

THE INFLUENCE OF DIETARY FIBER FROM RICE BRAN AND DEFATTED SESAME FLOUR ON FERMENTED MILK DRINK

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Key words:

Rice bran
Sesame flour
Kefir
Dietary fiber

Article history:

Received 04.01.2023
Received in revised form
18.01.2023
Accepted 01.02.2023

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ABSTRACT

Both rice bran (RB) and defatted sesame flour (DSC) are by-products of agricultural processing, they are rich in dietary fiber. In modern society, dietary fiber is increasingly being paid attention to for its health benefits. This paper is aimed at studying the properties of kefir added with RB and DSC, including content of dietary fiber, pH, titratable acidity, viscosity, amount of *Lactococcus spp.*, *Lactobacillus spp.* and yeast. Three different kefirs were investigated (control sample, kefir with 0.1% RB, kefir with 2% DSC) and stored for 28 days at 4±1 °C. The content of dietary fiber in samples of kefir, pH, titratable acidity, viscosity, amount of *Lactococcus spp.*, *Lactobacillus spp.* and yeast were determined at 1st, 7th, 14th and 28th days of storage. Results showed that the introduction of RB and DSC increased the content of dietary fiber in kefir samples by 300% and 680%, compared to control sample, improved the viscosity by 6 times and 25 times compared to control sample, and acidity — by 9% and 75%, stimulated growth of probiotics including *Lactococcus spp.* (content increased by 6% and 8% compared to control sample), *Lactobacillus spp.* (content increased by 16% and 4% compared to control sample) and yeast (content increased by 7% and 1% compared to control sample on the 7th day of storage). The increase of dietary fiber content and probiotics quantity could improve the health properties of kefir, the increase viscosity make kefir more popular with consumers (according to the data from a survey of consumers, they tend to prefer thicker kefir) and the increase of acidity would shorten fermentation time and reduce production cost. To reach better quality of kefir, RB and DSC should be pretreated: crushing, decolorization, deodorization. And adding larger amount lead to bad taste, so the urgent problem which needs to be resolved is to decrease the adverse effect of RB and DSC on properties of kefir.

ВПЛИВ ХАРЧОВИХ ВОЛОКОН РИСОВИХ ВИСІВОК І ЗНЕЖИРЕНОГО КУНЖУТНОГО БОРОШНА НА КИСЛОМОЛОЧНИЙ НАПІЙ

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Такі побічні продукти переробки сільськогосподарської продукції, як рисові висівки (РВ) і знежирене кунжутне борошно (ЗКБ) багаті на харчові волокна, корисні для здоров'я людини. У статті вивчається вплив РВ і ЗКБ на кефір. Було досліджено три різні зразки кефіру (контрольний зразок, кефір з 0,1% РВ та кефір з 2% ДСК), що зберігалися 28 днів при температурі 4 ± 1 °C. Вміст харчових волокон у зразках кефіру, рН, титровану кислотності, в'язкість, кількість *Lactococcus* spp., *Lactobacillus* spp. і дріжджів визначали на 1-й, 7-й, 14-й та 28-й дні зберігання. Результати показали, що введення РВ і ДСК підвищує вміст харчових волокон у зразках кефіру на 300% і 680%, в'язкість — у 6 і 25 разів, кислотність — на 9% і 75%, якщо порівняти з контрольним зразком. Крім того, додавання РВ і ДСК стимулює зростання пробіотиків, включаючи *Lactococcus* spp. (на 6% і 8%), *Lactobacillus* spp. (на 16% і 4%), і дріжджів (на 7% і 1% на 7-й день), порівняно з контрольним зразком. Збільшення вмісту харчових волокон і кількості пробіотиків підвищує цінність кефіру для здоров'я. Підвищення в'язкості зробить кефір більш популярним у споживачів (згідно з даними опитування споживачів, як правило, віддають перевагу більш густому кефіру), а підвищення кислотності скоротить час бродіння і знизить собівартість продукції. Щоб досягти кращої якості кефіру, РВ і ДСК повинні бути попередньо оброблені (дроблення, знебарвлення, дезодорація). З'ясовано, що додавання більшої кількості РВ і ДСК погіршує смак продукції, тому нагальною проблемою, яка потребує вирішення, є зменшення несприятливого впливу добавок на кефір.

Ключові слова: рисові висівки, кунжутне борошно, кефір, харчові волокна.

Formulation of the problem. With the improvement of people's living conditions, the health problems brought by excessive intake of products especially high-calorie are becoming more and more seriously. Obesity, hypertension, hyperlipidemia and hyperglycemia seriously threaten people's health, it is necessary to control the excessive intake of products to keep health.

Dietary fiber is known as "the seventh nutrient", it has several physiological functions. According to well-documented studies, dietary fiber plays a significant role in risk reduction of several chronic diseases. Diet with a high content of fiber has a positive effect on health and its consumption has been related to a better management of blood sugar, lowering blood cholesterol, improving health, and reducing risk of coronary heart disease, as well as several types of cancer, particularly colon cancer (Aune et al., 2012). These functions enable dietary fiber to meet the health needs of people.

Rice bran (RB) and defatted sesame cake (DSC) are by-products of agricultural processing, they are rich in dietary fiber. In view of benefits of dietary fiber, the introduction of RB and DSC into kefir would be a very significant attempt. But there is little study about the introduction of DSC or RB into kefir.

Thus, innovative facets regarding the usage of these wastes as co-products for further production of food additives, supplements or value-added products with high nutritional value are gaining increasing interest. Furthermore, their recovery and utilization are economically and ecologically attractive.

Analysis of recent research and publications. Dietary fibers are naturally present in a variety of grains, legumes, fruits, vegetables. Based on intestinal solubility (fermentability), dietary fibers are also classified as insoluble and soluble fiber. Soluble dietary fiber (SDF) partially dissolves in water. It undergoes metabolic processing via fermentation, yielding end products with significant health effect such as modulation of blood glucose and lipids, as well as improvement of colonic medium and regulation of immune responses (Ötles, & Ozgoz, 2014).

Dietary fiber could not be digested and absorbed by human body, and their intake could give the feeling of fullness, these characteristics could avoid the excessive intake of energy. Studies have shown that dietary fiber could promote the growth of probiotics (Ozcan, & Kurtuldu, 2014), which is also beneficial to human health. Dietary fiber had a strong water holding capacity, which helped to improve the consistency of fermented dairy products, so that dairy products would be more favored by consumers.

The effect of substitution of skim milk powder and milk fat by high performance and native inulin on rheological, textural and sensory properties of kefir were studied (Glibowski, & Zielińska, 2015). Results showed that both native and high performance inulin can be successfully applied as a skim milk powder substitute. Kefirs with different types of inulin revealed similar rheological and sensory properties. Rotational rheometry revealed that the studied kefir exhibited thixotropic and shear thinning behavior. Substitution of skim milk powder by inulin allowed reducing 12% and 35% of caloric value in comparison with skim milk powder and whole milk powder kefir, respectively.

Kefir enriched with apple fiber (AF) and lemon fiber (LF) was studied (Goncu, Celişkel, Guler-Akin, & Serdar Akin, 2017). Results showed that the water activity values of kefir samples were between 0.93–0.99, control sample had the highest water activity (A_w). Addition of DF reduced A_w of kefir. Fiber concentration affected A_w negatively ($p < 0.01$). Lactic acid bacteria (LAB) counts were between 9.79–10.89 log cfu/mL⁻¹ during storage time. The number of LAB were found to be higher in samples with DF than in the control sample ($p < 0.01$). Addition of DF had significant effect on the sensory characteristics of kefir ($p < 0.01$).

The effects of quinoa flour and inulin on some properties of kefir, including pH, syneresis, viscosity, *Streptococci* and *Lactobacilli* counts, total phenolic content, antioxidant activity, titratable acidity were investigated (Kef, & Arslan, 2021). In samples produced with both types of milk, inulin addition significantly ($p < 0.05$) increased viscosity and decreased syneresis. The lowest pH value was obtained in kefir with cow milk produced with 0.5% quinoa flour. Various formulations of kefir samples had significant effects on the syneresis, viscosity, *Streptococci* and *Lactobacilli* counts, total phenolic content, antioxidant activity and titratable acidity. Antioxidant activities in ke-

fir with cow milk and goat milk varied between 15.27 and 33.71 and between 9.00 and 20.45 ($\mu\text{mol Trolox Equivalent/L}$), respectively. Quinoa flour addition led to a decrease in the general appreciation score. The highest taste-aroma and general acceptability scores were obtained with the sample that included 1% inulin in both milk types.

The effects of milk supplementation with mango peels on the growth rates of kefir microorganisms during fermentation as well as the antioxidant properties of the obtained fermented products were evaluated (Vicenssuto, & de Castro, 2020). The results were comparatively evaluated between fermented samples with the presence of mango peels (KMP) and without them (KC). Significant increases ($p < 0.05$) in antioxidant activities for KMP were observed for all methods: 113% for DPPH-radical scavenging (10 h of fermentation), 153% for the Ferric Reducing Antioxidant Power (FRAP) assay (8 h of fermentation), 21% for ABTS-radical scavenging (6 h of fermentation) and 43% for Total Reducing Capacity (Folin-Ciocalteu method) (8 h of fermentation) compared to those of KC fermented samples. According to microbiological analyses, the KMP samples allowed microbial growth of up to 3-fold higher than the growth of KC samples, reaching counts of up to 108 CFU/mL for lactic acid bacteria.

DSC is a kind of by-product commonly discarded or utilized for feeding animals. The process of sesame oil extraction leads to the production of semidefatted sesame flour (SDSC), which containing 50% protein, high content of calcium (1.5 g/100 g), and crude fiber (10.8 g/100 g) (Maset et al., 2022).

RB is a by-product of rice milling industry and constitutes around 10% of the total weight of rough rice. It is primarily composed of aleurone, pericarp, sub aleurone layer and germ. Each year 90% of the RB produced in the world is utilized cheaply as a feed stock for cattle and poultry, and the rest is used for extraction of RB oil (Sohail, Rakha, Butt, Iqbal, & Rashid, 2017). The improper handling lead not only environmental pollution, but also wasting of resources.

The purpose of the study is to determine the impact of DSC and RB, which are rich in fiber, on properties of kefir, including physicochemical properties (pH, total titratable acidity, apparent viscosity) and microbial viability (*Lactobacillus*, *Lactococcus* and yeast).

Materials and methods. Kefir grains were obtained from private households in Tibet, China. Cow milk was supplied from Mengniu Dairy Group Co, Neimenggu, China. All chemicals were of analytical grade.

Sesame seeds (white) were procured from local market (Hezhou, Guangxi, China). DSC was obtained from sesame seed which was roasted at 120–130 °C for 10 min and pressed the fat. The flour was finely powdered and homogenized by sieving through fine screen (100 mesh). RB were procured from local market (Xingtai, Hebei, China). RB was homogenized by sieving through fine screen (200 mesh). RB was steam sterilized at 121 °C for 15 minutes before used.

Kefir was used as start culture at a ratio of 10% (v/v). In view of the significant negative influence of DSC and RB on the sensory quality of kefir, a small introduction ratio of DSC and RB were selected, namely 2% and 0.1%, respectively. Three samples were investigated: cow milk without additives (control sample), cow milk added with 2% amount of DSC, cow milk added with 0.1% amount of RB. All mixtures were fermented at 28 °C for 22 h until pH reached to 4.7, kefir samples were stored at 4 °C for 28 days.

The content of dietary fiber in all samples was studied. Microbial viability (*Lactobacillus*, *Lactococcus* and yeast) and physicochemical properties (total titratable acidity, apparent viscosity and pH) were studied for all samples. All the indexes were studied after 7, 14, 21, 28 days.

Proximate composition. The content of dietary fiber was measured according to (AOAC, 2000). The dried samples were digested with heat-stabilized α -amylase, protease and glucosidase to remove protein and starch. After ethanol precipitation and filtration, the residue was washed with ethanol and acetone, dried and weighed, which was the total dietary fiber residue. The total dietary fiber content in the sample can be calculated by deducting the corresponding protein, ash and reagent blank contents in the dietary fiber residue. The set for the test is dietary fiber determination system (CSF6, Italy).

The pH value was determined by a pH meter (METTLER TOLEDO LE438, Switzerland). The titratable acidity was measured by titrating 10 g of sample with 0.1 N NaOH using phenolphthalein indicator. The apparent viscosity of the samples was measured with a digital viscometer (NDJ-8S, Shanghai, China) (Putri, Setiani, & Warya, 2020). An appropriate rotor was selected, according to the viscosity of kefir, then the rotor was inserted into the kefir samples, and then an appropriate speed was selected. The torque generated by the rotor during rotation will be displayed by the instrument in the form of force, then the data obtained.

Microbiological analysis: 25 ml of sample was suspended in 225 ml sterile normal saline (0.85%, v/v) solution in a ratio of 1:9 (w/w) in a sterile bag. The mixture was homogenized in orbital shaker (Guansen 200JR, Shanghai, China) at 400 rpm for 1.0 min. Then, serial dilutions in normal saline (0.85%, w/v) were prepared, and proper dilution was transferred onto the appropriate media for each microorganism. The microbial loads of *Lactobacillus* were determined on Man-Rogosa-Sharpe agar, *Lactococcus* on M17 agar, yeast and molds on Potato Dextrose Agar. The plates were then incubated at 30 °C for 72 h for the detection of *Lactobacillus*, at 37 °C for 48 h for *Lactococcus*, at 28 °C for 5 days for yeast and molds. All microbiological incubations were carried out in incubators (BPC-150F, Shanghai, China). Colony counts were converted to log CFU/ml. Each sample for all measurements was replicated in twice (Goncu, Celikel, Guler-Akin, & Serdar Akin, 2017).

Statistical analysis. All experiments were replicated in three flasks and the data are presented as the mean and standard deviation of three independent experiments. Tukey's multiple range test (Du, 1985) was used to determine the significant differences among mean values at the $P = 0.05$ level. Statistical analyses were carried out using the Data Processing System soft-ware (DPS version 7.05, SAS Institute, Inc., 2000).

Presentation of the main results of the study. The introduction of RB and DSC could significantly improve the content of dietary fiber in kefir. Research data are shown in table 1.

Table 1. Content of dietary fiber in samples with additives of fiber

Samples	Control sample	Sample with 0.1% RB	Sample with 2% DSC
Content of fiber, %	0.01±0.00	0.03±0.002	0.68±0.11

The content of dietary fiber improved significantly ($P < 0.05$). Compared to control samples, the dietary fiber content in kefir added with 0.1% RB increased 3 times, and in kefir added with 2% RB increased 6.8 times.

The changes of pH and titratable acidity in different samples during 28 days are shown at figure 1 and figure 2.

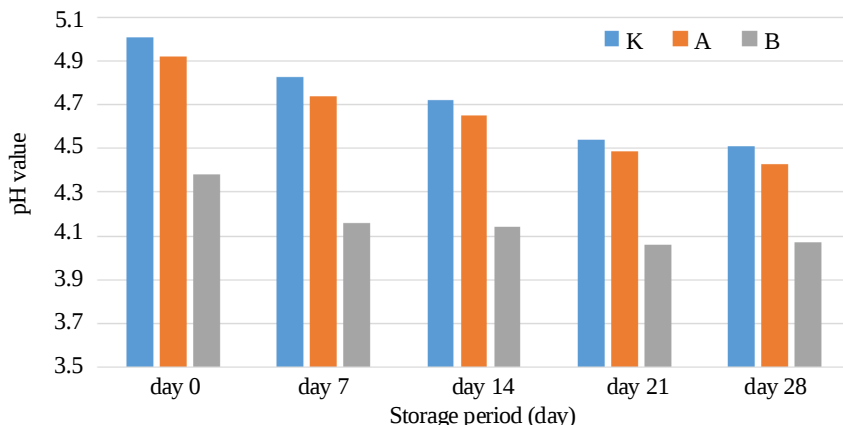


Figure 1. The changes of pH in samples with additives of fiber during 28 days

In the figure 2, K presented control samples, A presented kefir added with RB, B presented kefir added with DSC. The more amount of additives was added, the lower the value of pH was, the higher the value of titratable acidity was. pH of samples ranged between 3.93 and 5.01.

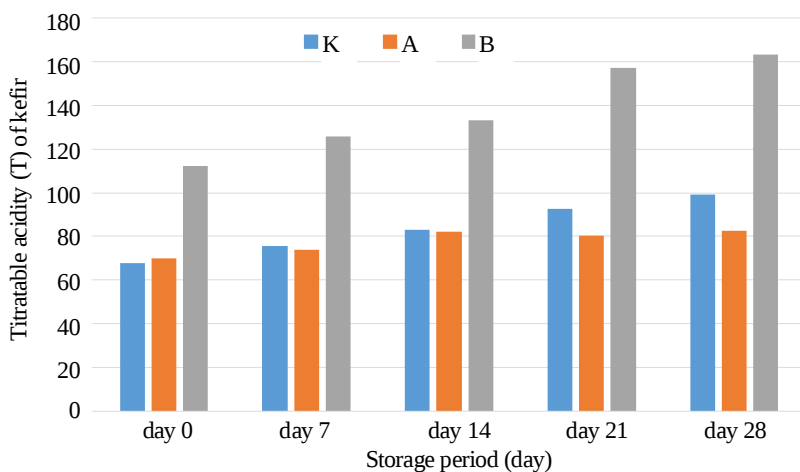


Figure 2. The changes of titratable acidity in samples with additives of fiber during 28 days

The titratable acidity ranged between 67.78 T and 163.52T. These dependences could be related to stimulation of lactic acid bacteria by fiber. The results have an agreement with previously obtained results by other scientists (do Espírito Santo, Perego, Converti, and Oliveira, 2012) who produced yoghurt with passion fruit fiber. Previous

studies have also demonstrated that kefir as a probiotic (Guzel-Seydim, Kok-Tas, Greene, & Seydim, 2011) and dietary fiber had a potential prebiotic effect (Shah, Li, Sabbah, Xu, & Mraz, 2020; Wu, et al., 2020).

The results of apparent viscosity are shown in figure 3. Compared to control sample, 2% amount of DSC increased the apparent viscosity of kefir ($p < 0.05$), so do as 0.1% amount of RB, which was caused by the adding amount of dry matter and the hydrophilicity of protein and fiber in DSC (Mudgil, & Barak, 2013; Ilyas, & Atalar, 2019). This is the most probably due to the effect of coverage of caseins with polysaccharides such as β -glucan, pectin etc. and stabilizing casein aggregates. These results are in agreement with findings of other scientists (Tseng, & Zhao, 2013) who reported that an increasing levels of addition wine grape pomace as prebiotic in yoghurts decreased apparent viscosity values. Also, there was stated (El-Batawy, Ashoush, & Mehanna, 2014) that increasing the concentration of pomegranate peel extracts decreased the viscosity values and these results are associated with effect of pomegranate peel extract on the aggregation of network in yoghurts via electrostatic interactions. On the other hand, there was reported (Güler-Akın, Ferliarslan, & Akın, 2016) that the yoghurts added with inulin had significantly higher apparent viscosity than the sample without it. Viscosity is an important technological property of fibers which provides rheological characteristics to food systems. SDF becomes viscous when mixed with water. Fibers, such as pectin, gums, β -glucans extracted from algae, form highly viscous solutions. They are used as thickening agents in foods at low concentrations. In the case of beverages and drinks, the addition of dietary fiber increases viscosity which leads to increased stability.

The apparent viscosity was the highest on the 7th day, and then kept steady during storage period ($p > 0.05$), this might be explained by the balance of sedimentation and hydrophilic interaction of DSC.

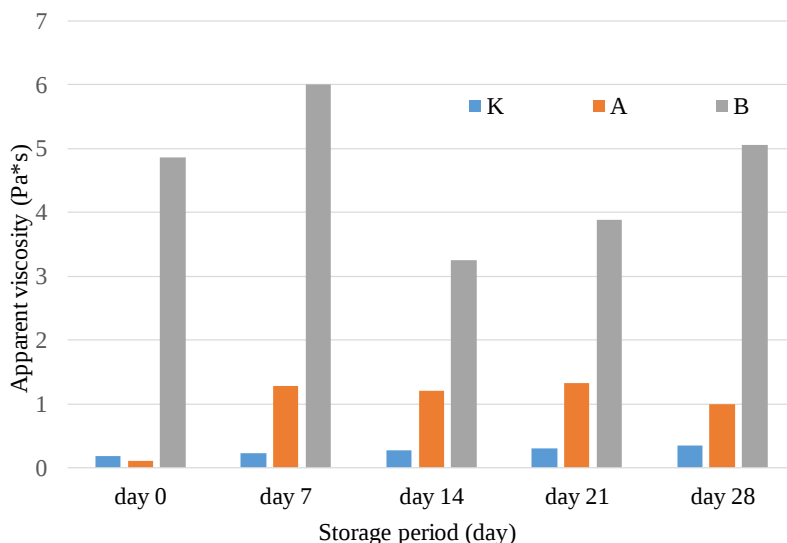


Figure 3. The changes of apparent viscosity in different samples in 28 days:
K — control samples; A — kefir added with RB; B — kefir added with DSC

The changes of the population of *Lactobacillus*, yeast and *Lactococcus* during 28 days are shown in figures 4–6. The *Lactobacillus* counts were between 8.04–9.15 log CFU mL⁻¹ during storage time; The *Lactococcus* counts were between 7.51–10.45 log CFU mL⁻¹ during storage time; yeast counts were between 7.95–10.11 log CFU mL⁻¹ during storage time. The introduction of DSC made no difference on the microbiological count, which may be due to the combined effect of the promotion of dietary fiber and the bacteriostasis of sesame phenolic substances on the growth of microorganisms. Scientists (Alfuryadi, Devanesan, Al-Ansari, AlSalhi, & Ranjitsingh, 2019) reported that DSC showed high activity against bacteria, authors also indicated that antimicrobial properties originate from phenolic compounds in DSC. Another reason of this reduction may be antagonistic effect of higher yeast content against lactic acid bacteria. The cocultured organism may compete for nutrients or produce metabolic products that stimulate or inhibit each other's growth (Santos, Libeck, & Schwan, 2014). Similar results were reported by (Montanuci, Pimentel, Garcia, & Prudencio, 2012).

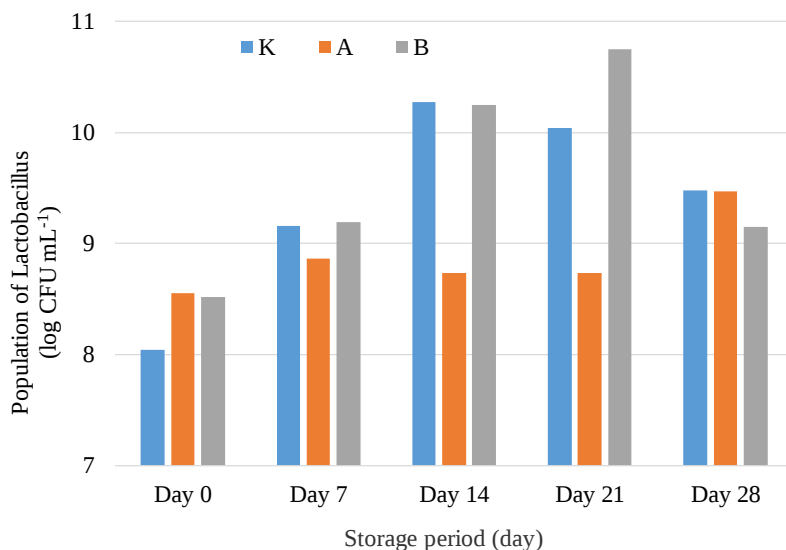


Figure 4. The population of *Lactobacillus* of kefir samples

Although there were differences in all samples for viable counts of lactococci during the first week of storage ($P < 0.05$), these differences weren't obvious, which indicated that the introduction of RB made little difference on the population of *Lactococcus*. The results were compatible with (Demirci, Aktaş, Sözeri, Öztürk, & Akın, 2017), who reported a slight decrease of *S. thermophilus* during storage of yoghurts added with RB. Storage period significantly affected the amount of *Lactococcus* ($P < 0.05$). As the prolonging of storage period, the amount of *Lactococcus* significantly decreased ($P < 0.05$) in the first two weeks, and kept steady in the following two weeks. The population of *Lactococcus* never declined less than 7.0 log CFU/mL.

Yeasts play an important role during kefir fermentation, because of end-products such as ethanol and carbon dioxide which would give kefir unique taste. The increase of population of yeast was more obvious in samples added with RB than control

sample during storage period. Such data indicated that RB positively stimulated growth of yeasts.

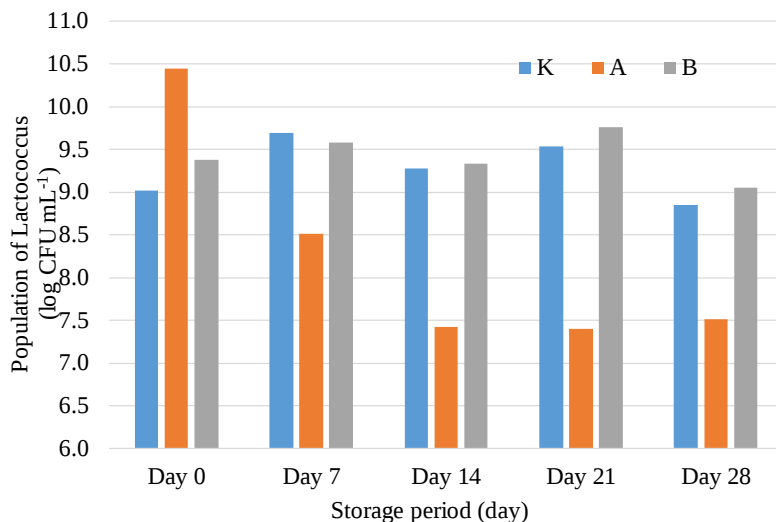


Figure 5. The population of Lactococcus of kefir samples

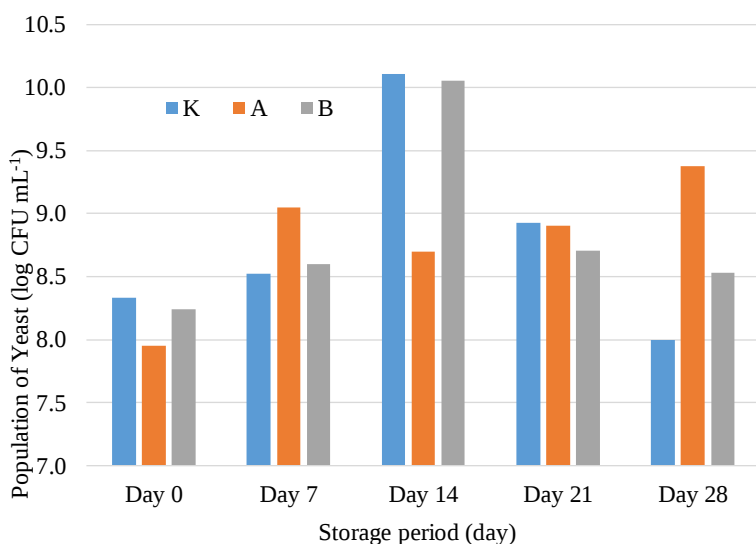


Figure 6. The population of yeast of kefir samples

Storage period apparently affected yeast counts in all samples ($P < 0.05$). Although insignificant differences were noted in samples during the first week, significant differences were found during the rest period of storage time ($P < 0.05$).

Conclusions

This study reported to application of DSC and RB in kefir formulations. Results showed that the introduction of DSC and RB improved the acidity by 9% and 75% and viscosity improved 6 times and 25 times, compared to control sample, mainly because of the dietary fiber in their recipe. Also, the introduction of DSC and RB improved the probiotics properties, *Lactococcus spp.* increased by 6% and 8% *Lactobacillus spp.* increased by 16% and 4%, and yeast increased by 7% and 1% compared to control sample on the 7th day. This study demonstrated that RB and DSC are appropriate by-products with desirable properties such as prebiotic potential and antioxidative contributions for functional dairy product manufacturers.

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