Grapevine and Horseradish Leaves as Natural, Sustainable Additives for Improvement of the Microbial, Sensory, and Antioxidant Properties of Traditionally Fermented Low-Salt Cucumbers

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Abstract: The agro-food industry produces large amounts of secondary by-products, which can act as a source of bio-active ingredients. These ingredients can be used as valuable additives to support the sustainable circular economy concept. This study aimed to analyze the potential application of horseradish and grapevine leaves in the fermentation process of low-salt pickled cucumbers to improve their sensory and functional properties. The pour plate technique, RT-qPCR, HPLC, and a nine-point hedonic scale test with penalty analysis were used to analyze the traditionally fermented product. The research showed that the addition of both horseradish and grapevine leaves did not negatively affect the kinetics of fermentation and had a positive effect on the overall desirability. Moreover, they contributed to an increase in the concentration of antioxidant compounds, namely gallic acid (grape leaves) and ellagic acid (grapevine and horseradish leaves). Bacterial metabiome analysis showed the positive effect of all analyzed additives on an increase in the relative expression of genes responsible for the synthesis of selected bacteriocins (plantaricin and acidocin). Research results indicated a high potential for sustainable use of by-products (horseradish and grapevine leaves) in the production of traditional low-salt fermented cucumbers with high health-promoting potential.

Keywords: traditional fermentation; plantaricin; acidocin; lactic acid bacteria; gallic acid; ellagic acid; sustainable food; agro-industrial by-products

1. Introduction

The latest trends in food sustainability include the concept of a circular bioeconomy, which aims to reduce socio-environmental and economic costs related to the production of agricultural residues and by-products. The directions of development include not only the improvement of productivity during growth, but also other issues: on-farm losses caused by problems with agricultural product distribution and agricultural waste management. For example, it is estimated that post-harvest losses of fruit and vegetables in the European Union reach nearly 20% [1–5]. Despite the implementation of Commission Regulation (EC) No. 1221/2008 of 5 December 2008, which repealed a number of European Commission regulations regarding the quality standards of many fresh fruits and vegetables, a significant fraction of crops are still considered “imperfect” for cosmetic and aesthetic defects and do not meet the market standards due to the visual defects in size, shape, and color. They are not accepted by retailers and are not admitted to trading [3]. The global consumers’ preference for vegetables and fruits that have an attractive appearance contributes to many losses, including valuable environmental resources such as land and fresh water. Despite many initiatives to introduce crops with visual defects to the market, selling them is still a major global problem [4–7].
The other issues in food sustainability that can increase economic competitiveness are agricultural by-products and waste management (such as the management of green leaves of widely cultivated crops), which can act as a valuable source of proteins and bioactive compounds [2,3]. Good examples, which are the subject of this research, are horseradish (Armoracia rusticana) leaves and domesticated grapevine (Vitis vinifera ssp. sativa) leaves.

Horseradish is a plant of the order Brassicales that has been cultivated for over 2000 years in many regions of the world, currently most widely in Europe and North America. It is a typical root vegetable, and the leaves are used in cooking on a much smaller scale [8–10]. Horseradish is considered a rich source of health-promoting compounds with antimicrobial, chemopreventive, anti-inflammatory, gastroprotective, and hypercholesterolemic properties. Due to the presence of phenolic compounds (flavonoids and phenolic acids), vitamins (C and B1), minerals (iron, potassium, magnesium, and calcium), and essential oils, it is a valuable traditional medical plant and is used for food [9–11].

The domesticated grapevine is a well-known plant native to the Mediterranean and Central Asian regions, that has gained worldwide popularity. It is cultivated mainly for fresh berries, which are the raw material of the wine industry [12,13]. Fresh leaves are a by-product of vineyards. They are applied in many traditional cuisines and traditional medicine due to their high health-promoting potential resulting from the presence of phenolic compounds (flavonoids, flavonols, and hydroxycinnamic acid derivates) [13,14].

Leafy crops, as well as leafy by-products of vegetable and fruit production, are raw materials that are relatively difficult to store, have a short shelf-life, and require special storage conditions, all of which are associated with high financial outlays [15]. An alternative for distributing fresh leaves is to pickle them or use them as an additive in fermented products. Both horseradish and grapevine leaves, due to their health-promoting properties described above, are considered in traditional Polish cuisine as valuable ingredients in pickles, in particular pickled cucumbers, used mainly in home-made fermentations [16]. It is also known that sensory descriptive analysis and consumer studies allow for obtaining valuable data that can be used to successfully improve the overall acceptability [17]. However, there is a lack of scientific research associated with the impact of horseradish and grape leaves on fermentation processes and consumer acceptability of the final product. Fermented foods are known for their health-promoting and unique sensory properties. The benefits result from the presence of probiotic lactic acid bacteria that have a positive effect on gut health, as well as the presence of biologically active compounds, including antimicrobials (organic acids, bacteriocins). Additionally, during the fermentation process, the elimination of compounds toxic to human health, such as phytates and tannins, and the formation of beneficial catabolites may occur; as a result, the fermented products are classified as super-foods [18–20].

The aim of this research was the analysis of the application potential of natural agricultural by-products, namely horseradish and grapevine leaves, in the low-salt fermentation of cucumbers regarding the improvement of the sensory and functional properties of the product. The analysis included a quantitative assessment of the total number of culturable mesophilic microorganisms, including the analysis of lactic acid bacteria, inspection of their genetic potential for bacteriocin synthesis, examination of concentration of the phenolic bioactive compounds, and consumer acceptability studies.

2. Materials and Methods

2.1. Characterization of Plant Raw Material and Fermentation Process

The experiment was carried out in the following variants: (a) control variant without additives (C), (b) variant with the addition of horseradish leaves in the amount of 7 g (H7), (c) variant with the addition of horseradish leaves in the amount of 14 g (H14), (d) variant with the addition of grapevine leaves in the amount of 7 g (G7), and (e) variant with the addition of grapevine leaves in the amount of 14 g (G14). The amounts of added leaves corresponded to a single and double dose of leaves found in traditional Polish recipes.
Disease-free cucumbers classified under EEC Commission Regulation No. 1677/88 as out of class and considered subjectively by the producer as not meeting market standards due to visual defects were obtained from local processors in Wielkopolska province (Poland). Horseradish leaves and grapevine leaves of the Aurora variety were also derived from local processors in Wielkopolska province. The plant material was washed with tap water. Cucumbers (300 g) and horseradish or grapevine leaves were placed in sterilized 900 mL glass jars in a set with a cap and a fermentation tube. The jars were filled with sterile 2% saline. The fermentation process was carried out for 14 days at 22 °C, with daily pH control using a simple electrochemical method (Elmetron, Zabrze, Poland). Samples were taken and analyzed on-line during the experiment (microbiology analysis), immediately after the end of fermentation (consumer test, chromatography analysis), or stored at −80 °C (before RT-qPCR analysis).

2.2. Microbiological Analysis

Microbiological analysis of raw materials and fermented cucumbers (brine samples) during the fermentation process (1, 7, and 14 days of fermentation) was carried out using the pour plate technique, as described in a previous publication [21]. Commercial microbiological media were applied. The count of the total number of culturable mesophilic microorganisms (TCM) was performed on standard nutrient agar medium (BTL, Warsaw, Poland) and the count of the total number of culturable mesophilic lactic acid bacteria (LAB) was performed on De Man–Rogosa–Sharpe (MRS) agar medium (BTL, Poland). The plates were incubated at 37 °C for 72 h.

2.3. Bacteriocin Gene Expression Analysis
2.3.1. RNA Isolation

Isolation and purification of total RNA from brine samples were performed using the Total RNA Mini Kit (A&A Biotechnology, Gdynia, Poland) by following the protocol recommended by the manufacturer. Three isolations were made from each sample, which were then pooled together after positive fluorometric quantitation by using the Qubit RNA BR Assay Kit (Thermo Fisher Scientific, St. Louis, MO, USA) on a Qbit 3.0 device (Thermo Fisher Scientific, St. Louis, MO, USA). The final RNA concentration was adjusted to 100 ng/µL.

2.3.2. RT-qPCR Analysis

cDNA synthesis was performed using the TranScriba noGenome Kit (A&A Biotechnology, Gdynia, Poland) with random hexamer priming according to the protocol recommended by the manufacturer. The qPCR was performed on the Mx3005P QPCR System (Agilent Technologies, Santa Clara, CA, USA) using Fast SG qPCR Master Mix (2×), plus ROX solution (EurX, Gdansk, Poland) and different dilutions of transcribed cDNA, according to the manufacturer’s protocol. Four genes responsible for the synthesis of plantaricins and one gene responsible for the synthesis of acidocins were selected for analysis. The list of primers and PCR reaction parameters used is presented in Table 1; the reference gene was the bacterial 16srRNA gene. The PCR reaction was carried out for 40 cycles.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Primer Name</th>
<th>Abbreviation</th>
<th>Sequence (5′-3′)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Plantaricin A</td>
<td>PlnAF</td>
<td>plnA</td>
<td>AAAATTTCAAATTAAAGGATGAAGCAA</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>PlnAR</td>
<td></td>
<td>CCCCCATCTGCAAAGAATACG</td>
<td></td>
</tr>
<tr>
<td>Plantaricin EF</td>
<td>PlnEFF</td>
<td>plnEF</td>
<td>GTTTTAATCGGGCGGTTAT</td>
<td>[22]</td>
</tr>
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<td></td>
<td>PlnEFR</td>
<td></td>
<td>ATACCACGAATGCTGCAAC</td>
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Table 1. Cont.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Primer Name</th>
<th>Abbreviation</th>
<th>Sequence (5′-3′)</th>
<th>Reference</th>
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<tr>
<td>Plantaricin J</td>
<td>PlnJF</td>
<td>plnJ</td>
<td>TAAGTTGAACGGGGTTGTTG</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>PlnJR</td>
<td></td>
<td>TAACGACGGATGGCTCTGC</td>
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<tr>
<td>Plantaricin K</td>
<td>PlnKF</td>
<td>plnK</td>
<td>TTCTGGTAACCGTCGGAGTC</td>
<td>[23]</td>
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<tr>
<td></td>
<td>PlnKR</td>
<td></td>
<td>ATCCCTTGAACCCACCAAGC</td>
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<tr>
<td>16srRNA</td>
<td>16srRNAF</td>
<td>16srRNA</td>
<td>GATGCATAGCCGACCTGAGA</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>16srRNAR</td>
<td></td>
<td>CTCCGTCAGACTTTCGTCGA</td>
<td></td>
</tr>
<tr>
<td>Acidocin LF221</td>
<td>LFAF</td>
<td>lf221</td>
<td>GTTGCGAGTATCATGTG</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>LFAR</td>
<td></td>
<td>TGTGCGAGCTCCGTGA</td>
<td></td>
</tr>
</tbody>
</table>

Each sample was analyzed in triplicate, a no-template control was used to exclude possible contamination, and melting curve analysis was used to verify the specificity of the PCR reaction. The relative expression level of each target gene was calculated using the comparative threshold cycle method ($2^{-ΔΔCt}$) [25].

2.4. Gallic Acid and Elagic Acid Chromatography Analysis

The analysis of gallic acid and ellagic acid concentrations in brine after 14 days of fermentation was performed on an Agilent 1260 Infinity II liquid chromatograph equipped with an automatic sample feeder (G7129A), a pump (G7111A), an SB-C18 column (50 mm × 4.6 mm) with a particle diameter of 1.8 µm, and a diode detector (G7115C) with a spectrum on transmission in the range of 190–400 nm (Agilent Technologies, Santa Clara, CA, USA). Analysis was performed at a wavelength of 280 nm (gallic acid) and 255 nm (ellagic acid). Trifluoroacetic acid was used as an eluent in acetonitrile at a flow rate of 1 mL/min, in a gradient: 0 min 10%, 1.6 min 20%, 10 min 100%, 15 min 100%, 15.1 min 10%, 18 min 10%. The samples were applied to the column in amounts of 2 mL. Quantitative calculations were performed using peak heights (measurement and computer integration using OpenLab CDS ChemStation Edition, Agilent Technologies, Santa Clara, CA, USA).

2.5. Consumer Test

Cucumbers were analyzed after 14 days of fermentation. They were taken out of the brine just before analysis, cut into 3 cm slices, and placed in plastic cups containing 15 mL of the brine. Samples were coded with 3-digit random numbers and presented in random order. Mineral water was provided to rinse the mouth between each trial. Participants (n = 64) aged 18 to 72 took part in the sensory evaluation (58% women and 42% men). All participants lived in Poland and declared regular consumption of pickled cucumbers in their diet. Before the test, participants were instructed about the testing procedure (using the scale according to the protocol and rinsing the mouth).

For assessing consumer acceptance, 9-point hedonic scale test was used (1 = “extremely dislike”, 5 = “neither dislike nor like” 9 = “extremely like”). Moreover, the suitability of selected attributes such as: firmness, color, astringency, salt, bitterness, and fermented cucumber aroma was evaluated by panelists on a 5-point ‘just-about-right’ (JAR) scale (1-much too little, 3-just about right, 5-much too much). The data collected during both tests were used for penalty analysis to determine how significantly rankings on the JAR scale are related to the consumer preference scores (acceptance). The mean drops were plotted against the percentage of consumers giving responses. Each attribute was considered significant if the minimum percentage of consumers was 20% and the drop in overall consumer acceptance was 1 point or more (upper right quadrant of a plot). Penalty analysis was done using the XLSTAT 2023 software (Addinsoft, New York, NY, USA).
2.6. Statistical Analysis

All experimental variants were performed in five repetitions. Results are expressed as means ± standard deviation. The statistical analysis of the results was based on multivariate analysis using one-way ANOVA and a post-hoc Turkey HSD test at a significance level of \( p < 0.05 \). Data were analyzed using Statistica 13.0 software (StatSoft, Krakow, Poland) and XLSTAT 2023 software (penalty analysis algorithm) (Addinsoft, New York, NY, USA).

3. Results

3.1. Microbiology and pH Analysis

In all variants, a gradual decrease in the pH level was detected during the experiment, up to 3.50–3.58 after 14 days of the fermentation process. None of the experimental variants showed statistically significant \( (p < 0.05) \) changes in the kinetics of pH compared to the control system.

The results of the quantitative analysis of TCM and LAB of raw materials (cucumber, horseradish leaves, and grapevine leaves) are shown in Figure 1. Both groups of microorganisms were most abundant in cucumber (TCM = \( 2.4 \times 10^3 \); LAB = \( 1.6 \times 10^2 \)). Horseradish and grape leaves can also be considered reservoirs of microorganisms, including lactic acid microorganisms.

![Figure 1](image)

**Figure 1.** Quantitative analysis of total culturable mesophilic microorganisms (TCM) and total culturable lactic acid bacteria (LAB) of raw materials used in the fermentation process.

The results of the pour-plate microbiological analysis of TCM and LAB in the brine of fermented samples are presented in Figures 2 and 3. In both cases, the highest number of microorganisms was detected after 7 days of the process. There were no statistically significant differences \( (p < 0.05) \) in the TCM counted between the experimental variants. The brines after 14 days of cucumber fermentation contained TCM in the range of \( 1.1–1.8 \times 10^8 \) CFU/mL of brine.
Figure 2. Quantitative analysis of total culturable mesophilic microorganisms (TCM) in brine of fermented cucumber samples with the addition of horseradish (H) or grapevine (G) leaves after 1, 7, and 14 days of fermentation.

Figure 3. Quantitative analysis of total culturable lactic acid bacteria (LAB) in brine of fermented cucumber samples with the addition of horseradish (H) or grapevine (G) leaves after 1, 7 and 14 days of fermentation.

In the group of lactic acid bacteria, after the first day of the process, significantly higher numbers of counts ($p < 0.05$) were found in the variants containing horseradish and grapevine leaves than in the control variant. After 7 and 14 days of the process, this tendency was maintained only in the variants containing a higher (14 g) addition of..
grapevine and horseradish leaves, and they did not differ significantly ($p < 0.05$) from each other.

3.2. Bacteriocin Gene Expression Analysis

The relative quantity (RQ) of bacteriocin genes in the microbiome of experimental variants after 14 days of fermentation is shown in Figure 4. The addition of horseradish and grapevine leaves significantly increased ($p < 0.05$) the expression of most of the analyzed genes, except for the *plnK* gene. In the case of the *plnK* gene, statistically significant ($p < 0.05$) higher expression than in the control sample was noted only for samples containing high doses of leaves (14 g). Moreover, in the case of most of the analyzed genes, except for the gene encoding acidocin, the amount of grapevine and horseradish leaves significantly ($p < 0.05$) increased gene expression.

![Figure 4. Relative quantity of selected bacteriocin genes (*plnA, plnEF, plnJ, plnK*, and *lf221*) in the microbiome of fermented cucumber samples after 14 days of fermentation with the addition of horseradish (H) or grapevine (G) leaves.](image)

3.3. Gallic Acid and Ellagic Acid Analysis

The addition of horseradish leaves contributed to a significant increase in the concentration of ellagic acid in the brine. The highest concentration of this acid was detected in the sample with the highest dose of leaves (14 g) and it was over 11 times higher than the control sample. The addition of horseradish leaves did not significantly affect the content of gallic acid in the brine.

The addition of grape leaves in both analyzed doses (7 g and 14 g) contributed to an increase in the concentration of ellagic acid in the brine by 105% and 148%, respectively, compared to the control sample. The addition of grape leaves also had a positive effect on the increase in gallic acid concentration by 91% and 131%, respectively, compared to the control sample (Figure 5).
3.4. Sensory Analysis

The average overall desirability of the cucumber samples fermented with the addition of horseradish and grapevine leaves was significantly higher \((p < 0.05)\) than the overall desirability of the reference sample (Table 2). Cucumber samples fermented with the addition of horseradish leaves were characterized by the highest overall desirability. However, no significant differences \((p < 0.05)\) were noted between samples with different doses of horseradish and grapevine leaves.

Table 2. Overall linking scores of fermented cucumbers in analyzed experimental variants based on the consumer test on a nine-point hedonic scale. Different superscript letters correspond to statistically significant differences between groups.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Overall linking score</th>
<th>Standard deviation (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.16 ⁰ ⁰ ⁰ ⁰</td>
<td>1.08</td>
</tr>
<tr>
<td>H7 Sample</td>
<td>8.16 ⁰ ⁰ ⁰ ⁰</td>
<td>1.05</td>
</tr>
<tr>
<td>H14 Sample</td>
<td>7.73 ⁰ ⁰ ⁰ ⁰</td>
<td>0.99</td>
</tr>
<tr>
<td>G7 Sample</td>
<td>6.17 ⁰ ⁰ ⁰ ⁰</td>
<td>1.23</td>
</tr>
<tr>
<td>G14 Sample</td>
<td>6.66 ⁰ ⁰ ⁰ ⁰</td>
<td>1.35</td>
</tr>
</tbody>
</table>

The penalty analysis allowed for the identification of attributes that most affected the linking score of the fermented cucumber samples (Figure 6). For the reference sample, it was: not enough color, not enough bitterness, not enough firmness, not enough astringency, and not enough fermented cucumber aroma. Samples that contained horseradish leaves (H7 and H14) were characterized by the same key attributes: not enough color, too much bitterness, and too much astringency. For the G7 sample, it was: much too firm, much too astringent, and not enough color, and for the G14 sample it was: much too firm, much too astringent, and not enough color.
4. Discussion

The use of plant products with low visual appeal and green leaves of widely cultivated crops is an area of interest both in the context of minimizing economic losses from plant production and obtaining bioactive substances. It favors the development of the sustainable “zero waste” vision by reducing agricultural waste and improving the efficiency of using natural resources for health-promoting food production. Moreover, it can decrease the costs associated with long supply chains and improve the resilience of sustainable, traditional food systems [2,4]. This research proved the possibility of using cucumbers that do not meet esthetic market standards, horseradish leaves, and grapevine leaves in the production of good quality pickled cucumbers with a high content of bioactive compounds and high consumer desirability.

The results of this study indicated that horseradish and grape leaves act as reservoirs of microorganisms, including lactic acid bacteria, which may be of key importance in the process of producing pickled cucumbers. The addition of unsterilized horseradish and grape leaves with their microbiome contributed to an increase in the number of LAB on the first day of fermentation. In the case of higher doses of leaves (H14 and G14 variants),
it also impacted the later stages of the process and the final product. The increase in the number of LAB in the experimental variants compared to the control sample could result from two factors. The first is the effect of inoculation with native LAB living on the leaves, which affected the interactions within the microbial metapopulation on the metabolic level. It ultimately determines specific taxonomic balance within the microbial system. Perhaps a larger initial number of these microorganisms contributed to the stabilization of their dominance in the further stages of fermentation. A similar effect was observed in other studies using LAB inocula during the fermentation of table olives [26]. However, the importance of the native leaf microflora cannot be confirmed, because there are no studies concerning the microbiome of grapevine and horseradish leaves grown in European regions. A study on the grape leaf microbiome in another geographical region focused on the dominant microbial groups and did not address lactic acid microorganisms directly [27].

The second factor that possibly impacts the number of LAB is the extraction phenomenon of various bioactive compounds from horseradish and grapevine leaves. It may affect the growth and activity of LAB and may have an effect on biotic interactions within the bacteria population. The most important bioactive compounds in horseradish leaves include rutin, quinic acid, and malic acid. A study by Mazzeo et al. (2015) [28] indicated the important role of rutin in the expression levels of proteins involved in the response to stress, like the integrity of cell walls and carbohydrate and amino acid metabolism. It is also well known that lactic acid bacteria can decompose quinic acid and malic acid, which may finally give them a metabolic predominance over other groups of microorganisms during the fermentation of plant raw materials [28–30]. Grapevine leaves are a rich source of bioactive compounds, including quercetin and resveratrol [20]. Quercitin has strong antimicrobial activity against both Gram-negative and Gram-positive bacteria; however, research indicates a low inhibitory effect in the case of microorganisms belonging to the Lactobacillus genus, which may result in a metabolic predominance of lactic acid bacteria in the microbial population in the presence of these compounds [31,32]. In previous studies, resveratrol favored adhesion and biofilm formation towards bacteria belonging to the Lactobacillaceae family, which could also contribute to the increased competitiveness and survival of these microorganisms in the fermentation environment [33]. It should be emphasized that due to the extraction time, the significant impact of biologically active compounds was probably most important in the later stages of fermentation, and the increase in the number of LAB on the first day resulted from the biotic factor and the effect of a larger inoculum.

It is worth emphasizing that all investigated samples contained more than 8.00 Log CFU LAB/mL. Following the guidelines proposed in recent literature, they can be considered a good source of probiotic microorganisms [34,35]. Following global trends, traditional probiotic foods are desired by consumers due to the growing interest in the microbiome health area. Thus, the traditional, sustainable food sector can be competitive with the global supplement market [36].

The presence of a stable population of lactic acid microorganisms in fermented cucumbers is crucial in the context of the potential for the synthesis of bacteriocins—compounds with bactericidal and bacteriostatic properties, acting as “natural antibiotics” and important in the food industry [37]. This study confirmed the positive effect of the addition of horseradish and grapevine leaves on the expression of genes responsible for the synthesis of plantaricins (Plantaricin A, Plantaricin EF, Plantaricin J, and Plantaricin K) and acidocin, which in previous studies showed activity against food spoilage microorganisms [38–40]. The higher gene expression levels can be explained (as in the case of the increase in the number of LAB) by the effect of inoculation of microorganisms living on the leaves, including those possessing bacteriocin genes. Another interesting explanation is the autoinduction mechanism highlighted in a previous publication. Maldonado et al. (2004) indicated that the presence of specific groups of microorganisms may act as a specific type of signal improving the production of bacteriocins from the plantaricin group. Perhaps the microbiome of horseradish and grapevine leaves, in addition to being a carrier of genes for the synthesis of bacteriocins, played the role of a carrier of microorganisms, stimulating the processes
of synthesis of these proteins [41]. Due to the need to minimize co-occurring factors, this topic requires further research using pure strains of microorganisms.

Results indicate that the addition of grapevine leaves may increase the health-promoting potential of fermented cucumbers, due to the increased concentration of gallic acid and elagic acid in the brine. Moreover, cucumbers pickled with horseradish leaves can be classified as a rich source of elagic acid. In the case of both additives, it is beneficial to add higher doses of leaves. Gallic acid is considered a compound with therapeutic effects in the treatment of gastrointestinal, neuropsychological, metabolic, and cardiovascular disorders. Ellagic acid has the potential to serve as an anti-atherogenic, anti-inflammatory, and neuroprotective agent [42,43]. Therefore, the consumption of cucumbers fermented with the addition of these leaves may be helpful in diet therapy and diet prevention of many diseases.

The overall desirability analysis showed that cucumbers fermented with the addition of horseradish and grapevine leaves were more attractive to consumers than the control samples. It can be concluded that these leaves have a high application potential, especially in the context of designing health-promoting foods with greater consumer acceptance. The optimal seems to be the variants containing the addition of horseradish leaves. The amount of leaves in the analyzed range, both in the case of horseradish and grapevine leaves, did not make any differences in the acceptance level.

Penalty analysis allowed for the identification of general features affecting consumer acceptance of cucumbers fermented with the addition of horseradish and grapevine leaves. Both lack (color in all cases) and excess of some features (astringency in all cases and bitterness in the case of horseradish leaves) negatively affected the evaluation of the product. However, the addition of both horseradish and grapevine leaves contributed to a decrease in the number of penalizing attributes compared to the control sample. Moreover, it resulted in the elimination of features that negatively affect consumer acceptance: too low firmness, too low astringency, too low bitterness, and too low fermented cucumber aroma. A quality feature that should be paid attention to during further optimization of the product is too low color, too much astringency, and too much bitterness. However, it is worth emphasizing that both overall desirability analysis and the penalty analysis were conducted on a one ethnic group of people (Polish nationality) probably with similar taste perceptions [44]. Introduction of the low-salt fermented cucumbers with grapevine and horseradish leaves and optimization of the product may require additional preference studies.

5. Conclusions

The research results indicate that the use of horseradish and grapevine leaves in the traditional low-salt fermentation of cucumbers which do not meet aesthetic market standards leads to the production of a high-quality final product that can be considered a source of probiotic microorganisms. The application of these sustainable by-products contributed to an increase in the concentration of antioxidant compounds: gallic acid (grape leaves) and ellagic acid (grapevine and horseradish leaves). Final products were characterized by high consumer acceptance and health-promoting properties. The most optimal variants seem to be those containing horseradish and grapevine leaves in higher doses (14 g/jar). Further analysis is recommended to improve the quality of the product, analyze its international acceptance, and investigate other groups of bacteriocins. During product improvement, the possibility of using additives that enhance color and minimize the feeling of astringency (horseradish and grapevine leaf variants of fermentation) as well as bitterness (horseradish leaf variant of fermentation) should be considered. Summarizing the conclusions of the manuscript, the application of horseradish and grapevine leaves in the process of traditional low-salt fermentation of cucumbers follows the circular bioeconomy concept and allows for obtaining nutritional, health-promoting, and socio-economic benefits.
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