Development of a novel white soft cheese using kefir starter cultures: Microbiological, physicochemical and sensory properties

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Abstract

Kefir health promoting effects have created increasing demands and research efforts to develop new kefir products. So, this study aimed at developing novel functional white-soft-cheeses (WSC) using kefir cultures. Kefir WSCs were produced from cow's milk with and without thyme. Microbiological, physicochemical and sensory criteria were examined during storage at 4°C for 15 days. Total aerobes, yeasts, and lactococci variably decreased in counts, whereas lactobacilli count significantly increased in both cheeses. Kefir addition significantly affected acidity, pH and proteolytic criteria. Kefir cheese without thyme scored highest sensory acceptability. Nevertheless, final counts of kefir cultures (>5x10^{7}CFU/ml) were far above the minimum therapeutic requirements (10^6 CFU/ml), and kefir cheeses sensory scored good acceptability, indicating the suitability of WSC as a potential vehicle of kefir. The current results indicate a new and interesting perspective in the development of new functional foods through using of kefir starters in various dairy and non-dairy products with acceptable sensorial criteria, and hence expand the range of kefir containing products for the consumers around the world.

Key words: Kefir, Starter cultures, White soft cheese, Lactobacilli, Probiotics, Proteolytic activity.

Introduction

Kefir is an ancient fermented milk beverage that has been originated and consumed for thousands of years in Central Asia and Eastern Europe (14). Kefir is considered as a natural probiotic product, made by inoculating milk with kefir grains. These grains are small clusters of microorganisms held together in a polysaccharide matrix named kefiran, resulting in acidic and slightly alcoholic beverage (5, 14). Kefir grains contain a consortium of lactic acid bacteria (LAB), acetic acid bacteria, and lactose- and non-lactose-fermenting yeasts (8, 9, 13).

Kefir's popularity is due to the long history of being beneficial to health, as it has been associated with several health benefits (antimicrobial, anti-cancer, hypocholesterolemic, lactose-intolerance alleviation, immunological, and improvement of intestinal conditions) (5, 25, 26). Accordingly, the interest in kefir has increased considerably in recent years creating more consumers demands on kefir products. Thus, numerous research efforts have been made to either improve traditional kefir beverage or develop a range of value-added kefir products by incorporating kefir cultures biomass into Feta-type cheese (19, 21), white pickled cheese (1, 15), whey cheese (13), whey-based beverages (3, 13), and baker's yeast (23).

In many Middle Eastern countries including Jordan, kefir beverage is not a popular product, and this limited popularity could be in part due to kefir tangent acidity and slight alcoholic taste. Instead, several other fermented products gained high popularity, including yoghurt, labaneh (concentrated yoghurt), and white soft cheese (WSC) (6). However, WSC is a semi hard, slightly acidic and salty cheese, with very pleasant sensorial criteria (6, 12). Traditionally, WSC is made by the addition of rennet enzymes to pasteurized milk with no addition of starter cultures, hence acid production and proteolytic activity are almost absent, and its pH is nearly the same of original milk pH (6.3-6.5). As a result of these criteria, WSC has been shown to be an ideal vehicle for probiotic bacteria delivery (6). This raises the possibility of using WSC as a vehicle to deliver kefir cultures to human with more pleasant and accepted sensorial criteria compared to traditional kefir beverage.

However, kefir cultures are very active proteolytic microorganisms, whereas WSC lacks this activity (5, 14). This raised the concern about the effect of addition of kefir cultures and thyme on different sensorial, quality and chemical characteristics of WSC. Accordingly, this study aimed to evaluate the effect of kefir cultures on the chemical (including proteolysis) microbiological and sensorial criteria of WSC, and the growth kinetics of different kefir cultures in WSC upon storage at 4 °C/15 days, in order to develop a novel kefir WSC.

Material and methods

Kefir culture biomass: Kefir cultures biomass used in the present study was isolated from commercial kefir grains obtained from local markets (Amman, Jordan). For culture resuscitation, kefir grains were grown on a synthetic medium consisting of 4% lactose, 0.4% yeast extract, 0.1% (NH4)2SO4, 0.1% KH2PO4 and 0.5% MgSO4-7H2O at 30◦C, which was sterilized at 130◦C for 15 min (19, 21). For further kefir starter cultures production, pressed wet cells were harvested by centrifugation at 3,000 g for 10 min. Harvested biomass (2-3 g) was resuspended directly in 500 mL of whey and incubated at 30°C for aerobic fermentation (19). Concentrated kefir cultures biomass was obtained by centrifugation of fermented whey at 3,000 g for 10 min.

Cheese making: Two types of kefir soft cheeses, with and without addition of thyme (Thymus vulgaris), were prepared from cow's milk according to a traditional Jordanian method (6), at the Department of Nutrition and Food Technology, Al-Balqa Applied University (Salt, Jordan). Cheese with no kefir nor thyme addition was prepared as a control. All of the cheeses were made with 3 L of pasteurized (82°C for 30 to 45 s) whole cow's milk. After cooling the milk to 38°C, 0.6 g of $CaCl_2$ and 0.1 ml of rennet enzyme (Chr. Hansen's, Copenhagen, DK) were added to milk. The mixture was then allowed to stand for 40 min. After curd formation, the curd was cut and mixed gently to enhance further curd formation and drain out whey (90% of total whey); then 1.5% NaCl was added to the curd. For the thyme cheeses, thyme was added as finely ground dried leaves into the curds before the final drainage. Drained semi-dry curd was poured into cheese vats (250 g each). For kefir cheeses, concentrated kefir cultures biomass was lightly suspended in 0.9% NaCl and added to the salted drained curds. These curds were mixed and allowed to set at room temperature for 2 h, then the curds were drained from the whey. The resulting cheeses were stored at 4°C for 15 d.

Physicochemical analysis: Cheese samples were tested for pH and acidity (g lactic acid/100 g cheese) Acidity was quantified by titration with 0.1 N NaOH using phenolphthalein as indicator (2) and expressed as lactic acid content. Cheese pH was measured using a digital pH meter (PH 610, Eutech Instruments, Singapore).

Proteolytic activity: Proteolytic activity was determined by ortho-phthalaldehyde (OPA) method (11). A 5 g portion of each cheese sample were mixed with 10 ml of 0.72 N trichloroacetic acid (TCA) and held for 10 min at room temperature. The mixture was centrifuged at 10,000 X g for 15 min and the supernatant was filtered through Whatman no. 4 filters. Two hundred microliters of each TCA filtrate was mixed with 3 ml of the OPA reagent and the mixture was held for 2 min before the absorbance was read at 340 nm with a Perkin-Elmer Lambda 3B spectrophotometer, (Perkin-Elmer Instruments, Norwalk, Conn.). The results were expressed as mmole of α-amino acids (α-AA). The OPA reagent was prepared by combining 25 ml of 100 mM sodium borate, 2.5 ml of 20% (wt/wt) sodium dodecyl sulfate, 100 µl of 2-β-mercaptoethanol, and 40 mg of OPA dissolved in 1 ml of methanol. The volume of the OPA reagent was adjusted to 50 ml with distilled water. All chemicals were obtained from Sigma-Aldrich (St. Lois, MI, USA). Analysis was carried out at days 0, 3, 9, and 15.

Impact of storage conditions on the viability of kefir cultures: The impact of storage conditions (15 d at 4°C) on the viability of different kefir cultures in WSC was determined as the following: representative 20 g cheese samples were diluted separately in 180 ml of 2% sodium citrate solution and homogenized for 5 min. Serial dilutions were made in quarter strength Ringer's solution and plated onto one of the following media: (i) plate count agar (Oxoid, CM0325) at 30 °C for 48 h for the determination of total aerobic counts (TAC), (ii) acidified MRS agar (Oxoid, CM0361) at 37 °C for 48 h anaerobically (Anerocult C anaerobic jar; Merck) for the enumeration of lactobacilli (gram positive, catalase negative), (iii) M-17 agar (Oxoid, Basingstoke, UK, CM0785) at 37 °C for 48 h for the enumeration of lactococci (gram positive, catalase negative), (iv) PDA agar (Oxoid, CM0139) (pH adjusted to 4.5 by sterile 10% lactic acid solution) at 30 °C for 48h for the enumeration of yeasts and moulds (7, 18, 20). Analysis was carried out at days 0, 3, 9, and 15.

Sensory evaluation: A hedonic scale (5-points, 1= dislike very much, and 5= like very much) test as described by Awaisheh (6) was used to investigate the degree of preference for soft cheese made by kefir cultures with and without thyme compared to control soft cheese. The following attributes were considered in evaluation: texture, flavor, odour, colour, and general acceptability. Twenty panellists from the staff of the Department of Nutrition and Food Technology at Al-Balqa Applied University, Salt, Jordan, made the sensory evaluation.

Statistical analysis: All analysis were performed in six replications unless otherwise stated. All data, except sensory evaluation data, were analysed using one-way analysis of variance of a Statistical Analysis System (SAS) software version 9.3 (SAS 2011). Tukey-Kramer test was performed to compare any significant differences in variables between groups. The normal distribution and log-transformation of microbiology data was carried out using SAS Univariate Procedure. Sensory evaluation data were analysed using Ordinal-scaled Chi-square test. Type I error was set at 0.05 significance level for all statistical tests.

Results and discussion

The impact of cheese processing and cold storage on different kefir culture microorganisms is illustrated in Table 1. The initial counts of all microbial types were greater than 6.87 $\log_{10}$ colony forming unit/g (CFU/g) in kefir cheeses and less than 4.27 $\log_{10}$ CFU/g in control. The TAC of kefir cheeses with thyme (KCT) and kefir cheese without thyme (KCWT) showed a mild decline during the first 9 ds followed by a strong significant increase in the following 6 ds reaching to 7.48 and 7.45 $\log_{10}$ CFU/g, respectively, whereas count of control cheese reached maximum count at day 9 (6.17 $\log_{10}$ CFU/g) and remained unchanged till the end of storage. For yeasts and molds, the initial and final counts were significantly higher in kefir cheeses than in control cheese. Yeasts and molds counts of kefir cheeses showed a mild decline during the first 3 ds, followed by a steady period till day 9 and a maximum at day 15 (6.52 and 7.28 $\log_{10}$ CFU/g for KCT and KCWT, respectively). For lactobacilli, the initial count was highest for kefir cheese alone followed by KCT and then control cheese, whereas the final counts were significantly equal for both kefir cheeses and lower for control (7.15, 7.26, and 5.5 $\log_{10}$ CFU/g, respectively). However, the lactobacilli count showed a general increase reaching maxima at day 3 and day 9 for control and kefir alone cheese, respectively, whereas the count of KCT showed a slight decrease during the first 3 ds, followed by a strong increase throughout the storage period. The lactococci initial counts were 7.99 and 8.11 $\log_{10}$ CFU/g for KCWT and KCT, respectively. The counts continued to decline till day 9 with a decline rate around 2 $\log_{10}$ units, followed by an increase in counts reaching to 6.80 and 6.47 $\log_{10}$ CFU/g at day 15 for KCWT and KCT, respectively.

Kefir is considered as a natural probiotic product, and for probiotic bacteria to be effective and exert their health-promoting effects, it must reach the intestine in high numbers (6, 14, 20). Thus, it is a crucial technological challenge to maintain probiotic survivability during processing and storage for the development of effective probiotic products. The suitability of WSC in maintaining probiotic viability and accordingly

development of effective probiotic products has been reported previously (6). The physicochemical attributes of WSC may represent the factors behind this suitability, including the suitable pH (app. 6), lack of acid production, availability of sugars, and high moisture content. Current results revealed that the lactobacilli final counts of both kefir cheeses at the end of storage period were well above the 'therapeutic minimum' level of probiotic (6 $\log_{10}$ CFU/g) (7). However, the addition of kefir cultures in cheese samples, as expected, significantly affected the counts of all microbial types. Up to our knowledge this is the first report in literature describing the development of a kefir WSC product. The initial pH values of kefir cheeses made without or with thyme were the same, but lower than control cheese (Table 2). During the storage period, pH values for all cheese types decreased significantly and at day 15, the pH values of both kefir cheeses remained lower than control cheese. Regarding acidity (Table 2), the initial acidity of kefir cheeses was the same and insignificantly lower than control cheese. During storage period, acidity for the 3 types of cheeses increased significantly (P<0.05), reaching at day 15 to 0.549, 0.445, and 0.442 g lactic acid /100g cheese for KCT and KCWT, and control cheeses, respectively. This increase in acidity and decrease in pH values are expected and are mainly due to lactose conversion to lactic acid by LAB. In agreement with our findings, various studies have reported the increased acidity and pH lowering effect of kefir cultures in traditional kefir beverage and products formulated with using kefir starter cultures such as cheeses and cheese whey-based beverages (13, 16, 19, 21).

Generally, LAB are proteolytic bacteria that produce a wide range of proteinase and peptidase enzymes capable of hydrolysing casein proteins (4, 22, 24). Current results showed that the addition of kefir cultures significantly increased proteolysis in WSC, with Kefir alone cheese had the highest significant (P<0.05) proteolysis, followed by KCT and control cheeses (0.654, 0.413, and 0.243 mmole of α-aa, respectively). It seemed that thyme, which is rich in antimicrobial compounds such as flavonoids, has significantly reduced kefir proteolytic activity, and this could explain the lower proteolysis in KCT compared to KCWT.

Table 3 showed the scores of the sensory evaluation of control and different kefir cheeses. The Chi-square analysis results showed insignificant difference (P>0.05) in overall acceptability, taste, colour, smell, and texture at day zero and 15 of production of KCT, KCWT and control

Table 1: Microbial counts of white soft cheeses made with kefir starter culture, with and without thyme addition upon storage at 4 °C/15 days.

Storage (day)	Total aerobic counts			Yeasts and molds			Lactobacilli			Lactococci		
	Control[1]	KCWT[2]	KCT[3]	Control	KCWT	KCT	Control	KCWT	KCT	Cont	KCWT	KCT
0	4.23±0.08 c[4,5](c)[6]	7.95±0.14 a(b)	8.43±0.20 a(a)	4.27+0.11 b(b)	6.95+0.15 a(a)	6.87±0.08 a(a)	4.14±0.12 b(c)	7.39±0.06 b(a)	6.89±0.02 b(b)	4.06±0.12 b(b)	7.99+0.08 a(a)	8.11+0.05 a(a)
3	5.83±0.08 b(b)	6.58±0.16 b(a)	6.26±0.05 b(a)	5.81+0.10 a(b)	6.08+0.03 b(a)	6.08±0.12 c(a)	5.62±0.03 a(c)	7.46±0.11 b(a)	6.13±0.07 d(b)	4.45±0.06 a(b)	6.08+0.10 c(a)	6.25+0.13 c(a)
9	6.17±0.10 a(a)	6.13±0.12 c(a)	6.14±0.01 c(a)	5.79+0.08 a(b)	6.06+0.08 b(a)	6.23±0.10 c(a)	5.54±0.01 a(c)	8.63±0.20 a(a)	6.61±0.15 c(b)	4.43±0.02 a(c)	6.60+0.01 b(a)	6.02+0.12 c(b)
15	6.18±0.11 a(b)	7.45±0.13 b(a)	7.48±0.05 b(a)	5.82+0.01 a(c)	7.28+0.18 a(a)	6.52±0.11 b(b)	5.50±0.10 a(b)	7.15±0.21 c(a)	7.26±0.18 a(a)	4.46±0.07 a(c)	6.80+0.17 a(a)	6.47+0.1 b(b)

1 Control: White soft cheese (rennet cheese) made with no kefir nor thyme addition.
2 KCWT: Kefir white soft cheese made with kefir starter culture and without thyme addition.
3 KCT: Kefir-thyme white soft cheese made with kefir and thyme addition.
4 All readings are means + Standard Deviation of six determinations.
5 Values with different letters in the same column are significantly different for the same cheese type (P < 0.05).
6 Values with different letters in brackets are significantly different for the same storage time for different cheese types (P < 0.05).

Table 2: Changes in physicochemical attributes of white soft cheeses made with kefir starter culture, with and without thyme addition upon storage 4°C for15 days.

Storage (day)	pH			Acidity as lactic acid (g/100 g cheese)			Proteolysis (mmole of α-AA)		
	Control[1]	KCWT[2]	KCT[3]	Cont	KCWT	KCT	Cont	KCWT	KCT
0	6.35+0.05a4,5(a)6	6.04+0.05a(b)	6.05+0.06a(b)	0.260+0.03d(a)	0.254+0.02c(a)	0.249+0.01c(a)	-	-	-
3	6.23+0.04b(a)	5.91+0.06b(b)	5.89+0.07b(b)	0.386+0.05c(a)	0.360+0.03b(a)	0.346+0.06b(a)	-	-	-
9	6.25+0.03b(a)	5.99+0.03ab(b)	5.95+0.05ab(b)	0.411+0.09b(a)	0.395+0.02b(a)	0.378+0.03b(a)	-	-	-
15	6.24+0.02b(a)	6.03+0.01a(b)	5.98+0.04ab(b)	0.442+0.10a(b)	0.445+0.02a(b)	0.549+0.05a(a)	0.243+0.07c	0.654+0.04a	0.413+0.05b

1 Control: White soft cheese (rennet cheese) made with no kefir nor thyme addition.
2 KCWT: Kefir white soft cheese made with kefir starter culture and without thyme addition.
3 KCT: Kefir-thyme white soft cheese made with kefir and thyme addition.
4 All readings are means + Standard Deviation of six determinations.
5 Values with different letters in the same column are significantly different for the same cheese type (P < 0.05).
6 Values with different letters in brackets are significantly different for the same storage time for different cheese types (P < 0.05).

Table 3: Sensory evaluation1 and acceptability attributes of control and kefir cheeses made with and without thyme addition upon storage at 4°C for15 days.

Sensory attributes	Storage time (day)	Control[2]		KCWT[3]		KCT[4]		X²[5]	Probability[6]
		Median	Range	Median	Range	Median	Range		
Color	0	4	3	3	3	4	3	12.70	0.120
	15	4	3	3.5	3	4.5	3	11.07	3.442
Smell	0	3	4	4	4	3	4	6.45	0.602
	15	4		4	4	3	4	12.99	0.120
Taste	0	3	4	4	4	3.5	4	4.12	0.853
	15	3.5	4	3	4	3	4	8.23	0.772
Texture	0	4	3	3.5	3	4	3	11.64	0.170
	15	4	3	3	3	4	3	10.99	0.39
General acceptability	0	3.5	4	4	3	4	3	1.22	0.976
	15	3	3	3.5	3	4	3	2.32	0.658

1 Sensory evaluation was done using 5-points hedonic scale (1= dislike very much, and 5= like very much) with 20 trained panelists.
2 Control: White soft cheese (rennet cheese) made with no kefir nor thyme addition.
3 KCWT: Kefir white soft cheese made with kefir starter culture and without thyme addition.
4 KCT: Kefir-thyme white soft cheese made with kefir and thyme addition.
5 X2: Chi-square values
6 Probability values more than 0.05 indicate insignificant difference between treatments for the sensory attribute at the same storage time.

cheese. However, the median and range of the overall acceptability at day 0 for control, KCWT, and KCT were as the following: 3.5 and 4, 4 and 3, 4 and 3, respectively; whereas, the median and range results at day 15 were: 3 and 3, 3.5 and 3, 4 and 3, for control, KCWT and KCT, respectively. Results of sensory evaluation clearly indicated that the addition of kefir culture did not negatively affect WSC sensorial criteria, instead, the addition of kefir culture gave better taste and odour evaluations, especially in KCWT. In addition, results revealed that storage time (up to 15 days) did not affect the sensory attributes of control, KCWT, and KCT, indicating the product stability upon refrigerated storage for 15 days; 15 days is the maximum permitted shelf-life for WSC in Jordan. In general, kefir cheeses were approved and accepted by the panel, as their preference scores were comparable to those of control cheese.

Conclusions

The present study demonstrated the suitability of WSC as a potential vehicle for kefir cultures to human, as the counts of kefir cultures remained above the minimum probiotic therapeutic counts during processing and cold storage, particularly for LAB, and the sensory evaluations were within the acceptable range until the end of storage. The use of kefir starters may have a major potential in probiotic food technology. Future studies using cheese inoculated with spoilage and/or pathogenic indicators may give better insight into the effectiveness of kefir starter cultures as an endogenous protecting shield in WSC.

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