II (2.6 log CFU/g), following a similar trend in yeast counts, which were 7.2 log CFU/g and 2.8 log CFU/g, respectively. A statistically significant difference ($p < 0.05$) was found between the mean counts of these 2 microbial groups in the tested samples. These findings confirm the beneficial effect of fermentation on the microbiological safety of rapeseed meal.

Physicochemical quality. The nutritional value of the 3 prepared variants of veggie burgers with the addition of a dried fermented rapeseed meal, was assessed (Tab. 3). The control sample – FDRSM is also included in Table 3. The protein content of the control samples was approximately 22 g/100 g, but the caloric content of 100 g of FDRSM was exactly 288 kcal. Apart from FDRSM, there was also high fibre content at 24.1 g/ 100g. Significant statistical differences were found between the control sample and the tested burgers for the parameters shown in Table 3.

Table 3. Nutritional value of veggie burgers with fermented dried rapeseed meal

PARAMETER [g/100g]	FDRSM (control)	FDRSM I	FDRSM II	FDRSM III
Protein (g/100g±SD)	21.1±0.2^{A}	7.8±0.1^{B}	4.8±0.2^{C}	7.4±0.1^{D}
Fat (g/100g±SD)	0.9±0.1^{A}	3.0±0.2^{B}	5.0±0.2^{C}	3.8±0.1^{D}
Water (g/100g± SD)	11.7±0.3^{A}	68.8±0.1^{B}	72.5±0.2^{C}	66.8±0.2^{D}
Ash (g/100g±SD)	5.3±0.1^{A}	2.1±0.1^{B}	1.8±0.1^{C}	1.8±0.1^{C}
Sugars (g/100g±SD)	2.0±0.1^{A}	3.4±0.1^{B}	2.1±0.2 ^{A}	3.4±0.2^{B}
Fibre (g/100g±SD)	24.1±0.2^{A}	10.0±0.1^{B}	8.4±0.2^{C}	11.2±0.3^{D}
Carbohydrates (g/100g±SD)	36.9± 0.3^{A}	8.3±0.1^{B}	7.5±0.3^{C}	9.0±0.3^{D}
kcal/100g	288	111	111	122
kj/100g	1212	464	461	509

FDRSM – fermented dried rapeseed meal. FDRSM I – 80g cooked carrots: 20g FDRSM. FDRSM II – 90g cooked carrots: 10g FDRSM. FDRSM III – 78g cooked carrots: 20g FDRSM+ 2g yeast flakes; The values are expressed as means±standard deviation (SD). Means in the same row followed by different uppercase letters (A– D) represent significant differences ($p < 0.05$)

The total carbohydrate content ranged from 7.5 g/100 g in FDRSM II to 9.0 g/100 g in FDRSM III, with sugar levels generally remaining low (<3.4 g/100 g). The average total fat content was 3.9 g/100 g, which is lower than the fat content reported for commercial plant-based burgers (4–15%) [13]. Statistically significant differences were observed between variant I and II and between variant II and III in terms of protein, fat, water, and fibre content. The highest protein content (7.8 g/100 g) and the lowest fat content (3.0 g/100 g) were recorded in FDRSM I, whereas the lowest protein content was found in FDRSM II. Analysis of dietary fibre content indicated that the developed burgers are a good source of this nutrient. The highest fibre content was observed in the samples FDRSMIII (11.20 g/100 g) and FDRSM I (10.0 g/100 g). These results highlight the significant impact of the amount of fermented product incorporated. Notably, both variant I and variant III contained the highest amount of FDRSM (20 g); however, variant I exhibited the highest protein content. The addition of yeast flakes, although generally recognized as a protein-rich ingredient, was limited to 2 g in variant III and was therefore insufficient to noticeably increase the overall protein content. While the difference in protein content between the 2 variants was statistically significant, the magnitude of the difference (0.4 g/100 g) was minor and unlikely to be of nutritional relevance, indicating that the yeast flakes had little impact on this parameter. In contrast, variant II, which contained less FDRSM (10 g), showed the highest fat content (5.0 g/100 g). This can be attributed to the lower proportion of the low-fat FDRSM (0.9 g/100 g), which increased the relative contribution of fat from the other ingredients. Moreover, the larger amount of carrot (90 g) and water loss during cooking concentrated the product mass, further elevating the fat content per 100 g.

DISCUSSION

The International Agency for Research on Cancer (IARC) has classified processed meat as carcinogenic to humans, driving the growing demand for plant-based meat alternatives (PBMAs) as a healthier dietary option [13]. Lactic acid fermentation is widely utilized in the development of new plant-based products [3] and is emerging as a powerful technology for enhancing the sensory, nutritional, and functional properties of next-generation plant-based foods. Lactic acid bacteria (LAB) play a crucial role in this process by producing secondary metabolites, such as bacteriocins, lactic acid, and acetic acid, which help inhibit or limit the growth of various intestinal pathogens [14, 15]. Additionally, the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have recognized numerous LAB species and their derived metabolites as safe [15]. Fermentation is a key technology for transforming plant-based ingredients into nutritionally and functionally attractive alternative protein sources. It enables the production of components that closely mimic meat products, making them viable substitutes for animal proteins [3]. High-quality plant-based meat alternatives could help reduce meat consumption and, consequently, mitigate environmental impact. However, as a novel food category, little is known about their nutritional and microbial characteristics. Soy remains a widely used replacement for animal protein, but the selection of more sustainable plant-protein sources is expanding. This includes oilseeds such as rapeseed and sunflower. While these plant sources are not new to the food industry, their applications as alternative protein sources continue to evolve [16]. Fermenting rapeseed products is a well-supported nutritional approach and offers a European alternative to soy, which is predominantly imported and genetically modified. To the authors' knowledge, research on plant-based meat alternatives has primarily focused on formulation and process development, while information regarding their safety and nutritional value remains limited.

The current study represents the first attempt to formulate veggie burgers using fermented rapeseed meal and to investigate their microbial and chemical safety, as well as their nutritional composition.

As reported in the literature, the safety of plant-based foods depends not only on the characteristics of the raw materials used, but also on such factors as soil quality, agricultural inputs, harvesting, storage, transport, processing, and post-processing handling. Therefore, monitoring the levels of potentially toxic inorganic elements is essential for ensuring the safety of these foods. The toxic effects of inorganic elements include cumulative toxicity as well as non-carcinogenic and/or carcinogenic risks, posing serious health hazards [12]. Inorganic elements, such as cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), and nickel (Ni), among others, are classified as toxic metals due to their harmful effects even at low exposure levels. Once in systemic circulation, these heavy metals can cause damage to various organs. Their primary risk, however, lies in their long-term accumulation, leading to adverse effects through mechanisms such as the disruption of enzymes, hormones, proteins, and cell membranes, as they serve no biological function [17]. In this study, the concentrations of 3 inorganic elements (Hg, Pb, Cd) were determined. All samples showed results below the limit of quantification (LOQ) for the tested elements.

Microbiological analysis of the veggie burgers in this study was a crucial first step in ensuring the high quality and safety of the final product while preventing food deterioration and food-borne disease. Since veggie burgers made from fermented rapeseed meal do not undergo heat treatment, identifying the microorganisms present in the raw material and determining their levels is essential. In terms of microbiological safety, plant-based foods are generally considered to pose a lower risk than animal-derived products. However, recent food-borne outbreaks caused by *Salmonella* and *Listeria monocytogenes* contamination have been linked to vegan cheese substitutes in the United States and Europe [18]. Spore-forming bacteria, such as *Bacillus* spp. and *Clostridium* spp., are commonly detected in many plant-based ingredients [19]. However, in the current study, the levels of both microorganisms were below the detection limit. *Listeria monocytogenes* were quantitatively not detected in any of the samples. The predominance of members of the lactic acid bacteria group in FDRSM may be relevant for the product stability. Moreover, lactic acid fermentation can reduce anti-nutrients in rapeseed products, further enhancing their nutritional value.

The results of this study confirm the benefits of this process, supporting the potential of fermented rapeseed meal as a sustainable protein source. The nutritional value of the veggie burgers was assessed by determining the content of key nutrients, including protein, fat, total carbohydrates, and dietary fibre. According to the Dietary Reference Intakes (DRI), the Recommended Dietary Allowance (RDA) for protein ranges from 34 – 56 g/day for individuals aged 9 – 70 years, depending on age and gender [Institute of Medicine Food and Nutrition Board]. The protein levels in the burgers ranged from 4.80 – 7.8 g per 100 g, which can be attributed to the different amounts of protein base incorporated into each variant. In commercially available vegan burgers, the protein content typically ranges from about 13 – 27 g/100 g. It should be noted that this value is similar to the energy value of PBBs available on the market in Poland, which usually ranges from 156 – 253 kcal/100 g (data obtained from product labels). A 250 g portion of FDRSM I could provide at least 50% of the RDA for adults. For vegetarian products, an optimal protein content is one of the key issues. Additionally, the RDA for carbohydrates is 130 g/day for individuals aged 9 – 70 years, and a 250 g serving of FDRSM II could provide approximately 15% of the RDA for adults. Consuming 250 g of FDRSM I would provide about 278 kcal, which could sustain an adult (70 kg, 2,000 kcal daily requirement) for approximately 3.0 – 3.5 hours [Institute of Medicine Food and Nutrition Board]

The growing shift toward plant-based diets for health, environmental, and ethical reasons is increasing the importance of dietary fibres from plant sources. These fibres provide essential nutrients and antioxidants that protect against various chronic conditions, promote overall health, and support sustainable eating. In this context, innovative fibre sources, such as agricultural byproduct of which rapeseed meal is a perfect example offer new and unconventional dietary fibre options that enhance nutrition while reducing food waste. The literature clearly indicates that most adults do not consume the recommended amounts of fibre (25–35 g/day) [20]. In this study, the rising consumer demand for clean-label and natural foods will further drive the use of dietary fibres as functional ingredients in plant-based products, including innovative burgers. In particular, rapeseed meal – an underutilized byproduct of oil production – can serve as a sustainable, fibre-rich ingredient, improving the texture, stability, and nutritional profile of plant-based burgers, while aligning with environmentally conscious food choices [21]. For this study, the DRI mentioned above can be fully met by consuming 250 grams of FDRSM I.

Plant proteins are often considered to have lower nutritional quality than animal proteins due to their lack of certain essential amino acids and lower digestibility. However, rapeseed meal boasts a high amino acid and protein content, as well as being a rich source of minerals and vitamins. Compared to soybean meal, rapeseed meal is particularly high in sulfur-containing amino acids, such as cystine and methionine, although it contains lower levels of lysine. Rapeseed proteins primarily consist of 11S globulins and albumins, which play important roles in immune function and the transport of fatty acids and hormones [8, 9]. The use of fermentation enhances the nutritional value of the product by increasing protein content and neutralizing the glucosinolate content [14]. Therefore, fermented rapeseed meal could serve as a viable alternative to animal protein, though further research and trials are necessary.

Glucosinolates and their derivatives may exhibit antifungal, antimicrobial, antioxidant, and anticancer properties, but they also have antinutritional effects [22]. The presence of glucosinolates in rapeseed significantly limits its use as a food ingredient due to these antinutritional properties. The most common glucosinolate derivatives in canola (*Brassica napus)* are sinigrin and progoitrin, which are largely responsible for its bitter taste and may also negatively affect thyroid function [23, 24]. The fermentation of rapeseed meal in the present study aimed to eliminate its bitter taste and enhance its nutritional value. Reducing glucosinolate content in the fermented product is crucial for its potential application in the food industry. Glucosinolate levels can be reduced during oil production through tissue structure disruption or via microbiological processes such as fermentation. In this study, the reduction of glucosinolates was specifically attributed to fermentation. The initial sample, prior to fermentation, had a low water content of approximately 11%. By analyzing this parameter, it was concluded that the observed reduction in glucosinolate content was primarily driven by fermentation. The significant decrease in glucosinolates in the fermented product indicates the effective reduction of antinutritional compounds, contributing to the elimination of the bitter taste [25]. The current study analyzed progoitrin, epiprogoitrin, and sinigrin- glucosinolates that serve as plant defense compounds and contribute to the bitter taste of rapeseed. A significant reduction was observed in their levels, from 7.5, 0.13, and 0.07 mmol/g in unfermented rapeseed meal to 0.09, 0.06, and <0.05 mmol/g in the fermented product. These results align with findings from other researchers. Literature reports indicate that microbial fermentation can reduce the glucosinolate content of rapeseed meal from 64.56 μmol/g to 3.47 μmol/g [23, 24]. However, studies detailing the exact effects of fermentation on individual glucosinolate derivatives remain limited. Moreover, Hao's 2020 study confirmed that fermentation significantly reduced not only glucosinolate, but also phytic acid, and crude fibre content. The concentration of glucosinolates decreased from 203.7 μmol/g in unfermented rapeseed meal to 111.5 μmol/g after 5 days of fermentation. Additionally, fermentation treatments effectively reduced phytic acid and crude fibre content by 58.7% and 68.8%, respectively. These findings support the potential of fermentation as a promising method for improving the nutritional profile of rapeseed meal.

When developing new plant-based foods, it is essential to gather information on the occurrence of relevant microbial contaminants and their properties to implement appropriate control measures [16]. Therefore, further research is needed to fully understand the potential and risks of the application of fermented rapeseed meal as a potential protein source.

CONCLUSIONS

Developing high-protein plant-based foods is a primary objective in the creation of new products, especially given the increasing demand for domestic plant proteins. This trend is partly driven by the heavy reliance on imported genetically modified soybean proteins, which make up to 90% of feed protein in Poland. Currently, there is limited evidence regarding the safety and health implications of consuming plant-based meat alternatives, particularly when compared with animal-derived products that differ in nutritional profiles. Therefore, further research is required to address this knowledge gap. The developed veggie burgers enriched with fermented rapeseed meal comply with consumer health and safety requirements and highlight the potential of incorporating fermented rapeseed meal into the human diet; nevertheless, additional investigations are warranted. In the near future, rapeseed-based products, such as rapeseed meal, are expected to obtain approval for human consumption within the European Union, as they are already authorized in countries such as Canada and Japan [6].

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