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THE VITAMIN C CONTENT IN FERMENTED MILK BEVERAGES OBTAINED FROM EWE'S MILK

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ABSTRACT

From ewe's milk four fermented milk products were made (yoghurt, bioyoghurt, sour milk, kefir) using the following starter culture: for yoghurt: YC-180 (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus),* bioyoghurt: ABT-1 (*S. thermophilus, L. acidophilus* and *Bifidobacterium* species), sour milk: CH-N-11 (*L .lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis*, *L. lactis* ssp. *diacetylactis*, *Leuconostoc mesenteroides* ssp. *cremoris*) and for kefir: DA (*Lactobacillus* ssp., *Lactococcus* ssp. and yeasts). Raw milk, pasteurised milk, as well as all the products were analysed when fresh, after 7 and 14 day of storage for determination of vitamin C and ascorbic acid content, titratable acidity and pH. The kind of starter culture affected the vitamin C content and ascorbic acid content in fresh fermented milks, while after 7 and 14 days of storage differences among fermented milks concerning vitamin C and ascorbic acid content were statistically non-significant. It was found that in both fresh bio-yoghurt and sour milk that were characterised by a higher vitamin C content, this content gradually decreased throughout the storage period. In fresh yoghurt and kefir vitamin C content was lower than in the rest of the fermented milks, but after 7 days of storage slightly increased.

Key words: ewe's milk, yoghurt, bioyoghurt, sour milk, kefir, vitamin C

INTRODUCTION

Vitamin C is considered as L-ascorbic acid, dehydroascorbic acid, and other forms that are inactive. L-ascorbic acid is produced from glucose in the animal and vegetable cell. To this synthesis, in the last stage, the presence of gulonolakton oxydase is required. Humans and some animals (i.e. guinea pig) do not posses this enzyme so that they are not capable of producing L-ascorbic acid [13], which performs very important functions such as:

taking part in the hydroxylation process of the proline residues in collagen, which stabilise the helix of this protein; participating in the synthesis of steryde hormones and in the hydroxylation of aroma compounds [11, 13]. This vitamin preserves structural and functional activity of DNA and lipoproteins and has been demonstrated as being an important antioxidant [8]. Lack of this vitamin in human nutrition may weaken the functions of this organism and in critical cases lead to diseases.

Vitamin C is a water-soluble vitamin presents in considerable amounts in fruits and legumes [9]. Milk and milk products are not good sources of this vitamin [14], although its content in milk varies, depending the effect of various factors. According to Haenlein [10]; Alichanidis and Polychroniadou [1], Steron [16] ewe's milk contain more vitamin C than cow's or goat's milk (4:1:1 respectively) and in comparison with human milk, vitamin C constitutes more than 80% its content in human milk. Vitamin C is among the least stable vitamins. Both L-ascorbic acid and dehydroascorbic acid are very heat-sensitive, especially in the presence of oxygen, iron and copper. They are not durable in alkali and neutral pH [9, 15]. The stability of this vitamin is affected by an acid environment. Some authors [5] indicate that a certain increase in the vitamin C content in fermented milk products may be associated with its synthesis by some lactic acid bacteria *(Lactococcus*).

The aim of the present study was to estimate the vitamin C content, especially that of ascorbic acid, in 4 fermented milks produced from ewe's milk.

MATERIALS AND METHODS

Materials

Ewe's milk was collected in the spring from Polish mountain ewes, bred at a farm in Opatkowice near Cracow. The treated milks were inoculated using direct-to-vat system (DVS) starter culture: yoghurt culture (YC-180; *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus),* probiotic culture (ABT-1; *S. thermophilus, L. acidophilus* and *Bifidobacterium* species) and sour milk culture (CH-N-11; *L .lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis*, *L. lactis* ssp. *diacetylactis*, *Leuconostoc mesenteroides* ssp. *cremoris*) obtained from Ch. Hansen and direct-to-vat inoculation (DVI) kefir culture (DA; *Lactobacillus* ssp., *Lactococcus* ssp. and yeasts) obtained from Rhodia Food Biolacta. The fermented milk beverages were evaluated when fresh and after storage at 4°C for 7 and 14 days.

Production of yoghurt, bioyoghurt, kefir and sour milk

Milk blended in the amount of 10 kg was strained using a cloth filter. 1kg of milk was used for analyses of raw milk, 1 kg was pasteurised (93°C/5 min) and subjected to analyses. The remained raw milk (8 kg) was divided into four equal portion (2 kg). Each batch of ewe's milk was heated to 93°C for 5 min, cooled: I batch to 45°C (yoghurt), II batch to 38°C (bioyoghurt), III batch to 22°C (sour milk), IV batch to 22°C (kefir) and inoculated with DVS cultures at a rate of: I batch 0.039g/kg, II batch 0.08g/kg, III batch 0.06g/kg and IV batch with DVI culture at a rate of 0.04g/kg of milk.

The inoculated milks were incubated to obtain soft curd: yoghurt at 43°C for 6 h, bioyoghurt at 38°C for 8 h, sour milk at 22°C for 16 h, kefir at 22C for 16 h. After the incubation period the fermented milks were transferred to cold storage (4°C) and stored for 14 days.

Analytical methods

Determination of acidity

The pH of the milk, yoghurt, bioyoghurt, sour milk and kefir was measured using a digital pH meter. The titratable acidity was determined according to the Soxhlet-Henkel method [6].

Density determination

The milk density was measured using a densitometer [6].

Determination of protein and dry matter content

The protein content of the ewe's milk was estimated from the crude nitrogen according to the Kjeldahl method. The dry matter content in milk was determined by oven drying methods [2].

Fat and lactose determination

The total fat and lactose contents of the milk were determined using the Gerber and Bertrand methods [6].

Vitamin C and ascorbic acid determination

The vitamin C and ascorbic acid contents in raw milk, pasteurised milk, yoghurt, bio-yoghurt, sour milk and kefir were determined by the Tilmans-Sharp method, as described by Zmarlicki [18].

Statistical analyses were carried out using computer's program Statgraphic 5.2.

RESULTS AND DISCUSSION

Ewe's milk was characterised by the following chemical composition (g/100 g): dry matter 17.08 (± 0.81), fat 6.4 (± 0.30), protein 5.3 (± 0.42), lactose 4.97 (± 0.13). Density of milk was 1.0319 g/ml (± 0.0026), pH 6.52 (± 0.06) and titratable acidity 10.32 °SH (±1.11). The physicochemical properties of raw milk were in agreement with those reported by Alichanidis & Polychroniadou [1] and Haenlein [10]. The vitamin C content in raw milk was 2.44 mg/100g (± 0.14) and ascorbic acid content 1.53 mg/100 g (± 0.18). Tamime & Robinson [17] reported lower value for this vitamin in ewe's milk (1 mg/100 g), but according to the other authors [2, 3, 16] the content of vitamin C may range from 3.4 mg/100 g to 5 mg/100 g of ewe's milk. Perhaps the presence of this vitamin in milk depends not only on the animal species (cow's and goat's milk have less vitamin C than ewe's milk) but also on the breed [10]. Ascorbic acid content in the present study constituted 62.7% of total vitamin C content in ewe's milk. In previous reports [4] this percentage was slightly higher (ascorbic acid content constituted 68.3% of total vitamin C content in ewe's milk).

After pasteurisation the vitamin C and ascorbic acid content in milk decreased by 13.5% and 17% respectively (Tab. 1). Losses were smaller than those found in previous research - over 35% [4]. The conditions of the two experiments were similar. Difference was only in the fact that previously as materials milk obtained from Polish Long Fleeces ewes was used. One of the factor contributing to the decrease in vitamin C content in the products is the presence of oxygen [15]. Perhaps differences in losses in both experiments are affected by the different content of oxygen in milk, that had not been degassed. Renner [15] indicated that pasteurisation process reduces vitamin C, especially the dehydroascorbic acid content 10-25%.

Table 1. The vitamin C and ascorbic acid content in raw ewe's milk, pasteurized milk and in the fresh fermented milk beverages

	Vitamin C			Ascorbic acid		
	x ± s [%]	% of raw milk content	% of pasteurized milk content	x ± s [%]	% of raw milk content	% of pasteurized milk content
Raw milk	2.44 ± 0.15[a]	100.0	115.6	1.53 ± 0.18	100.0	120.5
Pasteurised milk	2.11 ± 0.14[A]	86.5	100.0	1.27 ± 0.14	83.0	100.0
Yoghurt	2.04 ± 0.10[B]	83.6	96.7	1.45 ± 0.25	94.8	114.2
Bioyoghurt	2.32 ± 0.15[b]	95.1	110.0	1.56 ± 0.03	102.0	122.8
Kefir	2.10 ± 0.21[C]	86.1	99.5	1.13 ± 0.16[a]	73.9	89.0
Sour milk	3.04 ± 0.18[aABC]	124.6	144.1	2.07 ± 0.37[a]	135.3	163.0

A,B,C – statistically high significant difference between averages in the columns ($p \leq 0.01$)
a, b - statistically significant difference between averages in the columns ($p \leq 0.05$)
x ± s - average ± standard error

As seen from Table 1 the vitamin C and ascorbic acid content in fresh sour milk and bioyoghurt were statistically significantly higher than those in pasteurised milk and in the case of sour milk also higher than in raw milk. The observed increase in vitamin C in both fermented milk beverages could be attributed to the synthesis of this vitamin by the micro-organisms, that were used in the inoculation. According to Pałładina, quoted by Budsławski [5] some of the lactic acid bacteria (*Lactococcus* ssp.) are capable of synthesising vitamin C. This could be used in completing losses of this vitamin during pasteurisation, by inoculating milk with cocci. As seen from Table 1, a reverse situation was observed in kefir: the decrease in both vitamin C and ascorbic acid content in comparison with the pasteurised milk, which might be the result of ascorbic acid oxidation by micro-organisms included in the kefir starter culture. Ascorbic acid as an antioxidant reduces substance that are formed during metabolic changes of micro-organism [12].

In order to verify the differences among 4 fermented milk beverages with regard to vitamin C content, ascorbic acid content, titratable acidity and pH, analysis of one factor variance was estimated. The results for fresh, 7 and 14-d fermented milks are presented in Table 2. Fermented milks differed significantly with regard to vitamin C content only in fresh products. The differences in 7 and in 14 days of storage among fermented milks were small and statistically non-significant. Significant differences concerning ascorbic acid content in fresh sour milk and fresh kefir were also found. Fermented milk beverages differed statistically significantly in pH (fresh and after 14 days of storage) and in titratable acidity (fresh and after 7 days of storage) (Tab. 2). Yoghurt and kefir in comparison with sour milk and bioyoghurt were characterised by lower pH and higher titratable acidity. In a previous paper [3] it was found that bioyoghurt in comparison with yoghurt had a higher pH in all 14 day storage periods.

Table 2. Yoghurt, bioyoghurt, sour milk and kefir properties after 1, 7 and 14 days of storage

Parameters	Days	Yoghurt	Bioyoghurt	Kefir	Sour milk
		x ± σ	x ± σ	x ± σ	x ± σ
pH	1	4.45 ± 0.18^{a}	4.97 ± 0.18^{ab}	4.37 ± 0.14^{b}	4.73 ± 0.12
	7	4.28 ± 0.09	4.60 ± 0.04	4.24 ± 0.04	4.63 ± 0.12
	14	4.19 ± 0.06^{AB}	4.54 ± 0.06^{Aa}	4.25 ± 0.01^{ab}	4.56 ± 0.10^{Bb}
Titratable	1	41.47 ± 1.70^{ab}	34.80 ± 1.44^{Aa}	47.87 ± 0.87^{bAB}	37.60 ± 1.51^{B}
acidity [°SH]	7	47.47 ± 1.27^{a}	42.67 ± 1.04^{A}	50.53 ± 1.48^{aAB}	43.60 ± 0.69^{B}
	14	50.67 ± 2.92	45.20 ± 1.67	51.07 ± 1.35	46.87 ± 3.78
Vitamin C [mg/100g]	1	2.04 ± 0.10^{A}	2.32 ± 0.15^{a}	2.10 ± 0.21^{B}	3.04 ± 0.18^{ABa}
	7	2.21 ± 0.17	2.10 ± 0.11	2.27 ± 0.11	2.27 ± 0.45
	14	1.65 ± 0.11	1.59 ± 0.48	1.70 ± 0.39	1.76 ± 0.41
Ascorbic acid	1	1.45 ± 0.25	1.56 ± 0.03	1.13 ± 0.16^{a}	2.07 ± 0.37^{a}
[mg/100g]	7	1.33 ± 0.54	1.27 ± 0.55	1.53 ± 0.47	1.41 ± 0.45
	14	0.62 ± 0.41	0.71 ± 0.58	0.73 ± 0.61	0.85 ± 0.68

A,B - statistically high significant difference between averages in the line ($p \leq 0.01$)
a,b - statistically significant difference between averages in the line ($p \leq 0.05$)

The changes in vitamin C and ascorbic acid content in fermented milks after 7 and 14 days of storage in comparison with the content in fresh products are given in the Table 3. As seen in sour milk and bioyoghurt, vitamin C content gradually decreased, while in yoghurt and kefir after 7 day slightly increased. After 14 days, vitamin C content in all fermented milks was lower than in fresh. The ascorbic acid content, except kefir, decreased gradually during the storage period. In kefir an increase in ascorbic acid content after 7 days was observed and then, after 14 day, its decrease. Considerable variations in vitamin C and ascorbic acid content in kefir and yoghurt, increase especially after 7 days, give evidence concerning changes in those products, perhaps even concerning synthesis of vitamin C by micro-organisms of starter culture. Hanisch, quoted by Klupsch [12] observed an increase in ascorbic acid content in buttermilk between 2 and 4 days of storage, which was 45% and 92%, depending on the storage condition.

Table 3. The changes in the vitamin C and of ascorbic acid content in fermented milk beverages during storage period in comparison with the contents in fresh fermented milk beverages

Kind of milk beverage		Vitamin C			Ascorbic acid		
		Fresh	7 days	14 days	Fresh	7 days	14 days
Yoghurt	x	2.04	2.21	1.65	1.45	1.33	0.62
	%	100.0	108.3	80.9	100.0	91.7	42.8
Bioyoghurt	x	2.32	2.10	1.59	1.56	1.27	0.71
	%	100.0	90.5	68.5	100.0	81.4	45.5
Kefir	x	2.10	2.27	1.70	1.13	1.53	0.73
	%	100.0	108.1	80.9	100.0	135.4	64.6
Sour milk	x	3.04	2.27	1.76	2.07	1.41	0.85
	%	100.0	74.7	57.9	100.0	68.1	41.1

The losses of vitamin C content in milk and milk products during the storage period are affected by light, oxygen, iron, copper, and depend also on the kind of packaging [7, 12, 15]. The analysed fermented milk beverages were all stored in the same conditions: in hermetically sealed jars without light access, at 4°C. Fermented milks differed significantly with regard to the pH and titratable acidity (Tab. 2). There are opinions that acid environment stabilizes the vitamin C [9]. The results presented in the Table 3 correspond with those opinions: in bioyoghurt and sour milk that showed a higher pH and lower titratable acidity during the whole storage period, vitamin C was less stable after 14 days than that in yoghurt and kefir. According to the results obtained by Dave & Shah [7] ascorbic acid has some effect on the viability of *L. acidophilus.* It is possible that in the present bioyoghurt, (starter culture consisting also of this micro-organism) ascorbic acid was utilised by bacteria or constituted an important factor as oxygen scavenger and thereby improved the viability of micro-organism.

It must be emphasized, that in all the fermented products (fresh, after 7 and 14 day of storage) there were considerable variations with regard to the vitamin C and ascorbic acid content. This affected the results of two-factorial analysis of variation. This analysis (Tab. 4) demonstrated that the kind of fermented milk had no significant effect on vitamin C and the ascorbic acid content, but affected by storage period was significantly. However the titratable acidity and pH of those fermented products depended on the kind of product and on the storage time. The decrease in vitamin C and ascorbic acid content in yoghurt during storage as well as a decrease in pH and increase in titratable acidity were observed in previous research [4].

Table 4. The lowest squared means of analyzed parameters depending on the kind of fermented milk and on the storage period (fresh, 7, 14 days)

Parameters	Total	Kind of fermented milk				Days of storage		
	x ± s	Yoghurt	Bioyoghurt	Kefir	Sour milk	1	7	14
		x	x	x	x	x	x	x
Vitamin C [mg/100g]	2.09 ± 0.08	1.97	2.00	2.02	2.35	2.38^{A}	2.21^{a}	1.67^{aA}
Ascorbic acid [mg/100g]	1.22 ± 0.12	1.13	1.18	1.13	1.44	1.55^{a}	1.39^{b}	0.73^{ab}
Titratable acidity [°SH]	44.98 ± 0.52	46.53^{Aab}	40.89^{AB}	49.82^{BCa}	42.69^{Cb}	40.43^{DE}	46.07^{D}	48.45^{E}
pH	4.48 ± 0.03	4.31^{AB}	4.71^{AC}	4.29^{CD}	4.64^{BD}	4.63^{Fa}	4.44^{a}	4.38^{F}

A,B - statistically high significant difference between averages in the line (p≤0.01)
a,b - statistically significant difference between averages in the line (p≤ 0.05)</FON

With an aim of studying the dependences between vitamin C and ascorbic acid content the correlation coefficients were calculated. Those coefficients were statistically highly significant: for yoghurt r = 0.78, for bioyoghurt r = 0.87, for kefir r = 0.70, and for sour milk r = 0.93. Differences in the coefficients' values may be the result of a different rate of transformation of ascorbic acid in other forms of vitamin C, depending on the kind of fermented milk.

CONCLUSIONS

1. It was found that the kind of starter culture significantly affected the vitamin C and ascorbic acid content in fresh fermented milks, but after 7 and 14 days of storage the differences between those fermented products with regard to the vitamin C and ascorbic acid content were statistically insignificant.
2. Variations in vitamin C content in fermented milks during the storage period may give evidence of its synthesis by the micro-organism of the starter culture.
3. With the lapse in storage period in fermented milk beverages the vitamin C and ascorbic acid content as well as pH decreased, while titratable acidity increased.
4. High, positive and different values in correlation coefficients between vitamin C content and ascorbic acid content in fermented milks show different rates of change of ascorbic acid in other form of vitamin C in analysed fermented milk beverages.

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