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## THE USE OF HAZELNUT SEED SKINS FOR THE FORTIFICATION OF FOOD WITH POLYPHENOLS AND TO INCREASE FOOD SAFETY

### S u m m a r y

**Background.** Nut skins are ones of the most common types of waste in the confectionery industry. They are rich in polyphenolic compounds, including phenolic acids, flavonoids and flavonols, which have a health-promoting effects. The study aimed to analyze the content of polyphenols and the antimicrobial properties of hazelnut seed skins and the possibility of using them to enrich the model snack with phenolic compounds.

**Results and conclusion.** The researched huzelnut skins contained a wide spectrum of polyphenolic compounds, in particular a high content of gallic acid, and also showed inhibitory properties on the growth of the selected strains of pathogenic bacteria. Their addition may contribute to increasing food safety. It was found that the addition of seed skins did not adversely affect the sensory quality of the products to which they have been added and the consumer acceptance. Hazelnut seed skins can be used to enrich food with polyphenols.

**Keywords:** hazelnuts, seed skins, bars, polyphenols, phenolic compounds, functional food, zero waste

### Introduction

The confectionery and dried fruits and nuts processing industry generates a lot of post-production waste, and one of its most common types is nut seed skins. Currently, solutions are being sought for the management of this raw material. The nut skins are most often recycled or used for fodder. A new approach is their secondary use in food production, due to the high content of dietary fiber and phenolic compounds, which

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have a positive effect on the human body [6]. Nut skins are used as an additive used e.g. for wheat bread, ice cream, yogurt or meat products [2, 9]. In addition, the management of post-production waste is a part of sustainable development goals.

Research indicates a high content of antioxidant compounds in hazelnut oils, peel, seeds, leaves and seed husk [13]. Hazelnuts are a very good source of antioxidant compounds, including polyphenols, while the seed skins of these nuts are rich in phenolic acids and flavonoids, which, apart from vitamin E, constitute the main chemical component with high antioxidant potential [13]. Additionally, products derived from the processing of hazelnuts may exhibit antimicrobial properties [11].

The study aimed to analyze the content of polyphenols and antimicrobial properties of hazelnut seed skins and the possibility of using them to enrich the model snack with phenolic compounds.

### **Material and methods**

The research material was hazelnut seeds skins (Ferrero; Poland) and snack bars. Seed skins were used to make three variants of bars with the addition of 1 %, 2 % and 3 % of skins having the weight corresponding, more or less, to the basic base of the snack. The control sample and the base for the preparation of the tested variants was a product without the addition of seed skins. It consisted of ground dates (8 0%) (Bakalland, Poland), blanched cut almonds (4 %) (Bakalland, Poland), coconut flakes (4 %) (Bakalland, Poland), basil seeds (4 %) (Przedsiębiorstwo Wielobranżowe "Rekord" Export-Import, Poland), raspberry juice concentrate (4 %) (Döhler, Poland), strawberry lyophilisate (2 %) (Lyofood, Poland), dried beetroot (2 %) (Przedsiębiorstwo Wielobranżowe "Rekord" Export-Import, Poland). The ingredients were weighed in line with the recipe and mixed until a homogeneous mass was obtained using a 300 W mixer - KitchenAid (Belgium). 25 g portions were formed, which constituted a single bar, the dimensions of which were: 80 mm x 30 mm x 15 mm. The snacks thus formed were analyzed after 7 days.

#### *Analysis of the content of polyphenolic compounds by high-performance liquid chromatography (HPLC)*

The content of polyphenols was analyzed by HPLC in line with the methodology of Król et al. [8]. Nut skins were minced with a Bosch grinder (Germany), 1 g was weighed and extracted with 5 ml of 80 % methanol by shaking in a Micro-Shaker 326 M (Poland). The samples were sonicated in an ultrasonic bath (10 min, 30 °C, 550 Hz). After 10 min, the samples were centrifuged (10 min, 6000 rpm, 0 °C). A 1 ml of the extract was put into vials and analyzed. For the analysis of phenolic compounds, HPLC systems were prepared. It comprised two LC-20AD pumps, CMB-20A system controller, SIL-20AC autosampler, UV-visible SPD-20AV detector, CTD-20AC furnace and

Phenomenex Fusion-RP 80A column (250 × 4.60 mm; column size 4 μm), from Shimadzu (Shimadzu, Tokyo, Japan). Two gradient phases were used: A-10 % (v:v) acetonitrile phase and HPLC grade demineralized water; phase B-55 % (v:v) acetonitrile and HPLC grade demineralized water. The phases were acidified with 85 % o<sub>3</sub>PO<sub>4</sub> (pH 3.0). The analysis time was 38 minutes. The phase flow program was as follows: 1.00-22.99 min 95 % Phase A and 5% Phase B, 23.00-27.99 min 50 % Phase A and 50 % Phase B, 28.00-28.99 min 80 % phase A and 20 % phase B, and 29.00-38.00 min 95 % phase A and 5 % phase B. Two wavelengths of 250 nm (flavonol) and 370 nm (phenolic acid) were used. Phenolic compounds were identified based on external standards (Sigma-Aldrich, Poznań, Poland) with a purity of at least 99.5 %. The analysis was performed in 4 replications.

#### *Analysis of bacteriostatic properties of seed skins*

The bacterial strains selected for the analysis are shown in Table 1. The bacteria were activated from a culture bank (-80 °C) in nutrient broth (Oxoid, UK) and incubated for 24 hours at 37 °C. The bacterial cultures were diluted in a geometric series to a concentration of 4 log CFU / ml. Cultures prepared in this manner were used for the analysis of bacteriostatic properties. The nut seed skins were ground and diluted in Miller-Hilton agar (Oxoid, UK) to concentrations of 1% (w/w), 2 % (w/w), and 3 % (w/w). Media without the addition of the nut skins were the control. 0.1 ml of the bacterial suspension was plated on a solidified agar medium and spread. The plates with growth medium were incubated at 37 °C for 24 h. The bacterial growth inhibitory effect was evaluated by the visual observation of the agar surface with hazelnut seed skins. The lack of visible colonies on the surface of the growth medium was considered to be an inhibitory effect on bacterial growth. The determinations were made in 3 replications.

#### *Analysis of the antioxidant potential using the ABTS<sup>•+</sup> method 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt*

The antioxidant activity was measured by the method of Re et al. [12] using the radicals ABTS<sup>•+</sup> (2,2'-azobis (3-ethylbenzothiazoline-6-sulfonate) (Sigma-Aldrich, Poznań, Poland). ABTS<sup>•+</sup> was prepared 24 hours before the determination by mixing inactive ABTS radicals (7 mM/L) with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> salt (2.45 mM/L) (Sigma-Aldrich, Poznań, Poland) and stored at room temperature. After preparation, the solution was stored for 24 hours. Immediately before the determination, the ABTS<sup>•+</sup> solution was diluted with PBS to obtain an absorbance of 0.7 ± 0.02. The bar samples were ground with a Bosch grinder (Germany). Then 10 g of each sample was weighed and put into 100 ml volumetric flasks, the mark was made up to the mark with 0.1 M phosphate buffer (PBS) (Chempur, Poland). Samples were extracted in a laboratory shaker (Thermo

Fisher Scientific; USA) (180 min, 200 rpm, 37 °C) After extraction, the samples were centrifuged using an Eppendorf Centrifuge 5804 R (Poland) (10 min, 10000 rpm, 0 °C). The supernatant was diluted. 50 µL of each of the test sample solutions and 150 µL of the ABTS<sup>•+</sup> radical solution were poured into a well of a 96-well polystyrene plate with a volume of 1 well of 300 µL. The reaction was run for 6 minutes and then measured at 734 nm with a SpectraMax iD3 reader (Molecular Devices, USA). Results are expressed as µM Trolox equivalent per 10 g product (µM TEAC/10 g product). The analysis was performed in 5 replications.

#### *Sensory analysis*

A semi-consumer sensory evaluation was performed (n = 40) [1]. The consumers were students and employees of the Warsaw University of Life Sciences, who declared that they were snack consumers and not allergic to the recipe ingredients of the snack. The scaling method was used with the use of a 9-point hedonic scale [1]. The evaluation consisted in tasting the bars and assigning them to one of nine designations according to the sensory impressions they gave. The designations were as follows: 1 – extremely undesirable, 2 – very undesirable, 3 – undesirable, 4 – somewhat undesirable, 5 – neither desirable nor undesirable, 6 – somewhat desirable, 7 – desirable, 8 – very desirable, 9 – extremely desirable [1]. Four characteristics were assessed: smell, texture, taste and overall acceptance. To obtain data on the perception of tartness and bitterness, a ranking analysis was performed, covering the same group of people. In this method, the consumer had to rank the samples from the least bitter/tart to the most bitter/tart. The result was presented as the mean of the ranks.

#### *Statistical analysis*

The statistical analysis of the results was performed in the Statistica 13.3 program (StatSoft, Poland). Mean values and standard deviations were calculated and one-way ANOVA was used. The HSD Tukey test was used to compare the post hoc mean values. The difference was statistically significant when  $p < 0.05$  for the results of all analyzes performed.

### **Results and discussion**

Table 1 presents the results of the analysis of the bacteriostatic properties of seed skins. In the experiment, seed skins showed properties that inhibited the growth of selected microorganisms. The strains of *Staphylococcus aureus* and *Listeria monocytogenes* showed the highest resistance to the effects of compounds contained in the skins. Nevertheless, despite the lack of bacteriostatic effect of seed skins on these strains at the lowest tested concentration (1 %), such an effect was seen at a higher concentration of skins. The addition of the skins effectively inhibited the growth of the remaining

pathogenic bacteria in all tested levels of additions. The research conducted by Lorenzo et al. (2018) confirms the obtained results. The peel of peanuts used in the research effectively inhibited the development of *Bacillus cereus* [10]. The literature provides information on the mechanism of phenolic compounds inhibiting the growth of bacteria. Such an effect may result from the modification of the permeability of cell membranes, changes in intracellular functions caused by the hydrogen bonding of phenolic compounds with enzymes or the loss of the integrity of the cell membrane [3], leading to the inhibition of bacterial growth or inactivation. Such mechanisms were probably present in the research discussed.

Table 1. Bactericidal properties of seed skins at specific concentrations; n = 3

Tabela 1. Właściwości bakteriobójcze okrywy nasiennych orzechów laskowych w określonych stężeniach; n = 3

Bacteria strain under research Badany szczep	Hazelnut seed skins content Zawartość okrywy nasiennych orzechów laskowych		
	1 %	2 %	3 %
<i>Bacillus cereus</i> ATCC 11778	-	-	-
<i>Staphylococcus aureus</i> ATCC 25923	+	-	-
<i>Listeria monocytogenes</i> ATCC 19111	+	-	-
<i>Salmonella enterica</i> ATCC 29631	-	-	-
<i>Escherichia coli</i> O157:H7	-	-	-

Explanatory notes / objaśnienia: "-" indicates no bacterial growth, "+" indicates bacterial growth; "-", oznacza brak wzrostu bakterii, "+", uzyskany wzrost bakterii; n = 3.

In turn, Table 2 shows the content of phenolic compounds in seed skins obtained as a result of the HPLC method. The total content of polyphenols was 8.259 mg/g. Phenolic acids constituted about 92 % of the determined compounds, with gallic acid constituting the largest share. It exhibits anti-inflammatory, anti-cancer, antioxidant, and bacteriostatic properties [7]. Flavonoids constituted about 4.5 % of polyphenols. The studies conducted by Del Rio et al. [5] obtained different results. They indicated that the largest group of polyphenols (95 %) were flavan-3-ols, while phenolic acids accounted for less than 1 % of the compounds determined. These differences could result from the origin of the raw material, growing conditions or the roasting method used, which could lead to the autolysis of flavonoid compounds.

On the other hand, Table 3 presents the results of the antioxidant properties of the analyzed snacks with the use of hazelnut seed skins. These properties increased with the greater share of seed skins in the composition of the products. This result shows a clear effect of the amount of hazelnut seed skins added to the antioxidant activity of the analyzed samples. The differences between the samples were statistically significant ( $p < 0.05$ ). The studies by Contini et al. [4] confirm the antioxidant properties of hazelnut seed skins. The antiradical effectiveness of the skins was three times higher

than in the case of butylated hydroxytoluene,  $\alpha$ -tocopherol, and about 10 times than in the case of vitamin C.

Table 2. The content of phenolic compounds in seed skins obtained from the HPLC separation; n = 4  
Tabela 2. Zawartość związków fenolowych w okrywkach nasiennych uzyskanych z rozdzielu HPLC; n = 4

Labeled phenolic compounds Oznaczone związki fenolowe	Average content in mg/g Średnia zawartość mg/g	Labeled phenolic compounds Oznaczone związki fenolowe	Average content in mg/g Średnia zawartość mg/g
Total polyphenols Polifenole ogółem	8.259	Catechin / Katechuna	0.105
Total phenolic acids Kwasy fenolowe ogółem	7.888	Epigallocatechin Epigallokatechina	0.021
Gallic acid Kwas gallusowy	7.586	Rutinoside-3-O-querctetin Rutinozyd-3-O-kwercytyna	0.018
Coffee acid Kwas kawowy	0.220	Glycoside-3-O-kaempferol Glikozyd-3-O-kemferol	0.018
Cumaric acid Kwas kumarynowy	0.060	Quercetin / Kwercytyna	0.011
Ferulic acid Kwas ferulowy	0.022	Apigenin / Apigenina	0.091
Total flavonoids Flawonoidy ogółem	0.371	Kaempferol / Kemferol	0.107

Table 3. Antioxidant activity of studied products with the addition of skin nuts  
Tabela 3. Właściwości antyoksydacyjne badanych produktów z dodatkiem okryw z orzechów

The content of the skin coat in the snacks tested (w/w) Zawartość okrywy nasiennej w badanych przekąskach (w/w)			
0 %	1 %	2 %	3 %
Average [ $\mu$ M TEAC / 10g] / Średnia [ $\mu$ M TEAC / 10g]			
330.06 $\pm$ 4.42 <sup>a</sup>	355.44 $\pm$ 7.06 <sup>b</sup>	367.72 $\pm$ 5.85 <sup>c</sup>	376.54 $\pm$ 7.36 <sup>d</sup>

Explanatory notes / objaśnienia: The table shows mean values  $\pm$  standard deviations/ W tabeli przedstawiono wartości średnie  $\pm$  odchylenia standardowe; a, b, c, d - mean values in the rows marked with different letters differ statistically at  $p < 0.05$ / a, b, c, d- wartości średnie w wierszach oznaczone różnymi literami różnią się statystycznie przy  $p < 0,05$

The results of the sensory evaluation are shown in Figure 1. Among all the tested determinants, except for the odor, the control sample was the most acceptable. The sample with the addition of 2% of seed skins proved to be the most acceptable. However, the differences between the samples were not statistically significant ( $p > 0.05$ ).

This demonstrates that increasing the share of hazelnut seed skins (to the level of 3%) in products does not affect the level of consumer acceptance for them. It is worth emphasizing that none of the products scored lower than 7 points on the 9-point scale used. This result indicates the high acceptance of the developed products. In the studies by Bertolino et al. [2] seed skins were added to yogurt, consumers indicated products with a 3% addition of skins as the most acceptable. No studies on the enrichment of sweet snacks with skins have been found in the available literature. Comparing the results of Bertolino et al. [2], it can be noticed that the products obtained high hedonic scores with the addition of seed skins.

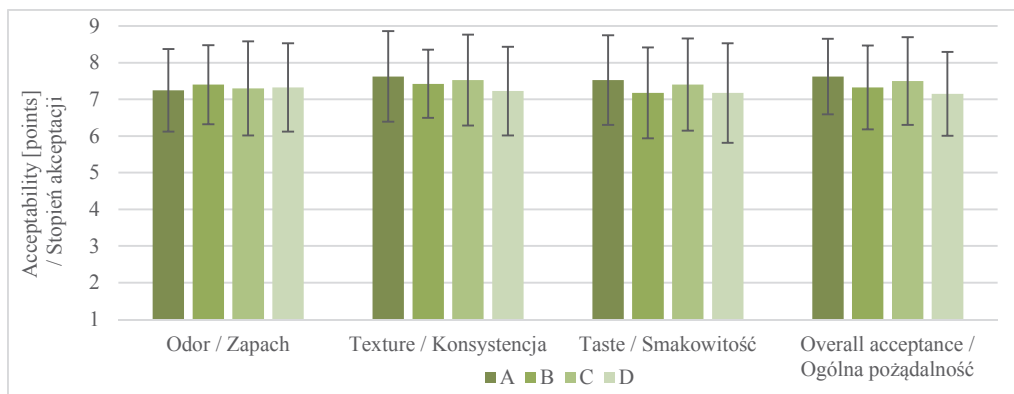


Figure 1 Assessment of the acceptability of the tested products

Rysunek 1. Ocena pożądalności badanych przekąsek

Explanatory notes/ objaśnienia: A- control test, B- test with the addition of 1% of seed skins, C- test with the addition of 2% of seed skins, D- test with the addition of 3% of seed skins/ A- próbakontrolna, B- próba z dodatkiem 1% okryw nasiennych, C- próba z dodatkiem 2% okryw nasiennych, D- próba z dodatkiem 3% okryw nasiennych; n=40; there were no statistical differences among the samples/ różnice statystyczne pomiędzy próbkami nie występowały

The scheduling method was used to evaluate the effect of the addition of hazelnut seed skins on the perception of astringency and bitterness in the products. The higher average rating the product had, the higher tart and bitter taste note it received. What could be observed was an increase in the perception of a bitter/tart taste with the addition of a seed skins (Tab. 5). The difference was significant ( $p < 0.05$ ) between the control sample and the one with 3% of nut skins. Despite the fact that this statistical significance existed, the results of the method using the 9-point hedonic scale proved that increasing the share of seed skins in the products did not reduce their acceptance. This result proves the possibility of using seed skins as an addition to sweet snacks. Polyphenolic compounds play an important role in shaping the sensory quality of food products. They increase the intensity of the bitter and tart taste sensation [15]. The

notes of bitterness and tartness are generally negative determinants. For this reason, masking these characteristics can be a key task when developing new products with nuts skins. On the other hand, literature data indicates that not all consumers have an aversion to bitter and tart taste notes and in some products these features may be highly accepted [1, 14].

Table 5. Results of ranking the bar samples in terms of astringency and bitterness

Tabela 5. Wyniki szeregowania próbek batonów pod względem wrażenia cierpkości i goryczy

Assessed sample / Oceniana próba	A	B	C	D
Average rank / Średnia rang	1,95 <sup>a</sup>	2,48 <sup>ab</sup>	2,45 <sup>ab</sup>	3,20 <sup>b</sup>

Explanatory notes/ objaśnienia:

Explanation of symbols as shown in figure 1./ objaśnienia symboli jak pod rysunkiem 1. The table shows mean values  $\pm$  standard deviations/ W tabeli przedstawiono wartości średnie  $\pm$  odchylenia standardowe; a, b - mean values in the rows marked with different letters differ statistically at  $p < 0.05$ / a, b - wartości średnie w wierszach oznaczone różnymi literami różnią się statystycznie przy  $p < 0,05$

## Conclusions

1. Hazelnut seed skins can be used to enrich sweet snacks due to the high content of phenolic compounds, especially gallic acid, which have health-promoting properties.
2. The addition of up to 3 % of hazelnut seed skins did not change the sensory value of the product and did not lower the consumer acceptance of the product, despite the increase in the perception of bitter and tart notes.
3. Chemical compounds contained in hazelnut seed skins show a bacteriostatic effect on pathogenic microorganisms that most often cause food poisoning.
4. Hazelnut seed skins have high application potential in the production of fortified food, due to bacteriostatic properties, chemical composition and the possibility of limiting bio-waste in the zero-waste idea.

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## WYKORZYSTANIE OKRYW NASIENNYCH ORZECHÓW LASKOWYCH DO WZBOGACENIA ŻYWNOŚCI W POLIFENOLE I ZWIĘKSZENIA BEZPIECZEŃSTWA ŻYWNOŚCI

### Streszczenie

**Wprowadzenie.** Jednym z najpowszechniejszych odpadów w przemyśle cukierniczym są okrywy nasienne orzechów. Są bogate w związki polifenolowe w tym kwasy fenolowe, flawonoidy i flawonole, które wykazują działanie prozdrowotne. Celem badania była analiza zawartości polifenoli i właściwości przeciwdrobnoustrojowych okryw nasiennych orzechów laskowych oraz możliwości ich wykorzystania do wzbogacenia modelowej przekąski w związki fenolowe.

**Wyniki i wnioski.** Okrywy nasienne zawierały szerokie spektrum związków polifenolowych, w szczególności wysoką zawartość kwasu galusowego, a także wykazywały właściwości hamujące wzrost wybranych szczepów bakterii patogennych. Ich dodatek może przyczynić się do zwiększenia bezpieczeństwa żywności. Stwierdzono, że dodatek okryw nasiennych nie wpływał negatywnie na jakość sensoryczną produktów z ich dodatkiem oraz na akceptację konsumentką. Okrywy nasienne orzechów laskowych mogą być stosowane do wzbogacania żywności w polifenole.

**Słowa kluczowe:** orzechy laskowe, okrywy nasienne, batony, polifenole, związki fenolowe, żywność funkcjonalna, zero waste ☒