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ORIGINAL RESEARCH PAPER

THE EFFECT OF HEMP PRESS CAKE FLOUR ON FATTY ACIDS COMPOSITION AND MICROBIOLOGICAL QUALITY OF GLUTEN FREE BREAD

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Abstract: The aim of this paper is to determine the impact of hemp press cake flour (HPCF) on fatty acids and the microbiological quality of gluten-free bread. Seven types of gluten-free bread with different amounts of HPCF (0, 5, 10, 15, 20, 25 and 30 %) were produced. Gas chromatography was used to determinate the amounts of individual fatty acids, as well as the groups of fatty acids (saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, branched fatty acids). HPCF contains significantly higher content of linoleic acid, α-linoleic acid, omega - 3 fatty acids and Omega - 6 fatty acids compared to rice flour, which indicates that replacing rice flour with HPCF will contribute to a statistically significant difference ($p < 0.05$) in the content of these essential fatty acids in different types of bread. The change in the microbiological quality of the gluten-free bread with different amounts of HPCF starts thirty-six hours after the production.

Keywords: *fatty acid, gluten free bread, hemp press cake flour, microbiology of bread*

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INTRODUCTION

The transition from the traditional (not effective) to a sustainable and circular bioeconomy is crucial for fulfilling the European Union's environmental policy [1]. One of the ways to set up a sustainable bioeconomy is through the reduction of waste from the food industry, i.e. valorization of by-products from the food industry. The reduction of this type of waste can be considered as an economic model that aims at maximum reuse and recycling of by-products to reduce the creation of new amounts of waste [2]. During the production of vegetable oils, significant amounts of waste and by-products are created [3].

Hemp (*Cannabis sativa* L.) is an ancient multipurpose crop which is mainly used in food, pharmaceutical, cosmetic and other industries [4]. Depending on the law in each country THC in hemp should not exceed the threshold limit permitted by government regulations, in Europe the national limit is 0.2 % [5]. The cultivation and use of hemp fully fits with the objectives of the European Green Deal, adhering to a clean, decarbonized circular economy and sustainable agriculture [6]. Hemp seeds, depending on the types, climatic conditions and region of cultivation, contain about 30 - 50 % oil [4], 27 - 36 g/100g fiber and 21 - 28 g/100g protein. Of the fatty acids, this type of seed contains linoleic acid, α-Linolenic acid (16 - 19 %), oleic acid (12 - 17 %), palmitic acid (5 - 8 %), γ-Linolenic acid (1- 3 %) and some other minor fatty acids [7 – 11]. In the last few years, interest in extra virgin vegetable oils has been increasing constantly as there are nutritional and health claims that these oils are quite healthy. When obtaining this type of oil, high temperature (higher than 45ºC) is avoided. By using this process, the yield of the obtained oil is quite small, but on the other hand, the nutritional values of the oil crops are preserved [12 – 15]. Oil cake which was mostly used as animal feed in the past [16], contains oil and other nutritional components [17]. The drying of this cake and its use in the bakery and confectionery industry as a component with an excellent nutritional composition has been demonstrated by several authors.

The increasing global demand for suitable food for people with gluten allergies has driven research efforts to develop gluten-free products. Since bakery items account for over 50 % of the food sources consumed by people, there has been a significant global interest in creating and diversifying gluten-free these products to benefit individuals with celiac disease [18]. Celiac disease affects about 1 % of the global population, with 83 % of cases remaining undiagnosed. The only effective treatment for celiac disease is to follow a strict gluten-free diet and avoid cross-contamination with foods containing gluten. This condition is primarily associated with nutrient malabsorption caused by damage to the intestinal villi due to gluten exposure [19].

Making bread from rice flour is challenging because rice does not contain gluten, which is the compound that gives bread its structure [20]. Therefore, it is necessary to add raw materials to the recipe that can help to obtain rice flour bread with good technological, nutritional and sensory characteristics. By replacing 20 % of wheat flour with Hemp press cake flour (HPCF) in the recipe of biscuits, there is an increase in the content of ash, proteins, phenolic components and antioxidant activity [16]. By partially replacing wheat flour with HPCF (up to 80 %), bread with an improved nutritional composition was obtained, and the stability of the dough remained unchanged when the replacement was up to 10 % HPCF [21]. Norajit *et al*. [22] analyzed extruded rice energy bars in which rice flour was replaced by 20 %, 30 % and 40 % HPCF flour. In addition to

improved nutritional characteristics, bars with 20 % HPCF had the most desirable color, taste and overall acceptability. On the other hand, due to the absence of the three-dimensional gluten network, the production of gluten-free products was considered a challenge [23] because most of the gluten-free products have poor sensory, textural and nutritional properties [24]. The use of HPCF due to its high protein content (mainly albumin an edestin) can be an excellent component in the production of gluten-free products. According to Korus *et al*. [25] 20 to 60 % of starch (the main component in a gluten-free product) can be replaced by HPCF. Moreover, HPCF biscuits showed significantly higher concentrations of total phenolic and flavonoid compounds. In the production of gluten-free crackers, an amount of 10 to 40 % of brown rice flour was replaced with HPCF. The replacement resulted in higher amounts of protein, fiber, minerals and essential fatty acids [25].
The aim of this paper is to determine the impact of replacing rice flour with HPCF in the amount of 5 %, 10 %, 15 %, 20 %, 25 % and 30 % on the composition of fatty acids and the microbiological quality of the types of bread produced.

MATERIALS AND METHODS

Materials

Gluten-free rice flour was used to make bread. HPCF was obtained after cold pressing hemp seeds under laboratory conditions (Yoda Classic, Nederland). The by-products were dried for 48 h at 50 ºC in a UFE 500 oven (Memmert GmbH, Schwabach, Germany). The dried press cake was finely ground (size < 0.5 mm) with an IKA MF10 grinder (IKA®- Werke GmbH & Co. KG, Staufen, Germany) and stored in sealed bags at 4 ºC for future analysis. The remaining raw materials to produce the gluten-free bread were purchased from a healthy food store in Sofia, Bulgaria.

Methods

Production of gluten free bread

Rice flour (100 %), guar gum (3 %), whey protein (3 %), oil (3 %), salt (1.8 %), sugar (2 %) dry yeast (1 %) and water were homogenized using a spiral mixer. The resulting dough was left to ferment (45 min. at 30 ℃). The bread was baked in a preheated oven. The baking time was 45 min. (5 min. at 200 ℃ and 40 min. at 175 ℃). Seven types of bread with different HPCF contents (0 %, 5 %, 10 %, 15 %, 20 %, 25 % and 30 %) were produced.

Determination of fatty acids

For the fatty acids determination, the fats of the analyzed samples were extracted. For this purpose, 25 g of each sample were used. Static extraction was done using chloroform and methanol (1:2) with total volume 80 mL. The extraction was repeated twice. The entire extract was then transferred to a separatory funnel and water was added to separate the phases. After separation of the aqueous phase, the non-polar phase was transferred to a vacuum evaporator to evaporate the remaining organic solvent [26]. Identification of fatty acids was performed using gas chromatograph Shimadzu-2010

gas chromatograph (Kyoto, Japan). The assay was performed with a CP7420 capillary column (100 m x 0.25 mm i.d., 0.2 m, Varian Inc., Palo Alto, CA) with carrier gas-hydrogen and make-up gas-nitrogen. Results was presented on g/100 g oil.

Determination of the Total Number of Yeasts and Molds

ISO 21527-2:2008 standard method was used [27]. At the intervals of 0, 12, 24, 36, 48, 60 and 72 h, an amount of 5 g sample was homogenized in 45 mL of peptone saline diluent for 1 min to prepare a 10^{-1} homogenate. Further decimal dilution (10^{-2}) was prepared as well in peptone saline diluent. All dilutions were inoculated in triplicate.
For quantification of microorganisms, 0.1 mL of the diluted sample (10^{-1} and 10^{-2}) were transferred into a sterile Petri dish covered with Dichloran-Glycerol Agar and spread using a Drigalsky spatula. The plates were incubated for 5 days at 25 °C. Typical colonies of yeasts and molds were counted after 5 days. Counts of visible colonies were made and expressed as log CFU·g^{-1} sample.

Determination of the Total Number of Aerobic Viable Bacteria

1 mL of appropriate decimal dilution was poured plated in Plate Count Agar (PCA, VWR Chemicals, Leuven Belgium). The plates were incubated at 30 °C for 72 h and counted according to ISO 4832:2006 [28].

Determination of Enterobacteriaceae and E. coli

1 mL of each appropriate dilution was plated onto Petrifilm plates (3MTM Petrifilm™, St. Minnesota, USA). Plates were incubated at 37 °C for 24 h and 48 h for *Coliforms* and *E. coli*, respectively, following the instructions of ISO 4832:2006 [29].

Determination of the Number of Bacillus spp.

Count of colonies was performed according to ISO 4832:2006 [29]. 0.1 mL of each appropriate dilution were plated onto Polymixin-Egg Yolk-Mannitol-Bromothymol-Blue Agar (PEMBA; Merck, Darmstadt, Germany) [30, 31]. Plates were incubated at 37 °C for 24 h.

Statistical analysis

Analysis of variance (ANOVA) and Fisher's Least Significant Difference test (LSD) at $p < 0.05$ were performed with the software's XLSTAT 2019 and Microsoft Office Excel 2019.

RESULTS AND DISCUSSION

Fatty acids

Fatty acids are important for the assessment and quality of raw materials from which a product was obtained [32]. The content of myristic and stearic acids in rice flour is higher by 2100 % and 172 % respectively, compared to the content of these fatty acids in HPCF. HPCF is a product obtained after cold pressing of hemp seeds that contain essential fatty acids, necessary for the normal functioning of the body. These fatty acids cannot be synthesized in the human body, and it is necessary to enter them through the

diet [33]. Table 1 shows that HPCF is richer by 165 %, 45 %, 65 % and 77 % respectively with palmitic, oleic, linoleic and α-linolenic acids compared to rice flour.

Table 1. *Composition of fatty acids in HPCF and rice flour*

Fatty acids [g/100g oil]	HPCF	Rice flour
Myristic acid (14:0)	0.30±0.00	6.60±0.24
Palmitic acid (16:0)	8.79±0.03	23.33±1.04
Stearic acid (18.0)	3.55±0.01	9.65±0.43
Oleic acids (18:1 *cis*-9)	13.17±0.04	19.10±0.94
Linoleic acid (18:2 (n-6))	53.25±0.16	18.57±0.83
α-linolenic acid	13.49±0.04	3.06±0.13
SFA	13.12±0.04	44.21±1.46
MUFA	14.96±0.04	23.47±0.68
PUFA	71.11±0.21	22.57±1.01
Omega- 3 fatty acids	13.51±0.04	3.06±0.14
Omega - 6 fatty acids	55.62±0.16	19.51±0.87
BFA	0.12±0.00	3.67±0.06

**SFA- Saturated fatty acids; MUFA - Monounsaturated fatty acids; PUFA - Polyunsaturated fatty acids; BFA - Branched fatty acids.*

Saturated and monounsaturated fatty acids are 237 % and 57 %, respectively, higher in content in rice flour compared to HPCF. The content of polyunsaturated fatty acids, as well as omega-3 and omega-6 fatty acids are in higher content in HPCF compared to rice flour (respectively 68 %, 77 % and 65 %). According to Lee *et al.* [34] hemp seeds yield a vegetable oil containing more than 89 % PUFAs. A HPCF analysis was made by Papatzimos *et al.* [35] and the obtained results were complemented by ours. HPCF was composed mainly of PUFAs and therefore can be used to produce gluten-free products [36]. Rice flour contains more unsaturated fatty acids in comparison with saturated fatty acids and is therefore considered as excellent dietary food [37]. Figure 1 presents the amounts of different fatty acids determined in gluten-free bread enriched with different amounts of HPCF.

The ANOVA (not shown) demonstrated the existence of significant differences among different fatty acids in bread enriched with different quantity of HPCF. The content of myristic, palmitic and stearic acid decreases by increasing the HPCF content in gluten-free bread. These fatty acids, like other saturated fatty acids, are associated with negative consequences for the human health. The consumption of food high in these fatty acids leads to an increase in cholesterol and mortality due to cardiovascular diseases [38]. Linoleic acid is polyunsaturated fatty acid (PUFA) which belongs to the family of essential fatty acids, which is mostly found in vegetable [39]. The content of linoleic acid in gluten-free bread with different amounts of HPCF ranges from 48.25 g/100 g oil (in control bread) to 50.56 g/100 g oil (in bread with 30 % HPCF). The α-linolenic acid content also increases with increasing the HPCF content in gluten-free bread (from 0.20 g/100g oil in gluten-free bread with 0 % HPCF to 4.88 g/100g oil in gluten-free bread with 30 % HPCF). The content of ω-3 and ω-6 increases respectively from 0.46 % and 49.11 % in the control bread to 4.90 % and 51.24 % in the bread with 30 % HPCF. Unsaturated fatty acids such as ω-3 and ω-6 are essential fatty acids and the human body is completely dependent on external sources for these fatty

acids. These fatty acids help in the normal growth and development of children, as well as in reducing the risk of heart diseases, because they prevent blood clotting [40].

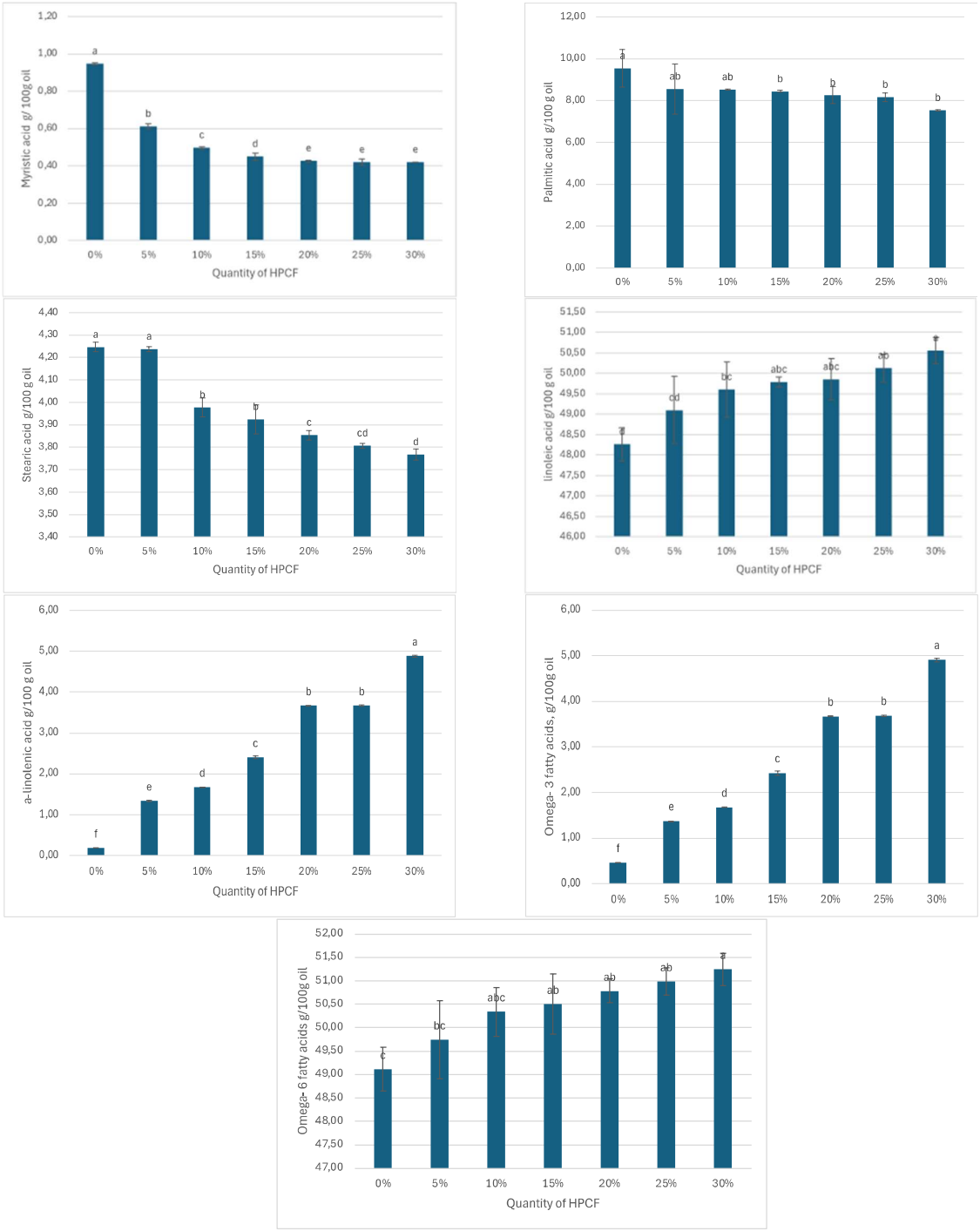

Figure 1. *Different fatty acids of gluten free bread enriched with HPCF*

The presented results are the average of three repetitions; the bars represent the standard deviation. Same letters in the above columns indicate that data are not significantly different ($p < 0.05$) following Fisher's LSD test.

The content of saturated fatty acids (SFA) decreases with increasing the content of HPCF in gluten-free bread. These fatty acids could increase the concentration of serum LDL cholesterol when present in the food consumed [41]. The amount of MUFAs also decreases, and the amount of PUFA and BFA increasing with HPCF content in gluten-free bread (Figure 2).

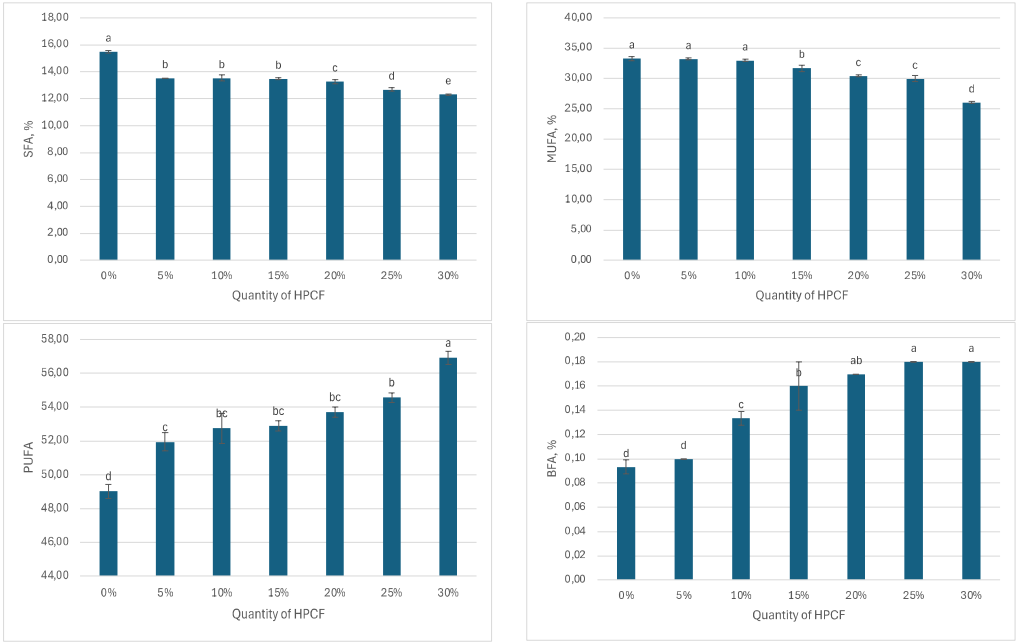

Figure 2. *Different fatty acids group in gluten free bread enriched with HPCF*
The results presented are the average of three repetitions; the bars represent the standard deviation. Same letters above columns indicate that data are not significantly different ($p < 0.05$) following Fisher's LSD test.

Microbiological Analyzes

The raw materials were analyzed in the same way as the bread samples, but presence of any microorganisms was not detected even after 72 hours of incubation (Table 2). This refers to the use of raw materials that are kept in appropriate packaging that is not contaminated.

Table 2. *Microbial counts ($CFU \cdot g^{-1}$) in raw materials used in an experiment*

	TNYM	**TNAVB**	***E. coli***	***Bacillus spp.***
Raw material/ hours	72	72	72	72
Rice flour	NG	NG	NG	NG
HPCF	NG	NG	NG	NG

TNYM= Total Number of Yeasts and Molds; TNAVB= Total Number of Aerobic Viable Bacteria; EEcoli= Enterobacteriaceae and E. coli; Bacillus spp.; NG= No Growth Detected

The number of yeasts and molds in the bread samples had a decreasing tendency with the addition of HPCF (Figure 3).

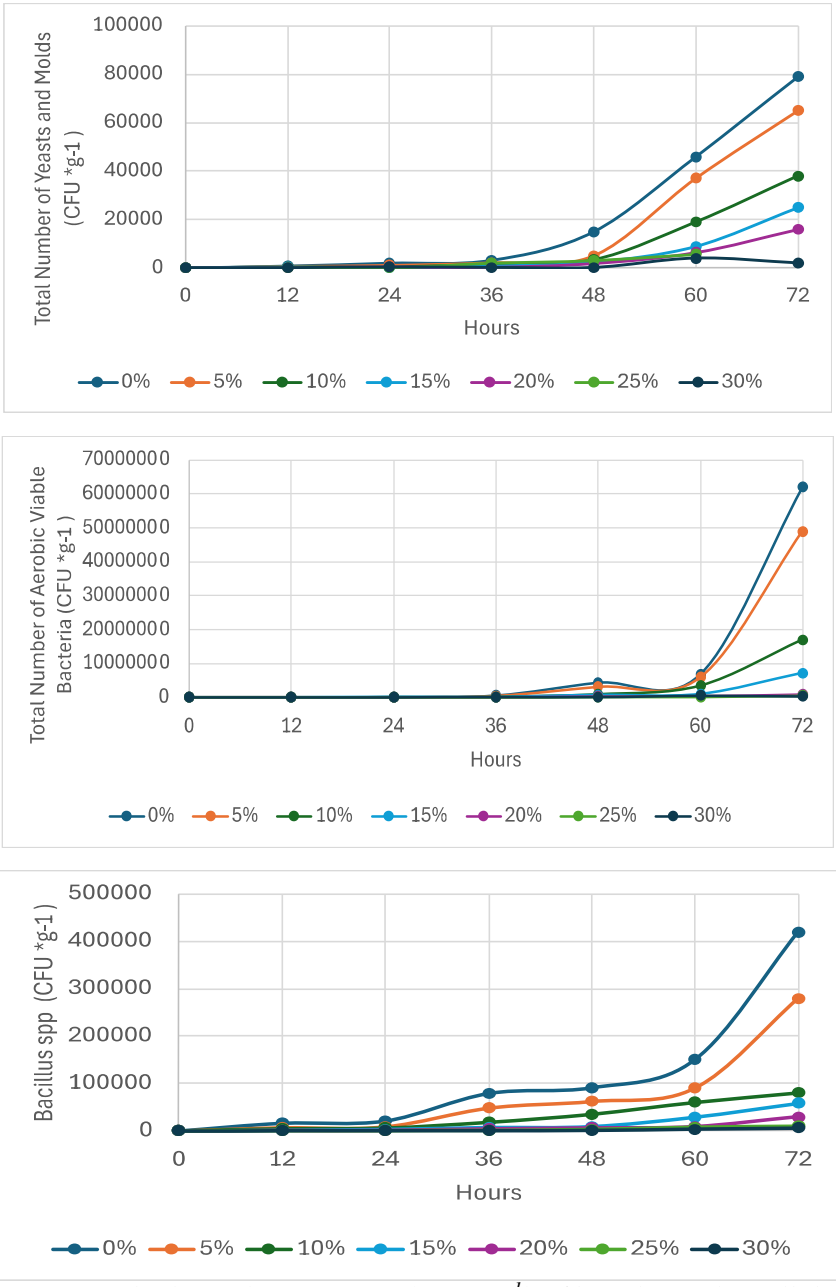

Figure 3. *Microbiological parameters (CFU ·g^{-1}) of bread samples. All data are presented as the mean from triplicate assays*

This decrease during storage was due to the low moisture content of the bread samples. Gluten-free foods can become stale so quickly because they are high in refined starches. Refined starches are partially damaged during the processing and extraction, causing the starch to behave differently. While wheat-based goods were naturally high in starch, gluten-free foods have a higher concentration of refined starches because they rely on refined starches to replace the structure and binding properties that gluten provides in foods. When starch is heated during baking, its structure starts to soften and melt. As the

starch heats up, it also absorbs moisture. Once the food was removed from heat, the structure of the starch starts to harden as it cools.

The decrease in the number of yeasts and molds was also supported by the fact that a major part of the bioactive substances in hemp flour have antifungal and antiviral properties [8, 41]. Fungal counts ranged from $0.2 \cdot 10^2$ CFU·g^{-1} to $7.9 \cdot 10^4$ CFU·g^{-1} with the highest counts recorded in bread samples with 0 % HPCF, after 72 hours, while the lowest counts ($0.2 \cdot 10^2$ CFU·g^{-1}) were observed in bread samples containing 30 % HPCF, after 12 hours (Figure 3). No fungi were detected in any bread samples tested immediately after baking.

The total number of aerobic viable bacteria in the bread samples was higher than the number of yeasts and molds, ranging from $1.3 \cdot 10^3$ CFU·g^{-1} to $6.2 \cdot 10^7$ CFU·g^{-1} with the highest recorded in bread samples with 0% HPCF, after 72 hours. In terms of aerobic counts, the results indicate that microbial load may increase with storage time. However, inadequate handling may increase microorganism levels posing health concerns [43]. Since *Enterobacteriaceae* and *E. coli* are heat-sensitive microorganisms, their presence in bread suggests potential cross-contamination via surfaces and hand contact after baking. The presence of these microorganisms in bread shows that it is necessary to handle and store bread properly to keep it safe [44]. In our experiments, this group of microorganisms was not detected in any of the analyzed bread samples.

A bread condition known as rope spoiling causes the bread to deteriorate due to germs. *Bacillus* genus-related bacteria with heat-resistant spores that can survive baking are referred to as spoilage organisms. These bacteria are commonly referred to as rope because they contaminate bread. The baking industry is greatly concerned about the economic impact of this. In terms of bread spoiling, ropiness ranks second only to moldiness in terms of importance. Rope spoilage is most common during summer due to favorable conditions for bacterial growth. While *Bacillus subtilis* is the main culprit, other bacteria such as *Bacillus licheniformis, Bacillus megaterium,* and *Bacillus cereus* have also been implicated in causing ropy bread [45].

In this study, the physicochemical conditions in bread facilitated the growth of sporulated microorganisms, such as *Bacillus* spp. According to Samapundo *et al.* [46], a heat treatment at 80 ºC for 10 min, a *p*H near 6.0 and water activity at 0.990 enabled the spores of *B. cereus* to germinate and grow spontaneously at 10 ºC. However, Smith et al. [44] mentioned that *B. cereus* spores exhibit high heat resistance (90 ºC for 10 min). Heat treatment into the bread reaches 100 ºC but only for a few minutes. *B. cereus* strains can produce one or more enterotoxins in the intestine or emetic toxin in the food. Enterotoxins that cause diarrhea are heat labile and cause symptoms such as abdominal pain and diarrhea. The toxins responsible for the emetic type are heat stable and cause nausea and vomiting [47]. Additionally, the *B. cereus* group ranks among the top ten reported causative agents of foodborne and waterborne outbreak [48]. In our study, counts of PEMBA (presumptive *Bacillus cereus)* were below the threshold (more than 10^5 CFU·g^{-1} cells or spores) known to cause diarrhea [49], except in the control group (bread samples without HPCF) and in bread samples containing 5 % and 10 % HPCF, after 60 h (Figure 3). Due to the baking process, most microorganisms present in bread were thermally destroyed. Therefore, observed microbial contamination is likely due to recontamination after baking.

CONCLUSION

The aim of this paper was to determine the influence of HPCF on the composition of fatty acids in gluten-free bread and its microbiological characteristics by extending the storage time of the bread. It was determined that HPCF contains significantly higher amount of essential fatty acids such as linoleic acid, α-linolenic acid, Omega - 3 fatty acids and Omega - 6 fatty acids, which means that a higher amount of HPCF in bread will also indicate a higher content of these essential fatty acids. From a microbiological point of view, bread with more HPCF with an extension of storage time up to 72 hours is characterized by a lower number of total yeasts and molds, aerobic viable bacteria and *Bacillus* spp. *Coliforms* and *E. coli* were not detected in any of the tested samples, which indicates excellent hygienic conditions during the production of the bread with different HPCF content.

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REFERENCES

1. Siegfried, K., Günther, S., Mengato, S., Riedel, F., Thrän, D.: Boosting Biowaste Valorisation-Do We Need an Accelerated Regional Implementation of the European Law for End-of-Waste?, *Sustainability*, **2023**, **15** (17), 13147;
2. Caponio, F., Piga, A., Poiana, M.: Valorization of Food Processing By-Products, *Foods*, **2022**, **11** (20), 3246;
3. Smeu, I., Dobre, A.A., Cucu, E.M., Mustățea, G., Belc, N., Ungureanu, E.L.: Byproducts from the Vegetable Oil Industry: The Challenges of Safety and Sustainability, *Sustainability*, **2022**, **14** (4), 2039;
4. Sundar, S., Singh, B., Kaur, A.: Infrared pretreatment for improving oxidative stability, physiochemical properties, phenolic, phytosterol and tocopherol profile of hemp (Cannabis sativa L.) seed oil, *Industrial Crops and Products*, **2023**, **206**, 117705;
5. Sunoj Valiaparambil Sebastian, J., Dong, X., Trostle, C., Pham, H., Joshi, M.V., Jessup, R.W., Burow, M.D., Provin, T.L.: Hemp Agronomy: Current Advances, Questions, Challenges, and Opportunities, *Agronomy*, **2023**, **13** (2), 475.
6. Pieracci, Y., Fulvio, F., Isca, V., Pistelli, L., Bassolino, L., Montanari, M., Moschella, A., Flamini, G., Paris, R.: The phenological stage of hemp inflorescences affects essential oil yield and its chemical composition, *Industrial Crops and Products*, **2023**, **197**, 116605;
7. Alonso-Esteban, J.I., Pinela, J., Ćirić, A., Calhelha, R.C., Soković, M., Ferreira, I.C.F.R., Barros, L., Torija-Isasa, E., Sánchez-Mata, M. de C.: Chemical composition and biological activities of whole and dehulled hemp (*Cannabis sativa* L.) seeds, *Food Chemistry*, **2022**, **374**, 131754;
8. Alonso-Esteban, J.I., González-Fernández, M.J., Fabrikov, D., Torija-Isasa, E., Sánchez-Mata, M. de C., Guil-Guerrero, J.L.: Hemp (*Cannabis sativa* L.) Varieties: Fatty Acid Profiles and Upgrading of γ-Linolenic Acid-Containing Hemp Seed Oils, *European Journal of Lipid Science and Technology*, **2020**, **122** (7);
9. Callaway, J.C.: Hempseed as a nutritional resource: An overview, *Euphytica*, **2004**, **140** (1-2), 65-72;
10. House, J.D., Neufeld, J., Leson, G.: Evaluating the Quality of Protein from Hemp Seed (Cannabis sativa L.) Products Through the use of the Protein Digestibility-Corrected Amino Acid Score Method, *Journal of Agricultural and Food Chemistry*, **2010**, **58** (22), 11801-11807;

11. Vonapartis, E., Aubin, M.-P., Seguin, P., Mustafa, A.F., Charron, J.-B.: Seed composition of ten industrial hemp cultivars approved for production in Canada, *Journal of Food Composition and Analysis*, **2015**, **39**, 8-12;
12. Kabutey, A., Herák, D., Mizera, Č.: Assessment of Quality and Efficiency of Cold-Pressed Oil from Selected Oilseeds, *Foods*, **2023**, **12** (19), 3636;
13. Nađeral, S., Škevin, D., Kraljić, K., Obranović, M., Papeša, S., Bataljaku, A.: Chemical Composition and Oxidative Stability of Roasted and Cold Pressed Pumpkin Seed Oils, *Journal of the American Oil Chemists' Society*, **2012**, **89** (9), 1763-1770;
14. Piravi-Vanak, Z., Dadazadeh, A., Azadmard-Damirchi, S., Torbati, M., Martinez, F.: The Effect of Extraction by Pressing at Different Temperatures on Sesame Oil Quality Characteristics, *Foods*, **2024**, **13** (10), 1472;
15. Rabiej-Kozioł, D., Momot-Ruppert, M., Stawicka, B., Szydłowska-Czerniak, A.: Health Benefits, Antioxidant Activity, and Sensory Attributes of Selected Cold-Pressed Oils, *Molecules*, **2023**, **28** (14), 5484;
16. Shen, P., Gao, Z., Fang, B., Rao, J., Chen, B.: Ferreting out the secrets of industrial hemp protein as emerging functional food ingredients, *Trends in Food Science & Technology*, **2021**, **112**, 1-15;
17. Cravotto, C., Claux, O., Bartier, M., Fabiano-Tixier, A.-S., Tabasso, S.: Leading Edge Technologies and Perspectives in Industrial Oilseed Extraction, *Molecules*, **2023**, **28** (16), 5973;
18. Vasilica-Alisa, A., Georgescu, A-N., Platon, N., Roșu, A-M., Muntianu, G., Teușdea, A.C., Vartolomei, N., Nistor, I-D. : Preliminary studies concerning the influence of buckwheat flour on the quality of white wheat bread, *Scientific Study & Research, Chimistry & Chemical Engineering, Biotechnology, Food Industry*, **2024, 25** (2), 157-167;
19. Ojha, P., Pathak, G., Maharjan, S., Manandhar, U., Maharjan, S., Karki, R.: Quality and textural properties evaluatin of gluten-free biscuit developed from maize, rice, buckwheat, and soybean, *Scientific Study & Research, Chimistry & Chemical Engineering, Biotechnology, Food Industry*, **2022, 23** (4), 295-305;
20. Nikolić, N., Radulović, N., Momcilović, B., Nikolić, G., Lazić, M., Todorovic, Z.: Fatty acids composition and rheology properties of wheat and wheat and white or brown rice flour mixture, *European Food Research and Technology*, **2008**, **227** (5), 1543-1548;
21. Pojić, M., Dapčević Hadnađev, T., Hadnađev, M., Rakita, S., Brlek, T.: Bread Supplementation with Hemp Seed Cake: A By-Product of Hemp Oil Processing, *Journal of Food Quality*, **2015**, **38** (6), 431-440;
22. Norajit, K., Gu, B.-J., Ryu, G.-H.: Effects of the addition of hemp powder on the physicochemical properties and energy bar qualities of extruded rice, *Food Chemistry*, **2011**, **129** (4), 1919-1925;
23. Peñalver, R., Nieto, G.: Developing a functional gluten-free sourdough bread by incorporating Quinoa, Amaranth, Rice and Spirulina, *LWT - Food Science and Technology*, **2024**, 116162;
24. Ua-Arak, T., Jakob, F., Vogel, R.F.: Influence of levan-producing acetic acid bacteria on buckwheat-sourdough breads, *Food Microbiology*, **2017**, **65**, 95-104;
25. Korus, J., Witczak, M., Ziobro, R., Juszczak, L.: Hemp (Cannabis sativa subsp. sativa) flour and protein preparation as natural nutrients and structure forming agents in starch based gluten-free bread, *LWT - Food Science and Technology*, **2017**, **84**, 143-150;
26. Bligh, E.G., Dyer, W.J.: A rapid method of total lipid extraction and purification, *Canadian Journal of Biochemistry and Physiology*, **1959**, **37** (8), 911-917;
27. ISO 21527-2:2008. Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for the Enumeration of Yeasts and Moulds-Part 2: Colony Count Technique in Products with Water Activity Less than or Equal to 0.95, *International Organization for Standardization*, **2008**;
28. ISO 4833-1. Microbiology of the food chain – horizontal method for the enumeration Colony count at 30 degrees C by the pour plate technique, *International Organization for Standardization*, **2013**;
29. ISO 4832. Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of coliforms-colony-count technique, International Organization for Standardization, **2006**;
30. Eglezos, S.: Microbiological Quality of Wheat Grain and Flour from Two Mills in Queensland, Australia, *Journal of Food Protection*, **2010**, **73** (8), 1533–1537;
31. Tallent, S.M., Knolhoff, A., Rhodehamel, E.J., Harmon, S.M., Bennett, R.W.: BAM chapter 14: Bacillus cereus FDA, In *Bacteriological analytical manual* (8th ed.), **2020**;

32. Reta, C., Atlabachew, M., Mehari, B., Hilawea, K. T., Asmellash, T.: Discrimination of the geographical origin of gluten-free teff grains from northwestern parts of Ethiopia by fatty acid analysis, *Heliyon*, **2024**, **10** (3), e24932;
33. Montero, L., Ballesteros-Vivas, D., Gonzalez-Barrios, A.F., Sánchez-Camargo, A. del P.: Hemp seeds: Nutritional value, associated bioactivities and the potential food applications in the Colombian context, *Frontiers in Nutrition*, **2023**, **9**, 10.3389/fnut.2022.1039180;
34. Lee, M.J., Park, S.H., Han, J.H., Hong, Y.K., Hwang, S., Lee, S., Kim, D., Han, S.Y., Kim, E.S., Cho, K.S.: The Effects of Hempseed Meal Intake and Linoleic Acid on Drosophila Models of Neurodegenerative Diseases and Hypercholesterolemia, *Molecules and Cells*, **2011**, **31** (4), 337-342;
35. Papatzimos, G., Mitlianga, P., Basdagianni, Z., Kasapidou, E.: Hemp Flour as a Functional Ingredient for the Partial Replacement of Nitrites in a Minced Meat Model: Effect on Nutrient Composition, Antioxidant Profile and Sensory Characteristics, *Applied Sciences*, **2024**, **14** (9), 3925;
36. Vivar-Quintana, A.M., Absi, Y., Hernández-Jiménez, M., Revilla, I.: Nutritional Value, Mineral Composition, Fatty Acid Profile and Bioactive Compounds of Commercial Plant-Based Gluten-Free Flours, *Applied Sciences*, **2023**, **13** (4), 2309;
37. Samaranayake, M.D.W., Abeysekera, W.K.S.M., Hewajulige, I.G.N., Somasiri, H.P.P.S., Mahanama, K.R.R., Senanayake, D.M.J.B., Premakumara, G.A.S.: Fatty acid profiles of selected traditional and new improved rice varieties of Sri Lanka, *Journal of Food Composition and Analysis*, **2022**, **112**, 104686;
38. Rioux, V., Pédrono, F., Legrand, P.: Regulation of mammalian desaturases by myristic acid: N-terminal myristoylation and other modulations, *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, **2011**, **1811** (1), 1-8;
39. Ephrem, E., Elaissari, H., Greige-Gerges, H.: Improvement of skin whitening agents efficiency through encapsulation: Current state of knowledge, *International Journal of Pharmaceutics*, **2017**, **526** (1-2), 50-68;
40. Devi, V., Khanam, S.: Study of ω-6 linoleic and ω-3 α-linolenic acids of hemp (Cannabis sativa) seed oil extracted by supercritical CO2 extraction: CCD optimization, *Journal of Environmental Chemical Engineering*, **2019**, **7** (1), 102818;
41. Grundy, S.M.: Cholesterol | Factors Determining Blood Levels, In *Encyclopedia of Human Nutrition Elsevier*, **1998**, 385-391;
42. Manach, C., Mazur, A., Scalbert, A.: Polyphenols and prevention of cardiovascular diseases, *Current Opinion in Lipidology*, **2005**, **16** (1), 77–84;
43. Garayoa, R., Abundancia, C., Díez-Leturia, M., Vitas, A.I.: Essential tools for food safety surveillance in catering services: On-site inspections and control of high risk cross-contamination surfaces, *Food Control*, **2017**, **75**, 48-54;
44. Smith, J.P., Daifas, D.P., El-Khoury, W., Koukoutsis, J., El-Khoury, A.: Shelf Life and Safety Concerns of Bakery Products-A Review, *Critical Reviews in Food Science and Nutrition*, **2004**, **44** (1), 19-55;
45. Saranraj P., Sivasakthivelan P.: Microorganisms Involved in Spoilage of Bread and Its Control Measures, In: *Bread and Its Fortification Nutrition and Health Benefits* (Ed. Rosell C.M., Bajerska J., Sheikha E.) **2015**, 132-149;
46. Samapundo, S., Heyndrickx, M., Xhaferi, R., De Baenst, I., Devlieghere, F.: The combined effect of pasteurization intensity, water activity, *p*H and incubation temperature on the survival and outgrowth of spores of Bacillus cereus and Bacillus pumilus in artificial media and food products, *International Journal of Foof Microbiology*, **2014**, **181**, 10-18;
47. Hariram, U., Labbé, R.: Spore Prevalence and Toxigenicity of and Isolates from U.S. Retail Spices, *Journal of Food Protection*, **2015**, **78** (3), 590-596;
48. EFSA: The European Union One Health 2018 Zoonoses Report, *EFSA Journal*, **2019**, **17** (12);
49. Cufaoglu, G., Ayaz, N. D.: Potential risk of Bacillus cereus in spices in Turkey, *Food Control*, **2022**, **132**, 108570.