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Microbial Growth Dynamics in Minced Meat Enriched with Plant Powders

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Abstract: Plant powders with antimicrobial properties can be used in food manufacturing and must comply with the demands of consumers regarding microbiological safety, nutritional value, and sensory properties of foods. The present study aimed to assess the microbial growth inhibitory ability of different plant powders, including by-products of horticultural primary processing (e.g., pomace) in raw and cooked minced pork. The total counts of aerobic mesophilic bacteria, pseudomonads, yeasts, and moulds were studied to assess the microbial growth dynamics in meat samples. Additionally, for the plant powders, which were able to suppress the microbial growth in a total counts dynamics study, the growth potential of *Listeria monocytogenes* in ready-to-eat (RTE) minced meat samples was estimated by challenge testing. The results showed that the most effective combinations of plant powders in raw minced pork, in relation to the total counts of microorganisms, were 3% apple+1% onion+2% blackcurrant berries (Apple+On+BCber); 3% apple+1% garlic+2% tomato (Apple+Ga+Tom); and 3% apple+2% tomato+1% rhubarb petioles (Apple+Tom+Rhub). However, challenge tests revealed that some plant powders were unable to inhibit the growth of *L. monocytogenes*. The lowest *L. monocytogenes* growth potential (δ = 2.74 log cfu/g) was determined for cooked minced pork samples enriched with 2% rhubarb petioles, followed by Apple+On+BCber (δ = 3.63 log cfu/g) and Apple+Tom+Rhub (δ = 3.74 log cfu/g). In minced pork samples without plant additives, the *L. monocytogenes* growth potential was 7.30 log cfu/g. In conclusion, blends of plant powders may have good potential for developing meat products with acceptable microbiological quality.

Keywords: plant powders; minced meat products; microbial growth inhibition; food safety

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1. Introduction

Meat and meat products contain valuable nutrients for human consumption; meat is an excellent source of protein, phosphorus, zinc, and iron [1]. Since raw minced meat is an easily perishable food, it is a suitable material for evaluating the inhibitory effect of various plant additives and their blends on microbial growth in the food matrix. European Commission Regulation No 2073/2005 for microbiological criteria in foodstuffs establishes criteria for aerobic colony count in minced meat [2]. According to the process hygiene criteria, the maximum number of aerobic mesophilic microorganisms can be 6.70 log cfu/g for two subsamples and 5.70 log cfu/g for three subsamples. These numbers will be considered when discussing the microbiological quality of the raw minced pork samples in the present study. The total viable count of microorganisms is often used in food durability studies to assess the microbiological quality of food within the determined time period.

Enumeration is one of the most commonly used forms of bacteriological analysis in food microbiology [3].

Various food preservation methods and technologies, such as modified atmosphere and vacuum packaging, marinating, and the use of preservatives are applied to enhance the safety and shelf-life of meat products by inhibiting microbial growth [4]. Nowadays, consumers are more aware of the substances used in food production and are also concerned about the worldwide problem of the multi-drug resistance of microorganisms. This has led to a growing trend to seek natural food additives that can be used for the inhibition of chemical and microbiological spoilage of foods [5].

It has been demonstrated that many plants are effective in microbial growth inhibition in foods [6–8]. In addition to an antibacterial effect, many plants rich in flavonoids, phenolic and other organic acids have antioxidant, nutritional, and sensory properties in foods [7]. Often, in vitro experiments have been used to study the antioxidant and antibacterial properties of plant materials. Results of in vitro studies are not directly correlated to the effects in different food matrices, because bioactive compounds can be bound to proteins, lipids, or other food ingredients. Moreover, other intrinsic factors can inhibit the antimicrobial effects of bioactive compounds in foods [7,9]. The use of plant materials containing bioactive compounds such as fruits, vegetables, berries, and their processing by-products is an opportunity to exploit these valuable resources for further use in different food matrices. Furthermore, the use of natural antimicrobials can prevent the negative impact of some synthetic food additives, such as hypersensitivity reactions and intolerance, and achieve the health-promoting effects of plant-derived bioactive compounds on consumers' health [7,10]. Previous studies [5,8] have indicated that garden rhubarb petioles (*Rheum rhaponticum* L.), blackcurrant berries (*Ribes nigrum* L.), chokeberries (*Aronia melanocarpa* Elliott), and tomato (*Solanum lycopersicum* L.) have antioxidant and antimicrobial properties both in vitro and in producto. Moreover, garlic (*Allium sativum* L.) and onion (*Allium cepa* L.), which are both rich in sulphur compounds and phenolic substances, have good antimicrobial properties [11,12]. Furthermore, allicin in pure form has been found to have antibacterial, antifungal, antiparasitic, and antiviral activity [13]. Garden rhubarb petioles have low total phenolic contents but contain organic acids in large amounts [5]. Blackcurrant berries have high contents of anthocyanins and phenolic acids [14]. Chokeberry berries have high contents of carotene, flavonoids, anthocyanins, and catechines [15]. Tomato is a good source of lycopene, phenolic acids, and flavonoids, and effectively inhibits the growth of yeasts and moulds [8]. Rowanberries (*Sorbus aucuparia* L.) have high contents of phenolics, flavonoids, and ascorbic acid [16]. Bombinaitė et al. [17] found that rowanberry pomace extracts inhibited the growth of pathogenic and spoilage microorganisms, especially Gram-positive bacteria.

The current study compared the impact of various plant powders, such as fruit, berry, and vegetable processing residues, on microbial growth in raw and cooked minced pork throughout the shelf-life. The growth potential of *Listeria monocytogenes* in samples of ready-to-eat (RTE) minced meat was also assessed by challenge testing in which the most efficient plant powders were used.

2. Materials and Methods

2.1. Meat and Plant Material

For the first test series, to get the freshest meat possible and to obtain low-fat content (13%), samples were brought directly from the meat industry and ground by the research group itself. The meat was stored in a refrigerator at a temperature not exceeding 5 °C. For the second test series, commercial minced pork with a fat content of 27% was used. Fresh ground pork in modified atmosphere packages was purchased from a retail outlet and transported immediately to the laboratory at refrigerated temperatures.

The plant materials used were mostly by-products of fruit, berry, or vegetable processing, e.g., press cakes from juice production. The plant materials were either convection-dried or freeze-dried and were added to the minced meat as a powder. The selection of

plant materials and the added amounts of powders was decided according to the results of preliminary sensory and chemical tests. Added plant powders changed the colour of the minced pork depending on the powders used. Overall acceptance was estimated in regard to taste, colour, odour, and appearance of the cooked samples.

Two test series were performed at different time periods with the same fruit, berry, and vegetable additives (Table 1). The pomace derived from juice pressing of apples (*Malus domestica* Borkh.), blackcurrants (*Ribes nigrum* L.), rowanberry (*Sorbus* sp.), and black chokeberry (*Aronia melanocarpa* (Michx.) Elliott), the petioles of rhubarb (*Rheum* sp.), fruit flesh of tomato (*Solanum lycopersicum* L.), onion (*Allium cepa* L.), and garlic (*Allium sativum* L.) were used as plant materials for powder preparation at the laboratories of the Polli Horticultural Research Center and the Food Hygiene laboratory of Estonian University of Life Sciences. The drying technology used was freeze-drying using a VirTis AdVantage 2.0 EL freeze-dryer (SP Industries, Warminster, PA, USA) and Alpfrigo CFD 1400 SS condensation fruit dryer (Logatec, Slovenia). The dried material was ground to obtain a fine powder using a Retsch GM 300 grinding mill (Retsch GmbH, Haan, Germany) and Retsch MM 400 mixer mill.

Table 1. Description of the samples used in experiments.

Abbreviation	Composition
Meat (Control)	Minced meat with 13% and 27% fat content
Apple	Minced meat with 3% apple
Apple+BCber	Minced meat with 3% apple+2% blackcurrant berries
Apple+Rber	Minced meat with 3% apple+2% rowanberries
Apple+Tom	Minced meat with 3% apple+2% tomato
Apple+On+BCber	Minced meat with 3% apple+1% onion+2% blackcurrant berries
Apple+Ga+Tom	Minced meat with 3% apple+1% garlic+2% tomato
Apple+CHber	Minced meat with 3% apple+2% chokeberries
Apple+CHber+Rber	Minced meat with 3% apple+2% chokeberries+2% rowanberries
Apple+Tom+Rhub	Minced meat with 3% apple+2% tomato+1% rhubarb petioles
Garlic	Minced meat with 2% garlic *
Onion	Minced meat with 2% onion *
Rhub	Minced meat with 2% rhubarb petioles *

* Only for challenge test series. Abbreviations: BCber, blackcurrant berries; Rber, rowanberries; Tom, tomato; On, onion; Ga, garlic; CHber, chokeberries; Rhub, rhubarb petioles.

2.2. Sample Preparation

A hand mixer, the Clatronic HM 2935 (Clatronic International GmbH, Kempen, Germany), was used to completely mix the minced meat with the plant powders (Table 1). Mixing was performed for three minutes at speed level one. As a control, pure minced meat was used. The samples were formed into meatballs (20 ± 1 g each) and half of them were cooked at 145 °C for 17 min reaching an internal temperature of 75 °C. A digital meat thermometer with a stainless-steel probe was used to measure the meatballs' core temperature. The samples were then cooled to room temperature. The final weight of the cooked samples was 15 ± 1 g. According to the challenge test guidance document [18], part of the samples was inoculated with a suspension of *L. monocytogenes*, and the rest with physiological saline to equalize the amount of liquid added to all samples. Samples were packed individually in sterile cups with a screw cap and stored in a cooling incubator (Binder GmbH, Tuttlingen, Germany) at 7 ± 1 °C for a maximum of 12 days for raw and 21 days for cooked samples. There were separate samples for pH and water activity analyses. Analyses of water activity, pH, and microorganisms were carried out simultaneously and in parallel on days 1, 5, 7, 9, and 12 with raw samples and on days 1, 12, and 21 with cooked minced pork samples.

2.3. pH Determination

The homogenates, made up of 10 g of sample and 100 mL of distilled water, were used to measure the pH values of the samples. A digital HandyLab680 pH meter (SI Analytics

GmbH, Mainz, Germany) was used to take the readings at room temperature. Regular checks of the pH meter calibration and duplicate analyses were made.

2.4. Water Activity (a_w) Determination

Water activity (a_w) was determined at 25 °C, using an Aqualab Decagon 3TE water activity meter (Decagon Devices Inc., Pullman, WA, USA) following the manufacturer's instructions. The analyses were carried out in duplicate.

2.5. Microbial Enumeration

For determination of total microbial counts, EVS-EN ISO 4833-2:2013 standard Part 2: Colony count at 30 °C by the surface plating technique; EVS-ISO standard 21527-1:2009 Horizontal method for the enumeration of yeasts and moulds—Part 1: Colony count technique in products with water activity greater than 0.95; and EVS-EN ISO standard 13720:2010 Meat and meat products—Enumeration of presumptive *Pseudomonas* spp. instructions were followed.

EVS-EN ISO 11290-2:2017 standard Microbiology of the food chain—Horizontal method for the detection and enumeration of *L. monocytogenes* and of *Listeria* spp. Part 2: Enumeration method was followed. Briefly, a Kern KB 2000-2N scale (Kern & SOHN GmbH, Balingen, Germany) was used to weigh 10 g of the material into a sterile Stomacher bag and diluted with 90 mL of sterile buffered peptone water (LAB204, Lab M, Lancashire, UK) to obtain an initial 10-fold dilution. Samples were blended for one minute at 230 rpm using a Stomacher™ 400 Circulator (Seward, UK). Plate Count Agar (PCA, LAB010, Lab M, Lancashire, UK) was used for the enumeration of microorganisms; DRBC Agar (ISO) (LAB217, Lab M, Lancashire, UK) for the enumeration of yeasts and moulds; *Pseudomonas* Agar Base (CFC, Biolife, Italiana S.r.l. Viale Monza, Milano, Italia) and Agar *Listeria* according to Ottaviani and Agosti for the enumeration of *L. monocytogenes* (ALOA, Biolife, Italiana S.r.l. Viale Monza, Milano, Italia).

The surface plating technique was used for all analyses. For small agar plates (90 mm), 100 µL and for large (120 mm), 1000 µL of initial dilution of the sample and further decimal dilutions were transferred onto the surface of the agar plates and spread evenly using a spatula. PCA plates were incubated under aerobic conditions at 30 °C for 72 ± 3 h; DRBC agar plates at 25 °C for 5 days; CFC agar plates at 25 °C for 44 ± 4 h; and ALOA plates at 37 °C for 24–48 h. Colonies were enumerated following incubation under the stated temperatures, and the results were represented as log10 colony-forming units per gram (cfu/g).

2.6. Challenge Test

Challenge testing was carried out in accordance with the technical guidance document version 4 of the European Union Reference Laboratory for *L. monocytogenes* (EURL Lm) in order to determine the growth potential (δ) of *L. monocytogenes* in cooked minced samples [18]. In short, three batches of samples for each day of analysis were artificially contaminated with a microbial suspension of *L. monocytogenes* strains using a syringe. The volume of the inoculum introduced into the food matrix did not exceed 1% of the whole mass [18]. Three different points of the sample were injected with 100 µL of the mixture at an approximate concentration of 100 cfu/g. The inoculated samples were packed separately into sterile cups and stored in an incubator at 7 ± 1 °C until the analysis point. Total microbial count, pH, and water activity (a_w) were also determined in two parallels on each analysis day. Based on the enumeration of *L. monocytogenes*, the growth potential of the pathogen was determined.

Bacterial Species and Strains

The microbial suspension of *L. monocytogenes* contained a mixture of two strains (12MOB045LM—serotype 1/2c and 12MOB089LM—serotype 4b) chosen from a set of 25 EURL Lm strains. The strains set was classified according to their growth rates related to

temperature, pH and a_w, genoserotypes, and origins [18]. Both strains with known growth characteristics were isolated from the meat. The suspension was prepared following the instructions of the challenge test guidance document [18]. In brief, two of the bacterial strains were grown in tryptone soya broth (TSB, Biolife, Italiana S.r.l. Viale Monza, Milano, Italia) at 37 ± 1 °C for 16 h to obtain subculture 1 in the early stationary phase (at about 9.20 $\log_{10}$ cfu/mL). Subculture 1 was transferred into another tube of TSB and was incubated at 10 °C for 3 days to obtain subculture 2. Subsequently, subcultures 2 of both strains were mixed in equal amounts. Successive dilutions of the mixed culture in physiological saline water were followed to obtain the inoculum at the desired concentration (100 cfu/g).

2.7. Statistical Analysis

Significant differences in counts between minced meat control and minced meat with added plant powders (day 5, day 7, day 9, and day 12 combined) were analysed using the Kruskal–Wallis rank sum test followed by multiple pairwise-comparisons between groups using the Wilcoxon rank sum test with Benjamini–Hochberg continuity correction.

3. Results

The enumeration of microorganisms able to grow at 30 °C, enumeration of yeasts and moulds, and enumeration of presumptive *Pseudomonas* spp. were made to determine the inhibitory effect of selected plant powder mixtures on microbial growth in raw and cooked minced pork. Additionally, for selected enriched ready-to-eat minced pork samples, *L. monocytogenes* growth potential was studied by using a challenge test.

3.1. Microbial Counts in Plant Powders

The results of the enumeration analyses of the tested plant powders showed that the total viable counts of mesophilic microorganisms and yeasts and moulds ranged from 100 to 4500 cfu/g and 20 to 1900 cfu/g, respectively (Table 2). The numbers of *Pseudomonas* spp. in fruit, berry, and vegetable powders were below the limit of detection (100 cfu/g).

Table 2. The microbial counts in plant powders.

Plant Powder	Total Viable Count (cfu/g)	*Pseudomonas* Count (cfu/g)	Yeast and Mould Count (cfu/g)
Apple	150	<100	30
Blackcurrant berry	680	<100	230
Rowanberry	100	<100	140
Tomato	200	<100	40
Onion	2100	<100	350
Garlic	1200	<100	1900
Chokeberry	4500	<100	20
Rhubarb petioles	400	<100	20

3.2. Total Viable Counts in Raw Meat Samples

Similar growth dynamics of microorganisms were observed in the raw minced pork samples. In the initial raw pork samples containing 13% of fat, total microbial counts were on average in the range of 4.30–4.75 log cfu/g (Figure 1A). In the following days, the number of microorganisms increased in most samples and was above 8 log cfu/g by day 12. Compared to the other samples, the total number of microbes in the Apple+On+BCber, Apple+Ga+Tom, and Apple+CHber+Rber samples were lower until day 9 of storage (4.48, 6.11 and 6.45 log cfu/g, respectively). In these samples, the rapid growth of microorganisms started after the 9th day of storage, when the numbers of mesophilic microorganisms were 5.06, 7.57, and 8.89 log cfu/g, respectively. However, a statistically significant difference ($p < 0.05$) compared to the control (raw minced meat) in mean microbial counts during the storage time was only found for raw minced meat with Apple+On+BCber.

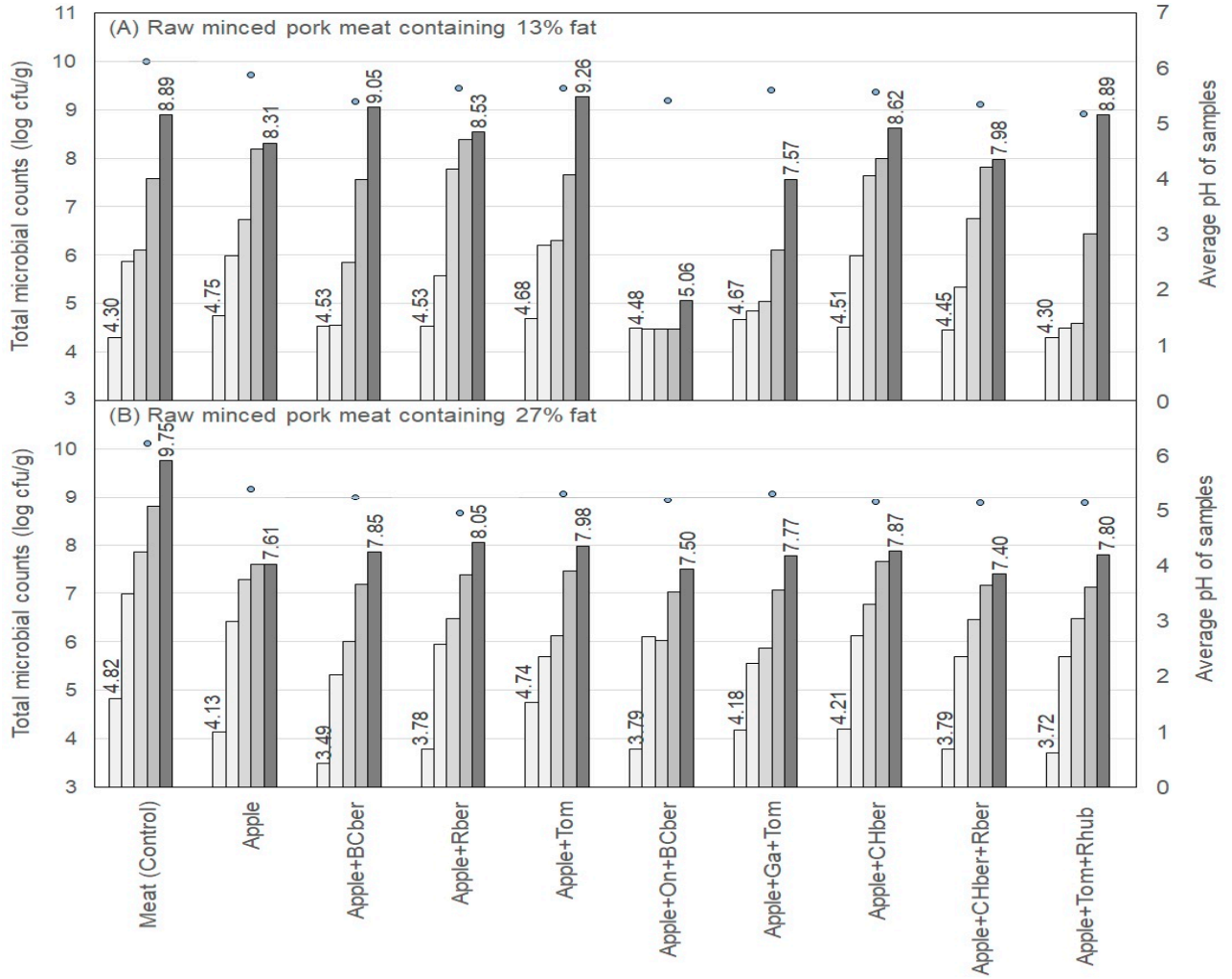

Figure 1. Total microbial counts and average pH (•) of raw samples containing 13% (**A**) and 27% (**B**) fat after 1, 5, 7, 9, and 12 days of storage (columns from left to right). The values on the first and the last day are presented numerically. pH (•) values are shown as an average throughout the storage period. Abbreviations: BCber, blackcurrant berries; Rber, rowanberries; Tom, tomato; On, onion; Ga, garlic; CHber, chokeberries; Rhub, rhubarb petioles.

In the series of experiments with a higher fat content of minced pork samples (27%), the total number of microorganisms increased from an initial range of 3.49 to 4.82 log cfu/g to 7.40 to 9.75 log cfu/g at the end of the storage period on day 12 (Figure 1B). Differences in total microbial counts between control and minced meat samples supplemented with plant powders were not statistically significant. Compared with 13% fat-containing minced pork samples, microbial growth inhibition was lower for 27% fat-minced pork samples enriched with plant powders.

3.3. Pseudomonas Counts in Raw Meat Samples

On day 1, the numbers of *Pseudomonas* spp. in the raw pork samples containing 13% fat were in the range of 4.19–4.74 log cfu/g and increased to 4.88–9.26 log cfu/g by day 12 (Figure 2A). However, in the samples of Apple+On+BCber and Apple+Tom+Rhub the total number of *Pseudomonas* spp. remained low throughout the study period, between 4–6 log cfu/g, and were significantly different ($p < 0.05$) compared to the control. Inhibition of microbial growth was also observed for Apple+Ga+Tom samples up to the 9th day of storage.

The control sample with a fat content of 27% had the highest concentration of *Pseudomonas* spp. during the entire storage period (Figure 2B). Compared to the control and other samples, the counts of pseudomonads in the Apple+Ga+Tom sample remained almost at the same level as in the initial phase of the food durability experiment. Inhibition of pseudomonads growth was also observed for Apple+On+BCber and Apple+CHber+Rber (Figure 2B) and was significantly ($p < 0.05$) different from the control.

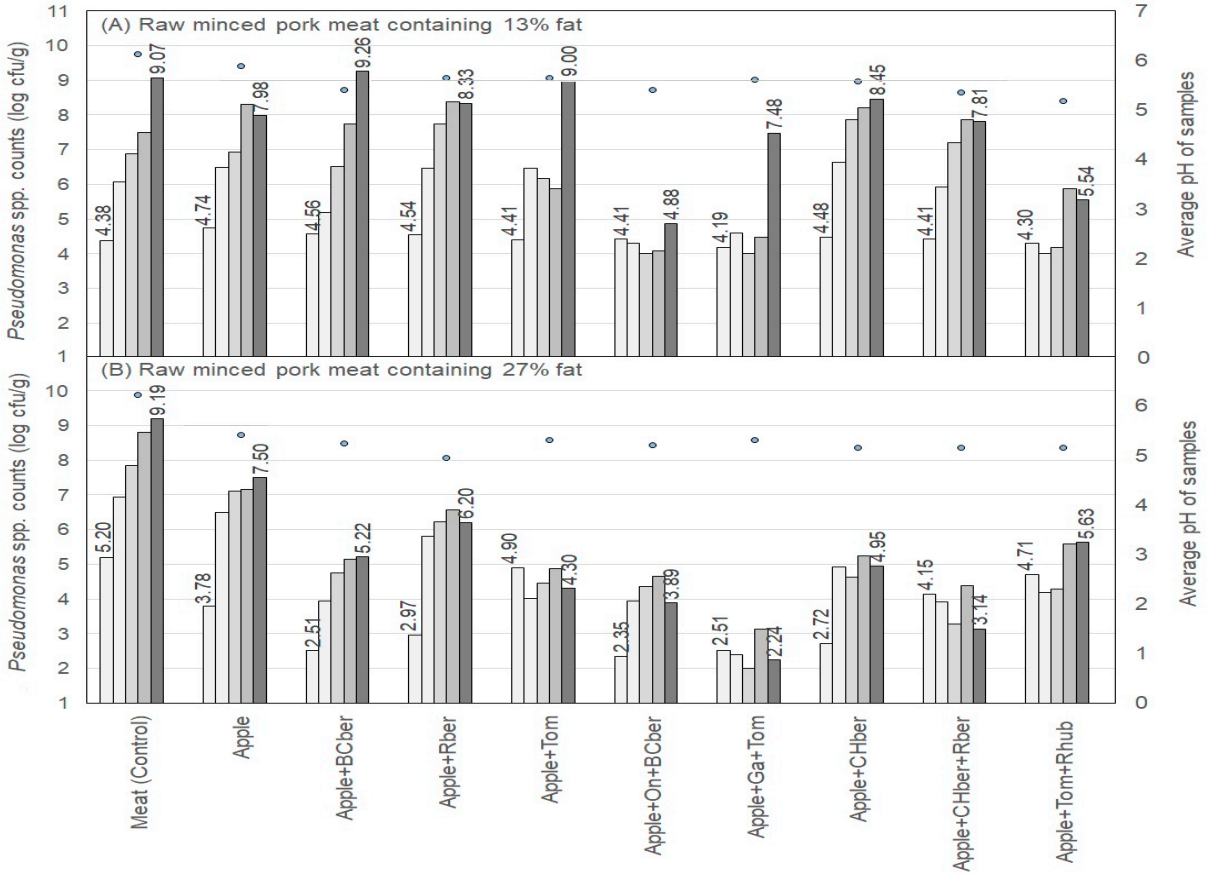

Figure 2. The number of *Pseudomonas* spp. and average pH (•) of raw samples containing 13% (**A**) and 27% (**B**) fat after storage times of 1, 5, 7, 9, and 12 days (columns from left to right). The values on the first and the last day are presented numerically. pH (•) values are averages throughout the storage period. Abbreviations: BCber, blackcurrant berries; Rber, rowanberries; Tom, tomato; On, onion; Ga, garlic; CHber, chokeberries; Rhub, rhubarb petioles.

3.4. Yeast and Mould Counts in Raw Meat Samples

The initial counts of yeasts and moulds in the raw minced pork samples containing 13% and 27% fat were 3.37–3.91 log cfu/g and 2.04–3.89, respectively (Figure 3A,B). In the subsequent days of storage, the numbers of moulds and yeasts increased in all of the 13% fat raw pork samples and were in the range of 4–6 log cfu/g on the last day of storage. Slight growth inhibition of yeasts and moulds was observed for meat samples containing blends of Apple+On+BCber and Apple+Ga+Tom, but only up to the 9th day of storage. Compared with low-fat samples, the high-fat-minced pork samples had higher yeast and mould counts on the last day of storage (day 12) (Figure 3B). However, compared with the control sample, yeast and mould growth was more inhibited in the higher-fat-minced pork samples enriched with plant powders. However, statistically significant differences ($p < 0.05$) in yeast and mould counts were found only for the raw minced pork samples of Apple+Ga+Tom and Apple+Tom.

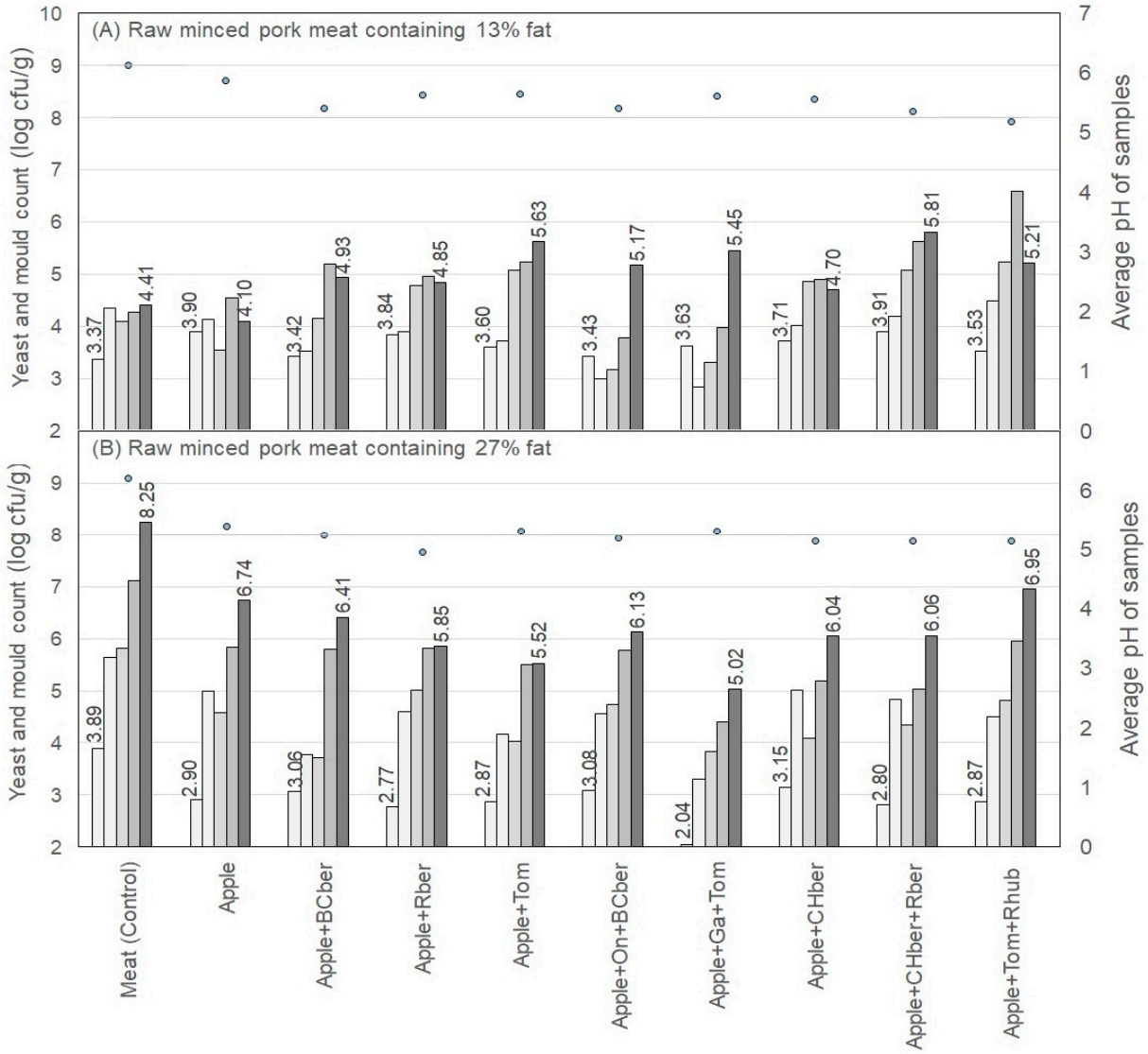

Figure 3. Yeast and mould counts and average pH (•) of raw samples containing 13% (**A**) and 27% (**B**) fat 1, 5, 7, 9, and 12 days of storage (columns from left to right). The values on the first and the last day are presented numerically. pH (•) values are shown as an average throughout the storage period. Abbreviations: BCber, blackcurrant berries; Rber, rowanberries; Tom, tomato; On, onion; Ga, garlic; CHber, chokeberries; Rhub, rhubarb petioles.

3.5. Differences in Microbial Counts in Raw Meat Samples

Statistically significant ($p < 0.05$) differences between controls (minced meat) and minced meat samples with plant powders to the average level of different microbial counts over storage time are shown in Table 3. The biggest inhibition of the growth of total microbes was found in the minced meat containing Apple+On+BCber. Regarding *Pseudomonas* spp. count, the most effective growth inhibition was found for the Apple+On+BCber and Apple+Tom+Rhub samples. Compared to controls, the best growth inhibition of yeasts and moulds was found for Apple+Tom+Rhub in the 13% fat-content meat samples, and for the Apple+Tom and Apple+Ga+Tom samples of minced meat with 27% fat content. Statistically significant differences ($p < 0.05$) were found for the total counts of *Pseudomonas* spp. between minced meat controls (13% and 27%) and minced meat samples with added plant powders.

Table 3. *p*-values of differences in microbial counts between minced meat controls (13% and 27% fat content) and minced meat samples with added plant powders on 5, 7, 9, and 12 storage days combined.

Group	Minced Meat 13%			Minced Meat 27%		
Plant Powder	**Total Microbial**	***Pseudomonas* spp.**	**Yeasts and Moulds**	**Total Microbial ***	***Pseudomnas* spp.**	**Yeasts and Moulds**
Apple	0.901	0.811	0.827	ND	0.030 **	0.142
Apple+BCber	0.892	1.000	0.421	ND	0.001 **	0.160
Apple+Rber	0.901	0.621	0.130	ND	0.001 **	0.160
Apple+Tom	0.586	0.630	0.190	ND	0.002 **	0.014 **
Apple+On+BCber	0.008 **	0.004 **	0.329	ND	0.002 **	0.142
Apple+Ga+Tom	0.315	0.053	0.421	ND	0.002 **	0.007 **
Apple+CHber	0.782	0.621	0.114	ND	0.002 **	0.067
Apple+CHber+Rber	0.964	1.000	0.087	ND	0.001 **	0.067
Apple+Tom+Rhub	0.915	0.004 **	0.022 **	ND	0.001 **	0.380

* Non-significant differences (Kruskal–Wallis rank sum test *p*-value = 0.099). ** Statistically significant differences (*p*-value < 0.05). Abbreviations: ND, multiple pairwise-comparison not performed; BCber, blackcurrant berry; Rber, rowanberry; Tom, tomato; On, onion; Ga, garlic; CHber, chokeberry; Rhub, rhubarb petioles.

3.6. Microbial Counts in Cooked Meat Samples

The length of durability studies for cooked samples was longer than for raw samples. Microbiological analyses were performed on days 1, 12, and 21 of the storage period. In general, the microbial counts were very low in the cooked samples throughout the storage period. In all tested samples, the *Pseudomonas* spp. counts as well as those of yeasts and moulds were below the limit of detection (10 cfu/g) throughout the storage period. The initial counts of total mesophilic microorganisms were below 10 cfu/g and increased to 10–400 cfu/g at the end of the storage period.

3.7. pH and Water Activity

Throughout the study period, the pH of the control samples containing either 13% or 27% fat was higher (pH = 5.88–6.84) compared to the minced meat samples enriched with plant powders (Figures 1–3). The lowest pH values were found in the Apple+Tom+Rhub samples in a range from 5.1–5.2, followed by the Apple+CHber+Rber and Apple+Ga+Tom in a range from 4.9–5.5, and the Apple+BCber and Apple+On+BCber samples in a range from 5.1–5.6. The water activity in the samples ranged from 0.977 to 0.987. The control had higher water activity throughout the study period compared to the samples enriched with plant powders.

3.8. Challenge Testing

The initial total viable counts of the cooked minced pork samples ranged from 10 to <100 cfu/g and increased to a range of 20–250 cfu/g by the end of the storage period. The total number of bacteria remained very low in the cooked samples supplemented with garlic, onion, and rhubarb petioles (20–55 cfu/g).

As shown in Table 4, all the cooked minced pork samples supported the growth of *L. monocytogenes* as the growth potential was higher than 0.5 log cfu/g during the specified shelf-life. The highest growth potential (δ = 7.92 log cfu/g) was found for the meat samples with onion, followed by garlic (δ = 7.85 log cfu/g), and in the meat sample without additive (δ = 7.3 log cfu/g). These samples had a higher pH (6.30–6.73) compared to the other samples (pH < 6).

During the 12-day storage period, the lowest *L. monocytogenes* growth potential was determined for RTE minced pork samples enriched with rhubarb (δ = 2.74 log cfu/g). Moreover, the blends of Apple+On+BCber (δ = 3.63 log cfu/g) and Apple+Tom+Rhub (δ = 3.74 log cfu/g) showed higher growth inhibition of *L. monocytogenes* compared to the other tested samples (Table 4).

Table 4. Results of challenge tests of cooked meat samples.

Samples	Storage Day	pH *	Water Activity * a_w	Total Count (cfu/g)	δ (log cfu/g) **
Meat ***	0	6.35 ± 0.048	0.986 ± 0.003	$<1.0 \times 10^2$	7.30
	6	6.30 ± 0.021	0.987 ± 0.003	4.3×10^2	
	12	6.35 ± 0.001	0.992 ± 0.005	2.5×10^2	
Apple+On+BCber	0	5.75 ± 0.071	0.987 ± 0.002	$<1.0 \times 10^2$	3.63
	6	5.70 ± 0.016	0.985 ± 0.001	$<1.0 \times 10^2$	
	12	5.72 ± 0.005	0.984 ± 0.004	1.0×10^2	
Apple+Ga+Tom	0	5.93 ± 0.066	0.986 ± 0.001	$<1.0 \times 10^2$	5.35
	6	5.87 ± 0.021	0.990 ± 0.001	1.0×10^2	
	12	5.95 ± 0.010	0.979 ± 0.001	2.0×10^2	
Apple+Tom+Rhub	0	5.51 ± 0.011	0.988 ± 0.002	$<1.0 \times 10^2$	3.74
	6	5.42 ± 0.013	0.987 ± 0.001	$<1.0 \times 10^2$	
	12	5.48 ± 0.012	0.977 ± 0.001	1.5×10^2	
Garlic	0	6.68 ± 0.015	0.986 ± 0.002	1.5×10^1	7.85
	6	6.73 ± 0.001	0.990 ± 0.003	5.5×10^1	
	12	6.72 ± 0.003	0.987 ± 0.002	2.0×10^1	
Onion	0	6.48 ± 0.011	0.964 ± 0.005	2.0×10^1	7.92
	6	6.58 ± 0.008	0.984 ± 0.001	3.6×10^1	
	12	6.60 ± 0.017	0.979 ± 0.001	3.0×10^1	
Rhub	0	5.24 ± 0.001	0.980 ± 0.001	1.0×10^1	2.74
	6	5.31 ± 0.013	0.987 ± 0.001	3.5×10^1	
	12	5.41 ± 0.059	0.979 ± 0.001	3.0×10^1	

* Values are mean (obtained from analyses in duplicate) ± SD (standard deviation). SD values are not shown when zero. ** Growth potential (δ) more than 0.5 log cfu/g indicates the samples which support the growth of *L. monocytogenes*. *** Purchased from a retail outlet (fat content 27%). Abbreviations: On, onion; BCber, blackcurrant berries; Ga, garlic; Tom, tomato; Rhub, rhubarb petioles.

4. Discussion

Most of the plant materials used in this study were selected based on the results of previous studies [5,8]. Raudsepp et al. [5] focused on in vitro experiments of selected plant–ethanol infusions against essential foodborne pathogens where the strongest inhibition of bacterial growth was observed in 96% ethanol infusions of the rhubarb roots, whereas the black chokeberry (*Aronia melanocarpa* (Michx.) Elliott), blackcurrant (*Ribes nigrum* L.), and edible honeysuckle (*Lonicera caerulea* L. var. *edulis* Turcz. ex Herder) berries had the highest antioxidative activity [5]. The plant powders and their mixes were added to minced meat in a study by Anton et al. [8] in order to examine their effects on the sensory properties, microbial growth, and lipid oxidation of meat during storage. The results revealed the high potential of rhubarb petioles (*Rheum* sp.), tomato (*Solanum lycopersicum* L.) powders, and their mixtures for the development of good quality meat products with lower salt content [8].

The current study used the most effective plant materials based on the aforementioned two studies with the aim to determine possible microbial growth inhibition in minced meat samples enriched with plant powders and their blends. Unlike the aforementioned previous studies, some of the powdered plant materials used for the enrichment of minced pork samples in the present study (e.g., apple, blackcurrant, rowanberry, and black chokeberry pomace) were derived from valuable juicing residues, which should not be wasted but recycled for valorization.

In the current study, the highest microbial growth inhibition effect was determined for the 13% fat content raw minced pork samples with Apple+On+BCber, Apple+Ga+Tom, and Apple+Tom+Rhub mixtures. Significant differences were not found between the samples with different fat contents, but, generally, the different microbial counts were higher in samples with 27% fat content. This is similar to the findings of Hurtado et al. [19], who found that microbial counts were significantly higher in pork samples with high-fat contents (20–30%) at low temperatures.

The current study's findings indicated that the combination of several different plant powders in raw minced pork samples was more effective against the growth of aerobic mesophilic microorganisms, pseudomonads, and yeasts and moulds compared to the samples containing only a single plant powder, or no plant additives (Control). Similarly, a study by Anton et al. [8] found that rhubarb petioles in raw meat samples and tomato, rhubarb petioles, and their combination in cooked meat samples had microbial growth inhibitory effects comparable to the effects of NaCl+$NaNO_2$. However, in the present study, the combination of Apple+BCber was effective only when it was supplemented with 1% onion. The combination of Apple+Tom was effective when 1% rhubarb petioles or 1% garlic was added to the meat samples. Onion and garlic are often used in recipes for meat products.

Many studies have found that various plant extracts (for example garlic *Allium sativum* L.), which are rich in sulphur compounds and phenolic substances have good antimicrobial properties against different microorganisms including pathogens [11,12]. Onion is one of the oldest cultivated plants with nutritious, antioxidant, and antimicrobial properties. The strong antibacterial activity of onion (*Allium cepa* L.) extracts against *Staphylococcus aureus* was found in the in vitro study of Ortiz [20]. Another study [21] found that onion had an inhibitory effect on bacterial growth, but this varied depending on the type of onion, its extraction concentration, and the bacteria tested.

The results of the present study indicate that plant powders processed under hygienic conditions have initially very low microbial counts, and, therefore, can be used for the enrichment of meat or other food products without irradiation. However, microbiological analyses of plant materials are necessary before their use in other foods because the high microbiological quality of plant powders cannot be assumed. The results of this study demonstrate that rhubarb petioles and selected blends of berries and fruits have an inhibitory effect on microbial growth in meat samples. A similar finding was made by Babaoğlu et al. [22], who demonstrated that beef patties containing berry pomace extracts had lower bacterial counts than the control. Additionally, it was found that the black chokeberry was the most promising preservative among the pomace extracts of other berries for samples under refrigerated conditions [22].

In the current study, very low total microbial counts were found in the cooked samples compared to the raw samples. Such a result was expected because effective heat treatment should destroy most of the initial content of microbes in meat products. The number of microorganisms was expected to grow slowly under refrigerated storage of cooked meat samples in screw-cap jars that were sterile prior to use.

Many studies [23–25] on *L. monocytogenes* have highlighted the need to change RTE food recipes to ensure food microbial safety including the inhibition of the growth of *L. monocytogenes*. Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs lays down the rules according to which the Food Business Operator (FBO) must comply and specifies microbiological criteria for *L. monocytogenes* in RTE foods. Applying the numeric *L. monocytogenes* criterion to RTE food products, food business operators need to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout its shelf-life [2]. According to the technical guidance document of the European Union Reference Laboratory for *L. monocytogenes*, the growth potential below the criterion 0.5 $\log_{10}$ cfu/g determines whether the RTE food is able to support the growth of *L. monocytogenes* or not [18]. In the current study, all samples supported *L. monocytogenes* growth. The highest growth potential of *L. monocytogenes* was in the meat samples with onion (δ = 7.92 log cfu/g) followed by garlic and meat samples without additives, and the lowest growth potential (δ = 2.74 log cfu/g) was in the samples enriched with 2% rhubarb petioles. This is in good agreement with the results of our previous studies, where rhubarb has been found to be one of the most effective plant materials against the growth of microorganisms.

The strong antibacterial effect of rhubarb roots has also been shown by Kosikowska et al. [26] and Raudsepp et al. [27]. In the current study, rhubarb petioles were selected because petioles are used as food and have good organoleptic properties in different food matrices. The second lowest growth potential of *L. monocytogenes* (δ = 3.63 log cfu/g) was

found in samples enriched with Apple+On+BCber. Additionally, blackcurrant berries, which are well-known for containing phenolic acids, anthocyanins, and other flavonoids, have shown good antioxidant and antimicrobial effects in both in vitro and *in producto* studies [8,27,28]. The growth potential value of 3.74 log cfu/g was found for cooked minced meat samples enriched with Apple+Tom+Rhub. Minced meat with Apple+Ga+Tom had a growth potential of 5.35 log cfu/g and in all other samples was higher than 7.00 log cfu/g. It is important to mention that the only additives used in the meat samples were plant powders and the samples were stored in sterile screw-cap jars, which means that no modification of the packaging atmosphere was used.

Our results indicate that plant powders alone cannot sufficiently inhibit the growth of *L. monocytogenes* in RTE minced meat samples. The combination with other food additives (e.g., preservatives) and/or modified packaging is often necessary to achieve the criterion of 0.5 log cfu/g. Comparing the results of the control and the treatment samples demonstrates that the plant powders do have some effect on *L. monocytogenes* growth, especially the blends of apple, rhubarb, tomato, onion, garlic, and blackcurrant. Other studies have also demonstrated that other plant materials including berries are effective against pathogenic microorganisms. Using electron microscopy, Wu et al. [29] showed that cranberry concentrate damaged the cell wall and cell membrane and induced cell lysis of *L. monocytogenes*. It was shown that plant extracts with high flavonoid contents can effectively inhibit the growth of Gram-positive bacteria by damaging the integrity of the cell wall and cell membrane, inhibiting the activity of the intracellular enzymes, changing the expression of associated genes, and inducing bacterial apoptosis [30]. Furthermore, Ravichandran et al. [31] showed that naturally occurring phenolic compounds in food matrices have antibacterial activity against *L. monocytogenes* and other pathogenic microorganisms.

Despite exceeding the *L. monocytogenes* growth potential criterion, the aerobic plate count levels in the specified storage period were very low, indicating that meat products with plant powders can have good microbiological quality and a sufficiently long shelf-life.

5. Conclusions

This shows that by-products obtained from the primary processing of some horticultural crops, such as fruits, berries, and vegetables, can be effectively used for the enrichment of meat products. Analyses demonstrated that rhubarb petioles and blends of selected berries and fruits have an inhibitory effect on microbial growth in meat samples. However, a combination of different plant powders may be necessary to achieve the microbial growth inhibition effect. The most effective combinations of plant powders in raw minced pork, against total counts of microorganisms, were 3% apple+1% onion+2% blackcurrant berries, 3% apple+1% garlic+2% tomato, and 3% apple+2% tomato+1% rhubarb petioles. Challenge tests demonstrated that plant powders could not fully inhibit *L. monocytogenes* from growing, therefore, in the technology of ready-to-eat foods, some additional intrinsic, extrinsic, or implicit factors need to be used. Nevertheless, blends of plant powders may have good potential for the development of meat products with acceptable microbiological quality. Moreover, the valorization of by-products generated during the primary processing of horticultural products is of great importance when considering the principles of circular bioeconomy and zero waste concepts.

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