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Short communication: Effect of whey protein addition and transglutaminase treatment on the physical and sensory properties of reduced-fat ice cream

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ABSTRACT

The effects of whey protein addition and transglutaminase treatment, alone and in combination, on the physical and sensory properties of reduced-fat ice cream were investigated. Adding whey protein with or without enzyme treatment decreased melting rate, overrun, and hardness of the reduced-fat ice cream; however, the enzyme-treated sample had a higher melting rate and overrun and softer texture. Whey protein-fortified samples showed higher melting resistance, but lower overrun and firmer texture compared with the enzyme-treated sample without added whey protein. Whey protein addition with or without transglutaminase treatment caused an increase in apparent viscosity and a decrease in flow index of the reduced-fat ice cream; nevertheless, the flow behavior of full-fat sample was most similar to the enzyme-treated reduced-fat sample with no added whey protein. Descriptive sensory analyses showed that neither whey protein addition nor transglutaminase treatment significantly influenced the flavor and odor of reduced-fat ice cream, but they both noticeably improved the color and texture of the final product. The results of this study suggest that whey protein addition with transglutaminase treatment improves the physical and sensory properties of reduced-fat ice cream more favorably than does whey protein addition or transglutaminase treatment alone.
Key words: reduced-fat ice cream, whey protein, transglutaminase, physical property

Short Communication

Awareness of the relationship between diet and health has stimulated interest in foods with less fat. However, reduced-fat foods do not enjoy wide popularity because of poor sensory properties (McClements, 2015). Reduced-fat ice creams usually suffer from undesirable flavor, low melting resistance, and high firmness (Roland et al., 1999). Whey proteins have been widely used as fat replacer in dairy products, including ice cream (Ohmes et al., 1998; Prindiville et al., 2000; Yilsay et al., 2006; Akalin et al., 2008). Whey proteins could simulate the role of fat in establishing the texture and flavor of ice cream, which is attributed to their ability to interact with water, proteins, and flavor compounds (Prindiville et al., 2000). Whey proteins, owing to their water-holding properties, could prevent increasing the ice phase volume of ice cream and thereby improve the texture (Ruger et al., 2002). The water-holding capacity of whey protein powders ranges from 70 to 147 g of water/100 g of powder, or 33 to 180 g of water/100 g of protein (Zayas, 1997); thermal denaturation of whey proteins may even lead to a 4-fold increase in their water-holding capacity (Zayas, 1997). Therefore, incorporation of whey proteins, especially in their denatured form, into the formulation of ice cream is expected to lower the free water available for freezing, and thus reduce the ice phase volume. The more ice in the ice cream, the greater the resistance to an applied force and the harder the sample (Muse and Hartel, 2004). In their denatured state, whey proteins interact with each other and caseins micelles, forming a protein network that, in turn, leads to increased viscosity of ice cream mix (Relkin and Sourdet, 2005). As the viscosity in ice cream mix is raised, the resistance to melting and the smoothness of body increases, whereas the whipping rate decreases (Ruger et al., 2002).

Transglutaminase treatment of milk proteins is another strategy to produce reduced-fat ice cream with improved textural properties. Transglutaminase (**TGase**, EC. 2.3.2.13) is an acyltransferase that catalyzes the cross-linking of most food proteins including milk proteins through formation of ε-(γ-glutamyl)-lysine intra- and intermolecular isopeptide bonds (Motoki and Seguro, 1998). The protein polymers formed via the catalytic action of TGase can perform similar functional properties as fat. It is believed that

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fat globules, as either individually distinct globules or clusters of globules, mechanically obstruct ice crystal growth in the ice cream matrix and thereby provide a smoother texture for ice cream (Marshall and Arbuckle, 1996). Daw and Hartel (2015) determined the particle size distribution of ice cream and pointed out that the peak appearing at 1 μm represents the individual fat globules in the ice cream mix and the peak beginning somewhere between 3 and 10 μm represents the fat clusters in the melted ice cream. Considering the mean size of milk proteins, which is in the range of 0.3 to 0.4 μm in ice cream mix (Daw and Hartel, 2015), it seems logical to hypothesize that the enzymatically formed protein polymers, which are most probably much larger than the individual whey protein molecules or casein micelles, could act as physical barriers, reducing the probability of collisions between ice crystals. These large protein polymers could also behave in the same way as hard spheres, showing high resistance to flow and thereby increasing the viscosity of ice cream (Rossa et al., 2012); the higher the viscosity, the slower the melting rate of ice cream. Rossa et al. (2012) reported that TGase-induced polymerization of milk proteins led to increased overrun and melting resistance and decreased hardness for the reduced-fat ice cream samples. No previous studies have reported on enzymatic incorporation of deliberately added whey proteins to milk into ice cream formulation by TGase. The aim of our study was, thus, to evaluate the effects of adding whey proteins and TGase on the physical and sensory properties of reduced-fat ice cream.

Raw skim bovine milk (protein 3.19 ± 0.05%, fat 0.52 ± 0.07%, moisture 91.23 ± 0.19%, and pH 6.65 ± 0.03), skim milk powder (protein 30.97 ± 0.19%, fat 0.1 ± 0.01%, and moisture 4.68 ± 0.1%) and pasteurized cream (fat 30.27 ± 0.29%) were supplied by Khuzestan Pegah Dairy Company (Shush, Iran). Whey protein isolate (**WPI**; protein 92 ± 2%, fat 0.2%, lactose 0.2%, ash 4%, and moisture content 6%, as determined by manufacturer) and microbial transglutaminase (Probind CH, nominal activity: 80–125 EU) were procured from Arla Foods Ingredients (Videbaek, Denmark) and BDF Natural Ingredients (Girona, Spain), respectively. Carboxymethyl cellulose and emulsifier E471 were purchased from Sunrose (Tokyo, Japan) and Puratus (Grand-Bigard, Belgium), respectively. Sucrose and vanillin were obtained from local markets.

Ice cream mixes were formulated to contain 10% milk fat (full-fat control); 5% milk fat (reduced-fat control), 5% milk fat plus whey protein isolate (**R-WPI**), 5% milk fat plus TGase (**R-TG**), and 5% milk fat plus whey protein isolate and TGase (**R-WPI+TG**). Formulas for the mixes are shown in Table 1. Raw skim milk was mixed with pasteurized cream at room temperature and warmed up to 50°C to ensure proper mixing. At this point, the mix was incorporated with 4 g/L of WPI, rapidly heated to 78°C and kept at this temperature for 15 min for denaturation of whey proteins. After cooling to 40°C, TGase was added to the mix at a ratio of 4 U/g of protein. The TGase concentrations were calculated considering the protein content of milk, quantified by the Kjeldahl method (AOAC International, 2005), plus that of the deliberately added WPI to milk. The enzymatic treatment was continued for 90 min and then stopped by heating the reaction mixture at 80°C for 2 min. Subsequently, dry ingredients, including skim milk powder, sucrose, vanillin, emulsifier, and stabilizer, were added and mixed. The skim milk powder was added to increase the DM content of the ice cream mix (Karaman et al., 2014). Each mix was pasteurized at 80°C for 25 s and then homogenized for 1 min using an Ultra-Turrax T25 homogenizer at 2,029 × g (IKA Instruments, Staufen im Breisgau, Germany). The mixes were rapidly cooled to 4°C and aged at the same temperature for 24 h. The aged mixes were frozen using a batch freezer (Feller ice cream maker, Model IC 100; Feller Technologic GmbH, Dusseldorf, Germany) for 35 min, packaged into 120-mL lidded plastic containers, hardened, and stored in a freezer at −25°C until analyses.

The flow behavior of unfrozen mixes was evaluated using a Physica MCR 301 rheometer (Anton-Paar GmbH, Graz, Austria) equipped with a circulating cooling bath at 4.0 ± 0.1°C. Samples were subjected to a shear rate linearly increasing from 2 to 100 s^{-1} in 14 min and the shear stresses were recorded at 30-s intervals. Consistency coefficient (k) and flow behavior index (n) were calculated by fitting shear rate and shear stress data to power law model. Apparent viscosity of the samples was computed at a shear rate of 50 s^{-1}.

A 120-g block of ice cream sample was placed on a wire screen located on top of a beaker and allowed to melt at room temperature (22 ± 1°C). The weight of drainage recorded at 10-min intervals was plotted against time and the melting rate (g/min) was calculated from the slope of the melting chart (Elsayed Metwally, 2007).

The overrun was calculated on a weight basis according to Adapa et al. (2000), as

$$\%\ \text{Overrun} = \frac{\text{weight of mix} - \text{weight of ice cream}}{\text{weight of ice cream}} \times 100.$$

The hardness of ice cream samples was measured using a TA.XT2i Texture Analyzer (Stable Micro Systems, Goldalming, UK) equipped with a 50 N load cell and a 6-mm diameter cylindrical probe. The ice creams were