RESEARCH ARTICLE

Milk protein and fat play different roles in affecting the bioavailability and the antioxidant activity of jujube juice phenolics in rats

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Scope: In this study, we intend to clarify the role that milk proteins and fat may play in affecting the bioavailability and the antioxidant activity of jujube juice phenolics.
Methods and results: Three milk preparations—skimmed milk, milk fat, and whole milk were produced to represent milk protein, milk fat, and milk protein and fat together, respectively. The bioavailability of phenolics and the rat plasma antioxidant capacity were measured for 8 h after the consumption of jujube juice with and without milk preparations. The addition of skimmed milk to jujube juice resulted in significant changes in the plasma kinetics profile of phenolics, rather than affecting the overall absorption. Milk fat did not interact with jujube juice phenolics. However, when jujube juice was ingested with whole milk, a significant reduction of the bioavailability of phenolics and the maximum increase in plasma antioxidant capacity was observed. Moreover, a consistent increase in the median diameters of the emulsions indicated the formation of complexes of proteins, fat, and phenolics during digestion.
Conclusion: The present study suggests that when ingested with jujube juice, milk proteins and fat play different roles in affecting the bioavailability and the antioxidant activity of jujube juice phenolics.

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1 Introduction

The natural phenolic compounds present in plants have received much attention because they may help the body to cope with oxidative stress [1, 2]. Epidemiological evidence shows that the consumption of fruits and vegetables can decrease the risk of heart disease and cancer [3]. However, it should be noted that the health effects of phenolics are critically determined by their bioavailability. Phenolic compounds are generally consumed in foods along with other macronutrients such as proteins and fat. These dietary components may have an impact on the bioavailability and the bioefficacy of phenolics [4].

In many countries, polyphenol-rich foods such as coffee or tea are usually consumed with milk [5]. It has been proposed that the linking of phenolics with milk proteins may impair their bioefficacy [6]. Lorenz et al. showed that the addition of milk to black tea blunted its vascular protective effects [7], and caseins were identified as the interfering agents via interactions with tea catechins *in vitro*. Further support is provided by an *in vitro* study that suggested that the addition of milk proteins could impair the antioxidant activity of tea and coffee polyphenols [8]. It has been reported that

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Abbreviations: AUC, area under the curve; **CA**, caffeic acid; $\mathbf{C_{max}}$, maximum plasma concentration; **J**, jujube juice with water; **JMF**, jujube juice with milk fat; **JSM**, jujube juice with skimmed milk; **JWM**, jujube juice with whole milk; **PA**, protocatechuic acid; **SM**, skimmed milk with water; **TAC**, total antioxidant capacity; $\mathbf{T_{max}}$, time to reach maximum plasma concentration; **TRAP**, total radical-trapping antioxidant parameter; **VA**, vanillic acid

phenolic compounds can bind to proteins through hydrogen bonding and hydrophobic interactions [9, 10]. Moreover, the polyphenols can also bind to protease and inhibit the enzyme activity [11]. Therefore, it has been postulated that the protein–polyphenol complexes are resistant to digestion and, thus, impair the bioavailability of polyphenols. However, some conflicting results were reported. Tagliazucchi et al. found that some polyphenols could enhance the enzymatic activity of pepsin [12]. This discrepancy is associated with many factors that affect the binding of the polyphenols to proteins, including the structure of the protein and the polyphenol [13], but also external conditions such as pH, temperature, and ionic strength [14, 15]. Therefore, the interactions between proteins and polyphenols may have different effect under different conditions. To date, most literature focuses on the interaction between milk proteins and polyphenols *in vitro*, but the information about the fate of the milk protein–polyphenol complexes *in vivo* and its effect on the bioavailability and the antioxidant activity of polyphenols is still limited.

With respect to milk fat, there may be a physiological effect on gastric emptying and small bowel transit time [16, 17]. This effect would delay but not decrease the absorption of phenolics, as in the case of strawberries consumed with cream [18]. In addition, Serafini et al. found that the addition of milk proteins and fat together to blueberry extracts was associated with a high degree of inhibition of the *in vitro* antioxidant activity of blueberry extracts [19]. An explanation for this observation is that the polypeptide chains located around the fat globule membrane provide a favorable environment for their linkage with phenolics [20]. Therefore, based on these results, we presumed that milk fat with and without milk proteins might have different patterns in affecting the antioxidant activity of phenolics.

Jujube (*Ziziphus jujuba Mill.*) belongs to the Rhamnaceae family and is distributed widely in Asia and southern Europe. It has been used as food and a traditional Chinese medicine for thousands of years. Our previous study and other studies have revealed that jujube contains abundant phenolic compounds with high antioxidant activity [21–26]. In recent years, jujube juice milk has been highlighted because it contains different food components that may provide health benefits beyond those arising from individual food components. The popularity of this beverage in the Chinese market indicates that consumers have incorporated it in their diet.

Therefore, the aim of the present study was to clarify the role that milk proteins and fat may play in affecting the bioavailability and the antioxidant activity of jujube juice phenolics. Three milk preparations were produced to represent the different components in milk: skimmed milk corresponding to milk protein, milk fat corresponding to the fat in milk, and whole milk corresponding to milk protein and fat together. The rat plasma antioxidant capacity and the bioavailability of phenolics were measured for 8 h after the consumption of jujube juice with and without milk preparations. Moreover, the median diameters of the emulsions in gastric and duodenal contents after the consumption of jujube juice with and without milk preparations were measured to investigate the physicochemical processes that occurred during *in vivo* digestion.

Table 1. Nutritional composition of the milk preparations used in present study[a)]

	Whole milk	Skimmed milk	Milk fat
Proteins (g)	3.30	3.30	0.1
Lipids (g)	3.85	0.1	3.80
Carbohydrates (g)	4.90	4.90	4.85
Calcium (mg)	120	120	115

a) One hundred milliliters of product.

2 Materials and methods

2.1 Chemicals and reagents

Chemicals and solvents were obtained from Sigma (St. Louis, MO, USA) unless otherwise stated. The commercial standards of protocatechuic acid (PA) and mandelic acid were purchased from Tokyo Chemical Industry (Tokyo, Japan). Double distilled water (Millipore; Billerica, MA, USA) was used throughout the study.

2.2 Experimental materials

Freeze-dried jujube (*Z. jujuba cv. Dongzao*) powder was prepared by the method described in our previous study [21]. The jujube powder was extracted by stirring with water (1:3, w/w) for 1 h. The resulting slurries were centrifuged for 10 min at 5000 × *g*. The supernatants were collected and the residue was re-extracted under the same conditions. The combined extracts were concentrated by a rotary evaporator under vacuum at 45°C to obtain the jujube juice.

Raw whole milk, skimmed milk, and milk ultrafiltrate were supplied by Beijing Sanyuan Food Corp. Ltd. (Beijing, China). The milk preparations were manufactured from a single milk batch. In brief, raw whole milk was creamed to obtain a stock cream and raw milk fat was obtained by blending the stock cream with the milk ultrafiltrate to obtain a desired fat content of approximately 3.8%. Then, the raw whole milk and milk fat were subjected to a commercial homogenization to form the same fat globule droplet size as commercial milk. All three milk preparations were pasteurized at 72°C for 15 s before further use. The milk fat droplet size did not vary due to pasteurization (data not shown). The nutritional composition of the milk preparations is shown in Table 1.

2.3 Analysis of jujube juice phenolics

Triplicate aliquots of the jujube juice were taken for quantitative analysis of their phenolic profile. Each aliquot was mixed with an equal volume of methanol. The mixture was

then centrifuged at 3000 × *g* at 4°C for 15 min. The supernatant was collected for analysis. The phenolic profile of the jujube juice was assessed by an HPLC system equipped with a diode array detector [27]. The HPLC analyses were run on an Inertsil ODS-3 column (4.6 × 250 mm, 5 μm) held at 30°C and eluted at a flow rate of 0.4 mL/min. The mobile phases consisted of 0.4% v/v of formic acid in water (solvent A) and ACN (solvent B). The linear gradient was as follows: 0–1–15–18–19–21 min, 5–5–35–50–80–5% of ACN. The phenolic compounds were identified by comparing the absorbance spectra and retention times with standards.

2.4 Animals and in vivo study design

Male Wistar rats (220 ± 12 g; Vital River Lab Animal Technology, Beijing, China) were housed two per cage in a temperature-controlled room (24 ± 1°C) with a 12-h light/dark cycle. The animals had free access to a commercial diet (Ke Ao Xie Li Feeds, Beijing, China) and deionized water. The study was approved by the China Agricultural University Institutional Animal Care Committee and was conducted in accordance with China Agricultural University guidelines for the care and use of laboratory animals (2011070121).

After a 1-week adaptation period, the rats were administered halothane anesthesia and a polyethylene catheter was inserted into the jugular vein for blood sampling. The experiment was conducted at least 5 days after surgery. Two days before consumption of the test drinks, rats were fed a purified diet that was free of any polyphenolic- or flavonoid-like compounds. Rats were randomly divided into four groups (eight rats in each group) and deprived of food the night before the experimental day. The experiments were begun in the morning and were performed on awake and unrestrained animals. Each group was assigned to one of the following treatments: jujube juice with water (water treatment, J), jujube juice with whole milk (whole milk treatment, JWM), jujube juice with skimmed milk (skimmed milk treatment, JSM), and jujube juice with milk fat (milk fat treatment, JMF). Each treatment contained two drinks in a 1:1 volume ratio, and the total volume of force-feeding was 2.5 mL. Blood samples of approximately 250 μL were collected at 0, 0.5, 1, 2, 3, 4, 6, and 8 h after the force-feeding. The blood samples were collected in heparin-containing tubes and centrifuged at 1500 × *g* at 4°C for 10 min. The aliquots of plasma were promptly frozen and stored at –80°C.

For the analysis of the droplet size of the emulsions in the gastric and duodenal contents, other rats were divided into seven groups (eight rats in each group). After a 1-week adaptation period, the rats were starved but were given free access to 50 g/L of glucose from 40 to 18 h before the experiment and were kept in restraining cages for the last 18 h, with only water provided ad libitum to avoid coprophagy. Therefore, the rat stomachs were free of any material [28]. On the experimental day, each group was assigned to one of the following treatments: jujube juice with water (J), jujube juice with whole milk (JWM), jujube juice with skimmed milk (JSM), jujube juice with milk fat (JMF), whole milk with water, skimmed milk with water (SM), and milk fat with water. Each treatment contained two drinks in a 1:1 volume ratio and the total volume of force-feeding was 2.5 mL. Four rats in each experimental group were anesthetized and killed by exsanguination at 1 h and 3 h after intragastric intubation. The stomach and duodenum were removed and their contents were collected on ice after rinsing with 2 mL of ice-cold 0.9% sodium chloride. Then, 50 μL of a methanolic solution of lipase inhibitors [29] (100 mM diisopropyl fluorophosphate, 50 mM acetophenone, and 250 mM phenylboronic acid) was added to per milliliter of the contents, and the sample was immediately used for particle size measurement. The particle size measurement was carried out by using a particle-size analyzer (Capa 700, Horiba, Kyoto, Japan). The accuracy of the data given by the Capa 700 was checked by using calibrated microparticles in the size range of 0.2–100 μm (polystyrene size-standard kit, Polyscience Inc., Warrington, PA, USA). Average particle size was calculated by the particle-sizer software. The lipase inhibitor cocktail did not change the droplet size of the emulsions (data not shown).

2.5 Extraction of phenolic acids in plasma

The phenolic acids in plasma were detected after enzymatic and acidic hydrolysis of the conjugated forms, as described by Azzini et al. [30]. Plasma samples (100 μL) containing 2,4,5-trimethoxycinnamic acid (5 μM) as an internal standard were hydrolyzed by adding 20 μL of ascorbic acid (1%), 20 μL of acetic acid (0.2 M), and 10 μL of β-glucuronidase type HP2 from *Helix pomatia* (165 100 U/mL). After incubation at 37°C for 2 h, proteins were precipitated with 200 μL of methanol:HCl (3 N) 1:1 v/v. Polyphenols were extracted with 2 mL of ethyl acetate, followed by stirring and sonication (2–3 min) before centrifugation at 2000 × *g* for 5 min. The extraction procedure was repeated twice, and the ethyl acetate extracts were combined, dried under nitrogen, and dissolved in methanol:H_2O (1:1) for analysis.

2.6 Quantitative analysis of phenolic acids in plasma

The phenolic acid analyses were conducted using an Agilent 1100 HPLC system including an autosampler, a binary pump, and a diode array detector (Agilent Technologies, Palo Alto, CA, USA), coupled with a 4000 QTRAP™ mass spectrometer (Applied Biosystems, Forest City, CA, USA). Separation was performed on a Phenomenex Synergi Max-RP column (3.0 × 150 mm, 4 μm) using a flow rate of 0.4 mL/min. The mobile phases were constituted with the following: solvent A, 0.4% v/v of formic acid in water, and solvent B, ACN. The 21-min linear gradient was as follows: 0–1–13–18–19–20–21 min, 5–5–31–80–80–5–5% of ACN, followed by 10 min of re-equilibration of the column before the next run. The mass

spectrometer used an electrospray interface in negative ionization mode. Major parameters were the following: 25 for curtain gas, –4200 V for potential of electrospray capillary, 500°C for source temperature, and 45 and 60 for nebulizing and turbo spray gas, respectively. The entrance potential was set at –10 V. The declustering potential, collision energy, and collision cell exit potential were optimized individually for each standard.

2.7 Total antioxidant capacity (TAC) assay

The total antioxidant capacity (TAC) *in vivo* was assessed using the total radical-trapping antioxidant parameter (TRAP) assays. The TRAP method is based on the protection provided by antioxidants on the fluorescence decay of R-phycoerythrin (lag phase) during a controlled peroxidation reaction [31]. In brief, 50 μL of the diluted sample was added to 75 μL of PBS (pH 7.4), 15 μL of R-phycoerythrin (4.30×10^{-3} μg/μL), and 60 μL of 2,2′-azobis (2-amidinopropane) dihydrochloride (7.5 mM). The reaction kinetics at 38°C was recorded for 60 min ($\lambda_{ex} = 495$ nm, $\lambda_{em} = 570$ nm) by a fluorescence plate reader spectrometer. The length of the lag phase, automatically calculated, was used to assess TRAP values, expressed as μmol/L for plasma.

2.8 Statistical analysis

The results obtained are expressed as means ± SD. The area under the curve from 0 to 8 h [$AUC_{(0–8\,h)}$] was calculated using the linear trapezoidal rule. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined directly from the data. Log transformations were performed on variables that were not normally distributed. A *t* test was used to compare the median diameters between the gastric and duodenal contents in the same treatment. For comparisons between treatments, data were analyzed by analysis of variance (ANOVA) and the Tukey multiple comparison test (SPSS, version 17.0; SPSS Inc., Chicago, IL, USA). Differences were considered significant at $p < 0.05$.

3 Results

3.1 Phenolic composition of jujube juice

The phenolic compounds identified in the jujube juice are presented in Table 2. Eight major phenolic compounds were identified: caffeic acid (CA), gallic acid, 4-hydroxybenzoic acid, 3-(4-hydroxyphenyl)propionic acid, mandelic acid, PA, quercetin, and quercetin galactoside. Of these, 4-hydroxybenzoic acid was the most abundant, and its concentration was 22.34 ± 1.32 mg/100 mL.

Table 2. Phenolic composition of jujube juice[a)]

Phenolic compound	Concentration (mg/100 mL)
Caffeic acid	3.30 ± 0.22
Gallic acid	19.71 ± 1.17
4-Hydroxybenzoic acid	22.34 ± 1.32
3-(4-Hydroxyphenyl) propionic acid	1.97 ± 0.15
Mandelic acid	3.35 ± 0.24
Protocatechuic acid	3.23 ± 0.15
Quercetin	14.45 ± 1.22
Quercetin galactoside	2.63 ± 0.25

a) The values are expressed as means ± SD ($n = 3$).

3.2 The effects of the addition of milk preparations on the bioavailability of jujube juice phenolics

After jujube juice consumption, CA, PA, and vanillic acid (VA) were identified in rat plasma. The quantities present 0.5–8 h after the ingestion of jujube juice are depicted in Fig. 1 and the pharmacokinetic parameters are presented in Table 3. Only one plasma concentration peak of the phenolic acids at 0.5–1 h postingestion was observed in the J, JWM, and JMF treatments. Interestingly, unlike the other three treatments, two plasma concentration peaks were found in the JSM treatment: peak 1, 0.5–1 h postingestion; and peak 2, approximately 4 h post-ingestion for CA and approximately 6 h postingestion for PA and VA. With respect to the pharmacokinetic parameters, the C_{max} of the phenolic acids did not differ significantly between the water and milk fat treatments. In contrast, the addition of whole milk and skimmed milk reduced the C_{max} for CA (−69.7% and −55.9%, respectively), PA (−25.6% and −35.5%, respectively), and VA (−47.4% and −43.9%, respectively) when compared to the levels reached after the ingestion of jujube juice with water. The T_{max} values of phenolic acids ranged from 0.6 to 1.6 h. Unlike C_{max}, the addition of milk preparations did not significantly ($p > 0.05$) affect the T_{max} values of phenolic acids. The AUC corresponded to the available plasma jujube juice phenolics against time. The addition of whole milk significantly ($p < 0.01$) reduced the $AUC_{(0–8\,h)}$ of CA and PA (−76.9% and −52.6%, respectively) compared with the water treatment. Unlike whole milk, the addition of skimmed milk to jujube juice did not significantly reduce the $AUC_{(0–8\,h)}$ of the PA and VA. Likewise, the addition of milk fat significantly reduced only the $AUC_{(0–8\,h)}$ of the PA (−21.2%, $p < 0.05$) when compared with the water treatment.

3.3 The effects of the addition of milk preparations on changes in rat plasma antioxidant capacity after the consumption of jujube juice

The changes in rat plasma antioxidant capacity over 8 h after the consumption of jujube juice with water or milk

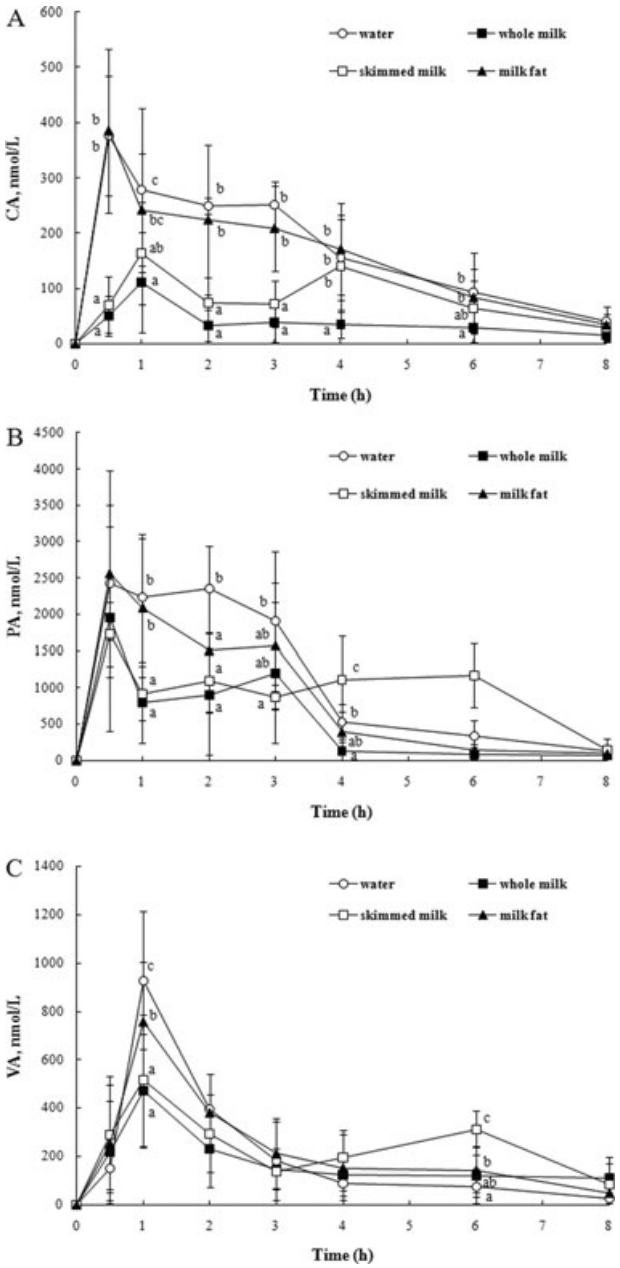

Figure 1. Changes in rat plasma concentrations of phenolic acids over 8 h after the ingestion of jujube juice with water (○), whole milk (■), skimmed milk (□), and milk fat (▲). The experimental details can be found in section Materials and methods. The values are expressed as means ± SD ($n = 8$). Different letters indicate a significant difference ($p < 0.05$) between treatments at a given time. (A) caffeic acid (CA); (B) protocatechuic acid (PA); (C) vanillic acid (VA).

preparations are shown in Fig. 2. The plasma markers of TAC increased significantly after jujube juice ingestion, reaching a maximum peak at 2 h. However, when jujube juice was ingested with whole milk or skimmed milk, the maximum increase in plasma antioxidant capacity fell from 38.1 ± 7.6 μM to 11.6 ± 1.8 μM and 24 ± 2.1 μM, respectively. Milk fat did not have a significant effect on changes in plasma antioxidant capacity compared with water.

3.4 The median diameters of the emulsions in gastric and duodenal contents after the consumption of jujube juice and milk preparations alone or together

The average particle size of the emulsions in gastric contents and duodenal contents 1 h and 3 h, respectively, after ingestion is shown in Fig. 3. When jujube juice was ingested alone, the median diameters of the emulsions in gastric and duodenal contents were 5.8 and 7.3 μm, respectively. However, when whole milk or skimmed milk was ingested with jujube juice, the average particle size of the emulsions was significantly ($p < 0.05$) higher compared to whole milk or skimmed milk ingested alone. In contrast, when jujube juice was ingested with milk fat, no significant difference was found for the median diameters of the emulsions compared with when milk fat alone was ingested. Compared to the gastric contents, the emulsion median diameters in the duodenal contents tended to be greater (1.5-fold higher) for the JWM treatments. Conversely, a decreasing trend was observed for the JSM and SM treatments.

4 Discussion

After consumption of jujube juice, three phenolic acids were detected in rat plasma. Among them, VA was not detected in jujube juice. It indicated that VA might be present in bound form in jujube juice. The result is similar with Russell et al. who found that VA was not available as a free acid in strawberries, but was detected in human plasma 1 h after consumption. It appears that this acid has been rapidly deconjugated in the blood [32]. When compared the initial time and 2 h after jujube juice consumption, an increase of 19% in the plasma antioxidant capacity was observed. Moreover, a good correlation between antioxidant capacity and phenolics levels was found ($r = 0.55$, 0.72, and 0.64 for CA, PA, and VA, respectively). Therefore, it indicated that the increased plasma antioxidant capacity was associated with the increase of polyphenols in the present study.

With respect to the effect of milk preparations, the results of our preliminary study have showed that milk ultrafiltrate did not show any significant affect on either the bioavailability or the antioxidant activity of jujube juice phenolics in rats (data not shown). Therefore, the effect of ingredients in milk preparations other than proteins and fats was neglected. No difference was found in the T_{max} values of phenolic acids in any of the treatments, and the T_{max} values ranged from 0.6 to 1.6 h postingestion (Table 3). Hence, phenolic compounds are most likely to be absorbed and metabolized by the small intestine. However, considering the kinetics profile shown in Fig. 1, we observed that even though skimmed milk

Table 3. The pharmacokinetic parameters of phenolic acids in rat plasma after the consumption of jujube juice with water or different milk preparations[a)]

	Water	Whole milk	Skimmed milk	Milk fat
C_{max} (nmol/L)				
CA	376 ± 136^{b}	114 ± 99^{a}	166 ± 116^{a}	390 ± 164^{b}
PA	2665 ± 413^{b}	1984 ± 1807^{ab}	1727 ± 549^{a}	2724 ± 1389^{b}
VA	927 ± 362^{b}	488 ± 274^{a}	520 ± 331^{a}	758 ± 311^{b}
T_{max} (h)				
CA	0.6 ± 0.8	0.9 ± 0.6	1.6 ± 3.7	0.8 ± 1.9
PA	1.4 ± 3.1	0.6 ± 0.8	1.4 ± 5.1	0.6 ± 0.8
VA	1.2 ± 1.1	0.9 ± 0.6	1.0 ± 1.7	1.2 ± 1.1
AUC (nmol/L·h)				
CA	1375 ± 277^{c}	317 ± 212^{a}	688 ± 368^{b}	1283 ± 226^{c}
PA	8824 ± 1561^{c}	4183 ± 2882^{a}	7735 ± 1278^{bc}	6951 ± 2845^{b}
VA	1673 ± 676^{ab}	1437 ± 851^{a}	2010 ± 523^{b}	1883 ± 639^{ab}

a) The values are expressed as means ± SD ($n = 8$).
Different superscript letters indicate a significant difference ($p < 0.05$) between treatment comparisons.
AUC, area under the curve; CA, caffeic acid; C_{max}, maximum plasma concentration; PA, protocatechuic acid; T_{max}, time to reach C_{max}; VA, vanillic acid.

did not modify the T_{max} values of phenolic acids, it modified the plasma appearance of those metabolites. The biphasic phenomenon observed may indicate that some of phenolic compounds are absorbed in the large intestine. Moreover, the absorption of phenolic compounds in the large intestine led to no significant differences in the AUC of PA and VA between the skimmed milk and water treatments. To date, few published studies have focused on understanding how milk proteins affect the *in vivo* bioavailability and bioefficacy of phenolics. Hassimotto et al. demonstrated that the addition of skimmed milk affected the bioavailability of phenolics from blackberry juice [33]. However, the postingestion plasma concentrations of phenolics were determined only for 4 h, and no pharmacokinetic parameters were calculated. Some *in vitro* studies have showed that polyphenols can bind to proteins with subsequent complexation [34, 35]. Moreover, it has been found that polyphenols have a significant affinity for proteins and peptides that contain a high proportion of proline residues in their sequences [36]. Accordingly, milk proteins have a high content of proline such as β-casein that is a 209 residue protein containing 35 prolines evenly distributed through the sequence [37]. Due to the rich proline present in milk proteins, it could be expected that additional interactions between polyphenols and peptides may present during the early digestion and which could be a hindrance to the polyphenols absorption through the intestinal brush border. Therefore, based on our present results, we proposed that when jujube juice was ingested with skimmed milk, the formation of milk protein/peptide–polyphenol complexes would decrease the absorption of polyphenols in the small intestine, and some polyphenols were not released from the complexes until it reached the large intestine. The present study suggests, for the first time, that the interaction between milk proteins and phenolics results in significant changes in the plasma appearance of phenolics, rather than affecting the overall absorption.

Milk fat did not significantly affect the T_{max} of phenolic acids and the plasma antioxidant capacity as compared to water. It has been previously reported that cream significantly delayed both gastric emptying and mouth-to-cecum transit time [18]. Cream contains fat content a tenfold higher compared to that of the milk fat used in the present study. Therefore, the explanation for the present result is that there was probably not enough fat in the milk fat to affect either gastric emptying or mouth-to-cecum transit time. However, the addition of milk proteins and fat together (whole milk) to jujube juice showed the highest degree of inhibition in both the bioavailability of the phenolics and the maximum increase in plasma antioxidant capacity. This finding is in accordance with an *in vitro* study, where the authors proposed that the polypeptide chains located around the fat globule membrane link with the phenolic compounds [20]. However, our results suggest that the situation during *in vivo* digestion is more complex.

Based on the results from the present study as well as from previous studies, we propose a schematic diagram of the physicochemical processes occurring before and after digestion when different milk preparations were added to jujube juice (Fig. 4). The median diameter of the emulsion was significantly higher when jujube juice with skimmed milk was consumed than when skimmed milk alone was consumed (Fig. 3). The finding suggests the formation of milk protein–polyphenol complexes. However, the decreasing trend of the median diameter found in the JSM treatments indicates the breakdown of the complexes during digestion. Combined with our *in vivo* study, the results suggest that phenolic compounds are released from the complexes and absorbed in the large intestine. No significant difference was found in the median diameters of the emulsions between the consumption of jujube juice with milk fat and milk fat alone, in accordance with the *in vivo* results that did not show any interaction between milk fat and phenolics. Nevertheless,

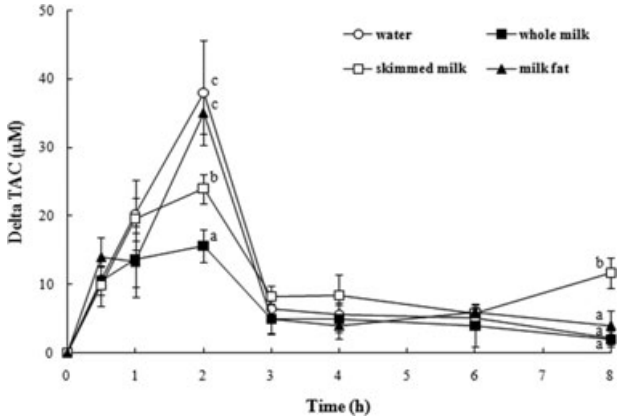

Figure 2. Changes in rat plasma antioxidant capacity over 8 h after the consumption of jujube juice with water (○), whole milk (■), skimmed milk (□), and milk fat (▲). The experimental details can be found in section Materials and methods. The values are expressed as means ± SD ($n = 8$). Different letters indicate a significant difference ($p < 0.05$) between treatments at a given time.

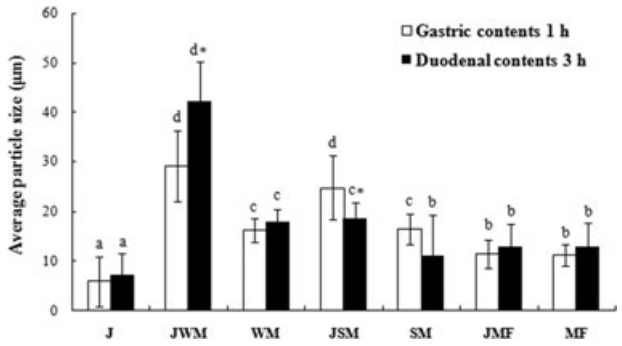

Figure 3. The average particle size of the emulsions in gastric contents and duodenal contents 1 h and 3 h, respectively, after ingestion. The experimental details can be found in the Materials and methods section. The values are expressed as means ± SD ($n = 4$). Different letters indicate a significant difference ($p < 0.05$) between treatment comparisons at a given time. *Significantly different from the average particle size of the emulsions in gastric contents in the same treatment, $p < 0.05$. J, jujube juice with water; JMF, jujube juice with milk fat; JSM, jujube juice with skimmed milk; JWM, jujube juice with whole milk; MF, milk fat with water; SM, skimmed milk with water; WM, whole milk with water.

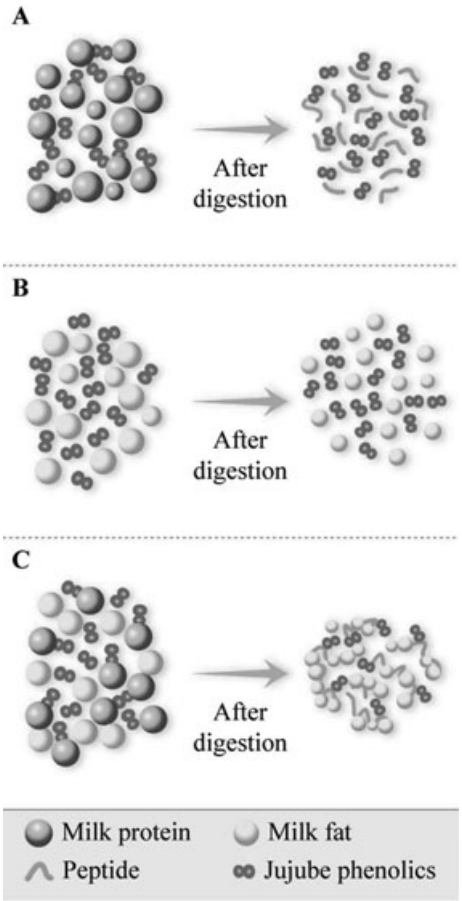

Figure 4. A simplified scheme of the physicochemical processes occurring before and after digestion when different milk preparations were added to jujube juice. (A) jujube juice with skimmed milk; (B) Jujube juice with milk fat; (C) jujube juice with whole milk.

when jujube juice and whole milk were ingested together, a significant further increase in the median diameter of the emulsion was found during digestion. Considering that phenolics can bind to milk proteins [34] and that proteins can be absorbed to the surface of lipid droplets, leading to the aggregation of fat globules during digestion [38], the data suggest a possible involvement of lipids in the interaction between proteins and phenolics during *in vivo* digestion. We presumed that protein–polyphenol complexes might be absorbed to the surface of the lipid droplets and create a more remarkable aggregation of the fat globules. Unlike protein–polyphenol complexes, the complexes with lipids did not break during *in vivo* digestion and thus impair the bioavailability and the antioxidant activity of jujube juice phenolics. One possible reason is that the aggregation of the fat globules affects the way gastrointestinal enzymes access substrates [39]. Another explanation is that lipase activity is inhibited by the phenolic compounds [40] and the long-chain fatty acids are generated during digestion [41–43].

In conclusion, in this study, we demonstrate that when ingested together with jujube juice, milk proteins and fat play different roles in affecting the bioavailability and the antioxidant activity of jujube juice phenolics. Milk proteins lead to significant changes in the plasma kinetics profile of phenolics, rather than affecting the overall absorption. Milk fat alone does not interact with jujube juice phenolics. However, milk fat along with milk proteins impairs the increase in plasma antioxidant capacity and the bioavailability of phenolics after the consumption of jujube juice. More in-depth mechanisms will require clarification in future investigations.

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