Journal of Home Economics

Volume 25, Number (3), 2015

Journal of Home Economics

http://homeEcon.menofia.edu.eg

ISSN 1110-2578

Nutritional Characterizations of Kefir Milk

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Abstract

Kefir is a cultured (fermented) milk drink. The beneficial bacteria and yeast is rich in amino acids, fatty acids, vitamins, minerals, enzymes, vitamin and minerals. The current study was designed to evaluate the nutritional value of kefir milk made from defatted cow milk, by determine chemical, physicochemical, amino acids, fatty acids, minerals contents and microorganisms. The results showed that the moisture, protein, fat, ash, lactose and energy value were 88.10 %, 4.71%, 1.96%, 0.70%, 4.53% and 54.60 kcal/g, respectively. The values of total solids, pH, titratable acidity, viscosity and total phenolic contents were 2.9%, 4.03, 0.88% as lactic acid, 9.4 mpa.s and 199.1 µg/ml GAE, respectively. The highest minerals content of kefir milk recorded with potassium, calcium and magnesium, while the lowest minerals content of kefir milk recorded with copper, ferric, and zinc. Kefir milk contains different amounts of *Lactococci* bacteria counts, *Lactobacili* bacteria counts and yeast counts. The highest amino acids composition of kefir milk recorded with glutamic acid, proline and lysine, while the lowest amino acids were valine, glycine and histidine. The major fatty acids in kefir milk were palmitic acid, stearic acid and myristic acid. kefir milk lipids have a higher content of monounsaturated fatty acids, similarly as for medium chain triglycerides. From obtained results it could be concluded that kefir milk has high nutritional value and good source of amino acid and fatty acid especially unsaturated and minerals content.

Key words: Kefir milk, Chemical composition, Physical properties and Nutritional value

Introduction

Traditional foods have been consumed locally or regionally for many generations around the world. They have been produced for ancient times and fermented foods had longer shelf -life and improved nutritional values compared to their unfermented equivalents **(European Commission, 2007; Euro FIR, 2009)**.

The name kefir is derived from the Turkish word keyif meaning "good food". In addition to this designation, kefir is also known by several other names, such as kefer, kefyr, kephir, kepi, kiaphur, kipi, and knapon **(Yerlikaya, 2014)**.

The most important fermented food products are fermented milk products which are dairy foods that have been fermented with yeast and lactic acid bacteria such as *Lactobacillus ssp.*, *Lactococcus ssp*. and *Leuconostoc* ssp. Fermenting milk provides many advantages to food. These advantages are the extension of shelf -life of products, improvement in taste and digestibility of products **(Rasic and Kurmann, 1978 and Pederson, 1979)**.

Kefir is one of the important fermented milk product which was originated in central Asia between the Caucasus Mountains and Mongolia, and is very popular in many countries nowadays, such as Turkey, Russia, Poland, Czech Republic, Slovakia, Hungary, Bulgaria, and Scandinavian countries, The United States, Brazil and Japan **(Dzwolak and Ziajka, 2000;Grønnevik *et al.*, 2011)**.

Kefir is a good source of many compounds that show antioxidant activity with their vitamins, phenolic acids, flavonoids, and sterols. Kefir milk also contains high percentage of fiber, vitamins A, D, E and B1, minerals such as calcium, potassium, sodium, magnesium and iron. This composition of milk provides more functionality to food such as improving beneficial effects for digestive system and preventing against colon cancer and helping to maintain an optimal weight due to high fiber content. Also kefir milk exhibits cholesterol and lipid-lowering effects **(Murphy *et al.*, 2004)**.

Kefir drink is a popular natural probiotic beverage in the Middle East. It is the fermentation product of a variety of probiotic microorganisms live microorganisms added to the diet in sufficient quantities to improve the health condition present in the kefir grain of the milk **(Hertzler and Clancy, 2003)**.

Otles *et al.*, (2003) mentioned that several studies suggest beneficial health effects of kefir grains, including antitumor, antimicrobial, antioxidant, immune-modulatory, anti-inflammatory and gastrointestinal regulatory activities.

The main mechanisms of action of probiotics include: an improved intestinal mucosal barrier increased adhesion to the intestinal lumen interface and concomitant inhibition of the adhesion of pathogens competition with pathogenic microorganisms for binding sites, nutrients, and colonization production of antimicrobial metabolites including the synthesis of bacteriocins, hydrogen peroxide(H_2O_2), and organic acids such as lactic acid responsible for the acidification of the environment promotion of innate and adaptive immune responses and many other unknown modes of action **(Ganguli *et al.*, 2013)**.

Gaware *et al.*, (2011) reported that kefir is a cultured (fermented) milk drink. It is a complex symbiosis of more than 30 microflora that form grains or cauliflower-like structures in the milk. In addition to beneficial bacteria and yeast it is rich in amino acids, vitamins, minerals, amino acids and enzymes. Particularly, containing calcium, phosphorus, magnesium, B2, B12, vitamin K, vitamin A, and vitamin D. It also has numerous antioxidant and therapeutic properties. In the present review we have discussed the origin, production, consumption and the health benefits of kefir.

Saad *et al.*, (2013) reported that foods presenting these microorganisms (kefir) may be considered functional foods, i.e., besides nourishing the body, these products have biologically active components that contribute to the maintenance of good health and wellness, while reducing the risk of diseases.

Materials And Methods

Materials

Source of kefir milk:

Live organic milk kefir grain was obtained in 2015 from Amazon Company, USA.

The chemicals and chemical kits

Solvents used were in an analytical reagent grade and purchased from Merck (Darmstadt, Germany).

Methods

Production of kefir milk

Kefir produced according to the method described by **(Otles and Cagindi, 2003)** as follow:

Suspension of kefir grains (50 g of kefir grains added to a broth of 1000 mL of defatted milk containing 3% of sugar) were incubated into glass flasks at 25°C during 15 days. After that, samples of this cultivated kefir were subdivided and incubated for 24 hours at room temperature. Flasks were not sealed to allow fermentation gas release. After 24 h fermentation, the suspension was carefully filtered in sterile quantitative (fast filtration) filter paper. This procedure was repeated each 24 hours, until the end of the experiment.

Analytical methods

Moisture content

The moisture, total nitrogen, fat and ash content while, carbohydrate was calculated by the difference as follows:

% Carbohydrates = 100 - (% moisture + % protein + % fat + % ash + % fiber).

were determined according to the method recommended by **A.O.A.C. (2005)**.

Energy value

Total calories were calculated by multiplying 1g protein and carbohydrates by 0.4 and 1g fat by 9.0 according to **FAO (1982)**.

Determination of total solids, titratable acidity and viscosity

Total solids (%), titratable acidity (%), and viscosity were determined as per standard procedures described by **Ranganna (2002)**. The viscosity of the sample was measured using a digital viscometer (model no. R 1:3M, Rheological Int.). The spindle number 3 was connected to the digital viscometer and its rotation was fixed at 100 rpm for 30 seconds. The viscosity of the sample was expressed as mPas.

pH measurement:

The pH value was measured using a pH meter of a glass electrode. The pH meter was allowed to stabilize for one minute and then the pH of the samples was directly reported according to the official method of analysis **(A.O.A.C., 1984).**

Determination of total phenolic content

Total phenolics in the selected extract samples were determined according to Mazza's method **(Mazza *et al*., 1999)**, with some modifications as described by **Radovanović and Radovanović,(2010)**. Briefly, 0.25 ml of the diluted sample was mixed with 0.25 ml of 0.1% Hcl in 95% ethanol and 4.55 ml of 2% HCl, approximately 15 min before reading the absorbance at 280 nm with a UV/ VIS spectrophotometer (Agilent 8453 spectrophotometer). The absorbance at 280 nm, A, was used to estimate total phenolics (gallic acid was used as standard).

Determination of minerals contents

The Atomic Absorption Spectrophotometry as described by **(Okwu & Ndu, 2006 and Odom *et al.,* 2013)** was used in the mineral analysis, magnesium and calcium was determined by complex ometric titration described by **(James, 1995 and Shimoyamada *et al.,* 1998)** whereas Potassium was by flame photometry explained in **(A.O.A.C. 1990)**.

Determination of Lactococci and Lactobacili bacteria counts:

MRS culture medium was used as method described by **(Oxoid Manual 1979).** While**,** malt – yeast extract agar medium used for determined mould and yeast according to method **(ICMSF, 1996)**.

Determination of amino acids composition

Amino acids were determined by HPLC (Knauer) according to **Marino *et al.,* (2010)** with some modification. Sample preparation: 5 g sample was weighed and put into a tube and then covered. Hydrochloric acid 6N was added as much as 10 mL and vortex until homogen. Hydrolysis of sample by using autoclave at 110°C for 8 h. Sample was cooled at room temperature and neutralized by NaOH 6 N. Solution of 40% Pb (CH3COO) 4 and 15% oxalic acid were added and put in vial and diluted with aquadest until 50 ml. Three ml of sample was taken and filtered with filter 0.45 µm. Sample was diluted by 10x and incubated for 3 minutes in o-phthaldehyde (OPA). Sample was injected as much as 30 µL to HPLC.

Determination of fatty acids

Fatty acid profile of kefir milk made from caw milk was analyzed by Gas Chromatography. This procedure is based on AOAC Official Method 969.33/963.22 **(A.O.A.C., 2000)** and analytical method validation according to **Gonzalez and Herrador (2007)**. Sample preparation: 350 mg sample was placed into 50 mlvolumetric flask and added 6 ml metanolic NaOH 0.5 M and boiling stones. Cooler was connected and reflux until removed of oil globule (5-10 min) and added 7 ml of boron trifluoride (BF3) through cooler and continue to reflux for 2 h. Heptana 2-5 ml was added through cooler and reflux for 1 min. Heater and cooler was removed and then added 15 ml saturated Nacl and shaken themixture for 15 sec. Nacl was added again until the heptane layer is in the neck of the bottle, take 1 ml of heptane layer and put in a tube. Na_2SO_4 was added into the tube and then filtered and injected into Gas Chromatography (Shimadzu GC-2010).

Statistical analysis

The data were analyzed using a completely randomized factorial design **(SAS, 1988)** when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ($P \leq 0.05$) were considered significant using Costat Program.

Results And Discussion

Nutritional Value Of Kefir Milk

Chemical Composition Of Kefir

Data presented in Table (1) show the chemical composition of kefir milk made from defatted cow milk. It is clear to be noticed that the moisture, protein, fat, ash, lactose and energy value were 88.10 %, 4.71%, 1.96%, 0.70%, 4.53% and 54.60 kcal/g, respectively. These results are in agreement with **(Zubillaga *et al*., 2001)**. They reported that the composition of kefir is variable and not well defined. It depends on the source and the fat content of milk, the composition of the grains or cultures and the technological process of kefir. **Sarkar (2007)** mentioned that kefir typically contains 89–90% moisture, 0.2% lipid, 3.0% protein, 6.0% sugar, 0.7% ash and 1.0% for lactic acid and alcohol. Also, **Liutkevicius and Sarkinas (2004)** reported that kefir grains contain 86.3% moisture, 4.5% protein, 1.2% ash and 0.03% fat.

Physicochemical properties of kefir milk

Tables (2) show the physicochemical properties results of kefir milk. It indicated that the values of total solids, pH, titratable acidity, viscosity and total phenolic contents were 2.9%, 4.03, 0.88% as lactic acid, 9.4 mpa.s and 199.1 µg/ml GAE, respectively. These results are in agreement with **(Wszolek *et al.,* 2001)** they found that the chemical composition of kefir ranged from 10.6% to 14.9% for total solids. **Fontan *et al.,* (2006)** found that the lactose content declined from 4.92% to 4.02%, the value of L(+)-lactic acid increased from 0.01% to 0.76% and pH fell to 4.24 during the first 24 h of fermentation. After 24 h, lactose was degraded more slowly, the rate of pH reduction decreased and the level of L(+)-lactic acid decreased marginally while that of D(−)-lactic acid had risen. **Öner *et al.,* (2010)** reported that starter culture type, storage period and mammalian species significantly affected changes in pH, the content of lactic acid ranged from 1.4 to 17.4 mg/mL and that of acetic acid increased from 2.10 to 2.73 mg/ml. **Sarkar, (2007)** mentioned that traditional kefir made from caprine milk was found to have a low viscosity and sensory properties unlike those of bovine kefir and contained 0.04–0.3% ethanol. Also, **Sabir *et al.,* (2010)** reported that the lactic acid and exopolysaccharide levels produced by eight strains of *Lactobacillus sp., Lactococcus sp*. and *Pediococcus sp.* were 8.1–17.4 and 173–378 mg/L, respectively. **Kök-Taş *et al.,* (2014)** found the phenolic content of plum- and molasses-enhanced kefir samples to be 2535.8 and 2357.6 mg/mL, respectively.

Minerals content of kefir milk

Data presented in Table (3) show the minerals content of kefir milk (µg/g). The obtained results indicated that the highest minerals content of kefir milk recorded with potassium, calcium and magnesium. The mean values were 1570, 1020 and 580 µg/g, respectively. On the other hand, the lowest minerals content of kefir milk recorded with copper, ferric, and zinc. The mean values were 0.75, 1.60 and 5.8 µg/g, respectively. These results are in agreement with those of **(Otles and Cagindi, 2003)** they reported that kefir is a good source of calcium and magnesium. Phosphorus, which is the second most abundant mineral in the human body and aids in the utilization of carbohydrates, fats and proteins for cell growth, maintenance and energy, is also abundant in kefir**.** Also, **Liutkevicius and Sarkinas (2004)** studied the macro- and micro-elements in kefir. They determined that the macro-elements present in kefir grain were: potassium, 1.65%, calcium, 0.86%, phosphorus, 1.45%, and magnesium, 0.30%, while the micro-elements found were: (mg/kg) copper, 7.32, zinc, 92.7, iron, 20.3, manganese, 13.0, cobalt, 0.16; and molybdenum, 0.33**.**

Total counts of kefir microorganisms

The total counts of kefir microorganisms as cfu/g X 10^6 are shown in Table (4). It is clear to notice that kefir milk contain different amounts of *Lactococci* bacteria counts, *Lactobacili* bacteria counts and yeast counts**.** The mean values were8.37, 6.18 and 4.07 cfu /g X 10^6, respectively. These results are in agreement with those of **Witthuhn *et al.*, (2005)** they reported that varying lactobacilli numbers during kefir production between 4.6 x10^3 and 2.6x10^8. These numbers reflect kefir produced commercially and traditionally, with the traditional kefir consistently representing the larger values, meaning that traditional kefir was consistently found to have a higher overall microbial load when compared to commercial kefir. **Guzel-Seydem, *et al.*, (2005)** showed the contents of Irish kefir grains to be 10^6 (cfu/ml) *lactococci*. **Beshkova *et al.*, (2002)** reported that the total number of yeasts in kefir made with pure cultures was 10^6-10^7 cfu/g.

Amino acids composition of kefir milk

Data given in Table (5) show the amino acids composition of kefir milk. It is clear to mention that the highest amino acids composition of kefir milk recorded with glutamic acid, proline and lysine. The mean values were 7.35, 3.42 and 3.38 g/16g N, respectively. On the other hand, the lowest amino acids composition of kefir milk recorded with valine, glycine and histidine. The mean values were 0.13, 0.59 and 0.95 g/16g N, respectively. These results are in harmony with findings of **Sarkar (2007)**. They found that kefir contains complete proteins that are partially digested, facilitating digestion by the body. The amino acid profile changes during the fermentation of milk, and kefir was found to contain higher levels of threonine, serine, alanine, lysine and ammonia than milk. Kefir also contains other amino acids, such as valine, isoleucine, methionine, lysine, phenylalanine and tryptophan. **Liutkevicius and Sarkinas (2004)** mentioned that the essential amino acid contents in kefir for mg/100 g were valine, isoleucine, methionine, lysine, threonine, phenylalanine and tryptophan. The values were 220, 262, 137, 376, 183, 231and 70, respectively. Also, **Kesenkas *et al.*, (2011)** reported tyrosine and leucine values of kefir were 0.009–0.016 mg/g and 1.89– 9.56 mmol/l, respectively after 28 d of storage. **Kesenkas *et al.*, (2013)** reported that tryptophan, one of the most important amino acids in kefir, is of key importance in the nervous system.

Fatty acids profile (% of total fatty acids) of kefir milk

The fatty acids profiles (% of total fatty acids) of kefir milk are shown in Table (6). It is clear to notice that the highest saturated fatty acids recorded with palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0). The mean values were 29.75, 17.64 and 10.07%, respectively. While, the lowest saturated fatty acids recorded with lignoceric acid (C24:0), heneicosanoic acid (C21:0) and behenic acid (C22:0). The mean values were 0.01, 0.03 and 0.04%, respectively. These results were consistent with the findings of **Marounek *et al*., (2012)** they found that the major FAs in kefir milk were palmitic and oleic acids, followed by stearic and myristic acids (23.6, 30.3, 13.6, 8.6%). On the other hand, highest unsaturated fatty acids results recorded with oleic acid (C18:1), palmitoleic acid (C16:1) and pentadecanoic acid (C15:1). The mean values were 27.16, 0.79 and 0.74 %, respectively. While, the lowest unsaturated fatty acids recorded with nevronic acid (C24:1), arachidonic acid (C20:4) and myristoleic acid (C14:1). The mean values were 0.02, 0.07 and 0.07 %, respectively. These results are in agreement with those of **Park *et al.,* (2007)** they reported that kefir milk lipids have a higher content of monounsaturated fatty acids (MUFA), Similarly as for medium chain triglycerides (MCT). **Haenlein (2004)** emphasized the beneficial properties of monounsaturated fatty acids (MUFA) and Polyunsaturated Fatty Acids (PUFA) for human health, especially for cardiovascular conditions conjugated linoleic acid (CLA) also has been identified as a significant nutrient for humans.

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Table (1): Chemical composition of kefir milk

Components	Wet weight (W/W %)
Moisture	88.10
Protein	4.71
Fat	1.96
Ash	0.70
Lactose	4.53
Energy value (Kcal/g)	54.60

W/W= Weight wet

Table (2): Physicochemical properties of kefir milk

Physicochemical properties	Amounts
Total solids	2.9 %
pH	4.03
Titratable acidity	0.88 % (as lactic acid)
Viscosity	9.4 mPa.s
Total phenolic content	199.10±7.73 (µg/ml GAE)

Mpa.s = Millipascal-second GAE= Gallic acid equivalents

Table (3): Minerals content of kefir milk (µg/g)

Minerals	µg/g
Calcium	1020
Phosphorus	118.5
Potassium	1570
Magnesium	580
Sodium	95.5
Zinc	5.80
Ferric	1.60
Copper	0.75

Table (4): Total counts of kefir microorganisms

Kefir organisms	CFU/g X 10^6
	Mean ± SD
***Lactococci* bacteria counts**	$8.37±0.43^a$
***Lactobacili* bacteria counts**	$6.18±1.23^b$
yeast counts	$4.07±0.59^c$

CFU/g = Colony form units/gram

Mean under the same column bearing different superscript letters are different significantly ($p < 0.05$).

Table (5): Amino acids composition of kefir milk

Amino acids	Amounts (g/16gN)
Aspartic acid	2.51
Glutamic acid	7.35
Serine	1.92
Histidine	0.95
Glycine	0.59
Threonine*	1.37
Arginine	1.00
Alanine	1.06
Tyrosine	1.50
Cystein	0.13
Valine*	2.06
Methionne*	0.70
Phenylalanine*	1.48
Isoleucine	1.56
Leusine*	3.38
Lysine*	2.84
Proline	3.42
Tryptophane*	1.13

*Essential amino acid.

Table (6): Fatty acids profile (% of total fatty acids) of kefir milk

Fatty acids	Amounts %
	Mean ± SD
Saturated fatty acids	
Caproic C6:0	1.27 ± 0.207^{f}
Caprylic C8:0	6.63 ± 1.331^{d}
Capric C10:0	0.06 ± 0.008^{a}
Lauric C12:0	3.90 ± 1.278^{e}
Myristic C14:0	10.07 ± 2.214^{c}
Palmitic C16:0	29.95 ± 4.294^{a}
Heptadecanoic C17:0	0.57 ± 0.023^{f}
Stearic C18:0	17.64 ± 7.197^{b}
Arachidic C20:0	0.10 ± 0.019^{f}
Heneicosanoic C21:0	0.03 ± 0.008^{f}
Behenic C22:0	0.04 ± 0.001^{f}
Lignoceric C24:0	0.01 ± 0.011^{f}
Unsaturated fatty acids	
Myristoleic C14:1	0.07 ± 0.0090^{d}
Pentadecanoic C15:1	0.74 ± 0.023^{b}
Palmitoleic C16:1	0.79 ± 0.419^{b}
Oleic C18:1	27.16 ± 4.006^{a}
Linoleic C18:2	0.44 ± 0.143^{b}
Linolenic C18:3	0.25 ± 0.025^{c}
Arachidonic C20:4	0.07 ± 0.022^{d}
Nevronic C24:1	0.02 ± 0.004^{d}

Mean under the same column bearing different superscript letters are different significantly ($p < 0.05$).

الخصائص التغذوية للبن الكفير

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الملخص العربى

لبن الكفير عبارة عن مشروب من مزرعة اللبن المتخمرة. يحتوى لبن الكافير على البكتيريا النافعة والخميرة وهى غنية في الأحماض الأمينية والأحماض الدهنية والفيتامينات والإنزيمات، والفيتامينات والأملاح المعدنية.وقد تم فى هذه الدراسة تقييم القيمة الغذائية للبن الكفير، من خلال تقدير التركيب الكيمياوى، والخواص الطبيعية، ومحتواه من الأحماض الأمينية والأحماض الدهنية والأملاح المعدنية ومحتواه من الكائنات الحية الدقيقة النافعة . وأظهرت النتائج المتحصل عليها على أساس الوزن الرطب أن قيم الرطوبة والبروتين والدهن والرماد واللاكتوز والطاقة كانت ٨٨,١٠٪، ٤,٧١٪، ١,٩٦٪، ٠,٧٠٪، ٤,٥٣٪ و ٥٤,٦٠ كيلو كالوري / جرام على التوالي .بينما كانت قيم المواد الصلبة ، الـpH ودرجة الحموضة واللزوجة، والفينولات الكلية ٩,٢٪، ٤,٠٣، ٠,٨٨٪ كحامض لاكتيك، ٩,٤مل باسكال و ١٩٩,١ ميكروجرام / مل مكافئ حامض جاليك، على التوالي.وكان أعلى محتوى من الأملاح المعدنية في لبن الكفير سجلت مع البوتاسيوم والكالسيوم والمغنيسيوم، في حين أن أقل محتوى سجل مع النحاس، الحديد، والزنك .يحتوي لبن الكافير على كميات مختلفة من بكتيريا حامض اللاكتيك الكروية والعصوية والخميرة .أعلى نسب للأحماض الأمينية من لبن الكفير سجلت مع حامض الجلوتاميك، البرولين والليسين، في حين كانت أقل قيمة مع الحمض الأميني الجلايسين. بينما كانت الأحماض الأمينية الدهنية الرئيسية في لبن الكفير هى حمض البالمتيك وحمض الأستياريك وحمض الميريستيك. وجد أن لبن الكفير ذات محتوى عالي من الأحماض الدهنية الاحادية غير المشبعة ، والأحماض الدهنية متوسطة السلسلة.الكربونية. من النتائج المتحصل عليها فإنه يمكن استنتاج أن لبن الكافير له قيمة غذائية جيدة لارتفاع محتواه من الأحماض الأمينية والأحماض الدهنية غير المشبعة والأملاح المعدنية.

الكلمات الدالة: لبن الكفير ـــ التركيب الكيماوى ـــ الخواص الطبيعية ـــ القيمة الغذائية