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Development of functional extruded crispbreads enriched with wild plant concentrates

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ABSTRACT

In the context of growing interest in functional nutrition, enriching food products with naturally active compounds is an important task. This research aimed to develop extruded cereal crackers enriched with concentrates from wild plants in Kazakhstan, such as hawthorn (*Crataegus laevigata*), chokeberry, rosehip, and sea buckthorn. The concentrates were obtained by ultrasound-assisted extraction and concentrated using vacuum evaporation. Wheat and buckwheat crispbreads were prepared by high-temperature extrusion. Afterwards, a plant-based syrup containing the concentrates was applied. This was a controlled experimental study. Samples were prepared using a fixed recipe and analyzed for bioflavonoid content and quality indicators. The bioactive composition was evaluated in the accredited laboratory of Nutritest LLP according to the methodology P 4.1.1672-2003. The highest content of rutin (157.8 ± 7.9 mg/100 g) was observed in hawthorn samples, dihydroquercetin (181.93 ± 9.10 mg/100 g) in chokeberry samples, and chlorogenic acid (42.75 ± 2.14 mg/100 g) in sea buckthorn samples. All samples met physicochemical standards (moisture, acidity, ash content). The study confirmed the high antioxidant activity and potential of wild plants for the development of functional foods. These products have attractive sensory characteristics and are promising for use in preventive nutrition.

Keywords: bioflavonoids, concentrates, crispbreads, ultrasound, wildplants

INTRODUCTION

In response to the growing global demand for functional foods and the promotion of healthy lifestyles, the development of novel food products enriched with biologically active compounds (BACs) has become increasingly relevant in the food industry **[1]**. These compounds, including flavonoids, polyphenols, and organic acids, are recognized for their antioxidant, anti-inflammatory, and immunomodulatory properties, contributing to the prevention of chronic non-communicable diseases **[2]**.

Several strategic initiatives reinforce this trend. Among them are the Strategy for the Development of the Food and Processing Industry of the Republic of Kazakhstan until 2030, which prioritizes innovation and health-focused product development **[3]**; the national project Healthy Nation for 2021–2025, aiming to improve public health through dietary approaches **[4]**; and international programs such as the United Nations Sustainable Development Goals, particularly Goal 3, which advocates for access to safe and nutritious food for all **[5]**.

The creation of functional food products based on regionally sourced raw materials and local wild plants (such as *Crataegus laevigata, Hippophae rhamnoides, Aronia melanocarpa,* and *Rosa canina*) aligns with these policy directions. It also supports sustainable agro-industrial development through resource efficiency and biodiversity conservation **[6]**.

Modern processing methods, including ultrasound-assisted extraction and vacuum evaporation, facilitate the recovery of thermolabile BACs while preserving their bioactivity **[7]** and **[8]**. At the same time, extrusion technology enhances the functional and structural qualities of cereal-based matrices, enabling the integration of bioactive concentrates into stable food formats **[9]**.

Although prior studies have demonstrated the benefits of these technologies individually—such as improving antioxidant stability in berry-enriched cookies **[6]**, optimizing polyphenol recovery via ultrasound **[10]**, or utilizing green evaporation systems for plant concentrates **[11]** — their combined influence on the nutritional, physicochemical, and sensory characteristics of ready-to-eat functional products has not been extensively explored **[12]**. Additionally, earlier research has validated the use of experimental-statistical modelling in optimising multistage food processes, including extrusion and drying **[13]**, the valorisation of biomass from agro-industrial byproducts **[14]**, and the stabilisation of polyphenol-enriched food matrices **[15]**.

Recent studies have further confirmed that integrated mild extraction and post-processing techniques (e.g., glazing and spray-coating) allow for enhanced retention of antioxidants and flavonoids in cereal matrices during high-temperature operations **[16]**. Investigations into polyphenol preservation under infrared and convective drying have demonstrated that short-duration thermal exposure, especially when combined with protective carriers, can significantly reduce degradation. Enhanced retention of phenolic content and antioxidant activity was observed in red dragon fruit peel subjected to far-infrared drying compared to conventional methods **[17]**. Moreover, textural and nutritional optimization in cereal-based crisps using fruit concentrates has proven effective for increasing consumer acceptability and health benefits **[18]**.

The practical relevance of using wild plant concentrates and functional glazing is also supported by advances in food engineering, which involve applying film-forming coatings to extruded snacks **[19]**, as well as by studies investigating regional plant species rich in dihydroquercetin and rutin. Furthermore, the nutraceutical significance of rutin has been comprehensively substantiated in recent research, highlighting its antioxidant and protective effects **[20]**. Accordingly, the present study proposes a unified processing strategy that combines ultrasound-assisted extraction, vacuum evaporation, and post-extrusion glazing. This approach aims to preserve and stabilize targeted bioactive compounds—such as rutin, dihydroquercetin, and chlorogenic acid—within wheat- and buckwheat-based crispbreads. The research supports the valorization of underutilized plant resources in Kazakhstan, enhances the nutritional and preventive value of extruded products, and contributes to innovation in the functional food sector.

Scientific Hypothesis

The type of wild plant concentrate (hawthorn, chokeberry, rosehip, sea buckthorn) and the cereal base (wheat or buckwheat) significantly affect the retention of bioflavonoids (e.g., rutin, dihydroquercetin, chlorogenic acid) and antioxidant activity in cereal crispbreads enriched by post-extrusion glazing. We hypothesize that the combination of ultrasound-assisted extraction and vacuum evaporation ensures statistically significant preservation of thermolabile bioactive compounds when applied to extruded matrices. This hypothesis was tested using multifactor statistical analysis.

Objectives

Primary objectives: To develop functional cereal crispbreads enriched with concentrates of wild plants of Kazakhstan (hawthorn, chokeberry, rosehip, and sea buckthorn) using ultrasound-assisted extraction and vacuum evaporation and to evaluate the content and stability of bioflavonoids in the final product.

Secondary objectives:

- To compare the retention of bioflavonoids (rutin, dihydroquercetin, chlorogenic acid) depending on the type of plant concentrate and cereal base (wheat vs. buckwheat).
- To determine the antioxidant activity of the enriched crispbreads.
- To assess the physicochemical properties of the products (moisture, acidity, ash content).
- To evaluate the sensory attributes and acceptability of the developed crispbreads.

MATERIAL AND METHODS

Samples

Samples description: The study used cereal crispbreads based on wheat or buckwheat flour. Functional enrichment was achieved by applying plant concentrates derived from hawthorn (*Crataegus laevigata*), sea buckthorn (*Hippophae rhamnoides*), chokeberry (*Aronia melanocarpa*), and rosehip (*Rosa canina*). A total of 20 samples were prepared using a central composite design that combined different base flours, types of plant extracts, and application methods (glazing or spraying).

Samples collection: Plant raw materials (hawthorn, sea buckthorn, chokeberry, rosehip) were harvested at full ripeness during the growing season in Kazakhstan. The samples were dried at temperatures not exceeding 45 °C, packed in airtight containers, and subsequently stored at 10 °C until further processing.

Samples preparation: Ground plant materials were subjected to ultrasound-assisted extraction (40 kHz, 35 °C, 30 min) using demineralized water as the solvent. The resulting extracts were concentrated by vacuum evaporation at 45–50 °C until a solids content of 40–42% was reached. Concentrates were stored at 4 ± 1 °C until further use. Cereal mixtures (wheat or buckwheat) were moistened to 17–18% and extruded at 250–290 °C to form crispbreads. After cooling, the surface of the crispbreads was coated with plant concentrates by glazing or spraying.

Number of samples analyzed: A total of 20 samples were prepared according to a central composite design (CCD), combining two cereal bases, four types of plant extracts, and two application methods. Each formulation was analyzed in triplicate (n = 3), yielding 60 analytical measurements for each target parameter.

Chemicals

All reference standards for rutin, dihydroquercetin, quercetin, catechin, gallic acid, chlorogenic acid, and anthraquinone derivatives were purchased from Sigma-Aldrich (Germany) and were of analytical grade quality. Demineralized water was used as the extraction solvent. No agar media were used in this study.

Animals, Plants and Biological Materials

Plant species used for concentrate production included *Crataegus laevigata* (hawthorn), *Hippophae rhamnoides* (sea buckthorn), *Aronia melanocarpa* (chokeberry), and *Rosa canina* (rosehip). All plants were harvested in the Almaty region of Kazakhstan.

No animals or microorganisms were used in this study.

Instruments

Ultrasound extractor (40 kHz, ULTRASONIC CLEANER VWR USC-TH, VWR International), vacuum evaporator (IKA RV 10, IKA-Werke GmbH & Co. KG, Germany), laboratory extruder (model Brabender E20), HPLC system (Shimadzu LC-20, Shimadzu, Japan) equipped with a ZORBAX Eclipse Plus C18 column (4.6 × 150 mm, 5 µm; Agilent Technologies, USA).

Laboratory Methods

Quantitative determination of bioactive compounds (rutin, dihydroquercetin, quercetin, catechin, gallic acid, chlorogenic acid, anthraquinone derivatives) was performed using a standard method based on R 4.1.1672-2003, Chapter 3, validated and conducted in accordance with ISO/IEC 17025:2019 requirements in an accredited laboratory (Accreditation Certificate No. KZ.T.02.E.1158). Measurements were performed in triplicate (n = 3) using HPLC with UV detection at 254–365 nm. Calibration was done using analytical-grade Sigma-Aldrich standards. Results were expressed in mg/100 g of dry matter.

Sensory evaluation was conducted with 15 trained panelists (aged 22–45 years, both male and female) under standardized conditions, employing a 5-point scale to assess taste, aroma, texture, appearance, and overall impression. Each sample was coded and served in randomized order. Taste neutralization (water and plain crackers) was used between samples.

Description of the Experiment

Study flow: The experimental study was conducted in four distinct phases:

Phase 1 — Preparation of Plant Extracts.

Wild plants (*Crataegus laevigata, Hippophae rhamnoides, Aronia melanocarpa,* and *Rosa canina*) were harvested at full ripeness, dried at temperatures below 45 °C, ground into powder, and stored at 10 °C in sealed containers.

Phase 2 — Extraction and Concentration.

Ultrasound-assisted extraction was performed using demineralized water at 40 kHz and 35 °C for 30 minutes. The resulting extracts were vacuum-evaporated at 45–50 °C until reaching a solids content of 40–42%. Final extracts were stored at 4 ± 1 °C.

Phase 3 — Crispbread Preparation and Coating.

Wheat and buckwheat grain mixtures were hydrated to 17–18% moisture and extruded at 250–290 °C. After cooling, the extruded crispbreads were coated with the plant extracts using two techniques: (1) glazing at 70-80 °C and (2) spraying at room temperature.

Phase 4 — Experimental Design and Evaluation.

A Central Composite Design (CCD) was applied to evaluate the effects of three independent variables:

Var1 = Cereal base (1 – wheat, 2 – buckwheat),

Var2 = Plant extract type (1 – hawthorn, 2 – sea buckthorn, 3 – chokeberry, 4 – rosehip),

Var3 = Application method (1 – glazing, 2 – spraying).

A total of 20 experimental runs were performed, including center points. All samples were analyzed for: bioflavonoid content (by HPLC), physicochemical properties (moisture, titratable acidity, ash), antioxidant activity (by DPPH assay), and sensory evaluation (using a 5-point hedonic scale). Statistical analyses were

conducted using Statistica 12.0 (StatSoft Inc., USA) and Microsoft Excel 2016. The data were first tested for normality using the Shapiro–Wilk test. Analysis of variance (one-way and multifactor ANOVA) was applied to determine the significance of experimental factors. Differences between group means were further examined using Tukey's HSD post hoc test. Correlation analysis was performed to evaluate relationships between bioactive compounds and sensory scores. No protocol deviations were recorded during the study.

Quality Assurance

Number of repeated analyses: All analytical measurements, including HPLC quantification of bioactive compounds, physicochemical parameters, and antioxidant activity, were performed in triplicate (n = 3) for each sample formulation to ensure repeatability and accuracy of results.

Number of experiment replication: Each experimental condition defined by the central composite design (CCD) was replicated once as an independent experimental run, resulting in a total of 20 experimental trials. Analytical measurements for each trial were conducted in triplicate (n = 3) to ensure statistical reliability.

Reference materials: Analytical standards of bioactive compounds, including rutin, dihydroquercetin, quercetin, catechin, gallic acid, and chlorogenic acid, were obtained from Sigma-Aldrich (Germany) and used as reference materials for calibration of the HPLC system. These standards were of certified purity and served to validate the accuracy and linearity of the chromatographic method. No secondary reference materials or commercial test kits were used.

Calibration: The HPLC system (Agilent 1260 Infinity) was calibrated using external standard calibration curves prepared from certified reference standards of bioactive compounds (Sigma-Aldrich). Calibration was performed at five concentration levels for each compound (rutin, dihydroquercetin, quercetin, catechin, gallic acid, and chlorogenic acid) within the expected range in the samples. Linearity was confirmed with correlation coefficients (R^2) above 0.998 for all analytes. Calibration was repeated every 10 sample runs to ensure accuracy and instrument stability.

Laboratory accreditation: The experiments were conducted in two accredited laboratories: the Food Safety Laboratory at Almaty Technological University and the accredited research laboratory of «Nutritest» LLP, both certified to the international standard ISO/IEC 17025:2019.

Data Access

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to institutional data protection policies and confidentiality agreements related to unpublished results and proprietary formulation details.

Statistical Analysis

All statistical analyses were performed using Statistica 12.0 (StatSoft Inc., USA) and Microsoft Excel 2016 (Microsoft Corporation, USA).

A central composite design (CCD) was used to structure the experiment and model the influence of three independent variables: cereal base type, extract type, and method of concentrate application.

Data were analyzed using one-way ANOVA to compare the effects of extract types and application methods on bioflavonoid content and antioxidant activity. Tukey's HSD post hoc test was applied to determine significant differences between treatment groups ($p < 0.05$).

To explore multidimensional patterns, principal component analysis (PCA) was performed. Additionally, response surface modeling and desirability function analysis were employed to identify the optimal combinations of factors. All outcomes were measured in triplicate (n = 3), and the degrees of freedom were appropriately adjusted based on replicate structure. No covariates were included in the model.

RESULTS AND DISCUSSION

1. Development of functional crispbread technology

The levels of phenolic compounds and the intensity of antioxidant activity in plant raw materials are strongly influenced by genotype, environmental conditions, and ripeness stage. Koczka et al. **[21]** showed that the antioxidant potential of rosehips significantly depends on species differences, while Mármol et al. **[22]** highlighted therapeutic applications of different Rosa species linked to their phytochemical profiles.

The extraction method also plays a decisive role. Hao et al. **[23]** demonstrated that ultrasonic-assisted extraction increases the recovery and antioxidant capacity of flavonoids. Vinatoru **[24]** emphasized guidelines for reproducibility and reliability in ultrasonic extraction. In our study, ultrasound yielded extracts of higher purity compared to conventional methods.

Thermal extrusion was used for producing crispbread. Sharma et al. **[25]** confirmed that extrusion reduces the antioxidant activity of barley due to thermal stress. Zahari et al. **[26]** demonstrated that the extrusion of plant-based protein systems significantly affects product structure, while Koppel et al. **[27]** highlighted the importance of extrusion conditions for texture and flavor in food matrices. Gu et al. **[28]** described extrusion as

a versatile food processing method, and Grasso **[29]** stressed the potential of using by-products in extruded snacks. Gondek et al**. [30]** further proposed valorization of crispbread waste for new snack formulations.

The effect of extrusion parameters has been widely studied. Paesani et al. **[31]** reported that extruding corn flour modifies the characteristics of gluten-free biscuits. Thiranusornkij et al. **[32]** observed changes in the functionality of rice flour when used in bread formulations. Han et al. **[33]** compared cereals and found substantial differences in antioxidant retention. Hossain and Jayadeep **[34]** demonstrated that extrusion alters the bioaccessibility of fat-soluble nutraceuticals. Pichmony and Ganjyal **[35]** provided a theoretical framework describing moisture, feed rate, and die geometry as critical input parameters.

Our results showed a reduction in thermolabile compounds (rutin, catechin, dihydroquercetin) when plant extracts were incorporated before extrusion. Similar findings were reported by Mangoale and Afolayan **[36]**, who observed stronger thermal degradation of wild plant polyphenols, and by ElGamal et al. **[37]**, who demonstrated loss of bioactivity during drying of horticultural products.

The biological effects of phenolics are closely linked to their bioavailability. Niedzwiecki et al. **[38]** highlighted anticancer effects of polyphenols, while Bié et al. **[39]** discussed their interactions with gut microbiota. Mironeasa et al. **[40]** reviewed the effects of extrusion on antioxidant compounds from vegetable sources. Di Lorenzo et al. **[41]** described the role of bioavailability in human health, and Rivero Meza et al. **[42]** demonstrated that extruding pigmented rice cereals alters their sensory and nutritional properties. Kratchanova et al. **[43]** compared extraction systems and demonstrated differences in the recovery of antioxidants.

The combined use of ultrasound-assisted extraction and vacuum evaporation in our study allowed retention of high flavonoid concentrations and improved antioxidant potential. This finding is consistent with that of Hao et al. **[23]**, who optimized ultrasonic extraction for the preservation of flavonoids.

Comparisons between wild and cultivated plants indicate that wild species are more susceptible to degradation. Mangoale and Afolayan **[36]** confirmed this trend in Alepidea species. Berga et al. **[44]** and Billowria et al. **[45]** emphasized that the stability of flavonoids strongly depends on formulation strategies and physicochemical properties.

Finally, extrusion itself generally reduces total phenolics compared with raw material. Mironeasa et al. **[40]** and Sharma et al. **[25]** confirmed this reduction, highlighting the need for protective application methods. In our study, post-extrusion glazing proved more effective than pre-extrusion addition. Xiao **[19]** described similar film-forming coatings that preserve sensitive compounds during processing.The experimental study included eight samples of extruded crispbreads, based on wheat and buckwheat, enriched with concentrates from hawthorn, sea buckthorn, rosehip, and chokeberry. The concentrates were applied in syrup form to pre-formed and cooled crispbreads. Glazing was conducted at 70-80 °C with standardized dosing of the functional ingredient (5.0 ± 0.2 g per 100 g of product).

According to technological assessments, glazing syrup temperatures ≤70-80 °C did not result in product deformation or loss of the characteristic crisp texture when applied to cooled extrudates. Short-term thermal exposure at this stage did not compromise the volumetric stability of the final product **[6]**.

The content of bioactive compounds in the plant extracts used for glazing varied significantly depending on the plant species (Table 1).

Table 1 Content of bioactive compounds in fruit concentrates of wild plants, mg/100 g.

Indicator	**Hawthorn, Mean ± SD**	**Sea buckthorn, Mean ± SD**	**Kainazar rosehip, Mean ± SD**	**Chokeberry, Mean ± SD**
Rutin	150.3 ± 7.52	103.9 ± 5.20	74.2 ± 7.42	152.2 ± 7.61
Quercetin	44.6 ± 2.23	36.5 ± 1.83	25.8 ± 2.58	43.8 ± 4.38
Catechin	6.04 ± 0.30	1.91 ± 0.10	18.4 ± 0.92	17.21 ± 0.86
Gallic acid	2.64 ± 0.13	3.53 ± 0.18	13.03 ± 0.65	12.25 ± 0.61
Hesperidin	ND	ND	0.471 ± 0.047	0.248 ± 0.025
Dihydroquercetin (Taxifolin)	27.89 ± 1.39	48.64 ± 2.43	147.47 ± 7.37	181.93 ± 9.10
Chlorogenic acid	44.69 ± 2.23	8.55 ± 0.43	ND	1.48 ± 0.07
Tannins (calculated as tannic acid)	162.5 ± 8.13	85.3 ± 4.26	ND	ND
Anthraquinone derivatives	1.48 ± 0.15	2.43 ± 0.24	2.10 ± 0.21	1.64 ± 0.16
Hydroquinone (Arbutin)	ND	1.69 ± 0.08	ND	0.20 ± 0.01

Note: ND - not detected

The highest rutin concentration was found in chokeberry (152.2 ± 7.61 mg/100 g) and hawthorn (150.3 ± 7.52 mg/100 g), indicating their pronounced vascular-protective potential. Rosehip concentrate exhibited elevated levels of dihydroquercetin (147.47 ± 7.37 mg/100 g), while chokeberry again showed the highest value among all samples (181.93 ± 9.1 mg/100 g), supporting its potential for developing antioxidant-enriched functional food products.

The highest rutin content was observed in chokeberry (152.2 ± 7.61 mg/100 g) and hawthorn (150.3 ± 7.52 mg/100 g), confirming their vascular-protective potential. Rosehip concentrate demonstrated high levels of dihydroquercetin (147.47 ± 7.37 mg/100 g). In contrast, chokeberry showed the highest level in the sample set (181.93 ± 9.1 mg/100 g), highlighting its potential for use in antioxidant-enriched functional food products.

To assess the influence of the type of grain base and the type of plant concentrate on the rutin content in crispbreads, an analysis of variance followed by multiple comparisons using the Tukey method (Tukey HSD, α = 0.05) was performed. The study encompassed eight experimental samples, which differed in both the type of cereal used (wheat or buckwheat) and the type of wild plant concentrate added (hawthorn, sea buckthorn, rosehip, or chokeberry). The results are presented in Table 2.

Table 2 Results of multiple comparisons (Tukey HSD) for rutin content in crispbread samples with various cereal bases and wild plant concentrates.

Cel l No.	Var 2	Var 3	{1} 160.45	{2} 36.337	{3} 49.093	{4} 22.800	{5} 51.257	{6} 44.163	{7} 77.820	{8} 45.560
1	1	1	–	0.000175*	0.000175*	0.000175*	0.000175*	0.000175*	0.000175*	0.000175*
2	1	2	0.000175*	–	0.197353	0.150575	0.090763	0.718496	0.000176*	0.545995
3	1	3	0.000175*	0.197353	–	0.001052*	0.999733	0.961206	0.000496*	0.993969
4	1	4	0.000175*	0.150575	0.001052*	–	0.000534*	0.006939*	0.000175*	0.003970*
5	2	1	0.000175*	0.090763	0.999733	0.000534*	–	0.801158	0.000960*	0.921166
6	2	2	0.000175*	0.718496	0.961206	0.006939*	0.801158	–	0.000213*	0.999986
7	2	3	0.000175*	0.000176*	0.000496*	0.000175*	0.000960*	0.000213*	–	0.000250*
8	2	4	0.000175*	0.545995	0.993969	0.003970*	0.921166	0.999986	0.000250*	–

Note: Var 1 – rutin content; Var 2 – cereal base (1 = buckwheat, 2 = wheat); Var 3 – type of concentrate (1 = hawthorn, 2 = sea buckthorn, 3 = rosehip, 4 = chokeberry).
*Statistically significant values ($p < 0.05$) are marked with *.

As shown in Table 2, the results of the Tukey HSD test ($p < 0.05$) revealed that the Wheat_hawthorn sample significantly differed from all other experimental groups. The Wheat-sea buckthorn, Wheat-chokeberry, and Wheat-rosehip samples did not differ significantly from one another ($p > 0.05$), but each showed statistically significant differences when compared to Buckwheat-rosehip and Buckwheat-chokeberry samples ($p < 0.01$). These results indicate that both the type of plant concentrate and the cereal base have a significant impact on the rutin content.

Figure 1 presents the predicted value profiles for rutin content (Var1) and overall desirability depending on the cereal base type (Var2) and the type of plant concentrate (Var3). The analysis was performed using a desirability function aimed at maximizing the rutin content of the product.

The highest rutin content (≈77.8 mg/100 g) was observed in the sample with a wheat base (Var2 = 2) and hawthorn concentrate (Var3 = 1). This combination also demonstrated the highest value of the desirability function (Desirability ≈ 0.60), making it optimal for developing a functional product with elevated rutin content.

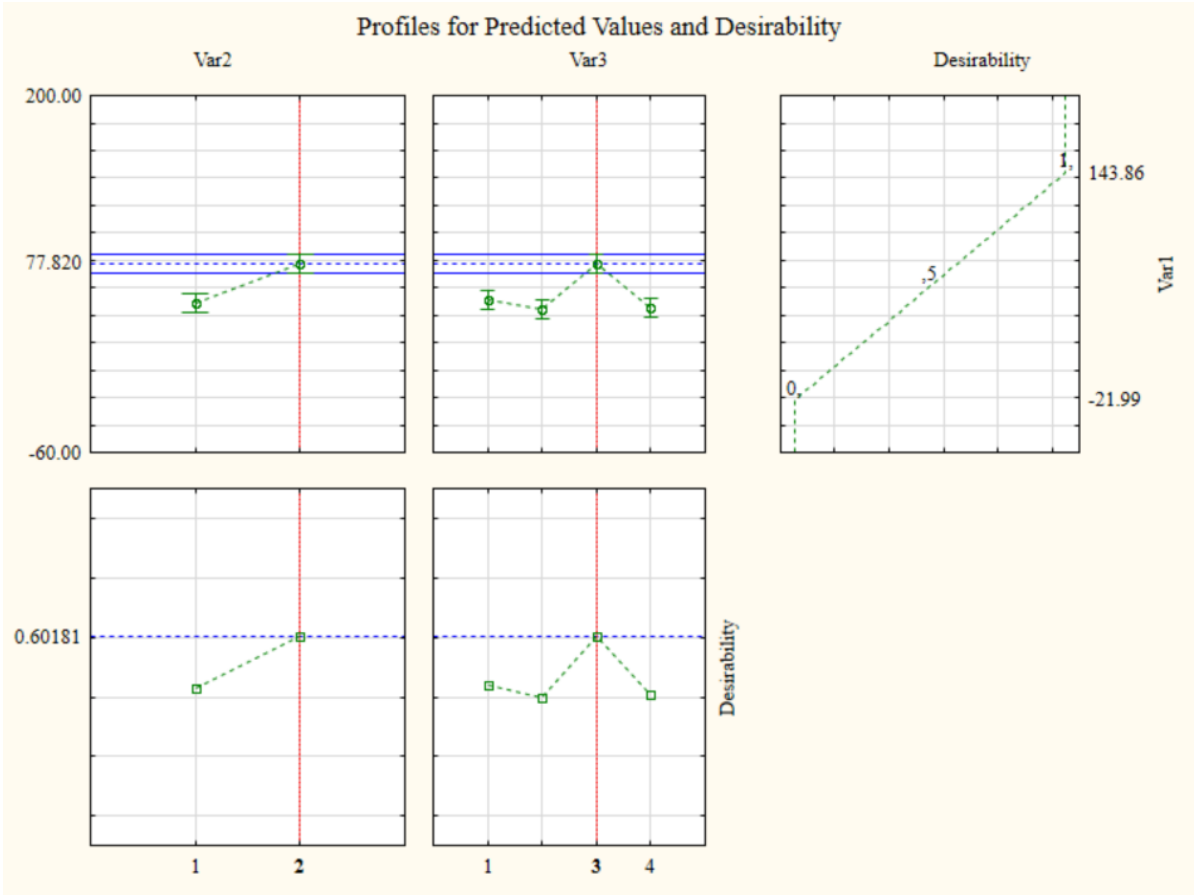

Figure 1 Predicted value and desirability profiles depending on factors Var2 (cereal base) and Var3 (type of plant concentrate.

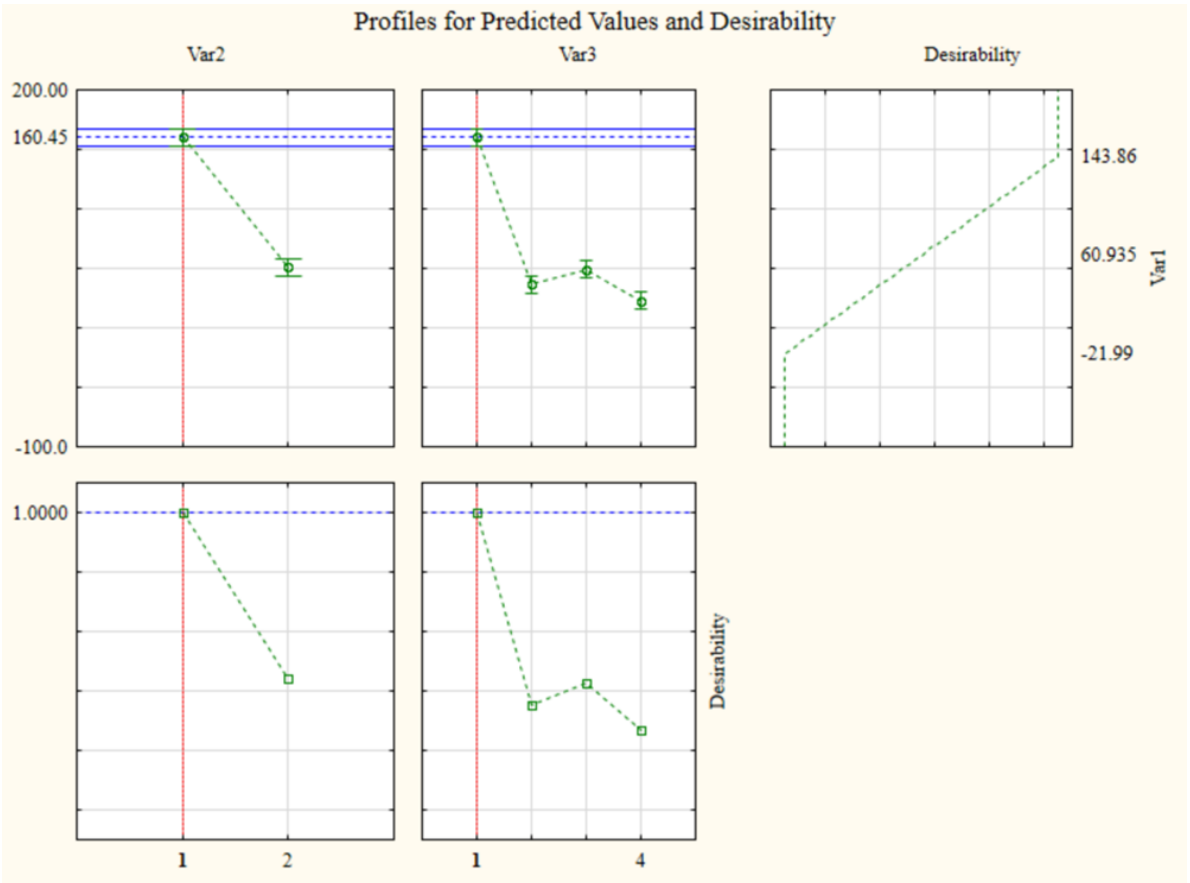

Figure 2 Predicted response and desirability profiles for dihydroquercetin content (Var1) as a function of cereal base (Var2) and plant concentrate type (Var3).

Figure 2 shows the response and desirability profiles for the variable Var1 (dihydroquercetin content) depending on the factors Var2 (type of cereal base) and Var3 (type of plant concentrate). The analysis was performed using the desirability criterion to identify optimal factor combinations.

The highest predicted content of dihydroquercetin (≈160.45 mg/100 g) was recorded in the sample based on buckwheat (Var2 = 1) with hawthorn concentrate (Var3 = 1). This combination resulted in the maximum desirability function value (Desirability = 1.0), indicating its high efficiency for enriching the product with the flavonoid DhQ. This result highlights the synergistic effect of this combination in ensuring the antioxidant activity and vascular-protective properties of the product. Other plant ingredients and the buckwheat-based formulations demonstrated significantly lower levels (Desirability < 0.3), reducing their priority in developing functional crispbreads aimed at vascular and metabolic stabilization.

ANOVA revealed significant differences among the samples the key bioactive compounds ($p < 0.05$). The model determined that the wheat–hawthorn combination is optimal for achieving maximum rutin content despite the higher mean values observed for the buckwheat–hawthorn sample in previous charts.

Following the assessment of rutin content, further analysis focused on dihydroquercetin - a compound with high antioxidant activity and notable thermal stability. Its inclusion in the technology optimization system broadens the functional profile of the product and substantiates its targeted action in cardioprotection and vascular regulation.

2. Evaluation of bioactive compounds (rutin, dihydroquercetin, etc.) in the obtained samples by HPLC. Comparison and justification of crispbread selection through statistical analysis

Table 3 Results of multiple comparisons (Tukey HSD) of dihydroquercetin (DhQ) content depending on the type of cereal base and plant concentrate.

Cel l No.	Var 2	Var 3	{1} 24.400	{2} 0.27333	{3} 0.29500	{4} 0.25700	{5} 0.21300	{6} 0.67333	{7} 0.95867	{8} 0.55600
1	1	1	–	0.000175*	0.000175*	0.000175*	0.000175*	0.000175*	0.000175*	0.000175*
2	1	2	0.000175*	–	1.00000	1.00000	1.00000	0.937429	0.537052	0.990248
3	1	3	0.000175*	1.00000	–	1.00000	0.999997	0.952431	0.573494	0.993899
4	1	4	0.000175*	1.00000	1.00000	–	1.00000	0.924315	0.509945	0.986536
5	2	1	0.000175*	1.00000	0.999997	1.00000	–	0.881119	0.439221	0.971338
6	2	2	0.000175*	0.937429	0.952431	0.924315	0.881119	–	0.989704	0.999966
7	2	3	0.000175*	0.537052	0.573494	0.509945	0.439221	0.989704	–	0.935396
8	2	4	0.000175*	0.990248	0.993899	0.986536	0.971338	0.999966	0.935396	–

Note: Var2 – cereal base (1 = buckwheat, 2 = wheat); Var3 – type of concentrate (1 = hawthorn, 2 = sea buckthorn, 3 = rosehip, 4 = chokeberry).
*Statistically significant values ($p < 0.05$) are marked with *.

The "buckwheat + hawthorn" sample (№1) exhibited statistically significant differences from all other combinations ($p < 0.0002$). All other combinations did not differ significantly from each other ($p > 0.05$), indicating that only hawthorn combined with a buckwheat base significantly increases the dihydroquercetin content. This confirmed the key role of the hawthorn phytocomponent in enriching the product with DhQ and demonstrated a pronounced interaction between the cereal matrix (buckwheat) and the plant extract.

3. Organoleptic evaluation of plant extracts samples: determination of the impact of the application on taste, aroma, texture, and overall perception, followed by statistical analysis of preferences.

Effects of extract type and base on taste evaluation

Figure 3 presents a 3D response surface model of taste scores depending on the variety of cereal base (wheat or buckwheat) and the type of plant concentrate (hawthorn, sea buckthorn, chokeberry, rosehip).

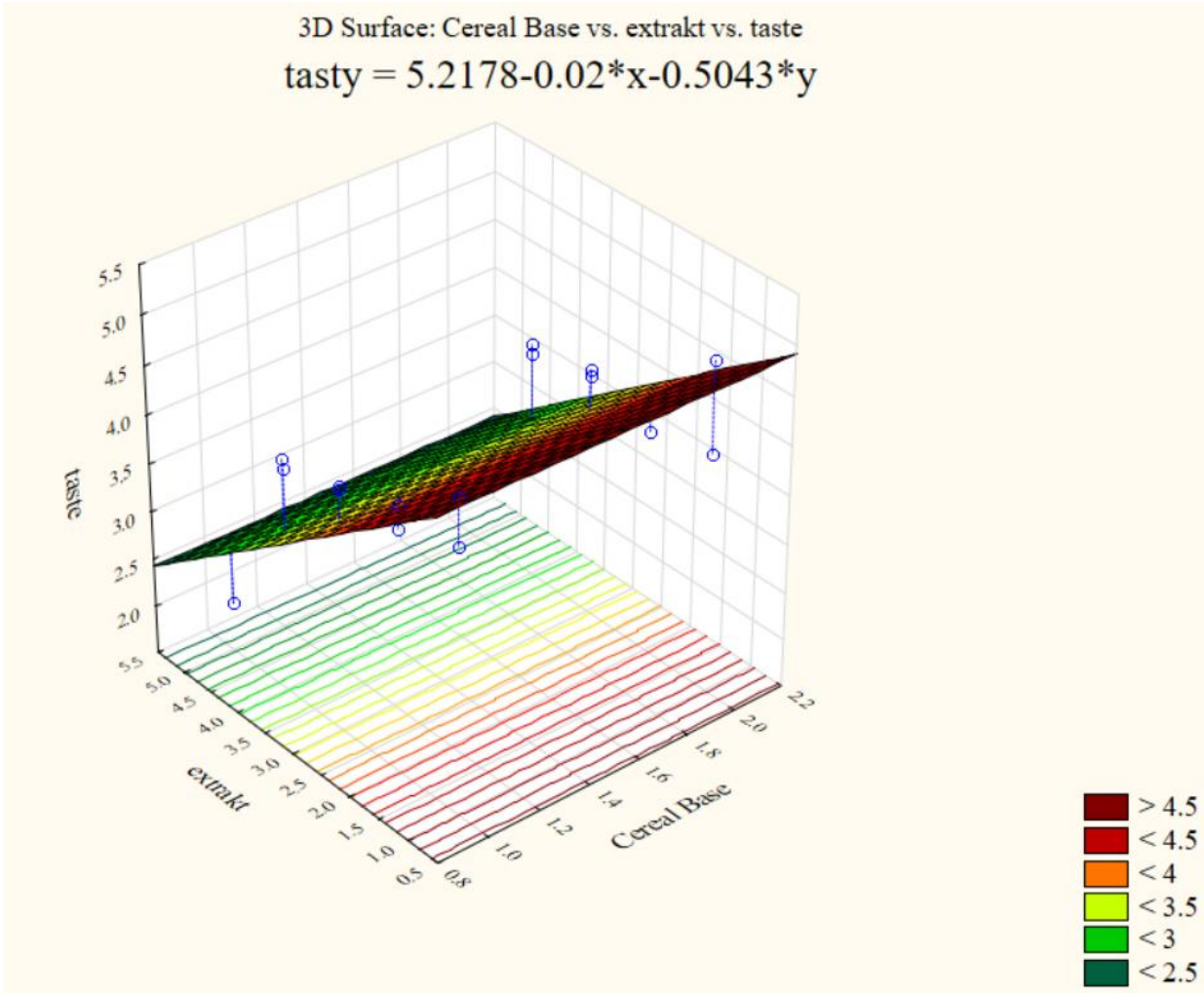

Figure 3 The response surface of the sensory taste evaluation depends on the type of base and plant extract.

As shown in Figure 3, the regression equation (tasty = 5.2178 – 0.02·x – 0.5043·y) indicates that the type of extract had a statistically significant effect on taste perception ($p < 0.05$), while the influence of the cereal base was not significant. An increase in the extract code (from hawthorn to rosehip) was associated with a notable decline in taste scores. This trend is likely due to the more pronounced sour or bitter flavor profiles of certain berries, such as chokeberry and rosehip, as confirmed by the results of the sensory evaluation protocol.

Evaluation of texture characteristics according to extract method of applying

Figure 4 presents the response surface for texture score as a function of the cereal base and extracts method of applying.

The model (texture = 4.706 – 0.17·x – 0.2·y) indicates a decrease in texture scores when using spraying compared to glazing. Glazing forms a denser and more stable coating that prevents deformation and moisture absorption, whereas spraying - especially when involving a liquid phase - leads to partial loss of crispness. The highest texture scores were recorded in samples based on wheat with the glazing method.

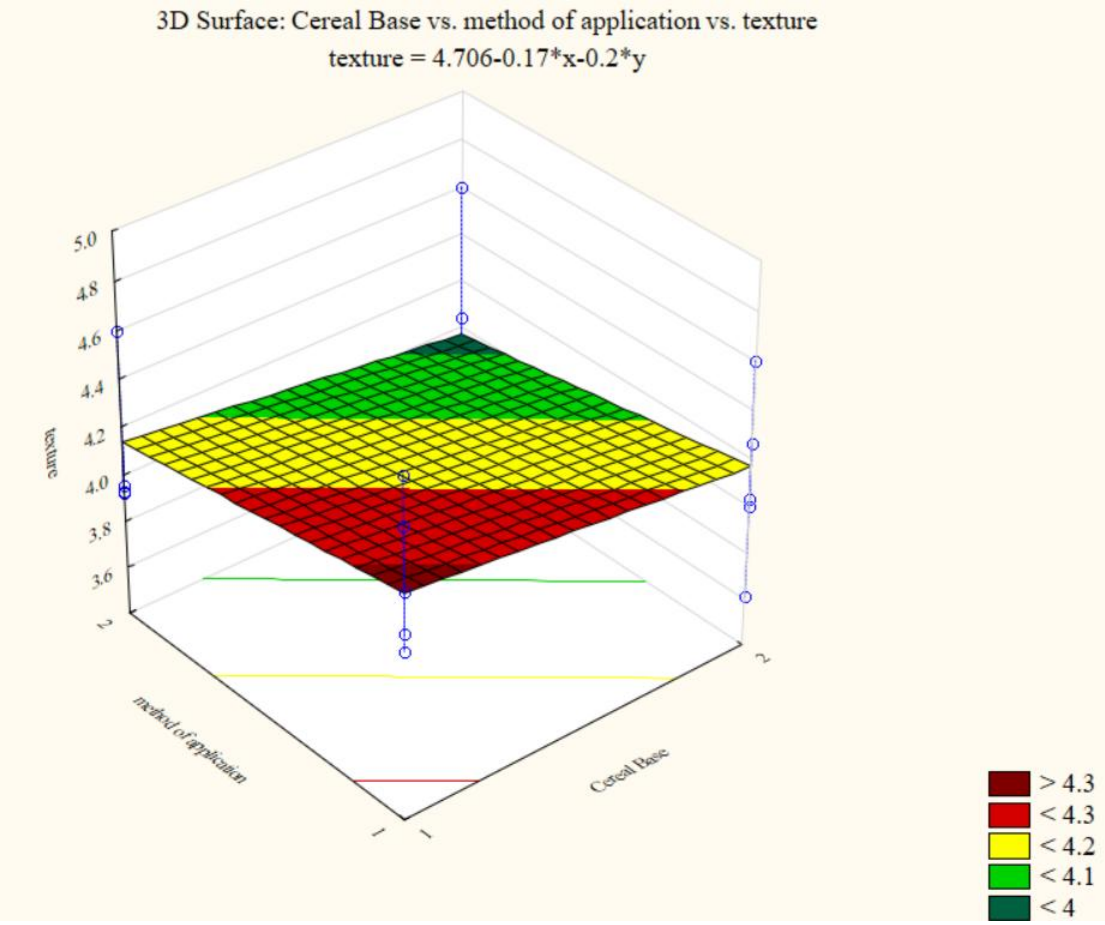

Figure 4 Response surface of texture characteristics according to extract application method and cereal base.

Pareto analysis of factor influence on taste evaluation

The results of the analysis of variance, shown in the Pareto chart (Figure 5), confirm the statistically significant influence of the "extract" factor in both linear and quadratic forms.

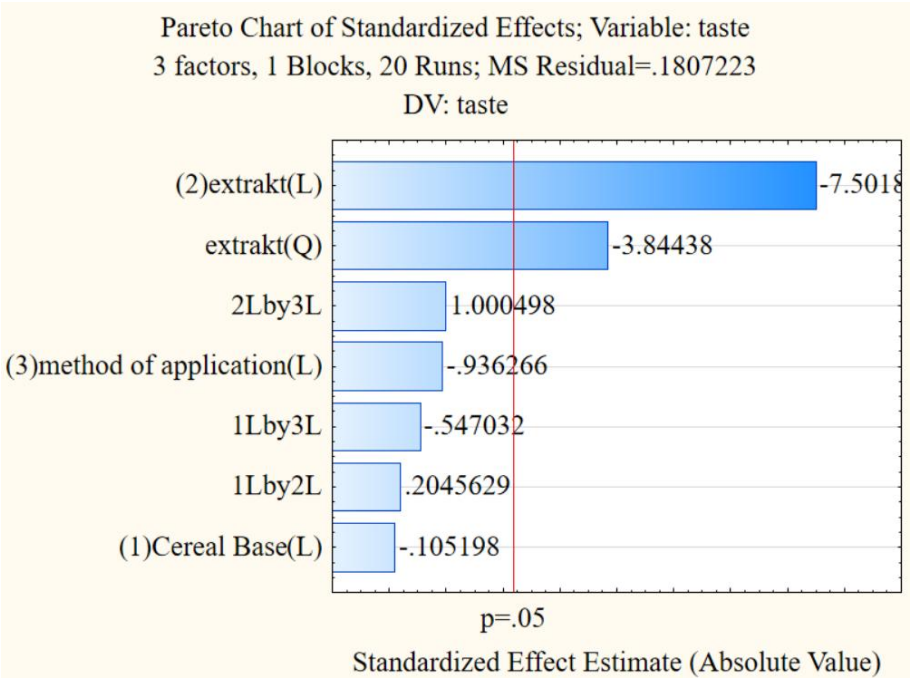

Figure 5 Pareto chart of the influence of technological factors on taste evaluation.

All other factors and interactions (base type, method of applying) did not exceed the critical significance level of p = 0.05, indicating their negligible influence on taste evaluation. Thus, the choice of the extract is a key parameter in shaping the product's flavor profile.

Desirability function and response profiles

Figure 6 presents the response profiles for taste, aroma, texture, appearance, and overall organoleptic evaluation.

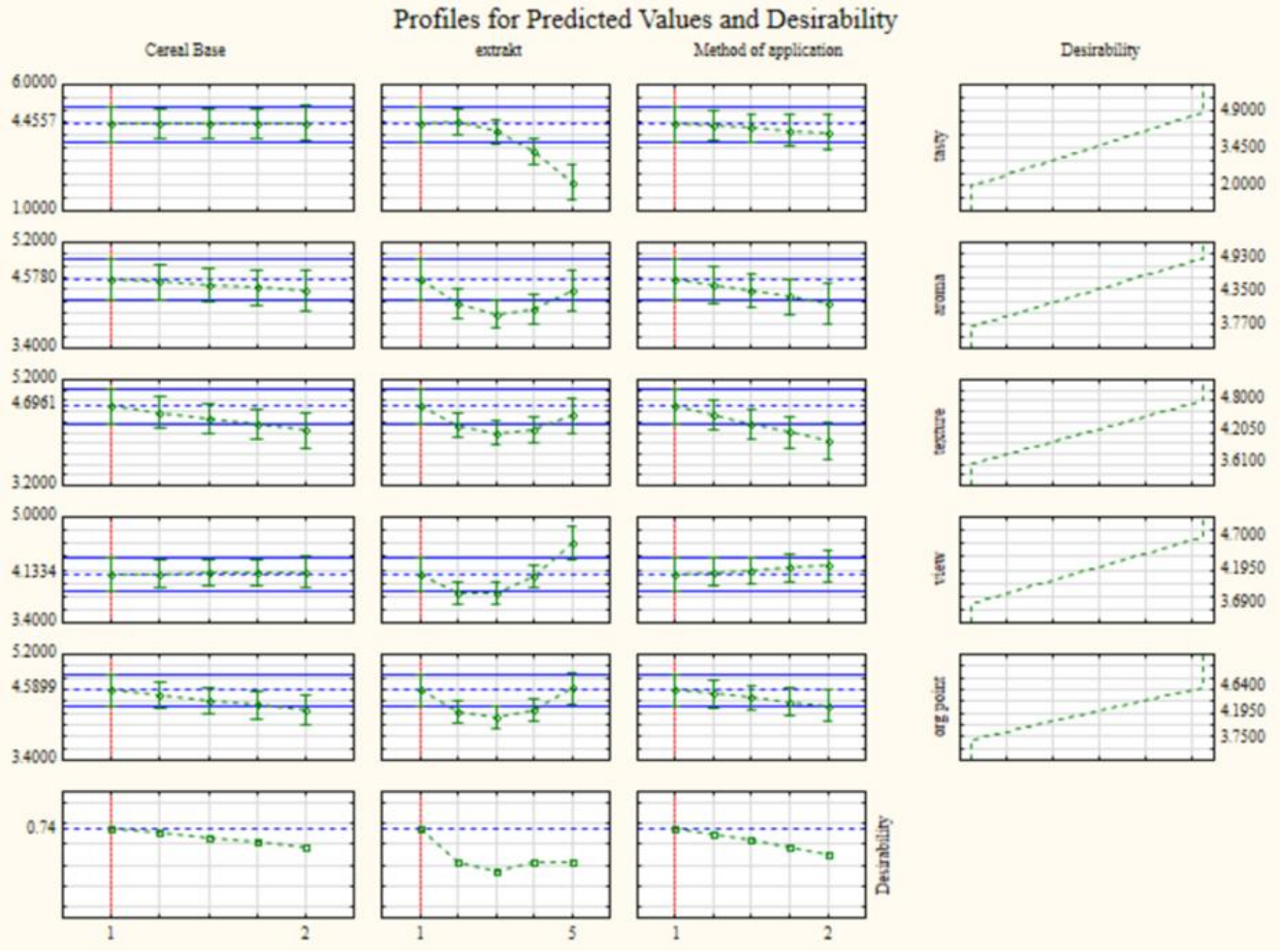

Figure 6 Response profiles and desirability function based on sensory attributes (taste, aroma, texture, appearance, overall evaluation).

The desirability function shows the highest values for a combination of wheat-based crackers, hawthorn extract, and the glazing method. This confirms the importance of a comprehensive approach to formulation and processing: hawthorn provides the most balanced combination of a neutral flavor and high levels of bioactive compounds (BACs) while maintaining acceptable sensory characteristics.

To visualize the differences between samples based on their bioactive compound content, principal component analysis (PCA) was performed. According to the scree plot (Figure 7), the first two principal components (PC1 and PC2) explain 63.08% of the total data variance (PC1 – 47.88%, PC2 – 15.20%).

The variable contribution plot (Figure 8) shows the distribution of BACs in the space of these components. The components are distributed as follows:

- Anthraquinones, gallic acid, and quercetin contributed most significantly to PC1, indicating the high variability of these compounds among the samples and their strong influence on inter-sample differentiation.

- Chlorogenic acid, hesperidin, and dihydroquercetin (DHQ) formed an opposing group, contributing to PC2, indicating a different biochemical profile in some samples (particularly those containing chokeberry and sea buckthorn).
- Rutin was located closer to the center of the coordinate space, possibly indicating a more uniform distribution of this compound across all samples.

In addition, *orgP* (overall sensory score) was included as a supplementary variable. The orgP vector is directed toward the region opposite the extracts rich in anthraquinones and gallic acid, which may indicate reduced sensory acceptability associated with high levels of these compounds, likely due to their bitterness or astringency.

Thus, PCA enables the identification of key biologically functional compounds (BFCs) that distinguish between samples and reveal their potential impact on consumer-relevant product attributes.

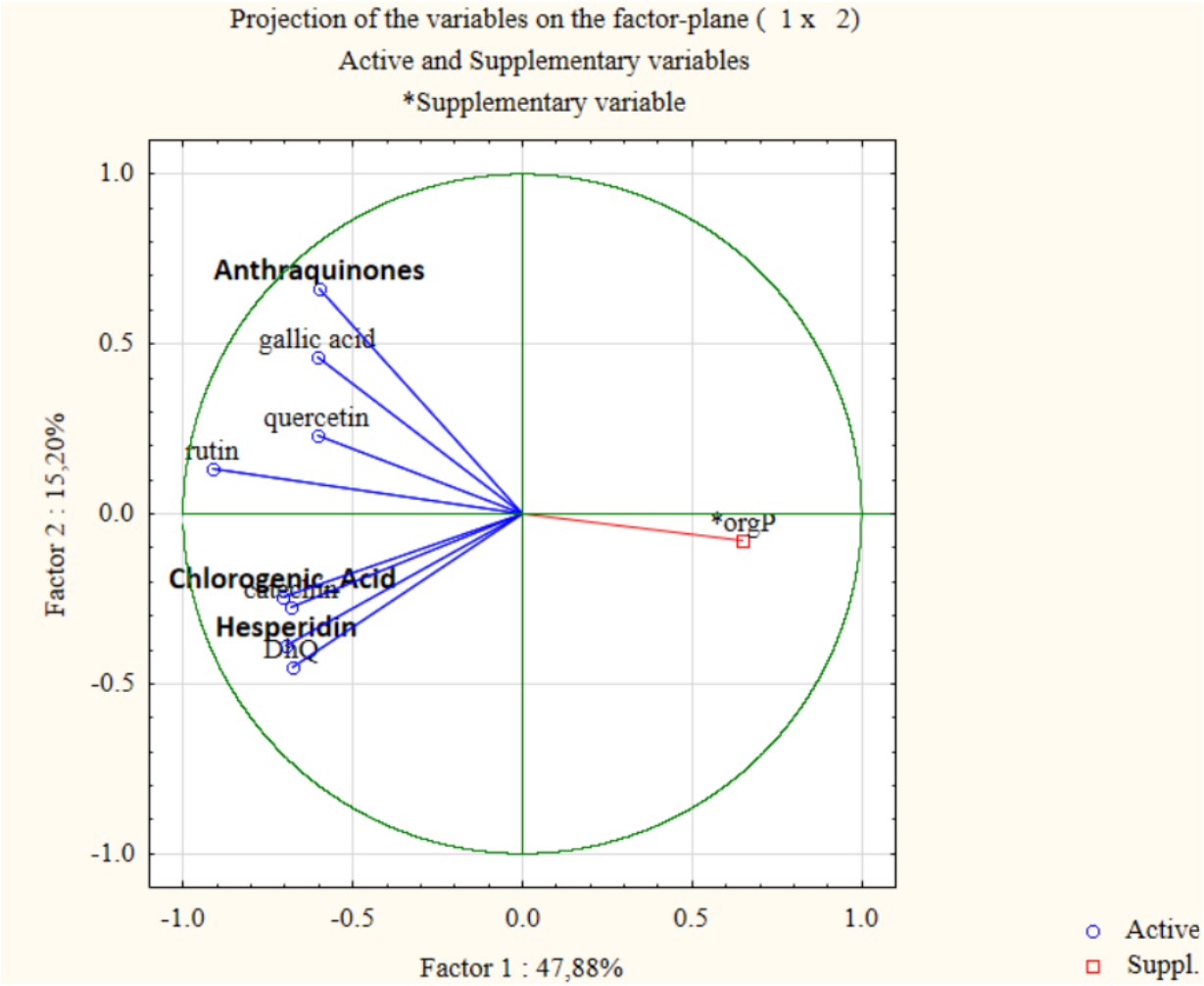

Figure 7 Loading plot: contribution of bioactive compounds to principal components PC1 (47.88%) and PC2 (15.20%).

The blue arrows in the PCA biplot represent the active variables, such as rutin, quercetin, and gallic acid. Their length and orientation indicate the magnitude and direction of their contribution to the respective principal components. The red arrow and point labeled orgP represent a supplementary variable, which was not used in constructing the components but was projected onto the factor plane for interpretative purposes. Figure 8 shows the scree plot of eigenvalues obtained from PCA using the correlation matrix of active variables. Each point corresponds to a principal component, where the X-axis denotes the component number and the Y-axis its eigenvalue. The percentages above the points indicate the share of variance explained.

Earlier works on functional plant ingredients mainly focused on quantifying phenolic content and antioxidant activity. For instance, Koczka et al. **[21]** analyzed total polyphenols in rosehips of different Rosa species, while

Mármol et al**. [22]** discussed therapeutic applications of rosehip extracts. Hao et al. **[23]** optimized ultrasonic-assisted extraction for flavonoids, and Sharma et al. **[25]** evaluated how extrusion alters antioxidant activity in barley.

However, none of these studies applied PCA to integrate polyphenol composition with sensory data. This constitutes the novelty of the present work: we combined correlation analysis with PCA to demonstrate how specific flavonoids (quercetin, rutin, dihydroquercetin) negatively align with sensory acceptance, whereas chlorogenic acid shows a negligible effect.

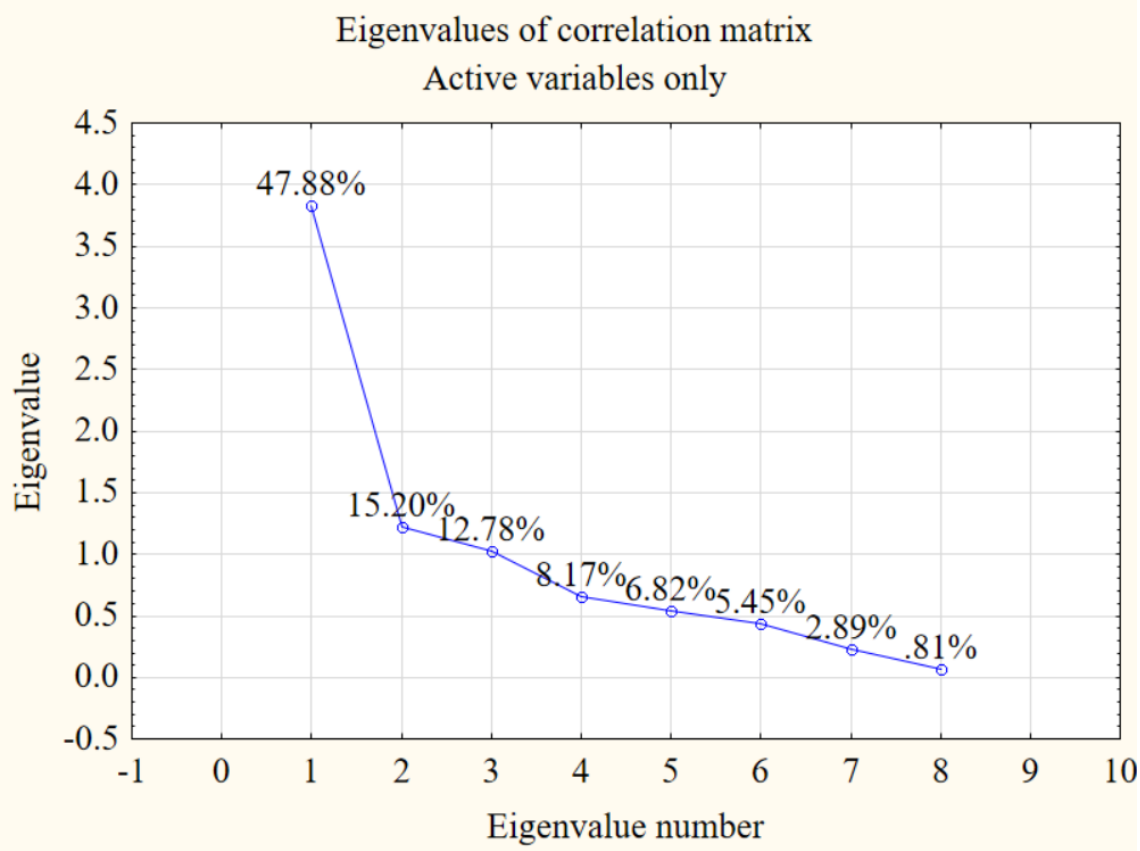

Figure 8 Scree plot - eigenvalues of the correlation matrix of active variables.

The scree plot demonstrates a marked decline in explained variance after the third principal component, indicating the presence of an elbow point beyond which additional components contribute minimally to the total variance.This justifies the selection of the first 2–3 principal components for further data interpretation.

Based on the correlation analysis (Table 4), statistically significant relationships were identified between the content of specific bioactive compounds (BACs) and the overall sensory score of the samples (*orgP*).

The strongest inverse correlations were observed between quercetin and *orgP* (r = −0.74), dihydroquercetin (DHQ) and *orgP* (r = −0.68), and rutin and *orgP* (r = −0.50).

These findings assume that high levels of these flavonoids are associated with a decrease in the sensory acceptability of the product, likely due to their characteristic bitterness or astringency.

Weaker, yet still negative, correlations with the overall sensory score were also identified for gallic acid (r = −0.43), catechin (r = −0.48), anthraquinones (r = −0.38), and hesperidin (r = −0.32).

An exception was chlorogenic acid, which showed only a weak negative correlation (r = −0.12), indicating a more neutral or less pronounced effect on taste and aroma.

These results highlight the importance of considering the potential impact of individual bioactive compounds on sensory characteristics when developing functional crispbreads. Correlation analysis can inform formulation strategies and technological approaches for minimizing the adverse effects of active compounds on consumer acceptability.

While the bitterness and astringency of polyphenols such as quercetin and rutin have been previously reported in plant-based matrices **[20]**, **[41]**, the novelty of the present study lies in establishing direct statistical

correlations between the concentrations of specific bioactive compounds and the sensory acceptance of extruded crispbreads enriched with wild plant concentrates. To our knowledge, this is the first quantitative evidence linking individual flavonoids (quercetin, dihydroquercetin, rutin) with decreased hedonic scores in cereal-based crispbreads, while also demonstrating that chlorogenic acid exerts minimal influence on sensory perception.

Table 4 Correlation matrix between bioactive compounds (BACs) and the overall sensory score (*orgP*).

Variable	DhQ	Rutin	Quercetin	Catechin	Gallic acid	Chloro-genic acid	Anthraqu inones	Hesperi-din	*orgP
DhQ	1.000000	0.467314	0.523109	0.524434	0.220906	0.433892	0.080686	0.474288	-0.676625
Rutin	0.467314	1.000000	0.429510	0.454513	0.584942	0.620019	0.654584	0.739345	-0.504587
Quercetin	0.523109	0.429510	1.000000	0.400469	0.298823	0.208327	0.457804	0.133352	-0.737619
Catechin	0.524434	0.454513	0.400469	1.000000	0.238550	0.442812	0.278525	0.420132	-0.481541
Gallic acid	0.220906	0.584942	0.298823	0.238550	1.000000	0.380830	0.441489	0.208516	-0.432853
Chlorogenic acid	0.433892	0.620019	0.208327	0.442812	0.380830	1.000000	0.227576	0.497237	-0.120937
Anthraquinones	0.080686	0.654584	0.457804	0.278525	0.441489	0.227576	1.000000	0.215794	-0.377105
Hesperidin	0.474288	0.739345	0.133352	0.420132	0.208516	0.497237	0.215794	1.000000	-0.318271
orgP	-0.676625	-0.504587	-0.737619	-0.481541	-0.432853	-0.120937	-0.377105	-0.318271	1.000000

Table 4 shows that dihydroquercetin and quercetin demonstrated the strongest negative correlations with sensory acceptance (r = –0.68 and r = –0.74, respectively). Such associations are typically linked with the bitter and astringent taste profile of flavonoids. Semwal et al. **[20]** described rutin as a flavonoid with both high nutraceutical value and sensory limitations due to bitterness. Di Lorenzo et al. **[41]** emphasized that the sensory perception of polyphenols is closely associated with their bioavailability and metabolic interactions. Berga et al. **[44]** further confirmed that the physicochemical properties of flavonoids strongly influence taste and formulation strategies.

Moderate negative correlations were also observed for rutin (–0.50), catechin (–0.48), gallic acid (–0.43), anthraquinones (–0.38), and hesperidin (–0.32). Koczka et al. **[21]** reported that high polyphenol concentrations in rosehips reduce acceptability due to astringency. Mármol et al. **[22]** confirmed that elevated rosehip extracts may impart an undesirable sour-bitter profile in food systems. Sharma et al. **[25]** observed a decline in sensory scores in barley extrudates linked to polyphenol degradation products. Han et al. **[33]** demonstrated that polyphenols from cereals significantly affect antioxidant activity but also influence taste perception. Hossain and Jayadeep **[34]** further found that extrusion of maize reduced consumer acceptance due to changes in phenolic composition.

Chlorogenic acid demonstrated only a weak association (–0.12), suggesting a limited contribution to taste perception. Rivero Meza et al. **[42]** observed a similar effect in extruded pigmented rice cereals, where chlorogenic acid had little influence on sensory acceptance.

Positive correlations between rutin, hesperidin, anthraquinones, and chlorogenic acid (r = 0.62–0.74) indicate the possibility of co-accumulation of these metabolites. Niedzwiecki et al. **[38]** described synergistic effects of polyphenol combinations in enhancing bioactivity. Bié et al. **[39]** discussed how polyphenols interact within food matrices and gut microbiota, forming complexes that modify functional properties. Billowria et al. **[45]** highlighted the analytical evidence of flavonoid–flavonoid interactions in complex formulations.

These findings align with recent reviews that emphasize the need for polyphenols to be balanced in functional formulations to achieve both biological efficacy and acceptable sensory quality. Bié et al. **[39]** underlined the need to consider gut-level interactions, while Berga et al. **[44]** stressed the importance of formulation strategies to avoid negative sensory outcomes.

CONCLUSION

This study confirmed that each wild plant used—hawthorn, sea buckthorn, rosehip, and chokeberry—formed a unique profile of bioactive compounds (BACs), influencing both the antioxidant potential and sensory attributes of cereal crispbreads. The use of ultrasound-assisted extraction and vacuum evaporation enabled the preservation of thermolabile polyphenols, while the glazing method ensured their uniform application without compromising texture. The highest rutin content (150–152 mg/100 g) was observed in hawthorn and chokeberry-enriched samples. Buckwheat proved to be a more protective matrix for flavonoids than wheat, with a significantly higher retention of rutin and dihydroquercetin. ANOVA and Tukey HSD confirmed statistically significant effects of both plant type and cereal base ($p < 0.05$), while PCA linked flavonoid concentration with sensory outcomes.

Desirability analysis identified buckwheat–hawthorn as the optimal combination in terms of both biochemical value and consumer acceptance (Desirability = 1.0). Sensory evaluation validated this result, showing high scores for taste, aroma, and texture. Conversely, chokeberry required flavor correction due to its bitterness, and spraying was less effective than glazing in preserving the product's structure and crispness. In conclusion, an integrated technological approach—combining mild extraction, targeted application, and statistical modeling—can successfully produce functional crispbreads with vascular-protective potential. These findings support the industrial development of flavonoid-rich snack products based on local plant biodiversity.

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AI Statement:

AI tools (ChatGPT, OpenAI, GPT-4, 2024 version) were used for text revision and language editing during the manuscript preparation stage. No AI tools were used for data collection, statistical analysis, or result interpretation.

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