Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2024, 30 (4), 366-373

Journal of Agroalimentary Processes and Technologies

The antimicrobial capacity of the fruits powders added to fortify the spreadable cream cheese

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Abstract

In this study, the spreadable cream cheese obtained from cow's milk has been fortified by fruit powders additions (aronia, sea buckthorn, rose hips, hawthorn) to improve the quality characteristics and increase the preservation degree. The physical-chemical properties after preparation and the microbiological ones at intervals of one, three, and ten days of refrigerated storage were analyzedin order to verify the antimicrobial properties of the added fruit powders. The results showed a slight increase in acidity and a decrease in moisture and fat content for all samples with fruit powders, compared to the control sample. The antimicrobial capacity was indicated by the decrease of the bacteria number, thus at the end of the 10 days monitorization interval the microbial count ($\log_{10}$ CFU g^{-1}) of TPC decreased by 19.25-21.80% compared to the control sample; also, EC was not detected at all in the samples with added powders, and CPS were reduced by 46.44-100%. Forest fruit powders can be used in the dairy products manufacturing as natural ingredients, offering resistance to contamination and consumer safety.

Key words: antimicrobial, fruit powder, spreadable cheese, shelf life

1. Introduction

Dairy producers want to extend the shelf life of their products in order to market them for longer periods of time. In this context, the integration of functional ingredients from plant sources in the cheese manufacturing process could be an effective strategy for improving the overall quality of the final products [1]. These functional compounds from cheeses satisfy the consumers needs and their lifestyles, aiming to maintain health and prevent the occurrence of diseases such as diabetes, obesity, hypertension, cardiovascular diseases, etc [2].

Cheeses, especially the unripenedones have a problem due to their relatively short shelf life, but this can be controlled by adding natural antimicrobial compounds to the main cheese formula [3]. Over the years, a series of vegetable extracts and by-productsresulting from the processing of citrus fruits, pomegranates, olives, mangoes, cucumbers, cumin, grape pomace, cranberries, garlic, etc. have been tested in the dairy sector [4-12], which added in the form of powders, oils or small pieces in cheeses showed antioxidant and antimicrobial properties improving the sensory profile, composition and shelf life. Specifically, obtaining powders from these fruits represents an alternative for consumption both by rehydration in the form of juice or infusion, as well as by adding them to desserts, dairy products, salads, ice cream, snacks, or even for enriching almost any food in bioactive compounds [13-14]. Among the various berries, aronia (*Aronia melanocarpa*) and sea buckthorn (*Hippophae rhamnoides*) are known to have an antibacterial effect on Gram-

DOI: 10.59463/JAPT.2024.2.35

positive pathogens [15-17]. Also, dog-rose (*Rosa canina*) and hawthorn (*Crataegus monogyna*) powders showed a good inhibitory activity against bacteria, such as *Staphylococcus aureus* - Gram-positive bacteria, followed by *Escherichia coli* and *Klebsiella pneumoniae* - Gram-negative bacteria [18]. In the hawthorn fruits extracts case, the antimicrobial activity was also observed for the strains of *Salmonella paratyphi A*, *Campylobacter jejuni*, *Klebsiella pneumoniae* and *Enteroc-occus faecalis* [19]. The fruits antimicrobial effect is due to their chemical composition, especially to the polyphenolic compounds, carotenoids, flavonoids, vitamins, etc. [20].

Microbiological hazards associated with cheese have been attributed to the consumption of soft cheeses with high moisture content, especially the raw milk cheeses [21], as well as to poor sanitation, which is the common route for pathogenic bacteria to contaminate the product during the manufacturing process [22]. The predominant bacterial pathogens that can be transferred through cheeses are *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, *Listeria monocytogenes* [23] and even if pasteurization destroys the potential pathogenic microorganisms, post-processing can lead to recontamination of the dairy products. Processed spreadable cheese is a ready-to-eat product made by mixing cheese, melting salts (eg sodium or potassium salts of phosphoric or citric acid) and other dairy and non-dairy ingredients, followed by heating and mixing to form a uniform mass [24-25]. Spreadable cream cheese belongs to the group of fresh cheeses, it has a white color and a mild, slightly acidic aroma. It is a product that will usually be kept cold and, for convenience, the most common presentation is in a casserole. The aim of this study was to evaluate the effect of berries powders addition to the spreadable cream cheese, considering the quality characteristics and the conservation capacity in time, by keeping the products in cold conditions. The influence of vegetable powders supplementation was investigated through physico-chemical and microbiological tests for each cheese sample, in comparison to the control sample (without the addition of powder).

2. Material and methods

2.1. Preparation of the cream cheese samples

The spreadable cream cheese samples used in the study were obtained from fresh cow's cheese. The rennet was added to the cow's milk at 35°C and this was incubated until complete acid coagulation (pH = 4.5–4.6). The formed clot was then mixed and placed in gauze and then allowed to drain overnight under refrigerated conditions (4 ± 1 °C) [26]. The obtained cheese was mixed with salt (0.2% g/g) and butter (2% g/g). After that, the spreadable cheese was divided into portions of 100 g and different fruit powders were added, such as aronia (Ar), sea buckthorn (SB), rose hips (RH), hawthorn (Ha) in a proportion of 5% (g/g). The control cheese was also prepared without any added powder. The cheese samples prepared for analysis are labeled as follows: CS (control sample), Ar-SCC (spreadable cream cheese with aronia powder), SB-SCC (spreadable cream cheese with sea buckthorn powder), RH-SCC (spreadable cream cheese with rose hips powder), Ha-SCC (spreadable cream cheese with hawthorn powder) (Figure 1).

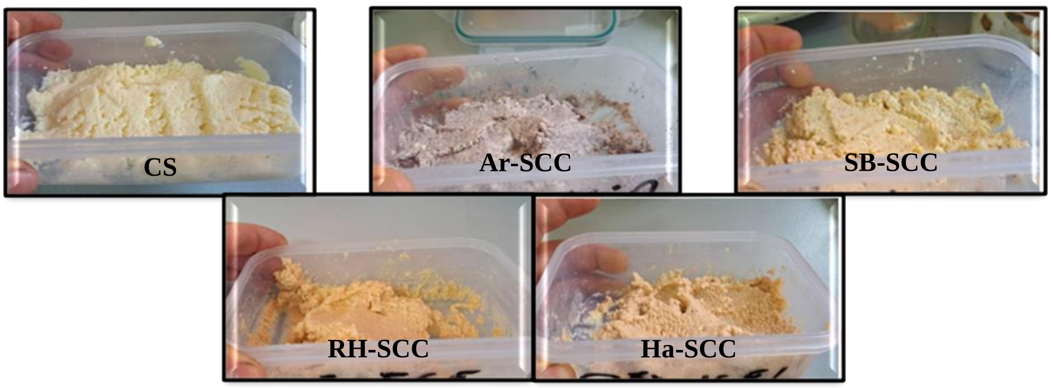

Figure 1. Images of the spreadable cream cheese samples (CS – control sample, Ar-SCC – with aronia powder, SB-SCC- with sea buckthorn powder, RH-SCC – with rose hips powder and Ha-SCC – with hawthorn powder)

All the five cream cheese samples were subjected to physico-chemical and microbiological evaluation on the first day after preparation, while the microbiological analyses continued also in the 3rd and the 10th day, respectively, by keeping the prepared casseroles in the refrigerator (4°C) (Figure 2).

Figure 2. Preserving the spreadable cream cheese samples by refrigeration

2.2 Physico-chemical and microbiological analysis methods

Cheeses acidity determination was carried out by the standard titration method. Each sample was titrated with 0.1 N NaOH solution, using phenolphthalein as an indicator. The titration took placeuntil a pale pink color was obtained, which must be maintainedfor at least 1 minute. The results were expressed in Thörner degrees (°T) [27].

Moisture content was determinedby the oven drying method. 3 g of each cheese sample was placed in the oven at 102 ± 2C °C for 2 hours drying, then the operation was repeated until the constant weight was reached. The moisture content (%) was recorded based on the difference between the initial weight and the weight after drying the sample [28].

Fat content determination was carried out by the Van Gulik -acid butyrometric method. 3 g of finely shredded cheese and sulfuric acid were placed in the beaker of the butyrometer. After the complete dissolution of the proteins while keeping the butyrometerin the water bath at 65-70°C for 30 minutes, 1 ml of isoamyl alcohol was added, shaken for 3 seconds and supplemented with sulfuric acid. The butyrometer was again placed in the warm water bath at 65 ± 2°C for 5 minutes, then shaken and centrifuged for 10 minutes at 1000-1200 rpm. After centrifugation, the butyrometer was once again placed in the water bath at 65-67°C for 5 minutes, and subsequently the number of divisions occupied by the fat column was read on the butyrometer column [28]. Fat content, expressed as a percentage of the dry matter, was calculated using the formula: $\%G = \frac{G}{100-A} \times 100$

The evaluation of the microbiological parameters was achieved by determining the total plate count (TPC) - mesophilic aerobic and facultative anaerobic microorganisms, detection and enumeration of *β*-glucuronidase-positive *Escherichia coli* count (EC) and isolation and enumeration of Coagulase-Positive Staphylococci (CPS), *Staphylococcus aureus* and other species.

For the analysis, 10 g of each cream cheese sample was homogenized with 90 ml of saline solution (9 g/l NaCl solution). Then the series of decimal dilutions followed and the inoculation in Petri dishes with the appropriate culture medium.

For the total plate count, decimal dilutions of 10^{-1} -10^{-5} were made, and later the method of distributing 1 ml of each dilution in Petri dishes (two for each dilution) and pouring the Plate count agar medium (PCA) was applied. This was followed by incubation at 30°C for 48 hours and reading the results for plates containing between 30 and 300 colonies [29].

Isolation of *Escherichia coli* was performed according to (ISO) 16649-2:2007 [30], with slight modifications. 1 mL of each dilution (10^{-1} -10^{-3}) was transferred into two sterile Petri dishes and then the selective medium pre-cooled at 44–47 °C, consisting of tryptone bile X-glucuronide (TBX) agar was poured (~15 mL). The solidified mixture was first incubated at 37 °C for 4 h and then at 43.5 °C for 24 h for the bacterial isolation. Blue-green *E. coli* colonies were enumerated and expressed as CFU/g product [31].

Isolation and enumeration of CPS (*S. aureus* and other species) were performed by preparing dilutions of 10^{-1} -10^{-4} and cultivation on the selective and differential Baird–Parker agar supplemented with egg yolk tellurite emulsion medium at 37 °C for 36–48 h, in accordance with (ISO) 6888-1:2021 [32]. Samples that produced typical colonies (convex black circular colonies surrounded by a transparent and/or opaque halo) were counted, being consideredas CPS colony forming units (CFU) [33]. Five colonies from each positive plate were randomly selected and subjected to the coagulase test by an agglutination reaction using rabbit plasma.

In all cases, the counted microorganisms were expressed as $\log_{10}$ colony-forming units per gram (CFU g−1). All results were expressed as the mean value of three determinations and standard error.

3. Results and Discussion

3.1. Physico-chemical Quality

After the preparation of the five varieties of spreadable cream cheese, both the simple sample (CS) and those with the berries powder addition (Ar-SCC, SB-SCC, RH-SCC, Ha-SCC) were monitored considering their acidity, moisture and fat content, in order to appreciate their quality. The titratable acidity (°T) of the fresh cream cheese samples was expressed by the average value calculated after performing three repetitions (Figure 3).

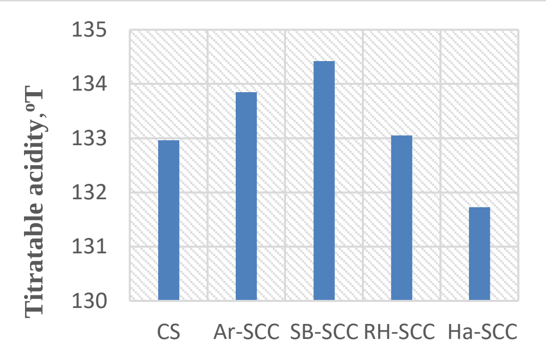

Figure 3. Average values regarding the titratable acidity of the spreadable cream cheeses (CS – control sample, Ar-SCC – with aronia powder, SB-SCC - with sea buckthorn powder, RH-SCC – with rose hips powder and Ha-SCC – with hawthorn powder)

The titratable acidity values vary depending on the type of the added powder, with higher values recorded for the samples with sea buckthorn powder (134.42°T), aronia (133.84°T) and rose hips (133.05°T) compared to the control sample (132.96°T), while in the hawthorn powder sample case (131.73^{0}T) the acidity is slightly lower. This can be influenced by the higher acidity of the fruits, which bring a benefit to the quality of the product, limiting a good part of the microorganisms that are dangerous for the consumers health [34].

The humidity of the cream cheeses is lower in the case of the samples with added powder, compared to the control sample (Figure 4). Fruit powders absorb water, increase the consistency of the product and reduce the possibility of rapid multiplication of the microorganisms.

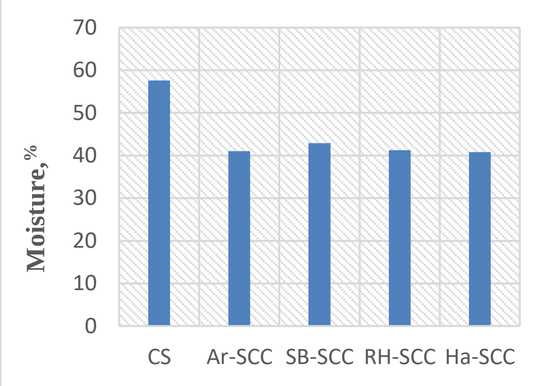

Figure 4. The average moisture values in the spreadable cream cheeses (CS – control sample, Ar-SCC – with aronia powder, SB-SCC- with sea buckthorn powder, RH-SCC – with rose hips powder and Ha-SCC – with hawthorn powder)

The fruit powders addition influences the fat content expressed as a percentage of the dry substance, the highest average value being recorded in the case of the control sample, 35.53%. For the other samples, the percentage of fat varies between 32.46%-34.15% due to reporting to a higher content of dry matter brought by the powders, which supplements the finished products with vitamins and minerals beneficial to the health of the consumer [35].

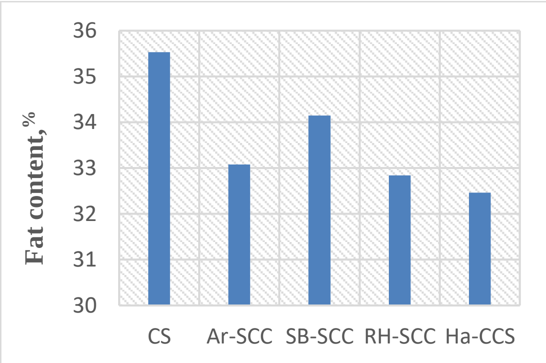

Figure 5. The average values of the fat content in the spreadable cream cheeses (CS – control sample, Ar-SCC – with aronia powder, SB-SCC- with sea buckthorn powder, RH-SCC – with rose hips powder and Ha-SCC – with hawthorn powder)

3.2.Microbiological parameters

The changes that occur in the case of the microbiological parameters for the spreadable cream cheese assortments, after 1, 3, respectively 10 days of refrigeration storage, are presented in table 1.

Table 1. Microbiological changes of the cream cheeses fortified with fruit powders during the storage at 4°C for 10 days (CS – control sample, Ar-SCC – with aronia powder, SB-SCC- with sea buckthorn powder, RH-SCC – with rose hips powder and Ha-SCC – with hawthorn powder)

Day	Sample	Parameter		
		TPC* Log10 CFU g⁻¹	**EC*** Log10 CFU g⁻¹	**CPS*** Log10 CFU g⁻¹
1	CS	3.87±0.12	1.35±0.04	3.25±0.03
	Ar-SCC	3.81±0.03	0.83±0.06	3.19±0.07
	SB-SCC	3.84±0.11	0.56±0.51	3.22±0.04
	RH-SCC	3.85±010	0.96±0.15	3.24±0.02
	Ha-SCC	3.83±0.08	0.93±0.11	3.13±0.06
3	CS	3.95±0.04	1.39±0.16	3.29±0.03
	Ar-SCC	3.62±0.05	0.15±0.27	1.98±0.12
	SB-SCC	3.69±0.03	0.1±0.17	2.13±0.02
	RH-SCC	3.77±0.09	0.38±0.35	2.15±0.23
	Ha-SCC	3.66±0.07	0.25±0.23	1.88±0.05
10	CS	4.31±0.08	1.06±0.23	2.39±0.15
	Ar-SCC	3.37±0.05	n.d	0.72±0.62
	SB-SCC	3.45±0.06	n.d	1.05±0.06
	RH-SCC	3.48±0.15	n.d	1.28±0.18
	Ha-SCC	3.42±0.09	n.d	n.d

TPC - total plate count
EC - β-glucuronidase-positive *Escherichia coli* count
CPS - coagulase-positive staphylococci (*S. aureus* and other species)
* mean values ± standard deviation (n=3)
n.d – not detected

The fruit powders proved to have the desired antimicrobial effect during the monitored interval (10 days), especially for bacterial strains with pathogenic potential. If in the control sample there is an increase in the total number of bacteria from 3.87±0.12 $\log_{10}$ CFU g^{-1} on the first day, to 3.95±0.04 $\log_{10}$ CFU g^{-1} after 3 days, respectively 4.31±0.08 $\log_{10}$ CFU g^{-1} in on the tenth day, in the samples with the addition of aronia, buckthorn, rose hips, and hawthorn powders, the number of microorganisms decreases during the refrigeration period. The most obvious reduction in the total number of bacteria is in the case of the aronia powder (3.81±0.03-3.37±0.05 $\log_{10}$ CFU g^{-1}), followed by hawthorn powder (3.83±0.08-3.42±0.09 $\log_{10}$ CFU g^{-1}), buckthorn powder (3.84±0.11-3.45±0.06 $\log_{10}$ CFU g^{-1}) and rosehips powder (3.86±010- 3.48±0.15 $\log_{10}$ CFU g^{-1}). Thus, at three days after the first determination, there is a decrease in TPC by 5.51% in Ar-SCC, 4.43% in Ha-SCC, 3.90% in SB-SCC and 2.07% in RH-SCC, respectively by 11.54% in Ar-SCC SCC, 10.70% in Ha-SCC, 10.15% in SB-SCC and 9.61% in RH-SCC after ten days of refrigeration storage. Compared to the control sample, the TPC reduction is between 0.51-1.55% on the first day, 4.55-8.35% on the third day and 19.25-21.80% on the tenth day of monitorization.

The antimicrobial effects of the powders addition in the spreadable cream cheeses are also manifested on *β*-glucuronidase-positive *E. coli* with values of 0.56±0.51 $\log_{10}$ CFU g^{-1} in SB-SCC, 0.83±0.06 $\log_{10}$ CFU g^{-1} in Ar-SCC, 0.93±0.11 $\log_{10}$ CFU g^{-1} in Ha-SCC and 0.96±0.15 $\log_{10}$ CFU g^{-1} in RH-SCC in the first day of determination, compared to the control sample where 1.35±0.04 $\log_{10}$ CFU g^{-1} were enumerated. After three days of refrigerated storage, there were decreases in *E. coli* CFU g^{-1} by 82.14% in SB-SCC, 81.92% in Ar-SCC, 73.11% in Ha-SCC and 60.41% in RH-SCC, compared with the control sample where a slight increase was registered. After ten days of refrigerated storage, β-glucuronidase-positive *E. coli* was not detected in any of the samples with added fruits, except in the control sample. Therefore, buckthorn powder has a high inhibitory capacity [36], but it can be observed that all berries powders additions had major effects on *E. coli*, a fact also described in the research of Sturza et al. 2019 [37]. *Aronia melanocarpa* powder has demonstrated antimicrobial effects, especially on the total number of bacteria and on *E. coli*, which indicates that it could be a useful food supplement used as a naturally derived additive to maintain the safety of various dairy products [15].

The presence of CPS with *S. aureus* and other species was detected on the first day of the determinations with values between 3.25±0.03 $\log_{10}$ CFU g^{-1} in CS, followed by 3.24±0.02 $\log_{10}$ CFU g^{-1} in RH-SCC, 3.22±0.04 $\log_{10}$ CFU g^{-1} in SB-SCC, 3.19±0.07 $\log_{10}$ CFU g^{-1} in Ar-SCC, 3.13±0.06 $\log_{10}$ CFU g^{-1} in Ha-SCC. After three days, the antimicrobial effect of the added powders can be observed, with decreases compared to the first day by 39.93% in the sample with hawthorn powder, 37.93% in the sample with aronia powder, 33.85% in the sample with hawthorn powder, 33.64% in the sample with rosehips powder. After 10 days of refrigerated storage, the rosehips powder had the most pronounced antibacterial effect, the presence of CPS being undetectable, instead the other samples recorded decreases between 60.49-77.42%, compared to the first day of the determinations. Considering the

CPS reduction compared to the control sample, on the first day there were reductions of 0.30-3.69%, on the third day 34.65-42-85%, and after ten days 46.44-100%. Thus, it was observed that the hawthorn powder shows marked effects on *Staphylococcus aureus* [37], followed by aronia, sea buckthorn and rose hips.

The results obtained in all the presented cases show a beneficial effect of the fruit powders in terms of the spreadable cream cheese conservability. As it appears from previous studies, fruits in different forms have desirable antimicrobial properties and are potential ingredients for enriching traditional food formulas [38-40]. The dairy industry can adopt sustainable strategies to improve the functional value of food, reduce microbial spoilage and increase shelf life.

4. Conclusion

Through the carried-out research, the fortifying and beneficial effects of the fruit powders addition in the spreadable cream cheese were confirmed. The fruit powders improve the quality of the product and possess antimicrobial activities highlighted by the microbiological analyzes undertakenduring the time interval of the refrigeration storage.

Titratable acidity increases slightly after adding aronia, sea buckthorn and rose hips powders compared to the control sample, while the humidity decreases in all samples with fruit powders addition, fact that reduces the possibility of microbial proliferation. Also, the fat content is reduced compared to the control sample, which indicates the dry substance increase by itssupplementation withfruit powders minerals and vitamins.

The antimicrobial capacity of the fruit powders was proven by the results obtained after monitoring the total number of bacteria, *β*-glucuronidase-positive *E. coli* and coagulase-positive staphylococci (*S. aureus* and other species), during 1, 3 and 10 days of refrigeration storage.There were decreases in the bacterial load with the increase of the storage period for all the samples with fruit powders addition (aronia, hawthorn, sea buckthorn and rose hips), compared to the control sample where the values were higher. *β*-glucuronidase-positive *E. coli* was best inhibited in the samples with the addition of sea buckthorn and aronia powders, followed by hawthorn and rose hips. Hawthorn powder had the major effect on coagulase-positive staphylococci, followed by aronia, sea buckthorn and rose hips.

It is important for the dairy industry to pay more attention to the microbial spoilage reduction, and the use of berries can be an effective alternative in increasing the nutritional value and satisfying the consumers needs.

Acknowledgements: The authors acknowledge to S.C. ZEA S.RL. for the raw milk provided.

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