FRM

Foods and Raw Materials, 2020, vol. 8, no. 2

E-ISSN 2310-9599
ISSN 2308-4057

Research Article
Open Access

Check for updates

DOI: http://doi.org/10.21603/2308-4057-2020-2-402-410
Available online at http://jfrm.ru/en

Kashk with caper (*Capparis spinosa* L.) extract: quality during storage

Ashkan Hematian, Marjan Nouri*, Saeed Safari Dolatabad

Islamic Azad University, Roudehen, Iran

* e-mail: M.nouri@riau.ac.ir

Received July 10, 2020; Accepted in revised form August 26, 2020; Published September 21, 2020

Abstract:

Introduction. Dairy products are an important part of the diet. Kashk is a traditional Iranian dairy product rich in protein. However, kashk has a high water content and is a good medium for the growth of microorganisms. The aim of this study was to investigate the effect of the ethanolic extract of caper fruit (*Capparis spinosa* L.) on reducing the microbial burden of kashk.

Study objects and methods. The study objects were three kashk samples. The control sample was kashk without caper extract. Two experimental samples included kashk with 0.211 and kashk with 0.350 mg/mL of ethanolic caper extract. All the samples were tested for pH, sensory and antioxidant properties, colorimetric parameters, and microbial population. The experiments were performed on days 0, 7, 14, 21 and 28 of storage.

Results and discussion. The results showed all the samples had pH within the standard values during the entire shelf life (3.96 to 4.53). The samples with 0.350 mg/mL of the caper extract had the lowest EC_{50} (12.05 µg/mL), i.e. the highest antioxidant activity. The increased concentration of the extract and storage time resulted in a decrease in L^* and increase in b^*, while did not impact a^*. *Staphylococcus aureus* population increased more rapidly than *Clostridium botulinum* during the storage time, and the overall sensory acceptability of the kashk samples on days 0 and 7 received the highest score.

Conclusion. The kashk samples containing 0.350 mg/mL of caper extract had an improved antimicrobial, antioxidant and antifungal properties and can be produced and consumed as a new functional product.

Keywords: Dairy products, plant extract, microbial population, antioxidant activity, sensory properties, shelf life

Please cite this article in press as: Hematian A, Nouri M, Dolatabad SS. Kashk with caper (*Capparis spinosa* L.) extract: quality during storage. Foods and Raw Materials. 2020;8(2):402–410. DOI: http://doi.org/10.21603/2308-4057-2020-2-402-410.

INTRODUCTION

Nowadays, a demand for healthier food containing essential nutrients is growing so that digestive health is considered a key factor in producing functional food products [1–3]. Dairy products are an important part of the diet [4–6]. Kashk, a by-product of milk processing, is traditionally obtained by boiling, condensing, or drying buttermilk after buttering or lean yogurt [7]. The chemical composition of kashk includes 84.25% of dry matter, 8.57% fat, 95.9% salt, 53.60% total protein, 11.08% ash, and 1.06% lactose. Kashk also contains amino acids and such minerals as calcium, magnesium, iron, sodium, and potassium [8, 9].

Generally, kashk is used as a flavoring agent [10]. Kashk results from soaking curd, adding water and salt, grinding and sanitizing [11]. Kashk has a high microbial contamination potential due to its high moisture and protein content, and if contaminated, it can be very dangerous and even lead to fatal cases. This product is mostly exposed to *Staphylococcus aureus* and *Clostridium botulinum* contamination [9, 12, 13].

Controlling foodborne pathogenic bacteria and ensuring food safety is the most important issue for those involved in food processing [14]. Due to the detrimental effects of chemical preservatives, the need for research into the antimicrobial effects of natural preservatives and plant essential oils on the growth of microorganisms in food models under laboratory conditions has increased [13]. On the other hand, any preservatives in kashk is prohibited to be used, while the use of natural aromatic extracts or plants as a flavoring agent is allowed [16].

Caper (*Capparis spinosa* L.) is a medicinal plant from the *Capparidaceae* family and different species of caper have different uses. Antimicrobial effects of some species of caper on *Staphylococcus aureus*, *Streptococcus pyogenes*, *Helicobacter pylori*, *Escherichia coli*, and *Bacillus cereus* have been

documented [15, 16]. The presence of stachydrine and spermidine alkaloids, such as capparispine and cadabicin, in the seeds, roots, flowers, and dried fruits of caper allows using this plant as a nutritional or pharmaceutical supplement worldwide [19–21].

The hydrophobic properties of caper extracts increase its permeability into the cell membrane of microorganisms, which disrupts all vital activities and ultimately causes cell death of the microorganisms. In addition, the extract can damage the enzymes involved in energy regulation and synthesize constituents that inactivate or destroy genetic materials [22, 23]. Its antimicrobial properties are also due to the presence of hydroxyl (OH) groups [24]. The aim of the present study was to investigate the effect of caper fruit extract on the quality and antimicrobial properties of kashk.

STUDY OBJECTS AND METHODS

Extraction of caper (*Capparis spinosa* L.). In this study, caper fruit was collected and identified in Khuzestan Province in southwestern Iran in 2020 (Fig. 1). The fruits were washed, dried and powdered by using an electric mill. Then, 7 kg of dried caper was extracted with 40 L of 70% ethanol for 24 h at room temperature using an electric mixer. The extract was then filtered with filter paper No.1 (repeated on the remaining sediment). Next, all solutions were concentrated in a vacuum rotary evaporator at 40°C, and extraction efficiency was calculated based on [25].

Composition and antioxidant properties of caper extract. The total amount of phenols was determined by the Folin & Ciocalteu's reagent, flavonoids by aluminum chloride method, and antioxidant activity by DPPH radical scavenging assay. Caper fruit extracts were analyzed for quantitative and qualitative determination of polyphenols and flavonoids using high performance liquid chromatography reversal and diode array detection. The apparatus was equipped with a detector, a C18 reverse phase column (Prodigy ODS-3, 4.6×150 mm, 5 µm; Phemomenex, Torrance, CA) and a linear converter unit. The column temperature was set at 30 ± 1°C. Rinsing with acetonitrile aqueous solution (97:3 ratio, both with 3% acetic acid) was performed as the initial step. The lyophilized extract was mixed with 1 mL of mobile phase prior to analysis. Further preparation was performed by centrifugation for 5 min at 12 rpm. Then, 20 µL of the solution was injected directly into the high-performance liquid chromatography system [26].

Figure 1 Caper fruit under study

Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of caper extract. *Staphylococcus aureus* stock culture (ATCC 9144) and dried *Clostridium perfringens* (ATCC13124) were taken from the Microorganism Collection Center of Iran Scientific and Industrial Research Organization. The stoke cultures were transferred into 10 mL Brain Heart Infusion (BHI) broth and incubated in a shaking incubator at 37°C for 24 h. Then, 0.1 mL of the culture was transferred into 10 mL of BHI medium and incubated at 37°C for another 24 h until the end of the progressive phase.

Subsequently, diluted cultures were used to inoculate plate agar and industrial kashk for subsequent target inoculations. In a 96-well plate, 100 µL of the culture medium (Müller Hinton broth) was added to all the wells, and then 100 µL of the sample extract was added to the first well. After mixing the culture medium and the sample in the first well, 100 µL of it was added to the second well and dilution continued until the concentration of the extract in the wells was reduced by half. Then, a uniform suspension of *Staphylococcus aureus* and *Clostridium perfringens* half McFarland 1.5×10^8 CFU/mL were added to all the wells and the 96-well plate was incubated at 37°C for 24 h. At the end, one well before the well in which turbidity was considered minimum bactericidal concentration and the first concentration in which turbidity was not observed was minimum inhibitory concentration [27].

Preparation and quality evaluation of kashk samples. Pasteurized kashk was randomly purchased from a local manufacturer in Karaj. The experimental samples were kashk with minimum bactericidal and minimum inhibitory concentrations of the caper extract. Kashk without the extract was used as control. Then, qualitative tests of the kashk samples were performed on days 0, 7, 14 and 28 of storage. The qualitative tests included pH (with the help of a pH meter based on AOAC 2000 standard), antioxidant properties (by using DPPH radical scavenging assay), and colorimetric test (by a HunterLab spectrophotometer based on CIELAB system) [26, 28]. Table 1 shows the kashk samples under study.

Microbial population in kashk during storage. In this method, each specimen was infected with *Staphylococcus aureus* and *Clostridium perfringens* with a microbial population of 10^5 CFU/mL and kept at refrigerator temperature. To determine the population of *Staphylococcus aureus*, a certain amount of sample was diluted and sterilized on a plate. Baird-Parker agar was added to the plate with egg yolk emulsion with tellurite and incubated at 37°C for 72 h. The number of

Table 1 Kashk samples under study

CK_0	Control kashk on day 0 (the day of production)
CK_7	Control kashk on day 7 of storage
CK_{14}	Control kashk on day 14 of storage
CK_{21}	Control kashk on day 21 of storage
Ck_{28}	Control kashk on day 28 of storage
EKI_0	Experimental kashk with MIC on day 0 (the day of production)
EKI_7	Experimental kashk with MIC on day 7 of storage
EKI_{14}	Experimental kashk with MIC on day 14 of storage
EKI_{21}	Experimental kashk with MIC on day 21 of storage
EKI_{28}	Experimental kashk with MIC on day 28 of storage
EKB_0	Experimental kashk with MBC on day 0 (the day of production)
EKB_7	Experimental kashk with MBC on day 7 of storage
EKB_{14}	Experimental kashk with MBC on day 14 of storage
EKB_{21}	Experimental kashk with MBC on day 21 of storage
EKB_{28}	Experimental kashk with MBC on day 28 of storage

MBC is minimum bactericidal concentration
MIC is minimum inhibitory concentration

Staphylococcus aureus per was calculated taking into account the dilutions and volumes used to determine the colony count in the plates. To count *Clostridium perfringens* in the samples, we used Sulfite Polymyxin Sulfadiazine (SPS) agar. Bacterial plates were incubated in an anaerobic chamber at 37°C for 72 h and finally colonies were counted [13].

Sensory properties of kashk samples. After basic training, 15 individuals (aged 25–30 years) evaluated the sensory characteristics of the kashk samples with the help of a five-point Hedonic scale. The characteristics under study were texture, odor, taste, oral sensation, adhesion, and general acceptance. The maximum (5) and the minimum (1) scores implied a high and low quality of a sample, respectively [29].

Statistical analysis. At first, normality test was performed for all the data, and then the data were analyzed by factorial test in a completely randomized design with the mean and standard deviation. Duncan's multiple range test was used to determine the difference between the mean values at the 0.05 level. Minitab software was used for statistical analysis.

RESULTS AND DISCUSSION

Extraction efficiency of caper (*Capparis spinosa* L.) extract. The extraction methods and the antioxidant activity of the extracts correlated with each other, due to the different polarity of the compounds. A reduced polarity of the solution undermines extraction efficiency. In particular, organic solvents are commonly used as antioxidants to extract phenolic compounds [30]. Two-component solvents are more effective in extracting phenolic compounds from plant samples than single-component solvents [31].

According to Table 2, the average extraction efficiency of the caper extract was 23.23%, which was similar to results obtained by Hosseini *et al*. (24%) who used water and ethanol solvents [25].

In our study, the total amount of phenolic and flavonoid compounds in the target extract was 20.01 (mg gallic acid per g) and 12.16 (mg catechin per g), respectively. M. Mahboubi and A. Mahboubi extracted phenolic and flavonoid compounds from caper with water, ethanol, methanol, and ethyl acetate solvents. The phenolic content was 17.2, 31.7, 34.2 and 30 mg gallic acid, and flavonoid content was 0.06, 1.4, 17.1, and 96.5 mg/kg catechin, respectively [17]. Stefanucci *et al.* reported that phenolic and flavonoid contents in caper extracts varied depending on the geographical area. The total phenols were in the range of 9.63 to 24.17 (mg gallic acid per g) and flavonoid compounds, 5.02 to 23.50 (mg catechin per g) [26]. Mollica *et al*. used three methods to obtain caper extract, namely sedimentation, microwave, and solvent application. The phenolic compounds were within the range of 14.27 to 17.96 (gallic acid per g), and the flavonoid compounds were within the range of 10.17 to 11.67 (mg catechin per gram) [34].

Low EC_{50} values indicate an increase in antioxidant activity. Vahid *et al*. found that the amount of phenolic and flavonoid compounds is an important factor in determining the antioxidant capacity of plant extracts [32]. Ehsanifar *et al.* reported that ethanol as a polar solvent extracted flavonoids, glycoside, catechol, and tannin from crude plant tissue [36]. Aqueous-alcoholic solvents soften the plant cell wall tissue and are associated with an increased solubility of bonded phenolic compounds. Since these compounds are highly soluble in polar solvents, they enter the solvent phase under the influence of concentration gradients.

Caper extracts contain phenolic and flavonoid compounds which can neutralize free radicals due to hydroxyl groups. A determining factor in antioxidant activity is not the number of hydroxyl groups present in the aromatic ring, but rather the position of the hydroxyl groups and the presence of other functional groups such as double bonds, the combination of hydroxyl groups and the presence of ketone groups play an important role. The negative correlation between total phenol

Table 2 Phenolic, flavonoid and EC_{50} properties of caper extract

Treatment	Extraction efficiency, %	Total content of phenolic compounds, mg gallic acid per g	Flavonoid content, mg catechin per g	EC_{50}, mg/mL
Extract with 70% of ethanol and 30% of water	23.20 ± 0.14	0.07 ± 2 0.01	0.20 ± 12.16	0.03 ± 1.48

Table 3 Phenolic compounds of caper (*Capparis spinosa* L.) extract

Name	Amount, μg/mg of extract
Gallic acid	0.06
Catechin	0.46
Chlorogenic acid	0.11
p-OH benzoic acid	0.27
Vanillic acid	0.12
Syringic acid	0.04
3-OH benzoic acid	0.08
p-coumaric acid	0.23
Rutin	6.32
t-Ferulic acid	0.03
Naringin	0.28
o-Coumaric acid	0.02
Quercetin	3.45

content and EC_{50} values in DPPH free radical scavenging assay indicates the direct effect of phenols on antioxidant activity [24]. M. Mahboubi and A. Mahboubi extracted *Capparis spinosa* extract with water, ethanol, methanol and ethyl acetate solvents and reported the EC_{50} values of 500, 560, 340 and 2000 (μg/mL), while in our study this value was 1.48 (mg/mL), indicating the antioxidant properties of caper. Other studies also showed that caper has a significant antioxidant activity [17, 26, 34].

Composition and antioxidant properties of caper extract. In recent years, phenolic compounds have received special attention because of their biological activities. We analyzed phenolic compounds by means of high-performance liquid chromatography and diode array detection (Table 3).

Four previously phenolic compounds of caper extract, such as catechin, gallic, chlorogenic, and o-coumaric acid, reported in [32] were also observed in this study. Previous studies have reported rutin as one of the main components of the extract, which was confirmed in this study as well. Rutin has a wide range of biological activities such as antioxidant, antimicrobial, antidiabetic and cholesterol-lowering effects [26, 33]. Therefore, the remarkable biological effects of caper can be attributed to rutin, which can be a source of bioactive compounds.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of caper extract. The microbial properties of kashk are defined by the limit of *Staphylococcus aureus* and sulfite reductive *Clostridia*. The results of MIC and MBC of *Capparis spinosa* extract for the two target microorganisms are shown in Table 4.

In the present study, the MBC level for *Staphylococcus aureus* (0.283 mg/mL) was lower than that for *Clostridium perfringens* (0.350 mg/mL). *Staphylococcus aureus* lacks capsules and spores but is resistant to drought and withstands salt up to 10%.

Table 4 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Target microorganisms	MIC, mg/mL	MBC, mg/mL
Staphylococcus aureus	0.148	0.283
Clostridium perfringens	0.211	0.350

Clostridium perfringens is a Gram-positive, sporozoic, and usually capsulogenic bacterium, with a higher resistance to the extract than *Staphylococcus aureus*. Various mechanisms have been proposed so far for the antimicrobial activity of the extracts. The most accurate theory suggests that the number and position of hydroxyl groups are a key factor in the antimicrobial activity of phenolic compounds, flavonoids, quercetin and their derivatives.

Other mechanisms, such as flavonoids as a result of the ability to form complexes with the cell wall and inhibit the growth of microorganisms, or phenolic compounds by enzymatic activity through reaction with sulfhydryl groups or nonspecific interactions with proteins prevent enzymatic and thus exhibit their antimicrobial activity. Polyphenols are also able to form high molecular weight soluble complexes with proteins, thereby attaching to the bacteria and destroying the receptors present on the bacterial cell surface [37]. Quercetin and its derivatives also inhibit bacterial growth through the DNA gyrase inhibition [27].

Rahnavard and Razavi showed that caper extracts demonstrated the antibacterial activity against a variety of Gram-positive and negative bacteria, including *Staphylococcus epidermis*, *Staphylococcus faecalis*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus cereus* [21]. The aqueous extract of caper fruit did not show any antibacterial activity, while the ethanolic extract had the antimicrobial effect on a variety of *Streptococcus* spp. and Gram-negative bacteria [17]. The comparing of results from different studies in this case seems complicated because the results are influenced by factors such as the composition and type of culture medium, microorganism growth phase, the volume of culture medium, pH, temperature, and incubation time. The chemical composition, type, and the mechanism of action of phenolic compounds also play a part in antimicrobial activity [38]. Since one of the aims of the present study was to increase the kashk shelf life, we used MIC and MBC of *Clostridium perfringens,* which were high, as the amount of extract used in the kashk samples.

pH determination. The pH test of the kashk samples is shown in Table 5. Statistical analysis of pH-dependent parameters showed that the concentration of extract, time, and extract × time had a significant effect on kashk pH ($P < 0.05$), i.e., pH reduced with time. On the other hand, the addition of the extract increased the pH value. Based on standard, pH higher than 4.5 is considered

Table 5 pH and DPPH radical scavenging ability of kashk samples during 28-day storage

Time, days	pH			EC_{50}, µg/mL		
	CK	EKI	EKB	CK	EKI	EKB
0	0.03 ± 4.53^{ab}	0.02 ± 4.54^{a}	0.04 ± 4.56^{a}	0.5 ± 32.74^{c}	0.7 ± 15.11^{j}	0.5 ± 12.05^{k}
7	0.02 ± 4.36^{c}	0.01 ± 4.51^{b}	0.03 ± 4.53^{ab}	0.2 ± 33.28^{c}	0.5 ± 17.10^{f}	0.2 ± 14.07^{h}
14	0.01 ± 3.99^{f}	0.03 ± 4.41^{c}	0.05 ± 4.42^{c}	0.7 ± 33.47^{c}	0.9 ± 17.40^{f}	0.4 ± 14.24^{h}
21	0.01 ± 3.29^{j}	0.04 ± 4.05^{e}	0.02 ± 4.11^{d}	1.1 ± 35.03^{b}	1.3 ± 18.05^{e}	0.5 ± 15.10^{j}
28	0.04 ± 2.96^{h}	0.01 ± 3.94^{f}	0.01 ± 3.96^{f}	0.8 ± 40.12^{a}	0.7 ± 20.15^{d}	0.8 ± 17.05^{f}

CK: control kashk, EKI: experimental kashk with MIC (0.211 mg/mL of caper extract), and EKB: experimental kashk with MBC (0.350 mg/mL of caper extract)
Letters a–h indicate significant differences

undesirable because it causes an undesirable taste in the product and reduces its customer acceptibility [39].

The pH of all samples was within the standard range (3.96 to 4.53) during 28-day storage. Samples without extract (control samples) had lower pH than the experimental samples, and samples with two different concentrations of extract increased pH. It was due to interaction between extract and lactic acid bacteria.

Faraji *et al*. used ethanolic extract of *Allium stipitatum* L. in different concentrations (0.5 to 2%) in kashk for 28 days. The results showed that the pH of samples increased with increasing the concentration of extract, and time had a diminishing effect on pHr, which is consistent with the present findings [40].

The extent of DPPH free radical scavenging ability. Statistical analysis showed that increasing the concentration of the extract, time and extract × time had a significant effect on EC_{50} in kashk samples ($P < 0.05$). The increased time and decreased amount of the extract resulted in an increase in EC_{50} in all the samples. The ability of the extracts to inhibit free radicals also increased as the concentration increased (Table 5).

Analysis of variance and comparison of mean EC_{50} of the samples showed that the kashk with the highest amount of extract (EKB, 0.350 mg/mL) had the lowest EC_{50} on day 0 of storage (the day of production) and thus had the highest potential for DPPH radical trapping. Since that sample contained the highest amount of the extract, the control sample assessed on day 28 of storage had the highest EC_{50} (40.12 mg/mL) and the lowest ability to trap DPPH radicals compared to the other samples. This is due to phenolic compounds present in kashk (EC_{50} 74.32 mg/mL) as scientists have found that kashk is capable of absorbing oxidative compounds due to its glutathione precursor (a type of protein) [41]. On the other hand, the effect of storage time on the DPPH trapping ability was significant ($P < 0.05$), which decreased in all the samples.

Jayasena and Jo reported a high correlation between the ability to trap free radicals and the amount of phenolic compounds in grains, fruits, vegetables, and beverages [24]. A review article by Mollica *et al*. showed the antioxidant activity of dairy products, namely of raw milk (EC_{50} 26.41 mg/mL), yogurt (32.4 mg/mL), and cheese (23.5 µg/mL), but no data on the antioxidant activity of kashk was found [34]. In this study, we determined that the antioxidant activity of the control sample on day 0 of storage was 32.74 mg/mL, and it significantly increased in the samples with the caper extract because the extract itself had an antioxidant activity (EC_{50} 1.48 mg/mL).

Colorimetric Analysis of Kashk Samples. The increased concentration of the extract on days 0 and 7 of storage had no significant effect on brightness (L^*) in the kashk samples, but with increasing the storage duration up to 28 days the effect of the extract on the samples was becoming significant ($P < 0.05$).

The samples showed the highest lightness on day 0, and the control sample had the lowest lightness (60.15) on day 28 (Table 6). The extract and time had also no effect on redness (a^*), and the addition of the extract and an increased storage time led to an increase yellowness (b^*) in the product. Thus all the three samples had the lowest yellowness on day 0, and the control sample had the most yellowness (27.20) on day 28.

Table 6 Colorimetric characteristics of kashk samples

Time, days	L^*			a^*			b^*		
	CK	EKI	EKB	CK	EKI	EKB	CK	EKI	EKB
0	0.4 ± 67.20^{a}	0.5 ± 67.17^{a}	1.2 ± 67.15^{a}	0.06 ± 3.05^{a}	0.06 ± 3.05^{a}	0.05 ± 3.05^{a}	1.2 ± 22.66^{e}	1.0 ± 22.55^{e}	1.4 ± 22.50^{e}
7	0.9 ± 67.08^{a}	1.4 ± 67.07^{a}	1.0 ± 67.07^{a}	0.10 ± 3.00^{a}	0.02 ± 3.09^{a}	0.01 ± 3.06^{a}	0.6 ± 24.39^{d}	0.4 ± 24.41^{ce}	0.5 ± 23.35^{de}
14	0.6 ± 65.17^{c}	0.8 ± 66.06^{b}	0.4 ± 66.26^{b}	0.04 ± 3.07^{a}	0.04 ± 3.11^{a}	0.03 ± 3.09^{a}	1.3 ± 24.99^{dc}	0.5 ± 25.20^{c}	0.9 ± 24.05^{d}
21	0.5 ± 65.10^{c}	0.9 ± 65.05^{c}	1.3 ± 65.07^{c}	0.03 ± 3.03^{a}	0.02 ± 3.12^{a}	0.05 ± 3.10^{a}	0.7 ± 25.15^{c}	0.9 ± 25.75^{bc}	1.1 ± 25.65^{bc}
28	1.1 ± 60.15^{e}	2.4 ± 61.10^{d}	7.1 ± 61.44^{d}	0.08 ± 3.10^{a}	0.03 ± 3.00^{a}	0.04 ± 3.04^{a}	1.0 ± 27.20^{a}	0.4 ± 26.04^{bc}	0.6 ± 26.20^{b}

CK: control kashk, EKI: experimental kashk with MIC (0.211 mg/mL of caper extract), and EKB: experimental kashk with MBC (0.350 mg/mL of caper extract)
Letters a–e indicate significant differences

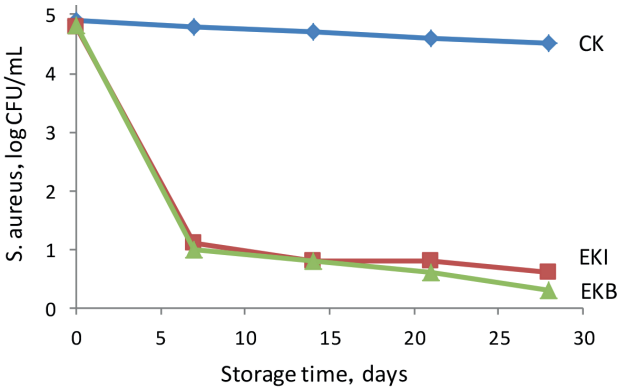

Figure 2 Dependence of *Staphylococcus aureus* concentration in kashk samples on storage time. CK: control kashk, EKI: experimental kashk with MIC (0.211 mg/mL of caper extract), and EKB: experimental kashk with MBC (0.350 mg/mL of caper extract)

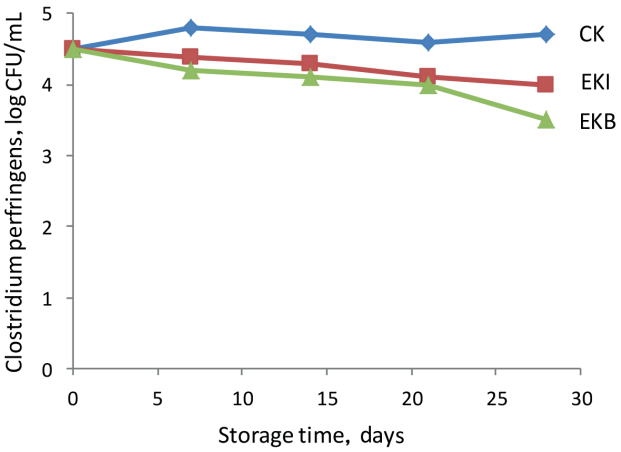

Figure 3 Dependence of *Clostridium perfringens* concentration in kashk samples on storage time**.** CK: control kashk, EKI: experimental kashk with MIC (0.211 mg/mL of caper extract), and EKB: experimental kashk with MBC (0.350 mg/mL of caper extract)

Faraji *et al.* stated that addition of 0.5–2% ethanolic extract of *Allium stipitatum* over a 21-day storage resulted in an increase in *L** (from 53.3 to 70.2), no change in *a** (around 3.49), and increased *b** (from 17.05 to 49.56) [40]. Hosseini and Ansari reported that the addition of modified Tapioca starch to kashk over a 60-day storage increased *L**, but did not impact *a** and *b** [29].

The effect of microbial population on shelf life of kashk samples. Bacterial population changes of *Staphylococcus aureus* and *Clostridium perfringens* in the control and experimental kashk samples (with the caper extract) during 28 days of storage at 4°C are shown in Fig. 2. The initial concentration of *Staphylococcus aureus* in the control sample was 4.91 CFU/mL, which remained unchanged until the end of storage. By the end of storage (on day 28), the initial amount of *Staphylococcus aureus* had reduced to 0.6 CFU/mL in the experimental sample with MIC (0.211 mg/mL of the caper extract), and to 0.3 CFU/mL in the kashk with MBC (0.350 mg/mL).

The initial bacterial count of *Clostridium perfringens* in the control was 4.50 CFU/mL, which had not increased significantly by day 28 of storage. In the kashk with MIC (0.211 mg/mL of the caper extract), the initial amount of *Clostridium perfringens* reduced to 4 CFU/mL, and in the kashkl with MBC (0.350 mg/mL), to 3.50 CFU/mL. The high susceptibility of *Staphylococcus aureus* to the caper extract can be related to phenolic compounds present in the extract [11].

Although the extract under study also reduced the initial amount of *Clostridium perfringens* in kashk, the reduction was less than a logarithmic cycle indicating its resistance to the extract. The decrease in microbial load in the whole system could be due to the intrinsic acidic pH of kashk, but after day 21 a different trend was observed for *Clostridium perfringens* in the control sample. The overall results showed that with the increasing of storage time of the extract-containing kashk samples, *Staphylococcus aureus* bacterial population decreased more rapidly than that of *Clostridium botulinum*, but none reached zero. Golestan *et al.* investigated the antimicrobial properties of the ethanolic extract of *Allium stipitatum* against *Clostridium botulinum* and *Staphylococcus aureus* in kashk. They found that *Staphylococcus aureus* count decreased more rapidly with increasing storage time, it had reached zero by the end of day 21 of storage [13].

Sensory properties of kashk. Figure 3 illustrates the effect of the caper extract on the sensory properties of the kashk samples based on the evaluation of panelists. The results showed that the highest scores of texture, smell, taste, oral feeling, adhesion, and general acceptability had the kashk samples on days 0, 7 and 14 of storage. With time, namely after 48 days of storage, the samples had the lowest overall acceptability.

The results also showed that the texture of the samples containing the extract received the highest score on day 14, while the control sample on day 14 had a lower score. However, the odor of the control sample had a higher score comparedc to that of the experimental kashk. There was no significant difference between the samples regarding taste and oral sensation. It is worth to note that the adhesion factor score remained maximal for all the samples during the storage time. The general acceptability of the kashk samples received the highest scores on days 0 and 7, and the lowest scores, on day 48 in all the three samples.

Golestan *et al.* demonstrated that peppermint essential oil at concentrations of 1500 and 2500 ppm and *Mentha pulegium* essential oil at a concentration of 2500 ppm had significant effects on taste of kashk [13]. Kashk samples containing 1500 ppm of *Mentha pulegium* essential oil and control samples were introduced as suitable samples, but kashk samples with

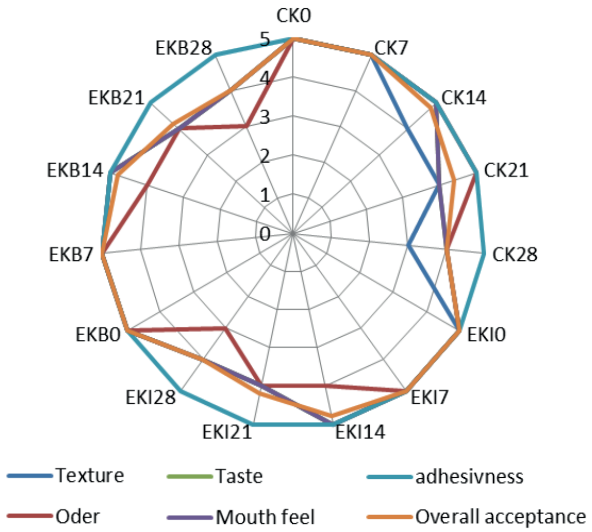

Figure 3 Effect of storage time (0 to 28 days) on sensory characteristics of kashk samples. CK: control kashk, EKI: experimental kashk with MIC (0.211 mg/mL of caper extract), and EKB: experimental kashk with MBC (0.350 mg/mL of caper extract)

Mentha pulegium essential oil at the concentrations of 1500 and 2500 ppm were acceptable for panelists.

Agulló *et al.* studied the effect of modified Tapioca starch on sensory properties (i.e., texture, smell, taste, oral sensation, adhesion and general acceptance) of kashk [29]. Their results showed that the type of starch affected the sensory properties. The sample with 1.5% hydroxypropylated tapioca starch had the best result and the control sample received the lowest score.

CONCLUSION

The results of the study showed that the caper (*Capparis spinose* L.) extract had no adverse effects on pH of kashk during storage time, and the kashk sample with the extract at the concentration of 0.350 mg/mL had the lowest EC_{50} (12.05 mg/mL), or the highest antioxidant activity on day 0 of storage. The increased extract concentration and storage time resulted in a decrease in *L** and an increase in *b**, while they did not influence *a**.

The number of bacteria had gradually decreased in the kashk samples with both concentrations of the extract by the end of a 28-day storage. With increasing the storage time, *Staphylococcus aureus* bacterial population declined compared to *Clostridium botulinum*.

The sensory evaluation results showed that the texture of the extract-containing samples had a higher score, which was even higher by day 14, and the control sample had a lower score on the same day. But in terms of smell the control samples were superior to the extract-containing samples.

In general, we can conclude that the kashk samples containing 0.350 mg/mL of caper extract had improved antimicrobial, antioxidant, and antifungal properties and can be considered as a new functional product.

CONTRIBUTION

Authors are equally related to the writing of the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Bolshakova LS, Lukin DE. Absorption of iodotyrosine from iodized milk protein in animals. Foods and Raw Materials. 2020;8(1):60–66. DOI: https://doi.org/10.21603/2308-4057-2020-1-60-66.
2. Winder CB, Churchill KJ, Sargeant JM, LeBlanc SJ, O'Connor AM, Renaud DL. *Invited review*: Completeness of reporting of experiments: REFLECTing on a year of animal trials in the *Journal of Dairy Science*. Journal of Dairy Science. 2019;102(6):4759–4771. DOI: https://doi.org/10.3168/jds.2018-15797.
3. Nouri M, Khodaiyan F. Green synthesis of chitosan magnetic nanoparticles and their application with poly-aldehyde kefiran cross-linker to immobilize pectinase enzyme. Biocatalysis and Agricultural Biotechnology. 2020. DOI: https://doi.org/10.1016/j.bcab.2020.101681.
4. Genis DO, Bilge G, Sezer B, Durna S, Boyaci IH. Identification of cow, buffalo, goat and ewe milk species in fermented dairy products using synchronous fluorescence spectroscopy. Food Chemistry. 2019;284:60–66. DOI: https://doi.org/10.1016/j.foodchem.2019.01.093.
5. Khramtsov AG. Traditions and innovations of dairy industry. Foods and Raw Materials. 2015;3(1):140–141. DOI: https://doi.org/10.12737/11247.
6. Nouri M, Ezzatpanah H. The quality of UHT skim milk as affected by addition of renneted skim milk. Nutrition and Food Sciences Research. 2017;4(3):39–46. DOI: https://doi.org/10.18869/acadpub.nfsr.4.3.39.
7. Song L, Aryana KJ. Reconstituted yogurt from yogurt cultured milk powder mix has better overall characteristics than reconstituted yogurt from commercial yogurt powder. Journal of Dairy Science. 2014;97(10):6007–6015. DOI: https://doi.org/10.3168/jds.2014-8181.
8. Pourjoula M, Picariello G, Garro G, D'Auria G, Nitride C, Ghaisari AR, et al. The protein and peptide fractions of kashk, a traditional Middle East fermented dairy product. Food Research International. 2020;132. DOI: https://doi.org/10.1016/j.foodres.2020.109107.

9. Rahpeyma E, Sekhavatizadeh S. Effects of encapsulated green coffee extract and canola oil on liquid kashk quality. Foods and Raw Materials. 2020;8(1):40–51. DOI: https://doi.org/10.21603/2308-4057-2020-1-40-51.
10. Shiroodi SG, Mohammadifar MA, Gorji EG, Ezzatpanah H, Zohouri N. Influence of gum tragacanth on the physicochemical and rheological properties of kashk. Journal of Dairy Research. 2012;79(1):93–101. DOI: https://doi.org/10.1017/S0022029911000872.
11. Iranmanesh M, Ezzatpanah H, Akbari-Adergani B, Torshizi MAK. SPME/GC-MS characterization of volatile compounds of Iranian traditional dried kashk. International Journal of Food Properties. 2018;21(1):1067–1079. DOI: https://doi.org/10.1080/10942912.2018.1466323.
12. Forghani F, Eskandari M, Oh D-H. Application of slightly acidic electrolyzed water and ultrasound for microbial decontamination of kashk. Food Science and Biotechnology. 2015;24(3):1011–1016. DOI: https://doi.org/10.1007/s10068-015-0129-5.
13. Golestan L, Seyedyousefi L, Kaboosi H, Safari H. Effect of *Mentha spicata* L. and *Mentha aquatica* L. essential oils on the microbiological properties of fermented dairy product, kashk. International Journal of Food Science and Technology. 2016;51(33):581–587. DOI: https://doi.org/10.1111/ijfs.13014.
14. Kareem RA, Razavi SH. Plantaricin bacteriocins: As safe alternative antimicrobial peptides in food preservation – A review. Journal of Food Safety. 2019;40(1). DOI: https://doi.org/10.1111/jfs.12735.
15. Oleynikov VV. Antioxidant and antimicrobial properties of oregano extract (*Origani vulgaris herba* L.). Foods and Raw Materials. 2020;8(1):84–90. DOI: https://doi.org/10.21603/2308-4057-2020-1-84-90.
16. Repajić M, Grudenić A, Levaj B. Functional and sensory properties of olives fortified spreadable cheese. Mljekarstvo. 2019;69(2):125–137. DOI: https://doi.org/10.15567/mljekarstvo.2019.0205.
17. Mahboubi M, Mahboubi A. Antimicrobial activity of *Capparis spinosa* as its usages in traditional medicine. Herba Polonica. 2014;60(1):39–48. DOI: https://doi.org/10.2478/hepo-2014-0004.
18. Samari F, Baluchi L, Salehipoor H, Yousefinejad S. Controllable phyto-synthesis of cupric oxide nanoparticles by aqueous extract of *Capparis spinosa* (caper) leaves and application in iron sensing. Microchemical Journal. 2019;150. DOI: https://doi.org/10.1016/j.microc.2019.104158.
19. Mishra SN, Tomar PC, Lakra N. Medicinal and food value of Capparis – a harsh terrain plan. Indian Journal of Traditional Knowledge. 2007;6(1):230–238.
20. Anwar F, Muhammad G, Ajaz Hussain M, Zengin G, Alkharfy KM, Ashraf M, et al. *Capparis spinosa* L.: a plant with high potential for development of functional foods and nutraceuticals/pharmaceuticals. International Journal of Pharmacology. 2016;12(3):201–219. DOI: https://doi.org/10.3923/ijp.2016.201.219.
21. Rahnavard R, Razavi N. A review on the medical effects of *Capparis spinosa* L. Advanced Herbal Medicine. 2017;3(1):44–53.
22. Demir Y, Güngör AA, Duran ED, Demir N. Cysteine protease (Capparin) from capsules of caper (*Capparis spinosa*). Food Technology and Biotechnology. 2008;46(3):286–291.
23. Mazarei F, Jooyandeh H, Noshad M, Hojjati M. Polysaccharide of caper (*Capparis spinosa* L.) Leaf: Extraction optimization, antioxidant potential and antimicrobial activity. International Journal of Biological Macromolecules. 2017;95:224–231. DOI: https://doi.org/10.1016/j.ijbiomac.2016.11.049.
24. Jayasena DD, Jo C. Essential oils as potential antimicrobial agents in meat and meat products: A review. Trends in Food Science and Technology. 2013;34(2):96–108. DOI: https://doi.org/10.1016/j.tifs.2013.09.002.
25. Fallah Huseini H, Hasani-Ranjbar S, Nayebi N, Heshmat R, Sigaroodi FK, Ahvazi M, et al. *Capparis spinosa* L. (Caper) fruit extract in treatment of type 2 diabetic patients: A randomized double-blind placebo-controlled clinical trial. Complementary Therapies in Medicine. 2013;21(5):447–452. DOI: https://doi.org/10.1016/j.ctim.2013.07.003.
26. Stefanucci A, Zengin G, Locatelli M, Macedonio G, Wang C-K, Novellino E, et al. Impact of different geographical locations on varying profile of bioactives and associated functionalities of caper (*Capparis spinosa* L.). Food and Chemical Toxicology. 2018;118:181–189. DOI: https://doi.org/10.1016/j.fct.2018.05.003.
27. Dehghani S, Nouri M, Baghi M. The effect of adding walnut green husk extract on antioxidant and antimicrobial properties of ketchup. Journal of Food and Bioprocess Engineering. 2019;2(2):93–100.
28. Nouri M, Ezzatpanah H, Abbasi S, Aminafshar M, Behmadi H. Effect of partial hydrolyzed k-casein on physicochemical and sensory properties of heated milk. Journal of Dispersion Science and Technology. 2012;33(8):1204–1209. DOI: https://doi.org/10.1080/01932691.2011.605637.
29. Hosseini F, Ansari S. Effect of modified tapioca starch on the physicochemical and sensory properties of liquid kashk. Journal of Food Science and Technology. 2019;56(12):5374–5385. DOI: https://doi.org/10.1007/s13197-019-04008-w.

30. Fernández-Agulló A, Pereira E, Freire MS, Valentao P, Andrade PB, González-Álvarez J, et al. Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. Industrial Crops and Products. 2013;42(1):126–132. DOI: https://doi.org/10.1016/j.indcrop.2012.05.021.
31. Chew KK, Khoo MZ, Ng SY, Thoo YY, Aida WMW, Ho CW. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Orthosiphon stamineus extracts. International Food Research Journal. 2011;18(4):1427–1435.
32. Vahid H, Rakhshandeh H, Ghorbani A. Antidiabetic properties of *Capparis spinosa* L. and its components. Biomedicine and Pharmacotherapy. 2017;92:293–302. DOI: https://doi.org/10.1016/j.biopha.2017.05.082.
33. Ganeshpurkar A, Saluja AK. The pharmacological potential of rutin. Saudi Pharmaceutical Journal. 2017;25(2): 149–164. DOI: https://doi.org/10.1016/j.jsps.2016.04.025.
34. Mollica A, Stefanucci A, Macedonio G, Locatelli M, Luisi G, Novellino E, et al. Chemical composition and biological activity of *Capparis spinosa* L. from Lipari Island. South African Journal of Botany. 2019;120:135–140. DOI: https://doi.org/10.1016/j.sajb.2018.02.397.
35. Ferreres F, Sousa C, Valentão P, Seabra RM, Pereira JA, Andrade PB. Tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC) seeds: Phytochemical characterization and antioxidant potential. Food Chemistry. 2007;101(2): 549–558. DOI: https://doi.org/10.1016/j.foodchem.2006.02.013.
36. Ehsanifar A, Kazemipoor N, Fouladi B. Anti-mycobacterial activity of *Capparis spinosa* l. extract against clinical isolates of *Mycobacterium tuberculosis*. Asian Journal of Pharmaceutical and Clinical Research. 2017;10(8):181–185. DOI: https://doi.org/10.22159/ajpcr.2017.v10i8.17629.
37. Wu J, Du G, Zhou J, Chen J. Systems metabolic engineering of microorganisms to achieve large-scale production of flavonoid scaffolds. Journal of Biotechnology. 2014;188:72–80. DOI: https://doi.org/10.1016/j.jbiotec.2014.08.016.
38. Tir M, Feriani A, Labidi A, Mufti A, Saadaoui E, Nasri N, et al. Protective effects of phytochemicals of *Capparis spinosa* seeds with cisplatin and CCl_4 toxicity in mice. Food Bioscience. 2019;28:42–48. DOI: https://doi.org/10.1016/j.fbio.2019.01.002.
39. Official methods of analysis of AOAC international. 16th ed. Washington: Association of Official Analytical Chemists; 1995.
40. Faraji M, Farhoodi M, Roozbeh Nasiraie L. Antibacterial effect of ethanolic extract shallot (Allium hirtifolium) on the Clostridium botulinum and Staphylococcus aureus and orgoanoleptic propertise of liquid curd. Iranian Journal of Nutrition Sciences and Food Technology. 2018;12(4):73–82.
41. Bahgaat WK, Ghani SAE. Comparison of amino acids and fatty acids profiles of egyptian kishk: Dried wheat based fermented milk mixture as functional food. American Journal of Food Technology. 2017;12(1):43–50. DOI: https://doi.org/10.3923/ajft.2017.43.50.

ORCID IDs
Marjan Nouri https://orcid.org/0000-0001-7028-8474