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Short communication

Effect of freeze–dried kefir culture on proteolysis in feta-type and whey-cheeses

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ABSTRACT

The effect of freeze–dried kefir culture on the proteolysis of feta-type and whey-cheese was investigated. All nitrogen fractions increased during ripening. Although no significant differences were observed in total nitrogen (TN), the levels of water-soluble nitrogen (WSN), pH 4.4-soluble nitrogen (SN), 12% trichloroacetic acid-soluble nitrogen (TCA-SN) and phosphotungstic acid-soluble nitrogen (PTA-SN) were significantly higher in cheeses produced by freeze–dried kefir culture during the later stages of ripening. Content of total free amino acids (FAA) was significantly affected by freeze–dried kefir starter culture and it was continuously increased in kefir-cheese while, in rennet-cheese it was increased up to 30 days of ripening and then slightly decreased. On the other hand, FAA content continuously decreased in kefir-whey-cheese whereas it increased in whey-cheese. The cheese samples produced by freeze–dried kefir as starter culture were characterised as high-quality products during the preliminary sensory evaluation and they were accepted by the panel. Overall, the use of freeze–dried kefir suggested acceleration of cheese ripening and resulted in improved sensory characteristics.

1. Introduction

Nowadays, an upsurge of interest in providing suitable starter cultures for cheese production has been observed (Boylston, Vinderola, Ghoddusi, & Reinheimer, 2004; Dimitrellou, Kourkoutas, Banat, Marchant, & Koutinas, 2007; Kourkoutas et al., 2006). In addition to improved quality, enhanced microbiological safety and health benefits, starter cultures have the advantage of offering consistent characteristics to the final products, which is important for commerce.

Feta cheese, one of the most significant and popular dairy products in Greece, with characteristic, slightly acid, salty taste, pleasant organoleptic properties and worldwide acceptance, is a soft, white cheese, usually ripened in brine. Traditionally, Feta cheese was prepared, either from thermized or raw ewe's milk, in small family premises with elementary equipment, using only rennet without the addition of any starter cultures. Its characteristic flavour and texture are developed by the action of the natural lactic acid microflora of milk. Today, most Feta cheese is produced from ewe's milk or a mixture of ewe's and goat's milk in contemporary cheese dairies, using yogurt culture for lactic acid production, followed by addition of rennet for completion of precipitation. The produced cheese is consumed after a 2 month ripening period. This maturation period is necessary for the sanitation of cheese products, especially those made from raw milk.

Another very popular cheese in Greece is Myzithra, which is produced from the whey remaining after Feta cheese production by heating to 80–95 °C until curd is formed. It is consumed either fresh (having a moisture content of about 70%), or after salting and water removal, leaving a maximum moisture content of 40% and ripening for at least 2 months (Kyriakopoulos, 1995). In industrial practice, ripening is usually carried out at ambient temperature (15–20 °C).

Kefir is a mixture of microbes that is mainly used in the production of the low alcoholic, traditional Russian fermented milk "kefir". This mixed culture consists of various yeasts (*Kluyveromyces*, *Candida*, *Saccharomyces* and *Pichia*), various lactic acid bacteria (LAB) of the genera *Lactobacillus, Lactococcus*, *Leuconostoc* and acetic acid bacteria (Garrote, Abraham, & De Antoni, 1997; Luis, Lopez, & Lema, 1993; Pintado, Lopes Da Silva, Fernades, Malcata, & Hogg, 1996; Witthuhn, Schoeman, & Britz, 2005). Yeasts and LAB co-exist in a symbiotic association and are responsible for the lactic–alcoholic fermentation. The consumption of kefir has been related to a variety of health benefits (Cevikbas et al., 1994; Rodrigues, Caputo, Carvalho, Evangelista, & Schneedorf, 2005). Kefir culture has been recently used as a starter in both feta-type (Kourkoutas et al., 2006) and whey-cheese (Dimitrellou et al., 2007) production.

Proteolysis is the principal and most complex biochemical event occurring during maturation of the majority of ripened

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cheese varieties (Fox et al., 1995a). In addition to softening the cheese body, proteolysis influences the development of cheese flavour via the formation of amino acids and peptides, which make a direct contribution to flavour (Fox, McSweeney, & Singh, 1995b).

Hence, the aim of the present study was to investigate the proteolytic activity of freeze–dried kefir starter culture in feta-type and whey-cheeses.

2. Materials and methods

2.1. Kefir culture

Kefir culture, isolated from commercially available kefir grains (MELITON S.A., Thessaloniki, Greece) usually used to produce kefir drink, was employed in the present study. It was grown on synthetic medium consisting of 4% lactose, 0.4% yeast extract, 0.1% $(NH_4)_2SO_4$, 0.1% KH_2PO_4 and 0.5% $MgSO_4 \cdot 7H_2O$ at 30 °C. The synthetic medium was sterilized at 130 °C for 15 min prior to use. Pressed wet weight cells (≈0.5–1.0 g dry weight) were prepared and employed directly in aerobic fermentations of whey for further production of kefir culture (Papavasiliou et al., 2008).

2.2. Production of freeze–dried kefir culture

Kefir culture, produced by aerobic fermentations of whey, was resuspended in fermented whey, used as a cryoprotecting agent, and the whole was then frozen to −45 °C. The frozen samples were freeze–dried overnight at 5×10^{-3} bar at −45 °C, using a model Freezone 4.5 freeze–dryer (Labconco) (Papavasiliou et al., 2008).

2.3. Cheese-making

Feta-type cheese was produced using pasteurised ovine milk, as described recently by Kourkoutas et al. (2006), with some modifications. In brief, cheese containing freeze–dried kefir culture, designated "kefir-cheese", was produced using milk heated at 37 °C to which freeze–dried kefir culture was added. Following the addition of commercial rennet (0.01%) after 30 min, the mixture was left undisturbed for 2 h for curd formation. Subsequently, the curd was cut into cubes (diameter ≈ 1 cm), left undisturbed for 10 min and then cloth-filtered overnight at room temperature (18–22 °C) for complete whey removal. The next day the surfaces of cheese samples (400 g each) were rubbed with 8 g of salt and after 4 days the cheese was immersed in 10% (w/v) brine solution. Cheese ripening was carried out at 15–18 °C for the first 15 days and then at 4–6 °C for up to 70 days. Feta-type cheese without kefir culture, designated "rennet-cheese", was also produced for comparison purposes.

Whey cheese was produced using milk whey derived after production of feta cheese, as described recently by Dimitrellou et al. (2007) with some modifications. In brief, milk whey was heated to 95 °C for 10–15 min until cheese curd was formed and then the whole was cloth-filtered. Cheese containing freeze–dried kefir culture, designated as "kefir-whey-cheese", was produced by spraying 50 ml of whey, in which 5 g of freeze–dried kefir culture were added, at cheese curd (400 g). The addition of kefir culture was carried out at ≈37 °C and then the produced cheese was re-filtered. Cheese without kefir culture, designated as "whey-cheese", was also produced for comparison purposes, by heating of milk whey, followed by cloth-filtration, as described above. The following day (after production), the surfaces of cheese samples (400 g each) were rubbed with 28 g of salt. Cheese ripening was monitored at 4–6 °C for 70 days.

Duplicate samples from each treatment were collected at various intervals and subjected to analysis, as described below.

2.4. Analysis

Moisture and ash content of cheese samples were determined according to the Association of Official Analytical Chemists (1995).

2.5. Nitrogen fractions

Total nitrogen (TN), expressed as crude protein on dry weight basis, was determined by using the Kjeldahl procedure (Kirk & Sawyer, 1991).

2.6. Water-soluble nitrogen (WSN)

Ten grammes of cheese were mixed with 50 ml of deionized water and homogenised. The homogenate was held for 1 h at 40 °C and then centrifuged at 3000*g* for 30 min at 4 °C. The suspension was finally filtered through Whatman No. 40 filter paper. The nitrogen content was determined using the Kjeldahl method (Bütikofer, Rüegg, & Ardö, 1993).

2.7. pH 4.4-soluble nitrogen (pH 4.4-SN)

Five grammes of cheese were dispersed in 90 ml of 0.1 M tri-sodium citrate solution (pH: 7.0), for 30 min at 30 °C by gentle stirring with a magnetic stirrer. The volume was adjusted to 100 ml with citrate solution and the pH adjusted if necessary to 4.4 at 30 °C with 1 M HCl acid solution (the volume of acid added was taken into account for the calculation of the dilution). The suspension was held at 30 °C for 30 min and then filtered through Whatman No. 40 filter paper. The nitrogen content was then determined using the Kjeldahl method (Bütikofer et al., 1993).

2.8. 12% trichloroacetic acid-soluble nitrogen (12% TCA-SN)

Twenty-five millilitres of WSN extract were added to 25 ml of 240 g/kg trichloroacetic acid solution. The suspension was held at room temperature for 2 h and then filtered through Whatman No. 40 filter paper. The nitrogen content was then determined using the Kjeldahl method (Bütikofer et al., 1993).

2.9. Phosphotungstic acid-soluble nitrogen (PTA-SN)

Ten millilitres of WSN extract were added to 7 ml of 3.95 M sulphuric acid solution and 3 ml 330 g/l phosphotungstic acid solution. The mixture was equilibrated overnight at 4 °C and then filtered through Whatman No. 40 filter paper. The nitrogen content was then determined using the Kjeldahl method (Bütikofer et al., 1993).

2.10. Determination of free amino acids (FAA)

Individual amino acids were analyzed in water-soluble extracts of the cheeses. Cheese samples (20 g each) were macerated with warm water (40 °C) to produce a total volume of 210 ml. Each sample was then filtered and the filtrate was used for FAA analysis.

2.11. Free amino acid (FAA) determination

Solid phase extraction (SPE) and the derivatization process were carried out according to the instructions of the EZ:faast kit (Phenomenex, USA). The derivatized FAA were separated, identified and quantified by gas chromatography of Fisons Instruments (GC 8000 series, model 8060) equipped with a split–splitless injector, FID detector and Chromcard software (CE Instruments). The oven temperature was programmed at 110 °C, and then raised to 320 °C at a rate of 15 °C/min. It was held at this temperature for

1 min. The injector and detector temperatures were 250 and 320 °C, respectively. A Zebron ZB-AAA GC column (60 m, 0.32 mm i.d.) for protein hydrolysate samples was used (Phenomenex, USA). For FAA determination, a total volume of 1.6 μl of derivatized samples was injected. The injector was operated in split mode (split ratio 1:15). The carrier gas used was helium at a flow rate of 1.5 ml/min. Concentration of FAA was determined using standard curves. Norvaline (2-aminovaleric acid) was used as internal standard.

2.12. Preliminary sensory evaluation

Kefir- and kefir-whey-cheese samples were tested for their sensory characteristics and compared to rennet- and whey-cheese respectively, as well as to similar types commercial products. Samples of approximately 25 g of cheese were presented in random order in 5 cm Petri dishes served at room temperature. Sensory evaluation was conducted in duplicate by 14 laboratory members (seven members previously trained and seven untrained) using locally approved protocols. The panel was asked to give scores on a 0–10 scale (0: unacceptable, 10: exceptional) for attributes grouped into three categories: aroma, taste and flavour (Dimitrellou et al., 2007; Kourkoutas et al., 2006). The overall score was calculated as the average of the above three categories of attributes. Panellists used water to clean their palates between samples and were unaware of the identity of the samples they tasted (samples were coded with randomly chosen 3-digit numbers).

2.13. Experimental design and statistical analysis

In the experiments conducted, the effects of the cheese-type, the starter culture and the ripening time on nitrogen fractions and free aminoacids were studied. All treatments were carried out in triplicate and the mean values are presented (standard deviation for all values was about ±5% in most cases). The experiments and the preliminary sensory evaluation were designed and analyzed statistically by ANOVA. Duncan's multiple range test was used to determine significant differences among results; coefficients, ANOVA tables and significance ($P < 0.05$) were computed using Statistica v.5.0).

3. Results and discussion

3.1. General

Since the use of freeze–dried kefir as starter culture in feta-type and whey cheese resulted in repression of spoilage, improved profile of aroma-related compounds and in overall improvement of quality (Dimitrellou et al., 2007; Kourkoutas et al., 2006), the strategy adopted in the present study was to investigate potential acceleration of cheese-ripening due to the proteolytic action of freeze–dried kefir culture.

3.2. Nitrogen fractions

The results are presented in Table 1. All nitrogen fractions increased during ripening. These results are in agreement with a previous study in which various adjunct cultures were used in feta-type cheese, in order to improve cheese flavour, through proteolysis (Michaelidou, Katsiari, Kondyli, Voutsinas, & Alichanidis, 2003). Cheese type, addition of freeze–dried kefir starter culture and the ripening time significantly affected moisture, dry matter, WSN, pH 4.4-SN, 12% TCA-SN and PTA-SN ($P < 0.05$). pH and ash content were affected by the freeze–dried kefir culture and the ripening time ($P < 0.05$), but TN by only cheese type ($P < 0.05$).

The cheese pH was reduced during ripening and was significantly lower ($P < 0.05$) in cheeses produced with freeze–dried starter culture (Table 1), probably due to production of lactic acid by the LAB present in kefir culture (Dimitrellou et al., 2007; Kourkoutas et al., 2006).

Extraction and quantification of WSN and cheese nitrogen soluble at pH 4.4 (pH 4.4-SN) are widely used as an index of proteoly-

Table 1
Effects of freeze–dried kefir starter culture on pH, acidity, ash and moisture content and nitrogen fractions during ripening of feta-type and whey-cheese.

Cheese type	Ripening time (d)	pH	Moisture (%)	DM (%)	Ash (% DM)	TN (% DM)	WSN (% TN)	pH 4.4-SN (%TN)	12% TCA-SN (% TN)	PTA-SN (% TN)
Rennet-cheese	0	6.53 ± 0.1	59.69 ± 2.2	40.31 ± 1.4	4.24 ± 0.2	5.95 ± 0.2	8.87 ± 0.3	6.93 ± 0.3	4.53 ± 0.3	1.96 ± 0.1
	1	6.58 ± 0.1	54.86 ± 2.1	45.14 ± 2.0	4.45 ± 0.2	5.87 ± 0.3	10.4 ± 0.4	9.28 ± 0.3	4.87 ± 0.2	2.09 ± 0.1
	4	5.56 ± 0.1	48.04 ± 2.0	51.96 ± 1.7	10.35 ± 0.4	5.66 ± 0.3	12.1 ± 1.0	13.6 ± 0.4	4.51 ± 0.2	2.34 ± 0.3
	15	5.39 ± 0.1	58.87 ± 1.2	41.13 ± 2.0	14.56 ± 0.7	5.57 ± 0.3	15.5 ± 1.1	17.8 ± 1.0	7.38 ± 0.3	2.98 ± 0.1
	30	5.38 ± 0.1	58.45 ± 2.3	41.55 ± 1.5	14.68 ± 0.6	5.51 ± 0.5	18.0 ± 1.5	19.3 ± 1.5	9.58 ± 0.5	3.46 ± 0.2
	70	5.66 ± 0.1	58.98 ± 1.9	41.02 ± 2.0	15.09 ± 1.0	5.61 ± 0.6	19.3 ± 1.4	21.3 ± 2.2	11.2 ± 0.4	3.85 ± 0.1
Kefir-cheese	0	6.49 ± 0.1	61.94 ± 1.8	38.06 ± 1.5	3.78 ± 0.2	5.83 ± 0.1	8.81 ± 0.3	6.89 ± 0.2	4.92 ± 0.1	2.01 ± 0.1
	1	5.20 ± 0.1	46.22 ± 1.0	53.78 ± 1.5	3.59 ± 0.2	5.45 ± 0.2	10.6 ± 0,3	9.89 ± 0.8	4.93 ± 0.1	2.16 ± 0.1
	4	4.98 ± 0.1	46.67 ± 1.1	53.33 ± 1.9	9.41 ± 0.3	5.53 ± 0.2	13.3 ± 1.4	13.1 ± 0.9	6.16 ± 0.2	2.76 ± 0.2
	15	4.78 ± 0.1	53.55 ± 1.2	46.45 ± 1.4	12.47 ± 0.4	5.36 ± 0.2	18.5 ± 1.4	18.6 ± 0.5	9.97 ± 0.3	4.04 ± 0.2
	30	4.80 ± 0.1	53.01 ± 2.0	46.99 ± 2.0	12.02 ± 1.0	5.43 ± 0.4	20.5 ± 1.0	21.5 ± 1.9	9.87 ± 0.4	4.57 ± 0.3
	70	4.75 ± 0.1	54.18 ± 1.1	45.82 ± 1.8	12.46 ± 0.9	5.37 ± 0.2	23.3 ± 1.1	25.6 ± 1.0	14.86 ± 1.0	5.05 ± 0.2
Whey-cheese	0	6.57 ± 0.1	57.93 ± 1.4	42.07 ± 1.2	2.04 ± 0.1	6.03 ± 0.2	6.72 ± 0.2	6.35 ± 0.2	3.14 ± 0.1	1.49 ± 0.2
	1	6.35 ± 0.1	55.09 ± 2.0	44.91 ± 1.8	2.18 ± 0.1	6.01 ± 0.2	8.05 ± 0.5	7.05 ± 0.7	4.01 ± 0.1	1.50 ± 0.2
	4	6.03 ± 0.1	49.20 ± 1.1	50.80 ± 3.3	7.62 ± 0.3	5.98 ± 0.2	8.96 ± 0.4	7.75 ± 0.6	4.47 ± 0.4	1.54 ± 0.1
	15	5.56 ± 0.1	60.93 ± 1.9	39.07 ± 2.0	14.82 ± 1.3	5.96 ± 0.2	11.9 ± 0.3	11.7 ± 0.7	5.39 ± 0.2	1.91 ± 0.1
	30	5.54 ± 0.1	66.33 ± 1.3	33.67 ± 1.6	17.37 ± 1.4	6.03 ± 0.2	12.1 ± 0.6	12.5 ± 1.0	6.03 ± 0.3	2.34 ± 0.2
	70	5.63 ± 0.1	66.15 ± 1.3	33.85 ± 1.9	17.64 ± 1.6	5.97 ± 0.2	12.9 ± 1.4	13.6 ± 1.1	6.47 ± 0.4	2.82 ± 0.2
Kefir-whey-cheese	0	6.18 ± 0.1	56.22 ± 1.1	43.78 ± 1.8	2.19 ± 0.1	6.01 ± 0.2	7.13 ± 0.2	6.90 ± 0.6	3.17 ± 0.2	1.51 ± 0.1
	1	5.04 ± 0.1	53.21 ± 2.1	46.79 ± 1.7	2.22 ± 0.1	5.96 ± 0.2	8.86 ± 0.2	7.43 ± 0.2	4.25 ± 0.2	1.60 ± 0.2
	4	5.07 ± 0.1	48.78 ± 1.7	51.22 ± 2.1	7.79 ± 0.2	5.90 ± 0.3	10.3 ± 0.2	9.48 ± 0.4	6.04 ± 0.5	2.04 ± 0.1
	15	4.79 ± 0.1	59.45 ± 1.4	40.55 ± 2.0	13.98 ± 0.5	5.90 ± 0.2	14.3 ± 0.3	14.7 ± 0.6	7.39 ± 0.3	2.90 ± 0.2
	30	4.77 ± 0.1	65.45 ± 2.5	34.55 ± 2.1	16.73 ± 1.3	5.88 ± 0.2	15.7 ± 0.6	16.0 ± 0.9	7.73 ± 0.7	3.07 ± 0.1
	70	4.60 ± 0.1	65.65 ± 1.6	34.35 ± 2.0	17.15 ± 1.5	5.85 ± 0.2	16.5 ± 1.4	17.0 ± 1.0	8.10 ± 0.6	3.60 ± 0.1

DM: dry matter, TN: total nitrogen, WSN: water-soluble nitrogen, pH 4.4-SN: pH 4.4-soluble nitrogen, 12% TCA-SN: 12% trichloroacetic acid-soluble nitrogen, PTA-SN: phosphotungstic acid-soluble nitrogen.

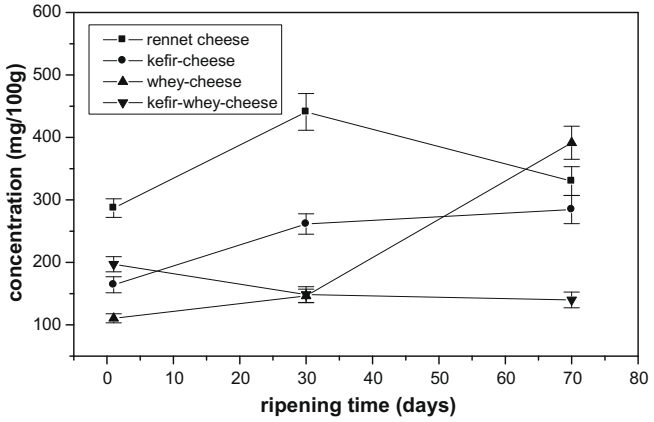

Fig. 1. Effect of freeze–dried kefir starter culture on total free aminoacids (mg/100 g dry matter) during ripening of feta-type and whey cheeses.

sis. In the present study, there was little difference between the levels extracted by water and pH 4.4 buffers (Table 1). Both WSN and pH 4.4-SN are mainly produced by rennet and usually increase during ripening. Whey proteins are also soluble at pH 4.4, but their contribution to pH 4.4-SN is relatively small. However, the contents of WSN and pH 4.4-SN were significantly higher ($P < 0.05$) in cheeses produced by freeze–dried kefir, indicating a high proteolytic activity of kefir culture.

The TCA-SN fraction in cheese contains small peptides (2–20 residues) and amino acids, resulting mainly from the proteolytic activity of bacteria (Christensen, Bech, & Werner, 1991) and, to a lesser extent, rennet (McSweeney & Fox, 1997). The TCA-SN fraction was more than doubled after 60 days of ripening in all cheese samples, while the increase was significantly higher ($P < 0.05$) in samples with freeze–dried kefir, due to the action of kefir culture (Table 1). Since no brine was added to the containers of the whey- and kefir-whey-cheeses, the expulsion of small molecular mass fractions was expected to be limited.

Phosphotungstic acid (PTA) is a very discriminating protein precipitant, since only free amino acids (apart from lysine and arginine), and peptides of less than about 600 Da are soluble in 5% PTA (Jarrett, Aston, & Dulley, 1982). PTA-SN has been used extensively as an index of free amino acids in cheese (Bütikofer et al., 1993; McSweeney & Fox, 1997; Pereira, Gomes, Gomes, & Malcata, 2008). The results obtained by PTA-SN showed that freeze–dried kefir culture had a significant effect ($P < 0.05$) on proteolysis at the level of small peptides, which could be attributed to the extended proteolytic activity of kefir microbial flora.

The significantly increased TCA-SN and PTA-SN, observed in cheeses produced by freeze–dried kefir as starter culture, are usu-

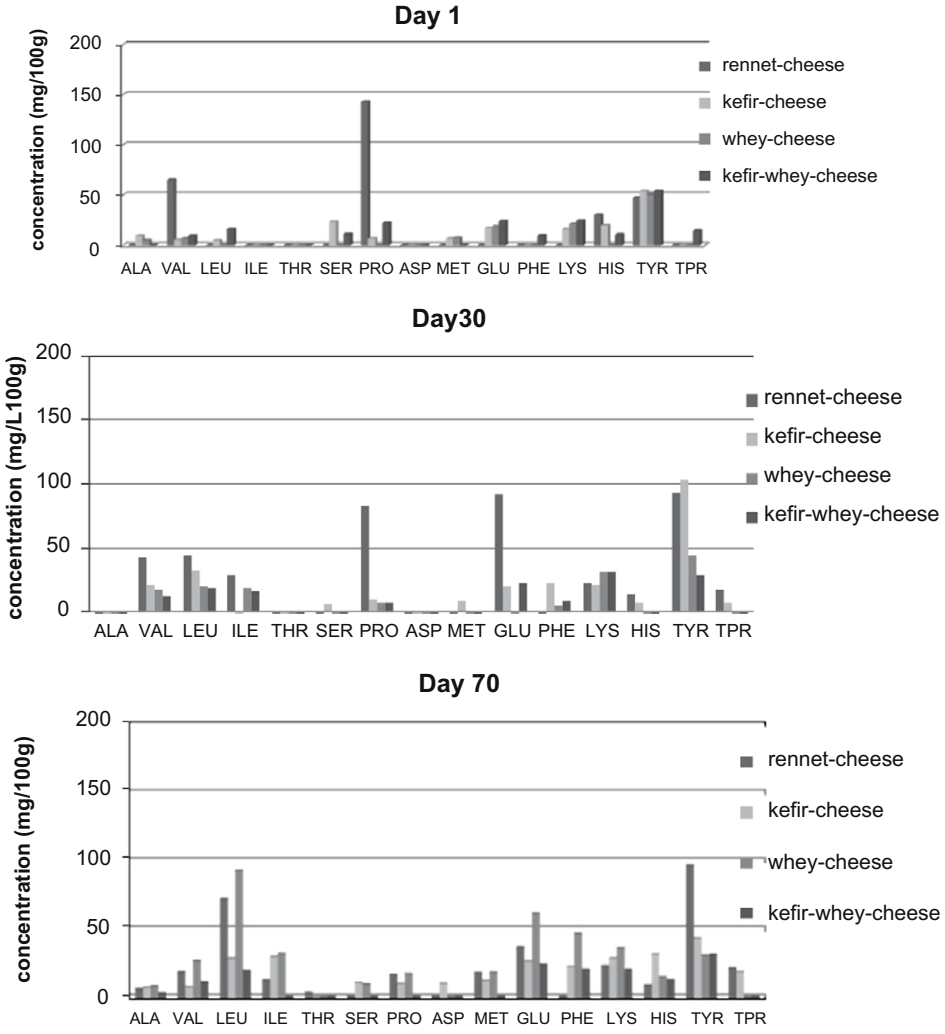

Fig. 2. Effect of freeze–dried kefir starter culture on levels of individual free aminoacids (mg/100 g dry matter) during ripening of feta-type and whey cheeses. ALA: alanine, VAL: valine, LEU: leucine, ILE: isoleucine, THR: threonine, SER: serine, PRO: proline, ASP: asparagine, MET: methionine, GLU: glutamic acid, PHE: phenylalanine, LYS: lysine, HIS: histidine, TYR: tyrosine, TPR: tryptophan.

ally associated with improved sensory characteristics, as the small peptides may act as precursors for aroma- and flavour-related compounds.

3.3. Free aminoacids (FAA)

Cheese proteolysis was also monitored by determination of free aminoacids during ripening. The results are presented in Figs. 1 and 2.

Concentrations of total FAA showed large deviations during ripening in the samples tested and were significantly affected by cheese type, freeze–dried kefir starter culture and ripening time ($P < 0.05$). Total FAA content continuously increased in kefir-cheese (Fig. 1), probably due to the proteolytic activity of kefir culture, while, in rennet-cheese it increased up to 30 days of ripening and then slightly decreased. On the other hand, in kefir-whey-cheese, it continuously decreased but increased in whey-cheese. The reduction of total FAA in kefir-whey-cheese could be attributed to metabolism of the nitrogen fraction by the kefir culture. A similar reduction of total amount of FAA, between 40 and 60 days of ripening of Feta-type cheese, probably due to decarboxylation, deamination and transamination reactions, has been previously reported (Katsiari, Alichanidis, Voutsinas, & Roussis, 2000). The different trends of total FAA observed in kefir and kefir-whey-cheese might be due to differences in the nature and content of proteins in the two cheese types.

Cheese-type, addition of freeze–dried kefir starter culture and the ripening time significantly affected concentrations of valine, threonine, serine, proline, asparagine, methionine, glutamic acid, phenylalanine, histidine and tyrosine ($P < 0.05$). Content of alanine, lysine and tryptophan were significantly affected by cheese-type and ripening time ($P < 0.05$), but not by freeze–dried kefir culture ($P > 0.05$). On the other hand, concentrations of leucine and isoleucine were significantly affected by freeze–dried kefir starter culture and the ripening time ($P < 0.05$), but not by the cheese-type ($P > 0.05$).

Serine content ranged in very low levels in cheese samples produced with no starter culture, while its concentration was reduced in kefir and kefir-whey-cheese during ripening. Likewise, threonine was found in traces in all cases. The very low concentrations of these two aminoacids are probably due to the action of lactobacilli (Irogoyen, Ortigosa, Juansaras, Oneca, & Torre, 2007; Marshall & Cole, 1983; Vescoso, Torriani, Dellaglio, & Botazzi, 1993), as they were present in the cheese samples studied (Dimitrellou et al., 2007; Kourkoutas et al., 2006) and are also the most common NSLAB that continue to grow during ripening (Mannu, Comunian, & Scintu, 2000). The branched-chain aminoacids (Leu, Ile, Val), along with aromatic aminoacids (Phe, Tyr, Trp) and Met, present in all cheese types are the main precursors of key aroma compounds (Yvon & Rijnen, 2001).

3.4. Preliminary sensory evaluation

The use of freeze–dried kefir as starter culture significantly affected ($P < 0.05$) the preferences of the tasters. Kefir- and kefir-whey-cheese scored the statistically higher values (data not shown) and gained the tasters' preference compared to the commercial products. They were characterised as novel, special cheese types with a characteristic flavour and a distinctive aromatic potential.

4. Conclusions

The use of freeze–dried kefir starter culture indicated acceleration of cheese ripening due to the higher content of nitrogen fractions and resulted in improved sensory characteristics. However, no conclusions could be drawn for the action of freeze–dried kefir culture on the concentrations of FAA, due to the interactions occurring between microorganisms, and to the complexity of the microbial ecosystem found in cheese. Hence, more research is required in the field, as our knowledge of the relationship between the microbial associations and the chemical compounds is still limited.

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