กิจกรรมการต้านอนุมูลอิสระของคีเฟอร์นมข้าวสี

Antioxidant Activities of Color Rice-Milk Kefir

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บทคัดย่อ

งานวิจัยนี้นำข้าวสายพันธุ์ไทย 3 สายพันธุ์ได้แก่ ข้าวแดง (ข้าวเจ้ามะลิแดง), ข้าวเหนียวกล่ำเปลือกดำ และข้าวดำ (ข้าวเหนียวดำ มข) มาผลิตเป็นนมข้าวแต่ละสีหมักด้วยหัวเชื้อคีเฟอร์และแปรผันสูตรนมที่มีการผสมและไม่ผสมนมโค หมักเป็นเวลา 72 ชั่วโมง วิเคราะห์หาคุณสมบัติทางกายภาพของผลิตภัณฑ์ ปริมาณฟีนอลิกทั้งหมดโดยวิธี Folin-Ciocalteau วิเคราะห์กิจกรรมการต้านอนุมูลอิสระของคีเฟอร์นมข้าวสีสูตรต่าง ๆ โดยใช้วิธี 2, 2,-diphenyl-1-pricrylhydrozyl (DPPH) scavenging, Ferric reducing antioxidant power (FRAP) และ Hydroxyl radical (OH) scavenging จากผลการทดลองพบว่า คีเฟอร์นมข้าวแดงที่ไม่ผสมนมโคเมื่อหมักเป็นเวลา 48 ชั่วโมงมีสารประกอบฟีนอลทั้งหมดสูงสุดเท่ากับ 1.60 มิลลิกรัมต่อลิตร และกิจกรรมการต้านอนุมูลอิสระของคีเฟอร์นมข้าวแดงที่ผสมนมโคซึ่งวิเคราะห์ด้วยวิธี DPPH ให้ค่ากิจกรรมสูงสุดเท่ากับ 64.37% ในขณะที่คีเฟอร์จากนมข้าวดำที่ผสมนมโคพบว่ามีกิจกรรมการต้านอนุมูลอิสระซึ่งวิเคราะห์โดยวิธี FRAP มีค่าสูงสุดเมื่อหมักเป็นเวลา 24 ชั่วโมง ให้ค่ากิจกรรมการต้านอนุมูลอิสระเท่ากับ 4.00 มิลลิกรัม Fe (II) / ลิตร และคีเฟอร์นมข้าวแดงที่ไม่ผสมนมโคให้ค่ากิจกรรมการต้านอนุมูลอิสระสูงสุด เมื่อวิเคราะห์ด้วยวิธี Hydroxyl radical เท่ากับ 99.37% จากผลการศึกษานี้ชี้ให้เห็นว่าคีเฟอร์จากนมข้าวสีแต่ละชนิดมีคุณสมบัติการต้านอนุมูลอิสระ ซึ่งน่าจะสามารถนำไปพัฒนาเป็นผลิตภัณฑ์สุขภาพในอนาคตได้

Abstract

In this study, different brans of Thai color rice cultivars namely, red rice (*mali dang*), brown rice (*gum pleuak dum*) and black rice (*dum moko*) was used to produce kefir by fermentation of kefir culture for 72 hours. The aim of this study was to find the difference between color rice-milk kefir with and without UHT milk addition on their total phenolic, antioxidant activities and physiochemical properties. The samples were analyzed using the Folin-Ciocalteau method for total phenolic content, 2, 2,-diphenyl-1-pricrylhydrozyl (DPPH) scavenging method, Ferric reducing antioxidant power (FRAP) and Hydroxyl radical (OH) scavenging activity. The results indicated that the highest total phenolic compound was found to be 1.60 mg/l for Kefir produced from 48 hours of fermentation of red rice milk without UHT milk. With the DPPH scavenging method, methanolic extract of kefir

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produced from fermented red rice milk with UHT milk for 0 hour showed the highest scavenging activity of 64.37% while FRAP values were higher for kefir produced from 24 hours fermented black rice milk with UHT milk, which was found to be 4.00 mg Fe (II)/l. Likewise with the hydroxyl scavenging method, Kefir produced from 24 hours fermented red rice milk without UHT milk showed the highest scavenging activity of 99.37%. However, all three color rice milk kefir showed the possibility of presence of polyphenols which have antioxidant properties. These results conclude that Kefir produced from fermented rice milk without UHT milk and rice milk with UHT milk could be a promising source of natural antioxidants with good potential for improving health.

Keywords: Kefir, colored rice, rice-milk, UHT milk, natural antioxidant.

Introduction

The word “Kefir” is derived from the Turkish word “keyif, which means “feeling good” after its ingestion (Lopitz *et al.,* 2006; Tamime, 2006). Kefir is defined as a beverage produced by the action of lactic acid bacteria (LAB) (lactobacilli, lactococci, leuconostocs), yeasts, and acetic acid bacteria (aceterobacteria) on milk (Farnworth and Mainville, 2008; Halle *et al.,* 1994). Kefir is characterized by its distinct flavour, typical of yeast, and an effervescent effect felt in the mouth (Lopitz *et al*., 2006; Rattray and Connell, 2011). The main products of Kefir fermentation are lactic acid, ethanol and carbon dioxide, which confer upon this beverage viscosity, acidity and low alcohol content. Minor components can also be found, including diacetyl, acetaldehyde, ethyl and amino acids contributing to the flavor composition (Rattray and Connell, 2011). Moreover, Kefir has antimicrobial, antihypertensive, antiinflammatory, anticarci-nogenic, antiallergic, and antioxidant activity; participates in immune-system modulation; reduces cholesterol levels; and alleviates lactose intolerance (Farnworth 2005).

In addition, nitrogen compounds and carotenoids, as well as ascorbic acids, are natural antioxidants which are obtained from plants (Laandrault *et al.,* 2001; Iqbal *et al.,* 2005). Similarly Lin & Yen (1999) showed that natural antioxidants from foods may reduce the oxidative damage on the human body. Moreover, there are many studies relating to antioxidant activity in rice, but few relating to rice milk. Furthermore, colored rice reported as potent sources of antioxidants and encouragements as viable sources of antioxidants for functional foods were made (Yawadio *et al.,* 2007).

Besides, the antioxidant activities of the brown rice extract were higher than BHA (Supasit Chooklin. 2013). But, a study by Wang *et al.,* (2006); Rekha and Vijayalakshmi, (2008) on soy milk showed that there is an increase in antioxidative activities of soymilk after fermentation with lactic acid bacteria, *bifidobacteria* and Probiotic Yeast. Similarly, Sirirat and Jelena, (2010) showed rice milk kefir has high

antioxidant activity compared to BHA. Moreover there are variations among antioxidant properties of Kefir samples produced from different cow/soy milk mixtures related to the soymilk ratio in kefir milk (Kesenkas *et al.,* 2011). Besides this, a study by Je Ruei *et al.,* (2005) proved that Kefirs are potential antioxidants that interact with a wide range of species directly responsible for oxidative damage. There are only few studies relating to the activity of rice milk kefir (Sirirat and Jelena, 2010).

This research has aimed to investigate the antioxidant activities and find the difference between Thai glutinous rice-milk kefir with and without UHT milk addition. Firstly, the total phenolic content (TPC) of rice milk Kefir, rice and cow milk kefir was measured following the Folin-Ciocalteu method, using gallic acid as the standard. Antioxidant activity was determined using Hydroxyl radical (OH) scavenging, the FRAP method and the DPPH method. The physiochemical properties were also determined.

Materials and methods

The experiments were carried out at the Department of Biotechnology, Faculty of Technology, Mahasarakham University, Thailand, during the period December 2012 to September 2013.

Materials

Thai rice cultivars used in this study were unpolished waxy color rice (*mali dang* (brown color), *gum pleuak dum* (red color) and *dum moko* (black color) from (Roi-et, and Udonthani Thailand).

Reagents and Chemicals

Folin-Ciocalteau reagent, 2, 2,-diphenyl-1-pricrylhydrozyl (DPPH), Gallic acid, Methanol, NaOH – phosphate buffer (pH7), Sodium carbonate.

Methods

Preparation of Rice Milk

Firstly, 500 g of (*mali dang, gum pleuak dum* and *dum moko*) rice was soaked in 1 L distillated water for 24 hrs. Then it was blended, filtrated with cotton sheet and cooked at 72 ºc for 15 min, after which the milk was cooled and ready for use.

Kefir Culturing

Kefir cultures DC 500 I from (Danisco Biolacta, Poland) was used as starter cultures. The starter cultures were grown in MRS medium and were incubated at 25˚c for 24 hrs then frozen at 4º C until they were used.

Kefir and Rice milk Fermentation

Rice milk prepared earlier and UHT milk was used to produce kefir. Three varieties of rice milk (*mali dang*, *gum pleuak dum* and *dum moko*) and UHT milk (total fat 9 % were used. Flask Fermentation was carried out at 24-26˚C for 0 (start), 24, 48 and 72 hrs by using 10% (v/v) of Kefir Starter culture. Time 0 hr is considered as the start or initiation time. The experiments were conducted in triplicate.

Physiochemical properties

The pH value was determined using a digital pH meter. The viscosity of the sample was determined using a viscometer. The

titratable acidity (ºTh) for the sample was determined by the method according to (Steffen, 1971; Sirirat and Jelena, 2010). Briefly, 20 ml of deionized water was added to 10 ml of milk and then 5 ml of alcoholic solution of phenolphthalein was added then the mixture was titrated with 1 M NAOH to the first persistent pink color. The amount of NAOH required was recorded. This amount was multiplied by 10 and gave the Th per 100 ml of milk.

Total Phenolic content

The total phenolic contents of Kefir produced from colored rice milk with and without UHT cow milk were determined by modified method of Singleton and Ross, (1965) using Folin-Ciocalteu's reagent. Briefly, 1 ml of colored rice milk kefir, color rice milk with and without UHT milk was added to 1 mL of gallic acid. Then 10 ml of water was added to the mixture after that, Folin-Ciocalteu's reagent (1:10) 0.5 ml was added and allowed to stand for 30 mins in room temp. At 20% (w/v) sodium carbonate (3 ml) and deionized water was added to the mixture and the volume was made to 50 ml. After being kept in darkness for 15 min, absorbance was measured at 725 nm using a spectrophotometer. The amount of total phenolic was calculated using the Gallic Acid Calibration Curve.

DPPH Free radical scavenging

The antioxidant activity of Kefir produced from colored rice milk with and without UHT milk was evaluated through a free radical scavenging effect on 2, 2-diphenyl-1- picrylhydrazyl (DPPH) radical. The determination was based on the method followed by Sirirat and Jelena, (2010). Briefly, 3 ml of DPPH solution was added to 0.1 ml of sample or 95% ethanol, which was used as control, mixed well and incubated for 30 min in a dark room at room temperature. Absorbance was measured at 517 nm using a spectrophotometer. The percentage of DPPH scavenging was calculated as:

$$\frac{(control\ absorbance - extract\ absorbance)}{(Control\ absorbance)} \times 100$$

Determination of Ferric Reducing /Antioxidant Power Assay (FRAP)

A FRAP assay was done with slight modification to the method of Benzie and Strain, (1999). FRAP reagent was prepared from acetate buffer (300 mM acetate buffer and adjusted to a pH of 3.6 by acetic acid and made up to 100 ml), 10 mM 2,4,6-Tripyridyl-s-Triazine TPTZ solution in 40 mM HCL and 20 mM iron (III) chloride solution and 300 mM acetate buffer in proportion of 1:1:10 (v/v) respectively. The prepared FRAP reagent was used for the experiment as follows: 300 µl sample or the standard was added to 1.7 ml of FRAP reagent. The mixture was mixed thoroughly and was incubated in the dark for 10 mins. The absorbance was measured at 595 nm using a spectrophotometer. The standard curve (r^2 = 0.9995) for FRAP was plotted with the absorbance at 595 nm and the values obtained were expressed in mM of ferrous equivalent Fe (II) per gram of sample.

$$\frac{(control\ absorbance - extract\ absorbance)}{(Control\ absorbance)} \times 100$$

Determination of Hydroxyl Radical (OH) scavenging activity

Hydroxyl radical (OH) scavenging activity was performed according to the method followed by Sirirat and Jelena, (2010).Briefly, 0.075 ml of sample was mixed with 0.2 M sodium phosphate buffer (pH of 0.7), 0.15 ml 2-Deoxyribose (10 mM), 0.15 ml of EDTA (10 mM), 0.15 mL of $FeSO_4$ (10 mM), 0.15 mL of hydrogen peroxide (10 mM), 0.525 ml of water. Samples were then incubated at 37ºC for 4 hrs. After incubation, 0.75 ml of trichloroactric acid (2.8%) acid and thiobarbituric (0.1%) acid were added. Then, samples were kept in a boiling water bath for 10 mins. The absorbance of each sample was measured at 520 nm and ethanol was used as a control. The percentage of hydroxyl scavenging was calculated as:

$$\frac{(control\ absorbance - extract\ absorbance)}{(Control\ absorbance)} \times 100$$

Statistical Analyses

Results obtained were reported as mean+ SD of triplicate measurements. Significant differences for multiple comparisons were determined by a two way analysis of variance (ANOVA) followed by a Duncan test with α = 0.05, significance level of $P < 0.05$ using SPSS (version 19).

Results and Discussion

Physiochemical properties

After the fermentation, a foamy drink was obtained. The viscosity of Kefir produced from 0, 24, 48 and 72 hrs fermented rice milk without UHT milk was slightly higher than the kefir produced from 0, 24, 48 and 72 hrs fermented rice milk with UHT milk. With regard to viscosity, the highest value was found to be 25 cps for kefir produced from 0 hrs fermented black rice milk without UHT milk. In terms of pH, the highest was found to be 4.23 for Kefir produced from fermented black rice milk with UHT milk. The highest acidity value was found to be 1.89 º Th for Kefir produced from 48 hrs fermented red rice milk without UHT milk. The values are presented in table 1. No significant difference was found in the acidity, pH and viscosity between the Kefir produced from fermented rice milk with and without UHT milk.

The results from physiochemical properties prove that, pH, viscosity (centipoises) and acidity (º Th) values decreased when the fermentation time was increased (Table 1). The decrease in viscosity may be due to the amount of polysaccharide content present in rice milk kefir during the start of fermentation till the end. Toba and group (1987) reported that, the viscosity of the fermented drink was directly proportional to the polysaccharide content. The decrease in acidity may be due to the growth of microorganisms and fermentation time. A study by Shiva *et al.,* (2011) suggests that the acid production in kefir depends on the growth of microorganisms and their ability for fermentation of the carbohydrates in milk and soymilk. Moreover, Sirirat and Jelena, (2010) stated that the decrease in pH may be due to increased lactic acid bacteria population at the beginning of fermentation.

Total Phenolic content

By the folin-Ciocalteu reagent method using gallic acid as standard, the average

quantity of total phenolic compounds (mg/l) was measured for Kefir produced from fermented brown, black and red rice milk with and without UHT milk. The measured values are represented in table 2. Values shown in all tables are means±standard deviation from triplicate experiments. The highest total phenolic content was found to be 1.60 (mg/L) for kefir produced from 48 hrs fermented red rice milk without UHT milk. Muntana and prasong (2010) also reported that red rice showed high total phenolic content and they also suggested that the higher color pigments may also be the main compounds for antioxidant activity. There was no significant difference at ($P<0.05$) between kefir produced from fermented rice milk without UHT milk and rice milk with UHT milk. Besides this, (Patrick and kalidas, 2005) proved soluble phenolic content of soymilk increased during the initial 40 hrs of culture time with active Kefir cultures.

According to Petti and Scully, (2009) phenolic compounds are plant metabolites characterized by the presence of several phenol groups. Some of them are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron, chelating metal ions in aqueous solutions. However, the phenolic compounds possess many biological properties such as antitumor, antimutagenic and antibacterial properties, and these activities might be related to their antioxidant activity (Shui and Leong, 2002). Some researchers have shown that pigmented rice, such as red and black rice are composed of high content of phenolic compounds (Oki *et al.,* 2002).

DPPH Radical Scavenging

The result presented in table 3 was expressed as % scavenging, while the highest % scavenging was found to be 64 % for Kefir produced from 0 hrs fermented brown rice milk and cow milk. On the other hand, kefir produced from 72 hrs fermented black rice milk showed the lowest % scavenging of 23.40 %. So, when the fermentation time was increased, the scavenging % decreased. According to our study the increased scavenging activity may be due to the milk protein or plant protein.

In addition, Liu and team (2005) proved that some antioxidant components were transferred from kefir grains to milk and soy-milk during fermentation. Besides this, Je Ruei Liu and group (2005) found that milks fermented by kefir grains demonstrated an enhanced activity as regards scavenging the DPPH radical as compared to unfermented milks, stating that kefir has got an influence on scavenging the DPPH radicals.

Moreover, (Villano *et al.,* 2007) proved that 1, 1-Diphenyl-2-picrylhydrazyl (DPPH; I) is a stable free radical. On accepting hydrogen from a corresponding donor, its solutions lose the characteristic deep purple (λmax 515–517 nm) colour. DPPH is very popular for the study of natural antioxidants.

Hydroxyl radical scavenging

The result presented in table 4 shows that Kefir produced from 24 hrs fermented red rice milk without UHT milk showed the highest scavenging activity of 99.37%. On the

contrary, when the fermentation time was increased to 72 hrs, the percentage of scavenging decreased to 50.12 for Kefir produced from fermented red rice milk with UHT milk. The higher % of hydroxyl scavenging may be due to the presence of polyphenolic substances in rice or kefir which can scavenge the free radicals. Gulcin and group (2010) stated that hydroxyl radical is the most highly reactive oxygen radical in the presence of transition metal ions and participates in free-radical reaction.

According to Boguslaw Lipinski, (2011) Antioxidant properties of polyphenols are based on their ability to be oxidized, polyphenols can scavenge hydroxyl radical (•OH) by virtue of their addition to double bonds with the formation of a corresponding hydroxyl derivative:

R–CH=CH–R + •OH ⟶ R–CH–(OH)–CH2–R.

(Patterson *et al.,* 2000)

Polyphenols are capable candidates for scavenging hydroxyl radicals which also indicates the uncertainity in the presence of polyphenols in rice milk kefir. Moreover, Q.D do and group (2013) found rice paddy herb contains high amount of antioxidants which play an important role in getting rid of hydroxyl radicals considered to be the free radicals causing several serious diseases such as cancer, heart disease, diabetes, etc.

Ferric Reducing antioxidant power

The result presented in table 5 shows that the FRAP values were higher for Kefir produced from 24 hrs fermented black rice milk with UHT milk, which was found to be 4.00 mg Fe (II) /L. The lower value was recorded for kefir produced from 24 hrs fermented brown rice milk without UHT milk, which was found as 1.33 mg Fe (II) /L. The higher FRAP values may be due to the active compounds present in rice, for the reason that , Je Ruei Liu and group (2005) found that milks fermented by kefir grains did not significantly affect their ferrous ion chelating ability However, Schafer and Buettner, (2001) stated that higher FRAP values give higher antioxidant capacity because FRAP value is based on reducing ferric ion, where antioxidants are the reducing agent and are compounds capable of donating a single electron or hydrogen atom for reduction.

Conclusions

The values obtained from physiochemical properties prove that the pH and acidity values increase with the addition of UHT milk. In relation to viscosity, without UHT milk the liquid was found more viscous. Moreover, the highest total phenolic content was found in kefir produced from 48 hours fermented red rice milk without UHT milk, and there were higher levels of phenolic compounds, which may act as antioxidant properties. There was no difference between the treatment and fermentation time. Furthermore, Kefir produced from both fermented rice milk without UHT milk and rice milk with UHT milk showed stronger antioxidant activity, provided that optimum fermentation time was maintained for greater antioxidant activity.

Kefir produced from 24 hrs fermented red rice milk without UHT milk showed the highest Hydroxyl scavenging

activity of 99.37%, which scavenged more hydroxyl radicals. This may be due to the phenolic content in the red rice. Red rice milk with UHT milk had higher DPPH radical scavenging with a percentage of 64.37 % which may be due to the presence or milk or plant protein. Our study proves that rice milk Kefir has greater antioxidant activity individually and also when combined with UHT milk. Study also indicates that the Rice used in this study certainly had a great influence towards antioxidant activity which may be be due to the presence of polyphenols. Colored rice milk Kefir can be a natural antioxidant supplement in the human diet. Further study should be conducted to identify the antioxidant compound and also processing of colored rice milk Kefir to be used as a food product.

Table1 Physiochemical properties (viscosity, pH and acidity) of Kefir produced from 0, 24, 48, 72 hrs fermented rice milk with and without UHT milk.

Treatment	**Viscosity(centipoises)**				**pH**				**Acidity(ºTh)**			
	0 hr	**24 hrs**	**48 hrs**	**72 hrs**	**0 hr**	**24 hrs**	**48 hrs**	**72 hrs**	**0 hr**	**24 hrs**	**48 hrs**	**72 hrs**
Kefir (Black rice milk without UHT milk)	25	14.58	10.41	8.33	4.09	3.96	3.75	3.70	0.351	0.51	0.387	0.342
Kefir (Black rice milk with UHT milk)	18.75	12.5	8.33	8.33	4.11	4.23	3.99	3.91	0.387	0.54	0.837	0.621
Kefir (Red rice milk without UHT milk)	16.67	8.33	12.5	2.08	4.1	3.69	3.59	3.54	1.71	1.602	1.89	0.603
Kefir (Red rice milk with UHT milk)	14.83	8.33	12.5	2.08	4.11	4.8	3.94	3.91	0.927	1.62	1.35	0.765
Kefir (Brown rice milk without UHT milk)	16.67	10.41	12.5	6.25	3.92	4.03	3.84	3.81	0.351	0.288	0.405	0.333
Kefir (Brown rice milk with UHT milk)	16.67	14.58	14.58	4.16	4.1	4.21	3.94	3.96	0.396	0.675	0.99	0.342

Data represented are mean values from triplicate experiments

Table 2 Total phenolic content of Kefir produced from 0, 24, 48, 72 hrs fermented rice milk with and without UHT milk

Treatment	Total phenolic content (mg/L)		
	0 hr	48 hrs	72 hrs
Kefir (Black rice milk without UHT milk)	0.26 ± 0.01 ^{bC}	0.63 ± 0.03 ^{bB}	0.44 ± 0.02 ^{bA}
Kefir (Black rice milk with UHT milk)	0.28 ± 0.01 ^{cC}	0.49 ± 0.01 ^{cB}	0.31 ± 0.01 ^{cA}
Kefir (Red rice milk without UHT milk)	0.26 ± 0.00 ^{aC}	1.60 ± 0.02 ^{aB}	0.55 ± 0.01 ^{aA}
Kefir (Red rice milk with UHT milk)	0.21 ± 0.01 ^{cbC}	0.44 ± 0.02 ^{cbB}	0.50 ± 0.02 ^{cbA}
Kefir (Brown rice milk without UHT milk)	0.23 ± 0.01 ^{dC}	0.31 ± 0.03 ^{dB}	0.28 ± 0.01 ^{dA}
Kefir (Brown rice milk with UHT milk)	0.23 ± 0.01 ^{abC}	1.20 ± 0.02 ^{abB}	0.62 ± 0.01 ^{abA}

Values with different lowercase letters in the same row and values with different uppercase letters in the same column have significant difference ($p < 0.05$). No significant difference was found.

Table 3 DPPH Free radical scavenging of Kefir produced from 0, 24, 48, 72 hrs fermented rice milk with and without UHT milk

Treatment (1000 ppm)	DPPH (% Scavenging)			
	0 hr	24 hrs	48 hrs	72 hrs
Kefir (Black rice milk without UHT milk)	57.80±0.39eA	22.94±0.16eB	53.57±0.37eA	23.40±0.48eC
Kefir (Black rice milk with UHT milk)	43.24±0.47dA	48.37±0.21dB	55.85±0.36dA	24.38±0.52dC
Kefir (Red rice milk without UHT milk)	58.50±3.59aA	56.65±0.26aB	48.20±0.32aA	34.57±0.33aC
Kefir (Red rice milkwith UHT milk)	64.37±0.67aA	44.21±0.24aB	53.05±0.58aA	45.96±0.47aC
Kefir (Brown rice milk without UHT milk)	52.53±0.42bA	46.07±0.41bB	50.12±0.75bA	45.86±0.74bC
Kefir (Brown rice milk with UHT milk)	42.57±0.52cA	42.82±0.21cB	54.62±0.45cA	44.81±0.47cC

Values with different lowercase letters in the same row and values with different uppercase letters in the same column have significant difference ($p < 0.05$). No significant difference was found.

Table 4 Hydroxyl radical scavenging of Kefir produced from 0, 24, 48, 72 hrs fermented rice milk with and without UHT milk

Treatment (1000 ppm)	**Hydroxyl radical (% Scavenging)**			
	0 hr	**24 hrs**	**48 hrs**	**72 hrs**
Kefir (Black rice milk without UHT milk)	98.11±1.46bcB	97.55±0.95bcA	51.13±0.31bcD	97.07±0.21bcC
Kefir (Black rice milk with UHT milk)	93.85±0.63eB	93.57±0.69eA	60.42±0.33eD	64.68±0.18eC
Kefir (Red rice milk without UHT milk)	92.22±4.81bB	99.37±0.27bA	58.85±0.16bD	95.91±0.10bC
Kefir (Red rice milk with UHT milk)	85.26±0.36dB	85.95±0.38dA	53.25±0.12dD	50.12±0.10dC
Kefir (Brown rice milk without UHT milk)	94.20±0.90cB	95.94±0.26cA	62.48±0.10cD	87.77±0.05cC
Kefir (Brown rice milk with UHT milk) milk	98.67±0.71aB	98.95±0.10aA	68.73±0.21aD	94.90±0.16aC

Values with different lowercase letters in the same row and values with different uppercase letters in the same column have significant difference ($p < 0.05$). No significant difference was found.

Table 5 FRAP of Kefir produced from 0, 24, 48, 72 hrs fermented rice milk with and without UHT milk

Treatment (1000 ppm)	**FRAP (mg Fe(II) /L)**			
	0 hr	**24 hrs**	**48 hrs**	**72 hrs**
Kefir (Black rice milk without UHT milk)	3.34±0.01aC	3.76±0.68aC	3.32±0.04aB	3.37±0.21aA
Kefir (Black rice milk with UHT milk)	3.17±0.01aC	4.00±0.03aC	3.83±0.24aB	3.11±0.17aA
Kefir (Red rice milk without UHT milk)	3.02±0.66bC	3.04±0.14bC	3.31±0.09bB	3.60±0.19bA
Kefir (Red rice milk with UHT milk)	3.15±0.00cC	3.26±0.01cC	2.44±0.13cB	3.34±0.03cA
Kefir (Brown rice milk without UHT milk)	2.91±0.00dC	1.33±0.23dC	3.48±0.02dB	3.34±0.06dA
Kefir (Brown rice milk with UHT milk) milk	2.55±0.01dC	2.60±0.61dC	2.69±0.18dB	3.43±0.13dA

Values with different lowercase letters in the same row and values with different uppercase letters in the same column have significant difference ($p < 0.05$). No significant difference was found.

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