Effect of commercial grape extracts on the cheese-making properties of milk

Denise Felix da Silva,* Paula T. Matumoto-Pintro,* Laurent Bazinet,†‡ Charles Couillard,‡ and Michel Britten§¹

*Departamento de Agronomia, Universidade Estadual de Maringá, Avenida Colombo 5790, Maringá, Paraná 87020-900, Brazil

†Dairy Research Centre (STELA), Université Laval, Quebec City, Quebec, Canada G1V 0A6

‡Institute of Nutrition and Functional Foods (INAF), Laval University, Quebec City, Quebec, Canada G1V 0A6

§Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Boulevard West, Saint-Hyacinthe, Quebec, Canada J2S 8E3

ABSTRACT

Grape extracts can be added to milk to produce cheese with a high concentration of polyphenols. Four commercial extracts from whole grape, grape seed, and grape skin (2 extracts) were characterized and added to milk at concentrations of 0, 0.1, 0.2, and 0.3% (wt/vol). The effect of grape extracts on the kinetics of milk clotting, milk gel texture, and syneresis were determined, and model cheeses were produced. Whole grape and grape seed extracts contained a similar concentration of polyphenolic compounds and about twice the amount found in grape skin extracts. Radical scavenging activity was directly proportional to the phenolic compounds content. When added to milk, grape extracts increased rennet-induced clotting time and decreased the clotting rate. Although differences were observed between the extracts, the concentration added to milk was the main factor influencing clotting properties. With increasing concentrations of grape extracts, milk gels showed increased brittleness and reduced firmness. In addition, syneresis of milk gels decreased with increasing concentrations of grape extracts, which resulted in cheeses with a higher moisture content. The presence of grape extracts in milk slightly increased protein recovery in cheese but had no effect on fat recovery. With whole grape or grape seed extracts added to milk at 0.1% (wt/vol), the recovery coefficient for polyphenols was about 0.63, and decreased with increasing extract concentration in milk. Better polyphenol recovery was observed for grape seed extracts (0.87), with no concentration effect. Commercial extracts from whole grape, grape seed, or grape skin can be added to milk in the 0.1 to 0.3% (wt/vol) concentration range to produce cheese with potential health benefits, without a negative effect on cheese yield.

Key words: grape extract, polyphenol, milk gel, cheese

INTRODUCTION

Polyphenols and flavonoids are known for their health benefits, especially for the prevention of diseases associated with oxidative stress such as cancer and cardiovascular, inflammatory, and neurodegenerative diseases (Hervet-Hernández et al., 2009). During their development, plants produce these phenolic compounds as secondary metabolites to protect themselves from oxidative stress. Oxidative stress has been implicated in several chronic disorders. The damaging effects of oxidative processes in living organisms can be diminished by dietary intake of polyphenols such as flavan-3-ols and procyanidins, which are present in grape seed extract (Chedea et al., 2010).

Phenolic compounds have been extracted from a variety of plant sources and used as ingredients in food matrices (Boroski et al., 2012). They have been shown to improve functional properties of dairy products such as storage and heat stability, as well as foaming properties (O'Connell and Fox, 2001). Enrichment with phenolic compounds has been proposed for various products such as dairy beverages (Boroski et al., 2012), yogurt (Najgebauer-Lejko et al., 2011), milk powder (Gad and El-Salam, 2010), and processed cheese (Chen et al., 2009).

Given that polyphenols interact with protein (Frazier et al., 2010), their addition to milk should result in high recovery in cheese. The interaction between phenolic compounds and proteins depends on the pH, molar ratio, and molecular properties of the polyphenols (Gad and El-Salam, 2010), and involves both hydrophobic and hydrophilic interactions (Hasni et al., 2011). Recent studies have shown, however, that rennet-induced coagulation is impaired by the addition of tea polyphenols to milk (Han et al., 2011; Haratifar and Corredig, 2014). Despite this limitation, Cheddar-type cheese has been produced from milk enriched with green tea extract (Giroux et al., 2013).

Whereas the use of tea extracts as a source of polyphenols in food formulations has been extensively stud-

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¹Corresponding author: michel.britten@agr.gc.ca

ied, grape extracts have received little attention. Yet grape extracts could be used in cheese making to avoid some of the problems associated with tea extracts, such as flavor and color defects (Giroux et al., 2013). Grape extracts are sold commercially and have generally recognized as safe (GRAS) status authorized by the US Food and Drug Administration in 2003. They have shown antioxidant activities both in vivo and in vitro in a variety of food formulations (Hu et al., 2004; Shaker, 2006; Brannan and Mah, 2007). Polyphenols from grape extracts exist mainly in polymeric form, whereas polyphenols from tea extracts exist mainly in monomeric form (Jeong and Kong, 2004). According to O'Connell and Fox (2001), the interaction between polyphenols and milk proteins and their effects on milk coagulation are related to polyphenol molecular size. The effect of grape extracts on the cheese-making properties of milk needs to be documented before they can be used for cheese fortification.

The objective of this study was to add various concentrations of commercial grape extracts to milk and assess their effects on cheese-making properties. Rennet-induced milk clotting kinetics, milk gel texture, and syneresis, cheese moisture, and mass balance for protein, fat, and polyphenols were determined.

MATERIALS AND METHODS

Materials

Four commercial grape extracts from *Vitis vinifera* were provided courtesy of Ethical Naturals (Sunnyvale, CA). The extracts were obtained from whole grape (W), grape seed (S), and grape skin (SK). In accordance with Ethical Naturals specifications, W extract was prepared using a mixture of ethanol and distilled water (50:1 volume ratio). S extract was prepared with ethanol and distilled water in a volume ratio of approximately 30 to 50:1. Two types of skin extract were used, one made with an ethanol/distilled water ratio of 6:1 (SK1 extract) and the other with a ratio of 100:1 (SK2 extract). The extracts were received in sealed aluminum bags and stored at 4°C until use. Low-heat skim milk powder (35% protein) and fresh cream (~40% milk fat) were obtained from Agropur (Granby, QC, Canada). Milk protein isolate powder, obtained by ultrafiltration/diafiltration (85% protein), was purchased from Idaho Milk Products (Jerome, ID). Rennet, Chy-Max Extra chymosin, was purchased from Chr. Hansen (Milwaukee, WI). Calcium chloride solution (45%, wt/vol) was obtained from Fromagex (Rimouski, QC, Canada). All other reagents were of analytical grade.

Commercial Extract Characterization

Total Phenolic and Flavonoid Compounds. The total phenolic compounds concentration was measured by the method of Singleton and Rossi (1965) with modifications. The extracts were dispersed in methanol (0.1 mg/mL). A 250-μL aliquot of the methanolic solution was mixed with 250 μL of Folin-Ciocalteu reagent (diluted 1:1 in water), 500 μL of a sodium carbonate solution (300 mg/mL), and 4 mL of deionized water. After 30 min in darkness at room temperature, the mixture was centrifuged at $4,600 \times g$ for 15 min. The absorbance of the samples was measured at 725 nm. Gallic acid was used to prepare the standard curve, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg of GAE/g).

Total flavonoid content was measured by the aluminum chloride (AlCl₃) colorimetric method (Woisky and Salatino, 1998) with modifications. Methanolic extract solutions (0.1 mg/mL) were prepared, and 500-μL aliquots were mixed with 250 μL of 5% aluminum chloride (wt/vol in methanol). The volume was then completed with methanol to 5,000 μL. The mixture was left at room temperature in darkness for 30 min and centrifuged at $4,600 \times g$ for 15 min. A standard curve was prepared with quercetin, and the results were expressed as milligrams of quercetin equivalents per gram of extract (mg of quercetin equivalents/g).

DPPH Scavenging. Grape extracts were evaluated for their free-radical-scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method of El-Massry et al. (2002) with modifications. Grape extracts were dispersed in methanol (0.1 mg/mL), and various volumes were mixed with 2.0 mL of DPPH methanolic solution (0.047 mg/mL), vortexed, and kept in darkness for 1 h. Absorbance was measured at 517 nm against pure methanol (blank) using a spectrophotometer. Percentage inhibition of the DPPH radical was calculated as follows:

$$\% \text{ inhibition DPPH} = \frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}} \times 100, \quad [1]$$

where A_{DPPH} is the absorbance of the methanolic solution of DPPH, and A_{sample} is the absorbance of DPPH solution mixed with the extract dispersion. Percentage inhibition was plotted against the extract dispersion concentration, and 50% of DPPH inhibition (IC₅₀) was determined by linear interpolation.

Solubility. Grape extracts were dispersed in water at a concentration of 1.0% (wt/vol), and the pH was then adjusted to 2.0, 4.0, 6.0, or 8.0 with HCl or 1 N NaOH.

The dispersions were then centrifuged at $1,000 \times g$ for 10 min at room temperature. Total solids content of the grape extract dispersion (before centrifugation) and the supernatant were determined by dehydration in a vacuum oven at 102°C for 5 h (AOAC International, 1995). Solubility was expressed as the percentage of solids recovered in the supernatant.

Rennet-Induced Milk Gelation

Milk Preparation. Milk samples were prepared from low-heat skim milk powder dispersed in deionized water (8.8 g/100 g) at room temperature with stirring for 30 min. The protein content was increased to 4.1% (wt/vol) with milk protein isolate. The grape extracts were added to standardized milk at concentrations of 0, 0.1, 0.2, and 0.3% (wt/vol) with stirring for 15 min. Milk preparations were kept overnight at 4°C .

Kinetics of Rennet-Induced Milk Clotting. Milk clotting was monitored on the basis of the change in turbidity at 650 nm according to the method developed by Robitaille et al. (2004). Milk preparations (10 g) were degassed under vacuum. The temperature was raised to 34°C in a water bath and the pH was adjusted to 6.50 with 20% lactic acid. Calcium chloride and rennet were added to obtain final concentrations of 0.02% (wt/vol) and 0.01% (wt/vol), respectively, and mixed carefully. Renneted milks were immediately transferred to a 96 multi-well microplate, and the optical density (OD) was recorded at 650 nm and 34°C for 60 min at 30-s intervals using a microplate reader (Ultra Microplate Reader LE 808, Bio-Tek Instruments, Winooski, VT). Optical density values were divided by the optical density at time 0 to calculate relative optical density (OD_{rel}). Sigma Plot (version 12.3, SPSS, Chicago, IL) was used to fit OD_{rel} and time (t) data to the sigmoidal equation:

$$OD_{rel} = \left[\frac{1 - c}{1 + \left(\frac{t}{b} \right)^a} \right]^d + c, \quad [2]$$

where a is the slope coefficient, b is the inflexion point coefficient, c is the OD_{rel} at infinitive time, and d is the symmetry parameter. Using these parameters, rennet clotting time and rennet clotting rate were calculated using the method of Pandey et al. (2003). Rennet clotting time is the time after renneting when OD_{rel} increases by 0.01 units, and the rennet clotting rate corresponds to the maximum of the first derivative of equation (2; maximum clotting rate).

Milk Gel Texture. Milk preparations (80 mL) were placed in 4.5 cm i.d. glass containers and renneted in the conditions described in the previous section. Gel texture was measured after 30 min of coagulation time. A 4-bladed stainless steel vane (14 mm in diameter and 50 mm long), fitted to a rotational Brookfield DV-I+ viscometer (Brookfield Engineering Laboratories, Middleborough, MA), was inserted to a depth of 30 mm under the gel surface. Rotational speed was set to 2 rpm. Torque was continuously recorded and converted to stress values using torsion/vane testing software (Gel Consultants Inc., Raleigh, NC). Yield stress and yield strain, corresponding to the peak value on the stress versus strain curve, were used to build the texture plot.

Milk Gel Syneresis. The syneresis of rennet-induced milk gels was measured in conditions representative of the cooking step of the cheese-making process. Renneted milks were prepared as previously described and 5-g portions were transferred to polyallomer tubes (14 × 95 mm; Beckman Coulter, Mississauga, ON, Canada). Polyallomer tubes were used to reduce gel adherence to the wall, which improves repeatability (Giroux et al., 2014). After a 30-min coagulation period at 34°C , the tubes were transferred to a water bath set to 42°C for 80 min. At the end of the cooking step, the whey was recovered by decantation and weighed. Syneresis was calculated by dividing the weight of whey by the weight of milk (5 g) and expressed as a percentage.

Laboratory-Scale Model Cheeses

The cheese-making process was carried out in 12×12 cm stainless-steel mini-vats with deep plexiglass covers layered with cheesecloth to facilitate whey separation and recovery (Morin et al., 2008). Cheese milk was prepared according to the previous section, but fresh cream was also used to standardize protein and fat concentrations at 4.1% (wt/vol) and 2.8% (wt/vol), respectively. Cheese milk preparations were batch pasteurized at 65°C for 30 min and kept overnight at 4°C . Before cheese making, milk temperature was brought to 34°C for 20 min, calcium chloride was added at a concentration of 0.02% (wt/vol), and the pH was adjusted to 6.20 with 20% lactic acid. After renneting (0.01%, wt/vol), 144 mL of milk was transfer to each mini-vat and placed in an orbital shaking water bath (model C-76, New Brunswick Scientific, Edison, NJ) at 34°C . Milk gels were cut 30 min after renneting using a cutting device with stainless steel knives spaced 1 cm apart to produce 1-cm^3 curd particles. Cheese curd was agitated at a low speed (50 rpm) for 15 min, and then the temperature was gradually increased to 42°C over 30 min with a faster (90 rpm) agitation speed. After cooking, the mini-vats were inverted to recover the

Table 1. Composition and properties of commercial grape extracts

Item ¹	Grape extract ²				
	W	S	SK1	SK2	SE
Solubility (%)	85.6 ^b	92.6 ^a	93.4 ^a	89.3 ^{ab}	1.8
TPC (mg of GAE/g)	962 ^a	935 ^a	512 ^b	431 ^c	8
Flavonoids (mg of QE/g)	112 ^a	83 ^b	41 ^c	77 ^b	4
DPPH (IC ₅₀ , µg/mL)	78 ^b	84 ^b	162 ^a	166 ^a	8

^{a-c}Means within a row with different superscripts are significantly different ($P < 0.05$).

¹TPC = total phenolic compounds; GAE = gallic acid equivalent; QE = quercetin equivalent; DPPH = 1,1-diphenyl-2-picrylhydrazyl; IC₅₀ = concentration for 50% inhibition.

²Grape extracts were W = whole grape extract; S = grape seed extract; SK1 = grape skin extract #1; SK2 = grape skin extract #2.

cheese whey in the plexiglass covers. Whey drainage was performed at 38°C for 60 min without agitation. To simulate the pressing step, curd was centrifuged at $10,000 \times g$ for 30 min at 32°C. Expressed whey was collected and pooled with drained whey, weighed, and kept for composition analyses. Cheese moisture was determined by dehydration in a vacuum oven at 102°C for 5 h (AOAC International, 1995). Milk and whey composition (total protein and fat) was determined using a MilkoScan FT-120 (Foss, Eden Prairie, MN). The phenolic compounds in the whey were measured at 420 nm using the reagent Fast Blue BB salt (Medina, 2011). Unlike the Folin-Ciocalteu reagent, Fast Blue BB salt avoids the interference caused by non-phenolic antioxidants or reducing substances.

The recovery coefficients (K) for protein, fat, and phenolic compounds in cheese were calculated using the following equation:

$$K = 1 - \frac{C_W}{C_M}, \quad [3]$$

where C_W and C_M are the concentrations of phenolic compounds, protein, or fat in whey (W) and milk (M), respectively.

Statistical Analysis

The experiments were carried out according to a 4×4 factorial design (4 commercial extracts and 4 concentrations) and repeated 3 times. Data analysis was conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC). Variance analysis was used to determine whether the factors (concentration and extracts) and their interaction had a significant effect on the measured parameters. Differences were considered significant when $P \leq 0.05$. Standard errors were obtained from the statistical model and are indicated in the tables or shown as error bars in the figures.

RESULTS AND DISCUSSION

Composition and Properties of Commercial Grape Extracts

The composition and main characteristics of grape extracts are presented in Table 1. The extracts were easily dispersible in water, and the solubility was over 90% for S extract and SK1 extract and slightly lower for SK2 extracts and W extracts. Solubility was tested over a pH range of 2 to 8 and no statistically significant effect of pH was detected ($P > 0.05$). Considering the average coagulation time of milk after renneting (<30 min), no sedimentation of grape extract was expected to occur during the cheese-making process.

Seed and whole grape extracts presented higher concentrations of total phenolic compounds (~950 mg of GAE/g) than the 2 skin extracts (511 mg of GAE/g for SK1 and 431 mg of GAE/g for SK2; $P < 0.05$). The proportion of flavonoids (on a total polyphenolics basis) ranged from 8 to 12%, except in the case of the SK2 extract, which showed a higher proportion of flavonoids (18%). The free-radical-scavenging activity of grape extracts was determined using the DPPH assay. The concentration required to inhibit 50% of DPPH radicals (IC₅₀) is given in Table 1. According to Perumalla and Hettiarachchy (2011), the free-radical-scavenging activity of grape extracts is primarily due to the presence of polyphenols. As expected, IC₅₀ was inversely correlated with the phenolic concentration in the extract ($R^2 = 0.99$). Expressed on a polyphenol concentration basis, IC₅₀ value averaged 77 µg of GAE/mL, and was similar for the 4 extracts ($P > 0.05$). In a previous study, (Katalinić et al., 2010) analyzed the free-radical-scavenging activity of red grape skin extracts from 7 different varieties and reported IC₅₀ values ranging from 58 to 239 µg of GAE/mL, with an average of 148 µg of GAE/mL. The grape extracts used in the present study therefore can be considered to have very good free-radical-scavenging activity.

Rennet-Induced Milk Gel Formation and Characteristics

Kinetics of Milk Clotting. The postrenneting change in the optical density of milk and milk with added grape seed extract (0.3%, wt/vol) is shown in Figure 1. The first derivative of the clotting curves, which relates to the clotting rate, is also shown. For control milk, the first sign of clotting was observed 7.4 ± 0.5 min after renneting. Optical density rapidly increased, and the maximum clotting rate ($0.282 \pm 0.007 \text{ min}^{-1}$) was observed 10.5 ± 0.5 min after renneting and then gradually decreased. Adding S extract to milk retarded milk clotting and reduced the clotting rate. Clotting time was increased by a factor of 2.5, and the maximum clotting rate was reduced by 65%. Similar trends were reported for milk with added tea catechins; they were attributed to the specific interaction between polyphenols and proline residues close to the point of cleavage of κ -casein, which reduces enzyme accessibility (Haratifar and Corredig, 2014). Although polyphenols from grape extracts exist mainly in polymeric form, they also seem to reduce enzyme accessibility.

The type of grape extract and the concentration added to milk had a significant effect on milk clotting parameters ($P < 0.05$). The average effect of grape extracts on milk clotting time is presented in Figure 2a. The S extract and SK2 extract severely retarded milk clotting, whereas W extract and SK1 extract had a limited effect. Large differences in the profile of polyphenols in grape skin, seed, and pulp have been found (Ivanova et al., 2011). Han et al. (2011) showed that the effect

on milk clotting varied with the structure and source of polyphenols. O'Connell et al. (1998) reported that milk clotting interference increased with the polymeric size of phenolic compounds. Surprisingly, no correlation seems to be present between milk clotting time and the total phenolic compounds concentration in the extract. The W extract showed the highest TPC concentration (Table 1), but little effect on milk clotting time was observed. Figure 2b shows the average effect of grape extract concentration on milk clotting time. Clotting time increased as the concentration of grape extract added to milk increased, which is in agreement with the findings of Haratifar and Corredig (2014).

The maximum milk clotting rate was significantly reduced by the grape extracts, and the average effects are shown in Figure 3a. The maximum clotting rate was inversely correlated with clotting time ($R^2 = 0.97$). Once again, the W extract showed the smallest detrimental effect. Interference with clotting properties is specific to the source of polyphenols and can be minimized by appropriate selection. Figure 3b shows the average effect of grape extract concentration on the maximum milk clotting rate. As expected, the maximum clotting rate decreased with the increase of grape extract concentration—another result that is consistent with the findings of Haratifar and Corredig (2014).

Rennet Gel Texture and Syneresis. Rennet gel texture is an important parameter used by experienced cheese makers to determine the appropriate cutting time (Hansen et al., 2010). Gel texture also determines the behavior of cheese curd during the cooking phase. Too soft or too brittle curds will result in excessive losses of fines in whey and lower cheese yield (Mateo et al., 2009). Yield stress and yield strain were determined on rennet-induced milk gels, and the grape extract concentration was found to be the main factor influencing texture ($P < 0.05$). The 4 grape extracts had a similar effect on texture parameters. A texture map was built from the yield stress and strain results and is presented in Figure 4. At 0.1% (wt/vol) concentration, grape extracts had no significant effect on yield parameters. At a concentration of 0.2% (wt/vol), only the yield strain was reduced, whereas at a concentration of 0.3% (wt/vol), both yield strain and yield stress were significantly reduced. Based on these results, the brittleness of milk gel starts increasing as the grape extract concentration approaches 0.2% (wt/vol) and the firmness starts decreasing at higher concentrations ($\sim 0.3\%$, wt/vol). Despite these changes, the practical effect on cheese making is expected to be limited. Adding 0.3% (wt/vol) grape extract to milk reduces gel yield strain and yield stress by only 4.6 and 2.5%, respectively. In contrast, Haratifar and Corredig (2014) observed a large decrease in rennet-induced gel elastic modulus (G')

Figure 1. Effect of grape seed extract (S) on rennet-induced milk clotting kinetics. Relative optical density OD_{rel} (solid line) and its first derivative dOD_{rel}/dt (dashed line) are presented as a function of time after renneting. Seed extract was added to milk at a concentration of 0.3% (wt/vol).

Figure 2. Effect of different commercial grape extracts (a) and their concentration in milk (b) on the rennet-induced milk clotting time (CT). The commercial extracts were whole grape extract (W), grape seed extract (S), grape skin extract #1 (SK1), and grape skin extract #2 (SK2). The dashed line indicates the clotting time for control milk. Because the statistical interaction between extract and concentration was not significant ($P > 0.05$), the symbols correspond to averaged values for the 3 concentrations (a) or the 4 extracts (b). Error bars represent the standard errors obtained from the statistical model.

when tea catechins were added to milk in the same range of concentrations as the grape extracts. In their study, cheese milk was not fortified with calcium chloride, and part of the milk-soluble calcium was probably chelated by polyphenols (Haratifar and Corredig, 2014). Calcium ions are required for both the enzymatic and aggregation phases of milk coagulation (Dalglish, 1992). Adding calcium chloride before renneting probably reduced the deleterious effect of polyphenol-rich extracts on milk coagulation.

During the cooking step of the cheese-making process, the increase in temperature induces milk gel contraction and syneresis. Syneresis is controlled by the balance between attraction and repulsion forces within the paracasein network and the rearrangement capacity of the network bonds (Matumoto-Pintor et al., 2011; Giroux et al., 2014). The rate and extent of syneresis has an effect on cheese moisture and may affect losses of protein and fat in whey (Everard et al., 2008). The effect of grape extracts on milk gel syneresis was measured after 80 min of cooking at 42°C. Gel syneresis was significantly affected by the grape extract concentration ($P < 0.05$), and the 4 extracts had a similar effect ($P > 0.05$). The average effect of the grape extract concentration on milk gel syneresis is shown in Figure 5. Gel syneresis decreased linearly with increasing concentrations of grape extract. Polyphenols are known to interact with caseins through hydrophobic interactions

(O'Connell and Fox, 2001; Hasni et al., 2011), which could reduce the available hydrophobic domains that drive gel contraction at the cooking temperature. Adsorption of polyphenols may also cause steric hindrance and reduce network rearrangement capacity. Han et al. (2011) reported results indicating that milk gel syneresis increased in the presence of commercial grape extract. However, in their study, milk syneresis was induced by centrifugation with no increase in temperature. Under those conditions, syneresis did not result from natural milk gel contraction and the contribution of hydrophobic interactions was reduced.

Cheese Composition

Model cheeses were produced from milk containing added grape extracts, and the effect on cheese moisture content and the recovery of protein, fat, and polyphenols was determined. Controlling cheese moisture content is critical because it contributes to cheese yield and economical profitability. The type of grape extract and the concentration added to milk had significant effect on cheese moisture content ($P < 0.05$). The average effect of grape extracts on cheese moisture is shown in Figure 6a. Whole grape and seed grape extracts increased cheese moisture by about 2.3%, whereas the grape skin extracts SK1 and SK2 increased moisture content by 4.4 and 3.3%, respectively. The average

Figure 3. Effect of different commercial grape extracts (a) and their concentration in milk (b) on the maximum milk clotting rate (CR_{max}). The commercial extracts were whole grape extract (W), grape seed extract (S), grape skin extract #1 (SK1), and grape skin extract #2 (SK2). The dashed line indicates the maximum clotting rate for control milk. Because the statistical interaction between extract and concentration was not significant ($P > 0.05$), symbols correspond to averaged values from the 3 concentrations (a) or the 4 extracts (b). Error bars represent the standard errors obtained from the statistical model.

effect of grape extract concentration is presented in Figure 6b, which shows a gradual increase of cheese moisture content. Grape extracts were found to inter-

fere with gel contraction and reduce syneresis during the cooking step (Figure 5). Cheese moisture content can therefore be expected to increase when grape ex-

Figure 4. Texture map (yield stress versus apparent yield strain) of rennet-induced milk gels. Grape extract concentration was 0 (○), 0.1 (□), 0.2 (Δ), and 0.3% (◇). Because no significant difference was found between the extracts ($P > 0.05$), symbols correspond to averaged values. Error bars represent the standard errors obtained from the statistical model.

Figure 5. Effect of commercial grape extracts concentration in milk on rennet-induced milk gel syneresis. Syneresis was measured after cooking the gels for 80 min at 42°C. Because no significant difference was found between the extracts ($P > 0.05$), symbols correspond to averaged values. Error bars represent the standard errors obtained from the statistical model.

tract is added to milk. Interestingly, the type of grape extract had no significant effect on milk gel syneresis, but differences in cheese moisture were observed. The skin extracts (SK1 and SK2) were more efficient than whole grape or grape seed extracts in retaining water in the cheese matrix. As shown in Table 1, the skin extracts contain 43 to 51% polyphenolic compounds, whereas whole grape and grape seed extracts contain more than 90% polyphenolic compounds. Nonphenolic material in grape skin extracts may bind water and contribute to higher cheese moisture content.

Protein recovery in cheese was affected by the presence of grape extracts in cheese milk ($P < 0.05$). The average effects of grape extracts and their concentrations on the protein recovery coefficient are presented in Figure 7a and 7b, respectively. The protein recovery coefficient was 0.768 for control milk and increased to 0.775 with the addition of grape skin extract SK1. The other grape extracts showed intermediate recovery coefficients. Protein recovery in cheese was proportional to the concentration of grape extract in the cheese milk (Figure 7b), which is consistent with the trend observed by Giroux et al. (2013) for Cheddar-type cheese enriched with green tea extract. Polyphenolic compounds interact with milk protein and can induce cross-linking and even precipitation (O'Connell and Fox, 2001). Polyphenolic compounds may therefore promote the fixation of soluble protein to the paracasein network or the formation of small protein aggregates, which will

increase milk protein recovery. The recovery of milk fat in cheese was also determined; however, the type of grape extract and its concentration had no statistically significant effect ($P > 0.05$). The average fat recovery coefficient was 0.945 ± 0.005 (data not shown). Considering that the grape extract had no influence on fat recovery and little influence on protein recovery, no practical effect on cheese yield is expected. Cheese yield in the presence of grape extracts is considered to be driven only by the effect on cheese moisture content.

The main purpose of adding grape extract to cheese milk is to produce cheese with a high concentration of polyphenolic compounds. The effect of grape extract type and concentration on the polyphenol recovery in cheese was determined (Figure 8). Significant differences were observed between the extracts, and these differences depended on the concentration added to cheese milk ($P < 0.05$). The coefficients of recovery of polyphenols from whole grape and grape seed extracts were, respectively, 0.60 and 0.66 at a concentration of 0.1% (wt/vol); the coefficients gradually decreased with increasing concentration falling to about 0.45 at a concentration of 0.3% (wt/vol). This result suggests a gradual saturation of interaction sites on casein micelles as the polyphenol concentration increases (Haratifar and Corredig, 2014). Giroux et al. (2013) reported a polyphenol recovery coefficient of 0.62 in Cheddar-type cheese made from milk enriched with a green tea extract; however, this value was not affected by the

Figure 6. Effect of different commercial grape extracts (a) and their concentration in milk (b) on the moisture content of model cheese. The commercial extracts were whole grape extract (W), grape seed extract (S), grape skin extract #1 (SK1), and grape skin extract #2 (SK2). The dashed line indicates the moisture content of cheese from control milk. Because the statistical interaction between extract and concentration was not significant ($P > 0.05$), symbols correspond to averaged values from the 3 concentrations (a) or the 4 extracts (b). Error bars represent the standard errors obtained from the statistical model.

Figure 7. Effect of different commercial grape extracts (a) and their concentration in milk (b) on the recovery coefficient of proteins (K_{PRT}) in cheese. The commercial extracts were whole grape extract (W), grape seed extract (S), grape skin extract #1 (SK1), and grape skin extract #2 (SK2). The dashed line indicates the recovery coefficient of proteins in cheese made from control milk. Because the statistical interaction between extract and concentration was not significant ($P > 0.05$), symbols correspond to averaged values from the 3 concentrations (a) or the 4 extracts (b). Error bars represent the standard errors obtained from the statistical model.

concentration of tea extract added to milk. In that study, the highest concentration of polyphenols added to milk was about 0.06% (wt/vol), which might be too low to observe a saturation effect. Cheeses made with the 2 grape skin extracts (SK1 and SK2) showed higher polyphenols recovery coefficients, with an average value of 0.87, which was fairly constant over the concentration range tested. One could argue that the enhanced recovery associated with skin extracts is attributable to the lower polyphenol concentrations of these extracts (Table 1). However, when recovery coefficient is plotted as a function of polyphenol concentration instead of extract concentration, the difference persists. Further analyses are required to better understand the reason for the higher recovery of polyphenols originating from grape skin extracts. Whereas the polyphenol profiles differ according to the parts of the grape they are extracted from (Ivanova et al., 2011), the nonpolyphenolic material in these extracts may also play a role.

The polyphenol concentration in the cheese was not analyzed, but it can be estimated from the polyphenol recovery coefficient and the cheese yield. Theoretical cheese yield can be calculated from the cheese milk composition (4.1%, wt/vol protein; 2.8%, wt/vol fat), the recovery of protein and fat (protein = 0.77, fat = 0.94), and the average moisture content of cheese (51%, wt/vol) using the modified van Slyke equation (Lou and Ng-Kwai-Hang, 1992). The theoretical adjusted

yield for the model cheeses produced in the present study is 13.2% and has been used to estimate their polyphenol contents. Figure 9 presents the polyphenol concentration in cheese as a function of grape extract concentration in cheese milk. Polyphenol concentrations ranged from 2.9 to 11.3 mg of GAE per gram of cheese, and interestingly, the slopes of the curves were similar. The lower polyphenol content in skin extracts (Table 1) is compensated by higher recovery in cheese (Figure 8), which explains the similar slopes. When grape skin extracts are added at a concentration of 0.3% (wt/vol), the polyphenol concentration in the resulting cheese is only about 15% lower than in cheeses made with whole grape or grape seed extracts.

Ratnasooriya et al. (2010) measured the concentration of total polyphenols in a selection of table and wine grape juices. Total polyphenol content in wine grape juices was about 5 times the concentration in table grape juices and averaged 11.3 mg of GAE/L. This means that just 1 to 4 g of cheese produced in the present study would provide an equivalent amount of grape polyphenols as 1 L of grape juice.

CONCLUSIONS

Commercial grape extracts can be added to milk to produce cheese with high concentrations of polyphenols. The grape extracts alter the milk clotting proper-

Figure 8. Effect of different commercial grape extracts and their concentration in milk on the recovery coefficient of polyphenols (K_{PP}) in cheese. The commercial extracts were whole grape extract (○), grape seed extract (□), grape skin extract #1 (Δ), and grape skin extract #2 (◇). Error bars represent the standard errors obtained from the statistical model.

ties, and the resulting milk gels are slightly more brittle and soft. Gel syneresis is also reduced, which results in cheeses with a higher moisture content. Despite

Figure 9. Effect of commercial grape extracts and their concentration in milk on the estimated total polyphenolic compound concentration [TPC] in cheese. The commercial extracts were whole grape extract (○), grape seed extract (□), grape skin extract #1 (Δ), and grape skin extract #2 (◇). GAE = gallic acid equivalents.

these differences, protein and fat recovery were not negatively affected, and polyphenol transfer to cheese depended on the type of extract and the concentration added to cheese milk. Commercial grape extracts can be used as functional ingredients to produce cheeses with high concentrations of polyphenolic compounds. A few grams of such cheeses can provide the same amount of polyphenols as 1 L of grape juice.

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