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Effect of Fiber and Insect Powder Addition on Selected Organoleptic and Nutritional Characteristics of Gluten-Free Bread

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Abstract: A wide range of gluten-free bakery products are already available on the market. However, they often have a low proportion of fiber and inferior sensory properties when compared to classic baked goods. The aim of this work was to evaluate the influence of the addition of different types of fiber and insect powder on selected organoleptic and nutritional properties of gluten-free bread and to reformulate a recipe for gluten-free bread. Twenty-four experimental samples were prepared with different types and percentages of fiber, either alone or in combination. Sensory analysis, instrumental texture analysis, and chemical analyses, including predicted glycemic index, were carried out. A total of 16 of the 24 fiber-enriched samples received an average or slightly above-average rating. The samples containing the fiber mixture without insect powder and the sample containing 9% flaxseed performed best in the overall evaluation. The combination of different types of plant fibers simultaneously with the incorporation of insect powder in a low concentration appears to be advantageous, both from the viewpoint of sensory acceptability and also from the viewpoint of the potential for increasing the polyphenol content and antioxidant capacity. This study lists the sensorially acceptable range of fiber concentrations, which can be a guide for the bakery industry.

Keywords: insect powder; *Tenebrio molitor*; sensory quality; antioxidant capacity; glycemic index

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1. Introduction

In recent years, we have seen a growing interest in gluten-free products due to the increased number of diagnosed cases of celiac disease, gluten allergy, non-celiac gluten sensitivity, or the voluntary exclusion or restriction of gluten in one's diet [1]. The reason for the exclusion can be the following: consumers suffering from other autoimmune diseases, e.g., type I diabetes, psoriasis, or rheumatoid arthritis [2].

Nowadays, a wider range of gluten-free bakery products are available on the market. However, these gluten-free bakery products are often based on starches and, compared to classic baked goods containing gluten, have a low proportion of fiber, which is a drawback for the consumer from a nutritional point of view [2–4]. The low proportion of fiber contained in the products represents one of the biggest shortcomings of gluten-free baking [5]. Fiber fortification in different forms can lead to an increase in the nutritional value of gluten-free bakery products [6].

In addition, gluten-free bread is often associated with a friable, incoherent crumb, a small volume, a light color, and often a bland taste [7–9]. These negatives can have a significant impact on sensory perception. This represents another reason to focus on enriching the recipe of gluten-free bakery products with suitable nutrients that will lead to an improvement in their nutritional as well as sensory profiles [10]. The technology of gluten-free bakery products, therefore, has certain disadvantages. Nevertheless, there are ways in which these shortcomings can be overcome. From a technological point of view, after adding fiber, the texture and elasticity of the dough are mainly improved [11]. It also contributes to the volume increase in the loaf and the softness of the crumb. The addition of fiber can positively affect sensory descriptors such as taste and color [8,12].

The recommended daily intake is between 22 and 30 g of fiber [13]. Bakery products are an important source of fiber in the Western diet. However, in the case of focusing on gluten-free bakery products without conscious supplementation from other sources, consumers may suffer from fiber deficiency; this has been confirmed by a number of studies in the case of patients with celiac disease [14–16]. There are a number of different types of fiber of vegetable origin with specific characteristics that are already used or could be used in the production of bakery products. Thanks to its properties, psyllium appears to be particularly suitable, as are flaxseed, chia seed, and apple fiber [17]. Insect powder is a less traditional but also attractive alternative ingredient for the fortification of gluten-free baked goods. Interest in insect products has been increasing, especially as suitable sustainable alternatives to other animal products, as the excessive production of animal products is usually not considered eco-friendly [18]. In addition, powder from ground insects represents a way to significantly enrich the product nutritionally with fiber, proteins, antioxidants, and vitamin B12 [19,20]. It has a brown color, and its taste is often described as slightly nutty [21]. The flavor and texture of insects appear to depend to some extent on the species, developmental stage, and processing method [22]. Several studies describe the use of insects, specifically in bakery products such as bread, as they represent a staple food. However, few of them deal with gluten-free pieces of bread; for example, Nissen et al. (2020) [23] or Kowalczewski et al. (2021) [24].

The aim of this work was to evaluate the influence of the addition of different types of fiber and insect powder on the organoleptic properties and selected nutritionally significant parameters of gluten-free bread. In order to determine the optimal addition of fiber and/or insect powder, different types of fiber as well as different proportions of them or their combination, either with or without the insect powder in the mixture, were evaluated.

2. Materials and Methods

2.1. Material

The basic recipe for the experimental gluten-free bread consisted of the following ingredients: semi-coarse rice flour (24%), semi-coarse corn flour (12.5%), fine buckwheat flour (11%), guar gum (1%), caraway seeds (0.5%), salt (1.5%), yeast (1%), sugar (0.5%), and lukewarm water (48%).

A total of five additional raw materials were chosen for the purpose of adding fiber, namely flaxseed, psyllium, apple fiber, chia seed, and insect powder. The amount of individual fiber and insect powder added was selected based on available studies. The range of values was expanded based on experimental baking during preliminary tests. All the raw materials used came from the local market network; only the insect powder, namely the yellow mealworm powder (*Tenebrio molitor*), was purchased from a specialized e-shop.

2.2. Preparation of Breads

Flaxseed, chia seed, psyllium, and apple fiber were allowed to swell in water for 15 h. To prepare each sample, yeast was weighed, to which 300 mL of lukewarm water and sugar were added. Subsequently, the yeast was allowed to activate in a bread proofer at a temperature of 40 °C and a humidity of 68%. Then, a loose mixture was prepared, which consisted of rice, corn, buckwheat flour, guar gum, salt, and caraway seed. Swollen fiber and a loose mixture were subsequently added to the activated yeast. A no-kneading method was used for the preparation of the dough, where, with an increased water content, a structure was formed without the use of mechanical energy [25]. After thorough mixing, the dough was put back into the bread proofer (Unox, Cadoneghe, Italy), where it was left to rise for 90 min. The dough was then transferred to silicone molds, where it was left to rise for another 30 min. Subsequently, the bread samples were placed in a preheated oven (Unox Elena, Cadoneghe, Italy). Baking in the mold took place for 20 min at 220 °C, followed by 20 min at 180 °C. The baking process was finished without the mold for another

20–30 min. The bread samples were then left to cool for 24 h (Figure 1). The sampling was performed in the following ways: (i) the loaf middle part was used for the textural analysis; (ii) the rest of the loaf was used for the sensory and chemical analyses; and (iii) the end slices were not used since they did not objectively represent the average characteristics of a sample.

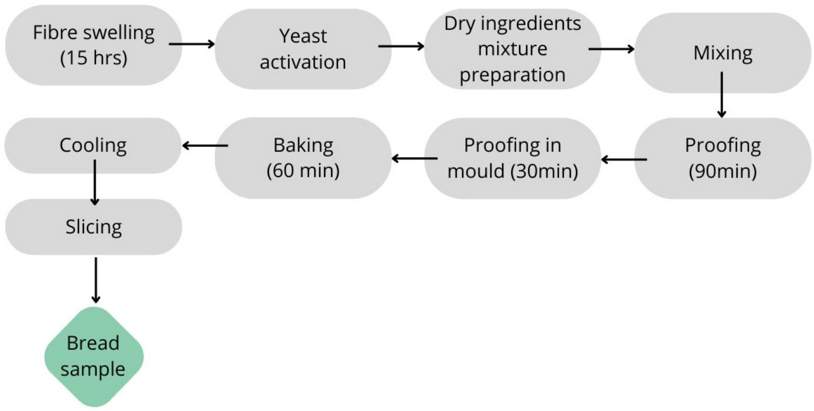


Figure 1. Gluten-free bread preparation flowchart.

2.3. Experimental Design

In the first phase, 20 experimental samples of gluten-free pieces of bread were prepared according to the same recipe, differing only in the type and percentage of fiber addition (see Table 1). The first sub-goal of the work was to evaluate the sensory quality of gluten-free bread samples compared to a control sample without fiber addition. Subsequently, a statistical evaluation of the partial results of the sensory acceptability of the samples containing the individual types of fiber used was carried out. The goal was to determine the highest percentage of added fiber that is still sensory-acceptable for consumers. Based on the statistical evaluation, the recipes for four compound gluten-free bread samples containing a combination of fiber types in different proportions were subsequently compiled in the second phase, as shown in Table 2. Sensory analysis and instrumental texture analysis were carried out for all samples; the influence of fiber on appearance was further monitored using images taken with a digital microscope; and chemical analyses were also carried out, specifically the determination of antioxidant capacity and glycemic index. In the third stage, all data were statistically evaluated (Figure 2).

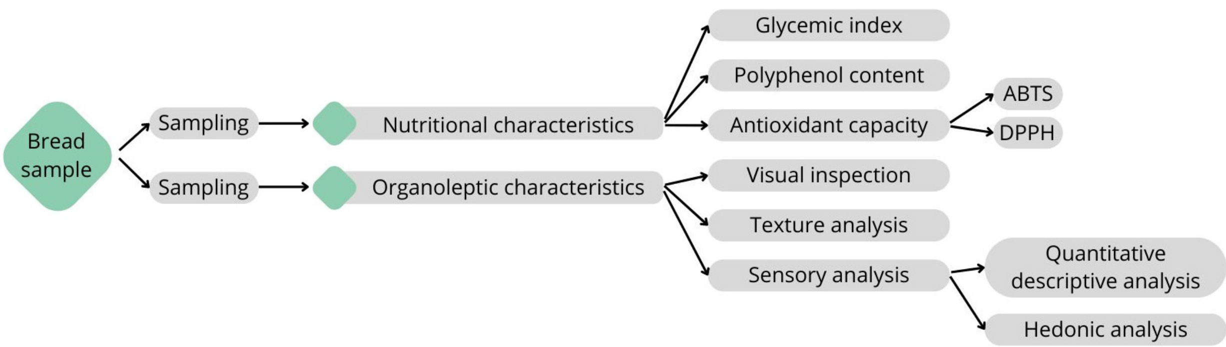


Figure 2. Experimental design flowchart.

Table 1. Percentage of individual fiber-type additions in experimental gluten-free bread samples.

Fiber Addition/Type	2%	5%	7%	9%	13%	17%	23%
Flaxseed	–	–	–	F_9	F_13	F_17	F_23
Psyllium	P_2	P_5	P_7	P_9	–	–	–
Apple fiber	A_2	A_5	A_7	A_9	–	–	–
Chia seed	CH_2	CH_5	–	CH_9	CH_13	–	–
Insect powder	I_2	I_5	–	I_9	I_13	–	–

Table 2. Percentage of individual fiber-type additions in experimental compound gluten-free bread samples.

Sample/Fiber Type	C_1	C_2	C_3	C_4
Flaxseed	5%	4%	8%	8%
Psyllium	2%	2%	2%	2%
Apple fiber	2%	2%	4%	4%
Chia seed	2%	2%	2%	2%
Insect powder	–	2%	–	2%

2.4. Sensory Analysis

Sensory analysis took place the day after baking. Quantitative descriptive analysis was used to describe key descriptors, followed by hedonic analysis to verify consumer acceptability. Analyses were performed at the Department of Plant Origin Food Sciences following the methods described in Tauferova et al. [26]. Briefly, for the purpose of quantifying attributes, 9-degree categorical ordinal scales with described extremes from 1 (minimum intensity) to 9 (highest intensity) were used. Accordingly, a hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) was used. The panel of trained evaluators (n = 19, age 22–50 years, both sexes) was made up of selected VETUNI academic staff and students who had previous experience in food sensory evaluation, specifically with evaluating bakery products. Both regular consumers of gluten-free bakery products and evaluators, who normally consume only classic bakery products with gluten content, were represented. A panel discussion regarding the most cited descriptors of gluten-free and plain white pieces of bread preceded the descriptor selection with the aim of identifying those that best characterized the product and excluding those that were not perceived by the majority of evaluators. All the evaluators clearly declared that they had no allergies or intolerances, and they performed the evaluations voluntarily. All the analyses were performed in a complete block design in six sessions (four samples per session, one session per day). The individual sessions were arranged to take place in the morning in a separate test room with off-white walls, natural lighting, and minimal distractions. The gluten-free bread samples in the form of 1.5 cm thick slices were presented on white plates identified by three-digit numerical codes; still water was used as a neutralizer.

2.5. Texture Analysis

Instrumental measurement of the sample texture was performed with a texture analyzer of TA.TX plus (Stable Micro Systems Ltd., Surrey, UK) using the standard method for the determination of bread firmness, AACC (74-09.01, The American Association of Cereal Chemists). The samples were manually cut into 2.5 cm thick slices. Slices from the middle of the loaf were used, and a cylinder probe (36 mm cylinder probe with radius* (P/36R)) was applied to determine the bread firmness. The pre-test speed was set to 1.0 mm/s, the test speed was 1.7 mm/s, and the post-test speed was 10.0 mm/s. The texture measurement was performed six times for each sample. To calculate results, Texture Exponent software (Exponent Connect, Stable Micro Systems Ltd., Surrey, UK) was utilized.

2.6. Visual Inspection by Optical Microscopy

A visual comparison of individual samples was made based on images taken with a digital USB microscope, the Dino-Lite AM4115T (AnMo Electronics, Taipei, Taiwan). First, the microscope was calibrated, and 20× magnification was used when taking pictures. Individual samples of gluten-free bread in the form of slices with a thickness of 1 cm were gradually placed in a dark chamber and uniformly illuminated by built-in LEDs. Four images were taken from each sample.

2.7. Determination of Total Polyphenol Content and Antioxidant Capacity

2.7.1. Extraction of Bioactive Substances

Samples of individual breads were processed according to the methodology described by Zielińska et al. [27]. After homogenization, 1 g was taken into a plastic centrifuge tube with 10 mL of 75% methanol, shaken, and left to extract for 120 min. The samples were then centrifuged (Witeg Labortechnik GmbH, Wertheim, Germany) for 10 min at 3000× *g*. The supernatant was collected and used for subsequent analyses.

2.7.2. Total Polyphenol Content (TPC)

The 96-well microplate method, according to Zhang et al. [28], was used to determine the total content of polyphenols. A total of 20 µL of the extracted sample was pipetted into a 96-well plate (Microtitration plate P No. V400917, Gama Group, České Budějovice, Czech Republic), 100 µL of Folin–Ciocalteu reagent diluted with distilled water 1:10 (Penta, Praha, Czech Republic), and 80 µL of 7.5% sodium carbonate solution. Gallic acid (MP Biomedicals, Shanghai, China) in the usual concentration range of 0–0.5 mmol/L was used for the calibration series. The samples were measured after 30 min at 765 nm with a 96-well plate reader (Tecan Austria GmbH, Grödig, Austria). From the measured absorbance, the content of total polyphenols was expressed by conversion from the regression equation of the calibration series.

2.7.3. ABTS Assay

The ABTS working solution was prepared according to the methodology of Xiao et al. [29] using ABTS (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 7 mmol/L and potassium persulfate at a concentration of 2.45 mmol/L in an acetate buffer of pH 4.5. The solutions were mixed in a ratio of 1:1 and left for 14 h in the dark. After incubation, 2.80 mL was taken, dissolved in 65 mL of acetate buffer, and left for 30 min in the dark at room temperature. Then, 10 µL of sample and 200 µL of ABTS working solution were pipetted into the 96-well plate. After 7 min of incubation in the dark, the absorbance was measured at 734 nm. The antioxidant capacity expressed as an inhibition ratio was calculated from the formula, where A0 corresponds to the absorbance of the control and A1 to the absorbance of the sample.

$$\text{The Inhibition Ratio } (\%) = (\text{A0} - \text{A1})/\text{A0} \times 100$$

2.7.4. DPPH Assay

The 10 µL of sample and 240 µL of methanolic DPPH solution (Sigma-Aldrich, USA) were pipetted into a 96-well plate. After incubation in the dark for 30 min, the absorbance at 517 nm was subsequently measured. The antioxidant capacity was calculated from the formula using the same formula as for the ABTS method.

2.7.5. Determination of Glycemic Index

Selected bread samples were subjected to enzymatic hydrolysis according to the methodology of Zielińska and Pankiewicz [30], with slight modifications. The following enzymes were used for enzymatic hydrolysis: pepsin (250 U/mg; Sigma-Aldrich, USA); amyloglucosidase (70 U/mg; Sigma-Aldrich, USA); and pancreatin (AppliChem, Darmstadt, Germany). From the beginning of the gastric phase, 1 mL was taken at 20, 30, 60, 90, 120, and 180 min. A total of 4 mL of 100% ethanol was immediately added to each and mixed to inactivate the enzymes. Samples were centrifuged (Witeg, Wertheim, Germany) to remove sediment. Subsequently, for each collection and sample, the glucose content was measured using a GOPOD assay kit (Megazyme, Wicklow, Ireland) in a 96-well plate at 510 nm using a reader (Tecan Austria GmbH, Grödig, Austria). Glucose was expressed as mg/g in the sample. A curve was constructed from the results, and the areas under the curves (AUC) were calculated. The ratio between the AUC of the sample and the AUC of the standard (reference white bread) was calculated as a hydrolysis index (HI). The glycemic index was then calculated according to the following formula from Goñi et al. [31].

$$\mathrm{GI}(\%) = 39.71 + 0.549 \times \mathrm{HI}$$

2.8. Statistical Analysis

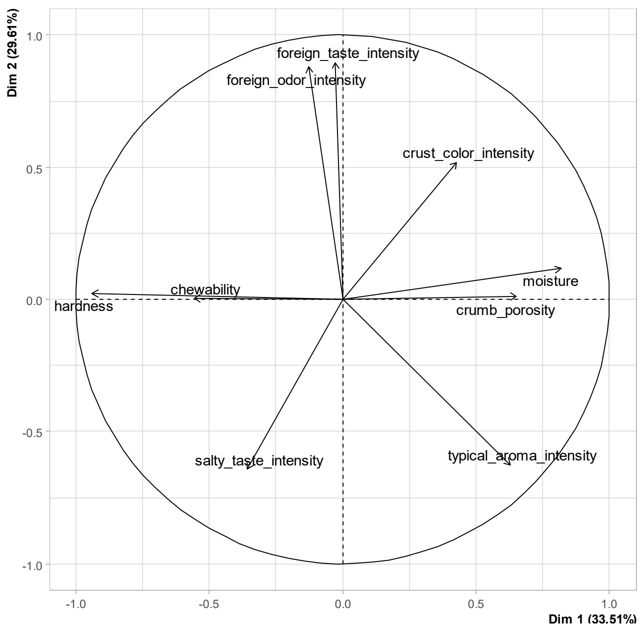
R software version 3.3.3 (The R Foundation for Statistical Computing, Vienna, Austria), namely the method of principal component analysis (PCA), was applied for the statistical evaluation of the sensory data. As for the texture analysis data, the presence of statistically significant differences ($p < 0.05$) between individual samples was evaluated using a one-factor analysis of variance followed by the Tukey HSD test using Unistat statistical software version 6.0 (Unistat Ltd., London, UK). The data for TPC do not follow a normal distribution by the Shapiro–Wilk test, so the Kruskal–Wallis multiple pairwise comparisons test with Dunn's procedure ($p < 0.05$) was used. Antioxidant capacity values (ABTS, DPPH) had a normal distribution, and therefore, the ANOVA variance analysis with post hoc Tukey's HSD test ($p < 0.05$) was used for statistical comparison. The XLSTAT software version 2023.5 (Addinsoft, Long Island City, NY, USA) was used for statistical analysis of chemical data.

3. Results and Discussion

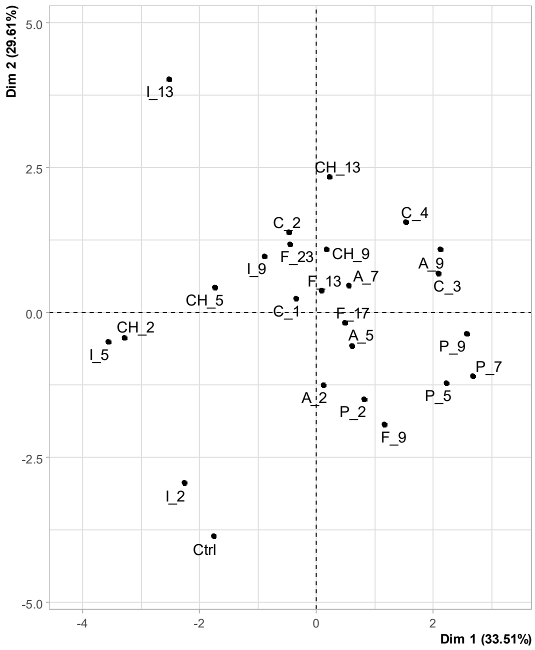
3.1. Effect of Fiber Addition on Key Organoleptic Properties of Experimental Samples of Gluten-Free Bread Samples

Quantitative Descriptive Analysis

Figure 3 presents the results of a quantitative descriptive analysis of the effect of fiber addition on the evaluated descriptors of individual samples. Charts showing the results of the principal component analysis describe 63.12% of the total variability, with the first component describing 33.51% and the second 29.61% of the variability. A number of statistically significant differences were confirmed between the experimental samples in terms of quantitative descriptive analysis. In accordance with the original assumption, the control sample without the addition of fiber achieved statistically significantly higher ($p < 0.05$) values of the intensity of typical bread taste and salty taste, statistically significantly lower intensity of crust color, moisture, and porosity of the crumb, and was not characterized by a noticeable foreign smell or taste. The bread sample containing 2% insect powder was the most similar to the control sample, but compared to the control, it had a statistically significantly lower typical aroma intensity and statistically significantly higher firmness and foreign taste intensity. On the contrary, the sample containing 13% insect powder stood out the most; it already showed a statistically significant ($p < 0.05$) lower intensity of the typical aroma and moisture and, at the same time, a statistically significantly higher intensity of crust color, firmness, foreign smell, and foreign taste. A larger proportion of added insect powder can significantly affect the color of the bread, as it is characterized by a brown color [21]. However, the addition of fiber, in general, can lead to an increase in the color intensity of baked goods [11].



(**a**)



(**b**)

Figure 3. The PCA results of quantitative descriptive analysis of individual experimental gluten-free bread samples are (**a**) variable factor map and (**b**) score plot for the mean points.

3.2. Hedonic Analysis

Figure 4 presents the results of the hedonic analysis of the effect of fiber addition on the evaluated descriptors of individual samples. Charts showing the results of the principal component analysis describe 83.92% of the total variability, with the first component describing 61.47% and the second 22.45% of the variability. Among the experimental samples, a number of statistically significant differences were also confirmed from the point of view of hedonic analysis. From the variable map (Figure 4a), there is a close correlation between the descriptors of the overall evaluation and the pleasantness of the texture, from which we can conclude that the overall evaluation of the samples was largely influenced by the evaluation of their textural properties. The specific values of each sample descriptor are shown in Table 3 below. From the point of view of hedonic analysis, the control sample without the addition of fiber achieved only slightly above-average values. The worst hedonically rated sample was the sample containing 9% psyllium (P_9). Its high percentage in the dough resulted in very low porosity, high humidity, and a pronounced shine to the crumb. A similar effect of psyllium on the texture of gluten-free dough is described by Filipčev et al. [32]. However, when the appropriate percentage of psyllium is added, the bread acquires an ideally smooth and elastic texture, which is key in gluten-free baking. Samples with a lower psyllium content were evaluated positively, and in the case of our samples, even a 7% addition of psyllium led to achieving a neutral or rather positive evaluation (≥5). Another advantage of psyllium is its positive effect on the shelf life of gluten-free bread samples [32]. The second overall worst-rated one was the sample containing 13% insect powder (I_13). Sample I_13 was the only one characterized by statistically significant lower aroma pleasantness (4.18). Among the other samples, the evaluators noted no statistically significant differences in aroma pleasantness. A total of 16 of the 24 fiber-enriched samples received an average or slightly above-average rating. Three samples, namely bread containing 9% flaxseed (F_9) and compound samples C_1 and C_3 without insect powder, were evaluated statistically significantly better in terms of the overall evaluation.

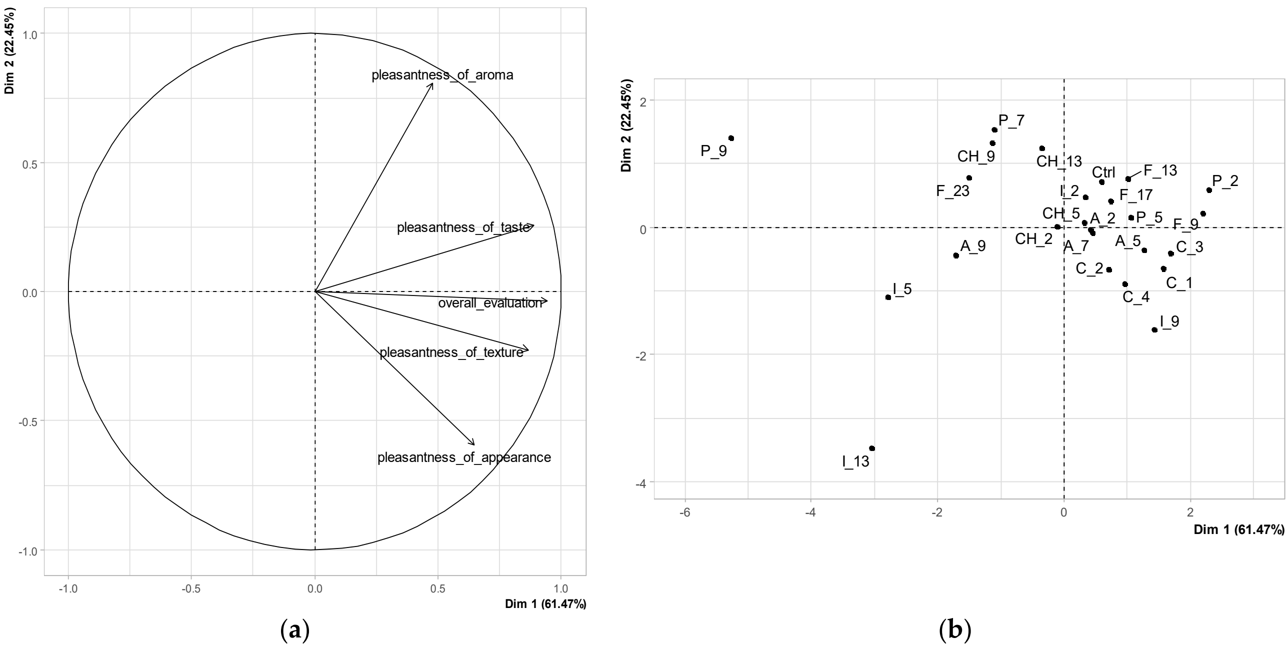


Figure 4. The PCA results of hedonic analysis of individual experimental gluten-free bread samples are (**a**) variables factor map and (**b**) score plot for the mean points.

Table 3. Adjusted mean of hedonic evaluation of experimental gluten-free bread samples.

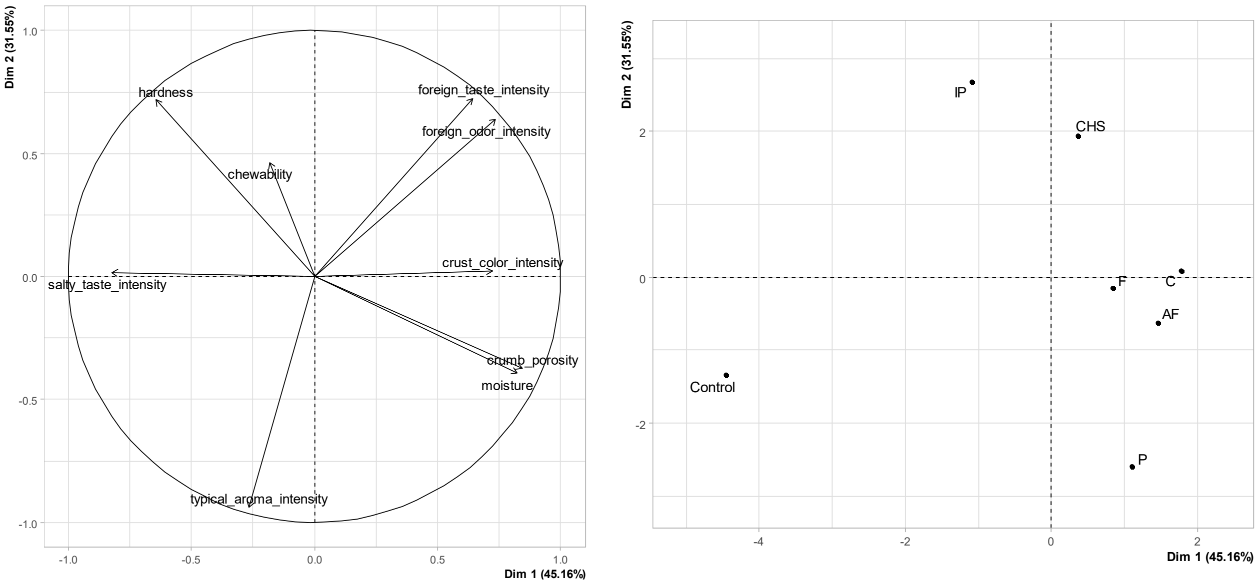
	Aroma	Appearance	Texture	Taste	Overall Evaluation
P_9	5.607	3.334	3.610	4.297	3.885
I_13	4.180	5.949	5.306	4.524	4.973
I_5	5.487	5.718	4.845	4.678	4.665
A_9	5.684	6.069	5.522	5.004	5.072
F_23	5.981	4.910	5.037	5.879	5.141
P_7	6.376	5.026	5.148	5.527	5.500
CH_9	5.859	4.475	5.123	6.315	5.717
CH_13	6.288	5.046	5.695	5.744	6.288
CH_2	6.002	7.046	4.980	6.315	5.717
CH_5	5.716	5.332	6.409	6.030	6.574
I_2	6.564	5.949	5.845	5.678	6.126
C_2	5.794	6.341	5.505	6.116	6.536
Ctrl	6.436	5.654	5.743	6.209	6.258
A_2	6.041	6.283	6.379	5.718	6.144
F_17	6.203	5.798	6.259	6.435	5.919
C_4	5.885	6.978	5.414	6.116	6.536
A_7	6.113	6.998	5.950	6.075	5.715
F_13	6.648	6.132	6.481	6.213	5.808
P_5	6.068	6.103	6.456	5.989	5.500
I_9	5.795	7.026	6.922	5.909	6.434
A_5	5.827	6.783	6.236	6.504	6.501
C_1	5.883	6.426	6.112	6.511	6.970
C_3	6.267	6.888	6.035	6.280	6.893
F_9	6.203	5.687	7.148	6.546	7.141
P_2	6.376	6.487	6.687	6.681	6.808

Statistically significant higher values are highlighted in blue; statistically significant lower values are highlighted in pink.

3.3. *Effect of Fiber Type on Key Organoleptic Properties of Groups of Gluten-Free Bread Samples*

3.3.1. Quantitative Descriptive Analysis

Figure 5 presents the results of a quantitative descriptive analysis of the influence of fiber type on the evaluated descriptors of sample groups. Showing the results of principal component analysis, charts (Figure 5a,b) describe 76.71% of the total variability, with the first component describing 45.16% and the second 31.55% of the variability. A number of statistically significant differences were confirmed between the groups of experimental samples according to the type of fiber from the point of view of quantitative descriptive analysis (Table 4).



(**a**) (**b**)

Figure 5. The PCA results of quantitative descriptive analysis of a group of experimental gluten-free bread samples according to the fiber type are (**a**) variables factor map and (**b**) score plot for the mean points (control, gluten-free bread without the addition of fiber; P, group of samples with added psyllium; AF, group of samples with added apple fiber; F, group of samples with added flaxseed; CHS, group of samples with added chia seeds; IP, group of samples with added insect powder; C, group of compound bread samples with multiple types of fiber).

Table 4. Matrix with the *p*-values of Hotelling's T2 tests for each pair of gluten-free bread groups according to the fiber type used (quantitative descriptive analysis).

	AF	C	Control	F	CHS	IP	P
AF	1	0.49	$p < 0.001$	0.34	$p < 0.01$	$p < 0.001$	$p < 0.05$
C	0.49	1	$p < 0.001$	0.18	$p < 0.01$	$p < 0.001$	$p < 0.001$
Control	$p < 0.001$	$p < 0.001$	1	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
F	0.34	0.18	$p < 0.001$	1	$p < 0.05$	$p < 0.001$	$p < 0.05$
CHS	$p < 0.01$	$p < 0.01$	$p < 0.001$	$p < 0.05$	1	$p < 0.01$	$p < 0.001$
IP	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	1	$p < 0.001$
P	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.001$	$p < 0.001$	1

Statistically significant differences between the groups are emphasized with green color. Control, gluten-free bread without the addition of fiber; P, group of samples with added psyllium; AF, group of samples with added apple fiber; F, group of samples with added flaxseed; CHS, group of samples with added chia seeds; IP, group of samples with added insect powder; C, group of compound bread samples with multiple types of fiber.

The sample map (Figure 5b) shows that the control sample stood out the most from all the other groups. It was characterized by statistically significantly lower values of foreign taste and foreign smell, statistically significantly lighter color of the crust, lower porosity of the crumb, lower humidity, and, at the same time, slightly more intense saltiness and slightly higher intensity of the typical aroma. The second group of samples that differed statistically significantly in a number of descriptors was the group of samples with insect powder (IP) content. This was characterized by statistically significantly lower values of the intensity of typical bread aroma, moisture, and porosity, and at the same time statistically significantly higher values of firmness, intensity of salty taste, and intensity of foreign taste, which in the case of this group reached the highest values ever. Pecova et al. [33] also demonstrated a negative effect of a higher concentration of insect powder in gluten-free products on some sensory parameters. González et al. [34] looked at the effect of a specific insect species as an additive on the organoleptic properties of bakery products, and significant differences in several descriptors were found after the addition of black soldier fly (*H. illucens*). Conversely, no statistically significant changes in selected organoleptic properties were detected in the house cricket (*A. domestica*) or the mealworm (*T. molitor*). The type of insect incorporated into the food can, therefore, have a different effect on the sensory quality of the resulting products. Certain types of fiber, in addition to their nutritional benefits, have the potential to affect the sensory characteristics of bakery products in a specific positive direction. For example, our results show that the addition of flaxseed and psyllium helps retain more moisture in the crust, thus contributing to a lower perceived firmness of the crumb. Apple fiber contributes to a more intense coloring of the crust and higher porosity (Table 5).

Table 5. Adjusted mean of the quantitative descriptive evaluation of a group of bread samples according to the fiber type.

	Hardness	**Foreign Taste**	**Foreign Odor**	**Salty Taste**	**Crust Color**	**Chewability**	**Crumb Porosity**	**Moisture**	**Typical Aroma**
IP	4.836	4.718	3.569	5.315	4.420	3.919	4.042	4.178	4.500
CHS	3.760	4.019	3.968	5.067	3.955	4.150	4.107	6.051	4.724
C	3.625	4.165	3.552	4.074	4.657	3.397	4.777	5.754	5.247
Control	4.266	2.126	1.455	5.765	2.658	3.922	3.645	4.064	6.150
F	3.391	3.633	3.464	4.224	3.296	4.574	4.690	6.484	5.438
AF	3.796	3.556	3.078	4.757	5.424	4.646	5.376	5.859	5.564
P	2.734	3.319	2.861	5.223	4.709	3.880	4.839	6.879	5.857

Statistically significant higher values are highlighted in blue; statistically significant lower values are highlighted in pink. Control, gluten-free bread without the addition of fiber; P, group of samples with added psyllium; AF, group of samples with added apple fiber; F, group of samples with added flaxseed; CHS, group of samples with added chia seeds; IP, group of samples with added insect powder; C, group of compound bread samples with multiple types of fiber.

3.3.2. Hedonic Analysis

Figure 6 presents the results of the hedonic analysis of the effect of the fiber type on the evaluated descriptors of the sample groups. Showing the results of principal component analysis, charts (Figure 6a,b) describe 97.46% of the total variability, with the first component describing 61.69% and the second 35.77% of the variability. Principal component analysis resulted in a reduction in factors, as only the descriptors pleasantness of taste, pleasantness of appearance, and overall evaluation contributed to the formation of the overall variability (Table 6). The group of samples with the addition of insect powder achieved slightly lower values in the pleasantness of taste and aroma. The group of samples with the addition of psyllium had a slightly worse appearance. However, it needs to be said that this applies in the case of a higher percentage addition since, in a lower concentration, the influence of psyllium on the appearance of bakery products can be positive. This is also confirmed by Aldughpassi et al. (2021) [35], who investigated the effect of adding psyllium to Arabic bread (pita). They report that the low percentage of added psyllium (3 and 5%) was rated very positively by the respondents due to the positive effect on the taste, smoothness, and color of the samples. Chia seeds (*Salvia hispanica*) have a similar effect. They have a pleasant, slightly nutty taste, and when mixed with water, they form a viscous gel similar to psyllium [36]. On the contrary, the group of samples with the addition of apple fiber was characterized by a slightly more pleasant appearance. The group of compound samples, with the addition of a combination of several types of fibers, achieved the best overall rating, also due to a more pleasant appearance. Consumers also usually have a better perception of complex flavors, where no sub-flavor overpowers the others significantly.

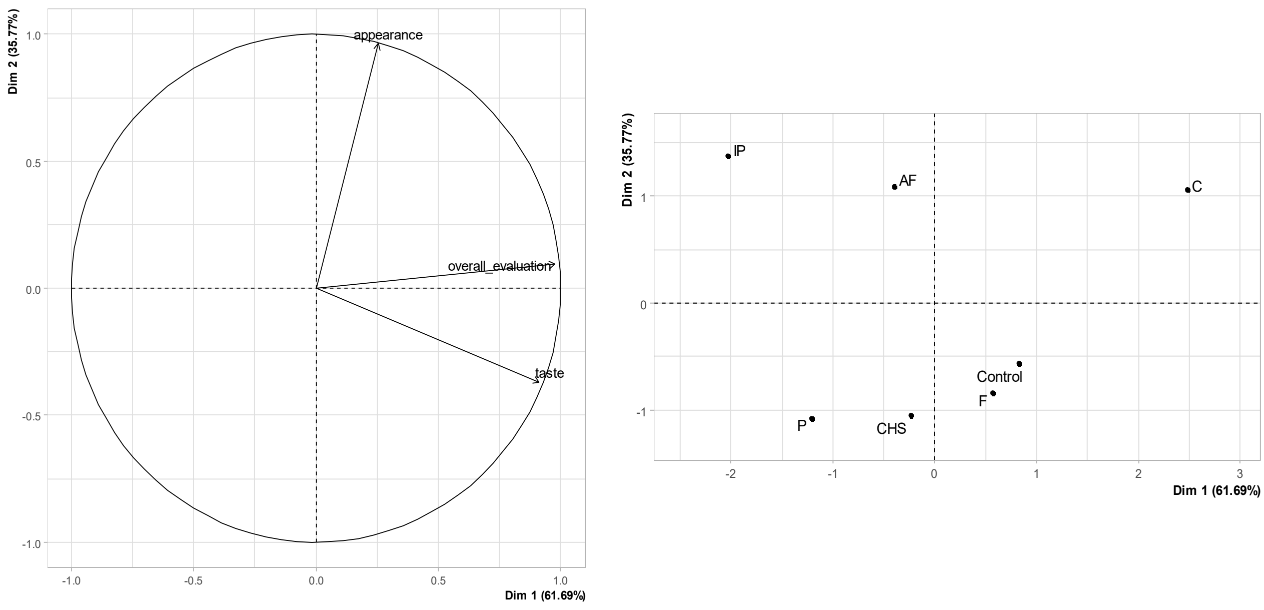


Figure 6. The PCA results of hedonic analysis of a group of experimental gluten-free bread samples according to the fiber type are (**a**) variables factor map and (**b**) score plot for the mean points (control, gluten-free bread without the addition of fiber; P, group of samples with added psyllium; AF, group of samples with added apple fiber; F, group of samples with added flaxseed; CHS, group of samples with added chia seeds; IP, group of samples with added insect powder; C, group of compound bread samples with multiple types of fiber).

Table 6. Adjusted mean of hedonic evaluation of a group of bread samples according to the fiber type.

	Appearance	Texture	Aroma	Overall Evaluation	Taste
IP	6.161	5.736	5.509	5.553	5.200
P	5.237	5.471	6.105	5.670	5.621
CHS	5.475	5.548	5.965	6.071	6.099
AF	6.533	6.019	5.915	5.856	5.824
Control	5.654	5.743	6.436	6.258	6.210
F	5.632	6.226	6.257	5.999	6.266
C	6.658	5.796	5.969	6.753	6.269

Statistically significant higher values are highlighted in blue; statistically significant lower values are highlighted in pink. Control, gluten-free bread without the addition of fiber; P, group of samples with added psyllium; AF, group of samples with added apple fiber; F, group of samples with added flaxseed; CHS, group of samples with added chia seeds; IP, group of samples with added insect powder; C, group of compound bread samples with multiple types of fiber.

3.4. Instrumental Texture Determination

The resistance of the bread crust and the consistency of the crumb are among the basic textural attributes and are referred to as firmness [37]. The addition of fiber shows a positive effect on firmness, especially during storage. This effect is attributed to fiber due to its ability to retain water [38]. From our results (see Table 7), it is evident that on the first day after baking the bread samples, no statistically significant differences were found in the texture, specifically the firmness parameter measured instrumentally, depending on the type and percentage of fiber addition. Firmness was also analyzed sensorially, with values on a scale from 1 to 9 ranging in a relatively narrow range from 2.118 to 5.491, yet several statistically significant differences were found. The absence of statistically significant differences in the case of instrumental texture analysis could have been caused by the choice of a specific textural parameter or the choice of method. It is clear that the degree of influence on textural parameters significantly depends on the nature of the additive used as a fiber source. In a study by Curti et al. [39], the addition of fiber in the form of bran in amounts of 16 and 23.5% based on the total amount of milled grain products used led to an approximately 2–2.5-fold increase in firmness determined on the first day after baking compared to the control sample from classic wheat flour. Our chosen sources of fiber in connection with the selected percentages of addition did not lead to more significant changes in the firmness of the bread samples on the first day after baking compared to the control.

Table 7. Results of instrumental texture determination.

Sample	Firmness (g)
F_9	3.680 ± 0.328
F_13	3.458 ± 0.503
F_17	3.803 ± 0.476
F_23	4.177 ± 0.680
P_2	3.765 ± 0.390
P_5	3.547 ± 0.404
P_7	3.700 ± 0.315
P_9	3.637 ± 0.334
A_2	4.435 ± 0.547
A_5	4.190 ± 0.415
A_7	3.805 ± 0.443
A_9	4.163 ± 0.554
CH_2	3.778 ± 0.225
CH_5	4.280 ± 0.400
CH_9	3.882 ± 0.625
CH_13	3.970 ± 0.583
I_2	3.893 ± 0.338
I_5	3.832 ± 0.353
I_9	3.792 ± 0.362
I_13	4.228 ± 0.657
C_1	3.918 ± 0.589
C_2	4.035 ± 0.380
C_3	3.935 ± 0.360
C_4	4.035 ± 0.215
Ctrl	4.048 ± 0.462

Mean ± standard deviation (SD).

3.5. Visual Inspection by Optical Microscopy

A digital microscope, Dino-Lite (AnMo Electronics, Taiwan), was used to capture the visual parameters of all experimental samples (1280 × 1024 resolution and ×20 magnification). Dino-Lite images of individual experimental gluten-free bread samples are shown in Figure 7.

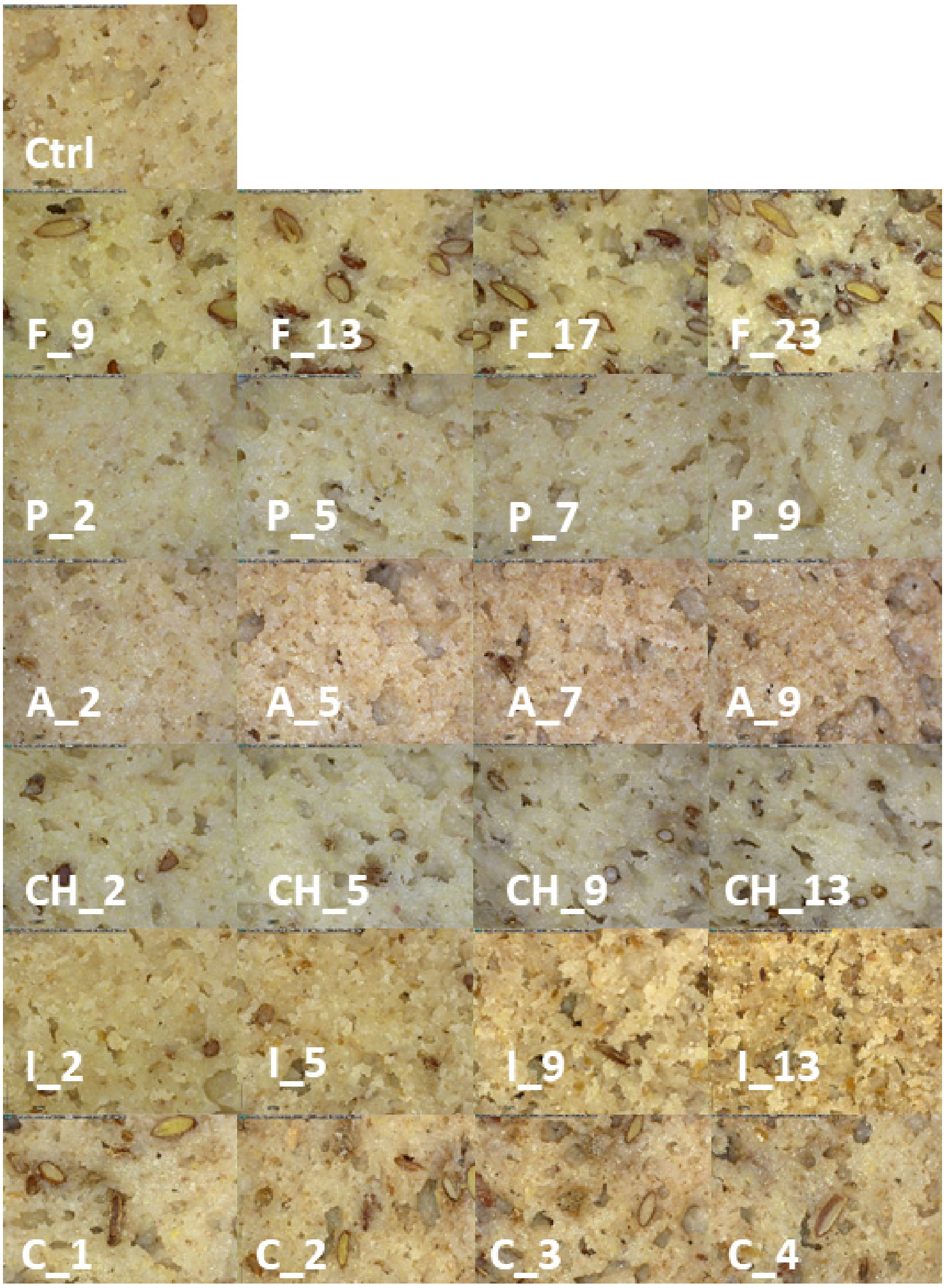


Figure 7. Dino-Lite images of individual experimental gluten-free bread samples.

For individual samples, differences can be noticed mainly in the shade of the crumb color. Slight differences can also be observed in the representation of differently colored particles visible in the crumb, in the crumb porosity, and in its gloss. The type and, to a lesser extent, the amount of added fiber had a demonstrable effect on the visual parameters of the experimental gluten-free bread samples.

Due to the ingredients used, gluten-free bread is often light, and according to Ozyigit et al. [11], the addition of fiber can, among other things, also lead to an improvement in sensory quality, mainly due to the change in color. However, the addition of psyllium resulted in an additional slight lightening of the crumb shade compared to the control sample without the addition of any fiber. As a consequence, the appearance of these samples was rated statistically significantly worse in the sensory analysis compared to the other samples. The samples were characterized by the highest gloss, indicating a higher percentage of moisture in the crumb. Similarly, the addition of chia seeds led to a lightening of the crumb color compared to the control sample. However, due to the dark brown color of the chia seed coat, these samples, in contrast to the psyllium samples, were characterized by the presence of dark particles in the crumb. At higher concentrations of the addition of chia seeds, the samples were characterized by a noticeable gloss on the cut surface. By sensory analysis, samples with a higher percentage of psyllium and chia seeds had a statistically significantly higher moisture content. Similar to psyllium, chia seeds, due to their high fiber and protein content, have a high ability to bind water and form a viscous gel with the potential to improve the technological properties of gluten-free doughs [40,41]. The addition of flaxseed had the least effect on the crumb color, but the presence of the seeds themselves in the crumb was the most pronounced for these samples. Only in the case of the highest concentration of the addition of 23% flaxseed was the sample characterized by a statistically significantly lower pleasantness of appearance. The addition of apple fiber significantly colored the mixture a red–brown color, giving the crumb the appearance of whole wheat graham bread. From a sensory point of view, the group of samples with the addition of apple fiber achieved a statistically significantly higher pleasantness of appearance, the highest of all monotype fiber additions. The crumb in the images showed a more pronounced gloss and, in the case of higher concentrations, also a significant porosity. We can observe an intense yellow–brown color in samples with insect powder. The yellowish color of the samples after the addition of insects was also described in the study by González et al. [34]. A different shade of darker color was observed in the compound samples, which is influenced by the addition of several different types of fiber. The control sample was the most visually similar to the flaxseed samples, specifically the F_9 sample. On the contrary, it appeared to be the most different from samples with insect powder and compound fiber.

3.6. Selected Nutritional Characteristics

3.6.1. Content of Polyphenols and Antioxidant Capacity

The total polyphenol content and antioxidant capacity determined by the ABTS and DPPH methods are shown in Tables 8 and 9. Current trends in the production of gluten-free foods speak of increasing their antioxidant capacity and enriching them with bioactive ingredients. Gluten-free cereals, minor cereals, and pseudocereals are widely used. Rice is often used in gluten-free products, especially in its white form, which is not so prominent. Colored rice varieties, however, show a higher antioxidant capacity than traditional white rice [42]. Corn grains also show similar characteristics [43]. Buckwheat flour, another component used, shows a high antioxidant capacity and a high content of polyphenols as well [44]. Flours from these three crops were used for the production of experimental bread samples and further enriched with selected types of fiber. The control bread sample alone contained 20.06 ± 0.06 mg GAE per 100 g, which is higher than the control gluten-free bread (rice) in the study by Alvarez-Jubete et al. [45] with a value of 8.8 ± 1.0 GAE per 100 g. The highest content of polyphenols was achieved by bread samples with the addition of insect powder (see Table 8). The control sample, as well as samples of F_13, CH_5, P_9, P_2, P_5, and CH_9, differed statistically significantly ($p < 0.05$) from all samples, while sample CH_9 had the lowest concentration of polyphenols. Samples with the addition of psyllium (P_9) and chia seeds (CH_9) showed the lowest antioxidant capacity values. The different values of the antioxidant capacity determined by the ABTS versus DPPH methods, e.g., noticeably in sample A_5, could be caused by the different representation of the crust and the crumb in the sample taken. It seems that the effect of high temperatures during baking and the formation of a dark color in the crust have a positive effect on the total content of polyphenols. This is probably due to their release during the baking process of bread-making. Positive changes in antioxidant capacity as a result of baking have also been reported [46]. A study by Abdel-Aal and Rabalski [47] describes a similar effect. The differences could, therefore, be due to, for example, the collection of a smaller amount of crumb compared to the crust, or vice versa.

Table 8. Polyphenol content in gluten-free bread samples.

Sample	TPC (mg GAE/100 g)
I_9	22.29 ± 2.36 [a]
I_13	21.11 ± 0.31 [ab]
I_5	21.06 ± 0.06 [ab]
I_2	20.64 ± 0.16 [abc]
C_4	20.53 ± 0.09 [abcd]
C_1	20.52 ± 0.01 [abcd]
A_2	20.74 ± 0.74 [abcd]
A_9	20.45 ± 0.10 [abcde]
C_2	20.46 ± 0.20 [abcdef]
F_23	20.33 ± 0.06 [abcdefg]
CH_13	20.31 ± 0.19 [abcdefgh]
C_3	20.31 ± 0.16 [abcdefgh]
CH_2	20.48 ± 0.69 [abcdefghi]
F_9	20.21 ± 0.09 [abcdefghi]
A_7	20.20 ± 0.05 [bcdefghi]
P_7	21.08 ± 2.35 [cdefghi]
A_5	20.09 ± 0.11 [cdefghi]
F_17	20.03 ± 0.14 [defghi]
Ctrl	20.06 ± 0.06 [efghi]
F_13	20.01 ± 0.17 [efghi]
CH_5	19.97 ± 0.06 [efghi]
P_9	19.69 ± 0.62 [fghi]
P_2	19.94 ± 0.08 [ghi]
P_5	19.81 ± 0.09 [hi]
CH_9	19.72 ± 0.02 [i]

Mean ± standard deviation (SD). Different index letters indicate statistically significant differences ($p < 0.05$).

Table 9. Antioxidant capacity of gluten-free bread samples.

Sample	ABTS (%)	Sample	DPPH (%)
A_9	20.39 ± 1.63	I_2	20.92 ± 0.51
A_2	19.32 ± 1.57	I_5	20.37 ± 2.33
A_5	18.20 ± 3.40	I_13	19.50 ± 1.90
I_13	18.10 ± 3.63	I_9	17.39 ± 2.17
I_9	17.57 ± 2.68	C_4	15.80 ± 0.95
C_2	17.50 ± 3.97	C_1	15.69 ± 0.72
I_2	17.44 ± 4.92	F_9	15.57 ± 1.13
I_5	17.08 ± 3.81	A_9	15.17 ± 2.39
C_1	15.81 ± 1.86	CH_2	14.56 ± 1.37
C_4	15.53 ± 2.24	F_13	14.54 ± 2.21
C_3	14.47 ± 3.54	F_23	14.51 ± 1.28
F_23	14.39 ± 0.97	C_2	14.37 ± 1.27
F_9	13.98 ± 0.97	CH_13	14.27 ± 0.87

Table 9. *Cont.*

Sample	ABTS (%)	Sample	DPPH (%)
A_7	13.92 ± 1.94	C_3	13.33 ± 1.84
F_13	13.81 ± 3.10	F_17	13.30 ± 0.40
F_17	12.93 ± 1.36	Ctrl	13.29 ± 0.80
CH_2	11.01 ± 2.25	P_2	12.80 ± 1.23
CH_13	10.55 ± 1.29	CH_5	11.86 ± 2.52
P_2	10.29 ± 0.34	P_5	11.16 ± 1.55
Ctrl	10.10 ± 1.59	P_7	9.32 ± 1.90
P_7	10.04 ± 2.54	A_2	9.03 ± 4.06
CH_5	9.49 ± 2.45	CH_9	9.01 ± 0.95
P_5	9.45 ± 1.59	P_9	8.06 ± 2.37
CH_9	8.25 ± 1.65	A_7	6.04 ± 4.19
P_9	7.90 ± 2.26	A_5	5.23 ± 4.04

Mean ± standard deviation (SD). Different colors show groups statistically different from each other at α = 0.05.

3.6.2. Glycemic Index

Based on the results of the sensory evaluation of the overall pleasantness descriptor, a total of five best-rated samples were selected, namely A_5, C_3, C_1, F_9, and P_2, which were subsequently analyzed for glucose content in order to express their GI. The results (see Table 10) show that the addition of different types of fiber does not lead to a significant change in GI values.

Table 10. Predicted glycemic index of selected samples of gluten-free bread.

Sample	GI (%)
A_5	40.61
C_3	40.40
C_1	40.49
F_9	40.72
P_2	40.66
Ctrl	40.69

Buckwheat, representing one of the main components for the production of gluten-free bread samples, is significantly involved in reducing the GI, mainly due to the fact that it contains a large number of resistant starches [48]. In addition to the amount of total and resistant starches, the content of amylopectin and amylose also plays an important role [49]. As argued by Kurek et al. [50], the addition of flaxseed and apple fiber can also significantly reduce GI. It is possible that buckwheat flour, which was one of the basic ingredients for the production of all experimental bread samples, reduced the availability of starches so much that the predicted GI was also significantly reduced due to this, and the proportions of added fiber we chose did not further lead to its further significant reduction. Kurek et al. [50] found low GI values of gluten-free bread samples containing flaxseed and apple fiber similar to our samples, namely GI values < 55, which are characteristic of low glycemic index foods [49]. Common white wheat bread reaches glycemic index values of 75, and specialty grain bread values are significantly lower, namely 53 [51].

4. Conclusions

Our results showed that both the type and percentage of fiber have a statistically significant effect on the sensory quality and consumer acceptance of gluten-free bread samples. In terms of sensory quality, experimental gluten-free bread samples with added fiber scored average to positive. The only exceptions were two samples containing mealworm powder and a sample with 9% psyllium content, which were evaluated negatively. In the case of the addition of a lower proportion of insect powder in combination with vegetable fiber, the risk of the presence of a noticeable specific smell and taste and, therefore, rejection by the consumer is significantly lower. The samples containing the fiber mixture without insect powder and the sample containing 9% flaxseed performed best in the overall evaluation. However, the use of a combination of different types of plant fibers simultaneously with the incorporation of insect powder in a low concentration appears to be advantageous both from the point of view of sensory acceptability and also from the point of view of the potential for increasing the polyphenol content and antioxidant capacity.

This study provides comprehensive information regarding the specific effect of individual types of fiber on organoleptic properties and selected nutritionally significant characteristics of gluten-free bread samples. The addition of an appropriate amount of fiber has an indisputably positive effect on the sensory quality and nutritional value of gluten-free bread samples and is, therefore, a beneficial option for both the consumer and the manufacturer. Given the current situation on the market for gluten-free products, it is desirable to reformulate recipes so that they achieve both higher nutritional and sensory quality.

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