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ORIGINAL RESEARCH PAPER

QUALITY AND SHELF LIFE OF SKUTA WHEY CHEESE PACKED UNDER VACUUM AND MODIFIED ATMOSPHERE IN PRESENCE OR ABSENCE OF THE HEMP SEED POWDER

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Abstract: *Skuta* is a traditional Croatian whey cheese, with a limited shelf life, produced by heat coagulation of ovine or cow's whey. To improve its quality and extend shelf life, samples were stored at 4 °C under vacuum (VP) and modified atmosphere conditions (MAP) (70/30 % N_2/CO_2) with or without the addition of organic hemp seed (HS) powder (2 % w/w) during 21 days storage. MAP and VP had been used to control the growth of spoilage microorganisms and foodborne pathogens, while HS powder was added because of its nutritional and possible antimicrobial properties. The quality characteristics and shelf life of both untreated and HS powder treated *Skuta* were assessed using microbiological, physicochemical and sensory parameters. Total aerobic mesophilic count and growth of yeast and molds were reduced in MAP samples. No foodborne pathogens were detected in samples, regardless of the treatment. The addition of HS powder had a significant impact on the nutritional value, the proportion of minerals and salt content of *Skuta*, but did not have impact on the microbial spoilage. MAP had positive impact on *Skuta* shelf life in sensory terms, while HS powder addition had no significant impact on shelf life extension but was still sensory acceptable.

Keywords: *hemp seed powder, modified atmosphere packaging, preservation, quality, whey cheese*

INTRODUCTION

Whey is a major by-product of the dairy industry, which has several biological and environmental implications. Whey is an excellent source of functional proteins and offers a great number of vitamins, minerals and lactose, whose amount depends on the technological processes of cheese manufacturing and the quality of the used milk [1]. Because of its great nutritional value, the attitude towards whey has changed over the years from being a by-product to value added raw material. Whey proteins are nutritionally the most valuable components in whey and possess a plethora of healthy components such as essential amino acids, bioactive peptides, antioxidants and immunostimulators, so studying opportunities to maximize whey utilization become a priority, focusing especially on its high-quality proteins [2]. The oldest way to utilize the whey is albumin or whey cheese production. In Croatia, the most popular whey cheese is *Skuta*. Traditional home-made *Skuta* is mainly produced from sheep's milk whey (*Istria* or *Pag Skuta*), cow's milk whey, and sometimes goat's milk whey. *Skuta* belongs to the group of soft cheese and have a high nutritional value which is the result of a great amount of whey proteins that are easy to digest and have a high level of utilization [3]. Principally, *Skuta* is obtained either through the concentration of whey and the molding of the concentrated product or through the coagulation of whey by heat with or without the addition of acid [4]. Croatian *Skuta* properties differ because of the type of whey used (sheep, goat or cow) and the type of additive employed (salt, vinegar, acid whey) during the processing [4]. In order to enhance product texture and better extraction of the residual proteins, a small amount of milk or cream can be added to the whey prior to heating. Also, to enhance its quality, flavor and nutritional value, *Skuta* can be mixed with herbs, nuts, dried fruits or other supplements that will enrich *Skuta* in its functional and nutritional properties (vitamins, minerals, proteins, etc.). Organic hemp seed powder was used in this work as a supplement, to enrich *Skuta* protein and essential oils content. Hemp seeds are considered as functional food, have excellent fatty acid profiles and protein qualities, and have been used for centuries to treat various disorders [5], also are an excellent nutritional source of high quality proteins [6], as they are easily digested, absorbed, and utilized [7]. Also, according to Nissen *et al.* [8], hemp seeds and its essential oils showed interesting antimicrobial activities and could be used against spoilage and food-borne pathogens. The chemical composition of traditional *Skuta* and its intrinsic properties, *p*H (6.10 - 6.80) and water activity (0.974 - 0.991), as well as the absence of preservatives in the formulation, make this product an excellent substrate for the growth of spoilage and pathogen psychotropic bacteria, molds and yeasts during refrigerated storage [9, 10]. To reduce the spoilage level and prolong the shelf life different biopreservatives may be used [10], as well as several packaging techniques. *Skuta* shelf life is generally limited to a few days due to the exposure of the product to the atmosphere prior to packaging. In addition to improving quality and extending shelf life, modified atmosphere packaging (MAP) has been used to control the growth of pathogenic microorganisms in whey cheeses [9, 11 – 13]. MAP is a packaging technique frequently used in the food industry in order to control the microbial growth [14]. Several studies demonstrated the effectiveness of MAP in shelf life, and the use of MAP for the shelf life extension was previously evaluated in different whey cheeses from sheep [15] or cow's whey milk and in similar products such as Myzithra Kalathaki [12] or Anthotyros [11]. Modification of the gas

composition that surrounds a food product during storage, including N_2 and CO_2, can reduce physiological changes, oxidation reactions, and microbial growth [16].
The objectives of present work were to: (1) determine the microbiological, physico-chemical and sensory changes *Skuta* during storage under vacuum and MAP conditions at 4 °C with and without addition of organic hemp seeds powder, (2) determine the shelf life of samples under the same packaging conditions and (3) determine the impact of addition of organic hemp seeds powder on *Skuta* nutritional value and shelf life.

MATERIALS AND METHODS

Sample preparation, packaging and addition of hemp seed powder

Skuta samples produced from the sweet whey remained after the production of traditional Croatian cow's milk cheese *Škripavac* was kindly supplied from local small dairy producer. Samples were transported to the laboratory in polystyrene boxes containing ice and were used within 3 h after production. Portions of supplied *Skuta* (200 ± 10 g) were individually packed into a cast polyamide with a polyethylene sealing layer (PA/PE) pouches of 85 ± 8 μm thickness with an oxygen permeability under 50 $cm^3 \cdot m^{-2} \cdot d^{-1} \cdot bar^{-1}$, carbon dioxide permeability under 180 $cm^3 \cdot m^{-2} \cdot d^{-1} \cdot bar^{-1}$ and nitrogen permeability less than 30 $g \cdot m^{-2} \cdot d^{-1}$ (Südpack, Germany). Four treatments were used: A: *Skuta* stored under VP (70 mbar) without the addition of organic HS powder; B: *Skuta* stored under VP (70 mbar) with the addition of organic HS powder (2 % w/w); C: *Skuta* stored under 70/30 % (N_2/CO_2) MAP without the addition of organic HS powder, D: *Skuta* stored under 70/30 % (N_2/CO_2) MAP with the addition of organic HS powder (2 % w/w). Mixture of gases was specially prepared and supplied as bottled compressed gas mixture consisting of 70 % N_2 and 30 % CO_2 (Messer Croatia Ltd., Croatia). Pouches were heat-sealed using the Multivac C100 Professional tabletop vacuum pack machine (Multivac, Sepp Haggenmüller SE Co. KG, Germany) connected to the bottle with compressed gas mixture.
Organic hemp seed powder from genus *Cannabis sativa subsp. sativa L.* was purchased from Herbio Plus Ltd. (Velika Gorica, Croatia). Hemp seed powder had the following nutritive value (in 100 g of powder): 48 % proteins, 5.16 % carbohydrates (4 % sugar), 13.25 % fibers, 15.31 % fat, and energy value 1506 kJ (360 kcal). HS powder was added to the *Skuta* samples after the draining of residual whey, and before molding (2 % w/w) (treatments B and D). Upon addition, HS powder was gently mixed with *Skuta* to achieve an even distribution.
Samples were stored in refrigerator at 4 °C for 21 days and analyzed microbiologically, physicochemically and by a sensory panel after 7, 14 and 21 days of storage. There were separate sample packaging for each analysis (physicochemical, microbiological and sensory analysis) and each sampling day.

Physicochemical analysis

Water activity analysis

Skuta samples were grounded in laboratory porcelain mortar with pestle and approximately 7 g of sample was placed into sample dish. Water activity was measured

using AQUALAB Pawkit Water Activity Meter with capacitance humidity sensor (Decagon Devices, Inc., Pullman, Washington, USA) which converts the humidity value into a specific capacitance, which is then measured electronically by the circuit, translated by the software and displayed as water activity on the instrument screen.

Acidity analysis

Acidity was monitored on each sampling day by measuring the *p*H value of samples. *p*H values of samples were measured using *p*H 3210 meter (Wissenschaftlich-Technische-Werkstätten GmbH & Co. KG WTW, Weilheim) connected to a Schott Blue Line 21 *p*H electrode for penetrating measurement.

Whey volume (syneresis)

Syneresis was monitored on each sampling day right after the opening of the packaging for physicochemical analysis. Volume of whey released from 200 g of *Skuta* sample during storage period was measured by pouring the released whey into the glass measuring jug and reading the value on the measuring scale.

Composition analysis

Moisture, fat, protein and salt content were monitored on each sampling day for each treatment (A, B, C, D) by Food Scan Analyzer LAB (NIT Analyzer) with Global cheese calibration for fat, moisture, protein, salt and total solids (Foss Analytical AB, Denmark).

Gas composition

Gas composition was measured before all other analysis on 7, 14, and 21 storage day by piercing the surface of packaging with a sterile needle connected to the hand-held Oxybaby headspace gas analyzer (WITT Gasetechnik GmbH & Co. KG, Germany). Measures of combined residual % O_2 and % CO_2 were read directly on the instrument, while % N_2 was obtained by difference.

Microbiological analysis

Microbiological analyses were conducted for all treatments (A, B, C, D) on 7, 14 and 21 days of storage according to international standard methods and included the following parameters: total aerobic mesophilic count [17], *Enterobacteriaceae* [18], yeasts and molds [19, 20], coagulase-positive staphylococci [21], *Escherichia coli* [18, 22], *Salmonella spp.* [23], *Listeria monocytogenes* [24, 25] and sulfite reducing *Clostridia* [26]. All the results were expressed as decimal logarithm of colony forming units per gram of whey cheese (log CFU·g^{-1}).

Sensory analysis

The sensory evaluation of the *Skuta* samples was performed on first and seventh day of production by a 6 assessors (panelist) from the Karlovac University of Applied Sciences (Karlovac, Croatia) that were familiar with whey cheese. The coded *Skuta* were removed from the refrigerator and out of the PA/PE bag right before the evaluation. About 30 g of cheese was presented to each member. Water was also provided to

panelists to rinse their mouths between samples. A scoring method with a sum of 20 ponderable scores was applied for the evaluation of sensory characteristics of whey cheeses (appearance, color, consistency, odor and flavor), ranging it from 0 to 5 using the significance factor for each parameter [27].

RESULTS AND DISCUSSION

Physicochemical changes during storage

Changes in physicochemical parameters (*p*H, water activity, syneresis, moisture, fat, protein and salt content) were monitored during storage of *Skuta* samples at 4 °C under VP and MAP conditions, both in absence or presence of HS powder. There were no significant differences between *p*H values regarding the storage conditions (MAP or VP) of *Skuta* samples ($P > 0.05$), while *p*H values were reaching the mean values in the range of 5.71 to 6.25. In contrary, *p*H values changed significantly throughout 21 days observation period (T_0-T_{21}) ($P < 0.05$). *p*H value of *Skuta* samples packed in VP increased in first 7 days of storage (T_7), while in those packed in MAP decreased. Between T_7 and T_{14} *p*H value slightly increased in VP and MAP packaging but decreased in samples with the addition of HS powder. Increase in *p*H value may be the result of increase of yeasts and molds activity in *Skuta* samples and alkaline components formed by their metabolism [28]. At the end of the storage period *p*H decreased significantly in all samples ($P < 0.05$) and the minimum value was noticed for the VP packed samples. Present results suggest that the addition of HS powder have a no significant effect on the *p*H values of *Skuta*, irrespective of the packaging conditions (Figure 1).

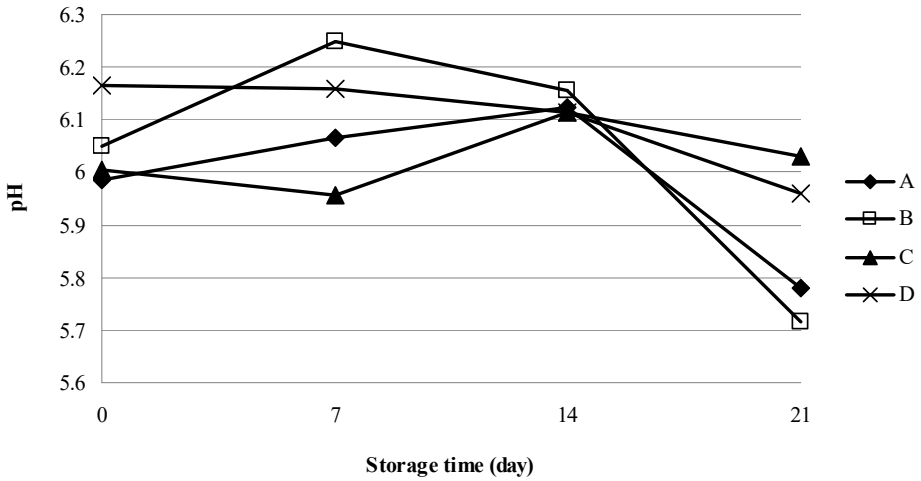

Figure 1. *pH values of Skuta packed under VP (A, B) or MAP (C, D) in presence (B, D) or absence (A, C) of HS powder*

High water activity values were measured in all *Skuta* samples, and there were no significant differences between a_w values regarding the storage conditions ($P > 0.05$) (Table 1). High values are in accordance with those for fresh and albumin types of cheeses, as well as in correspondence with high moisture content.

Table 1. *Water activity (mean values of two batches) of Skuta packed under VP (A, B) or MAP (C, D) in presence (B, D) or absence (A, C) of HS powder*

Treatment	Sampling time			
	T_0	T_7	T_{14}	T_{21}
A	1.01	0.97	0.98	1.01
B	0.97	0.99	0.99	0.98
C	1.01	0.97	0.98	0.99
D	0.97	0.97	0.98	1.00

*The sampling times T_0, T_7, T_{14} and T_{21} refer to the days (0, 7, 14, and 21, respectively) elapsed during shelf life

Whey release (syneresis) during storage period is characteristic for high moisture content cheeses. Present results showed the higher whey release in *Skuta* samples packed in vacuum during first week of storage (T_7) than those packed in MAP. After the T_4 whey release slightly increased in all samples, except the sample D (packed in MAP with addition of HS powder) where increased rapidly and reached a maximum value in last sampling day (T_{21}) at 6.2 mL. HS powder treated *Skuta* samples showed higher whey release than untreated samples in both VP and MAP (Figure 2).

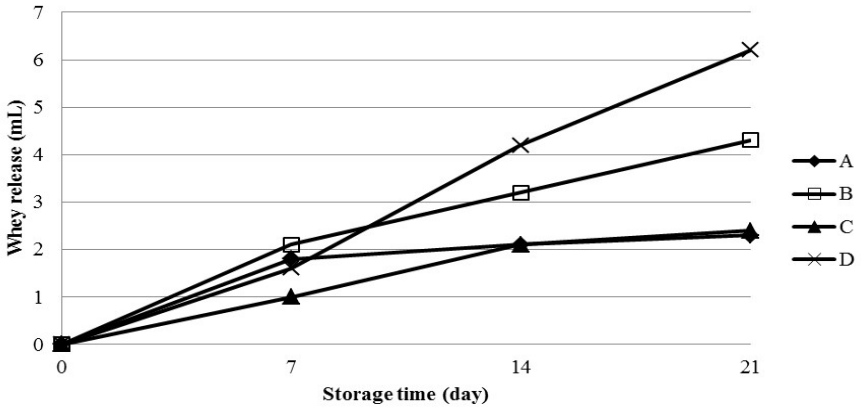

Figure 2. *Whey release (syneresis) during Skuta shelf life under different packaging conditions*

Analysis of the nutritional composition revealed that there was no significant difference in the water, fat, protein and carbohydrates content between treatments with addition of HS powder (B, D) compared to treatments without addition of HS powder (A, C) ($P > 0.05$). Nevertheless, the addition of HS powder had a significant effect on the nutritional value of *Skuta* ($P < 0.05$), the proportion of minerals ($P < 0.05$) and salt content ($P < 0.01$). The applied atmosphere had no effect on the nutritional composition of *Skuta* ($P > 0.05$). Results of composition analysis are given in Table 2.

Table 2. *Composition of Skuta packed under VP (A, B) or MAP (C, D) in presence (B, D) or absence (A, C) of HS powder (mean values (%) ± standard deviation) during shelf life*

Composition	Treatment	T_7	T_{14}	T_{21}
Moisture	**A**	70.60 ± 0.05	71.59 ± 0.03	70.65 ± 0.01
	B	71.76 ± 0.01	69.93 ± 0.05	69.71 ± 0.10
	C	70.32 ± 0.04	72.52 ± 0.09	70.47 ± 0.10
	D	70.20 ± 0.14	68.94 ± 0.06	72.10 ± 0.33
Fat	**A**	13.37 ± 0.07	12.74 ± 0.04	13.23 ± 0.03
	B	11.40 ± 0.11	12.50 ± 0.04	13.00 ± 0.02
	C	13.12 ± 0.09	11.69 ± 0.03	13.40 ± 0.08
	D	12.59 ± 0.02	13.17 ± 0.04	10.89 ± 0.08
Protein	**A**	9.71 ± 0.01	9.73 ± 0.01	10.04 ± 0.01
	B	8.92 ± 0.05	10.12 ± 0.08	10.18 ± 0.02
	C	9.91 ± 0.01	9.93 ± 0.03	10.23 ± 0.03
	D	9.96 ± 0.04	10.47 ± 0.01	9.08 ± 0.03
Salt	**A**	0.66 ± 0.04	0.59 ± 0.02	0.71 ± 0.02
	B	0.83 ± 0.03	0.91 ± 0.03	0.909 ± 0.01
	C	0.64 ± 0.03	0.61 ± 0.02	0.75 ± 0.04
	D	0.69 ± 0.03	0.92 ± 0.07	0.81 ± 0.10

*The sampling times T_7, T_{14} and T_{21} refer to the days (0, 7, 14, and 21, respectively) elapsed during shelf life

The gas compositions in headspace for each *Skuta* sample at each sampling time are reported in Figure 3. Efficacy of MAP is conditioned by several factors, like the relationship between the product volume and the headspace volume, the residual O_2 rate contained in the product, the level of vacuum and the correct selection of packaging materials [29]. The gas mixture used in treatment C and D was 70 % N_2 and 30 % CO_2, while treatments A and B were packed under vacuum (70 mbar). In MAP samples (C, D) at T_0 CO_2 rate was 5.70 % and 12.70 %, notably lower than in the applied gas mixture. Through the observation period, % CO_2 increased in all treatments (Figure 3b). The highest % CO_2 was reached in treatment B, while treatment D had the lowest increase trend. Increase in CO_2 is associated with high aerobic mesophilic bacteria level resulting in CO_2 release in the headspace, as a microbial fermentation product, as well as O_2 reduction during storage [15]. O_2 concentration showed a progressive reduction trough observation period regardless of the treatment (Figure 3a). VP samples (A, B) showed expectedly lower O_2 rate (2 % and 1 %) than MAP samples (8.10 % in C and 5.6 % in D) at T_0 due to applied vacuum. There was increase in % O_2 in treatment B at T_{14}, and also in treatments A and C after the T_{14}, probably due to the packaging permeability, or the microbial growth especially yeast growth (similar reported by Pala *et al.* [9] and Kizilirmak Esmer *et al.* [28]). The changes in the relative concentrations of % CO_2 and % O_2 during observation period affected the N_2 levels in headspace. In MAP samples (C, D) at T_0 % N_2 was 86.20 and 81.70, slightly higher than in the gas mixture. The nitrogen percentage decreased trough whole observation period for all samples, reaching the minimum value at 14.20 % for treatment B (Figure 3c).

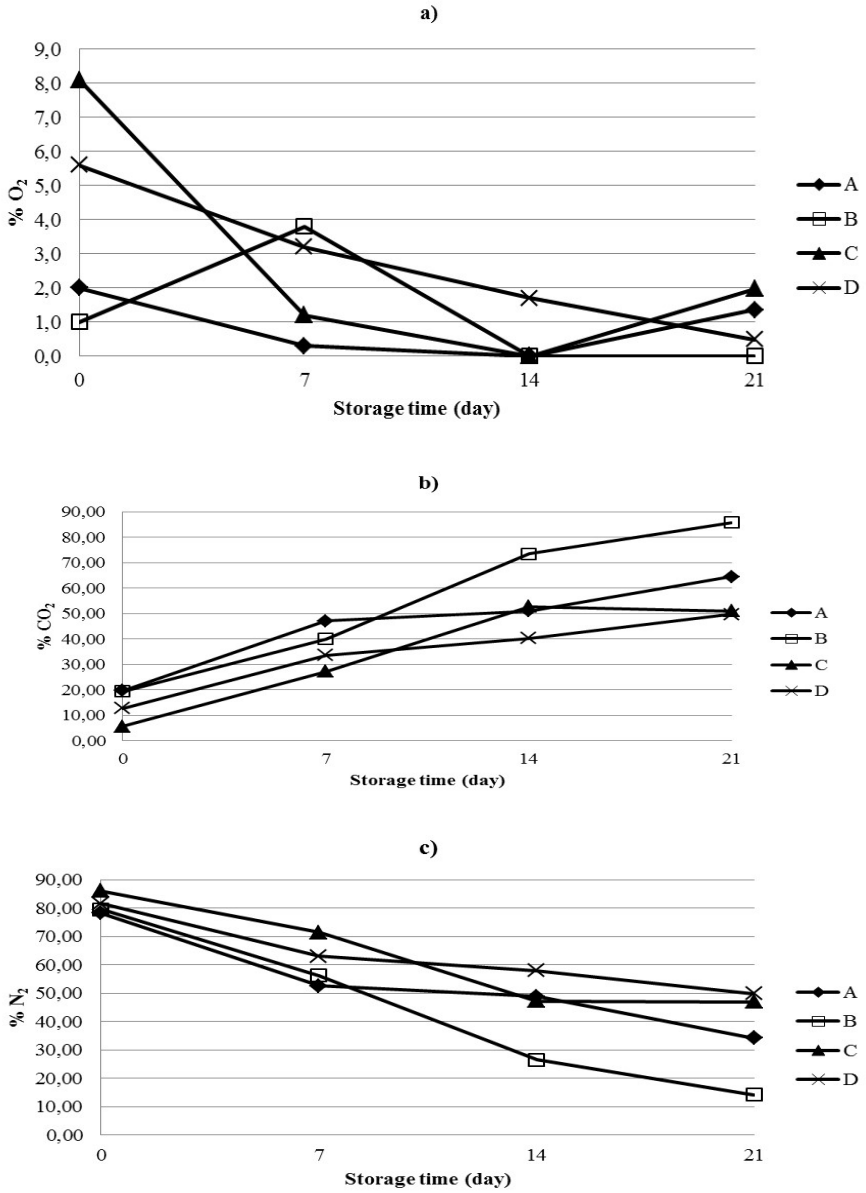

Figure 3. *Evolution of % O_2 (a), % CO_2 (b) and % N_2 (c) in the headspace of Skuta packed under VP (A, B) or MAP (C, D) in presence (B, D) or absence (A, C) of HS powder*

Microbiological changes during storage

Microbiological changes during storage are shown in Table 3. Total aerobic mesophilic count (TAMC) increased in all treatments but did not exceed the spoilage limit throughout the observation period (T_{21}) (mesophilic counts of 7 log CFU·g^{-1}; [30]). Samples from treatments C and D (MAP) prove to have lower TAMC than samples in A and B (VP).

Table 3. *Microbiological profile of Skuta packed under VP (A, B) or MAP (C, D) in presence (B, D) or absence (A, C) of HS powder (log_{10} colony forming unit g^{-1}) during shelf life*

Parameter	Treatment	T_0	T_7	T_{14}	T_{21}
Total aerobic mesophilic count	A	3.00	4.48	5.48	5.48
	B	3.47	4.46	5.26	4.88
	C	2.9	2.93	4.65	4.72
	D	3.3	4.37	4.38	4.58
Enterobacteriaceae	A	0.00	4.16	4.18	4.18
	B	2.00	2.38	4.49	4.99
	C	0.00	1.60	3.13	4.65
	D	1.95	2.91	4.28	5.18
Yeasts	A	<1	3.18	4.30	4.40
	B	2.41	2.60	2.74	4.18
	C	<1	<1	3.83	4.76
	D	<1	<1	4.13	2.91
Molds	A	<1	2.90	2.56	2.26
	B	<1	<1	2.43	<1
	C	<1	<1	2.66	<1
	D	<1	2.26	2.56	<1

*The sampling times T_7, T_{14} and T_{21} refer to the days (0, 7, 14, and 21, respectively) elapsed during shelf life

Montone *et al.* [31] also reported higher values of TAMC in their research of microbiological and chemical parameters of *Campania buffalo ricotta cheese*, as well as higher *p*H and water activity values, and pointed out the need of better control of the supply chain. Soft cheeses in general are of limited durability, because of high moisture content and high *p*H value, which support the proliferation of some spoilage microorganism (especially *Enterobacteriaceae*) under cold storage [32]. Total count of *Enterobacteriaceae* (TCE) in treatment A reached 4.16 log CFU·g^{-1} in T_7 and remained constant throughout the rest of the observation period, while in treatments B, C, and D increased constantly. Treatments B and D, samples with addition of HS powder, had higher TCE than A and C (absence of HS powder). That implies that HS powder did not have antimicrobial impact on *Enterobacteriaceae* growth in those samples, as was considered. Yeasts and molds prove to be an important microbial contaminant in the dairy industry [33]. VP does not remove all the O_2 from package, so yeast and mold growth may still occur [34]. Yeast count increased trough storage period for all samples and did not reach the stationary phase over the entire observation period, except the treatment D where yeast count decreased after the T_{14}. On the other hand, the yeast count in *Skuta* samples packed under VP conditions increased earlier (before T_7) than those packed in MAP (just after T_{14}). Molds were detected at sampling time T_7 for treatments A and D and at T_{14} for treatments B and C (< 1 log CFU·g^{-1}). The mold growth may be the result of high O_2 content in headspace (between 2-8 % in first 7 days, depending on the treatment) which probably contributed to the mold growth. After the T_{14} decrease in molds were observed in all samples probably because of the lower rates of O_2 and inhibitory effect of higher concentration of CO_2 in headspace. Other authors also reported similar effects for various types of cheese [12, 13, 15, 34]. *Skuta* is not heat treated before consumption, and because of its chemical properties and absence of competitive microflora is an excellent substrate for growth of foodborne

pathogens such as *Salmonella spp.* or *L. monocytogenes* [35, 36]. Same is also evident by the results in current research (*p*H values 5.715-6.250, water activity 0.97-1.00 and NaCl content 0.585-0.915 %). *Salmonella spp.* and *L. monocytogenes* were absent in all samples analyzed during the shelf-life, while *E. coli*, sulfite reducing *Clostridia* and coagulase positive *Staphylococci* were below 1 log CFU·g^{-1}. None of analyzed *Skuta* samples had the presence of pathogenic bacteria and had no significantly increased bacterial contamination indicators. These results represent a great microbiological accuracy of the *Skuta* samples. According to the results obtained, there is no significant effect on the microbiological safety of the product between the methods of packaging of the used *Skuta* samples.

Sensory changes during storage

Appearance, color, consistency, odor and flavor of *Skuta* samples with and without addition of HS powder were observed right after the production (T_0) and after seven days of storage at 4 °C (T_7) under VP (A, B) and MAP conditions (C, D). Samples stored fourteen (T_{14}) and twenty-one day (T_{21}) at 4 °C under VP (A, B) and MAP conditions (C, D) were not included in sensory evaluation due to the growth of molds and yeast. Samples with added HS powder obtained lower grades than those without the HS powder addition, regardless of the packaging conditions, but were still acceptable. At T_0, results showed significant impact of HS powder addition on appearance, color, consistency and flavor of *Skuta* ($P < 0.01$), but there was no significant impact on odor ($P > 0.05$). After seven days storage (T_7) at 4 °C, results of organoleptic evaluation showed significant difference between samples stored in VP (A) and MAP conditions (C) ($P < 0.05$). *Skuta* samples packed under MAP conditions obtained higher score than those packed under VP (Table 4). Samples with addition of HS powder stored for seven days (T_7) at 4 °C under VP (B) and MAP conditions (D) were excluded from the taste observation due to the intensive odor (intensive sour and hemp scent), unacceptable appearance (stickiness, sliminess) and color (grayish top surface with dark spots).

Table 4. *Overall quality score based on the sensory evaluation*

Sampling time	Sample	Average score	Maximum score	Relative [%]	Quality
T_0	*Skuta* without addition of HS powder	19.13	20.00	95.65	excellent
	Skuta with addition of HS powder	12.80	20.00	64.00	still acceptable
T_7	A	15.73	20.00	78.65	good
	B	N/A	N/A	N/A	N/A
	C	18.07	20.00	90.35	excellent
	D	N/A	N/A	N/A	N/A

Dermiki *et al.* [12] and Papaioannou *et al.* [13] also reported the effectiveness of MAP in prolonging the sensory acceptability of cheeses in terms of taste and odor. *Skuta* samples with the addition of HS powder (B, D) were evaluated based on the odor and appearance as not acceptable for human consumption by all assessors.

CONCLUSIONS

To the best of our knowledge, this is the first study reporting on the use of MAP and addition of the organic HS powder in production of Croatian whey cheese *Skuta*. HS powder addition had significant impact on increase in the nutritional value and the proportion of minerals in *Skuta* samples regardless of the packaging conditions, but did not have positive impact on shelf life, nor did have positive influence on sensorial characteristics. Compared to VP, MAP prove to have positive impact on *Skuta* shelf life in sensory terms, as well on microbiological stability, in reduction of total aerobic mesophilic count (TAMC) and growth of yeast and molds. There was no significant difference between packaging conditions regarding the microbiological safety of *Skuta*, while foodborne pathogens were absent in both VP and MAP. Based on the results obtained it is evident that addition of HS powder in production of *Skuta* did not extend the shelf life as expected, but shortened it to less than 7 days. Further research is needed to accurately determine microbiological and sensorial changes during second week of storage under MAP and VP, especially for samples with the addition of HS powder. While no antimicrobial activity of HS powder was confirmed, further studies regarding the preservation and quality of *Skuta* involving other hemp-based products, such as essential hemp seed oils, are also planned.

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