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ABSTRACT

In this study, the effects of plant process and storage conditions as well as composition on the viability of kefir microbiota (Lactobacillus spp., Lactococcus spp., acetic acid bacteria and yeasts) physico-chemical and textural properties of plain and fruit flavoured kefirs were investigated. During refrigerated storage of 28 days the microbiological and textural characteristics of kefir samples were found to be affected significantly (P<0.05;<0.01) by the manufacturer-dependent milk type, starter culture, formulation and processing conditions. Furthermore, the storage time had a significant impact on the viability of microorganisms which were positively improved by fruit inclusion in the formula.

Keywords: *Kefir. storage. cell viability and texture*

INTRODUCTION

Kefir is, a traditional fermented milk beverage, originated in the Caucasus Mountains with a creamy consistency. It is highly consumed in Balkans, Eastern Europe and Turkey since being marketed as a unique beverage that has been related to various health benefits (Fontán et al., 2006; Prado et al., 2015) not only depending on its health promoting bacteria, but also on the presence of a variety of functional metabolites as organic acids and nutritional value (Wouters et al., 2002; Rodrigues et al., 2005 a,b; Leite et al., 2013). The word 'kefir' is derived from the Turkish word 'kevif' which means 'good feeling' after drinking it (John and Deeseenthum, 2015).

Kefir has a characteristic viscous, slightly carbonated and acidic taste due to 1% lactic acid and 0.3-1% ethanol content (Garrote et al., 2001; Ozcan, 2010; Guzel-Seydim et al., 2011). Kefir grains are gelatinous and variable in shape and size, having a white or lightly yellow color. The microorganisms in the grains are a mixture of lactic acid bacteria (Lactobacillus, Lactococcus, Leuconostoc and Streptococcus spp.), acetic acid bacteria (Acetobacter aceti and Α. rasens) and veasts (Kluvveromyces Torula kefir, *Saccharomyces* marxianus, exiguus and Candida lambica), and exist in a relatively stable and specific balance in a complex symbiotic relationship. Of these microbial population Lactobacilli are the dominant species (65-80%). Both the bacteria and the veasts are embedded in а polysaccharide-protein matrix, named kefiran, which is a water-soluble branched glucogalactan that co-exist in an association and are responsible for lactic-alcoholic fermentation (Garrote et al., 2001; Simova et al., 2002; Wouters et al., 2002; Farnworth, 2005; Witthuhn et al., 2005; Wszolek et al., 2006).

There are many studies on immune modulatory, anti-inflammatory, anti-neoplastic, pro-digestive and antioxidants effects of kefir (Rodrigues et al., 2005(a,b); Vinderola et al., 2005, 2006; Yilmaz-Ersan et al., 2018). It was shown that kefir consumption on daily basis could help lactose intolerance; interact with cholesterol metabolism; reveal therapeutic activity against colon carcinogens; and delay breast cancer development (Hertzler and Clancy, 2003; de Moreno de LeBlanc et al., 2006; Van Wyk et al., 2011). On the other hand, many studies reported anti-bacterial, anti-tumour, the antiinflammatory, gut immune-systems modulating and epithelial cell protecting against pathogenic factors activities of kefiran (Tada et al., 2007; Cheirsilp and Radchabut, 2011; Wang et al., 2008, 2012). It has been claimed that kefir could counteract the pathogenic genera of Salmonella, Helicobacter, Shigella, Staphylococcus, and Escherichia coli; and thus could be applied to

promote food safety by inhibiting coliforms and numerous pathogens (Meydani and Ha, 2000; Kim et al., 2016).

Kefir can be produced traditionally or commercially either by fermenting milk with commercial freeze-dried kefir starter cultures, kefir grains, and the product that remains after the removal of kefir grains (Farnworth and Mainville, 2003; Farnworth, 2005; Chandan, 2006; Bensmira et al., 2010; Gul et al., 2015; Prado et al., 2015). The artisanal kefir production begins with the inoculation of pasteurized milk of 20°C to 25°C with kefir grains. After incubating for 18 to 24 hours and filtration the mixture is ready to use. Nowadays, direct-to-vat inoculation (DVI) of freeze-dried kefir starter cultures is favored due to time-saving and hygienic reasons, and the final product has consistent properties. Any type of milk, i.e. cow, goat, sheep, coconut, rice and soy, can be used for production: however, in general cow milk is preferred (Oner et al., 2010; Kesenkas et al., 2011; Walsh et al., 2016).

Some of the recent studies evaluated the microbiological stability of kefir grains and changes in microbial population during storage (Simova et al., 2002; Irigoven et al., 2005; Witthuhn et al., 2005, Grønnevik et al., 2011; Leite et al., 2012), or quality attributes (Beshkova et al., 2002; Fontán et al., 2006; Sarkar, 2007; Magalhães et al., 2011; Barukčić et al., 2017). Rheological and textural properties of kefir are of major importance, since they affect the sensory perception of the final product which ultimately assign its acceptance by the The major factors affecting consumers. rheological and textural properties of kefir are the chemical composition and type of the milk used for its production, the thermal processing of milk, the starter culture type preffered, the incubation temperature, etc (Koroleva, 1988; Wszolek et al., 2001; Bensmira and Jiang 2012; Glibowski and Kowalska, 2012; Montanuci et al., 2012; Affane et al., 2016; Barukčić et al., 2017). In that regard, the objectives of the present study were to evaluate the technofunctional properties and microbiologic viability of plain and fruity kefir during refrigerated storage.

MATERIAL AND METHODS

Material

In this study, a total of twenty four kefir samples (11 plain and 13 fruit flavored) have been

analyzed, of which were collected from different retail markets and producers of Bursa, Turkey. The samples were transported to the laboratory under refrigerated conditions $(4\pm1^{\circ}C)$ and analyzedduring cold storage of 28 days.

Methods

Lactobacilli (LB) were counted on MRS (De Man, Rogosa and Sharpe agar) medium (pH 6.5±0.2) at 30°C under anaerobic conditions for 72 h, while Lactococci (LC) were enumarated on M17 medium (pH 7.2 ± 0.2) under same conditions for 48 h (Irigoyen et al., 2005; Cetinkaya and Elal Mus, 2012). In order to inhibit yeast growth cycloheximide (200 mg/L) was added to both above media. Viable yeast counts were obtained on OGYE (Oxytetra Glucose Yeast Agar Base) medium (pH 7.0 ± 0.2) to which 1% oxytetracycline was added at 25°C for 120 h (Suriasih et al., 2012). Acetic acid bacteria (AAB) were grown on Acetobacter peroxydans medium (APM) aerobically for 3-5 days at 25°C (Witthuhn et al., 2005). All evaluations were performed in duplicate.

Viability proportion index (VPI) of microorganisms was calculated as following (Ahmadi et al., 2012):

VPI = Final cell population $(\log_{10} \text{ cfu mL}^{-1}) / \text{initial cell population} (\log_{10} \text{ cfu mL}^{-1})$

For the determination of the pH of the kefir samples a Model 315i/SET (WTW Germany) pH meter was used. The colour was measured using a Minolta Spectrophotometer CM-3600d (Osaka. Japan) and expressed as L. a. and b values. The syneresis index was expressed as the volume of drained whey in mL per 100 mL sample (Akin and Ozcan, 2017). Texture profile analysis (TPA) was conducted on 100 mL kefir by Universal TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, U.K.) equipped with a 5-kg load cell. The operating conditions were selected according to Agata and Jan (2012). Textural measurements were evaluated for triplicate using three sample containers using a back extrusion cell with a solid rod of 35 mm diameter (P/25, Stable Micro Systems, Surrey, UK). The probe was inserted into the cylindrical container (48 mm diameter) holding 100 mL of sample using a 5-kg load cell. Probe speed was fixed at 1 mm s^{-1} , and the compression and relaxation with a penetration depth of 20 mm was used. The textural

parameters of firmness, cohesiveness, consistency and index of viscosity were tested.

Statistical comparisons were performed using the Statistica software package for Windows version 7.0 (Statsoft, Tulsa, OK). Pearson's correlation coefficient was performed to measure theassociation betweenvariables (SPSS Statistics).

RESULTS AND DISCUSSION

Viability of Kefir Microbiota

The complex microbiota of kefir is a symbiotic mixture of Lactobacilli, lactic streptococci, yeasts and acetic acid bacteria. Of the microbiota present the lactic acid bacteria and yeasts were reported as the predominating species (Loretan et al., 2003; Magalhães et al., 2010, 2011). The counts of *Lactobacillus* spp., *Lactococcus* spp., acetic acid bacteria and yeasts in plain and fruit flavored kefirs were given in Table 1.The differences in viability of microbial species in kefir samples during storage might be due to manufacturer- and process-dependent chemical composition and microbial growth variations.

As seen in Table 1. Lactobacillus spp. were the predominant flora in fresh and stored kefirs, and in plain kefir samples their viability decreased throughout storage as similar for Lactococcus spp., AAB and yeasts. Considering from the point that composition of milk and starter culture used in production has a significant effect on microbial population counts, at the beginning of storage plain kefir reported as having high viable counts. However, all enumerated microbial species were higher in fruit flavored kefirs than plain samples at the end of storage. Producers could apply either artisanal production with kefir grains or industrial production with commercial starter culture, and could use any kind of milk, and hence the composition and microbiota of kefir might vary. Several studies emphasized that the environment (culture maintenance, preservation and storage conditions) was the principal factor leading to the microbial diversity of kefir (Latorre-Garcia et al., 2007; Magalhães et al., 2011; Biadała and Jan, 2012), is responsible for the physicochemical features and biological activities (Guzel-Seydim et al., 2000a,b; Jianzhong et al., 2009; Gao et al., 2012; Altay et al., 2013).

There is a symbiotic relation between the microorganisms present in kefir grains or

commercial cultures. The bacteria and yeasts utilize their bioproducts as growth factors resulting lactic and alcoholic fermentation for the characteristic flavor and texture of the final product (Simova et al., 2002; Witthuhn et al., 2005; Wang et al., 2012; Hamet et al., 2013).

The microorganisms isolated from kefir are usually various homo- and heterofermentative lactic acid bacteria species of Lactobacillus, Lactococcus, Leuconostoc and Streptococcus such as L. paracasei ssp. paracasei, L. acidophilus, L. delbrueckii ssp. bulgaricus, L. plantarum, L. kefiranofaciens, and L. kefiri (Yuksekdag et al., 2004a,b; Zhou et al., 2009; Garofalo et al., 2015); acetic acid bacteria species such as Acetobacter aceti and A. rasens (Gao and Li, 2016); and lactose and non-lactose assimilating yeast species such as Saccharomyces cerevisiae, S. unisporus, Candida kefyr, and Kluyveromyces marxianus ssp. marxianus (Cheirsilp and Radchabut, 2011: Diosma et al., 2014; Zanirati et al., 2015). Lactobacillus kefiri However, is the predominant species in the final drink with 80% of the microbiota (Wouters et al., 2002; Jianzhong et al., 2009). Furthermore, kefir contains high levels of vitamins, such as folic acid, pantothenic acid, riboflavin and niacin, which are considered to be essential for the growth of some Lactobacillus species (Ahmed et al., 2013).

AAB were found in the kefir beverages at levels between 10^{8} - 10^{9} cfu mL⁻¹ (Loretan et al., 2003). Magalhães et al., (2011) stated that AAB represent only 20% of the total microbial population in kefir with usually lower counts (< 10^{5} cfu g⁻¹), but counts as high as 108 cfu g-1 wereobserved by Garrote et al. (2001; 2010). Rea et al. (1996), mentioned that AAB might stimulate the growth of other organisms since they are vitamin B₁₂ producers. Koroleva (1988) reported that the consistency, and thus sensory quality, of kefir could be improved by using a starter containing AAB, but not at levels higher than 10^{6} cfu g⁻¹.

The number of yeasts present in kefir is usually lower than that of the LAB, and specifically around 10^4 – 10^5 cfu g⁻¹(Latorre-García et al., 2007), but in some grains higher yeast counts than LAB counts have been reported (50 and 31.2%, respectively) (Zajsek and Gorsek, 2010). In general, *Saccharomyces cerevisiae* was reported to be the dominant yeast species (Leite et al., 2012; 2013). Once in milk kefir

grain/starter culture microorganisms are released they continue to grow and multiply by metabolizing the available nutrients in the milk, and especially lactose, that serves as the carbon and energy source.

Milk and	Lactobacilli		Lactococci		Acetic Acid Bacteria		Yeasts	
Fruit Type	1 st day	End of storage	1 st day	End of storage	1 st day	End of storage	1 st day	End of storage
Plain/Cow	$4,00 \times 10^8$	$4,00 \times 10^7$	$3,72 \times 10^{8}$	$4,00 \times 10^7$	$4,00 \times 10^5$	$2,70 \times 10^5$	$3,00 \times 10^2$	0,00
Plain/Cow	$3,20 \times 10^8$	$8,00 \times 10^7$	$2,10 \times 10^8$	$4,00 \times 10^7$	$7,28 \times 10^7$	$5,70 \times 10^{6}$	$2,00 \times 10^2$	$1,00 \times 10^{1}$
Plain/Cow	$6,50 \times 10^7$	$4,00 \times 10^7$	$1,00 \times 10^8$	$2,00 \times 10^7$	$8,00 \times 10^5$	$1,72 \times 10^7$	$2,00 \times 10^4$	$2,00 \times 10^3$
Plain/Cow	$3,00 \times 10^7$	$1,00 \times 10^7$	$1,30 \times 10^{8}$	$1,00 \times 10^7$	$4,00 \times 10^5$	$7,00 \times 10^5$	$1,50 \times 10^5$	$2,97 \times 10^{5}$
Plain/Cow	$2,16 \times 10^8$	$7,00 \times 10^7$	$4,19 \times 10^{8}$	$4,00 \times 10^7$	$6,00 \times 10^6$	$3,70 \times 10^{6}$	$6,00 \times 10^3$	$4,00 \times 10^2$
Plain/Cow	$3,15 \times 10^{8}$	$2,90 \times 10^8$	$4,50 \times 10^{8}$	$3,00 \times 10^8$	$1,72 \times 10^{8}$	$8,40 \times 10^5$	$2,00 \times 10^3$	$1,00 \times 10^4$
Plain/Cow	$1,35 \times 10^{9}$	$5,00 \times 10^{6}$	$1,30 \times 10^{9}$	$1,00 \times 10^7$	$3,60 \times 10^7$	$2,32 \times 10^7$	$1,00 \times 10^4$	$8,00 \times 10^3$
Plain/Cow	$3,00 \times 10^9$	$6,40 \times 10^8$	$1,00 \times 10^{10}$	$8,10 \times 10^{8}$	$6,70 \times 10^{6}$	$7,00 \times 10^{6}$	0,00	$4,00 \times 10^3$
Plain/Cow	$5,50 \times 10^8$	$1,90 \times 10^{8}$	$4,30 \times 10^{8}$	$5,80 \times 10^7$	$6,00 \times 1^7$	$2,94 \times 10^7$	$1,50 \times 10^5$	$5,18 \times 10^{5}$
Plain/Goat	$1,60 \times 10^9$	$1,60 \times 10^8$	1,36x10 ⁹	$4,10 \times 10^{8}$	$7,80 \times 1^{6}$	$1,49 \times 10^7$	$1,20 \times 10^{5}$	$2,13 \times 10^{5}$
Plain/Goat	$3,00 \times 10^7$	$1,04 \times 10^9$	$2,00 \times 10^7$	$1,11 \times 10^{9}$	$1,50 \times 1^{6}$	$3,90 \times 10^5$	$1,80 \times 10^3$	$3,30 \times 10^4$
AVERAGE	7,60x10 ⁸	$2,33 \times 10^{8}$	1,35x10 ⁹	2,58x10 ⁸	3,31x10⁷	9,39x10 ⁶	$2,53 \times 10^5$	9,86x10 ⁴
Apricot/Peach	$3,52 \times 10^8$	$8,70 \times 10^8$	$2,99 \times 10^8$	$1,48 \times 10^9$	$4,00 \times 10^5$	$6,60 \times 10^6$	0,00	$8,32 \times 10^{5}$
Strawberry	$5,28 \times 10^8$	$2,00 \times 10^7$	$2,87 \times 10^{8}$	$4,30 \times 10^{8}$	$2,80 \times 10^{6}$	$1,00 \times 10^5$	0,00	$2,00 \times 10^2$
Apple/Cinnamon	$2,50 \times 10^8$	9,60x10 ⁸	$4,9x10^{8}$	$1,00 \times 10^8$	$2,70 \times 10^5$	$1,00 \times 10^5$	1×10^{2}	$4,00 \times 10^3$
Blueberry	$1,63 \times 10^9$	$1,23 \times 10^{9}$	$1,36 \times 10^{9}$	$2,00 \times 10^7$	$8,30 \times 10^{6}$	$5,90 \times 10^{6}$	$1,3x10^4$	$6,00 \times 10^3$
Strawberry	$6,28 \times 10^8$	$4,40 \times 10^{7}$	$5,04 \times 10^8$	$1,10 \times 10^{8}$	3,35x10 ⁷	$1,78 \times 10^{7}$	0,00	$9,00 \times 10^2$
Forest fruit	$2,03 \times 10^8$	$3,20 \times 10^7$	$3,84 \times 10^8$	$3,90 \times 10^7$	$5,00 \times 10^{6}$	$1,82 \times 10^{7}$	$1,00 \times 10^{5}$	$4,00 \times 10^2$
Honey/Banana	$2,30 \times 10^8$	$3,60 \times 10^8$	$4,50 \times 10^{7}$	$3,10 \times 10^{8}$	$7,00 \times 10^{6}$	$6,60 \times 10^6$	$2,80 \times 10^4$	$9,00 \times 10^4$
Strawberry	$2,03 \times 10^8$	$3,70 \times 10^8$	$2,60 \times 10^8$	$9,50 \times 10^{8}$	$2,73 \times 10^{7}$	$2,82 \times 10^7$	0,00	$1,00 \times 10^{1}$
Forest fruit	$3,50 \times 10^8$	$3,20 \times 10^8$	$3,74 \times 10^{8}$	8,90x10 ⁸	$4,00 \times 10^7$	$2,68 \times 10^7$	0,00	0,00
Fig/Cereal	$2,30 \times 10^8$	$1,70 \times 10^{8}$	$3,20 \times 10^8$	$4,60 \times 10^8$	$2,90 \times 10^7$	$2,30 \times 10^7$	$2,00 \times 10^2$	$1,55 \times 10^4$
Banana	$2,20 \times 10^8$	$7,00 \times 10^8$	$2,70 \times 10^8$	$6,50 \times 10^8$	$5,00 \times 10^5$	$8,00 \times 10^5$	$1,00 \times 10^2$	$2,99 \times 10^5$
Strawberry	$3,20 \times 10^8$	$6,00 \times 10^8$	$3,00 \times 10^8$	$4,80 \times 10^8$	$1,20 \times 10^5$	$6,00 \times 10^5$	$1,00 \times 10^2$	$6,32 \times 10^{5}$
Forest fruit	$1,47 \times 10^{9}$	$3,80 \times 10^8$	$7,50 \times 10^8$	$5,10 \times 10^8$	$4,90 \times 10^{6}$	$3,30 \times 10^{6}$	$2,00 \times 10^{1}$	$2,29 \times 10^{5}$
AVERAGE	5,08x10 ⁸	4,65x10 ⁸	4,34x10 ⁸	4,94x10 ⁸	9,71x10 ⁶	1,06x10⁷	1,08x10 ⁴	1,62x10 ⁵

Table1. Microbial properties of kefir samples



Figure.1 VPI values of kefir samples

Figure 1 showed the viability of kefir microbiota during 28 days' cold storage. It was found that VPI values were higher in fruit flavored kefir than plain kefir, indicating that the nutrients present in fruits, like fructose, glucose, dietary fiber, phenolic compounds, vitamins, improved the viability of microorganisms. The VPI values of yeasts were significantly high in fruit flavored kefirs during storage.

Physico-Chemical Properties

The pH, syneresis and colour values of kefirs samples during storage at 4 °C were given in Table 2. There were no significant differences

in pH values among plain and fruit flavoured kefir samples throughout storage indicating the possible buffering capacity of ingredients in milk. Buffering refers to resistance to pH change by the total activity of individual ionizable acid-base groups and proteins. Dairy products contain several constituents that are responsible for buffering capacity. These constituents are the small compounds (inorganic phosphate, citrate, organic acids) containing one or several acid–base groups and proteins (caseins and whey proteins) that have several acid–base groups (Salaün et al., 2005).

The major compounds formed by LAB are characterized as lactic acid, acetaldehyde, diacetyl, acetoin, acetone, ethanol, CO_2 and acetic acid. Lactic acid is a non-volatile, odorless compound and responsible for the characteristics acidity of fermented products. The total lactic acid content of kefir varies from

 Table2. Physico-chemical properties of kefir samples

0.80 to 1.15% and originates from the degradation of lactose by the homofermentative and heterofermentative LAB present in kefir grains/starter cultures (Garrote et al., 2001).

Syneresis values increased during storage for all plain and fruit flavored samples, however, in fruit flavored kefirs depending on water holding capacity of dietary fibers present in fruits the syneresis was lower than plain samples. The consistency and water-holding capacity of acidified milk gels strongly related to the quality of fermented milk product (Table 2).

The colour values changed depending on milk and fruit type. L values, corresponding brightness/whiteness, were lower in fruit flavored kefirs as a result of different fruits used in the process. Plain kefirs were greenish according to a values, whereas for b values all samples were represented as blue (Table 2).

14:11 1	pH		Syneresis		L		a		b	
Milk and Fruit Type	1 st day	End of Storage								
Plain/Cow	4,50	4,42	10,50	10,5	75,32	76,81	-3,17	-3,58	4,70	5.83
Plain/Cow	4,16	4,24	4,50	7,50	70,89	72,24	-3,37	-3,45	6,48	6,26
Plain/Cow	4,55	4,40	4,00	9,00	74,65	72,46	-3,28	-3,26	5,88	7,09
Plain/Cow	4,55	4,51	7,00	20,50	69,13	68,79	-3,81	-3,77	6,54	6,46
Plain/Cow	4,49	4,48	3,50	6,50	74,26	71,05	-3,25	-3,32	5,71	7,18
Plain/Cow	4,25	4,27	12,00	16,00	74,08	74,10	-3,11	-2,93	4,91	5,30
Plain/Cow	4,27	4,25	6,50	16,00	69,50	68,58	-3,25	-3,50	7,43	7,33
Plain/Cow	4,45	4,40	2,00	5,00	78,44	72,17	-3,72	-3,48	7,12	6,72
Plain/Cow	4,34	4,41	27,00	19,50	73,61	71,71	-2,89	-2,54	4,37	4,81
Plain/Goat	3,81	3,83	14,50	29,00	70,12	76,69	-2,72	-2,64	6,90	6,91
Plain/Goat	4,14	4,09	6,00	17,50	71,82	71,03	-3,25	-3,29	6,45	6,46
AVERAGE	4,30	4,30	8,86	14,27	72,89	72,33	-3,25	-3,25	6,04	6,39
Apricot/Peach	4,30	4,25	3,00	6,50	64,00	72,51	-1,11	-2,18	8,93	10,62
Strawberry	4,30	4,23	1,50	17,00	66,50	59,91	0,43	3,54	5,93	5,39
Apple/Cinnamon	4,28	4,36	7,00	8,00	69,17	65,77	-0,72	-0,87	7,57	8,40
Blueberry	4,54	4,62	3,50	7,50	70,89	72,34	-3,15	-3,27	6,51	6,46
Strawberry	4,50	4,44	3,00	7,00	67,90	66,59	1,28	0,00	5,75	6,50
Forest fruit	4,31	4,52	3,50	5,50	69,65	64,02	1,71	1,00	4,97	5,63
Honey/Banana	4,43	4,44	4,00	11,50	69,30	71,35	-3,00	-2,87	7,46	7,23
Strawberry	4,33	4,23	14,00	17,00	59,52	59,91	4,00	3,54	5,27	5,39
Forest fruit	4,43	4,27	8,50	12,50	62,99	68,77	1,90	1,21	3,45	4,09
Fig/Cereal	4,20	4,21	7,50	15,00	69,59	78,20	-3,04	-3,35	6,94	7,23
Banana	4,30	4,18	2,00	3,00	68,08	67,56	-3,21	-3,23	7,74	7,74
Strawberry	4,27	4,20	4,00	6,50	50,52	50,80	5,21	4,78	5,31	5,50
Forest fruit	4,39	4,32	2,00	5,50	71,60	65,51	0,47	0,55	7,32	6,71
AVERAGE	4,35	4,33	4,89	9,42	66,13	66,40	0,06	-0,09	6,40	6,68

The textural properties of each type of kefir were shown in Table 3. All analyzed textural parameters of plain and flavoured kefir samples decreased throughout storage, whereas at the end of storage cohesiveness and index of viscosity were higher in fruit flavored kefirs than plain samples. The fresh plain and fruit flavored kefirs had higher firmness values. At the end of storage fruit flavored kefirs displayed higher values for firmness depending on dietary fibers in fruits resulting in differentiation in chemical composition and effecting the waterbinding capacity. Most research studies published on the rheological and textural

properties of kefir reported that the major factors affecting textural properties of kefir were the size and type of the starter culture, the chemical composition of the milk and the fermentation (incubation temperature and time, agitation) and storage conditions (Wszolek et al., 2001; Farn worth, 2005; Irigoyen et al., 2005; Sady et al., 2009; Bensmira et al., 2010). Viscosity and firmness are the most important rheological parameters of fermented milk products affecting the quality of the final product and consumer acceptability. Firmness as the force required to attaina certain deformation that is the level to which a material can be deformed before it is ruptured and is the measure of the strength of internal bonds (Mudgil et al., 2017). Cohesiveness is defined as forces of internal bonds, which keep the product as a whole. Domagala et al. (2006) stated that with increasing the fat content, depending on milk origin, could cause a significant decrease in cohesiveness.

Milk and Fruit	Firm	nness	Consistency		Cohesiveness		Index of Viscosity	
Туре	1 st day	End of						
		Storage		Storage		Storage		Storage
Plain/Cow	17,24	19,67	403,34	551,78	-7,97	-8,45	-2,31	-1,85
Plain/Cow	20,42	18,48	746,24	648,74	-8,01	-9,04	-2,07	-2,92
Plain/Cow	25,93	29,81	829,80	178,95	-5,87	-9,99	-0,96	1,37
Plain/Cow	17,10	16,98	587,13	577,70	-7,72	-8,07	-1,80	-2,01
Plain/Cow	24,63	20,20	691,61	667,45	-9,54	-8,69	-7,84	-1,16
Plain/Cow	15,94	19,40	341,89	662,48	-5,93	-5,25	-0,91	-0,72
Plain/Cow	99,66	22,72	749,11	777,35	-13,17	-10,99	-2,40	-4,09
Plain/Cow	18,70	15,70	642,22	348,08	-9,78	-7,27	-2,91	-1,36
Plain/Cow	15,62	18,15	359,99	655,12	-7,63	-9,68	-1,79	-4,14
Plain/Goat	48,86	18,39	802,61	429,11	-9,19	-7,58	-2,54	-1,92
Plain/Goat	18,34	16,86	649,48	572,87	-6,45	-7,91	-1,70	-1,56
AVERAGE	30,41	19,67	615,39	551,78	-8,48	-8,45	-2,55	-1,85
Apricot/Peach	23,69	24,21	772,32	842,16	-12,30	-13,47	-7,94	-14,94
Strawberry	30,50	24,11	817,67	828,90	-11,21	-13,54	-6,73	-12,29
Apple/Cinnamon	18,23	21,24	327,36	728,52	-14,42	-11,94	-3,21	-3,01
Blueberry	22,42	19,923	691,74	668,64	-9,68	-10,316	-3,21	-3,266
Strawberry	23,10	18,676	690,08	644,906	-8,41	-8,783	-2,32	-2,423
Forest fruit	22,66	20,33	771,74	698,863	-8,55	-10,43	-2,32	-3,266
Honey/Banana	19,62	17,403	681,13	422,595	-9,33	-7,723	-2,55	-0,22
Strawberry	19,01	20,15	640,26	723,59	-6,46	-5,79	-1,73	-0,91
Forest fruit	19,14	21,10	687,89	690,64	-7,14	-6,92	-1,50	-1,52
Fig/Cereal	19,57	18,16	650,17	619,26	-8,24	-9,11	-1,83	-2,97
Banana	20,20	19,75	673,05	663,81	-10,36	-9,84	-2,96	-2,96
Strawberry	21,64	25,41	731,65	707,12	-10,72	-11,07	-2,92	-9,26
Forest fruit	20,86	17,59	718,47	388,12	-10,69	-10,28	-2,89	-2,82
AVERAGE	21.59	20.61	681.04	663.62	-9.81	-9.94	-3.24	-4.60

 Table3. Textural properties of kefir samples

 Table4. Pearson's correlation coefficients between physico-chemical and textural parameters

	pН	Syneresis	Firmness	Consistency	Cohesiveness	Index of Viscosity
pH	1					
Syneresis	-0.417*	1				
Firmness	-0.273	0.106	1			
Consistency	-0.213	-0.236	0.405*	1		
Cohesiveness	0.018	0.389	-0.399*	-0.455*	1	
Index of	0.104	0.237	-0.150	-0.636**	0.726**	1
Viscosity						

**Correlation is significant at P<0.01; *Correlation is significant at P<0.05

In Table 4 the Pearson's correlation coefficients between properties of kefir samples were presented. There was considerably high positive correlation between the index of viscosity and cohesiveness (r=+0.726, P<0.01). However, the pH had negative correlation with syneresis (r=-0.417, P<0.05). Firmness values were positively correlated with consistency (r=+0.405, P<0.05),

whilst negatively with cohesiveness (r=-0.399, P<0.05). Consistency values were negatively affected by the index of viscosity (r=-0.636, P<0.01) and cohesiveness (r=-0.455, P<0.05).

CONCLUSION

For the majority of consumers, the desirability of food products depends on their nutritional health-promoting properties. Based on and the focus on health awareness among consumers in order to ensure the maximum beneficial effects food products with high microbial cell viability and appealing organoleptic characteristics have been commercialized. In the present study one could say that the microbial, sensorial and textural properties of commercial plain and fruit flavoured kefirs varied according to microorganisms present, milk type, fruit flavor addition and firm-dependent processing conditions, of which were the key product success factors. Kefir samples were evaluated to contain sufficient levels of health beneficiary microorganisms at the end of storage.

REFERENCES

- [1] Affane ALN, Muller N, Van der Wijst M, Sigge G, Britz TJ. Metabolite profiles and acceptability by clusters of different kefir types for South African consumers. Journal of Microbiology, Biotechnology and Food Sciences.(2016);5(4):364-368.
- [2] Agata L, Jan P. Production of fermented goat beverage using a mixed starter culture of lactic acid bacteria and yeasts. Engineerin in Life Sciences. (2012);12:486–493.
- [3] Ahmadi E, Mortazavian AM, Fazeli MR, Ezzatpanah H, Mohammadi R. The effects of inoculants variables on the physico-chemical and organoleptic properties of Doogh. *International* Journal of Dairy Technology. (2012);65(2):274–281.
- [4] Ahmed Z, Wang Y, Ahmad A, Khan S T, Nisa M, Ahmad H, Afreen A. Kefir and health: a contemporary perspective.Critical Reviews in Food Science and Nutrition. (2013);53(5): 422–434.
- [5] Akin Z, Ozcan T. Functional properties of fermented milk produced with plant proteins. LWT - Food Science and Technology.(2017); (5): 25–30.
- [6] Altay F, Karbancioglu-Güler F, Daskaya-Dikmen C, Heperkan D. A review on traditional Turkish fermented nonalcoholic beverages: Microbiota, fermentation process and quality characteristics. International Journal of Food Microbiology. (2013);167:44-56.
- [7] Barukčić I, Gracin L, Jambrak AR, Božanić R.Comparison of chemical, rheological and

sensory properties of kefir produced by kefir grains and commercial kefir starter. Mljekarstvo. (2017);67(3):169–176.

- [8] Bensmira M, Jiang B.Rheological characteristics and nutritional aspects of novel peanut-based kefir beverages and whole milk kefir. International Food Research International.(2012);19(2):647–650.
- [9] Bensmira M, Nsabimana C, Jiang B.Effects of fermentation conditions and homogenization pressure on the rheological properties of Kefir. Food Science and Technology. (2010);43(8): 1180-1184.
- [10] Beshkova DM, Simova ED, Simov ZI, Frengova GI, Spasov ZN.Pure cultures for making kefir. Food Microbiology. (2002);19:537–544.
- [11] Biadała A, Jan P.Production of fermented goat beverage using a mixed starter culture of lactic acid bacteria and yeast.Engineering in Life Sciences.(2012);12(4):486–493.
- [12] Chandan RC. History and consumption trends. In: Chandan RC. Manufacturing yogurt and fermented milks. Oxford, Blackwell Publishing;(2006).p.3–16.
- [13] Cetinkaya F, Elal Mus T. Determination of microbiological and chemical characteristics of kefir consumed in Bursa. Veterinary Journal of Ankara University.(2012);59:217–221.
- [14] Cheirsilp B, and Radchabut S.Use of whey lactose from dairy industry for economical kefiran production by *Lactobacillus kefiranofaciens* in mixed cultures with yeasts. New Biotechnology. (2011);28(6):574–580.
- [15] Diosma G, Romanin DE, Rey-Burusco MF, Londero A, Garrote GL.Yeasts from kefir grains: isolation, identification, and probiotic characterization. World Journal of Microbiology and Biotechnology. (2014);30:43–53.
- [16] Domagala J, Sady M, Grega T, Bonczar G.Rheological properties and texture of yoghurts when oat-maltodextrin is used as a fat substitute. International Journal of Food Properties.(2006);9(1):1–11.
- [17] de Moreno de LeBlanc A, Matar C, Farnworth E, Perdigon G.Study of cytokines involved in the prevention of a murine experimental breast cancer by kefir.Cytokine. (2006); 34(1-2):1–8.
- [18] Glibowski P, Kowalska A.Rheological, texture and sensory properties of kefir with high performance and native inulin. Journal of Food Engineering. (2012);111(2):299–304.
- [19] Farnworth ER. Kefir a complex probiotic.Food Science and Technology Bulletin Functional Foods.(2005);2(1):1–17.
- [20] Farnworth ER, Mainville I. Kefir: "a fermented milk product," in Handbook of Fermented

Functional Foods, ed. E. R. Farnworth (Boca Raton, FL: CRC Press); (2003).p.77-112.

- [21] Fontán MCG, Martínez S, Franco I, Carballo J. Microbiological and chemical changes during the manufacture of kefir made from cows' milk, using a commercial starter culture. International Dairy Journal. (2006);16(7):762– 767.
- [22] Gao J, Gu F, Abdella NH, Ruan H, He G.Investigation on culturable microflora in Tibetan kefir grains from different areas of China. Journal of Food Science. (2012);77:425– 433.
- [23] Gao X, Li B.Chemical and microbiological characteristics of kefir grains and their fermented dairy products: A review. Cogent Food & Agriculture.(2016);2:1272152.
- [24] Garofalo C, Osimani A, Milanovic V, Aquilanti L, De Filippis F, Stellato G, Di Mauro S, Turchetti B, Buzzini P, Ercolini D, Clementi F. Bacteria and yeast microbiota in milk kefir grains from different Italian Regions. Food Microbiology.(2015);49(14):123–133.
- [25] Garrote GL, Abraham AG, De Antoni GL. Chemical and microbiological characterization of kefir grains. Journal of Dairy Research.(2001);68(4):639–652.
- [26] Garrote GL, Abraham AG, De Antoni GL. Microbial interactions in kefir: A Natural Probiotic Drink.İn: Biotechnology of Lactic Acid Bacteria.(eds.) Mozzi F,Raya RR, and Vignolo GM (Ames, IO: Wiley-Blackwell).(2010);p.327–340.
- [27] Grønnevik H, Falstad M, Narvhus JA. Microbiological and chemical properties of Norwegian kefir during storage. International Dairy Journal. (2011);21(9):601–606.
- [28] Gul O, MortasM, Atalar I, Dervisoglu M, Kahyaoglu T.Manufacture and characterization of kefir made from cow and buffalo milk, using kefir grain and starter culture. Journal of Dairy Science. (2015);98:1–9.
- [29] Guzel-Seydim ZB, Seydim AC, Greene AK, Bodine AB. Determination of organic acids and volatile flavor substances in kefir during fermentation. Journal of Food Composition and Analysis. (2000a);13:35–43.
- [30] Guzel-Seydim Z, Seydim AC, Greene AK. Organic acids and volatile flavor components evolved during refrigerated storage of kefir. Journal of Dairy Science. (2000b); 83:275–277.
- [31] Guzel-Seydim ZB, Kok-Tas T, Greene AK, Seydim AC. Functional properties of kefir. Critical Reviews in Food Science and Nutrition.(2011);51:261–268.
- [32] Hamet MF, Londero A, Medrano M, Vercammen E, Van Hoorde K, Garrote GL, Huys G, Vandamme P, Abraham AG.

Application of culture-dependent and culture independent methods for the identification of *Lactobacillus kefiranofaciens* in microbial consortia present in kefir grains. Food Microbiology.(2013);36:327–334.

- [33] Hertzler SR, Clancy SM. Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. *Journal of the American Dietetic Association*. (2003);103(5):582–587.
- [34] Irigoyen A, Arama I, Castiella M, Torre P, Ibanez FC. Microbiological, physicochemical, and sensory characteristics of kefir during storage. Food Chemistry. (2005); 90(4):613– 620.
- [35] Jianzhong Z, Xiaoli L, Hanhu J, Mingsheng D. Analysis of the microbiota in Tibetan kefir grains using denaturing gradient gel electrophoresis. Food Microbiology.(2009);26(8):770–775.
- [36] John SM, Deeseenthum S. Properties and benefits of kefir -A review. Songklanakarin Journal of Science and Technology.(2015);37(3):275–282.
- [37] Kesenkas H, Dinkci N, Seckin K, Kinik O, Gonc S, Ergonul PG, Kavas G. Physicochemical, microbiological and sensory characteristics of soymilk kefir. *African* Journal of Microbiology Research. (2011);5:3737–3746.
- [38] Kim DH, Kim H, Jeong D, Kang IB, Chon JW, Kim HS, Song KY, Seo KH. Antimicrobial activity of kefir against various food pathogens and spoilage bacteria. *Korean* Journal for Food Science of Animal Resources.(2016);36(6):787–790.
- [39] Koroleva NS. Technology of kefir and koumys. IDF Bulletin 227b.(1988);96–100.
- [40] Latorre-García L, del Castillo-Agudo L, Polaina J. Taxonomical classification of yeasts isolated from kefir based on the sequence of their ribosomal RNA genes. World Journal of Microbiology & Biotechnology.(2007);23(6):7 85–791.
- [41] Leite AMO, Mayo B, Rachid CTCC, Peixoto RS, Silva JT, Paschoalin VMF, Delgado S. Assessment of the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing analysis. Food Microbiology.(2012);31(2):215–221.
- [42] Leite AMO, Miguel MAL, Peixoto RS, Rosado AS, Silva JT, Paschoalin VMF. Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. Brazilian Journal of Microbiology.(2013);44(2):341–349.
- [43] Loretan T, Mostert JF, Viljeon BC. Microbial flora associated with South African household kefir. South African Journal of Science.(2003);99(1):92–94.

- [44] Magalhães KT, Pereira GVM, Dias DR, Schwan RF. Microbial communities and chemical changes during fermentation of sugary Brazilian kefir. World Journal of Microbiology & Biotechnology. (2010);26(2):1 241–1250.
- [45] Magalhães KT, Dragone G, de Melo Pereira GV, Oliveira JM. Comparative study of the biochemical changes and volatile compound formations during the production of novel whey-based kefir beverages and traditional milk kefir. Food Chemistry.(2011);126(1):249– 253.
- [46] Meydani SN, Ha WK. Immunologic effects of yogurt. American Journal of Clinical Nutrition. (2000);71(4):861–872.
- [47] Montanuci FD, Pimentel TC, Garcia S, Prudencio SH. Effect of starter culture and inulin addition on microbial viability, texture, and chemical characteristics of whole or skim milk Kefir. Ciência Tecnologica Alimentaria Campinas. (2012);32(4):850–861.
- [48] Mudgil D, Barak S, Khatkar BS. Texture profile analysis of yogurt as influenced by partially hydrolyzed guar gum and process variables. Journal of Food Science and Technology. (2017);54(12):3810– 3817.
- [49] Oner Z, Karahan AG, Cakmakci ML. Effects of different milk types and starter cultures on kefir. G1da.(2010);35:177–182.
- [50] Ozcan T. Determination of minerals in kefir by inductively coupled plasma-optical emission spectrometer (ICP-OES). Asian Journal of Chemistry. (2010);22(8):6589–6594.
- [51] Prado MR, Blandón LM, Vandenberghe LP, Rodrigues C, Castro GR, Thomaz-Soccol V, Soccol CR. Milk kefir: composition, microbial cultures, biological activities, and related products. Frontiers in Microbiology.(2015);6:1177.
- [52] Rea MC, Lennartsson T, Dillon P. Irish kefirlike grains: their structure, microbial composition and fermentation kinetics. Journal of Applied Bacteriology. (1996);81(1):83–94.
- [53] Rodrigues KL, Caputo LRG, Carvalho JCT, Evangelista J, Schneedorf JM. Antimicrobial and healing activity of kefir and kefiran extract. International Journal of Antimicrobial Agents. (2005a);25(5):404-408.
- [54] Rodrigues KL, Carvalho JCT, Schneedorf JM. Anti-inflammatory properties of kefir and its polysaccharide extract. Inflammopharmacology. (2005b);13(5-6):485-492.
- [55] Sady M, Domagala J, Najgebauer-Lejko D, Grega T. Effect of whey protein concentrate addition on texture and rheological properties

of kefir produced from skimmed milk. Biotechnology in Animal Husbandry. (2009);25(5-6):763–771.

- [56] Salaün F, Mietton B, Gaucheron F. Buffering capacity of dairy products. International Dairy Journal.(2005);15:95–109.
- [57] Sarkar S. Potential of kefir as a dietetic beverage - a review. British Food Journal. (2007);109(4):280–290.
- [58] Simova E, Beshkova D, Angelov A. Lactic acid bacteria and yeast in kefir grains and kefir made from them.Journal of Industrial Microbiology and Technology. (2002);28(1):1– 6.
- [59] Suriasih K, Aryanta WR, Mahardika G, Astawa NM. Microbiological and chemical properties of kefir made of Bali Cattle milk. Food Science and Quality Management. (2012); 6:12–22.
- [60] Tada S, Katakura Y, Ninomiya K, Shioya S. Fed-batch coculture of *Lactobacillus kefiranofaciens* with *Saccharomyces cerevisiae* for effective production of kefiran. Journal of Bioscience and Bioengineering.(2007);103(6):557–562.
- [61] Van Wyk J, Witthuhn RC, Britz TJ. Optimisation of vitamin B12 and folate production by *Propionibacterium freudenreichii* strains in kefir. International Dairy Journal. (2011); 21(2):69–74.
- [62] Vinderola CG, Duarte J, Thangavel D, Perdigon G, Farnworth E, Matar C. Immunomodulating capacity of kefir. Journal of Dairy Research. (2005);72(2):195–202.
- [63] Vinderola CG, Perdigon G, Duarte J, Farnworth E, Matar C. Effects of the oral administration of the products derived from milk fermentation by kefir microbiota on immune stimulation. Journal of Dairy Research.(2006);73(4):472– 479.
- [64] Walsh AM, Crispie F, Kilcawley K, O'Sullivan O, O'Sullivan MG, Claesson MJ, Cotter PD. Microbial succession and flavor production in the fermented dairy beverage kefir. Applied and Environmental Science. (2016);1(5):1–16.
- [65] Wang Y, Ahmed Z, Feng W, Li C, Song S. Physicochemical properties of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir. International Journal of Biological Macromolecules. (2008);43(1):283– 288.
- [66] Wang SY, Chen KN, Lo YM, Chiang ML, Chen HC, Liu JR, Chen MJ. Investigation of microorganisms involved in biosynthesis of the kefir grain. Food Microbiology. (2012);32 (2):274–285.
- [67] Witthuhn RC, Schoeman T, Britz TJ. Characterisation of the microbial population at

different stages of kefir production and kefir grain mass cultivation. International Dairy Journal. (2005);15(4):383–389.

- [68] Wouters J T M, Ayad E H E, Hugenholtz J, Smit G. Microbes from raw milk for fermented dairy products. International Dairy Journal.(2002);12(2-3):91–109.
- [69] Wszolek M, Tamime AY, Muir D D, Barcly M N I. Properties of kefir made in Scotland and Poland using bovine, caprine and ovine milk with different startercultures. *Lebensm Wiss Technology*. (2001);34(4):251–261.
- [70] Wszolek M, Kupiec-Teahan B, Skov Gulard H, Tamime AY. Production of kefir, koumiss and other related products. In Fermented Milks.(ed.) A.Y. Tamime (Oxford: Blackwell Publishing; 2006.p.174–216.
- [71] Yuksekdag Z N, Beyatli Y, Aslim B. Determination of some characteristics coccoid forms of lactic acid bacteria isolated from Turkish kefirs with natural probiotic. LWT-

Food Science and Technology. (2004a);37:663–667.

- [72] Yuksekdag ZN, Beyatli Y, Aslim B. Metabolic activities of Lactobacillus spp. strains isolated from kefir. Nahrung. (2004b);48: 218–220.
- [73] Yilmaz-Ersan L, Ozcan T, Akpinar-Bayizit A, Sahin S. Comparison of antioxidant capacity of cow and ewe milk kefirs. Journal of Dairy Science. (2018);101(5):3788–3798.
- [74] Zajsek K, Gorsek A. Modelling of batch kefir fermentation kinetics for ethanol production by mixed natural microbiota. Food Bioproducts Processing.(2010);88(1):55–60.
- [75] Zanirati DF, Abatemarco M, Cicco Sandesb SH, Nicolia JR, Nunes AC, Neumanna E. Selection of lactic acid bacteria from Brazilian kefir grains for potential use as starter or probiotic cultures. Anarobe. (2015);32:70–76.
- [76] Zhou J, Liu X, Jiang H, Dong M. Analysis of the microbiota in Tibetan kefir grains using denaturing gradient gel electrophoresis. Food Microbiology. (2009);26:770–775.

Citation: Tulay Ozcan, Lutfiye Yilmaz-Ersan, Arzu Akpinar-Bayizit, Saliha Karaman, Tugce Ozdemir, Esra Topcuoglu, Cheima Mansri." The Shelf Life Characteristics of Plain and Fruit Flavored Kefir: Microbiological and Techno-Functional Properties Journal of Animal Husbandry and Dairy Science, 2(4), pp 9-18.

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