Food Sci. Biotechnol. 24(1): 99-106 (2015)
DOI 10.1007/s10068-015-0015-1

RESEARCH ARTICLE

Effect of *Gluconacetobacter* spp. on Kefir Grains and Kefir Quality

Nilgün Özdemir, Tuğba Kök-Taş, and Zeynep Guzel-Seydim

Received October 28, 2013; revised May 31, 2014; accepted July 18, 2014; published online February 28, 2015

Abstract Microbial, chemical, physical, and sensorial analyses of kefir samples produced using kefir grains embedded with *Gluconacetobacter* spp. were investigated using kefir samples with *Gluconacetobacter* spp. inclusion (KA), and regular kefir grains (control, KC) lacking *Gluconacetobacter* spp. The genus *Gluconacetobacter*, (identified using PCR) isolated from apple cider vinegar, was embedded in kefir grains. Inclusion of *Gluconacetobacter* spp. provided a significant biomass increase ($p<0.01$). The *Lactobacillus* spp., *Lactococcus* spp., yeast, *Lactobacillus acidophilus,* and *Bifidobacterium* spp. contents of KA were similar to KC. The acetic acid bacterial content of KA was 3.86 log CFU/mL. A significant ($p<0.05$) increase was observed in the exopolysaccharide content and viscosity of KA compared with KC. Inclusion of a different useful bacterium not naturally present in kefir grains is herein reported. Kefir grains can be an important carrier for microorganisms.

Keywords: kefir, *Gluconacetobacter* spp., biomass, exopolysaccharide

Introduction

Kefir is a fermented dairy product obtained from milk using a natural starter culture known as kefir grains. The microflora in kefir grains is mostly composed of lactic acid bacteria (LAB) and yeast, in addition to some acetic acid bacteria (AAB) (1,2). Kefir produced from fermentation by this microflora is a functional product because kefir delivers positive effects to humans (3,4).

Variations in the microfra of kefir grains may cause variations in the aroma, composition, and organoleptic characteristics of a final product (4). The microflora may vary based on the milk, the production techniques, the inoculation ratio, and the origin of kefir grains, which are a unique biomass composed of predominantly lactic acid bacteria and yeast.

In recent years, fermented natural vinegar has increased in importance. Vinegar is produced when bacteria produce acetic acid via oxidization of ethanol produced from fermentable sugar by yeast. When acetic acid bacteria oxidize alcoholic liquids to form acetic acid and water, the process is called acetic acid fermentation (5,6). Acetic acid bacteria are rod-shaped, Gram-negative, and obligate aerobes (7). The new species *Gluconacetobacter sacchari* sp. nov. of the family Acetobacteriaceae was isolated from the leaf sheath of sugar cane in Queensland and northern New South Wales, Australia (8). Vinegar exhibits the health-related properties of anti-tumor, anti-microbial, anti-oxidant, and cholesterol-lowering effects, and promotes cardiovascular health (5,9,10). The positive health effects of vinegar might originate from ingredients and/or the AAB culture, more studies of which have appeared in recent years. The anti-microbial effect of AAB has been used in dermatology as AAB positively affects neural transmission and enhances the barrier function of the skin. The use of cellulose produced by acetic acid bacteria during wound treatment is an example of an application (11,12).

The naturally occurring AAB in kefir grains have been identified (13,14). However, many studies have reported that only lactic acid bacteria and yeast are present in the kefir grain structure. Kefir grains from Turkey do not contain *Gluconacetobacter* spp. The aim of this research was to investigate inclusion of *Gluconacetobacter* spp. in kefir grains since acetic acid bacteria exhibit health-related properties. The microflora of kefir was investigated after

Nilgün Özdemir (✉)
Department of Food Engineering, Engineering Faculty, Ondokuz Mayıs University, Kurupelit Samsun 55139, Turkey
Tel: +90-537-543-0383, Fax: +90-362-457-6035
E-mail: nilgun.ozdemir@omu.edu.tr

Tuğba Kök-Taş, Zeynep Guzel-Seydim
Department of Food Engineering, Engineering Faculty, Suleyman Demirel University, Isparta 32260, Turkey

Gluconacetobacter spp. inclusion, and the quality of kefir was evaluated.

Materials and Methods

Kefir grain and vinegar production Kefir grains were obtained from Danem Ltd. in the Lakes District Technopark at Suleyman Demirel University (Isparta, Turkey). The vinegar used to isolate *Gluconacetobacter* spp. was produced using traditional techniques (15) and natural cider and cider vinegar obtained from the Department of Food Engineering, Suleyman Demirel University, Isparta, Turkey were mixed in a 3:1 ratio at 24°C and allowed to stand for 20 days (15).

Isolation and amplification of *Gluconacetobacter* spp. *Gluconacetobacter* spp. isolated and identified using yeast glucose chloramphenicol agar (YGC agar; Merck, Darmstadt, Germany) was obtained from natural cider vinegar. First, 25 mL samples of cider vinegar were centrifuged (NF 048 BENCH-TOP Centrifuge; Nuve Inc., Ankara, Turkey) at 2,100×*g* for 10 min to collect the cell pellet of approximately 1 mL in volume. Then, 100 μL of each cell pellet sample was incubated for 5 days at 30ºC with enrichment using yeast glucose broth (YG broth; 5% glucose, 1% yeast extract) (13,16). The YG broth was incubated for 72 h at 30ºC. The content of *Gluconacetobacter* spp. was determined by spread plate method using YGC agar.

Kefir grains produced using *Gluconacetobacter* spp. Pasteurized milk containing 2% kefir grains were inoculated with isolated *Gluconobacter* spp (4%). and incubated at 25ºC for 22 h. The incubation procedure was repeated for 15 days. After the 15 day period, kefir grains were produced with *Gluconacetobacter* spp. over 25 days (KGA) (Fig. 1).

Kefir production Kefir was produced by inoculating 2% natural kefir grains containing *Gluconacetobacter* spp. (7.36 log CFU/mL) (KGA) isolate into pasteurized milk at 25°C for 20 h. The fermentation was ended at pH 4.6. The kefir grains were removed under aseptic conditions and washed with sterile water (Fig. 1). Kefir (KA) was produced with KGA (Fig. 1). The same process was used to produce the control sample of kefir (KC): Control group kefir grains (KGC) were inoculated into pasteurized milk, followed by fermentation under the conditions described in Fig. 1.

PCR analyses of kefir grains containing *Gluconacetobacter* spp. DNA from *Gluconacetobacter* spp. obtained from vinegar, and kefir grains containing *Gluconacetobacter* spp. were isolated using the modified procedure described by Trcek *et al.* (17). Bacterial genomic DNA was extracted using a QIAgen Mini Stool Kit in accordance with the manufacturer's instructions.

The 16S rDNA genes from the kefir bacterial population were captured using culture-dependent approaches and amplified from purified genomic DNA using the primers NuniADHfw 5'-TGG(T/C)(A/T)CGG(C/T)AT(T/C)CC(G/C)GG-3' and NuniADHrev 5'-GT(G/C/A)GCGTC(A/G)TA(A/G)GC(A/G)TGGAA-3' (17). PCR reactions were carried out in a thermocycler (Thermal Cycler; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Each PCR reaction tube contained PCR Master Mix (Progema) composed of a 2× concentrated solution of 0.05 u/μL of *Taq* DNA polymerase (pH 8.5), reaction buffer, 4 mM $MgCl_2$, 0.4 mM dNTP, dATP, dCTP, dGTP, and dTTP, 1 μL of isolated DNA, and 1 μM of each primer. Reaction mixtures were adjusted to a total of 50 μL using water.

For bacteria, the initial 4 min denaturation step at 94ºC was followed by 30 cycles of a 3 stage program (30 s at 94ºC, 1 min at 55ºC, 2 min at 72ºC, and a final 7 min of elongation at 72ºC). The sizes of the PCR products (approximately 200 bp) were confirmed using 2% (w/v) agarose gel electrophoresis (Sub-Gel GT Agarose Gel Electrophoresis System; Bio-Rad Laboratories, Inc.). Amplified fragments were purified using an Applied Biosystems Bigdye Cycle sequencing kit and sequenced at RefGen-Gene Research and Biotechnology (Ankara, Turkey). Sequencing results were analyzed based on comparison of each sample base sequence with rDNA sequences in the nucleotide sequence database (3130 XL Genetic Analyzer; ABI, Applied Biosystems, Foster city, CA, USA) at the National Centre for Biotechnology Information (Bethesda, MD, USA) using the basic local alignment search tool (BLAST) search program (18).

Determination of the kefir grain biomass Kefir grains containing *Gluconacetobacter* spp. (KGA) and control grains (KGC) were activated for 15 days without interruption. Increases in the kefir grain biomass were determined gravimetrically (19). Grains were aseptically separated from fermented milk using stainless steel sieves (Ø 5 cm). Grains were washed using sterile distilled water and transferred into autoclaved sterile aluminum weighing dishes containing sterilized paper towels to remove excess water. After the paper towels were removed, the kefir grains were weighed using an analytical balance. Biomass was expressed as a percentage of the difference between final and initial grain weights to initial grain weights.

Microbiological analysis Lactobacilli counts were determined using de Man, Rogosa, and Sharpe (MRS) medium (Accumedia 7543; Accumedia, East Lansing, MI, USA) after incubation at 37ºC under CO_2 (5%) for 48 h. *Lactococcus* and *Streptococcus* spp. were plated on M17 medium (Oxoid, Basingstoke, UK) and incubated under

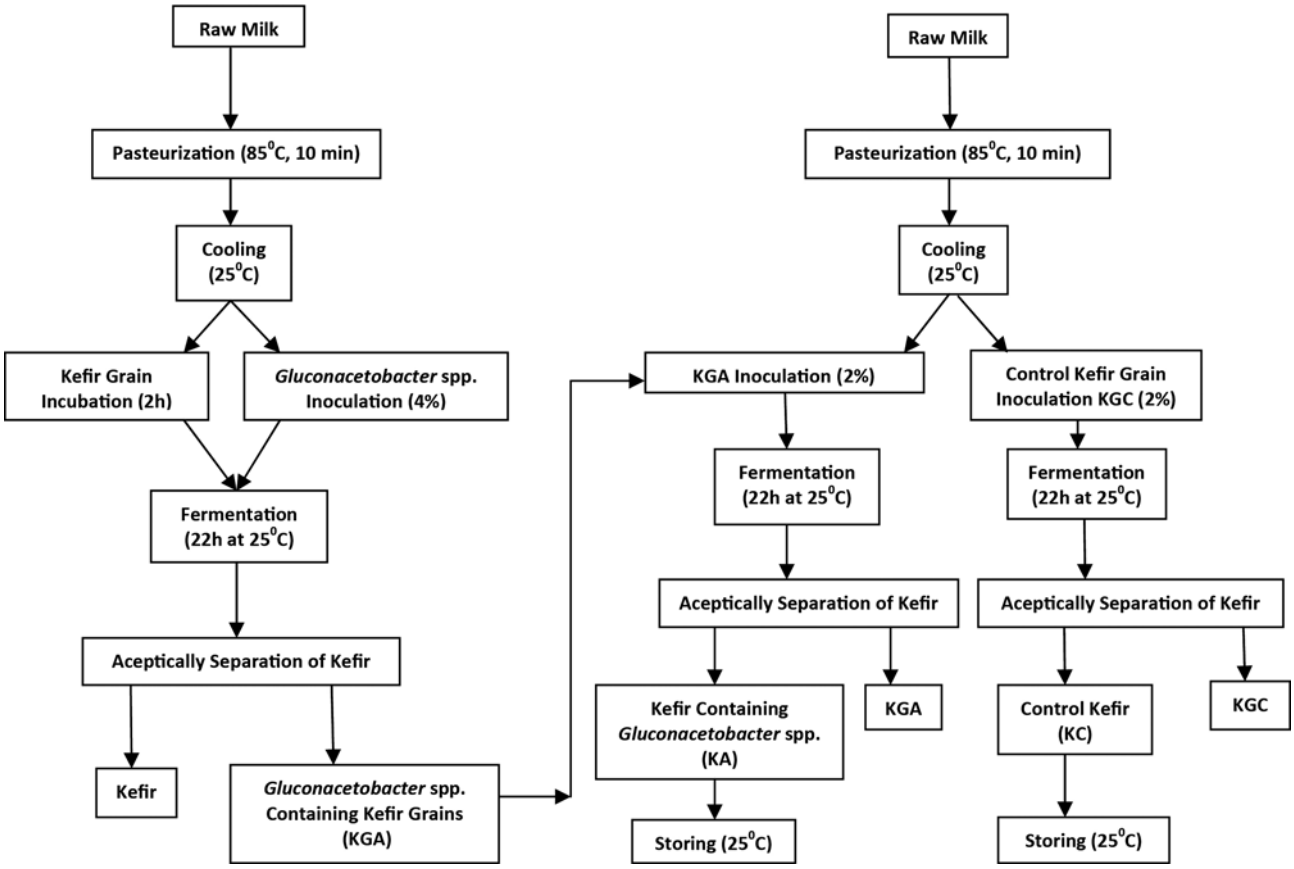

Fig. 1. Flow chart for kefir sample production and experimental design. KA, kefir produced from grains embedded with *Gluconacetobacter* spp. (KGA); KC, kefir produced from control grains (KGC)

5% CO_2 at 37°C for 48 h. Yeasts were grown on Potato Dextrose Agar (Merck, Darmstadt, Germany) with 0.14% added lactic acid at 25°C for 5 days (20). *Lactobacillus acidophilus* and *Bifidobacterium* spp. were cultured on MRS with sorbitol (10%) and on MRS with NNLP (20%) containing neomycin sulfate (100 mgL), nalidixic acid (50 mgL), lithium chloride (3,000 mgL), and paramycin sulfate (200 mgL) (21). Acetic acid bacteria were grown on YGC (Merck) with 4 mg/L of cycloheximide (Sigma) added to inhibit yeast growth (13,22) at 30°C for 5 days (16).

Chemical analyses of kefir The pH, titratable acidity expressed as the lactic acid content, ash content, and dry matter in kefir samples were determined according to previously described methods (23).

Purification and quantification of exopolysaccharide Separation and quantification of exopolysaccharide (EPS) were carried out as reported by Zisu and Shah (24). Proteins from 50 mL of a diluted kefir sample were precipitated using 2 mL of 20% (w/v) trichloroacetic acid (TCA) and separated by centrifugation (Sorvall RT7; Kendro Instruments Australia Pty Ltd., Sydney, Australia) at 3,313×*g* for 30 min at 4°C. The pH of the supernatant was adjusted to 6.8 using 40% (w/v) NaOH. A supernatant sample was heated (Schutzart DIN, Schwabach FRG, Germany) in a sealed container to 100°C for 30 min to denature whey proteins, which were removed by centrifugation (NF 048 BENCH-TOP Centrifuge; Nuve Inc.) at 3,313×*g*, 30 min, 4°C. The carbonhydrate pellet was resuspended in 10 mL of distilled water and treated for 1 h at room temperature in a sonication bath (FX 14PH sonication bath; Unisonics Pty Ltd., Sydney, Australia). The suspension was dialyzed (Spectrum Laboratory Products Inc., CA, USA) against tap water at 4°C for 2 weeks using a membrane tube (spectra/por dialysis Mebrane) with a 12,000 Da molecular weight (M_w) cut-off (Carolina Biological Supply Company, Burlington, NC, USA). Water was changed twice per day. The EPS concentration in the dialyzed suspension was quantified using the phenol-sulfuric method and was reported as glucose equivalents (25).

Rheological analysis A Brookfield Rotational Rheometer (DV-II Pro LV; Brookfield Engineering Laboratories, Middleboro, MA, USA) was used to determine the rheological properties of kefir samples. Viscosity was

determined at 100 rpm every 20 s at 4ºC using a DV-4 spindle (26).

Sensory analyses The sensory characteristics of kefir samples were evaluated using descriptive analyses of 12 staff and student panelists from the Department of Food Engineering, Suleyman Demirel University, based on interest, availability, and consumption of kefir made from cow's milk. Panelists included 5 males and 7 females aged 20-42 years who had used descriptive sensory methodology on a regular basis over the previous 2 years. Panelists were trained using traditional kefir with the sensory descriptive method established by Coggins *et al.* (27). Samples were evaluated using a 5 point intensity scale where 0=not detected and 5=extremely strong in respect to the sensory attributes of homogeneous structure, mild foam, white yellowish color, mounth coating, viscosity, structure without separation of serum, typical odor, fermented odor, sourish odor, non-animal odor, typical taste, refreshing taste, fermented taste, sourish taste, mild sweetish taste, and non-foreing taste. A total of 2 kefir samples were presented to the panel group at each session. Kefir samples (KA and KC) were served and presented to panelists in cups bearing a random 3 digit number. Sensory data were measured using a sensory assessment form.

Statistical analysis All analyses were replicated 3 times and performed in duplicate. Data were analyzed using SPSS Base 16.0 software (SPSS, Inc., Chicago, IL, USA). Rheological and EPS data were statistically evaluated using the repeated measures analysis of variance (ANOVA) technique. Tukey's multiple comparison test was used to evaluate differences within groups. Significance was defined as $p<0.05$. More significance was defined as $p<0.01$. And no significance was defined as $p>0.05$.

Results and Discussion

PCR analysis of *Gluconacetobacter* spp. isolated from vinegar and kefir grains *Gluconacetobacter* spp. was identified based on analysis of DNA sequences using the BLAST database (28). Isolated DNA was most similar to *Gluconacetobacter saccharivorans*. Identification to the species and genus levels in kefir grain samples was defined as 100% similarity of the 16S rDNA gene to relative sequences in the database (Fig. 2). Fernández-Pérez *et al.* (16) isolated *G. europaeus, Ga. xylinus, Ga. hansenii*, and *Acetobacter pasteurianus.* These AAB produce vinegar from red and white grapes and from apple cider. The genus *Gluconacetobacter* is usually isolated from apple cider vinegar, implying that *Gluconacetobacter* is important for apple cider vinegar fermentation. *Gluconacetobacter* has a high tolerance toward acid environments. Therefore, this genus was suitable for incorporation into kefir grains to resist conditions of kefir production.

G. europaeus is resistant toward 10% acetic acid, and *G. intermedius* is resistant toward 6% acetic acid (17). Yang *et al.* (29) studied interactions between kefir and the microflora of combou tea, which is a fermented product containing LAB, AAB, and yeasts. *Gluconobacter* spp. A4 isolated from combou tea produced D-saccharide 1,4 lactan (DSL). There is a reported symbiotic relationship between LAB isolated from kefir and *Gluconobacter* spp. A4 (29). DSL production increased due to fermentation.

Changes in the kefir grain biomass Kefir grains are used as a natural starter culture to make kefir. During fermentation, kefir grains grow from pre-existing kefir grains and the amount of biomass is slowly increased. The amount of milk used in fermentation increases as the kefir grain biomass increases. Increases in the kefir grain biomass

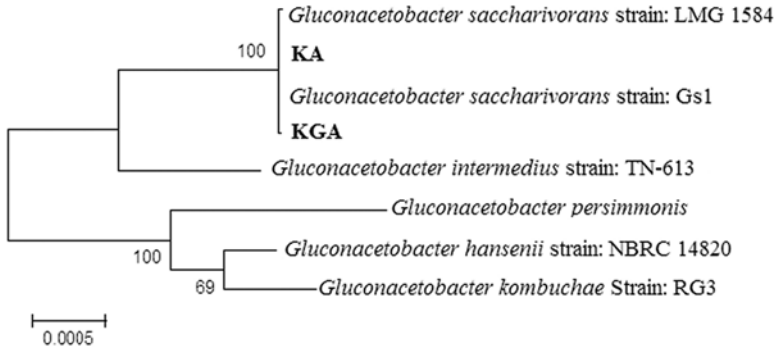

Fig. 2. Phylogenetic tree for species of *Gluconacetobacter* spp. and related taxa. This tree was based on 16S rRNA gene sequencing data and constructed using the neighbor-joining method (KA, kefir sample; KGA, kefir grain).

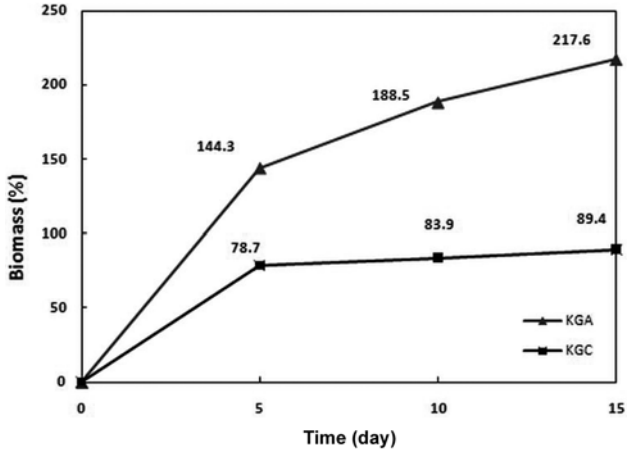

Fig. 3. Biomass increases in kefir grains.

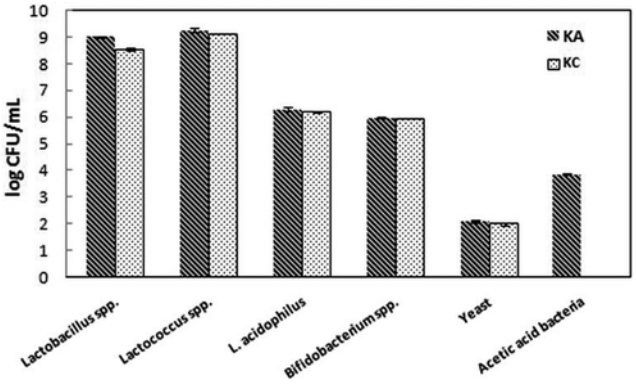

Fig. 4. Microbiological content of the kefir samples.

were determined on the 1st, 5th, 10th, and 15th days for KGA and KGC samples. The KGA grain biomass increased very significantly ($p<0.01$) more than the KGC grain biomass. The biomass increase was significantly ($p<0.05$) different between the KGC and KGA samples beginning on the 10th day (Fig. 3). The biomass increased by 89.4 and 217.6% for KGC and KGA, respectively. Valepyn *et al.* (30) reported that the genera *Gluconacetobacter* and *Acetobacter* produced extracellular polysaccharides of interest. Therefore, inclusion of *Gluconacetobacter* spp. in kefir grains may increase the kefir grain biomass because *Gluconacetobacter* spp. produced exopolysaccharides. Guzel-Seydim *et al.* (19) reported that biomass increases for kefir grains varied when the different fermentation parameters of atmospheric CO_2 content and whey protein content in the growth medium were changed. The biomass increased by 392% for kefir grains grown in a medium enriched with whey proteins. Inclusion of a whey protein isolate or modified whey protein accelerated kefir biomass growth with preservation of microflora.

Microbiological analysis *Lactobacillus* spp. and *Lactococcus* spp. contained in kefir samples (KA, KC) ranged from 9.02 to 8.53 log CFU/mL, and 9.28 to 9.27 log CFU/mL, respectively (Fig. 4). These results were similar to values reported by Kök-Taş *et al.* (31). *Lactobacillus* spp. content of KA were significant ($p<0.05$) compared with KC. *Lactococcus* spp. content of KA were not significant ($p>0.05$) compared with KC. The yeast contents of KA and KC were 2.08 and 2.0 log CFU/mL, respectively. The yeast contents of KA was not significant ($p>0.05$) compared with KC.

The *L. acidophilus* and *Bifidobacterium* spp. contents in kefir samples (KA, KC) ranged from 6.31 to 6.19 log CFU/mL and 5.98 to 5.95 log CFU/mL, respectively. The *L. acidophilus* and *Bifidobacterium* spp. contents of KA was not significant ($p>0.05$) compared with KC. Contents of *L. acidophilus* and *Bifidobacterium* spp. were between 5.78 and 6.43 log CFU/mL, respectively (31). Therefore, inclusion of *Gluconacetobacter* spp. in kefir grains did not negatively affect the existing probiotic kefir microflora. The *Gluconacetobacter* spp. content was 3.86 log CFU/mL in KA. The *Gluconacetobacter* spp. content was not determined for KC (control kefir sample). Microbial compositions of kefir grains originating from Taiwan, Russia, Ireland, Turkey and some other countries have been identified (14,22,32). Marquina *et al.* (22) reported that kefir grains were present at a level of 4.1 log CFU/mL of acetic acid bacteria. Guzel Seydim *et al.* (32) reported only lactic acid bacteria and yeasts in Turkish kefir grains. Kefir grains in Turkey do not contain *Gluconacetobacter* spp. (32). Furthermore, AAB inoculation did not adversely affect the grain microflora, particularly the existing probiotic kefir microflora. Therefore, the therapeutic properties of kefir were enhanced with inclusion of *Gluconacetobacter* spp.

The *Gluconacetobacter* spp. contents of KGA were measured for 25 days (at 1, 5, 10, 15, 20, and 25 days) to assess continuity. In this study, *Gluconacetobacter* spp. was grown with a balanced, native, kefir grain microflora. The *Gluconacetobacter* spp. content was continuous at a level from 2.91 to 2.56 log CFU/mL (Fig. 4).

Chemical analysis of kefir The pH values, lactic acid, dry matter, and ash contents of KA and KC samples were 4.70, 4.68; 1.18, 1.53; 12.56, 12.18, and 1.18, 1.26%, respectively (Table 1). Chemical analytical results were consistent with previous reports (13,26). The titratable acidity values (as lactic acid equivalents) of KA sample were statistically more significant ($p<0.01$) than the titratable acidity values (as lactic acid equivalents) of KC sample. This difference arose due to inclusion of *Gluconacetobacter* spp. in kefir. *Gluconacetobacter* spp. generates the organic acids, acetic acid and carbon dioxide from organic molecules in the surrounding environment. The reduction in the lactic acid content might have been caused by competition

Table 1. Chemical analysis of kefir samples

Kefir Samples	pH	Lactic Acid (%)	Dry Matter (%, w/w)	Ash Content (%, w/w)
KA	4.70±0.02	1.18±0.02	12.56±0.06	1.18±0.12
KC	4.68±0.01	1.53±0.04	12.18±0.05	1.26±0.06

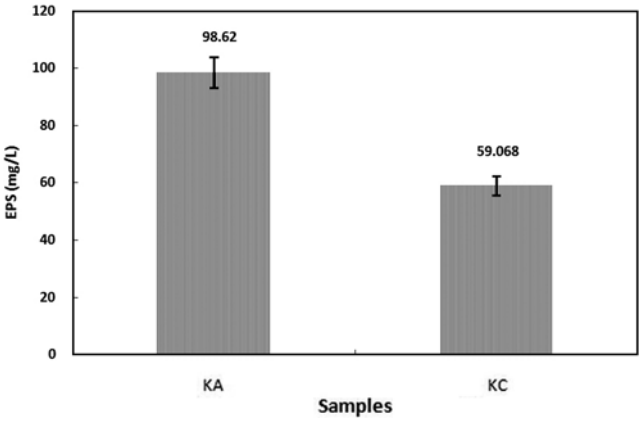

Fig. 5. EPS contents of the kefir samples.

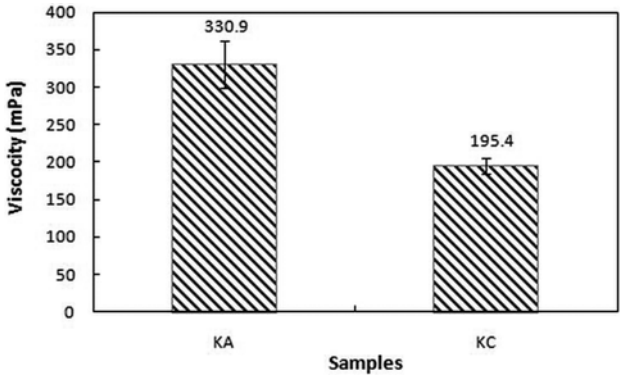

Fig. 6. Viscosity values of the kefir samples.

between LAB and AAB for the lactose bacterial substrate.

Evaluation of the exopolysaccharide contents of kefir EPS contents of KA and KC sample were 98.62 and 59.07 mg/L, respectively (Fig. 5). The EPS content of KA was increased significantly (p<0.05) compared with KC, due to increased biomass production of EPS in the presence of *Gluconacetobacter* spp. (33). The EPS content is important for the physico-chemical, rheological, and organoleptic properties of kefir. EPS is produced by native microorganisms within kefir grains, such as *L. kefiranofaciens*. The content and characteristics of EPS depend on biodiversity in the microflora, the fermentation conditions, and the growth environment (34). Kök Taş *et al.* (31) reported that the EPS content of kefir grains ranges between 79-130.15 mg/L. *Gluconacetobacter* spp. produces EPS and cellulose (35). Therefore, the EPS produced by *Gluconacetobacter* spp. contributed to the kefir EPS levels.

Rheological analysis The viscosity values of KA and KC samples were measured at 100 rpm (Brookfield Rotational Rheometer, DV-II Pro, LV; Brookfield Engineering Laboratories, Middleboro, MA, USA) as 330.9 and 195.5 mPa, respectively (Fig. 6). The viscosity values of KA samples that included *Gluconacetobacter* spp. was significantly (p<0.05), higher than KC samples. Growth of *Gluconacetobacter* spp. in kefir improved the product viscosity. Irigoyen *et al.* (13) reported that the viscosity values of kefir samples produced using different concentrations of kefir grains (1 and 5%) were 293 and 372 mPa, respectively, at 100 rpm. The viscosity values increased with an increase in the kefir grain concentrations used for inoculation. Kök Taş *et al.* (31) reported that viscosity values of kefir samples ranged between 225-315 mPas at 10 rpm. Ertekin and Güzel-Seydim (26); however, reported viscosity values of kefir samples produced using skimmed milk to be 180 mPa at 10 rpm. Similarly, Tratnik *et al.* (36) reported that the viscosity values for kefir samples prepared from cow and goat milk with additives, including insulin, whey protein concentrate, and dried milk, ranged between 42.2-166.6 mPa. Babina and Rozhokova (37) noted that both LAB and AAB increased kefir viscosity. Moreover, some studies have suggested that *A. aceti* and *A. rancens* both increase kefir viscosity and consistency (38). KA samples in this study exhibited high viscosity values because a high EPS content contributed to an increase in viscosity.

Sensory analysis Sensory evaluation data are reported in Fig. 7. KA and KC samples were evaluated according for texture, odor, and taste attributes. For taste, KA samples that included *Gluconacetobacter* spp. scored significantly (p<0.05), higher than KC samples, particularly for a mild, sweetish flavor. This result would be satisfactory for consumers. KA samples scored significantly (p<0.05) higher than KC samples for foam formation. Foaming is a desirable appearance attribute. No significant (p>0.01) difference between KA and KC samples was observed for odor. Generally, KA samples scored higher for all attributes than KC samples. Acetic acid bacteria are rarely present in unmodified kefir grains. A recent report describing the microflora of kefir grains indicated that the grains did not contain acetic acid bacteria (39). The inclusion of a useful microorganism that is not naturally present in kefir grains is first reported herein. The structure of the kefir grain can be used to carry useful bacteria.

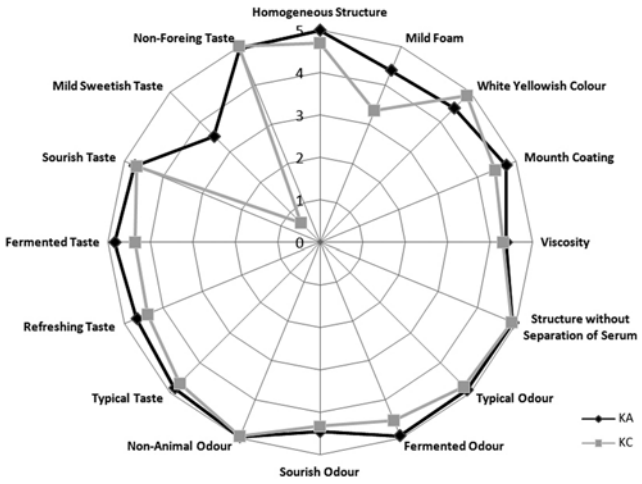

Fig. 7. Sensory data for the kefir samples.

In this study, *Gluconacetobacter* spp. was successfully incorporated into kefir grain microflora without disrupting the native lactic acid bacteria or yeast. Acetic acid bacteria contributed to production of exopolysaccharides and increased the grain biomass without negatively affecting the natural microflora or the sensory properties of kefir. Moreover, the sensory properties of kefir were improved with inclusion of *Gluconacetobacter* spp. Kefir consumption rates may improve when products generated from grains incorporating acetic acid bacteria become more widely known for enhanced qualitative characteristics, including appearance-texture, smell, and taste.

Acknowledgments This project was financially supported by the Scientific Research Commission of Suleyman Demirel University (Project#2721-YL-11). Danem Co. (Isparta, Turkey) provided natural kefir grains.

Disclosure The authors declare no conflict of interest.

References

1. Ottogalli G, Galli A, Resmini P, Volonterio G. Composizione microbiologia, chimica ed ultrastruttura dei ganuli di kefir. Annali. Di. Microbiolgia 23: 109-121 (1973)
2. Garrote GL, Abraham AG, De Antoni GL. Chemical and microbiological characterization of kefir grains. J. Dairy Res. 68: 639-652 (2001)
3. Farnworth ER. Kefir-a complex probiotic. Food Sci. Technol. Bull. Funct. Foods 2: 1-17 (2006)
4. Güzel-Seydim ZB, Kök-Tas T, Greene AK, Seydim, AC. Review: Functional properties of kefir. Food Sci. Nutr. 51: 261-268 (2011)
5. Budak NH, Aykin E, Seydim, AC, Greene AK, Guzel-Seydim ZB. Functional properties of vinegar. J. Food Sci. 79: 57-64 (2014)
6. De Ory I, Romeo LE, Cantero D. Optimum starting-up protocol of a pilot plant scale acetifier for vinegar production. J. Food Eng. 52: 31-37 (2002)
7. Swings I. The Genera Acetobacter and Gluconobacter. In: The Prokaryotes. Balows A, Triiper HG, Dworkin M, Harder W, Schleifer KH (eds). Springer, New York, NY, USA. pp. 2268-2286 (1992)
8. Franke HI, Fegan M, Hayward C, Graham Leonard G, Stackebrandt E, Sly LI. Description of *Gluconacetobacter sacchari* sp. nov., a new species of acetic acid bacterium isolated from the leaf sheath of sugar cane and from the pink sugar-cane mealy bug. Int. J. Syst. Bacteriol. 4: 1681-1693 (1999)
9. Nishikawa Y, Takata Y, Nagai Y, Mori T, Kawada T, Ishihara N. Antihypertensive effects of korusu extract, a traditional vinegar produced from unpolished rice, in the SHR rats. Nippon. Kagaku. Kaishi. 48: 73-75 (2001)
10. Costa AO, Thomaz-Soccol V, Paulino RC, Castro AE. Effect of vinegar on the viability of Giardia duodenalis cysts. Int. J. Food Microbiol. 128: 510-512 (2009)
11. Fukami H, Kobayashi S, Tachimoto H, Kishi M, Kaga T, Waki H, Iwamoto M, Tanaka Y. Effect continuous ingestion of acetic acid bacteria on memory retention and the synaptic function in aged rat. Biosci. Biotech. Bioch. 74: 1498-1500 (2010)
12. Oda T, Tachimoto H, Kishi M, Kaga T, Ichihash M. Effect of oral intake of ceramide-containing acetic acid bacteria on skin barrier function. Anti-Aging Med. 7: 50-54 (2010)
13. Irigoyen A, Arana I, Castiella M, Torre P, Ibanez FC. Microbiological, physicochemical, and sensory characteristics of kefir during storage. Food Chem. 90: 613-620 (2005)
14. Da Cruz Pedrozo Miguel MG, Gomes Cardoso P, de Assis Lago L, Schwan RF. Diversity of bacteria present in milk kefir grains using culture-dependent and culture-independent methods. Food Res. Int. 43: 1523-1528 (2010)
15. Budak HN, Guzel Seydim ZB. Antioxidant activity and phenolic content of wine vinegars produced by two different techniques. J. Sci. Food Agr. 90: 2021-2026 (2010)
16. Fernández Pérez R, Torres C, Sanz S, Ruiz Larrea F. Rapid molecular methods for enumeration and taxonomical identification of acetic acid bacteria responsible for submerged vinegar production. Eur. Food Res. Technol. 231: 813-819 (2010)
17. Trcek J, Toyama H, Czuba J, Misiewicz A, Matsushita K. Correlation between acetic acid resistance and characteristics of PQQ-dependent ADH in acetic acid bacteria. Appl. Microbiol. Biotech. 70: 366-373 (2006)
18. Kök-Taş T, Ekinci Y, Güzel-Seydim ZB. Identification of microbial flora in kefir grains produced in Turkey using PCR. Int. J. Dairy Technol. 65: 126-131 (2012)
19. Guzel-Seydim Z, Kök-Taş T, Ertekin B, Seydim, AC. Effect of fat replacers on biomass increase in kefir grains. J. Dairy Sci. 94: 1239-42 (2011)
20. Mossel DAA, Corry JEL, Stuijk CB, Baird RM. Essential of the Microbiology of Food: A Texbook for Advance Studies. John Wiley & Sons, Chichester, UK. pp. 224-227 (1995)
21. Özer, BH, Uzun YS, Kırmacı HA. Effect of cell immobilization on viability of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 in Kasar cheese. Int. J. Dairy Technol. 61: 237-244 (2008)
22. Marquina D, Santos A, Corpas I, Munoz J, Zazo J, Peinado JM. Dietary influence of Kefir on microbial activities in the mouse bowel. Appl. Microbiol. 35: 136-140 (2002)
23. AOAC. Official Method of Analysis of AOAC Intl. 16th ed. Official Methods of Analysis (OMA). Association of Official Analytical Communities, Arlington, VA, USA (1996)
24. Zisu B, Shah NP. Effects of pH, temperature, supplementation with whey protein concentrate, and adjunct cultures on the production of exopolysaccharides by *Streptococcus thermophilus* 1275. J. Dairy Sci. 86: 3405-3415 (2003)
25. Cerning J, Bouillanne C, Landon M, Desmazeaud M. Isolation and characterization of exopolysaccharides from slime forming mesophilic lactic acid bacteria. J. Dairy Sci. 75: 692-699 (1973)
26. Ertekin B, Guzel Seydim ZB. Effect of fat replacers on Kefir quality. J. Sci. Food Agr. 90: 543-548 (2010)
27. Coggins PC, Schilling MW, Kumari S, Gerrard PD. Development of

a sensory lexicon for conventional milk yogurt in the United States. J. Sens. Stud. 5: 671-687 (2008)
28. The Basic Local Alignment Search Tool. BLAST finds regions of similarity between biological sequences. Available from: http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi. Accessed Jun. 18, 2011.
29. Yang Z, Zhou F, Ji B, Li B, Luo Y, Yang, L, Li T. Symbiosis between Microorganisms from Kombucha and Kefir: Potential Significance to the Enhancement of Kombucha Function. Appl. Biochem. Biotech. 160: 446-455 (2010)
30. Valepyn E, Berezina N, Paquot M. Optimization of production and preliminary characterization of new exopolysaccharides from *Gluconacetobacter hansenii* LMG1524. Adv. Microbio. 2: 488-496 (2012)
31. Kök Taş T, Seydim AC, Özer B, Guzel-Seydim ZB. Effect of different fermantation parameters on quality characteristics of kefir. J. Dairy Sci. 92: 780-789 (2013)
32. Guzel-Seydim, ZB, Seydim AC, Greene AK. Turkish kefir and kefir grains: Microbial enumeration and electron microscopic observation. J. Int. Dairy Technol. 58: 25-29 (2005)
33. Park JK, Jung JY, Park YH Cellulose production by *Gluconacetobacter hansenii* in a medium containing ethanol. Biotechnol. Lett. 25: 2055-2059 (2003)
34. Wang Y, Li C, Liu P, Ahmed Z, Xiao P, Bai X. Physical characterization of exopolysaccharide produced by *Lactobacillus plantarum* KF5 isolated from Tibet Kefir. Carbohyd. Polym. 82: 895-903 (2006)
35. Dutta D, Gachhui R. Nitrogen-fixing and cellulose-producing *Gluconacetobacter kombuchae* sp. nov., isolated from Kombucha tea. Int. J. Syst. Evol. Micr. 57: 353-357 (2007)
36. Tratnik L, Rajka B, Herceg Z, Draglic I. The quality of plain and supplemented kefir from goat's and cow's milk. Int. J. Dairy Technol. 59 (2006)
37. Babina NA, Rozhokova IV. Quantitative composition of Kefir grains and Kefir microflora at different of the year. Food Chem. 2: 15-17 (1973)
38. Libudzisz Z, Piatkiewicz A. Kefir Production in Poland. Dairy Ind. Int. 55: 31-34 (1990)
39. Witthuhn RC, Schoeman T, Britz TJ. Isolation and characterization of the microbial population of different South African Kefir grains. Int. J. Dairy Technol. 57: 33-37 (2004)