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The Effect of Some Fermentation Conditions on the Production of Kefiran by Kefir Grains in Fermented Milk

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Abstract
Kefir is produced from the fermentation of milk by microorganisms in kefir grains. Kefir grains include lactic acid bacteria, yeast, and acetic acid bacteria surrounded by a protein matrix and polysaccharide called kefiran. One of the most important antibacterial compounds of this beverage is microbial exopolysaccharide of kefiran. The present study aimed to investigate the effect of milk type, fermentation time, temperature, and stirring conditions on the production of kefiran by kefir grains. Activated kefir grains were added to full-fat and non-fat milk. Fermentation was carried out at 25 and 37 °C under stirred and non-stirred. After 24, 48, 72, and 120 h of fermentation, the grains were separated from kefir extract and kefiran exopolysaccharide was extracted from kefir grains. The effect of these variables and their interaction on the production of kefiran using Design-Expert software and full factorial design have been analyzed. Also, the MIC and MBC of extracted kefiran were determined against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus,* and *Shigella dysenteriae.* All fermentation conditions and their interactions had a significant effect on kefiran production. Considering all fermentation conditions and interaction of all factors, more kefiran were produced in fermented kefir grains at 37 °C compared to 25 °C, in full-fat milk compared to non-fat milk, under stirred conditions compared to non-stirred and fermented beverages for 48 and 120 h. MIC and MBC of extracted kefiran for tested bacteria were determined in the range of 1.4-11.25 mg/mL.

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Introduction

Today, the consumption of fermented milk as probiotic products has raised interest due to its beneficial properties, including improved digestion (Hertzler & Clancy, 2003), antibacterial effect (Rodrigues, Caputo, Carvalho, Evangelista, & Schneedorf, 2005), hypocholesterolemia effect (Taylor & Williams, 1998), control of plasma glucose (Hadisaputro, Djokomoeljanto, & Soesatyo, 2012), antihypertensive effect (Maeda, Zhu, Omura, Suzuki, & Kitamura, 2004), anti-inflammatory effect (Lee *et al.*, 2007; Rodrigues *et al.*, 2005), antioxidant activity (Guzel-Seydim, Seydim, & Greene, 2003), anti-carcinogenic activity (Gao *et al.*, 2013) and anti-allergenic activity (Lee *et al.*, 2007).

Kefir beverage is one of the fermented milk products produced from the fermentation of milk by microorganisms in kefir grains. One of the characteristics that have been introduced for this beverage is its antimicrobial properties (Leite *et al.*, 2013; Prado *et al.*, 2015).

Numerous factors including the production of organic acids, peptides (bacteriocins), exopolysaccharide of kefiran, hydrogen peroxide, carbon dioxide, ethanol, and diacetyl are involved in this antimicrobial activity (Leite *et al.*, 2013; Moradi & Kalanpour, 2019; Prado *et al.*, 2015).

Kefir grains are gelatinous granules, which have irregular shape, and white to yellow consisting of a mixture of microorganisms including lactic acid bacteria, yeasts, and acetic acid bacteria that coexist in a complex symbiotic relationship (Farnworth, 2006; Prado *et al.*, 2015).

One of the important features of kefir grains is that after numerous cultivations in suitable conditions, their physiological and functional characteristics are preserved and their properties are recovered with the growth of the grains (POP *et al.*, 2014).

Due to the different growth requirements of microorganisms in kefir grains, cultivations conditions such as fermentation time, temperature, stirring conditions, and substrate type (milk type) can have a significant impact on the microorganism proportions in the grains, the growth of each of these microorganisms, and thus the biological characteristics of the final product (Rattray & O'connell, 2011).

The microorganisms in kefir grains are covered by a protein matrix and polysaccharide called kefiran. kefirn is a branch glucogalactane of water-soluble which consists of the equal amounts of D-galactose and D-glucose. The most important species producing this polysaccharide in kefir grains are *Lactobacillus kefiranofacience* and *Lactobacillus kefir*. Scientific reports have attributed the biological activities of this beverage, including its antimicrobial and antitumor activities, to this polysaccharide (Frengova, Simova, Beshkova, & Simov, 2002; Zajšek & Goršek, 2011). Also, kefiran has significant potential as a food gum in the food industry, in the production of new packaging materials, or as a food enhancer due to its health benefits (Piermaria, Pinotti, Garcia, & Abraham, 2009; Prado *et al.*, 2015).

Adjusting the fermentation conditions and composition of milk can play a role in the kefiran production and kefir grains (Frengova *et al.*, 2002; Zajšek & Goršek, 2011).

In the usual and traditional method of kefir production, fermentation is performed at 20-25 °C for 18-24 h (Farag, Jomaa, El-Wahed, & R El-Seedi, 2020).

Many studies have produced kefir and extracted of kefiran from kefir grains in different culture media and different fermentation conditions and have pointed to the effect of different fermentation conditions on kefiran production. (Dias *et al.*, 2012; Florence *et al.*, 2012; Harta *et al.*, 2004; Ismaiel, Ghaly, & El-Naggar, 2011; Kim *et al.*, 2016; Kukhtyn *et al.*, 2018; Paraskevopoulou *et al.*, 2003; POP *et al.*, 2014; Rahimzadeh, Golnar, Bahar, & Amir Mozaffari, 2012; Rahimzadeh, G, Fazeli, Mozafari, & Mesbahi, 2015; Taniguchi, Nomura, Itaya, & Tanaka, 2001; Weschenfelder, Paim, Gerhardt, Carvalho, & Wiest, 2018; Yeesang, Chanthachum, & Cheirsilp, 2008; Yokoi & Watanabe, 1992; Zajšek & Goršek, 2011).

This study aimed to investigate the effect of some fermentation conditions including type of milk, fermentation time, temperature, and stirring conditions on the production of kefiran by kefir grains in fermented milk. Furthermore, the interactions of these variables using Design-Expert software have been analyzed. Also the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of extracted kefiran against four pathogenic gastrointestinal bacteria were determined.

Materials and methods

The process of fermentation of kefir grains

The method presented by Ajam & Koohsari (2020) was used to perform the fermentation process. Briefly, Kefir grains were recovered in milk. Kefir grains were recovered by sequential subcultures in milk for 4 days at 25 °C. The milk was replaced every 24 h. Then, the 5 g of kefir grains added to 50 mL of full-fat (Fat content=3.25%) and non-fat milk (Skim milk powder + Water) and samples were incubated at 25 and 37 °C under stirred (80 rpm) and non-stirred conditions. At 24, 48, 72, and 120 h intervals, kefir grains were separated from the fermentation product with a sieve and were used for the extraction of kefiran.

Extraction of kefiran

50 mL of boiling water was added to kefir grains and placed on a magnetic heater for 3 hours to dissolve the kefiran polysaccharide and inactivate the hydrolyzing enzymes. Upon reaching ambient temperature, 8.5 mL of 80% trichloroacetic acid was added to each of the treatments, and after overnight the samples were centrifuged at 4 °C for 20 min at 10,000 Xg. The same volume of supernatant was added to ethanol 97% and to precipitate of kefiran, the samples were stored overnight, at -20 °C. The solution was centrifuged at 4 °C for 20 min at 10,000 Xg and the precipitate was washed with boiling water. The washing procedure of the precipitate was repeated 3 times in boiling water, and the final precipitate was dried at 42 °C for 48 h. Finally, the dry weight of kefiran was measured by percentage (Rimada & Abraham, 2003; Zajšek & Goršek, 2011).

Preparation of bacterial strains

The strains of the tested bacteria were 2 gram-negative bacteria of *Escherichia coli* (PTCC 1338) and *Shigella dysenteriae* (PTCC 1188), and 2 gram-positive bacteria of *Staphylococcus aureus* (PTCC 1112) and *Bacillus cereus* (PTCC 1154). They were prepared from the Iranian Research Organization for Science and Technology (IROST) in a lyophilized form. Then, they were recovered in the Brain Heart Infusion (BHI) medium (Merck) for 24 h at 37 °C in the microbiology laboratory of the Azadshahr Branch, Islamic Azad University. The 24-hour culture of each bacterium was inoculated into Nutrient Broth culture medium (Merck) and it was incubated at 37 °C to obtain turbidity equal to 0.5 McFarland $=1.5\times10^8$ CFU/mL (Clinical and Laboratory Standards Institute [CLSI], 2018).

Determination of MIC and MBC of extracted kefiran

The determination of MIC of extracted kefiran was carried out based on turbidimetric assay and by using the macrodilution tube method. For this purpose, the method presented by CLSI (2018) was used. with some modifications serial dilutions of extracted kefiran (1000 mg/mL) were prepared in Nutrient Broth then to each of these tubes from was added, suspensions of 5×10^5 CFU/mL from each of the bacteria and incubated for 24 h at 37 °C. There were also control tubes negative controls (without bacterial suspension) and positive controls (without extracted kefiran). The results after 24 h of incubation for microbial turbidity were recorded. The last dilution in which microbial turbidity was not observed, as the MIC was considered. For the determination of MBC, from the tube that contained extracted kefiran concentrations higher than the MIC were cultured onto the Nutrient agar medium. The MBC was defined as the lowest concentration that allowed no visible growth on the agar (CLSI, 2018).

Statistical design and data analysis

The study was conducted in factorial template with randomized subtype and the full factorial design. Factors included two levels of the type of milk, 2 levels of stirring conditions, 2 temperature levels, and 4 levels of fermentation time. The responses include the kefir extraction percentage (%) (Supplementary file).

Design-Expert software (Version 12.0.3.0) was used for data analysis and draw charts.

Results and discussion

In Table (1) presents the mean of the kefiran extraction amounts (%) included 32 treatments, related to the effect of different fermentation conditions i.e. 2 levels of the type of milk, 2 levels of stirring conditions, 2 temperature levels and 4-time levels on mean of the kefiran extraction amounts by percentage. According to the analysis of variance, the results of the mean of the kefiran extraction amounts showed that all fermentation conditions including temperature, stirring conditions, time and milk type had significant effect on kefiran extraction amounts from kefir grains (Table 2). The interactions of these variables also had a significant effect on kefiran extraction from kefir grains ($P \leq 0.0001$).

The interaction of three factors, temperature with stirring conditions and time and three factors, temperature with time, and type of milk had significant effects on kefiran extraction amounts from kefir grains. Even the interaction of the four factors tested had a significant effect on kefiran extraction (Table 2). Given that the difference between Predicted R^2 (0.8) and Adjusted R^2 (0.87) is less than 0.2 and Adeq Precision equals 18.88 (greater than 4), indicates the model's desirability and them navigation (Table 2).

Table 1. The mean of the kefiran extraction amounts (%) in different fermentation conditions

Treatment	Temperature (°C)	Type of milk*	Stirred/Non Stirred**	Time (h)	Extraction amounts (%)
1	25	NFM	NS	24	0.367 ± 0.19
2	25	NFM	NS	48	0.720 ± 0.16
3	25	NFM	NS	72	1.773 ± 0.22
4	25	NFM	NS	120	2.760 ± 0.09
5	25	NFM	S	24	0.447 ± 0.31
6	25	NFM	S	48	0.913 ± 0.35
7	25	NFM	S	72	2.080 ± 0.24
8	25	NFM	S	120	2.973 ± 0.37
9	25	FFM	NS	24	2.033 ± 0.28
10	25	FFM	NS	48	3.660 ± 0.14
11	25	FFM	NS	72	1.807 ± 0.36
12	25	FFM	NS	120	2.017 ± 0.28
13	25	FFM	S	24	2.390 ± 0.10
14	25	FFM	S	48	2.967 ± 0.37
15	25	FFM	S	72	1.993 ± 0.03
16	25	FFM	S	120	0.753 ± 0.16
17	37	NFM	NS	24	0.293 ± 0.09
18	37	NFM	NS	48	2.260 ± 0.10
19	37	NFM	NS	72	1.917 ± 0.03
20	37	NFM	NS	120	2.090 ± 0.24
21	37	NFM	S	24	2.677 ± 0.09
22	37	NFM	S	48	2.283 ± 0.10
23	37	NFM	S	72	1.907 ± 0.38
24	37	NFM	S	120	2.633 ± 0.09
25	37	FFM	NS	24	2.793 ± 0.09
26	37	FFM	NS	48	1.927 ± 0.03
27	37	FFM	NS	72	1.517 ± 0.10
28	37	FFM	NS	120	1.767 ± 0.61
29	37	FFM	S	24	2.990 ± 0.37
30	37	FFM	S	48	1.853 ± 0.35
31	37	FFM	S	72	1.327 ± 0.08
32	37	FFM	S	120	2.460 ± 0.31

*NFM: Non Fat Milk, FFM: Full Fat Milk
**NS: Non-Stirred, S: Stirred
***Data are means of 3 replicates

Table 2. P-value and other parameters extracted from Analysis of Variance Table

Source	P-value
Model	<0.0001
A: Temperature	0.0037
B: Type of Stirring (Stirred/Non Stirred)	0.0048
C: Time (Hour)	<0.0001
D: Type of milk (Full fat/Non fat)	<0.0001
AB	<0.0001
AC	<0.0001
AD	<0.0001
BC	<0.0001
BD	<0.0001
CD	<0.0001
ABC	0.0001
ABD	0.9161
ACD	<0.0001
BCD	0.1599
ABCD	<0.0001
R^2	0.9131
Adjusted R^2	0.8710
Predicted R^2	0.8044
Adeq Precision	18.8844

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$

Effect of milk type on kefiran extraction from kefir grains

Milk type had a significant effect on kefiran extraction amounts from grains kefir ($P<0.0001$) (Table 2). As the highest amounts of kefiran were extracted from fermented kefir grains in full-fat milk (Table 1) also, considering all factors and their interactions, more kefiran was extracted from fermented kefir grains in full-fat milk compared to non-fat milk (Fig. 1 and 2).

Zajšek & Goršek (2011) also proposed full-fat cows' milk as the best environment for the production of kefiran from kefir grains.

Weschenfelder *et al.* (2018) reported more antibacterial activity of prepared kefir samples in standard commercial pasteurized milk (1.5-2% Fat) compared to prepared kefir samples in from skimmed milk.

This may be related to the production of compounds of the antimicrobial nature by the microorganisms present in kefir grains. Kefiran is one of the most important factors in the antibacterial activity of kefir beverage and adjusting the fermentation conditions and composition of milk can play a role in the kefiran production (Frengova *et al.*, 2002; Zajšek & Goršek, 2011). More antibacterial activity of produced kefir samples in pasteurized milk, which has more fat than skimmed milk, indicates the production of more kefiran in these samples.

The growth of kefir grains in milk with different composition depends on the existence and amount of substrates needed for the growth of microorganisms in kefir grains, including protein and lactose.

The Effect of fermentation time on kefiran extraction from kefir grains

Fermentation time had a significant effect on kefiran extraction amounts from grains kefir ($P<0.0001$) (Table 2). Although the highest amount of kefiran was extracted from fermented kefir grains for 48 h (Table 1), considering all factors and their interactions, the highest amount of kefiran was extracted from fermented kefir grains for 48 and 120 h (Fig. 1 and 2).

In a study of several physicochemical analyzes of produced kefir under different fermentation conditions, Ismaiel *et al.* (2011) concluded that the highest produced kefiran and biomass of kefir grains were found in samples of fermented kefir in non-fat cows' milk at 30 °C for 120 h.

Of course, there are various reports on the optimum of fermentation time for kefiran extraction from kefir grains. Some studies have reported short 24-hour fermentation times (POP *et al.*, 2014; Zajšek & Goršek, 2011). On the other hand, some studies reported longer times, such as 7 days (Maeda, Zhu, Suzuki, Suzuki, & Kitamura, 2004) and 10 days (Taniguchi *et al.*, 2001) for producing the highest amount of kefiran by *Lactobacillus kefiranofaciens*.

In the present study, the highest kefiran extraction was observed after 48 hours of fermentation and then, with increasing fermentation time, we observed a decrease in kefiran production.

It has also been observed in the study of Pop et al. That the mass of kefir grains increased after 24 h, but it decreased at 48 and 72 h at 25 °C. Reduced nutrient or excess acidity for biological activities due to lactic acid production may be the reason. Of course, there are also reports of lower levels of exopolysaccharides even during cold storage (Kök-Taş, Seydim, Özer, & Guzel-Seydim, 2013; Ramchandran & Shah, 2009). Logical justification for the decrease in extracted kefiran content may be the hydrolysis of the polysaccharide in its monomers by enzymatic degradation (POP *et al.*, 2014).

Effect of stirring conditions on kefiran extraction from kefir grains

Stirring conditions had a significant effect on kefir extraction amounts from grains kefir (P=0.0048) (Table 2). Although the highest amounts of kefiran were extracted from fermented kefir grains in non-stirred conditions (Table 1), considering all factors and their interactions, more kefiran was extracted from kefir samples prepared with stirred conditions compared to non-stirred samples (Fig. 1 and 2).

Studies have indicated the role of stirring in increasing kefiran production (POP *et al.*, 2014; Zajšek & Goršek, 2011).

Stirring with increasing mixing preserves homogeneous fermentation conditions as well as enhances nutrient and air mass transfer, which justifies more kefiran extraction in stirred media than non-stirred media (Zajšek & Goršek, 2011). Of course, there is also research that has reported higher amounts of kefiran extracted in non-stirring conditions (Ismaiel *et al.*, 2011).

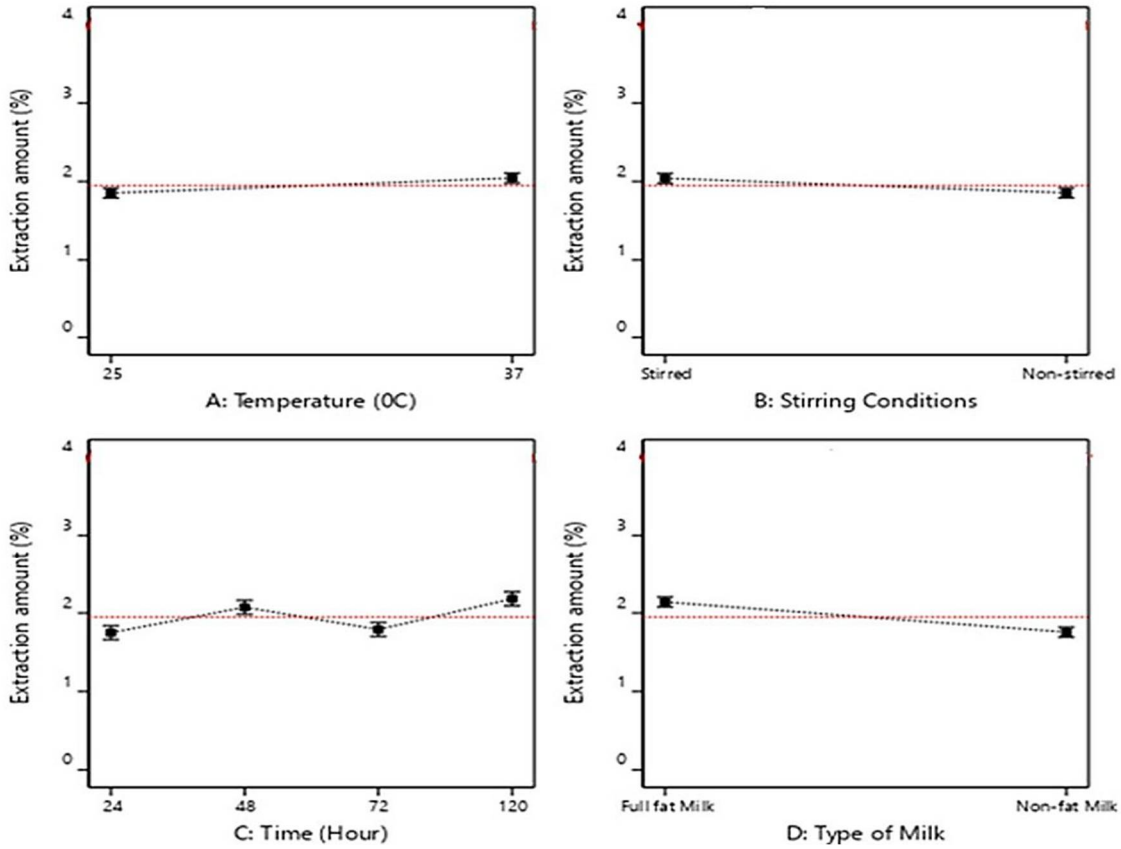

Fig. 1. Effects of fermentation conditions on extraction amounts (%) of kefiran (Factors involved in multiple interactions)

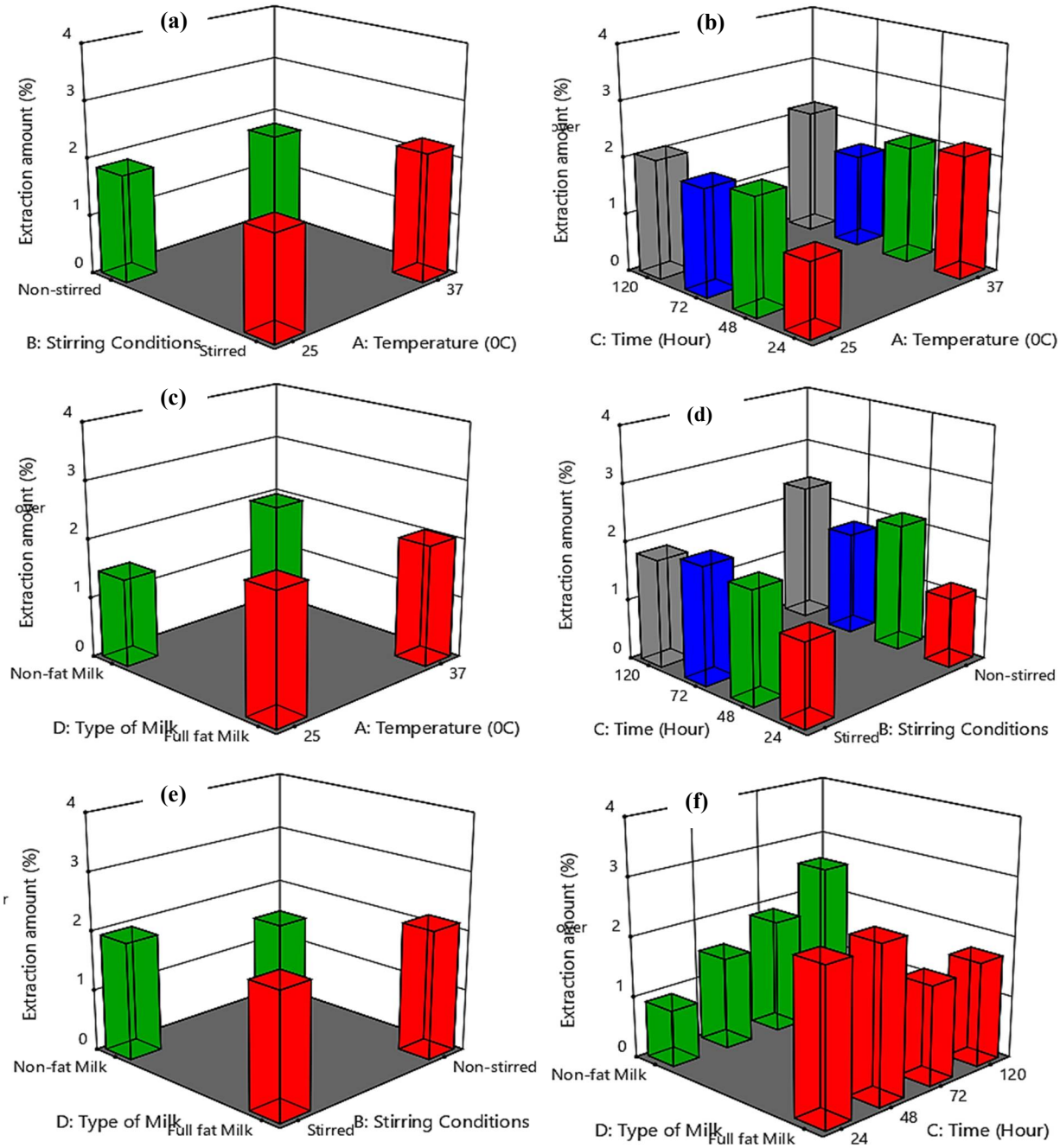

Fig. 2. Extraction amounts (%) of kefiran in samples (Stirred and Non-stirred in 25 and 37 °C (a), Different times in 25 and 37 °C (b), Full fat and Non-fat milk in 25 and 37 °C (c) Stirred and Non-stirred in different times (d), Full fat and Non-fat milk stirred and Non-stirred (e) and Full fat and Non-fat milk in different times (f)

The effect of temperature on kefiran extraction from kefir grains

Temperature had a significant effect on kefir extraction amounts from grains kefir (P<0.0001) (Table 2). Although the highest amounts of kefiran were extracted from fermented kefir grains at 25 °C (Table 1), considering all factors and their interactions, more kefiran were extracted from kefir samples prepared at 37 °C compared to the samples prepared at 25 °C (Fig. 1 and 2).

Since kefir traditional beverages are prepared at 25 °C for 18-24 h (Farnworth & Mainville, 2008). Several studies have indicated the highest amounts of kefiran extraction from kefir grains at 25 °C (POP *et al.*, 2014; Zajšek & Goršek, 2011).

Maximum production of kefiran at 25 °C is due to the fact that microorganisms protect themselves against environmental

effects by increasing kefiran production (Zajšek & Goršek, 2011).

Studies have also reported the highest amounts of kefiran extraction from kefir grains at 30 °C (Ismaiel *et al.*, 2011).

Kefiran production is mostly done by *L. kefiranofaciens* and increases with control of culture conditions (Frengova *et al.*, 2002; Zajšek & Goršek, 2011). Numerous studies have reported temperature of 30 °C as the optimum temperature for the most production of kefiran by *L. kefiranofaciens* (Taniguchi *et al.*, 2001; Yeesang *et al.*, 2008; Yokoi & Watanabe, 1992).

Decreasing of kefiran extraction from kefir grains at higher temperatures can be attributed to the dissolving of this exopolysaccharide at high temperatures.

Rimada & Abraham (2001) investigated kefiran production from kefir grains in whey and concluded that at 43 °C the reduction in kefiran production and the growth rate of kefir grains reached their maximum, which may be because the kefir produced at high temperature dissolves and is transferred to the culture medium.

With all these interpretations, the amount of extracted kefiran from kefir grains also depends on the extraction method. Rimada & Abraham (2003) showed that the amount of extracted exopolysaccharide from the culture medium of kefir grains was highly dependent on method of exopolysaccharide extraction. Extraction of exopolysaccharide with one step of heat treatment resulted in more exopolysaccharide than non-heat treatment. The use of heat treatment in addition to isolation and dissolving the polysaccharides attached to the cell wall of the microorganisms and fermented product proteins also inactivates potential enzymes that are potentially capable of decomposing the polymers (Rimada & Abraham, 2003).

MIC and MBC of extracted kefiran

To determination of MIC and MBC, were used from extracted kefiran samples under fermentation conditions in full-fat milk, under stirred conditions, at 25 °C and fermentation time of 48 h. MIC and MBC of extracted kefiran samples were determined for the tested bacteria in the range of 1.4-11.25 mg/mL (Table 3).

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracted kefiran (mg/mL)

Bacteria	MIC	MBC
S. dysenteriae	1.40	1.40
S. aureus	5.60	5.60
E. coli	11.25	11.25
B. cereus	2.80	2.80

Rodrigues *et al.* (2005) reported MIC and MBC levels of extracted kefiran from kefir grains of 4.62 and 4.94 mg/mL, respectively. The bacterium of Gram-positive of *Streptococcus pyogenes* was reported to be the most susceptible and *S. aureus*, *Streptococcus salivarius*, *Salmonella typhimurium*, *Candida albicans,* and *Listeria monocytogenes* were in the next susceptibility class. *Pseudomonas aeruginosa* and *E. coli* were identified as the most resistant to extracted kefiran from kefir grains (Rodrigues *et al.*, 2005). In the present study, *E. coli* with MIC and MBC of 11.25 mg/mL was identified as the most resistant to the extracted kefiran.

One of the particular cases in the study of Rodrigues *et al.* (2005) is the proximity of the MIC and MBC that were also observed in the present study. The similarity of MIC and MBC levels or the proximity between the two indicates the Lethal activity of the tested compound (Rodrigues *et al.*, 2005).

Rezaei, Zaghian, & Emtiazi (2012) reported the antibacterial activity of extracted kefiran from kefir grains against gram-positive bacteria of *B. cereus*, *S. aureus,* and *L. monocytogenes*. They also observed resistance of *E. coli* and *P. aeruginosa* to extracted kefiran from kefir grains (Rezaei *et al.*, 2012).

Conclusions

The highest amounts of kefiran (3.66%) was extracted from fermented kefir grains in full-fat milk under non-stirred conditions, at 25 °C after 48 h. However,

considering all factors and their interactions, more kefiran were extracted from fermented kefir grains at 37 °C, compared to 25 °C, in full-fat milk compared to non-fat milk, under stirred conditions compared to non-stirred and fermented beverages for 48 and 120 h. MIC and MBC of extracted kefiran for tested bacteria were determined in the range of 1.4-11.25 mg/mL.

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References

Ajam, F., & Koohsari, H. (2020). Effect of some fermentation conditions on antibacterial activity of fermented milk by kefir grains. *Journal of Food Processing and Preservation, 44*(12), e14913. doi:https://doi.org/10.1111/jfpp.14913

Clinical and Laboratory Standards Institute [CLSI]. (2018). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. In (11th ed. CLSI standard M07 ed.): Clinical and Laboratory Standards Institute, 950 West Valley Road, Suit 2500, Wayne, Pennsylvania 19087 USA.

Dias, P. A., Silva, D. T. d., Tejada, T. S., Leal, M. C. G. M., Conceição, R. d. C. d. S. d., & Timm, C. D. (2012). Survival of pathogenic microorganisms in kefir. *Revista do Instituto Adolfo Lutz (Impresso), 71*(1), 182-186 .

Farag, M .A., Jomaa, S. A., El-Wahed, A., & R El-Seedi, H. (2020). The many faces of kefir fermented dairy products: Quality characteristics, flavour chemistry, nutritional value, health benefits, and safety. *Nutrients, 12*(2), 346. doi:https://doi.org/10.3390/nu12020346

Farnworth, E. R. (2006). Kefir–a complex probiotic. *Food Science and Technology Bulletin: Fu, 2*(1), 1-17 .

Farnworth, E. R., & Mainville, I. (2008). Kefir-A fermented milk product. In E. R. Farnworth (Ed.), *Handbook of Fermented Functional Foods* (Second ed.): CRC Press.

Florence, A. C. R., Oliveira, R. P., Silva, R. C., Soares, F. A., Gioielli, L. A., & Oliveira, M. N. (2012). Organic milk improves Bifidobacterium lactis counts and bioactive fatty acids contents in fermented milk. *LWT, 49*(1), 89-95 .doi:https://doi.org/10.1016/j.lwt.2012.04.023

Frengova, G. I., Simova, E. D., Beshkova, D. M., & Simov, Z. I. (2002). Exopolysaccharides produced by lactic acid bacteria of kefir grains. *Zeitschrift für Naturforschung C, 57*(9-10), 805-810. doi:https://doi.org/10.1515/znc-2002-9-1009

Gao, J., Gu, F., Ruan, H., Chen, Q., He, J., & He, G. (2013). Induction of apoptosis of gastric cancer cells SGC7901 in vitro by a cell-free fraction of Tibetan kefir. *International Dairy Journal, 30*(1), 14-18. doi:https://doi.org/10.1016/j.idairyj.2012.11.011

Guzel-Seydim, Z., Seydim, A., & Greene, A. (2003). Comparison of amino acid profiles of milk, yogurt and Turkish Kefir. *Milchwissenschaft-Milk Science International, 58*, 158-160 .

Hadisaputro, S., Djokomoeljanto, R & ,.Soesatyo, M. (2012). The effects of oral plain kefir supplementation on proinflammatory cytokine properties of the hyperglycemia Wistar rats induced by streptozotocin. *Acta Med Indones, 44*(2), 100-104 .

Harta, O., Iconomopoulou, M., Bekatorou, A., Nigam ,P., Kontominas, M., & Koutinas, A. (2004). Effect of various carbohydrate substrates on the production of kefir grains for use as a novel baking starter. *Food Chemistry, 88*(2), 237-242. doi:https://doi.org/10.1016/j.foodchem.2003.12.043

Hertzler, S. R & ,.Clancy, S. M. (2003). Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. *Journal of the American Dietetic association, 103*(5), 582-587. doi:https://doi.org/10.1053/jada.2003.50111

Ismaiel, A. A., Ghaly, M. F., & El-Naggar ,A. K. (2011). Some physicochemical analyses of kefir produced under different fermentation conditions. *Journal of Scientific and Industrial Research, 70*(5), 365-372 .

Kim, D.-H., Jeong, D., Kim, H., Kang, I.-B., Chon, J.-W., Song, K.-Y., & Seo, K.-H. (2016). Antimicrobial activity of kefir against various food pathogens and spoilage bacteria. *Korean journal for food science of animal resources, 36*(6), 787. doi:https://doi.org/10.5851/kosfa.2016.36.6.787

Kök-Taş, T., Seydim, A. C., Özer, B., & Guzel-Seydim, Z. B. (2013). Effects of different fermentation parameters on quality characteristics of kefir. *Journal of dairy science, 96*(2), 780-789. doi:https://doi.org/10.3168/jds.2012-5753

Kukhtyn, M., Vichko, O., Kravets, O., Karpyk, H., Shved, O., & Novikov ,V. (2018). Biochemical and microbiological changes during fermentation and storage of a fermented milk product prepared with Tibetan Kefir Starter. *Archivos Latinoamericanos De Nutricion, 68*(4), 336-343 .

Lee, M.-Y., Ahn, K.-S., Kwon, O.-K., Kim, M.-J ,.Kim, M.-K., Lee, I.-Y., . . . Lee, H.-K. (2007). Anti-inflammatory and anti-allergic effects of kefir in a mouse asthma model. *Immunobiology, 212*(8), 647-654. doi:https://doi.org/10.1016/j.imbio.2007.05.004

Leite, A. M. d. O., Miguel, M. A. L., Peixoto, R .S., Rosado, A. S., Silva, J. T., & Paschoalin, V. M. F. (2013). Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. *Brazilian Journal of Microbiology, 44*(2), 341-349 .

Maeda, H., Zhu, X., Omura, K., Suzuki ,S., & Kitamura, S. (2004). Effects of an exopolysaccharide (kefiran) on lipids, blood pressure, blood glucose, and constipation. *Biofactors, 22*(1-4), 197-200. doi:https://doi.org/10.1002/biof.5520220141

Maeda, H., Zhu, X., Suzuki, S., Suzuki, K., & Kitamura, S. (2004). Structural characterization and biological activities of an exopolysaccharide kefiran produced by Lactobacillus kefiranofaciens WT-2BT. *Journal of agricultural and food chemistry, 52*(17), 5533-5538. doi:https://doi.org/10.1021/jf049617g

Moradi, Z., & Kalanpour, N. (2019). Kefiran, a branched polysaccharide: Preparation, properties and applications: A review. *Carbohydrate polymers, 223*, 115100. doi:https://doi.org/10.1016/j.carbpol.2019.115100

Paraskevopoulou, A., Athanasiadis, I., Kanellaki ,M., Bekatorou, A., Blekas, G., & Kiosseoglou, V. (2003). Functional properties of single cell protein produced by kefir microflora. *Food research international, 36*(5), 431-438. doi:https://doi.org/10.1016/S0963-9969(02)00176-X

Piermaria, J. A., Pinotti ,A., Garcia, M. A., & Abraham, A. G. (2009). Films based on kefiran, an exopolysaccharide obtained from kefir grain: Development and characterization. *Food hydrocolloids, 23*(3), 684-690. doi:https://doi.org/10.1016/j.foodhyd.2008.05.003

POP, C., Apostu, S ,.Salanţă, L., Rotar, A. M., Sindic, M., Mabon, N., & SOCACIU, C. (2014). Influence of different growth conditions on the kefir grains production used in the kefiran synthesis. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca: Food Science and Technology, 71*(2), 147-153. doi:https://doi.org/10.15835/buasvmcn-fst:10802

Prado, M. R., Blandón, L. M., Vandenberghe, L. P., Rodrigues, C., Castro, G. R., Thomaz-Soccol, V., & Soccol, C. R. (2015). Milk kefir: composition, microbial cultures, biological activities, and related products. *Frontiers in microbiology, 6*, 1177. doi:https://doi.org/10.3389/fmicb.2015.01177

Rahimzadeh, G., Bahar, M. A., & Amir Mozaffari, N. (2012). Antimicrobial activity Kefir on different time fermentation. *Iranian Journal of Medical Microbiology, 5*(4), 35-41 .

Rahimzadeh, G., Fazeli, M., Mozafari, A. N., & Mesbahi, M. (2015). Evaluation of anti-microbial activity and wound healing of kefir. *International Journal of Pharmaceutical Sciences and Research, 6*(1), 286-293.

Ramchandran, L., & Shah, N. P. (2009). Effect of exopolysaccharides and inulin on the proteolytic, angiotensin-I-converting enzyme-and α-glucosidase-inhibitory activities as well as on textural and rheological properties of low-fat yogurt during refrigerated storage. *Dairy science & technology, 89*(6), 583-600. doi:https://doi.org/10.1051/dst/2009039

Rattray, F., & O'connell, M. (2011). Fermented Milks| Kefir. In P. F. F. John W. Fuquay, P. L. H. McSweeney (Ed.), *Encyclopedia of Dairy Sciences* (2 ed., pp. 518-524): Elsevier.

Rezaei, M., Zaghian, S., & Emtiazi, G. (2012). Purification, Characterization, and Determination of Antimicrobial Activity of Kefiran. *Journal of Isfahan Medical School, 30*(202), 1277-1283 .

Rimada, P. S., & Abraham ,A. G. (2001). Polysaccharide production by kefir grains during whey fermentation. *The Journal of dairy research, 68*(4), 653-661. doi:https://doi.org/10.1017/S0022029901005131

Rimada, P. S., & Abraham, A. G. (2003). Comparative study of different methodologies to determine the exopolysaccharide produced by kefir grains in milk and whey. *Le Lait, 83*(1), 79-87. doi:https://doi.org/10.1051/lait:2002051

Rodrigues, K. L., Caputo, L. R. G., Carvalho, J. C. T., Evangelista, J., & Schneedorf, J. M. (2005). Antimicrobial and healing activity of kefir and kefiran extract. *International journal of antimicrobial agents, 25*(5), 404-408. doi:https://doi.org/10.1016/j.ijantimicag.2004.09.020

Taniguchi, M., Nomura, M., Itaya, T., & Tanaka, T. (2001). Kefiran production by Lactobacillus kefiranofaciens under the culture conditions established by mimicking the existence and activities of yeast in kefir grains. *Food science and technology research, 7*(4), 333-337. doi:https://doi.org/10.3136/fstr.7.333

Taylor, G. R., & Williams, C. M. (1998). Effects of probiotics and prebiotics on blood lipids. *British Journal of Nutrition, 80*(S2), S225-S230. doi:https://doi.org/10.1017/S0007114500006073

Weschenfelder, S., Paim, M. P., Gerhardt, C., Carvalho, H. H. C., & Wiest, J. M. (2018 .(Antibacterial activity of different formulations of cheese and whey produced with kefir grains. *Revista Ciência Agronômica, 49*(3), 443-449. doi:https://doi.org/10.5935/1806-6690.20180050

Yeesang, C., Chanthachum, S., & Cheirsilp, B. (2008). Sago starch as a low-cost carbon source for exopolysaccharide production by Lactobacillus kefiranofaciens. *World Journal of Microbiology and Biotechnology, 24*(7), 1195-1201. doi:https://doi.org/10.1007/s11274-007-9592-3

Yokoi, H., & Watanabe, T. (1992). Optimum culture conditions for production of kefiran by Lactobacillus sp. KPB-167B isolated from kefir grains. *Journal of fermentation and bioengineering, 74*(5), 327-329. doi:https://doi.org/10.1016/0922-338X(92)90069-7

Zajšek, K., & Goršek, A. (2011). Experimental assessment of the impact of cultivation conditions on kefiran production by the mixed microflora imbedded in kefir grains. *Chemical Engineering Transactions, 24*(April), 481-486 .

تأثیر برخی شرایط تخمیر بر تولید کفیران توسط دانه‌های کفیر در شیر تخمیرشده

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چکیده

کفیر از تخمیر شیر توسط میکروارگانیسم‌های موجود در دانه‌های کفیر تولید می‌شود. دانه‌های کفیر شامل باکتری‌های اسید لاکتیک، مخمر و باکتری‌های اسید استیک است که توسط یک ماتریکس پروتئینی و پلی‌ساکاریدی به نام کفیران احاطه شده‌اند. یکی از مهم‌ترین ترکیبات ضدباکتریایی این نوشیدنی پلی‌ساکارید اگزوپلی ساکارید میکروبی کفیران است. مطالعهٔ حاضر با هدف بررسی تأثیر نوع شیر، زمان تخمیر، دما و شرایط هم‌زدن بر تولید کفیران توسط دانه‌های کفیر انجام شد. دانه‌های کفیر فعال به شیر پرچرب و بدون چربی اضافه شدند. تخمیر در دماهای ۲۵ و ۳۷ درجهٔ سانتی‌گراد در شرایط هم‌زده و غیرهم‌زده انجام شد. بعد از ۲۴، ۴۸، ۷۲ و ۱۲۰ ساعت، دانه‌ها از عصارهٔ کفیر جداشده و کفیران از دانه‌های کفیر استخراج گردید. تأثیر این متغیرها و اثرات متقابل آنها بر تولید کفیران با نرم‌افزار Design-Expert و طراحی فاکتوریل کامل مورد تجزیه‌وتحلیل قرار گرفت. همچنین کمترین غلظت مهارکنندگی (MIC) و کمترین غلظت باکتری‌کشی (MBC) کفیران استخراج‌شده علیه *اشریشیاکلی*، *استافیلوکوکوس اورئوس*، *باسیلوس سرئوس* و *شیگلا دیسانتری* تعیین شد. تمام شرایط تخمیر و اثرات متقابل آنها تأثیر معناداری بر تولید کفیران داشت. با درنظرگرفتن تمام شرایط تخمیر و اثرات متقابل فاکتورها، کفیران بیشتری در دانه‌های کفیر تخمیرشده در دمای ۳۷ درجهٔ سانتی‌گراد در مقایسه با ۲۵ درجهٔ سانتی‌گراد، در شیر پرچرب در مقایسه با شیر بدون چربی، در شرایط هم‌زده نسبت به غیرهم‌زده و نوشیدنی‌های تخمیرشده برای ۴۸ و ۱۲۰ ساعت تولید شد. MIC و MBC کفیران استخراج‌شده برای باکتری‌های آزمایش‌شده در محدودهٔ ۱۱/۲۵-۱/۴ میلی‌گرم بر میلی‌لیتر تعیین شد.

واژه‌های کلیدی: شرایط تخمیر، کفیر، کفیران، کمترین غلظت باکتری‌کشی، کمترین غلظت مهارکنندگی