

Research Article

Development of a Novel Whey Date Beverage Fermented with Kefir Grains Using Response Surface Methodology

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The aim of this study was to develop a novel kefir beverage using date syrup, whey permeate, and whey. The levels of the kefir grain inoculum (2–5% w/v), fruit syrup (10–50% w/v), and whey permeate (0–5% w/v) on pH, total phenolic content, antioxidant activity, lactic acid bacteria and yeast counts, and overall acceptability were investigated using central composite design. The use of response surface methodology allowed us to obtain a formulation with acceptable organoleptic properties and high antioxidant activities. The obtained beverages had total phenolic content, % DPPH scavenging activity, and overall acceptability ranging from 24 to 74 mg GAE/mL, from 74.80 to 91.37 mg GAE/mL, and from 3.50 to 6 mg GAE/mL (based on a 1 to 9 preference scale), respectively. Date syrup of 36.76% (w/v), whey permeates of 2.99%, and kefir grains inoculum size of 2.08% were the optimized process conditions achieved.

1. Introduction

The dairy industry is one of the most polluting industries in regard to the large volume of whey produced. Whey disposal represents a serious environmental problem. On the contrary, the use or the valorisation of whey may be advantageous not only for the environment but also for a sustainable economy. Whey is a highly nutritious product, and it is easily digested and assimilated [1]. It contains proteins with a very high biological value, lactose, minerals, and water-soluble vitamins [2, 3]. Moreover, it has a number of health-promoting effects [4, 5].

The use of whey in food production is one of the most attractive valorisation methods. Recently, plenty of new products containing whey have been developed. Whey-based beverage is one type of such products. Fruit addition

to whey improves both its flavor and its nutritional value. Among fruits that can be used are date fruits due to their high nutritive quality. They contain a complex mixture of saccharides, amino acids, antioxidants, polyphenols, and carotenoids [6, 7]. The consumption of beverages rich in antioxidants may reduce the oxidative damage on the human body [8, 9]. Antioxidants can also enhance the shelf life of the final products and prevent the development of off-flavors [10]. Moreover, date constituents are known to play an important role due to their multiple biological activities such as antidiabetic, anti-inflammatory, antitumour, and hepatic and renal protection properties [11].

In recent years, much attention has been paid to the development of probiotic whey beverages, since their benefits are more and more recognised. The beneficial health properties of kefir have been associated with the presence of

probiotic microorganisms and their metabolic products, mainly organic acids, that inhibit pathogenic and food-spoilage microorganisms [12–15]. Romanin et al. [16] showed that certain strains present in kefir grains have a potential for eliminating the intestinal inflammatory response and preventing pathogen adhesion and invasion into intestinal cells. Kefir grains include lactobacilli (*L. brevis*, *L. acidophilus*, *L. casei*, *L. helveticus*, and *L. delbrückii*), streptococci (*Streptococcus salivarius*), lactococci (*L. lactis ssp. thermophilus*, *Leuconostoc mesenteroides*, and *L. cremoris*), yeast (*Kluyveromyces*, *Candida*, *Torulopsis*, and *Saccharomyces* spp.), and a small amount of acetic acid bacteria [15, 17].

Kefir-based beverages also possess an antioxidant activity as reported by many scientific researches [18–22]. Furthermore, the hydrolysis of whey bioactive peptides such as β -lactoglobulin by probiotic bacteria is an alternative to reduce its allergenicity [23–25].

However, the challenge in the production of a probiotic beverage is to provide nutritional benefits ensuring good sensory properties. The response surface methodology (RSM) has been used for the development of fruit whey fermented beverages. To the best of our knowledge, only three studies have treated the fermentation optimization by kefir grains using RSM [19, 26, 27].

The purpose of this research was to optimise the formulation of a functional whey beverage (containing whey, fortified with whey permeates and date syrup) fermented by kefir grains. RSM employing mixture design was used to determine the optimum ratio of whey permeates, date syrup, and kefir grains on pH, lactic acid bacteria, yeast viability, antioxidant activity (% DPPH scavenging activity), total phenolic content, and overall acceptability.

2. Materials and Methods

2.1. Samples. Kefir grains collected from Tunisia were evaluated in this study [28]. Kefir grains were inoculated (5%, w/v) and propagated in sterilized milk at 25°C for 24 h. The grains were retrieved by filtration, reinoculated into sterilized milk, and incubated again at 25°C for 24 h. Milk was replaced every 2 days to maintain the grain viability.

Liquid cheese whey was collected from cheese making artisan in the Béja region. Its composition was lactose (5.4% w/v), proteins (2.5% w/v), fat (0.26% w/v), and ash (0.7%), with pH 6.4.

Whey permeates were supplied from a cheese making company. It had the following characteristics: proteins 3%, lactose 85%, and ash 7%.

Date syrup was prepared from date fruits (Deglet Nour). Fruits were washed, then manually sliced to pieces and then used for extraction with water. Date pulps (100 g) were put in an Erlenmeyer flask (1 L), and water was added. The date pulp/water ratio (D/W) was 1 : 3, and the sample was blended using a blender (Moulinex, France). The pH was adjusted to 6.0. The sample was placed in a water bath at 70°C for 2 h. After heating, the syrup was filtered to remove impurities and insoluble matters. Date syrup had

the following characteristics: 25° Brix, pH 6.12, dry matter (%) 76.43 \pm 0.22, and proteins (g/100g FW) 1.74 \pm 0.14.

2.2. Experimental Design. RSM was used to determine the effects of three variables: kefir grains inoculum (2–5% w/v), date syrup (10–50% w/v), and fortification with whey permeates (0–4% w/v), on pH (Y1), lactic acid bacteria (Y2) and yeasts' growth (Y3), total phenolic content (Y3), antioxidant capacity (Y4), and overall acceptability (Y5).

Nineteen treatments were conducted based on a central composite design (CCD) at 5 coded levels of –1.68, –1, 0, 1, and 1.68 (Tables 1–3) with the central point used at five replicates.

Response function (Y) was related to coded independent variables (x_i , $i = 1, 2, 3$) and β estimated regression coefficients using the second-order polynomial equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3. \quad (1)$$

Coefficients were b_0 (constant), b_1, b_2, b_3 (linear), b_{11}, b_{22}, b_{33} (quadratic), $b_{12}, b_{13},$ and b_{23} (interaction) of the model, calculated by software NEMROD-W (version 99901, LPRAI Company). Experiments were performed in triplicate, and each beverage sample was analyzed in duplicate. Results were expressed as mean values \pm standard deviation. Optimum levels were determined by optimizing the responses. The validation of the optimum model was made five times.

2.3. Date Whey Beverage Preparation. 300 mL of the beverage was prepared by mixing cheese whey with date syrup and whey permeates in the ratios provided in Tables 2 and 3 (Supplementary Materials available here).

2.4. pH Measurement. The pH was determined directly with a pH meter (Mettler-Toledo EL20).

2.5. Determination of Lactic Acid Bacteria and Yeast Cell Viability. Lactic acid bacteria (LAB) counts (\log_{10} CFU/mL) were carried out on MRS agar with cycloheximide (to avoid yeast development) after aerobic incubation at 37°C for 48 h. PDA containing chloramphenicol (to inhibit bacteria growth) was used for yeasts' enumeration. The plates were incubated at 30°C for 2–3 days.

2.6. Determination of Total Phenolic Content. The total phenolic content (TPC) of each sample was determined according to the Folin–Ciocalteu method [29], modified by Karaaslan et al. [30]. Briefly, 0.03 mL of beverage was added to 2.730 mL distilled water and shaken well. The mixture was added to 0.15 mL of Folin–Ciocalteu reagent. Then, 0.45 mL of sodium carbonate (20%) was added to the mixture, shaken, and left at room temperature (30°C) for 30 min. The absorbance was measured at 750 nm. The TPC was assessed by plotting the gallic acid calibration curve and expressed as

TABLE 1: Independent variables and coded levels for optimization.

Variable	Parameter (% w/v)	Ranges and levels				
		-1.68	-1	0	1	1.68
X_1	Whey permeate fortification	0	1	2.5	5	5
X_2	Date syrup	10	18	30	42	50
X_3	Kefir grains	2	2.6	3.5	4.4	5

TABLE 2: Matrix of the central composite design (CCD) and observed responses for the response variables (pH, LAB, and yeasts).

Exp. no.	X_1 (whey permeate % w/v)	X_2 (date syrup % w/v)	X_3 (kefir grains % w/v)	Response variables		
				pH (Y1)	LAB (\log_{10} CFU/mL) (Y2)	Yeasts (\log_{10} CFU/mL) (Y3)
1	1.0	18	2.6	4.02 ± 0.01	6.77 ± 0.10	4.35 ± 0.23
2	4.0	18	2.6	4.19 ± 0.02	5.47 ± 0.02	4.77 ± 0.31
3	1.0	42	2.6	4.53 ± 0.02	5.10 ± 0.16	4.50 ± 0.24
4	4.0	42	2.6	4.60 ± 0.02	7.00 ± 0.16	5.20 ± 0.28
5	1.0	18	4.4	4.00 ± 0.02	6.47 ± 0.07	5.70 ± 0.14
6	4.0	18	4.4	4.23 ± 0.02	3.27 ± 0.21	2.10 ± 0.02
7	1.0	42	4.4	4.08 ± 0.02	2.32 ± 0.14	2.60 ± 0.28
8	4.0	42	4.4	4.44 ± 0.07	2.38 ± 0.10	2.10 ± 0.01
9	-0.0	30	3.5	4.11 ± 0.17	4.27 ± 0.12	2.90 ± 0.26
10	5.0	30	3.5	4.37 ± 0.04	2.32 ± 0.10	2.10 ± 0.09
11	2.5	10	3.5	4.02 ± 0.17	5.90 ± 0.18	4.10 ± 0.28
12	2.5	50	3.5	4.64 ± 0.08	3.39 ± 0.15	2.21 ± 0.22
13	2.5	30	2.0	4.32 ± 0.04	8.70 ± 0.14	6.56 ± 0.38
14	2.5	30	5.0	4.19 ± 0.01	5.30 ± 0.28	5.78 ± 0.24
15	2.5	30	3.5	4.10 ± 0.01	4.07 ± 0.19	3.56 ± 0.41
16	2.5	30	3.5	4.19 ± 0.28	3.38 ± 0.20	3.31 ± 0.06
17	2.5	30	3.5	4.21 ± 0.14	3.56 ± 0.16	3.49 ± 0.12
18	2.5	30	3.5	4.20 ± 0.02	4.01 ± 0.12	3.41 ± 0.19
19	2.5	30	3.5	4.17 ± 0.02	3.92 ± 0.09	3.39 ± 0.28

Whey permeate fortification ranged from 0 to 5% (w/v); date syrup ranged from 18 to 50% (w/v); kefir grains ranged from 2 to 5% (w/v).

TABLE 3: Matrix of the central composite design (CCD) and observed responses for the response variables (DPPH scavenging activity, TPC, and overall acceptability).

Exp. no.	X_1 (whey permeates % w/v)	X_2 (date syrup % w/v)	X_3 (kefir grains % w/v)	Response variables		
				DPPH scavenging activity (Y4)	Total phenolic content (mg GAE/mL) (Y5)	Overall acceptability (Y6)
1	1.0	18	2.6	90.70 ± 0.89	27.00 ± 0.89	4.10 ± 0.14
2	4.0	18	2.6	74.80 ± 0.26	41.00 ± 0.69	4.20 ± 0.01
3	1.0	42	2.6	91.37 ± 0.96	67.00 ± 0.02	6.80 ± 0.12
4	4.0	42	2.6	89.05 ± 0.76	74.00 ± 0.27	6.00 ± 0.00
5	1.0	18	4.4	91.12 ± 0.83	36.00 ± 0.55	4.10 ± 0.02
6	4.0	18	4.4	90.02 ± 0.38	25.00 ± 0.00	4.00 ± 0.00
7	1.0	42	4.4	83.02 ± 0.88	45.00 ± 0.14	6.50 ± 0.67
8	4.0	42	4.4	90.19 ± 0.28	34.00 ± 1.11	6.20 ± 0.17
9	-0.0	30	3.5	90.44 ± 0.49	40.00 ± 0.70	5.00 ± 0.18
10	5.0	30	3.5	84.43 ± 0.82	44.00 ± 0.96	4.70 ± 0.35
11	2.5	10	3.5	89.99 ± 0.00	25.00 ± 0.01	3.30 ± 0.42
12	2.5	50	3.5	86.71 ± 0.41	55.00 ± 0.31	6.60 ± 0.01
13	2.5	30	2.0	87.68 ± 0.43	60.00 ± 0.11	6.80 ± 0.23
14	2.5	30	5.0	91.46 ± 0.67	28.00 ± 0.19	5.10 ± 0.05
15	2.5	30	3.5	90.68 ± 1.08	25.00 ± 0.93	3.80 ± 0.13
16	2.5	30	3.5	91.06 ± 0.10	24.00 ± 0.00	3.70 ± 0.28
17	2.5	30	3.5	90.56 ± 0.67	24.00 ± 0.56	3.50 ± 0.14
18	2.5	30	3.5	91.10 ± 0.16	25.00 ± 0.42	3.60 ± 0.12
19	2.5	30	3.5	91.02 ± 0.06	23.00 ± 0.00	3.70 ± 0.20

Overall acceptability scored on the nine-point Hedonic scale.

milligrams of gallic acid equivalents per mL of the sample (GAE per milliliter of sample), and the correlation coefficient was $R^2 = 0.991$.

2.7. Measurement of Total Antioxidants. The antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according to Balakrishnan and Agrawal with modifications [31]: 700 μL of each sample was added to 700 μL of a 0.035 mg/mL methanolic

solution of DPPH. The mixtures were shaken well. The control sample was prepared with the same volume of methanol instead of beverage. The mixtures were left at room temperature (in a dark place) for 30 min, and after that, the absorbance was measured at 517 nm using a spectrophotometer (Jenway 63200 UV/Vis). The antioxidant activity was determined as DPPH radical scavenging activity (%):

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{control absorbance} - \text{sample absorbance}) \times 100}{\text{control absorbance}} \quad (2)$$

2.8. Sensory Evaluation. Each sample was tested for overall acceptability by a panel of 20 trained testers. The panelists first participated in four 1 h training sessions, during which time descriptors were developed. The final descriptors were adopted by the panelists after discussion during training. After the training period, samples were evaluated in duplicate. In all cases, the samples were presented randomly.

Overall acceptability was scored on an increasing scale from 1 (extremely disliked) to 9 (extremely liked). An aliquot of 10 mL was served in transparent bottles. Mean scores of overall acceptability of each beverage were used as responses for optimization.

3. Results and Discussion

This study was carried out to optimise the formulation of kefir- whey- date beverage with different levels of inoculum size, whey permeate (WP), and date syrup (DS) using RSM (Table 1). WP was added to improve the beverage appearance and texture. Moreover, WP will fortify the fermented beverage by prebiotic components such as galactooligosaccharides [32]. The combination whey liquid-WP has not yet been used for beverage fortification or microbial growth.

Nineteen formulations of beverage were prepared, with varying kefir grain concentration from 2 to 5%, DS concentration from 1 to 50%, and WP concentration from 0 to 5% (Tables 2 and 3). The Central Point was repeated five times (experiment nos. 15, 16, 17, 18, and 19). The effect of these parameters was studied on pH, LAB and yeasts' growth, DPPH radical scavenging activity (%), TPC, and overall acceptability.

The effects of the WP, DS, and inoculation size on these variables at linear, quadratic, and interaction levels are presented in Table 4. The sign and magnitude of the coefficients indicate the effect of the variable on the response. Negative sign of a coefficient at the linear level indicates the antagonistic effects, whereas the positive sign indicates the synergistic effects. However, at the interaction level, the level of one variable could be increased while that of the other decreased to achieve the same response. The adequacy was calculated by *F*-ratio, mean, standard deviation, coefficient correlation, and lack of fit test.

3.1. Effect of Variables on Microbial Growth. The number of LAB and yeasts in the fermented products varied from 2.32 to 8.70 and from 2.1 to 6.56 \log_{10} CFU/mL, respectively (Table 2). The highest growth was observed at run no. 13 with an inoculum size of 2% in a medium containing 30% DS and 70% cheese whey fortified with 2.5% WP. Whey and date syrup are a suitable substrate for kefir grains. In fact, as shown in many studies [33–35], whey and date syrup offer nutritional elements that stimulate the microbial growth.

R^2 was 0.983 and 0.929 for LAB and yeast counts (Table 5), respectively, indicating that the model was able to understand more than 90% of the data variability and lack of fit was highly nonsignificant.

All linear, quadratic, and interaction variables had significant effects on LAB and yeasts' growth (Table 4). The variance analysis of the number of LAB and yeasts in the final products showed negative linear effects ($b_1, b_2 < 0$), suggesting that initial sugar concentration in the medium was inhibitory for the growth in the studied range. In fact, many previous studies found that the rate of kefir growth is reduced when the substrate concentration was too high [36]. This inhibition can be due to lactic acid or ethanol produced during the fermentation. Kashket [37] reported that lactic acid accumulation can impair the transmembrane pH gradient that is essential for energy generation in LAB, resulting in biomass inactivation. Thus, a greater concentration of substrate should be avoided when the target of fermentation is biomass production.

This result is in contradiction with those found by Harta et al. [38], who reported that addition of carbohydrate significantly increases the biomass yield. According to Gorsek and Tramsek [39], glucose concentration and medium temperature had not an important effect on the biomass increase in kefir grains. Therefore, the different carbon sources might have different effects of catabolic repression on the cellular secondary metabolism [40]. It is known that the recommended viable cell count in fermented food products is 10^6 to over 10^8 CFU/mL, in order to exert positive health impact on the target host [41] and in this study, the LAB count was more than 7 \log_{10} CFU/mL in the experiment nos. 4 and 13.

The quadratic effect of DS (b_{22}) and KG (b_{33}) showed positive effects for LAB growth. High amount of date syrup may offer important quantity of amino acids and mineral

TABLE 4: Analysis of variance as a linear, quadratic, and interaction for each response variable.

	Coefficient	Standard deviation	t.exp	Signif. %	Coefficient	Standard deviation	t.exp	Signif. %
	<i>pH</i>				<i>LAB (log₁₀ CFU/mL)</i>			
<i>b</i> ₀	4.175	0.024	175.17	***	3.796	0.144	26.44	***
<i>b</i> ₁	0.093	0.014	6.43	***	-0.426	0.087	-4.90	***
<i>b</i> ₂	0.165	0.014	11.43	***	-0.688	0.087	-7.92	***
<i>b</i> ₃	-0.059	0.014	-4.10	**	-1.144	0.087	-13.15	***
<i>b</i> ₁₁	0.019	0.014	1.32	21.7	-0.218	0.087	-2.51	*
<i>b</i> ₂₂	0.051	0.014	3.53	**	0.259	0.087	2.98	*
<i>b</i> ₃₃	0.024	0.014	1.69	12.3	1.092	0.087	12.55	***
<i>b</i> ₁₂	0.004	0.019	0.20	84.1	0.807	0.114	7.11	***
<i>b</i> ₁₃	0.044	0.019	2.32	*	-0.467	0.114	-4.11	**
<i>b</i> ₂₃	-0.079	0.019	-4.17	**	-0.613	0.114	-5.39	***
	<i>Yeasts (log₁₀ CFU/mL)</i>				<i>DPPH scavenging activity (%)</i>			
<i>b</i> ₀	3.434	0.043	80.07	***	90.914	0.110	826.23	***
<i>b</i> ₁	-0.317	0.026	-12.19	***	-1.630	0.067	-24.45	***
<i>b</i> ₂	-0.417	0.026	-16.06	***	0.108	0.067	1.62	18.0
<i>b</i> ₃	-0.559	0.026	-21.51	***	1.083	0.067	16.24	***
<i>b</i> ₁₁	-0.342	0.026	-13.15	***	-1.384	0.067	-20.75	***
<i>b</i> ₂₂	-0.110	0.026	-4.24	*	-1.060	0.067	-15.90	***
<i>b</i> ₃₃	0.956	0.026	36.77	***	-0.629	0.067	-9.43	**
<i>b</i> ₁₂	0.422	0.034	12.45	***	2.731	0.087	31.36	***
<i>b</i> ₁₃	-0.652	0.034	-19.22	***	3.036	0.087	34.86	***
<i>b</i> ₂₃	-0.460	0.034	-13.55	***	-2.856	0.087	-32.80	***
	<i>Total phenolic content (mg/mL)</i>				<i>Overall acceptability (scores)</i>			
<i>b</i> ₀	24.179	0.374	64.70	***	3.664	0.051	71.95	***
<i>b</i> ₁	0.419	0.226	1.85	13.7	-0.117	0.031	-3.81	*
<i>b</i> ₂	10.358	0.226	45.75	***	1.073	0.031	34.77	***
<i>b</i> ₃	-8.993	0.226	-39.72	***	-0.231	0.031	-7.50	**
<i>b</i> ₁₁	6.409	0.226	28.30	***	0.397	0.031	12.88	***
<i>b</i> ₂₂	5.702	0.226	25.18	***	0.433	0.031	14.03	***
<i>b</i> ₃₃	7.117	0.226	31.43	***	0.786	0.031	25.48	***
<i>b</i> ₁₂	-0.875	0.296	-2.96	*	-0.138	0.040	-3.41	*
<i>b</i> ₁₃	-5.375	0.296	-18.17	***	0.037	0.040	0.93	40.7
<i>b</i> ₂₃	-6.875	0.296	-23.24	***	0.013	0.040	0.31	76.7

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

content. Date proteins contain 23 amino acids; some of them are not present in other fruits [42]. The same result was obtained by increasing KG size. In fact, an increase in inoculum size enhances the growth of LAB and yeasts. This finding agrees with that of Sabokbar et al. [26].

It can be seen that the interaction effect between WP and DS (b_{12}) was positive ($p < 0.1\%$) (Table 4). However, negative interaction was seen between WP and kefir grains (KG) (b_{13} , $p < 1\%$ (Figure 1(a))) and between DS and KG (b_{23} , $p < 0.1\%$ (Figure 1(b))). The 3D response surface and the 2D contour plots are the graphical representations of the regression equation and are plotted to understand the interaction of the variables. Interactions between variables were considered negligible when circular contour plots are observed; nevertheless, a perfect interaction between the variables is indicated by the presentation of elliptical contours of which the smallest eclipse in the contour represents the maximum level [43]. The 2D and 3D plots presented maximum LAB number (about 8.70 log₁₀ CFU/mL) for higher kefir inoculum and low date syrup concentration.

The following equations can be used:

$$\begin{aligned} \text{LAB} = & 3.796 - 0.426X_1 - 0.688X_2 - 1.144X_3 - 0.218X_1^2 \\ & + 0.258X_2^2 + 1.092X_3^2 + 0.807X_1X_2 \\ & - 0.467X_1X_3 - 0.613X_2X_3, \quad R^2 = 0.983, \end{aligned} \quad (3)$$

$$\begin{aligned} \text{yeasts} = & 3.434 - 0.317X_1 - 0.417X_2 - 0.559X_3 \\ & - 0.342X_1^2 - 0.111X_2^2 + 0.956X_3^2 + 0.422X_1X_2 \\ & - 0.652X_1X_3 - 0.460X_2X_3, \quad R^2 = 0.929. \end{aligned} \quad (4)$$

The LAB and yeasts' counts in the obtained kefir beverage meet the specifications of probiotic beverages suggested by the FAO/WHO [44].

TABLE 5: Analysis of variance and regression analysis.

Source	Sum of squares	DF	Mean squares	F value	Signif. %
<i>pH</i>					
Model	0.6427	9	0.0714	25.0873	***
Residual	0.0256	9	0.0028		
Lack of fit	0.0179	5	0.0036	1.8550	28.4
Pure error	0.0077	4	0.0019		
Total	0.6684	18			
$R^2 = 0.962$; Adj- $R^2 = 0.923$					
<i>LAB (log₁₀CFU/mL)</i>					
Model	55.3596	9	6.1511	59.5603	***
Residual	0.9295	9	0.1033		
Lack of fit	0.5648	5	0.1130	1.2390	42.9
Pure error	0.3647	4	0.0912		
Total	56.2891	18			
$R^2 = 0.983$; Adj- $R^2 = 0.967$					
<i>Yeasts (log₁₀CFU/mL)</i>					
Model	30.7575	9	3.4175	370.6613	***
Residual	2.3358	9	0.2595		
Lack of fit	2.2989	5	0.4598	49.8674	**
Pure error	0.0369	4	0.0092		
Total	33.0932	18			
$R^2 = 0.929$; Adj- $R^2 = 0.859$					
<i>DPPH scavenging activity (%)</i>					
Model	288.9329	9	32.1037	529.0648	***
Residual	17.2440	9	1.9160		
Lack of fit	17.0013	5	3.4003	56.0359	**
Pure error	0.2427	4	0.0607		
Total	306.1769	18			
$R^2 = 0.944$; Adj- $R^2 = 0.887$					
<i>Total phenolic content (mg/mL)</i>					
Model	4.50380E + 0003	9	5.00422E + 0002	714.8885	***
Residual	3.82025E + 0001	9	4.24472E + 0000		
Lack of fit	3.54025E + 0001	5	7.08050E + 0000	10.1150	*
Pure error	2.80000E + 0000	4	7.00000E - 0001		
Total	4.54200E + 0003	18			
$R^2 = 0.992$; Adj- $R^2 = 0.983$					
<i>Overall acceptability (score)</i>					
Model	27.4714	9	3.0524	234.7983	***
Residual	0.9654	9	0.1073		
Lack of fit	0.9134	5	0.1827	14.0530	*
Pure error	0.0520	4	0.0130		
Total	28.4368	18			
$R^2 = 0.966$; Adj- $R^2 = 0.932$					

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.2. *Effect of Variables on pH.* The pH value varied from 4.6 ± 0.02 to 4.02 ± 0.01 after 48 h of fermentation (Table 2). This decrease is the result of organic acid production by LAB and yeasts [22, 45]. The pH values obtained were similar to those obtained by previous studies [34, 46]. Pereira et al. [46] reported that the pH of whey kefir drinks varied between 4.2 and 4.5 because of the buffer effect of whey proteins. Acidic pH is important to preserve food from food-spoilage microorganisms [47].

Furthermore, the pH is an important factor that could strongly influence the quality of a fermented product. It has been noted that pH affects the amount of carbon dioxide obtained during the manufacture of kefir drink by Clementi et al. [48].

From the coefficient of individual variables, an increase in the inoculum size of KG induced a more important

decrease in the pH. However, only the quadratic effect of date syrup concentration (b_{22}) is significant, and the others are not. Concerning the interaction effects, only the effect of WP and syrup date (b_{12}) is not significant. R^2 value was 0.962 (Table 5), and lack of fit was highly nonsignificant.

The regression equation given the level of pH as a function of WP, DS addition, and inoculation size:

$$\begin{aligned} \text{pH} = & 4.175 + 0.093X_1 + 0.165X_2 - 0.059X_3 + 0.051X_2^2 \\ & + 0.044X_1X_3 - 0.079X_2X_3, \quad R^2 = 0.962. \end{aligned} \quad (5)$$

3.3. *Effect of Variables on Phenolic Compounds and Antioxidant Activity.* The effects of these parameters on total phenols and antioxidant activity are shown in Table 3. The

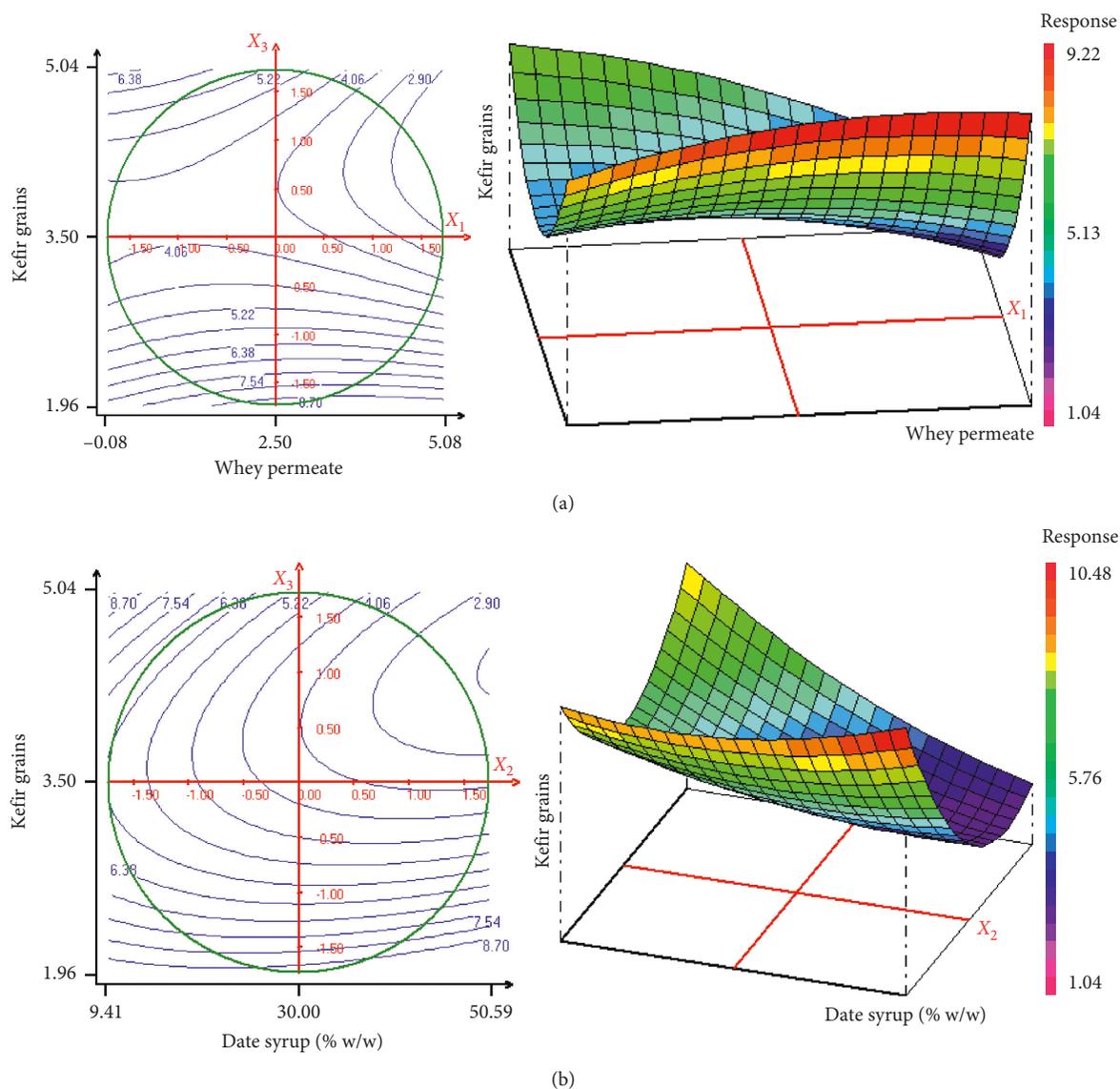


FIGURE 1: Response surface and contour plots that represent the effect of WP and kefir grains (a) and the effect of kefir grains and date syrup on LAB counts (b) (X_1 : whey permeate (% w/v); X_2 : date syrup (% w/v); X_3 : kefir grains (% w/v)).

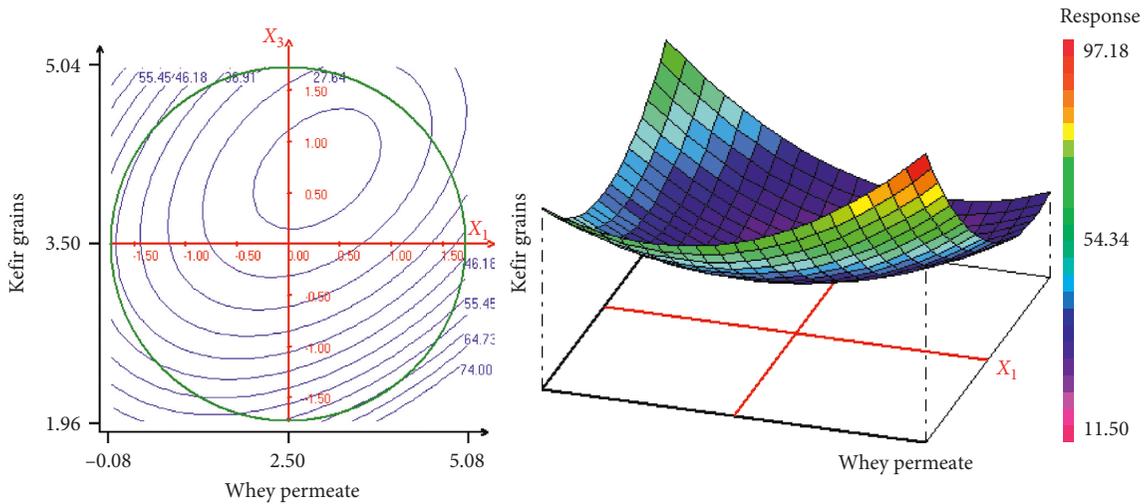
TPC in the beverages varied from 23 to 74 ± 0.27 mg GAE/mL and the % DPPH scavenging activity from 74.8 ± 0.26 to 91.46 ± 0.67 at the end of the fermentation (48 h). At the linear level, KG had a significant effect on the TPC and antioxidant activity of the end product ($p < 0.001$).

It was observed that the quadratic effect of KG is positive and significant on the TPC ($p < 0.001$). As discussed in the literature by Sabokbar and Khodaiyan [49], an increase in KG induced an increase in the TPC. In fact, the fermentation enhances the levels of phenolic compounds [19, 50].

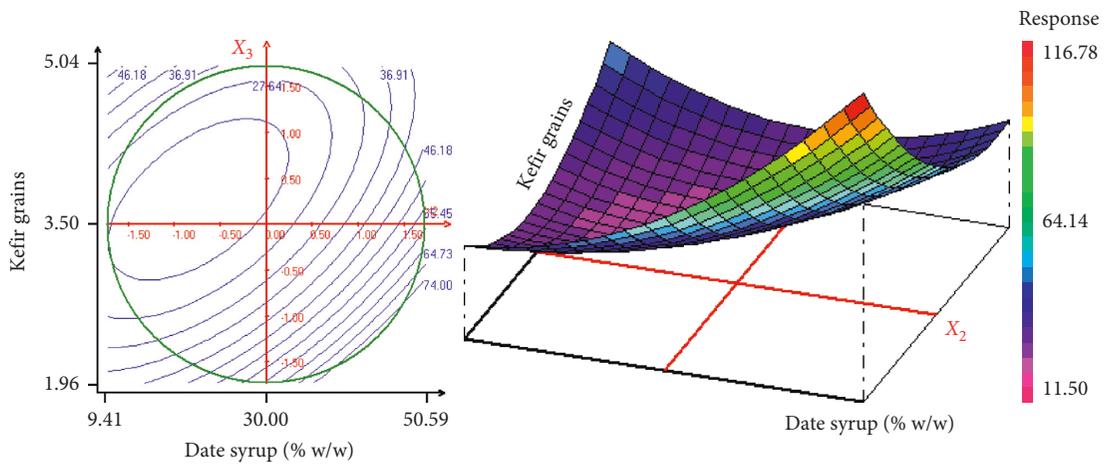
At synergistic level, WP and DS (b_{12}) affected the TPC significantly at $p < 0.05$ and DPPH scavenging activity at $p < 0.001$. Both interaction effect of DS and KG and interaction effect of KG and WP were significant on phenols and DPPH at $p < 0.001$. WP and KG (b_{13}) interaction had a negative effect on TPC (Figure 2(a)). The same interaction

was observed with DS and KG amount (b_{23}) (Figure 2(b)). Maximum TPC was obtained (74 mg GAE/mL) with a low level of kefir inoculum and high level of date concentration. As reported by Rao et al. [51], the convex response surfaces suggest that there are well-defined optimum variables. If the surfaces are rather symmetric and flat near the optimum, the optimized values may not change widely from the single variable conditions. This shape was observed both of these variables (interactions b_{13} and b_{23}).

The interaction effects of the variables WP and DS and WP and KG were positive but the interaction effect of DS and KG was negative on DPPH scavenging activity ($p < 0.1\%$). Maximum values of scavenging activity (91.46%) were obtained with a date level of about 40% and 2% kefir grain inoculum size as seen by 2D contour plots and also with 30% DS and 5% kefir (experiment no. 14, Table 3) (Figure 3). The multiple coded equations in terms



(a)



(b)

FIGURE 2: Response surface and contour plots that represent the effect of WP and kefir grains (a) and the effect of kefir grains and date syrup on polyphenol content (mg GAE/ml) (b).

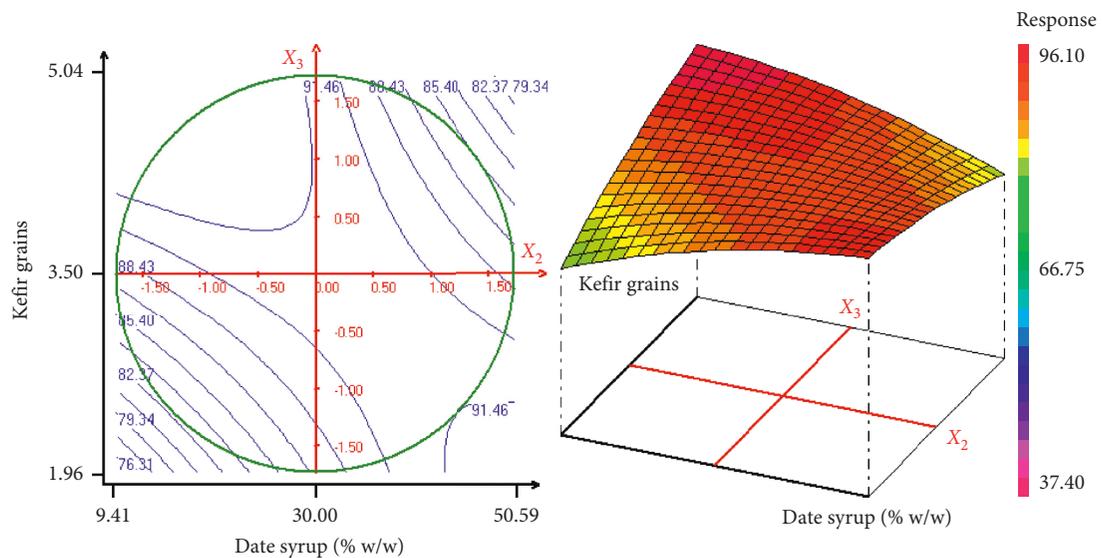


FIGURE 3: Response surface and contour plots that represent the effect of kefir grains and date syrup on % DPPH scavenging activity.

of coded factors generated for these responses are shown as follows:

$$\begin{aligned} \text{TPC (mg GAE/ml)} = Y5 = & 24.179 + 10.358X_2 - 8.993X_3 \\ & + 6.409X_1^2 + 5.702X_2^2 + 7.117X_3^2 \\ & - 0.875X_1X_2 - 5.375X_1X_3 \\ & - 6.875X_2X_3, \quad R^2 = 0.992, \end{aligned} \quad (6)$$

$$\begin{aligned} \% \text{DPPH scavenging activity} = Y4 = & 90.914 - 1.63X_1 \\ & + 1.083X_3 - 1.384X_1^2 \\ & - 0.11X_2^2 - 0.629X_3^3 \\ & + 2.731X_1X_2 + 3.036X_1X_3 \\ & - 2.856X_2X_3, \\ & R^2 = 0.944. \end{aligned} \quad (7)$$

Similar results have been noted on the improvement of phenolic contents and antioxidant capacities of fermented beverages (Table 6) like kefir and kombucha or by probiotic LAB [21, 52–54]. The TPC increase can be explained by the changes in the structure of phenolic compounds present in the date syrup due to acidic conditions and to the production of new substances by yeasts and LAB. Flavonoids and phenolic components such as cinnamic acid and its derivatives are responsible for the antioxidant activity of date palm [55]. In fact, cinnamic acid is considered as a suitable H-donor and scavenges the radicals and active oxygen species to obtain resonance stabilization [56]. The peptide chains present in whey are also known to have antioxidant properties [2]. Ozcan et al. [22] reported that their antioxidant effects depended upon the type of amino acids, the sequence, the distribution of hydrophobic residues, the structure and the length of the polypeptide, and the position of amino acids in the chains.

Abbas [57] reported that yeasts could produce a number of antioxidant compounds. Cruz et al. [58] reported that these substances were produced only under stressing conditions or in response to toxic medium ingredients such as phenolics or additives. LAB are also known to have enzymes responsible for the release of small molecules with high antioxidant activities during fermentation such as tyrosine and cysteine [59–61].

3.4. Effect of Variables on Overall Acceptability. The influences of all three independent variables (X_1 , X_2 , and X_3) on the overall acceptability (OA) of the fermented samples are shown in Table 3. All linear, quadratic, and interaction variable terms of the (X_1) and (X_2) had significant effects on the OA (Table 4).

Using the response surface statistical method, the following equation was obtained for OA response:

$$\begin{aligned} \text{OA} = & 3.664 - 0.117X_1 + 1.073X_2 - 0.231X_3 + 0.397X_1^2 \\ & + 0.433X_2^2 + 0.786X_3^2 - 0.138X_1X_2. \end{aligned} \quad (8)$$

The value of the determination coefficient ($R^2 = 0.966$) points out the goodness of the regression, which can be used to explain 96.6% of the total variation in this response (Table 5). The OA scores of kefir beverages were ranged from 3.3 ± 0.42 to 6.8 ± 0.23 . Date-based beverages or beverages supplemented with dairy products are generally well appreciated by consumers [35, 62].

The effect of the WP and the size of KG inoculum were significant ($p < 0.05\%$) and negative at linear level (Table 4). However, DS affected positively at linear (b_2) ($p < 0.001\%$) and quadratic levels (b_{22}) ($p < 0.001\%$) (Table 4). Growth of kefir grains induced a release of volatile compounds able to improve the flavor of whey. Athanasiadis et al. [63, 64] mentioned that fructose fermentation by kefir culture leads to the formation of volatile compounds that contribute to a fine aroma. Fruity aroma was also associated with kefir yeasts as reported by Randazzo et al. [65] and Nambou et al. [66].

At the interaction level, WP and DS (b_{12}) affected negatively the OA (Figure 4). An increase in DS with decrease in WP induced an increase in OA. Recently, Arsić et al. [67] and Abdulalim et al. [68] reported that the addition of fruit juices improves sensory profiles of fermented whey-based beverages. Evaluating the response surface obtained for overall liking (Figure 4), the formulations (3 and 13) received the same levels of acceptance, which corresponded to the highest scores (average 6.8). It means that it is possible to find more than one optimal point during the product optimization process. Similar result was noticed by Rebouças et al. [69].

3.5. Optimization of Independent Variables. Optimization was performed in order to achieve the levels of independent variables, which led to the best formulation of date-whey-kefir beverage. The optimum formulation was the sample with 2.99% WP and 36.76% DS and inoculated with 2.08% kefir grains.

The optimum mixture was produced in triplicate for validation of the predicted model. As shown in Table 7, the optimized results were LAB counts $8.85 \log_{10} \pm 0.10$ CFU/mL, yeast counts $7.12 \log_{10} \pm 0.27$ CFU/mL, pH 4.48 ± 0.01 , TPC 74.20 ± 0.3 mg GAE/mL, antioxidant activity $86.77 \pm 0.24\%$, and OA 6.70 ± 0.1 .

4. Conclusion

Kefir beverage made with whey permeate, cheese whey, and date syrup had acceptable organoleptic properties. RSM could be successfully used for the kefir beverage formulation. RSM predicted that level of 2.99% whey permeates; 36.76% date syrup; and 2.08% kefir grains would be the best formulation. This study provides an attractive alternative for developing a new fermented fruit whey beverage, which is nutritious, and with low cost.

TABLE 6: Comparison of scavenging activity % and total phenolic content (mg GAE/mL) between fermented beverages and control.

Independant variables	Antioxidant activity (%)		Total phenolic content (mg GAE/mL)	
	Unfermented	Fermented	Unfermented	Fermented
D1	86.39 ± 0.45	90.70 ± 0.89	22.58 ± 0.43	27.00 ± 0.89
D2	79.36 ± 0.56	74.80 ± 0.26	15.33 ± 0.43	41.00 ± 0.69
D3	79.81 ± 0.69	91.37 ± 0.96	33.65 ± 0.37	67.00 ± 0.02
D4	80.49 ± 0.96	89.05 ± 0.76	43.03 ± 0.60	74.00 ± 0.27
D5	66.89 ± 0.75	91.12 ± 0.83	21.72 ± 0.43	36.00 ± 0.55
D6	70.06 ± 0.78	90.02 ± 0.38	21.72 ± 0.67	25.00 ± 0.00
D7	72.78 ± 1.09	83.02 ± 0.88	32.38 ± 0.95	45.00 ± 0.14
D8	73.46 ± 0.61	90.19 ± 0.28	34.93 ± 0.62	34.00 ± 1.11
D9	86.84 ± 0.55	90.44 ± 0.49	38.77 ± 0.45	40.00 ± 0.70
D10	61.90 ± 0.31	84.43 ± 0.82	40.05 ± 1.03	44.00 ± 0.96
D11	75.05 ± 0.80	89.99 ± 0.00	21.72 ± 0.94	25.00 ± 0.01
D12	77.32 ± 0.28	86.71 ± 0.41	41.32 ± 0.93	55.00 ± 0.31
D13	85.94 ± 0.56	87.68 ± 0.43	28.54 ± 0.36	60.00 ± 0.11
D14	76.19 ± 0.23	91.46 ± 0.67	29.39 ± 0.24	28.00 ± 0.19
D15	75.27 ± 0.34	90.68 ± 1.08	28.12 ± 0.70	25.00 ± 0.93
D16	75.28 ± 0.50	91.06 ± 0.10	27.22 ± 0.75	24.00 ± 0.00
D17	74.78 ± 0.35	90.56 ± 0.67	27.46 ± 0.64	24.00 ± 0.56
D18	75.12 ± 0.37	91.10 ± 0.16	27.40 ± 0.42	25.00 ± 0.42
D19	75.33 ± 0.55	91.02 ± 0.06	28.10 ± 0.33	23.00 ± 0.00

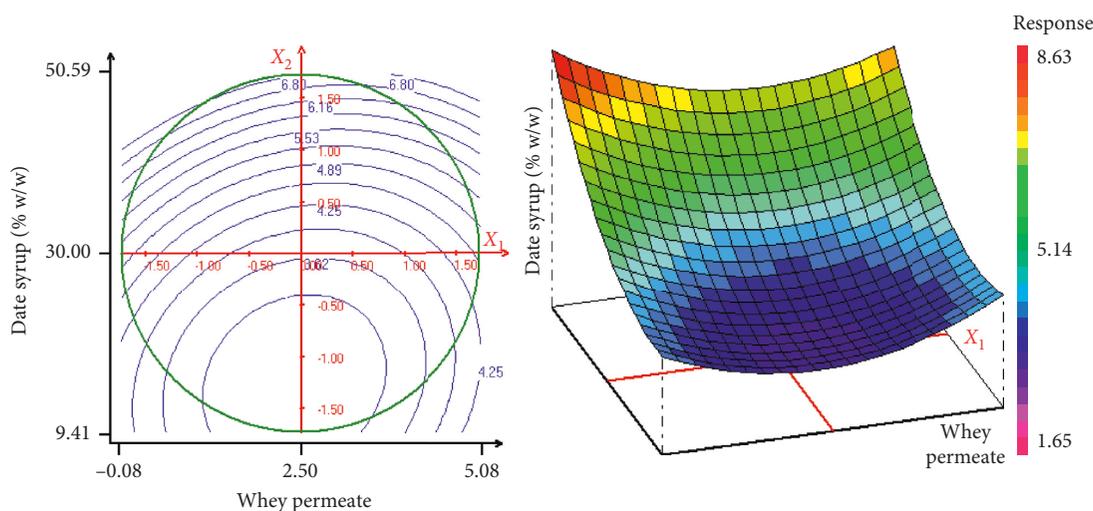


FIGURE 4: Response surface and contour plots that represent the effect of whey permeates and date syrup on overall acceptability score.

TABLE 7: Predicted and experimental values of responses under optimum conditions.

Response variable	Optimum condition	
	Experimental	Predicted
pH	4.48 ± 0.01	4.52
LAB (log10 CFU/mL)	8.85 ± 0.10	8.89
Yeasts (log10 CFU/mL)	7.12 ± 0.27	7.19
Antioxidant activity (%)	86.77 ± 0.24	88.11
Total phenolic content (mg/mL)	73.5 ± 0.30	74.20
Overall acceptability*	6.70 ± 0.10	6.74

*9-point hedonic test.

Data Availability

The data used to support the findings of this study are included within the article (Tables 2, 3, 6, and 7).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

Kefir beverages formulation made with cheese whey, whey permeate, and date syrup. (*Supplementary Materials*)

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