

## OPTIMIZATION OF MILK TYPE AND PHYSICAL FACTORS FOR REDUCTION OF ALCOHOL CONTENT IN KEFIR

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### ABSTRACT

Milk kefir beverage has enormous health benefits. However, alcohol content in kefir may represent a barrier for some consumers, so this work attempts to reduce its alcohol content. Three main factors, i.e., the type of milk, inoculum size and incubation temperature were investigated. Results showed that cow's milk inoculated with 2% kefir grains (KG) and incubated at 24°C for 48h had the lowest ethanol concentration (EC), however, ewe's milk and soymilk contained the highest EC. According to EC in the previous experiment, buffalo's, cow's and goat's milk were selected to prepare milk kefir. Cow's milk kefir (CMK) had the lowest EC (0.062± 0.041), while, goat's milk kefir (GMK) had the highest EC (0.093±0.021) at zero time. Moreover, buffalo's milk kefir (BMK) had the highest pH, total solids, acetaldehyde, lactose and viscosity. Statistically, after 10 d BMK won the greatest Overall quality (5.825) while CMK came in the second place (5.355) then GMK (5.310).

**Keywords:** kefir, ethanol content, buffalo's, cow's, goat's, ewe's milk, soy milk.

### INTRODUCTION

Kefir is of Caucasus origin, being made in Mountains of Russia. It has been credited with various health promoting properties (Liu et al., 2005). This fermented milk beverage is a result of microbial action of a wide combination of lactic and acetic acid bacteria in addition to various yeasts exist in gelatinous white or yellow particles known as “kefir grains” that can ferment mammal's milk, milk substitutes and other sugary liquids. The attributes of kefir are creamy consistency, viscous, a slightly acidic taste and some effervescence, the final product contain lactic and acetic acid, acetaldehyde, diacetyl, acetoin, ethanol (< 2%), carbon dioxide and free fatty acids (Leite et al., 2013a).

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 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). Despite the enormous health benefits of kefir, the presence of alcohol is an obstacle to use it in Arab countries because of the traditions and religion habits that deplore the presence of alcohol in food and drinks.

Hence the main goal of this work was to study certain nutritional and physical factors affecting reducing alcohol content in kefir for making milk kefir beverage with as little as possible of alcohol with retaining nutritional and healthy benefits of kefir.

### MATERIALS AND METHODS

Fresh whole buffalo's, cow's, ewe's and goat's milk were obtained from El-Serw experimental station, Animal Production Research Institute, Agricultural Research Center, Egypt. Soybeans (Giza 111) were obtained from Tagelez Research Station, Crops Research Institute, Agricultural Research Center, Egypt. Soy milk was prepared according to Kasenkas et al. (2011) with slight modifications. The soybeans seeds were washed and soaked overnight in distilled water. After decanting the water, the soaked soybeans were mixed with 3 times of their weight of distilled water, blended and filtered. All milks used in this study were analyzed for gross chemical composition as indicated in (Table, 1).

**Table 1: Gross chemical composition of used milks.**

Type of milk	Ingredient, %					
	Fat	Lactose	Protein	TS	Acidity	pH
Buffalo	5.7	4.46	4.42	15.38	0.16	6.51
Cow	3.9	4.37	3.59	12.66	0.17	6.68
Ewe	6.3	5.61	4.7	17.39	0.17	6.53
Goat	3.8	4.46	3.28	12.32	0.15	6.57
Soy	2.18	2.91 (As carbohydrate)	3.53	10.3	0.12	6.74
Skimmed	0.2	4.8	4.6	10.5	Not measured	Not measured

Kefir grains were kindly provided by the Department of Food Engineering, Suleyman Demirel University, Isparta, Turkey. For activation, sterilized skim milk was inoculated with fresh washed kefir grains (with sterile water) at level of 5% w/v, then incubated at 25 °C for 24 h and the medium was exchanged daily, this process being necessary to maintain the grains' viability.

For fermenting and detecting of kefir ethanol content, 50 ml of each milk type (standardized 3% fat) was sterilized (121 °C for 15 min), cooled to 25 °C and individually inoculated with active KG (2 or 4%) mixed well, and one ml of each was added in test tube (15×125mm). The tubes were hung in airtight 250-ml flasks containing 25 ml of potassium dichromate (33.768 g of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> dissolved in 400 ml of distilled water with 325 ml of sulfuric acid and volume raised to 1L) solution that acted as recipient for the formed alcohol. All flasks were incubated at 24 or 28 °C for 48 hours.

Alcohol was determined in the fermented milks according to Caputi et al. (1968) with slight modification. Briefly, after incubation, every test tube was removed then 30 ml of distilled water was added on the inner wall of flask and mixed well with K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, about 20 ml of distillate was collected from each sample and the flasks were kept in a water bath at 62.5 °C for 20 minutes, cooled to room temperature and the volume was raised to 50 ml., the optical density was spectrophotometrically measured at 600 nm, the data were calibrated by various standard curves made from ethanol for each condition.

For examining milk type, temperature and time of incubation vs KG biomass, 25ml of each type of milks was sterilized (121°C for 15 min) and inoculated with 2% KG (A). The inoculated flasks were incubated at 24 or 28 °C for 24 or 48 h. After each experiment, all formed KG were separated by a narrow colander, washed and left in air for 5 min then weighted (B). The variation in biomass (BM %) was calculated as follows;  $BM\% = (B-A)/A \times 100$ .

The KG grown in various milks were separated under sanitization condition, two slices from each grain were stained using Safranin simple staining. The microbial structure of the KG were light microscopically examined using oil-immersion lens.

For making of kefir, milks (standardized 3% fat), heated to 95 °C for 15 min and immediately cooled to 23 °C then, inoculated with active KG (2% w/v) and incubated at 24 °C until pH fall to ~4.7. After

incubation, the grains were separated by a narrow colander from kefir beverages. The Kefir samples were chemically, microbiologically and organoleptically analyzed at the zero, 7<sup>th</sup> and 15<sup>th</sup> day of storage period. The trials were performed in triplicates. Milk protein, milk fat, pH, acidity (TA), total solids (TS), lactose (L) of milk kefir beverages were determined according to AOAC (2003). Soymilk fat was determined by method described by Pearson (1981). Acetaldehyde (A) was measured (mg/L) using a Shimadzu (240 UV-vis) spectrophotometer (Japan) as described by Lees and Jago (1970), alcohol content (EC) was determined using spectrophotometer according to Caputi et al., (1968) with some modifications. To measure viscosity (V) of Kefir, each sample (100 mL) was placed in a viscometer (LVDV11+P, Brookfield, USA) and the viscosity (cPs) was measured between 5 to 8 min with a one minute period at 12 rpm.

Yeast counts were enumerated according to Van der Walt and Yarrow (2009), ten grams of each sample was taken, diluted in 90 ml of sterile solution of 2% (w/v) sodium citrate and homogenized in a Stomacher for 30 s. to obtain tenfold dilutions. yeast were determined by surface plating on yeast potato dextrose agar (PDA) with 0.01% of chloramphenicol, after incubated at 25 °C for 3 days.

Lactic acid bacteria (LAB) were determined on De Man, Rogosa (MRS) agar and incubated at 37 °C for 48h.

For the sensory analysis, ten of dairy science specialists, who received an explanation of the goal of test, the evaluation methods used and the test parameters. The sensory properties included appearance, flavor & odor, sourness, texture and overall quality. The score ranges were from 1 (poor) to 7 (excellent). The samples were presented in a triplicate cups in random order and coded with three digit random numbers. The organoleptic properties of fresh obtained kefir samples and after 10 days were evaluated.

## RESULTS AND DISCUSSION

Alcohol content in kefir is the critical point in this work, Three main factors that are expected to play an important role to reduce kefir alcohol content were studied, i.e. the type of milk, inoculum size and incubation temperature. Data presented in Table 2 show the ethanol concentration % (EC) as a result of the previous mentioned factors.

**Table 2: Effect of milk type, inoculum size and temperature on kefir alcohol content.**

Inoculum, %	The type of milk	Ethanol, % ±2SD	
		Incubation at 24 °C	Incubation at 28 °C
2	Buffalo	0.180±0.035	0.194±0.061
	Cow	0.125±0.045	0.181±0.040
	Goat	0.207±0.068	0.221±0.040
	Ewe	0.222±0.045	0.254±0.061
	Soy	0.252±0.093	0.308±0.046
4	Buffalo	0.187±0.068	0.207±0.046
	Cow	0.155±0.093	0.214±0.061
	Goat	0.207±0.093	0.254±0.023
	Ewe	0.244±0.090	0.294±0.061
	Soy	0.267±0.162	0.361±0.040

In general, all factors studied affected on kefir ethanol content. Regarding the type of milk, Cow's milk inoculated with 2% KG and incubated at 24°C for 48 h contained the lowest EC (0.125%), followed by buffalo's milk (0.180%). On the other hand, increasing the incubation temperature from 24 °C to 28°C at the same inoculum size (2%) resulted in to slight increase in the ethanol content of kefir. However, an obvious difference was observed at 4% inoculum size when rising incubation temperature from 24 °C to 28 °C. Therefore, the type of milk as a growth medium, the incubation temperature and inoculum size were influential factors on the ethanol content of kefir.

kefiran plays an important role in the kefir quality (slimy and gummy) as well as the therapeutic effects. It has a great role in KG biomass (BM), in this respect, three important factors were investigated, i.e. the type of milk, incubation temperature and incubation time. Data listed in Table 3 indicate that BM% directly affected by the type of milk, after 24 h of incubation at 24 °C, buffalo's and ewe's milk produced the highest increase in BM% (34), soy milk (30) then cow's and goat's milk (24) came next. With the prolongation of incubation time up to 48 h, lower BM% were recorded by 28, 20, 30, 20 and 26 % for buffalo's, cow's, ewe's, goat's milk and soy milk, respectively. Also, raising the incubation temperature resulted in a decline in BM%

significantly, whither for 24 or 48h, but prolong the incubation period to 48h at 28 °C dropped BM% sharply. Hence, 24°C for 24 h were the most suitable conditions to produce maximum biomass.

Milk type and its chemical composition (proteins, lipids, carbohydrates, minerals and vitamins), impacted on KG biomass (Table 3), leading to variation in the resulted KG biomass. In this respect, Pop et al., (2014) reported that biomass of KG greatly affected by the type of milk, and the organic skim milk incubated at 25°C for 24 h with a rotation rate of 125 rpm were the optimal conditions for produce the highest KG biomass. These results might be attributed to the kind of substrates, necessary for the growth of KG. Surely, the incubation temperature at 28 °C affected negatively on BM%, and positively on EC% more than 24°C. This means that at high incubation temperature the milk compounds are consumed by KG microorganisms for the formation of alcohol rather than KG kefiran. Additionally, casein is also important factor that reported to increase KG biomass by its positive effect on kefiran which increased by rising casein in buffalo's and ewe's milk, according to Wang and Bi (2008) who confirmed that biomass include kefiran reached the maximum value in the presence of casein.

**Table 3: Effect of milk type, incubation time and temperature on the biomass of Kefir grains.**

The type of milk	Incubation at 24 °C				Incubation at 28 °C			
	24 h		48 h		24 h		48 h	
	BM, gm	BM, %	BM, gm	BM, %	BM, gm	BM, %	BM, gm	BM, %
Buffalo	0.67	34	0.64	28	0.64	28	0.61	22
Cow	0.62	24	0.60	20	0.59	18	0.57	14
Ewe	0.67	34	0.65	30	0.65	30	0.62	24
Goat	0.62	24	0.60	20	0.60	20	0.58	16
Soy	0.65	30	0.63	26	0.64	28	0.61	22

Because of lack of clarity in the relationship between EC and biomass, the microbial community of KG were microscopically examined. All KG contained diversity of microbes, the microbial structure of KG grown in buffalo's milk contained lower load of microorganisms, they were short and long rods, single or double cocci, about three yeast cells/field and small-sized masses of granules. The KG of cow's milk composed from 30-50 cells of yeasts/field in various budding state in masses, various di-, tri- and streptococci, short and long rods in equal numbers. KG grown in ewe's milk showed three cells of yeasts/field, di- and tri-cocci, short and long rods, some masses of granules could also be seen. Very low yeast cells (about two/field), single- or di- cocci, rods (short and long), beside some masses of granules appeared in KG grown in goat's milk. Finally, nearly 5 yeasts cell/ field, single- or di- cocci, rods (single, di, chain short and long) beside some masses of granules could be seen in the film of KG cultivated in soy milk, its grains seemed to be more fragmented.

The increment of yeast counts in case cow's milk may be due to that outer grain portions is suitable for

yeasts, unlike bacteria which often located in the inner grain portions, our observation is in consistent with the notes of Leite et al., (2013b).

Generally, Kefir composition is not uniform and not well described (Otlés and Cagindi, 2003). Fermented milks usually are stored at low temperatures to prolong the validity period and maintaining its quality. Measurement of pH and TA of fermented milks is important to determine the quality. The changes of pH and TA during the storage are shown in Table 4. The initial pH values were 4.62, 4.58 and 4.66 for BMK, CMK and GMK, respectively. Gradual decreases were observed to reach 4.48, 4.51 and 4.51 after 15 d of cold storage. Chemically, TA occupied an opposite trend. 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 yogurt (Kang et al., 2013). These results for kefir are in agreement with the findings of Yoo et al., (2013).

Normally, TS kefir content affected by the TS of milks, so BMK had the highest TS followed CMK then GMK. Data shown in Table 4 reveal that TS of Kefir samples compatible with TS of milks and gradually increased along storage. Kefir contains 10.6% - 14.9% TS (Wszolek et al., (2001), while Magalhaes et al., (2011a) confirmed that Brazilian Kefir contain 9.62% dry matter after a day of keeping. The increment of TS may be due to declining of moisture content during storage.

Lactose concentration in fermented dairy products is strongly related to pH and acidity due to the activity of microorganism, which have the ability to metabolize lactose as energy source. Initial lactose quantities were 3.45, 3.35, and 3.34, then gradually decreased to reach 3.22, 3.15 and 3.16 after 15 day of cold storage for BMK, CMK and GMK, respectively.

Nutty and pungent aromas are usually detected in fermented dairy products; acetaldehyde is responsible for that aroma. Experimental kefir samples contained limited levels of acetaldehyde (Table 4); initial quantities were 7.7, 6.8 and 6.6 mg/L, gradually increased to reach 9.4, 9.1 and 8.73 mg/L after 7 d then decreased to reach 8.83, 8.7 and 8.2 for BMK, CMK and GMK, 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 GüzelSeydim, 2010). Acetaldehyde is considered the major yogurt or fermented milks flavor. 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  $\alpha$ -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 and Geroyiannaki et al. (2007).

Change of viscosity during storage (Table 4) in the different kefir fermented milks was measured along the storage period. The viscosity affected the palatability of fermented milks so it is an important factor in quality of yogurt and kefir. BMK had the highest viscosity (1510 cPs) followed by CMK (1390 cPs), while GMK had the lowest viscosity (1270 cPs). The viscosity of all samples tended to increase slightly at the end of the storage period to reach 1612, 1472 and 1360 cPs after 15 d of cold storage. Similar results were reported by Yoo et al., (2013), who made kefir by two-step fermentation. The total solids content of the yogurt mixture, the degree of hydrolysis of proteins, the slime-producing capacity and acid producing capacity of the strain are important factors affect the viscosity of fermented milks (Tamime and Robinson, 1999) as well casein micelles and fat globules most affect the viscosity of milk. The previous factors that increase the viscosity maybe explain our results. Surely, polysaccharides, Mucoïd substances, kefiran produced lactic acid bacteria and other microbes increase the kefir viscosity. Casein also plays an important role in the increment of viscosity.

**Table 4: Physicochemical properties of experimental kefir samples  $\pm 2SD$ .**

Item	Storage period (days)								
	BMK			CMK			GMK		
	0	7	15	0	7	15	0	7	15
pH	4.62 $\pm$ 0.08	4.51 $\pm$ 0.05	4.48 $\pm$ 0.04	4.58 $\pm$ 0.31	4.56 $\pm$ 0.09	4.51 $\pm$ 0.05	4.66 $\pm$ 0.05	4.56 $\pm$ 0.07	4.51 $\pm$ 0.06
TA	0.80 $\pm$ 0.04	0.85 $\pm$ 0.07	0.91 $\pm$ 0.04	0.80 $\pm$ 0.16	0.82 $\pm$ 0.05	0.88 $\pm$ 0.04	0.76 $\pm$ 0.07	0.83 $\pm$ 0.06	0.88 $\pm$ 0.07
TS	16.25 $\pm$ 0.03	16.42 $\pm$ 0.03	16.54 $\pm$ 0.04	13.15 $\pm$ 0.01	13.24 $\pm$ 0.03	13.38 $\pm$ 0.02	12.27 $\pm$ 0.04	12.42 $\pm$ 0.03	12.55 $\pm$ 0.02
L	3.45 $\pm$ 0.43	3.35 $\pm$ 0.37	3.22 $\pm$ 0.05	3.35 $\pm$ 0.06	3.23 $\pm$ 0.07	3.15 $\pm$ 0.14	3.34 $\pm$ 0.08	3.30 $\pm$ 0.04	3.16 $\pm$ 0.19
A	7.7 $\pm$ 0.92	9.4 $\pm$ 1.22	8.83 $\pm$ 1.30	6.80 $\pm$ 0.92	9.1 $\pm$ 0.80	8.7 $\pm$ 0.92	6.6 $\pm$ 1.11	8.73 $\pm$ 0.9	8.2 $\pm$ 1.44
EC	0.082 $\pm$ 0.041	0.134 $\pm$ 0.021	0.168 $\pm$ 0.043	0.062 $\pm$ 0.041	0.103 $\pm$ 0.041	0.137 $\pm$ 0.024	0.093 $\pm$ 0.021	0.156 $\pm$ 0.021	0.206 $\pm$ 0.041
V	1517 $\pm$ 13.1	1550 $\pm$ 15.0	1614 $\pm$ 5.3	1389 $\pm$ 8.1	1435 $\pm$ 8.3	1475 $\pm$ 6.0	1273 $\pm$ 6.1	1322 $\pm$ 4.0	1362 $\pm$ 4.0

TA: Titratable acidity, TS: total solids, L: lactose, A: Acetaldehyde, EC: ethanol, V: Viscosity. BMK: buffalo's milk kefir, CMK: cow's milk kefir, GMK: goat's milk kefir

Cow's, buffalo's and goat's milk kefir showed the lowest level of ethanol content, particularly when inoculated with 2% inoculum at 24 °C; therefore, these treatments were chosen to produce milk kefir beverages. EC of the kefir samples (BMK, CMK and GMK) during storage is shown in (Table 4). The fresh CMK had the lowest EC (0.062) followed by BMK (0.082) then GMK (0.093), these concentrations tended to increase slightly during the storage period to reach the maximum levels, being 0.137, 0.168 and 0.206% after 15 d of cold storage, respectively. Typically, Kefir contains 1.0% alcohol, comparing to other studies, the final ethanol concentrations were 8.7  $\pm$  1.6 g/l, 8.3  $\pm$  0.2 g/l and 7.8  $\pm$  0.3 g/L for milk kefir, cheese whey kefir and de-proteinised cheese whey kefir, respectively (Magalhães et al., 2011b). Kefir products fermented by a conventional method contained a high concentration of

alcohol, starting from 1.3% and up to 1.36% (Sarkar, 2007 and Yoo et al., 2013). 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<sub>2</sub> produced during the fermentation of kefir depend on the production conditions. Moreover, concentration of ethanol in fresh yogurt samples ranged 1.38 - 4.61 ppm and increased to reach 3.17-8.88 and 4.26-8.75 ppm after 10 d at 4°C and 20 °C, respectively (Hruskar and Milana Ritz, 1995 and Farnworth, 2005)

Yeast and lactobacilli are mutually dependent and grow in balanced proportions in kefir grains, and symbiosis between yeast, lactobacilli and streptococci

were observed during the production of kefir (Sarkar, 2008). Data presented in (Table 5) show the changes in LAB counts in the experimental kefir samples during cold storage. In fresh samples, LAB recorded 9.13, 8.82 and 9.20 log cfu/mL, these numbers slightly increased to reach 9.19, 9.01 and 9.28 log cfu/mL after 7d, then limitedly decreased to become 9.17, 8.88 and 9.18 on the fifteenth day of storage of BMK, CMK and GMK, respectively. The differences of these counts

might be attributed to the type of milk and their available nutrients. LAB did not change in the first 9 d of storage, but increased slightly afterwards Leite et al. (2013b); 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).

**Table 5: Lactic acid bacteria and yeast counts during cold storage of buffalo's, cow's and goat's milk kefir.**

Microorganism	Counts (Log CFU/ml) $\pm$ 2SD during storage								
	BMK			CMK			GMK		
	0	7	15	0	7	15	0	7	15
LAB	9.13 $\pm$ 0.031	9.19 $\pm$ 0.04	9.17 $\pm$ 0.69	8.82 $\pm$ 0.061	9.01 $\pm$ 0.07	8.88 $\pm$ 0.07	9.20 $\pm$ 0.061	9.28 $\pm$ 0.06	9.18 $\pm$ 0.06
Yeasts	4.31 $\pm$ 0.042	4.61 $\pm$ 0.08	4.82 $\pm$ 0.05	5.18 $\pm$ 0.061	5.38 $\pm$ 0.05	5.54 $\pm$ 0.031	4.46 $\pm$ 0.092	4.74 $\pm$ 0.092	4.91 $\pm$ 0.061

Yeast counts in experimental kefir samples were 4.31, 5.18 and 4.46 log cfu/mL increased gradually to record 4.82, 5.54 and 4.91 at the end of cold storage for BMK, CMK and GMK, respectively. This variation due to contrast milk composition, kefir microflora composition 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 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).

Table 6 shows the results of descriptive sensory analysis of BMK, CMK and GMK. Major factors determining the quality of kefir samples are generally appearance, flavor & odor taste, texture, and overall

acceptability. Generally, the evaluation degrees gradually slightly increased during storage. BMK was rated favorably in all sensory parameters to achieve the maximum overall quality at zero (5.290) and after 10 d (5.825). In spite of cow's milk Kefir was the lowest in appearance; it achieved the second place in overall quality, this perhaps because of increase the other sensory parameters (flavor or odor, sourness and texture). The important note was the disappearance the alcoholic odor, and yogurt aroma which is dominant in the most samples. Raising clean acidity (lactic acid), increase acetaldehyde and viscosity, all these attributes led to high quality, hence increase acceptance rates in all the samples. In spite of the lower evaluation of GMK compared to CMK and BMK, it gains the acceptance. This evaluation nearly harmonized sensory evaluation reported by Yoo et al., (2013).

**Table 6: the sensory evaluation of fresh experimental kefir samples and after 10 days.**

Kefir samples	The time of evaluation(d)	Sensory evaluation $\pm$ 2SD					Overall quality
		Appearance	Flavor or odor	sourness	texture		
BMK	0	5.70 $\pm$ 0.40	5.57 $\pm$ 0.70	4.55 $\pm$ 0.46	5.34 $\pm$ 0.46	5.290	
	10	6.22 $\pm$ 0.74	5.90 $\pm$ 0.40	5.59 $\pm$ 0.48	5.59 $\pm$ 0.48	5.825	
CMK	0	5.32 $\pm$ 0.35	5.43 $\pm$ 0.51	4.35 $\pm$ 0.59	4.35 $\pm$ 0.59	4.863	
	10	5.64 $\pm$ 0.43	5.75 $\pm$ 0.27	4.71 $\pm$ 0.29	5.32 $\pm$ 0.24	5.355	
GMK	0	5.53 $\pm$ 0.54	5.21 $\pm$ 0.54	4.40 $\pm$ 0.52	4.75 $\pm$ 0.19	4.973	
	10	5.77 $\pm$ 0.31	5.60 $\pm$ 0.52	4.61 $\pm$ 0.34	5.26 $\pm$ 0.27	5.310	

The range of each parameter is 1-7

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 rates. Cow's milk inoculated with 2% KG and incubated at 24°C until pH fall to ~4.7 is suitable for controlling kefir alcohol content. Buffalo's milk kefir had the best quality but contained slightly higher alcohol than cow's milk Kefir.

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### التحسين من نوع اللبن والعوامل الفيزيائية للحد من محتوى الكفير من الكحول.

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برغم الفوائد الصحية الكثيرة لمشروب لبن الكفير، إلا أن وجود نسبة كحول به تتثنى كثير من المستهلكين عن تناوله، لذا هدفت هذا البحث لدراسة تحسين نوع اللبن وبعض العوامل الفيزيائية للحد من محتوى الكحول فى الكفير. أظهرت النتائج أن اللبن البقرى المُلَفَّح بـ ٢% حبيبات الكفير والمُحَضَّن على ٢٤ م لمدة ٤٨ ساعة احتوى أقل نسبة إيثانول عكس لبن الصويا ولبن النعاج اللذان احتويا أعلى نسبة إيثانول. لذا تم اختيار اللبن البقرى والجاموسى ولبن الماعز لإنتاج لبن الكفير. احتوى كفير اللبن البقرى الطازج أقل نسبة إيثانول (٠.٠٦٢%) ونال المرتبة الثانية حسيًا، أما كفير اللبن الجاموسى ورغم احتوائه على (٠.٠٩٣%) إيثانول إلا إنه نال أعلى درجات التقييم الحسى.