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Enzymatic modification of milk protein concentrate and characterization of resulting functional properties

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ABSTRACT

Milk protein concentrates are suitable ingredients for high-protein beverages, but are underutilized due to poor solubility at ambient temperature and neutral pH. The other functional properties of milk protein concentrate, such as emulsification and foaming, depend on its solubility. Milk protein concentrate with 80 g protein 100 g^{-1} was hydrolyzed with three digestive enzymes – chymotrypsin, trypsin, and pepsin – and one cysteine protease – papain – to improve solubility and functionality. Two hydrolysates were produced with each enzyme at targeted levels of hydrolysis to help prevent the development of bitterness. Reduced urea sodium dodecyl sulfate polyacrylamide gel electrophoresis showed that casein subunits were more susceptible to hydrolysis compared with the whey proteins. Enzyme hydrolysis improved the solubility of the milk protein concentrate in the pH range of 4.6–7.0 inclusive. All enzyme hydrolysates had reduced surface hydrophobicity and gel strength. Hydrolysis with chymotrypsin and trypsin improved emulsion activity and stability whereas emulsification capacity was improved with all enzymes. Foaming properties depended on enzyme and hydrolysis time. The hydrolysis of milk protein concentrate with food enzymes can improve solubility and alter resultant functional properties.

1. Introduction

Milk protein concentrates (MPCs) are dairy powders produced by concentrating the protein in skim milk with ultrafiltration followed by evaporative concentration, and spray drying to about 5 g water 100 g^{-1} (Oldfield & Singh, 2005). Membrane filtration partially removes lactose and minerals such that the final spray dried product contains 42–85 g protein 100 g^{-1}. Diafiltration removes more lactose and minerals from the retentate and is utilized when producing MPCs that contain equal to or greater than 70 g protein 100 g^{-1} (Baldwin & Pearce, 2005; Mistry, 2002). Protein content (g protein 100 g^{-1}) in an MPC powder is distinguished by its numerical accompaniment, but the ratio of casein to whey (80:20) is consistent with that typical in fluid skim milk (Kelly, 2011).

MPCs are used in processed cheese applications, cultured dairy products, nutritional products, and protein standardizations (Anema, Pinder, Hunter, & Hemar, 2006; Havea, 2006; Mistry, 2002). MPCs with high-protein (e.g., ≥70 g protein 100 g^{-1}) have limited use in some applications because they have poor solubility at ambient temperature (De Castro-Morel & Harper, 2002; Havea, 2006; Singh, 2011). Insolubility, which increased with storage time and temperature, was attributed to fusion of adjacent powder particles through protein–protein interactions during storage of MPC80 (Le, Bhandari, & Deeth, 2011). Insoluble protein material in reconstituted MPC85 consisted predominantly of casein and the minor whey proteins (Havea, 2006). Although MPCs contain less lactose than other powder milk products (e.g., nonfat dry milk), lactosylation or the binding of lactose to an amino acid, specifically lysine, can initiate Maillard browning reactions which contribute to undesirable color change and loss of nutritional value during storage (Le et al., 2011; Thomas, Scher, Desobry-Banon, & Desobry, 2004). The presence of advanced Maillard browning products may increase protein–protein crosslink formation during storage and can contribute to reduced MPC solubility (Anema et al., 2006; Le et al., 2011). Hydrolyzed whey protein concentrate (WPC) had reduced lysine availability which may suggest decreased Maillard reactions during storage of hydrolyzed dairy proteins (Sinha, Radha, Prakash, & Kaul, 2007). Protein solubility largely determines its use in foods, especially in beverage applications, and is one of the most important factors in determining other functional properties (Kilara & Panyam, 2003).

The emulsification and foaming properties of MPCs are reported to be relatively poorer in comparison with sodium caseinate, WPC, and whey protein isolate (WPI) which limits their use in coffee creamers, whipped toppings, soups, and processed meats (Singh,

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2011; Srinivasan, Singh, & Munro, 2002). Although functionality may be expected to correlate with protein content, the study of thirty-seven internationally produced MPCs did not show correlation between functional properties and MPC protein percentage (De Castro-Morel & Harper, 2002). MPCs with equivalent nominal protein content and milk protein isolates produced in the United States exhibited large differences in solubility which were attributed to differences in processing conditions, mineral composition, and storage conditions (Sikand, Tong, Roy, Rodriguez-Saona, & Murray, 2011). Since solubility is a prerequisite for various functional properties, it is possible that different processing conditions may produce some high-protein MPCs with emulsification and foaming properties comparable with sodium caseinate (Kelly, 2011; Singh, 2011). MPC85 compared with sodium caseinate and WPC had poorer emulsifying ability, but provided greater stability against creaming (Euston & Hirst, 1999).

Controlled enzymatic hydrolysis has been used to improve protein functionality, including soybean proteins (Jung, Murphy, & Johnson, 2005; Lamsal, Reitmeier, Murphy, & Johnson, 2006) and egg proteins (Wang & Wang, 2009). Endo-peptidase treatment of soy protein concentrate (77 g protein 100 g^{-1}) and soy protein isolate (93 g protein 100 g^{-1}) increased protein solubility, emulsion capacity, and surface hydrophobicity (Jung et al., 2005). Egg yolk protein solubility, emulsion stability, and all foaming properties except for capacity improved with hydrolysis (Wang & Wang, 2009).

Literature on enzyme hydrolysis of MPC is limited, especially on resulting functionality. Hydrolysis of MPC is complicated by initial compositional differences in the MPC, the ability to obtain a reproducible degree of hydrolysis (DH), and by the different hydrolysis rates of its individual proteins (Urista, Fernández, Rodriguez, Cuenca, & Jurado, 2011). The casein protein in MPC is synthesized for easy digestion and is more susceptible to enzyme hydrolysis than the compact globular structure of whey protein, namely β-lactoglobulin (β-lg) (Guo, Fox, Flynn, & Kindstedt, 1995). Rather, purified dairy proteins, which are easily obtained commercially, are often hydrolyzed separately as casein (e.g., caseinates, α-casein, β-casein, κ-casein) or whey (e.g., WPC, WPI, β-lg, α-la) and have been reported (Augustin & Udabage, 2007; Chobert, 2003; Kilara & Panyam, 2003; Urista et al., 2011).

An understanding of the hydrolysis of the two main protein fractions simultaneously present in MPC is necessary to increase applicability in food systems (Urista et al., 2011). The objective of this work was to evaluate the functional properties of MPC80 hydrolyzed with three digestive enzymes – chymotrypsin, trypsin, and pepsin – and one cysteine protease – papain. Our hypothesis was that enzyme modification of MPC80 will result in enhanced solubility and improved functional properties.

2. Materials and methods

2.1. Materials and reagents

MPC80 was purchased from Idaho Milk Products (Jerome, ID) and protein content was determined to be 80.41 g protein 100 g^{-1} in our lab. Trypsin (≥75 USP units mg^{-1} dry weight), chymotrypsin (≥75 USP units mg^{-1} dry weight), pepsin (3200 FCC units mg^{-1} dry weight), and papain (≥12,000 USP units mg^{-1} dry weight) were obtained from American Laboratories Incorporated (Omaha, NE). Bovine serum albumin (BSA), sodium pyrophosphate tetrabasic (TSPP), and 8-Anilino-1-napthalene-sulfonic acid (ANS) were purchased from Sigma–Aldrich Inc. (St. Louis, MO). Sodium dodecyl sulfate (SDS) and tris were purchased from Biorad (Hercules, CA). β-Mercaptoethanol and the bicinchoninic acid (BCA) protein assay kit where obtained from VWR International (Radnor, PA). All other chemicals were obtained from Thermo Fisher Scientific (Waltham, MA). Hydrochloric acid and sodium hydroxide used in pH adjustments were diluted to 2 mol L^{-1} or 4 mol L^{-1} with distilled water.

2.2. Preparation of enzyme hydrolysates of MPC80

MPC80 was mixed into Millipore water, agitated by an overhead mixer to a final concentration of 5 g protein 100 g^{-1}. Mixing ceased when all protein powder was visibly hydrated and the MPC80 dispersion was then stored overnight at 4 °C in a sealed vessel to allow for complete hydration.

MPC80 dispersion was weighed into 4-L Erlenmeyer shake flasks, equilibrated to room temperature, and adjusted to the optimum hydrolysis pH using hydrochloric acid and sodium hydroxide. For trypsin and chymotrypsin hydrolysis, the pH of the MPC80 dispersion was adjusted to 8.0. MPC80 dispersions for papain hydrolysis were adjusted to pH 6.8 and those for pepsin hydrolysis were adjusted to pH 2.0. After pH adjustment, the MPC80 dispersion temperature was equilibrated to 37 °C for hydrolysis by trypsin or pepsin, 50 °C for chymotrypsin, and 60 °C for papain.

Enzyme solutions were prepared separately in Millipore water for chymotrypsin, trypsin, and papain, and in 0.01 mol L^{-1} hydrochloric acid for pepsin at concentrations of 100 g L^{-1}. Enzyme solution was added to provide 1 g enzyme for every 100 g protein being hydrolyzed. The sealed 4-L flask was agitated in a shaker incubator at controlled temperature and times (Table 1). Two hydrolysis times were selected for each enzyme based on preliminary hydrolysis work (data not shown). At the end of hydrolysis, the reactions were stopped by placing the flasks in a 95 °C water bath for 10 min. A non-hydrolyzed MPC80 control solution prepared at 5 g protein 100 g^{-1} was also heated at 95 °C for 10 min (MPCFD). MPCFD and the MPC80 hydrolysates were freeze dried and ground

Table 1
MPC80 hydrolysis conditions and resultant analytical properties.

Sample	Hydrolysis condition				Analytical properties		
	Enzyme	pH	Temperature (°C)	Time (min)	Moisture (g 100g $^{-1}$)	Protein (g 100g $^{-1}$)	DH (%)
MPC80	–	–	–	–	5.0	80.4	–
MPCFD	–	–	–	–	4.4	80.9	2.9
Chy-5	Chymotrypsin	8.0	50	5	2.6	80.5	24.4
Chy-10	Chymotrypsin	8.0	50	10	6.5	76.6	24.3
Try-10	Trypsin	8.0	37	10	5.5	78.1	14.8
Try-60	Trypsin	8.0	37	60	4.5	79.3	15.1
Pep-240	Pepsin	2.0	37	240	5.5	74.2	5.0
Pep-720	Pepsin	2.0	37	720	4.2	75.2	5.7
Pap-30	Papain	6.8	60	30	5.3	78.5	7.2
Pap-180	Papain	6.8	60	180	5.6	78.3	9.8

MPC80, unmodified MPC80; *MPCFD*, MPC80 reconstituted at 50 g protein kg^{-1} solution, heated at 95 °C and freeze dried.