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

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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 (Hervert-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).

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Whereas the use of tea extracts as a source of polyphenols in food formulations has been extensively studied, 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 × *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 per gram of extract (mg of **GAE**/g).

Total flavonoid content was measured by the aluminum chloride ($AlCl_3$) 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 × *g* for 15 min. A standard curve was prepared with quercitin, and the results were expressed as milligrams of quercitin equivalents per gram of extract (mg of quercitin 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 ($\mathbf{IC_{50}}$) was determined by linear interpolation.