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Antifungal Activity of Peptides Derived From Iranian Traditional Kefir

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Abstract

Background: Probiotic products contain metabolites that have positive effects on the intestinal microorganisms and well-being of the host as well as possess antimicrobial properties.

Objective: This study aimed to investigate the antifungal activities of the water-soluble peptides (WSPs) found in ewe and cow milk kefir fermented by the traditional kefiran of Semnan (Semnan province, Iran).

Materials and Methods: Kefir samples were prepared by inoculating Iranian traditional kefiran into pasteurized milk. WPSs were extracted and antifungal activity was evaluated.

Results: During the 28-day storage, the concentration of the amino acids increased except for a decrease detected in the concentration of arginine, aspartic acid, cysteine, and glycine; and the total amino acid concentration in ewe milk and kefir was higher than that in cow's milk and kefir. The WSPs of both kefir samples showed considerable inhibitory activities against the growth of *Candida albicans* and *Aspergillus niger*, but *Penicillium* sp. had the lowest sensitivity when treated with WSPs. The antifungal activity of WSPs of ewe kefir was significantly higher than that of cow kefir. The highest growth inhibitory potential of WSPs between two kefir samples was found for *C. albicans*. The antifungal potential of WSPs of ewe kefir was considerably higher than that of cow kefir.

Conclusion: The higher antifungal potential of ewe kefir was likely associated with the high concentration of protein, extensive degradation of proteins, and diversity of amino acids produced during the fermentation.

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Background

Probiotic products contain metabolites that have positive effects on the intestinal microorganisms and well-being of the host as well as possess antimicrobial capacities. Kefir is characterized as a probiotic dairy beverage prepared by the fermentation of milk with a sour taste and a creamy texture. Milk is fermented by probiotic microorganisms accumulated in kefir grain which consists of a symbiotic mixture of lactic acid bacteria (*Streptococcus* spp., *Lactobacillus*, *Lactococcus* (LC), and *Leuconostoc*), acetic acid bacteria (*Acetobacter*), yeasts (*Saccharomyces* spp., *Candida*, *Torula*, and *Kluyveromyces*), and mycelial fungi aggregated in a glucogalactan matrix called kefiran.[1,2] Previous studies have indicated that regular consumption of some fermented products like kefir in the daily diet controls sugar level of blood plasma, regulates blood pressure, improves immunity in the gut system,[3,4] exerts a protective effect on the gastric ulcers,[3] prevents cancers and allergies, and develops antimicrobial capacities.[2,5] According to the findings from these studies, bioactive functional peptides resulted from hydrolysis of milk protein by kefir microorganisms are mostly generated from as_1-, as_2-, β-, and κ-casein.[6-8] Ebner et al [9] reported that peptide profiling of cow's milk kefir revealed 236 unique peptides derived from caseins during the fermentation by commercial starter culture or kefirans. They identified 16 bioactive peptide fractions with antioxidant, antimicrobial, antithrombotic, immunomodulating, and opioid activities in kefir. Their results showed that many of the kefir peptides were not endogenously present in the unfermented milk, but were produced from milk caseins through the proteolytic activity of kefir microbiota and, therefore, they were specific for this product. Also, the antimicrobial potential of some bioactive peptides originated from αs_2-casein was observed in sheep milk.[10]

Fungal contamination and mycotoxin secretion occurring in food products cause health and economic losses. Different studies have demonstrated the antifungal effects of lactic acid bacteria (LAB) and their potential to bind mycotoxins.[11,12] Different types of metabolites like bacteriocins, hydrogen peroxide, short-chain peptides, diacetyl, organic acids (formic, propionic, acetic, lactic, and phenyllactic acids) released during the milk fermentation have displayed antifungal properties.[12-14]

Though the proteolysis of milk-originated proteins and the bio-functions of the produced peptides in milk products such as cheese and cream have been already investigated,[15] the fermented dairy-based drinks have not been given sufficient research attention. Since the microbiological species of the traditional kefir grains (kefiran) vary from region to region, the metabolites and products of the fermentation are versatile.

Therefore, this study aimed to extract the water-soluble peptides (WAPs) of ewe and cow's milk fermented by local kefir grains from Semnan province, Iran, and to investigate their antifungal activities against some common food contaminating fungi.

Materials and Methods

Preparing Kefir Inoculums

Traditional kefiran was obtained from a rural mountainous part around the city of Semnan (Semnan province, Iran). Raw cow and ewe milk samples were purchased from a rural farm in Bandpei village located in Mazandaran province, Iran. The milk samples were heat-treated at 85±1°C for 10 minutes and cooled to 25°C which was the appropriate temperature for inoculation. To recover active microorganisms of the kefiran, kefir grains were first washed with lukewarm sterile water, inoculated to the pasteurized milk samples, and stored at 25±1°C for about 24 hours; this process was repeated on a daily basis for a week.[16] Using a plastic sieve, then the kefir grains were filtered and the milk curd was separated, rinsed, added into the pasteurized milk and, finally, kept at 25±1°C until investigations.

Preparation of Kefir

The kefir samples were produced by inoculating 5% w/v of kefiran to the pasteurized milk followed by incubation at 25±1°C for 42-48 hours (Memmert Incubator 400, Switzerland) until the pH of 4.6±0.1 was achieved. Then, the kefir grains and curds were separated by filtration, and the filtrates were transferred to glass bottles. The samples were stored at cold room (4±1°C) until analysis. Sample analysis was conducted on the basis of kefir's shelf life (about 1 month) on days 1, 7, 14, 21 and 28 in the cold storage.[17]

Microbiological Composition

The microorganisms in kefir samples were investigated according to the procedure explained by da Cruz Pedrozo Miguel et al.[18] The culture media, chemicals, and reagents used in this study were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (Steinheim, Germany). Serial dilutions of all kefir samples were inoculated on de Man-Rogosa-Sharpe (MRS) agar, followed by incubation in aerobic conditions (surface culturing, pH 7) for 48 hours at 30±1°C for LC sp. enumeration and under anaerobic conditions (plate culturing, pH 5.5) at 37±1°C for 5 days to count *Lactobacillus* (LB) sp.

The yeast enumeration was performed using the method adopted by Grønnevik et al. The serial dilutions of the kefir samples were plated on Sabouraud dextrose agar (SDA) and incubated at 30±1°C for 5 days.[19] The results were expressed as a logarithm of the number of colony-forming units per milliliter of the sample ($\log_{10}$ CFU/mL).

Titratable Acidity and pH

The pH of kefir samples was determined by a pH-meter (model 913, Metrohm, Switzerland). The titratable acidity (g/L) of the samples was measured according to Azizkhani et al.[20]

Extraction of Water-Soluble Peptides

The WSPs were extracted on days 1, 7, 14, 21, and 28 of the shelf-life period at 4± 1°C.[21] Two test tubes (for each kefir sample) containing distilled water (20 mL) and kefir (20 mL) were vortexed for 10 seconds. The tubes were kept for 1 h in a water bath at 40±1°C and then centrifuged (14560 g, 20 min, model Z206A, Hermle, Germany) at 4±0.5°. The pellets were removed and the supernatant was re-centrifuged and filtered using a Whatman N° 4 filter paper. The final filtrate was stored at –20±1°C until next step. The total protein content was measured applying a protein assay kit (TP0100, Sigma Aldrich, Germany). The bovine serum albumin was used as the standard. The concentration of 20 mg of WPSs per mL of distilled water was used for evaluating antifungal activity.

Concentration of Amino Acids

The amino acid composition and concentration of cow and ewe's milk and kefir were determined by applying a Sykam amino acid analyzer (model S-433) equipped with an integrated solid-state column oven with fast heating and cooling capability (Biokal, the Netherlands). The post-column ninhydrin labeling technique was used to detect and quantify the eluted chemical compounds according to the manufacturer's procedure.

Antifungal Activity Assay

Antifungal activity was investigated by applying the method developed by Gamba et al[22] and Eddine et al.[23] *Penicillium* sp., *Fusarium* sp., and *Aspergillus niger* (ATCC 9142) as the commonest foodborne pathogen or food spoiling fungi were purchased from the Organization of Scientific and Industrial Research (Tehran, Iran). *Candida albicans* (ATCC 76615) was kindly donated by the Department of Mycology, Faculty of Veterinary Medicine, University of Tehran (Tehran, Iran). The molds were cultured on the potato dextrose slant agar (PDA) and incubated for 7 days at 30±1°C. To prepare monospore suspension, 10 mL of sterile sodium lauryl sulfate (0.01% w/v in sodium chloride 1%) was added to the slant PDA.

Then, the suspensions were filtered through Whatman paper (pore size: 180 µm). The conidia were enumerated using a Neubauer chamber and the fungal count was set at 5×10^5 conidia/mL. The basal medium to perform the antifungal assay consisted of yeast extract (2% w/v), malt extract (1% w/v), and agar (2% w/v). The culture medium was sterilized in an autoclave (121˚C for 15 minutes), cooled to 45˚C, and mixed with 20 mg of WPSs per mL of distilled water.[21] Then, about 20 mL of the supplemented media was transferred into the Petri dishes (diameter: 90 mm) and 10 µL of conidia suspensions (5×10^5 conidia/mL) was inoculated on the center of the solidified culture medium by micro-pipetting. Agar plates without WSP were considered as the positive controls, inoculated with the fungal suspension. The negative control was agar media containing the same amount of WSP without fungal inoculation. The initial diameter of the fungal colony was considered as the diameter of the inoculums. The plates were packed in the polypropylene boxes containing jars of water to reduce moisture loss, and then incubated for 7 days at 25±1˚C. The average diameter of the colonies (mm) was measured and the diameter of the inhibition zone was calculated as follows:

Growth inhibition zone = $D_{control} - D_{sample}$

$D_{control}$ was the diameter of the control fungal colony, and D_{sample} was the diameter of the samples.[21,22]

C. albicans was cultured in Yeast Potato Dextrose (yeast extract, peptone, and glucose/dextrose) broth until obtaining the McFarland optical density of 0.5 and the adjusted final count of 5×10^5 conidia/mL. The antifungal test was carried out on a solid medium prepared from 1% w/v malt extract, 2% w/v yeast extract, and 2% w/v agar. One mL of the yeast suspension was inoculated on this medium in 90 mm diameter Petri dishes. Wells with 6 mm depth size and 8 mm diameter size were made in the inoculated medium, 100 µL of the WSP was transferred into the wells and incubated at 37±1˚C for 48 hours, and then the inhibition zone was measured. The antifungal activity was investigated on days 0, 1, 7, 14, 21, and 28 of the storage time and ketoconazole at a concentration of 50 µg/mL was applied as the control antifungal agent.[23]

Statistical Analysis

All assays were repeated three times. Statistical analyses were carried out using SPSS statistical software (version 22.0) and one-way ANOVA. The significance level of 95% was used and the data were presented by mean ± standard error of the mean.

Results and Discussion

Microbiological Composition of Kefir Samples

The data obtained for the microbial population of cow and ewe kefir samples are shown in Table 1. According to our results, no significant change was detected in the population of LAB during the storage time ($P>0.05$). The count of LC ranged from 11.5 to 12.3 log10 CFU/mL for cow milk kefir and 11.3 to12.6 log10 CFU/mL for ewe milk kefir during 28 days of storage. The population of LB varied between 11.8 to 12.5 log10 CFU/mL for cow milk kefir and 11.5 to 12.1 log10 CFU/mL for ewe kefir during the storage time. According to the data, the population of LB decreased slightly ($P>0.05$) while the number of LC colonies increased ($P>0.05$) during cold storage. Also, no significant difference was found between the LAB cell count of cow milk kefir and that of ewe milk kefir ($P>0.05$). The population of yeasts reduced to 1.6-1.7 log10 CFU/mL during the cold storage ($P<0.05$), and no considerable difference was found between cow and ewe kefir samples ($P>0.05$).

As for the population of lactic acid-producing bacteria, our study results were similar to the findings of previous studies that had related the resistance of LAB in cold storage of the fermented dairy products to their proteolytic activity. One of the determining factors in considerable survival of LAB and maintaining their metabolism in high rate is the fact that these bacteria extensively hydrolyze the protein compounds to peptide fragments and a variety of amino acids. These hydrolyzed products are considered

Table 1. Population of Lactic Acid Bacteria and Yeasts in Kefir Samples During Incubation at 4±1°C

Storage period (Day)	1	7	14	21	28
LB (log_{10} CFU/mL)					
Cow kefir	12.5 ± 1.93^{aA}	11.8 ± 0.55^{bB}	12.2 ± 1.37^{aC}	12.5 ± 1.68^{aD}	11.9 ± 0.83^{bE}
Ewe kefir	12.1 ± 2.05^{aA}	11.5 ± 0.86^{bB}	12.0 ± 0.95^{aC}	12.1 ± 1.14^{aD}	11.7 ± 0.69^{bE}
LC (log_{10} CFU/mL)					
Cow kefir	11.5 ± 1.07^{aA}	12.1 ± 1.50^{bB}	12.3 ± 1.48^{bC}	12.05 ± 1.25^{bD}	12.15 ± 1.30^{bE}
Ewe kefir	11.4 ± 1.53^{aA}	11.9 ± 0.85^{bB}	12.1 ± 1.76^{bC}	12.18 ± 1.44^{bD}	12.6 ± 0.55^{cE}
Yeasts (log_{10} CFU/mL)					
Cow kefir	9.1 ± 0.75^{aA}	8.6 ± 0.45^{bB}	8.1 ± 0.90^{cC}	7.6 ± 0.82^{dD}	7.4 ± 0.22^{dE}
Ewe kefir	9.0 ± 0.61^{aA}	8.4 ± 0.76^{bB}	8.2 ± 0.54^{bC}	7.5 ± 0.30^{cD}	7.3 ± 0.59^{cE}

Data are shown as the mean ± standard error of the mean

[a]Different lowercase superscripts in rows express significant difference between means for kefir samples during the storage period ($P<0.05$).

[A]Different uppercase superscripts in columns express significant difference between means of cow kefir and ewe kefir about population of each microorganism (LB, LC, and yeast) ($P<0.05$).

as rich nutrient supplies that provide the culture viability, growth, and maintenance of the cells as well as provoke the metabolism which leads to producing antimicrobial metabolites.[21,24-26]

The yeast population in cow and ewe kefir samples was found 7.3-9.1 $\log_{10}$ CFU/mL which was in agreement with the study results of de Lima et al determining 6.6-8.2 $\log_{10}$ CFU/mL for sheep kefir, and those of Montanuci et al recording 8 $\log_{10}$ CFU/mL of yeasts for cow kefir. The fermented product prepared in the study by Lima et al contained a yeast count of 8 log10 CFU/mL on the first day of storage, which decreased during storage to 6.6 log10 CFU/mL at the end of 28 days.[21,27] Grønnevik et al reported the yeast count in kefir ranging from 3 to 7 log10 CFU/ mL, which was slightly lower than the count detected in our study.[19] Also, the yeast population in the cow milk kefir studied by Montanuci et al was at the same level as that obtained by our study (8 log10 CFU/mL).[27]

Chemical Characteristics

Changes in chemical values of kefir samples as the indicators of acid-forming activity were investigated during storage at 4°C (Figure 1). Before fermentation, the pHs of cow and ewe milk were 6.60±0.10 and 6.50±0.07, respectively; and the obtained titratable acidity were 1.70±0.22 g lactic acid/L and 1.88±0.19 g lactic acid/L for cow and ewe milk, respectively. As presented in Figure 1, the acidity increased to 13.92 and 13.46 g/L in cow and ewe kefir during the fermentation as the result of kefir grain microorganisms' activities. Also, reductions of 1.04 and 1.06 were observed in pHs of cow and ewe kefir samples, respectively. There was no considerable difference between pH values and acidity of cow milk and ewe milk kefir ($P > 0.05$).

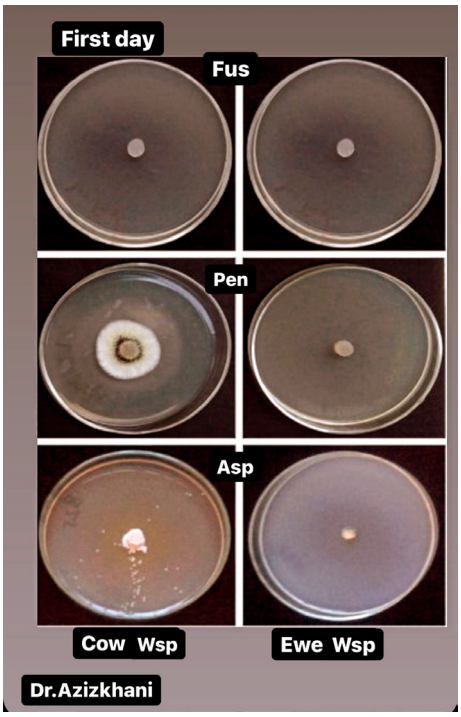

Figure 1. The pH Value and Titratable Acidity of Kefir Samples (♦: Cow Kefir; ◊: Ewe Kefir) During Storage at 4°C.

The metabolic activity of the microorganisms in kefir grains causes a decrease in lactose content, production of CO_2, the formation of organic acids, alcohols like ethanol, aromatic compounds and volatile components, descending pH value, and enhancing the acidity.[26] In our study, the lactose level of cow and ewe milk fermented by kefir microorganisms decreased significantly during the fermentation to 47.00% and 42.60% in cow and ewe milk, respectively. Since lactose is the significant source of carbohydrate for microorganisms in the kefir grain, it is degraded during the fermentation process and, therefore, a considerable acid production is resulted.[27] Also, our findings were in line with the results from other studies reporting the degradation of lactose and carbohydrates, decrease in pH, and increase in the acidity during storage of different types of kefir sample.[20,21]

Amino Acid Composition

According to the amino acid analysis data presented in Table 2, the concentration of the amino acids increased ($P < 0.05$) during the fermentation and storage period of kefir samples; however, the concentrations of arginine, aspartic acid, cysteine, and glycine decreased ($P < 0.05$). Moreover, the total amino acid concentration in ewe's milk and kefir was significantly higher than that in cow's milk and kefir ($P < 0.05$). During the fermentation process, proteins are partially hydrolyzed which facilitates their digestion in the body.[29] Obviously, the concentration of amino acids changes during milk fermentation, and it has been reported that kefir contains higher levels of alanine, threonine, lysine, serine, and ammonia than unfermented milk. Also, kefir contains other types of amino acids, such as methionine, valine, lysine, isoleucine, tryptophan, and phenylalanine.[29,30] According to Arslan, the essential amino acids in kefir include valine 220 mg/100 g, isoleucine 262 mg/100 g, methionine 137 mg/100 g, lysine 376 mg/100 g, threonine 183 mg/100 g, phenylalanine 231 mg/ 100 g, and tryptophan 70 mg/100 g.[1] Some studies have demonstrated the high proteolytic activities of kefir microorganisms like LC strains.[31,32] In a study by Kesenkaş et al, the concentrations of tyrosine and leucine in the kefir sample after 28 days of storage were reported to be 1.89– 9.56 mmol/L and 0.009–0.016 mmol/L, respectively.[32]

Antifungal Activity

Table 3 presents the antifungal potential of the WSPs extracts from cow and ewe milk kefir (at the concentration of 20 mg/mL) during the shelf-life period. The WSPs of unfermented cow and ewe milk (Day 0) had no antifungal effect on the tested fungi. As seen in the table, the antifungal activity increased during 28 days of

Table 2. Amino Acid Composition of Cow's and Ewe's Unfermented Milk and Kefir

Amino Acid Composition and Concentration (mg/100 g)	Cow's Unfermented Pasteurized Milk	Cow's Kefir	Ewe's Unfermented Pasteurized Milk	Ewe's Kefir
Ala	105.5± 9.66[a]	214.0±15.73[a]	279.3± 6.81[c]	545.8±8.11[d]
Arg	112.8±3.57[a]	58.2±4.10[a]	198.4±7.26[c]	88.5±6.14[d]
Asp	258.4±4.17[a]	13.5±1.01[a]	320.9±4.46[c]	29.1±1.30[d]
AspNH2	ND	ND	ND	ND
Cys	22.5± 3.30[a]	ND	35.6± 2.91[c]	ND
Glu	755.2±30.75[a]	910.4±22.27[a]	1032.5±45.01[c]	1288.1±31.45[d]
GluNH2	ND	ND	ND	ND
Gly	62.5±5.85[a]	47.2±6.77[a]	44.6±3.90[c]	31.9±5.05[d]
His	98.6±8.55[a]	856.0±10.21[b]	159.2±23.55[c]	1440.6±18.73[d]
Ile	145.0±3.01[a]	271.6±8.11[b]	335.1±7.45[c]	742.5±14.10[d]
Leu	285.3±5.95[a]	1540.5±7.45[b]	580.0±6.02[c]	2025.0±10.31[d]
Lys	275.3±6.02[a]	369.5±2.31[a]	520.6±3.38[c]	750.3±5.85[d]
Met	59.9±4.15[a]	133.1±4.76[a]	151.6±5.80[c]	298.0±3.45[d]
Phe	161.5±3.56[a]	231.9±15.05[b]	284.5±10.69[c]	405.7±2.55[d]
Pro	317.2±4.15[a]	1540.0±7.98[b]	583.4±6.25[c]	2032.5±11.37[d]
Ser	163.1±5.08[a]	450.7±2.25[a]	498.3±5.04[c]	1162.0±5.13[d]
Thr	150.0±4.25[a]	187.5±3.55[b]	268.8±2.07[c]	301.4±3.11[d]
Trp	50.5± 1.13[a]	70.6± 1.10[a]	86.1± 2.90[c]	110.4± 3.63[d]
Tyr	150.4± 2.50[a]	520.0±2.08[a]	281.6± 2.35[c]	1030.0±5.18[d]
Val	165.5±1.06[a]	227.3±2.52[b]	451.0±3.16[c]	645.9±1.85[d]
Total	3188.8±5.75	7410.2±9.83	6111.5±7.25	12927.7±12.29

ND,No Detected

[a]Different lowercase lowerscripts in rowd express significant difference between means of different sampls (unfermented milk and kefir) ($P<0.05$).

Table 3. Antifungal Potential (mm of Growth Inhibitory Zone) of WSPs (20 mg/mL) of Cow and Ewe Kefir Samples

Storage time (Day)	1	7	14	21	28
WSP of cow kefir					
Aspergillus niger	8.35±0.73[a]	10.22±0.91[b]	13.50±1.23[c]	15.11±0.65[d]	18.05±1.72[e]
Penicillium sp.	6.21±0.45[a]	7.40±0.55[b]	10.08±0.86[c]	11.35±0.97[d]	12.93±0.75[e]
Fusarium sp.	7.84±0.90[a]	9.91±1.03[b]	11.25±0.67[c]	13.86±1.21[d]	16.63±1.28[e]
Candida albicans	14.75±1.31[a]	18.32±1.50[b]	20.69±2.05[c]	21.04±1.85[d]	23.50±2.65[e]
WSP of ewe kefir					
Aspergillus niger	14.38±0.97[a]	16.47±1.24[b]	19.21±1.14[c]	20.95±2.33[d]	24.58±1.36[e]
Penicillium sp.	8.98±0.65[a]	9.65±0.85[a]	12.33±0.96[b]	14.74±1.58[c]	15.20±1.39[d]
Fusarium sp.	11.03±1.15[a]	12.85±1.10[b]	14.25±0.75[c]	15.80±1.43[d]	18.46±1.07[e]
Candida albicans	19.61±1.44[a]	24.57±1.46[b]	26.45±2.10[c]	27.78±2.50[d]	29.54±2.48[e]

[a]Different lowercase superscripts in rows indicate significant difference between means for kefir samples during the storage period ($P<0.05$).

the cold storage. The WSPs of both kefir samples showed considerable inhibitory activity against the growth of *C. albicans* and *A. niger*, but *Penicillium* sp. had the lowest sensitivity when treated with WSPs. Generally, the antifungal activity of WSPs of ewe kefir was significantly higher than that of cow kefir ($P<0.05$). The greatest inhibitory potential of WSPs between two types of kefir was found against *C. albicans* ($P<0.05$).

McNair et al detected a new peptide (DMPIQAFLLY; 1211 Da) with antifungal activity in sour cream treated with two bioprotective strains of LAB (i.e., *L. para*casei and *L. rhamnosus*). The proteolytic function of these LAB strains led to a 4-fold higher concentration of the peptides and amino acids during storage. The peptide inhibited the growth of *Debaryomyces hansenii* at concentrations of ≥35 µM. This newly identified peptide derived from a casein fragment was present in milk and milk products fermented by traditional starter culture, but in lower amounts compared to fermentations by commercial strains.[33] Bovine and ovine milk include a complex mixture of hundreds of protein fragments like caseins.[34] It has been demonstrated that fermentation of

milk with LAB results in hydrolysis of caseins to peptides with a length of 4-18 amino acids.[35] In this study, the antifungal effect of kefir was significantly higher than that of unfermented milk ($P < 0.05$). Amino acid composition data showed a significant difference between the extracts of milk and those of kefir due to the different peptides produced by kefir grain microorganisms.[9] Since the protein content of ewe milk is twice higher than that of cow milk [20], moreover, the diversity and concentration of peptides and amino acids in ewe milk fermented products are higher compared to those in cow milk fermented products. Ebner et al identified more than 230 peptide fragments originated from casein during fermentation by kefir grains.[9] In this regard, de Lima et al identified 17 peptide fractions in sheep kefir. These studies indicated the antimicrobial activities of the peptides. The fermentation process makes versatile changes in the peptide profile of milk and, therefore, milk products fermented by local starters like kefir grains (with different species of microorganisms) have various bio-functional capacities.[36] In our study, the fermentation process was discovered to alter the profile of the peptide fractions. The ewe kefir WSP had greater diversity of peptides compared to cow kefir WSP due to higher protein content and higher hydrolysis rate of proteins in ewe milk. The WSP antifungal activity of ewe milk kefir was considerably greater than that of cow milk kefir.

Conclusion

In sum, the fermentation process significantly changed the composition and concentration of the amino acids. The WSPs of both *ewe and cow* kefirs showed considerable inhibitory capacity against the growth of *C. albicans* and *A. niger*, but *Penicillium* sp. exhibited the lowest sensitivity when treated with WSPs. Moreover, the antifungal potential of WSPs of ewe kefir was considerably higher than that of cow kefir. The highest inhibitory potential of WSPs extracted from both kefir samples was found for *C. albicans*. It seemed that the higher antifungal potential of ewe kefir was associated with higher protein concentration, more extensive hydrolysis of peptides and proteins, as well as the diversity of amino acids produced during the fermentation.

Authors' Contribution

MA: Supervision, conception and design of the study, software, validation, writing- original draft, writing-reviewing and editing preparation. AS: Conceptualization, investigation, methodology, data analysis.

Conflict of Interests Disclosure

The authors declare that they have no conflict of interests.

Ethical Approval

Not applicable.

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