Textural Properties of Cheese Analogs Containing Proteolytic Enzyme-Modified Soy Protein Isolates

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Cheese analogs were prepared from untreated or proteolytically modified soy protein isolates (SPIs), replacing 60% of casein, to explore their potential to replace higher-priced milk proteins. Quality attributes of cheese analogs were evaluated by texture profile analysis with the Instron and melting spread. Compared with commercial milk-based cheeses, ranging from hard-type (Cheddar) to soft-type products (Mozzarella), textural properties of cheese analogs were markedly different; they were harder and more fracturable with no measurable adhesiveness. The use of enzyme-modified SPI significantly ($P < 0.05$) lowered both hardness and fracturability of cheese analogs and also brought about adhesiveness, all of which fell within the range observed for dairy cheeses. Although melting spread of cheese analogs was improved by the use of enzyme-modified SPI, it was still inferior to those of dairy cheeses and needed further improvement. Treatments of SPI with alcalase and trypsin were more influential in modifying textural properties of the resulting cheese analogs than those with other proteases studied.

KEY WORDS: Cheese analogs, proteolytic enzyme-modified, soy protein isolate, textural properties.

The growing need for low-cost food proteins has created extensive research interest in utilizing soy protein in various products. Cheese is among products in which higher-priced milk protein (*i.e.*, casein) can be partly replaced with soy protein (1,2). The history of cheese-like products based on vegetable proteins (cheese analogs) dates back to ancient Chinese days with sufu, a fermented soy curd (3). However, modern activities in making cheese analogs remain at the research level; information concerning the utilization of soy protein in analog cheese-making is scarce due in part to its proprietary nature. In contrast, imitation cheese products utilizing caseinates as the sole protein source have come to command a respectable share of the process cheese market (4).

Only a few studies have been published on the utilization of vegetable proteins in analog cheese-making. Jonas (5) reviewed cheese analogs prepared from lactic bacteria-fermented or rennet-treated soy milk. The effects of processing variables such as soy milk proportion, soy protein isolate (SPI) dispersibility, and rennet level on coagulation time and curd strength have been examined (6–8). The effects of added soy proteins on analog cheese texture have been unfavorable due to the curd being non-elastic and clumps of soy protein in the rennet curds (9,10). Instead of forming curds by rennet-induced coagulation, a different formula and process for making a cheese analog were also reported in which gel was formed by heating (11,12).

When utilizing soy proteins in dairy food systems, one should be aware that soy proteins are considerably different from milk proteins in molecular and functional properties. Soy proteins, mostly 7S and 11S protein fractions, are much larger in molecular size than milk proteins, possess complex quaternary structures, and unlike casein, they are not phosphoproteins (2,13). It is generally believed that the less-than-desirable functionalities of soy protein have limited its expanded usage in foods (14). To improve functionalities for food applications, the authors modified SPI with proteases and observed improvement in functional properties including solubility, emulsifying capacity and heat coagulability (15). Such modifications are expected to affect the quality attributes of food products containing proteolytically modified SPI. The objective of our study was to gain insight into the performance of enzyme-modified SPI in cheese analogs as evaluated by their textural properties.

MATERIALS AND METHODS

Materials. Two kinds of commercial SPIs, Ardex F (AF) and Supro 710 (SP), were obtained from Archer Daniels Midland Co. (Decatur, IL) and Ralston Purina Co. (St. Louis, MO), respectively. Both are neutral, nongelling and low-viscosity SPIs recommended for use in imitation dairy products. AF was chosen for proteolytic modification because it would be comparable to laboratory-prepared SPI; it was prepared by alkali extraction of defatted soy flour, followed by acid precipitation, neutralization and drying. SP was used as received because it was provided as partially hydrolyzed by the supplier with the degree of hydrolysis (DH) and nitrogen solubility (NS) being estimated at 9.1 and 76.3%, respectively (16). Preparation of enzyme-modified SPIs used in this study was described elsewhere in detail, together with their molecular and functional properties (15). Briefly, AF dispersion (15–20%, w/v) was treated for 10 min with five different proteases (2% of SPI, w/w): trypsin (T2395) and α-chymotrypsin (C4129) from Sigma Chemical Co. (St. Louis, MO), alcalase 0.5L and liquozyme 120L from Novo Laboratories, Inc. (Wilton, CT), and rennet (Emporase EL-400) from Dairyland Food Laboratories, Inc. (Waukesha, WI). The proteolysis was stopped by heating the reaction mixture at 87°C for 5 min. After pH adjustment to 7.0, the enzyme-modified SPI was freeze-dried and then stored at 4°C until used. Both DH and NS at pH 7.0 (% in parentheses) were about 1.2 (80.4), 16.3 (89.2), 14.5 (86.8), 11.4 (84.0), 2.0 (84.2), and 1.8 (86.1) after treating AF with none, trypsin, alcalase, α-chymotrypsin, liquozyme and rennet, respectively (15). Sodium caseinate was obtained from New Zealand Milk Products, Inc. (Petaluma, CA). Partially hydrogenated soybean oil (Wesson) and five cheese samples (sharp Cheddar, extra sharp Cheddar, mild Colby,

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Mozzarella, and Monterey Jack) were purchased from a local supermarket. All chemicals were reagent grade.

Preparation of cheese analogs. Experimental cheese analogs were prepared according to the procedure of Chen *et al.* (11) who made cheese analogs from peanut protein and oil. The preparation formula (Table 1) called for about 29% protein, 20% oil, 47% water, and 4% additives with the ratio of SPI to sodium caseinate being 6:4 (w/w). The procedure used to make the cheese analogs is shown in Figure 1. Distilled water and oil were preheated to 90°C before their use. A Kitchen-Aid mixer (Model K5-A, Hobart Corp., Troy, OH) with a water bath was used for mixing. The mixing speeds and times were as shown in Figure 1. The experimental cheese analogs produced were placed in air-tight containers and kept under refrigeration until analysis.

Texture profile analysis (TPA). Textural parameters were measured with an Instron Universal Testing Machine (Model 1122, Instron Corporation, Canton, MA). The cheese analogs and commercial cheeses were cut into

TABLE 1

Cheese Analog Preparation Formula

Ingredient	Grams	Percent
Soy protein isolate[a] (untreated or treated)	54.15	17.27
Sodium caseinate	36.10	11.51
Soybean oil	63.25	20.17
Distilled water I	106.25	33.88
Distilled water II	13.75	4.38
Distilled water III	27.75	8.85
Sodium chloride	5.75	1.83
Sodium citrate	1.25	0.40
Sodium pyrophosphate	1.00	0.32
Lactic acid	4.35	1.39
Total	313.60	100.00

[a]60% replacement of caseinate.

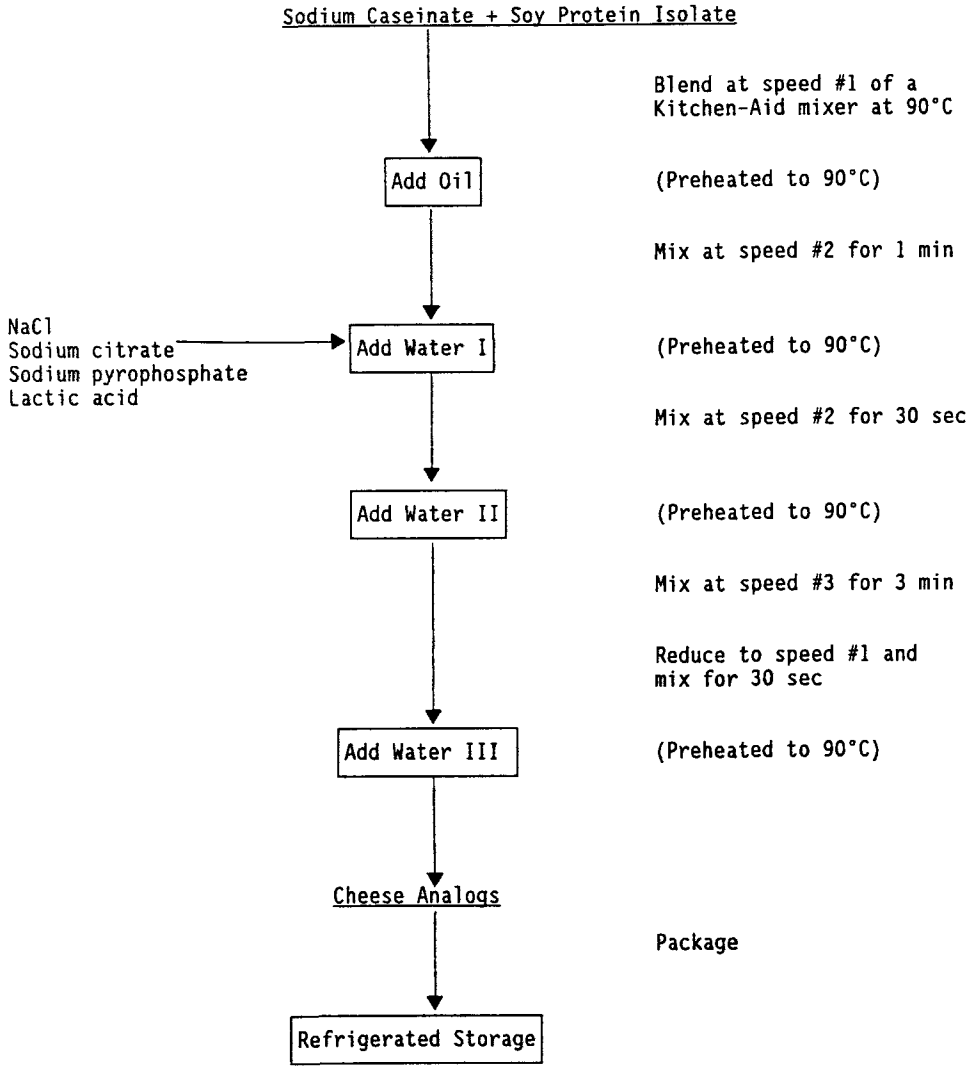

FIG. 1. Schematic procedure of cheese analog preparation.

cubes (about 1.3 cm), which were subjected to a double-compression test with a 454-kg reversible load cell. The samples were compressed to 75% of their original height with a 7.62-cm diameter plunger. The full-scale load of 9.07 or 22.7 kg was used. The crosshead speed was 2 cm/min with a chart speed of 5 cm/min. From the force-time curve (TPA curve) of Instron data, five textural parameters were obtained: hardness, cohesiveness, adhesive force, springiness and fracturability. Gumminess was computed as the product of hardness × cohesiveness and chewiness as the product of gumminess × springiness (17,18). The areas under the force-time curves during the first and second downstrokes were measured with a digitizer.

Melting spread measurement. Melting spread of commercial cheeses and cheese analogs were determined according to the method of Chang (19). Cheese plugs, 0.64 cm thick and 1.90 cm in diameter, were heated at 232°C for 3 min, allowed to reach room temperature, and assessed for increased diameter.

Statistical analysis. A randomized block design for the three replications was used. Data were statistically analyzed by ANOVA (20). Where significant F-values were obtained, Duncan's multiple-range test was applied to locate the differences among means (21). Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Cheese analogs were produced with SPI before and after proteolytic modification, which replaced 60% of caseinates. This substitution level was set arbitrarily rather than as an optimal level, although caseinates could be successfully replaced up to 60% with peanut protein for cheese analogs (11). To compare textural properties of cheese analogs containing SPI with those of commercial dairy cheeses, both products were subjected to TPA. TPA curves (not shown) of AF and SP cheese analogs were similar to each other but different from those of commercial cheeses, including sharp Cheddar and Mozzarella which represented hard- and soft-type cheeses, respectively. The differences in TPA curves were quantitatively evaluated by comparing textural parameters obtained from TPA curves.

Hardness was the most obvious difference, which was found to be much higher in the AF cheese analog than in the five commercial cheeses (Fig. 2). SP, available as a partially hydrolyzed SPI, produced cheese analogs softer than the AF cheese analog but harder than dairy cheeses except sharp Cheddar. It is interesting to note that no differences in hardness were observed by TPA between extra sharp Cheddar and mild Colby, both hard cheeses, and also between Mozzarella and Monterey Jack, both soft cheeses. A less pronounced difference was observed in cohesiveness and springiness between AF cheese analog and the three hard-type dairy cheeses. AF cheese analog was high in gumminess (the product of hardness and cohesiveness) due to its high hardness, whereas the high gumminess in Mozzarella was due to its high cohesiveness. The AF cheese analog did not display any measurable adhesion, which is the negative force that represents the work necessary to overcome attractive forces between the surface of the food and the surfaces of tongue and teeth, *etc.*, coming in contact with it (17,18). In contrast, SP cheese analog showed some adhesion, which was not lower than Mozzarella ($P > 0.05$). Fracturability, measured as the force at the first significant break in the TPA curve, was not observed in soft cheeses such as Mozzarella and Monterey Jack. The AF cheese analog displayed the highest fracturability.

Since SP cheese analog possessed adhesion and also showed somewhat reduced hardness and fracturability compared with AF cheese analog, the effects of prior hydrolytic modification of SPI on textural properties were further investigated, with the AF cheese analogs containing proteolytic enzyme-modified AF. Proteolytic modification of AF brought about reduction of the cheese analog hardness to 40–60 newtons, which fell within the hardness range observed for dairy cheeses (Fig. 2). For decreasing the hardness, treatment of AF by alcalase was most effective. Although modification with trypsin accomplished about the same DH as that with alcalase (15), it resulted in a less pronounced decrease in hardness of cheese analogs than with alcalase and α-chymotrypsin. Modification with liquozyme and rennet, which showed only slight increase in the DH (15), was not effective in decreasing the hardness.

Whereas hardness was effectively lowered, cohesiveness was not affected by enzymatic modification of SPI. Modification with alcalase and α-chymotrypsin increased springiness. Gumminess and chewiness of cheese analogs became lower and seemed dependent mainly upon hardness because hardness was greatly affected by enzymatic modification of SPI. Cheese analogs acquired some adhesion after proteolytic modification of AF and moved into the range observed for dairy cheeses (Fig. 2). Alcalase, α-chymotrypsin, and trypsin treatments effectively decreased the fracturability that was rendered even lower than those of dairy cheeses. However, liquozyme and rennet were not effective in altering fracturability of cheese analogs.

Melting spreads of cheese analogs and commercial dairy cheeses were determined by measuring the increased diameter of cheese plugs after oven-heating at 232°C. Figure 2 compares melting spreads of cheese analogs with those of five commercial cheeses. The decreasing order of melting spread was: extra sharp Cheddar, sharp Cheddar, Monterey Jack, mild Colby, Mozzarella, SP cheese analog and AF cheese analog. There were differences in melting spread among the products except between Monterey Jack and mild Colby cheeses. The melting spreads of AF and SP cheese analogs, 1.9 and 2.3 cm, respectively, were much inferior to those of commercial dairy cheeses, which ranged from 3.5 to 4.7 cm. The enzymatic modification of SPI changed melting spread of cheese analogs, the extent of change depending on proteases (Fig. 2). Alcalase treatment of AF was most effective in improving the melting spread, followed by trypsin and α-chymotrypsin treatments. Liquozyme and rennet treatments seemed to have no effect on improving melting spread. Although melting spread of AF cheese analogs was improved up to 2.5 cm upon prior enzymatic modification of AF, it was still far below the ranges observed for commercial cheeses.

Considering that cheese products have unique textural properties such as elasticity and stretchability (22), our initial purpose was to gain some insights into the implication of proteolytic modification of SPI in cheese analogs' textural properties. It was not our objective to study other sensory attributes such as taste or flavor, which can be

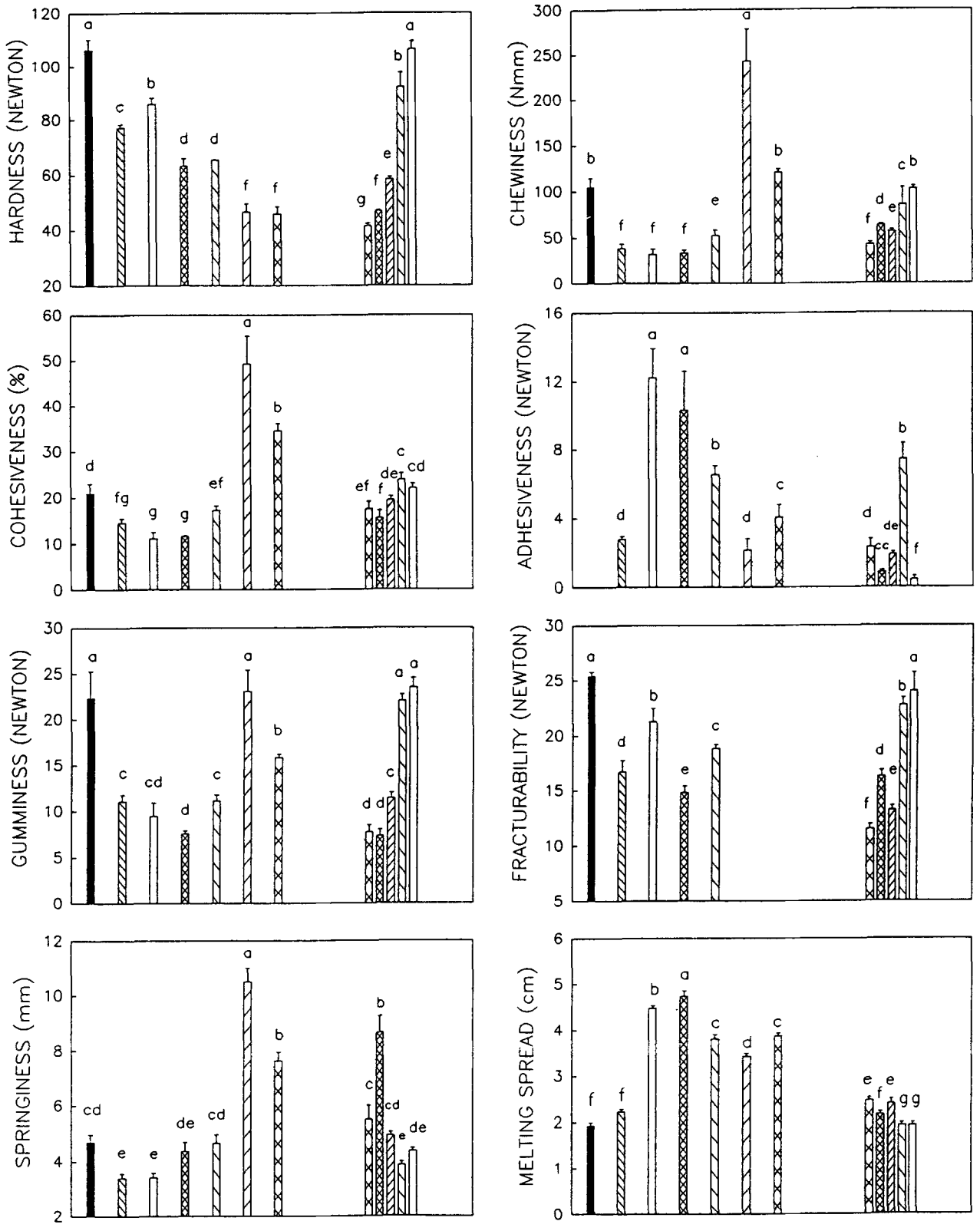

FIG. 2. Comparison of textural properties by TPA and melting spread between cheese analogs and commercial dairy cheeses: Individual bars from left to right—Ardex F cheese analog, Supro 710 cheese analog, sharp Cheddar, extra sharp Cheddar, mild Colby, Mozzarella, and Monterey Jack. Cluster bars from left to right—cheese analogs containing Ardex F proteolytically treated with alcalase, α-chymotrypsin, trypsin, liquozyme, and rennet. Error bars indicate standard deviations. Data in bars headed by a common letter do not differ significantly ($P > 0.05$).

a subject of a separate investigation. Compared with commercial dairy cheeses, cheese analogs containing SPIs displayed higher hardness, fracturability, gumminess and chewiness but no measurable adhesion. Meltability of cheese analogs, as measured by melting spread, was much inferior to that of dairy cheeses. These differences in textural properties might be partly accounted for by the differences in molecular size and structure between soy and milk proteins (2,13), because such protein molecular properties would influence the characteristics of the resulting gels or curds. Such inference is well supported by the observations of the significant reduction in hardness and fracturability and the creation of adhesion in cheese analogs containing proteolytically modified SPI instead of regular SPI. These observations suggest that textural properties of cheese analogs may be manipulated through proteolytic modification of SPI to better simulate those of dairy cheeses.

In general, SPI treated with alcalase most effectively modified textural properties of cheese analogs. Although the DH by trypsin was as high as the DH by alcalase (15), modification of SPI with trypsin resulted in less pronounced alteration in textural parameters of cheese analogs. The cheese analogs produced in the present study left much to be desired with regard to melting spread, even when prepared with enzyme-modified SPI. It would be worthwhile in future studies to evaluate the influence of individual protein fractions, such as 7S or 11S proteins and also their partial hydrolysates with varying DH, on the textural properties of cheese analogs. Such information will enable us to better understand the effectiveness of altering molecular properties of SPI on textural properties of cheese analogs.

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