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# KEFIR PRODUCTION FROM COW'S AND BUFFALO'S MILK UNDER EGYPTIAN CONDITIONS

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**ABSTRACT:** In this work, the growth and activity of natural kefir starter and kefir grains starter cultures were evaluated in fermented cow and buffalo kefir. Viable cell counts of natural kefir starter and kefir grains starter cultures, physicochemical properties, viscosity, concentration of ethanol and sensory evaluation of the kefir samples were determined during refrigerated storage at 5±1°C for 21 days. Kefir made from buffalo milk by kefir grains cultures (KG-B) showed better retention of viability, ethanol content, viscosity and decreased the sensory properties comparing with another samples. The best sensory properties were noticed in kefir made from cow milk by natural kefir (KS-C), then after kefir made from cow milk by kefir grains (KG-C) respectively. Thus, the study demonstrates that the kefir production from cow milk using natural kefir starter culture may be preferred under Egyptian conditions which had the lowest ethanol concentration and will be accepted by Egyptian consumer.

**Key words***:* Kefir, lactic acid bacteria, yeast, ethanol, viscosity.

## INTRODUCTION

Kefir is a fermented dairy product that originates from the mountains of the Caucasus (**Tratnik, *et al.,* 2006**). The term is derived from the word kef, which means 'pleasant taste' in Turkish (**Guzel-Seydim, *et al.,* 2000; De Oliveria *et al.,* 2013**). Kefir is also known variously as kefyr, kephir, kefer, kiaphur, knapon, kepi or kippi (**Sarkar, 2007**). The beverage itself typically has a slightly viscous texture with tart and acidic flavour, low levels of alcohol, and in some cases slight carbonation. Kefir is traditionally made with cow's milk but it can be made with milk from other sources such as goat's, sheep's, buffalo's, or soymilk (**Wszolek *et al.,* 2001; Liu *et al.,* 2006**). Although it has become a popular drink in many parts of the world, from Japan to eastern and northern Europe (**Otles and Cagindi, 2003**), it is unknown in Egypt as other fermented dairy products, such as yoghurt and cheese.

Kefir is generally consumed with meals and alone as a probiotic drink. It is recommended for consumption because of its **probiotic bacteria and yeast mixture (Simova** *et al.,* 2002). Kefir's probiotic property comes from kefir grains or cultures containing various species of lactobacilli, lactococci, Leuconostoc spp., acetic acid bacteria, and yeasts, among others (**Wszolek *et al.,* 2001; Witthuhn *et al.,* 2005**). The usual daily consumption of the fermented dairy foods known as probiotics, such as kefir, has tremendous health benefits including; (1) therapeutic effects such as prevention of urogenital infection, synthesis of vitamins (B2, B6, and B12), prevention of diarrhea and prevent skin problem; (2) immunomodulation including prevention of respiratory diseases, and improve resistance to allergies; (3) improving intestinal microbial structure leading to prevention of irritable bowel syndrome, support digestive process, prevention of exogenous pathogen (*e.g.* traversal's diarrhea) and prevention

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endogenous (*e.g.* antibiotic associated diarrhea); and (4) metabolic effects include lactose hydrolase (improve lactose digestion), bile salt de-conjugation (bile salt hydrolase), cholesterol reduction, lower the toxigenic / mutagenic reduction in gut, anti-carcinogenic activity, enhance calcium metabolism and prevent osteoporosis (**Anandharaj *et al.*, 2014**).

In this study, Kefir was made under Egyptian conditions from cow's and buffalo's milk using kefir grains or natural kefir starter culture. The microbiological, chemical characteristics, rheological, and sensory properties were investigated during storage at 5±1°C for 21 days.

# MATERIALS AND METHODS

## Materials

Fresh whole buffalo's and cow's milk were obtained from Dairy Technology Unit, Food Science Department, Faculty of Agric., Zagazig University. For kefir production, two types of starter cultures were used:

a) Kefir grains (KG) were kindly provided by the Department of Food Engineering, Suleyman Demirel University, Isparta, Turkey.

b) Natural kefir starter (KS) was obtained from kefir grains by straining after first fermentation at 25°C for about 22 hr.

## Manufacturing of Kefir

Buffalo's and cow's milk were heated to 95°C for 15 min and cooled to 25°C. Then each heat treated milk was divided into two equal portions and used for manufacturing of kefir.

Kefir A. Kefir grains were re-activated three times in heat treated milk. After each growth cycle at 25°C for 18 hours, the grains were separated by using a sieve. Active kefir grains were inoculated into milk cooled to 25°C with a (3% *W/V*) inoculation rate, and incubated at 25°C for 22 hours. After incubation, the grains were separated by a narrow colander from kefir and washed with sterile water, then maintained at 4 °C until the next production.

Kefir B. Natural kefir starter culture was used to ferment milk for kefir production was inoculated into milk cooled to 25°C with a 3% inoculation rate, and incubated at 25°C for 22 hours. The kefir samples were taken into the plastic bottles (100 ml). After incubation of kefir A and kefir B, fermented products were stored for 21 days at 5±1°C and analyzed when fresh (after the incubation), and then after 7, 14, and 21 days for all analyses. Cow's (C) and buffalo's (B) milk kefir made using kefir grains (KG) and natural kefir starter culture (KS) were named KG-C, KG-B, KS-C, and KS-B, respectively.

## Chemical Analyses

Kefir products were analyzed for moisture, total solids (TS), fat, total nitrogen (TN), total protein (TP), pH and titratable acidity (TA) contents according to **AOAC (2000)**.

Acetaldehyde content of kefir treatments was determined by **Less and Jago (1969)** method. Acetaldehyde reacts with semi-carbazide to form semi-carbazone which has absorption value at wave length of 224 nm. Viscosity of kefir was determined by the method of **Aryana (2003)** using Rotational viscometer Type Lab. Line Model 5437. Results expressed as (mpa.s). The ethanol content in kefir milk samples was determined following the method of College of Science, University of Canterbury. Transfer 10 ml of the acid dichromate solution to a 250 ml conical flask with matching rubber stopper, Pipette 1 ml of the diluted sample into the sample holder. This can in 5 ml beaker or glass vial, prepare three samples as the entire contents of the flask are used in the titration. Suspend the sample holder over the dichromate solution and hold in place with the rubber stopper, Store the flask overnight at 25–30°C (an incubator is ideal), Next morning allow the flask to come to room temperature, then loosen the stopper carefully and remove and discard the sample holder. Rinse the walls of the flask with distilled water, then add about 100 ml of distilled water and 1 ml of potassium iodide solution. Swirl to mix, prepare 3 blank titrations by adding 10 ml of acid dichromate solution to a conical flask, adding 100 ml of water and 1 ml of potassium iodide solution and swirling to mix. Fill a burette with sodium thiosulphate solution and titrate each flask with sodium thiosulphate. When the brown iodine colour fades to yellow, add 1 ml of starch solution and keep titrating until the blue colour disappears, Titrate the blank flasks first, and repeat until concordant

results are obtained (titres agreeing to within 0.1 ml). Then titrate each of the alcohol samples. The alcohol concentration was calculated using the generated standard curve.

## Sensory Evaluation

The sensory evaluation was done by panelists from the staff members of Food Science Department, Faculty of Agriculture, Zagazig University. The scores attributed to each of the parameters evaluated: flavour (45 points), texture (35 points), acidity (10 points), appearance (10 points), total (100 points) All samples of kefir were organoleptically evaluated after refrigerated storage for 1, 7, 14 and 21 days. According to the scheme described by **Farag *et al.* (2007)**.

**Microbiological examination**:

**Total viable bacterial count**

Total viable bacterial count was estimated by plating suitable dilution in duplicates on the basel medium according to the method suggested by the American Public Health Association (**APHA, 1992**).

**Enumeration of lactobacilli strain**

MRS agar (Oxoide Ltd. Asingstoke, UK) with pH 6.2±0.1 was used for enumeration of lactic acid bacterial according to **Dave and Shah (1996)**. The plats were incubated at 37ºC for 48 hrs.

**Enumeration of Streptococcus strain**: Lactic streptococci were enumerated on M17 medium ((Oxoide Ltd. Asingstoke, UK) the inculated plates were incubated at 37˚C for 48 hr., under aerobic conditions. According to **Dave and Shah (1996)**.

**Enumeration of moulds and yeasts**

Moulds and yeasts were enumerated on sabaraud dextrose agar as recommended by the **APHA (1992)**. Plates were incubated at 25˚C for 4-5 days.

## Statistical Analysis

Data were statistically analyzed using a computer program "SPSS system for windows version 22 (**SPSS v.20, 2012**) for analysis of variance (ANOVA) by one way and comparison of means by LSD multiple comparison test where $P<0.05$ was considered for significant difference

# RESULTS AND DISCUSSION

## Chemical Composition

Moisture and total solids (TS) contents during the storage of the kefir samples are shown in Tables 1 and 2. Normally, TS of kefir affected by the TS of milk. KG-B had the highest TS (17.19%) followed by KS-B (16.24%) then KG-C (13.56%) while KS-C had the lowest TS (13.31%). The TS of Kefir samples were compatible with TS of milks and gradually increased along storage (**Wszolek *et al.,* 2001**). reported that kefir contains 10.6% - 14.9% TS, the increment of TS may be due to declining of moisture content during storage period.

Tables 1 and 2 show that fat levels didn't affect by storage period, while the percentage of fat didn't change at all and didn't register any significant differences.

Protein contents of fresh samples ranged between 3.53% and 4.29% (Tables 1 and 2) and did not differ significantly among kefir samples ($P > 0.05$). Being dependent on the protein content of milk, protein values of kefir samples were found to be 3.3 g/100 g in the previous studies (**Renner and Renz-Schaven, 1986; Hallé *et al.,* 1994**).

Measurement of pH and titratable acidity (TA) (%) of fermented milks is important to determine the quality. The changes of pH and TA% during the storage are shown in Tables 1 and 2 and Fig. 1 The pH values of KG-C, KS-C, KG-B, and KS-B were found to be 4.40, 4.35, 4.62 and 4.37, respectively, when fresh (after the incubation), Gradual decreases were observed to reach 4.31, 4.25, 4.58 and 4.34 after 21 days of cold storage. Chemically, these ranges of pH and TA (%) are considered to be in the acceptable range of probiotic fermented milks. The pH decreases due to increasing acidity in the early stage of storage caused by continued metabolic activity of the fermentation bacteria, *e.g.* LAB. The pH and TA values found in this study are considered to be in the acceptable range of a commercial yoghurt (**Kang *et al.,* 2013**). These results for kefir are in agreement with the findings of **Yoo *et al.* (2013)**.

**Table 1. Change in chemical composition of Kefir made from cow's milk during cold storage**

| Sample | Storage period (Day) | Moisture (%) | TS (%) | Total protein (%) (TN×6.38) | Fat (%) | pH | Titratable Acidity (%) | Acetaldehyde (mg/l) | Ethanol (%) |
|---|---|---|---|---|---|---|---|---|---|
| KG-C | Fresh | 86.44 ±0.106$^{A}$ | 13.56 ±0.106$^{C}$ | 3.59 ±0.025$^{C}$ | 3.40 ±0.100 | 4.40 ±0.010$^{A}$ | 0.84 ±0.020$^{C}$ | 8.833 ±1.232$^{B}$ | 0.0180 ±0.0115$^{C}$ |
| | 7 | 86.31 ±0.076$^{AB}$ | 13.69 ±0.076$^{B}$ | 3.67 ±0.026$^{B}$ | 3.40 ±0.100 | 4.38 ±0.020$^{A}$ | 0.92 ±0.020$^{AB}$ | 25.678 ±1.481$^{A}$ | 0.0830 ±0.0356$^{B}$ |
| | 14 | 86.27 ±0.061$^{B}$ | 13.73 ±0.061$^{AB}$ | 3.74 ±0.020$^{A}$ | 3.40 ±0.100 | 4.27 ±0.025$^{B}$ | 0.95 ±0.030$^{A}$ | 31.977 ±6.167$^{A}$ | 0.1223 ±0.0665$^{A}$ |
| | 21 | 85.94 ±0.242$^{C}$ | 14.06 ±0.242$^{A}$ | 3.47 ±0.045$^{D}$ | 3.40 ±0.100 | 4.31 ±0.020$^{B}$ | 0.88 ±0.040$^{AB}$ | 29.102 ±8.483$^{A}$ | 0.1550 ±0.0400$^{A}$ |
| LSD | | 0.1711 | 0.1711 | 0.0414 | | 0.0214 | 0.0535 | 9.456 | 0.0314 |
| KS-C | Fresh | 86.69 ±0.147$^{A}$ | 13.31 ±0.127$^{B}$ | 3.53 ±0.015$^{C}$ | 3.40 ±0.100 | 4.35 ±0.030$^{A}$ | 0.82 ±0.020$^{C}$ | 7.737 ±1.255$^{C}$ | 0.0163 ±0.0144$^{C}$ |
| | 7 | 86.48 ±0.191$^{AB}$ | 13.52 ±0.195$^{B}$ | 3.62 ±0.050$^{B}$ | 3.40 ±0.100 | 4.25 ±0.025$^{C}$ | 0.87 ±0.030$^{BC}$ | 20.200 ±1.014$^{B}$ | 0.0310 ±0.0900$^{C}$ |
| | 14 | 86.23 ±0.242$^{B}$ | 13.77 ±0.241$^{AB}$ | 3.70 ±0.049$^{A}$ | 3.40 ±0.100 | 4.29 ±0.015$^{B}$ | 0.92 ±0.030$^{AB}$ | 25.541 ±1.741$^{A}$ | 0.0637 ±0.0665$^{B}$ |
| | 21 | 86.16 ±0.057$^{B}$ | 13.84 ±0.055$^{A}$ | 3.72 ±0.023$^{A}$ | 3.40 ±0.100 | 4.25 ±0.030$^{BC}$ | 0.93 ±0.053$^{A}$ | 23.624 ±5.147$^{AB}$ | 0.1210 ±0.0888$^{A}$ |
| LSD | | 0.3256 | 0.3823 | 0.0837 | | 0.0418 | 0.0541 | 5.318 | 0.0212 |

KG-C= kefir made from cow milk by kefir grains, KS-C= kefir made from cow milk by natural kefir starter. Means of three replications ± standard deviation. $^{ABCD}$ Letters indicate significant differences between storage times ($P<0.05$)

**Table 2. Change in chemical composition of Kefir made from buffalo's milk during cold storage**

| Sample | Storage period (Day) | Moisture (%) | TS (%) | Total protein (%) (TN×6.38) | Fat (%) | pH | Titratable acidity (%) | Acetaldehyde (mg/l) | Ethanol (%) |
|---|---|---|---|---|---|---|---|---|---|
| KG-B | Fresh | 82.81 ±0.115$^{A}$ | 17.19 ±0.165$^{B}$ | 4.29 ±0.015$^{B}$ | 4.70 ±0.100 | 4.62 ±0.015$^{A}$ | 0.67 ±0.030$^{C}$ | 16.776 ±3.917$^{B}$ | 0.0637 ±0.0133$^{D}$ |
| | 7 | 82.45 ±0.060$^{B}$ | 17.55 ±0.060$^{B}$ | 4.36 ±0.020$^{A}$ | 4.70 ±0.100 | 4.61 ±0.026$^{AB}$ | 0.79 ±0.030$^{B}$ | 30.334 ±3.110$^{A}$ | 0.0857 ±0.0665$^{C}$ |
| | 14 | 82.36 ±0.125$^{B}$ | 17.64 ±0.145$^{B}$ | 4.41 ±0.015$^{A}$ | 4.70 ±0.100 | 4.53 ±0.015$^{C}$ | 0.82 ±0.020$^{B}$ | 37.729 ±1.018$^{A}$ | 0.1387 ±0.0665$^{B}$ |
| | 21 | 82.27 ±0.070$^{B}$ | 17.73 ±0.080$^{A}$ | 4.37 ±0.045$^{A}$ | 4.70 ±0.100 | 4.58 ±0.030$^{B}$ | 0.93 ±0.030$^{A}$ | 30.197 ±10.707$^{A}$ | 0.1667 ±0.0709$^{A}$ |
| LSD | | 0.2612 | 0.2707 | 0.0569 | | 0.0314 | 0.0541 | 9.856 | 0.0185 |
| KS-B | Fresh | 83.76 ±0.046$^{A}$ | 16.24 ±0.066$^{D}$ | 4.27 ±0.025$^{C}$ | 4.70 ±0.100 | 4.37 ±0.010$^{B}$ | 0.87 ±0.020$^{B}$ | 13.490 ±1.320$^{B}$ | 0.0210 ±0.0655$^{D}$ |
| | 7 | 83.43 ±0.048$^{B}$ | 16.57 ±0.068$^{C}$ | 4.33 ±0.020$^{B}$ | 4.70 ±0.100 | 4.29 ±0.020$^{C}$ | 0.92 ±0.041$^{A}$ | 28.006 ±3.727$^{A}$ | 0.0503 ±0.0929$^{C}$ |
| | 14 | 83.26 ±0.073$^{C}$ | 16.74 ±0.077$^{B}$ | 4.39 ±0.020$^{AB}$ | 4.70 ±0.100 | 4.43 ±0.025$^{A}$ | 0.96 ±0.020$^{A}$ | 33.484 ±3.261$^{A}$ | 0.0917 ±0.0929$^{B}$ |
| | 21 | 82.97 ±0.122$^{D}$ | 17.03 ±0.102$^{A}$ | 4.42 ±0.037$^{A}$ | 4.70 ±0.100 | 4.34 ±0.010$^{B}$ | 0.93 ±0.060$^{A}$ | 28.417 ±8.033$^{A}$ | 0.1507 ±0.0135$^{A}$ |
| LSD | | 0.1125 | 0.1547 | 0.0529 | | 0.0421 | 0.0611 | 9.218 | 0.0211 |

KG-B= kefir made from buffalo milk by kefir grains, KS-B= kefir made from buffalo milk by natural kefir starter. Means of three replications ± standard deviation. $^{ABCD}$ Letters indicate significant differences between storage times ($P<0.05$)

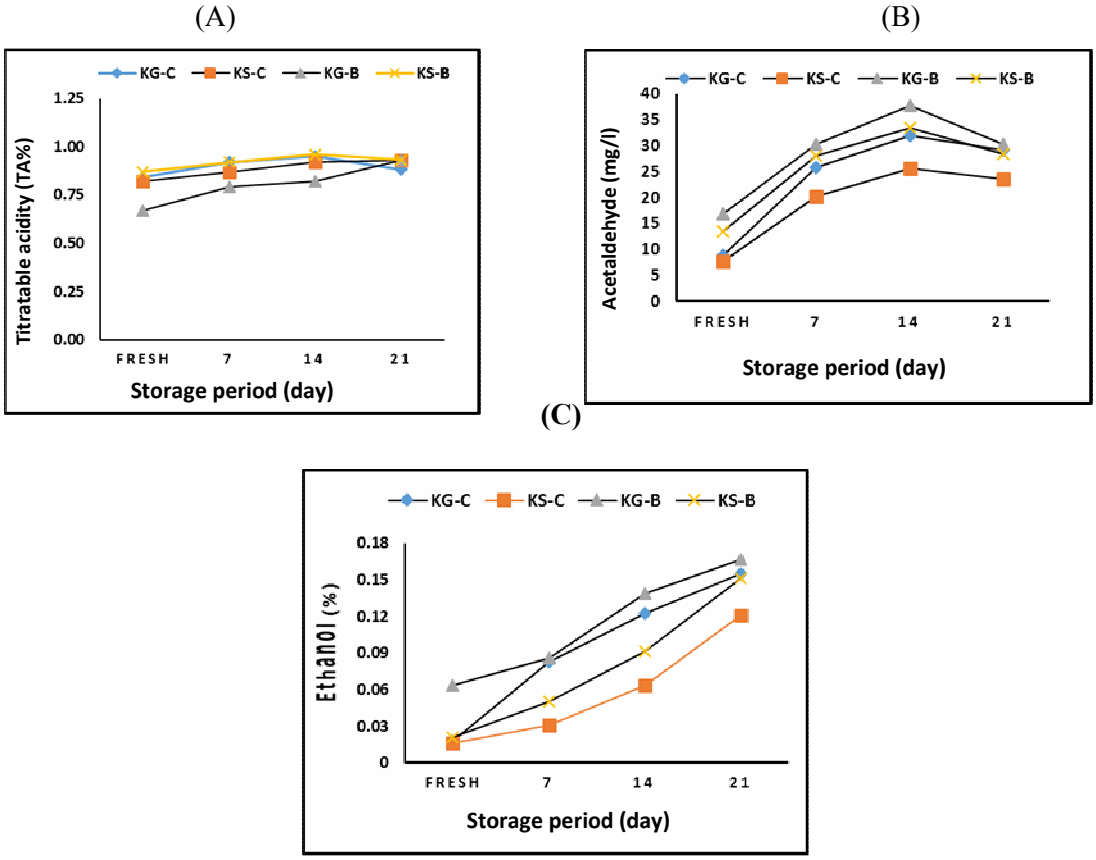


**Fig. 1. The change of titratable acidity (A), acetaldehyde (B) and ethanol contents (C) in kefir samples during cold storage. KG-C= kefir made from cow milk by kefir grains, KS-C= kefir made from cow milk by natural kefir starter, KG-B= kefir made from buffalo milk by kefir grains, KS-B= kefir made from buffalo milk by natural kefir starter.**

Nutty and pungent aromas are usually detected in fermented dairy products; acetaldehyde is responsible for that aroma. Experimental kefirs contained limited levels of acetaldehyde (Tables 1 and 2 and Fig. 1), initial quantities were 8.833, 7.737, 16.77 and 13.499 mg/l, gradually increased to reach 31.977, 25.541, 37.729 and 33.484 mg/l after 14days then decreased to reach 29.102, 23.624, 30.197 and 28.417 mg/l for KG-C, KS-C, KG-B and KS-B, respectively. The low concentrations of acetaldehyde in Kefir beverages probably due to the metabolism of a part of it to alcohol by alcohol dehydrogenase enzyme (**Ertekin and Güzel-Seydim, 2010**). Acetaldehyde is considered the major yoghurt or fermented milks flavour. It can be formed by the group of N- streptococci. These microorganisms degrade lactose to galactose and glucose; glucose can be metabolized by the homofermentative Embden-Meyerhof-Parnas pathway to pyruvate, where 2 mol of lactate is formed per glucose molecule residual pyruvate, catalyzed by an α-carboxylase, is then converted to diacetyl and acetaldehyde. An aldehyde dehydrogenase may also generate acetaldehyde from Acetyl-CoA, which is formed from pyruvate by the action of a pyruvate dehydrogenase (**Yuksekdag *et al.*, 2004; Geroyiannaki *et al.*, 2007**). Ethanol content (EC) of the kefir samples (KG-C, KS-C, KG-B and KS-B) during storage is shown in Tables 1, 2 and Fig. 1. The fresh KS-C had the lowest EC (0.0163%) followed by KG-C (0.0180%) then KS-B (0.0210%) and KG-B (0.0637%) These concentrations tended to increase slightly during the storage period to reach the maximum levels, being 0.1210%, 0.1550%, 0.1507% and 0.1667% after 21 days of cold storage, respectively. Typically, Kefir contains 1.0% alcohol, comparing to other studies, the final

ethanol concentrations were 8.7 ± 1.6 g/l, 8.3 ± 0.2 g/l and 7.8 ± 0.3 g/l for milk kefir, cheese whey kefir and deproteinised cheese whey kefir, respectively (**Magalhães *et al.,* 2011**). Kefir products fermented by a conventional method contained a high concentration of alcohol, starting from 1.3% and up to 1.36% (**Sarkar, 2007 ; Yoo *et al.,* 2013**). The highest ethanol content in KG-B compared with KG-C could be due to high yeast counts in KG-B ($P < 0.05$). It is well known that, there are lot of microbes responsible for the production of ethanol in Kefir; yeasts such as *Kluyveromyces marxianus* var. *lactis, Saccharomyces cerevisiae, Candida inconspicua* and *Candida maris* and Heterofermentative bacteria, e.g. *Lactobacillus kefiri* and *Leuconostoc* spp. The amounts of ethanol and $CO_2$ produced during the fermentation of kefir depend on the production conditions. Moreover, concentration of ethanol in fresh yoghurt samples ranged from 1.38 to 4.61 ppm and increased to reach 3.17-8.88 and 4.26-8.75 ppm after 10 d at 4ºC and 20ºC, respectively (**Hruškar and Milana Ritz, 1995 ; Farnworth, 2005**)

Change of viscosity during storage (Table 3) in the different kefir samples was measured along the storage period. The viscosity affected the palatability of fermented milks so it is an important factor in quality of yoghurt and kefir. KG-B had the highest viscosity (1340 mPa.s) followed by KS-B (1080 mPa.s) then KG-C (1125 mpa.s) while KS-C had the lowest viscosity (890 mPa.s). Viscosity values significantly increased until reached to the maximum values after 14 days and then sharply decreased to record the lowest values after 21 days. Moreover, kefir samples made using kefir starter KS had viscosity less than milk kefir made with kefir grains. Similar results were reported by **Yoo *et al.* (2013)** who made milk kefir by two-step fermentation.

Kefir samples were analyzed for their microbiological properties when fresh (after the incubation), and then after 7, 14, and 21 of storage at 5±1°C. The counts of total bacterial count, *lactobacilli, lactococci* spp., and yeast in all samples were determined. Table 4 and Fig. 2 show the mean changes in total bacterial counts. The KG-B samples had the highest counts ($P < 0.05$) followed by KG-C, KS-C and KS-B.

*Lactobacilli* counts in fresh kefir samples recorded 8.376, 8.264, 8.443 and 8.293 log cfu/ml. These numbers slightly increased to reach 8.411, 8.274, 8.729 and 8.375 log cfu/ml after 7days, then limitedly decreased to 8.192, 8.155, 8.370 and 8.292 log cfu/ml on the 21 day of storage of KG-C, KS-C, KG-B and KS-B, respectively. The differences of these counts might be attributed to the type of milk and their available nutrients. *Lactobacilli* did not change in the first 9 days of storage, but increased slightly after wards. Similar results were reported by **Leite *et al.* (2013)**. These numbers are within the scope of probiotic in fermented therapeutic products. Therapeutic LAB counts must be $\geq 10^6$ in probiotic products so the experimental kefir has therapeutic effects (**Yoo *et al.,* 2013**). Although lactobacilli counts of the kefir samples produced from buffalo milk were found higher than cow milk kefir samples on fresh ($P < 0.05$). Milk type had no effect on lactobacilli counts of all kefir samples at the end of storage ($P > 0.05$). However, **Han *et al.* (2007)** stated that microorganisms can grow rapidly due to the high nutritional properties of buffalo milk.

Lactococci counts of the kefir samples made from buffalo milk using kefir grains (KG-B) were higher than the samples made from cow milk at the end of storage period ($P < 0.05$). Lactococci counts in other kefir samples showed a significant increase ($P < 0.05$) until d7; after d 7 days of the storage period the lactococci populations of all kefir samples slightly decreased. This could be attributed to cell proteolysis due to the yeast counts of different kefir samples. The highest yeast counts were observed in KG-B kefir sample ($P < 0.05$) after incubation. Yeast counts increased significantly ($P < 0.05$) gradually at the end of cold storage. This variation due to milk composition, kefir microflora composition which varies according to culture medium and production method (**Sarkar, 2008**). Yeast levels present in KG vary widely, ranging from $1.5 \times 10^5$ to $3.7 \times 10^8$ cfu/ml. These results agree with **Witthuhn *et al.* (2004)**. A total of 66 yeast colonies were isolated from 5 Tibet kefir samples, yeast isolates were classified into 8 groups belonging to the genera: Saccharomyces, Pichia, Debaryomyces, Rhodotorula, Candida, Kluyveromyces and Kazachstania (**Li *et al.,* 2015**).

The sensory evaluation results of kefir samples in fresh, and after 7, 14, 21days are given in Table 5. The KS-C sample in fresh had

**Table 3. Viscosity (mpa.s) of Kefir samples during cold storage**

| Sample[1] | Storage Period (Day) | | | | LSD |
|---|---|---|---|---|---|
| | Fresh | 7 | 14 | 21 | |
| **KG-C** | 1125±28.00$^{C}$ | 1170±50.00$^{A}$ | 1235±38.18$^{AB}$ | 985±38.18$^{B}$ | 83.885 |
| **KS-C** | 890±38.18$^{C}$ | 920±38.18$^{A}$ | 950±38.18$^{B}$ | 730±38.18$^{C}$ | 79.256 |
| **KG-B** | 1340±76.37$^{C}$ | 1385±38.18$^{A}$ | 1420±25.00$^{B}$ | 1280±62.91$^{D}$ | 48.249 |
| **KS-B** | 1080±52.04$^{B}$ | 1100±38.18$^{A}$ | 1120±38.18$^{B}$ | 840±38.18C | 92.727 |

Means of three replications ± standard deviation. KG-C= kefir made from cow milk by kefir grains, KS-C= kefir made from cow milk by natural kefir starter. KG-B= kefir made from buffalo milk by kefir grains, KS-B= kefir made from buffalo milk by natural kefir starter

$^{ABCD}$ Letters indicate significant differences between storage times ($P<0.05$)

**Table 4. Microbial counts of kefir samples during cold storage (log CFU/ml)**

| Microorganisms | Storage period (Day) | KG-C | KS-C | KG-B | KS-B |
|---|---|---|---|---|---|
| **Total viable bacterial count (TVBC)** | **Fresh** | 8.679±0.023$^{B}$ | 8.563±0.030$^{BC}$ | 8.681±0.043$^{A}$ | 8.562±0.012$^{B}$ |
| | **7** | 8.731±0.041$^{A}$ | 8.678±0.042$^{A}$ | 8.723±0.031$^{A}$ | 8.633±0.055$^{A}$ |
| | **14** | 8.661±0.025$^{C}$ | 8.607±0.012$^{B}$ | 8.621±0.169$^{B}$ | 8.535±0.024$^{BC}$ |
| | **21** | 8.617±0.039$^{D}$ | 8.527±0.018$^{C}$ | 8.463±0.119$^{C}$ | 8.494±0.026$^{C}$ |
| **LSD** | | 0.0949 | 0.057 | 0.2038 | 0.061 |
| ***Lactobacillus* spp.** | **Fresh** | 8.376±0.012$^{A}$ | 8.264±0.067$^{A}$ | 8.443±0.045$^{C}$ | 8.293±0.0129$^{A}$ |
| | **7** | 8.411±0.055$^{A}$ | 8.274±0.045$^{A}$ | 8.729±0.072$^{A}$ | 8.375±0.067$^{A}$ |
| | **14** | 8.276±0.072$^{AB}$ | 8.199±0.054$^{A}$ | 8.570±0.081$^{B}$ | 8.345±0.095$^{A}$ |
| | **21** | 8.192±0.115$^{B}$ | 8.155±0.048$^{A}$ | 8.370±0.053$^{C}$ | 8.292±0.108$^{A}$ |
| **LSD** | | 0.1512 | 0.102 | 0.0661 | 0.085 |
| ***Lactococci* spp.** | **Fresh** | 8.483±0.026$^{B}$ | 8.374±0.041$^{C}$ | 8.532±0.139$^{B}$ | 8.419±0.028$^{B}$ |
| | **7** | 8.635±0.039$^{A}$ | 8.577±0.015$^{A}$ | 8.711±0.010$^{A}$ | 8.614±0.066$^{A}$ |
| | **14** | 8.573±0.045$^{AB}$ | 8.488±0.018$^{B}$ | 8.590±0.019$^{AB}$ | 8.565±0.061$^{A}$ |
| | **21** | 8.502±0.083$^{B}$ | 8.336±0.034$^{C}$ | 8.480±0.054$^{B}$ | 8.448±0.091$^{B}$ |
| **LSD** | | 0.1014 | 0.075 | 0.1675 | 0.020 |
| **Yeasts** | **Fresh** | 4.512±0.136$^{D}$ | 4.144±0.121$^{C}$ | 4.568±0.068$^{C}$ | 4.171±0.080$^{C}$ |
| | **7** | 5.083±0.014$^{C}$ | 4.502±0.061$^{B}$ | 5.077±0.100$^{B}$ | 4.711±0.055$^{B}$ |
| | **14** | 5.203±0.070$^{B}$ | 4.612±0.028$^{B}$ | 5.218±0.147$^{AB}$ | 4.710±0.109$^{B}$ |
| | **21** | 5.349±0.020$^{A}$ | 5.120±0.026$^{A}$ | 5.360±0.094$^{A}$ | 5.166±0.012$^{A}$ |
| **LSD** | | 0.1127 | 0.1341 | 0.2405 | 0.1134 |

KG-C= kefir made from cow milk by kefir grains, KS-C= kefir made from cow milk by natural kefir starter. KG-B= kefir made from buffalo milk by kefir grains, KS-B= kefir made from buffalo milk by natural kefir starter. Means of three replications ± standard deviation. $^{ABCD}$ Letters indicate significant differences between storage times ($P<0.05$)

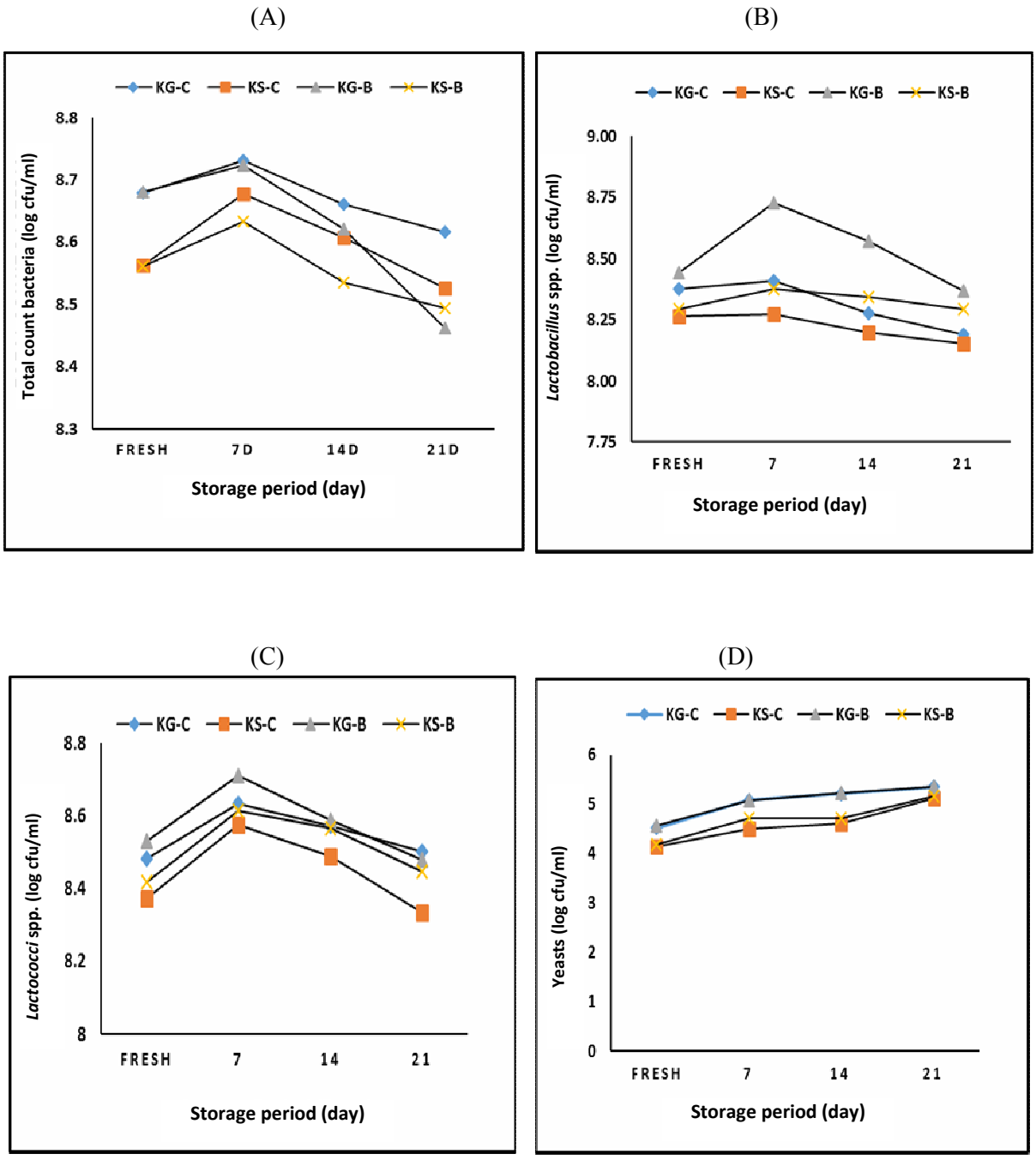


**Fig. 2. The change of total count bacteria (A), *lactobacilli* (B), *lactococci* (C), and yeasts (D) counts in kefir samples during cold storage. KG-C= kefir made from cow milk by kefir grains, KS-C= kefir made from cow milk by natural kefir starter, KG-B= kefir made from buffalo milk by kefir grains, KS-B= kefir made from buffalo milk by natural kefir starter**

**Table 5. The Sensory evaluation of kefir samples during cold storage at 5±1°C for 21 days**

| Sensory property | Storage period (Day) | KG-C | KS-C | KG-B | KS-B |
|---|---|---|---|---|---|
| | **Fresh** | 42.16±0.76$^{A}$ | 42.93±0.11$^{A}$ | 41.41±1.32$^{A}$ | 41.73±1.41$^{A}$ |
| **Flavour** | **7** | 41.00±1.01$^{AB}$ | 41.66±0.57$^{AB}$ | 41.33±1.37$^{A}$ | 42.33±0.57$^{A}$ |
| **(45 points)** | **14** | 39.33±1.15$^{B}$ | 39.66±1.52$^{B}$ | 39.66±1.65$^{A}$ | 40.33±0.57$^{A}$ |
| | **21** | 30.66±0.57$^{C}$ | 31.00±1.00$^{C}$ | 30.66±1.15$^{B}$ | 30.66±1.15$^{B}$ |
| **LSD** | | 1.811 | 2.034 | 2.745 | 2.285 |
| | **Fresh** | 32.14±0.79$^{A}$ | 31.33±1.52$^{A}$ | 31.66±1.52$^{A}$ | 31.66±1.52$^{A}$ |
| | **7** | 31.66±0.57$^{A}$ | 32.00±1.00$^{A}$ | 31.50±1.32$^{A}$ | 31.66±0.57$^{A}$ |
| **Texture** | **14** | 30.66±1.15$^{A}$ | 29.66±1.52$^{A}$ | 29.33±1.15$^{A}$ | 29.33±1.15$^{A}$ |
| **(35 points)** | **21** | 24.00±2.00$^{B}$ | 24.00±2.00$^{B}$ | 22.33±2.51$^{B}$ | 24.33±2.08$^{B}$ |
| **LSD** | | 2.735 | 3.325 | 3.631 | 2.859 |
| | **Fresh** | 8.79±0.36$^{A}$ | 9.00±0.00$^{A}$ | 8.70±0.50$^{A}$ | 9.00±0.00$^{A}$ |
| **Acidity** | **7** | 8.33±0.57$^{AB}$ | 8.33±0.57$^{B}$ | 8.00±0.00$^{AB}$ | 8.00±0.00$^{B}$ |
| **(10 points)** | **14** | 8.00±0.00$^{B}$ | 8.00±0.00$^{B}$ | 7.66±0.57$^{BC}$ | 7.66±0.57$^{B}$ |
| | **21** | 7.00±0.00$^{C}$ | 7.00±0.00$^{C}$ | 7.00±0.00$^{C}$ | 6.66±0.57$^{C}$ |
| **LSD** | | 0.619 | 0.562 | 0.823 | 0.798 |
| | **Fresh** | 8.95±0.08$^{A}$ | 9.00±0.00$^{A}$ | 8.75±0.43$^{A}$ | 9.00±0.00$^{A}$ |
| **Appearance** | **7** | 8.00±0.00$^{B}$ | 8.33±0.57$^{B}$ | 8.33±0.57$^{AB}$ | 8.00±0.00$^{B}$ |
| **(10 points)** | **14** | 8.00±0.00$^{B}$ | 8.00±0.00$^{B}$ | 8.00±0.00$^{B}$ | 8.00±0.00$^{B}$ |
| | **21** | 7.00±0.00$^{C}$ | 7.00±0.00$^{C}$ | 7.00±0.00$^{C}$ | 6.66±0.57$^{C}$ |
| **LSD** | | 0.0814 | 0.236 | 0.642 | 0.587 |
| | **Fresh** | 92.05±1.61$^{A}$ | 92.26±1.41$^{A}$ | 90.54±3.51$^{A}$ | 91.40±1.44$^{A}$ |
| **Total** | **7** | 89.00±1.76$^{AB}$ | 90.33±1.52$^{A}$ | 88.83±2.85$^{A}$ | 90.00±1.00$^{A}$ |
| **(100 points)** | **14** | 86.00±2.10$^{B}$ | 85.00±2.64$^{B}$ | 84.66±2.51$^{A}$ | 85.33±1.52$^{B}$ |
| | **21** | 68.66±2.18$^{C}$ | 69.00±2.64$^{C}$ | 67.00±3.20$^{B}$ | 68.33±0.57$^{C}$ |
| **LSD** | | 4.227 | 4.789 | 6.787 | 2.198 |

KG-C= kefir made from cow milk by kefir grains, KS-C= kefir made from cow milk by natural kefir starter, KG-B= kefir made from buffalo milk by kefir grains, KS-B= kefir made from buffalo milk by natural kefir starter. Means of three replications ± standard deviation. $^{ABCD}$ Letters indicate significant differences between storage times ($P<0.05$)

the best flavour and appearance, with a mean score of 42.93 and 9.00 respectively. The KG-C and KS-C samples had mean texture scores of 32.14 and 31.33, KG-B and KS-B samples had mean texture scores of 31.66 and 31.66 points in fresh, respectively. The sample KS-C had the highest acidity score, while the lowest value was remarked in the KG-B, Statistically, after 21days KS-C had the greatest total (69.00) while KG-C came in the second order (68.66) the third was KS-B (68.33). then KG-B (67.00). Studying two kefir samples over a 5-day storage period, Kilic *et al.* (1999) found that the scores of all the sensory attributes decreased significantly with time.

## Conclusion

Kefir was made from cow's and buffalo's milk under Egyptian conditions. Two methods were used of the manufactured, the first was using kefir grains and the second was using natural kefir starter. Comparison study between the two milks showed no difference between the two methods except for ethanol. The type of milk used in kefir production affected microbiological characteristics and rheologlogical properties of the kefir samples. The highest ethanol content and yeast growth was detected in KG-B at the end of storage period. The microbiological and chemical composition of kefir provide a complex probiotic effect due to the inherent lactic acid bacteria and yeast. The present results refer to the possibility of producing milk kefir contains very limited alcohol content with good physicochemical, microbial properties as well as high acceptance rate. Kefir production from cow milk using natural kefir starter culture may be suitable or preferred under Egyptian conditions because it had the lowest ethanol, although Buffalo's milk kefir had the best quality but contained slightly higher alcohol than cow's milk Kefir. Comparison overview, likeness of chemical properties was very clear between kefir produced by kefir grains and kefir produced by KS in fresh samples or during storage. It could be concluded, from these results, that milk kefir can be produced either using kefir grains or KS where the final product is highly similar.

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## إنتـــاج الكيفيـــر مـــن اللبـــن البقـــري والجامـــوســـــى تحـــت الظـــــروف المصـــــــرية

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يهدف هذا العمل الى دراسة تقييم نمو ونشاط مزارع كيفير الطبيعية ومزارع بادئات حبوب الكيفير في تخمر اللبن البقري والجاموسى لإنتاج الكيفير، تم تقييم عدد الخلايا الحية فى مزارع كيفير الطبيعية ومزارع بادئات حبوب الكيفير، والخصائص الفيزيائية والكيميائية، واللزوجة، وتركيز الإيثانول والتقييم الحسي للعينات أثناء التخزين على ٥ ± ١°م لمدة ٢١ يوما، أظهرت النتائج أن الكيفير المصنع من اللبن الجاموسى باستخدام حبوب الكيفير (KG-B) أفضل في بقاء عدد الخلايا الحية، ومحتوى الإيثانول، واللزوجة وانخفاض في الخواص الحسية في حين ان عينات المقارنة كانت أفضل فى الخصائص الحسية وحظت عينات الكيفير المصنوعة من اللبن البقري الطبيعي عن طريق مزارع الكيفير الطبيعية (KS-C) على أفضل درجات التقييم الحسى، يليها الكيفير المصنع من اللبن البقرى باستخدام حبوب الكيفير(KG-C) على التوالي، تبين من الدراسة أن إنتاج الكفير من حليب الأبقار باستخدام مزارع كفير الطبيعية يكون المفضل في ظل الظروف المصرية، ونستخلص من هذه النتائج أن الكيفير يمكن أن ينتج إما باستخدام حبوب الكيفير أو مزارع الكيفير الطبيعية حيث أن المنتج النهائي كان أقل تركيز من الايثانول ويقبله المستهلك المصري.

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