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Quality Characteristics of Cheese Analogs Containing Lipoxygenase-Defected Soymilk and α-Chymotrypsin Modified Soy Protein Isolate

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ABSTRACT: Cheese analogs using lipoxygenase-defected soymilk and α-chymotrypsin modified soy protein isolate (SPI) were prepared. Color, textural properties, sensory attributes and melting spreadability of cheese analogs were evaluated and compared with mozzarella cheese, and relationships between textural properties, sensory attributes and melting spreadability of cheese analogs were analyzed. Off-flavors were not mostly discriminated. Cheese analogs containing 10% SPI untreated and containing 6% and 8% SPI treated by α-chymotrypsin in ΔE value of color were the most similar to mozzarella cheese. Quality characteristics and melting spreadability of cheese analogs were highly affected and improved by α-chymotrypsin modification. Sensory attributes and melting spreadability of cheese analogs containing 6% SPI treated by α-chymotrypsin were the most similar to mozzarella cheese, while in textural properties, cheese analogs containing 10% SPI were the most similar with mozzarella cheese. Hardness in sensory attributes was highly positively correlated with hardness ($r > 0.65$), adhesiveness ($r > 0.56$), chewiness ($r > 0.77$) and gumminess ($r > 0.76$) in textural properties, while it was highly negatively correlated with melting spreadability ($r > -0.68$).

Keywords: cheese analog, soy protein isolate, textural properties, sensory attributes, melting spreadability

A number of dairy analogs including soymilk, soy cheese, soy yogurt, soy ice cream, margarine and coffee whitener have been developed using soy protein or oil. Cheese analogs are being used increasingly due to the lower cost, the efficient manufacturing process, and the replacement of certain milk ingredients by soy protein (Bachmann, 2001). However, although soy protein is available as a cheese substrate, use of soy protein in processing of cheese analogs was inferior because soy proteins are much larger in molecular size than milk products, possess complex quaternary structures, are not like casein and are not phosphoproteins (Brooks & Morr, 1985). Nevertheless, these problems of soy protein have been effectively improved by enzyme modification. The functional properties such as solubility, foaming properties, emulsifying capacity and thermal aggregation of SPI were remarkably improved by proteolytic enzyme (Kim(Lee) *et al.*, 1990; Ortiz & Wagner, 2002) as well as the size of particles in the soymilk was considerably reduced, showed better sensory attributes and higher physical stability by the enzymatic treatments (Rosenthal *et al.*, 2003).

Mozzarella cheese is one of the most popular cheeses in the world, its consumption is greatly increasing due to popularity of the pizza. Mozzarella cheese differs from most cheeses and it is usually consumed in the melted state on pizza, and thus mozzarella cheese is required the functional properties such as meltability, stretchability and elasticity (Kindstedt *et al.*, 1989). Natural mozzarella cheese exhibits a pseudoplastic flow characteristic in the melted state. It was found that a SPI gel with gum arabic and gelatin formed a cheese-like product that exhibited a pseudoplastic flow in the melted state (Taranto & Yang, 1981). And mozzarella cheese analogs have been produced from soy protein, gelatin, gum arabic and other ingredients, and the textural and stretching properties were similar to natural mozzarella cheese (Yang & Taranto, 1982) as well as a mixture of sodium caseinate, SPI and corn starch has been used to prepare cheese analog (Lee & Son, 1985). And cheese analogs have been made from modified starches or SPI and gelatin as a caseinate replacement (Kiely *et al.*, 1991). As well SPI has induced the strongest gel network structure with mozzarella cheese in the rheological behaviors of mozzarella cheese filled with various proteins such as whey protein, caseinate, egg white, SPI and gelatin (Hsieh *et al.*, 1993). Moreover, in preparation of cheese analogs replaced with 60% of casein to SPI, textural properties and melting spreadability were improved by proteolytic modification (Kim(Lee) *et al.*, 1992). However, these studies on cheese analogs were performed to improve the textural properties of cheese analogs.

Although there are many advantages to soy protein as dairy substitutes, the use of soy protein is restricted due to three lipoxygenase isozymes, L-1, L-2 and L-3 that cause off-flavors in soy products, and thus flavor quality without off-flavor is still required. For example, increasing the per-

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centage of cow's milk was better marked in sensory attributes of cheese analog made with a mixture of cow's milk and soymilk (Abou El-Ella, 1980). In comparison of milk-based and soymilk-based yogurt, soymilk-based yogurt compared well with milk-based yogurt except soy off-flavors remained (Lee *et al.*, 1990). Therefore, many types of studies have been carried out to improve the flavor, one of which is genotype selection method (Hajika *et al.*, 1991; Hammond *et al.*, 1992; Kitamura, 1993; Kim *et al.*, 1994; Narvel *et al.*, 1998; Narvel *et al.*, 2000; Son *et al.*, 2002). Soybean cultivars that have lipoxygenase-defected genotype have been found and applied to soy products. One of such genotypes is Jinpum-kong (L-2,3 defected genotype). Jinpum-kong is very similar with soybean cultivars that have normal nutritional value, physical and chemical properties as well as the yield of product and sensory attributes are excellent in processing of soycurd, soymilk and soy yogurt (Kim *et al.*, 1994; Park & Lee, 1997a, b). This lipoxygenase-defected genotype soybean could be used to obtain very acceptable quality without off-flavors in a manufacturing process of other soy products.

However, there is no study on quality characteristics of cheese analogs using lipoxygenase-defected soybean and other ingredients, therefore, this study was carried out to gain improved textural properties, sensory attributes and melting spreadability of cheese analog using lipoxygenase-defected soymilk and α-chymotrypsin modified SPI. Cheese analogs were contained 2%, 4%, 6%, 8% or 10% of SPI, and the content ratio of soymilk and SPI was 65.0% and the soybean oil was 20.0%. Moisture and crude protein contents, color, instrumental textural properties, sensory attributes and melting spreadability of cheese analogs were evaluated and compared with commercial mozzarella cheese. Relationships between sensory attributes, instrumental textural properties and melting spreadability of cheese analogs were analyzed using a Pearson correlation.

MATERIALS AND METHODS

Materials

Soybean used to prepare soymilk was lipoxygenase-defected soybean (Jinpum-kong; L-2,3 defected genotype) which were grown and selected at National Institute of Crop Science, Suwon. Commercial SPI (Supro 500E) was supplied by Purina Co., Seoul. Protease used was α-chymotrypsin (type II. Bovine pancrease) from Sigma Chemical Co., all chemicals used were reagent grade. Mozzarella cheese used to compare with experimental cheese analogs was purchased from a local supermarket.

Preparation of soymilk

Soymilk was prepared by method of Lee & Lee (1997). Sorted, washed and dried 100 g of Jinpum-kong was soaked at room temperature for 20 h, and added 8 times volume of distilled water after drained, and then ground for 5 min using a grinder (M-1000, LG, Korea). This soymilk was filtered through four layers of cheese cloth and autoclaved at 121°C for 15 min.

Enzyme modification of SPI

Supro 500E was modified by α-chymotrypsin following the method of Kim(Lee) *et al.* (1990). A 20% (w/v) SPI suspension was adjusted to pH 7.8 using 6 N NaOH, and after adding the enzyme (2% of SPI, w/w), the suspension was dispersed in distilled water with a water bath using a disperser (GTR-1000, Tokyo Rikalikai Co., Japan) at 37°C for 30 min. After incubation with α-chymotrypsin, the suspension was heated to inactivate the enzyme at 87°C for 5 min, and then the hydrolysate was neutralized to pH 7.0, freeze-dried and placed –20°C until analyzed.

Na-caseinate

↓ ← Add soybean oil at 90°C

Mixing at speed # 1 of egg beater 1 min

↓ ← Add SPI and soymilk (1/2) at 90°C

lactic acid, disodium phosphate,

sodium chloride

Mixing at speed # 2 of egg beater for 5 min

↓

Stretch and smooth emulsion formation

↓ ← Add soymilk (1/2) at 90°C

Mixing (I) at speed # 1 of egg beater for 1 min

(II) at speed # 3 of egg beater for 5 min

↓

Cheese analog

↓ Package

Refrigerated storage

Fig. 1. Procedure of cheese analog preparation.

Preparation of cheese analogs

Cheese analogs were prepared according to the modified method of Kim(Lee) *et al.* (1992). Cheese analogs were partial dairy cheese analogs which replaced a part of caseinate to SPI. Each of cheese analogs was added with 2.0%, 4.0%, 6.0%, 8.0% or 10.0% of SPI. The content ratio of soymilk (Jinpum-kong) and SPI (Supro 500E) was 65.0%, and 20.0% soybean oil, 1.5% lactic acid, 11.7% Na-caseinate, 0.5% disodium phosphate and 1.3% NaCl were used. Cheese analogs containing 2%, 4%, 6%, 8% or 10% of untreated SPI or α-chymotrypsin treated SPI were symbolized to U2, U4, U6, U8 and U10 or T2, T4, T6, T8 and T10, respectively. The procedure of cheese analog preparation is shown in Fig. 1. Cheese analogs were stored in 5°C refrigerator and analyzed after being placed at room temperature for 1 h.

Moisture and crude protein contents

Moisture content was measured by moisture content measuring machine (LJ 16, Mettler, Switzerland). A 2 g sample of cheese analog was placed on a plate, dried at 110°C for 30 min, and then was calculated. The weight difference between pre and post drying was considered. Crude protein content was determined by the micro-Kjeldahl method (AOAC, 1975).

Color

Color was determined by L, a' and b'-based color difference meter (CQ-1200X, Hunter Lab, USA) which has been calibrated with a standard white plate (L = + 94.81, a' = – 0.96, b' = + 0.43). Cheese analog was cut into 4 cm × 4 cm × 1 cm, and then the L, a' and b' values were determined. The ΔE value was then defined by this formula; $\Delta E = 100 - (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$.

Texture profile analysis (TPA)

Textural properties were measured by Rheometer (CR-100D, Sun Scientific, Japan). Cheese analog was cut into 3 cm^3 cube, and were measured for hardness, cohesiveness, adhesiveness and springiness. The gumminess and chewiness were calculated using the following formula; gumminess = hardness × cohesiveness, chewiness = gumminess × springiness. The assay conditions of Rheometer used were as follow; probe adapter: 3 cm round type, maximum load: 10 kg/cm, table speed: 180 mm/min, chart speed: 100 mm/min and compression ratio: deformation 35%.

Sensory evaluation

Sensory evaluation was carried out in a sensory evaluation room, which has individual booths by selected and trained 10 of female graduated students. Each panelist received samples of 3 cm^3 cube that were coded and served on white plates. And rinse water was provided, and samples were evaluated individually (Stone & Sidel, 1993; Bergara-Almeida *et al.*, 2002). Panelists were asked to evaluate hardness, mouth-feel, bitterness, beany flavor, roasted nutty flavor and overall quality based on a 5-point hedonic scale that were hardness: 1 = very hard, 5 = very soft; mouth-feel: 1 = very sandy, 5 = very smooth; bitterness: 1 = very strong, 5 = very slight; beany flavor: 1 = very strong, 5 = very slight; roasted nutty flavor: 1 = very strong, 5 = very slight; overall quality: 1 = very poor, 5 = very good. Panelists were also asked to comment on what they liked or disliked about the samples.

Melting spreadability

Melting spreadability was determined according to method of Kim(Lee) *et al.* (1992). Sample was cut into 0.5 cm thick and 1.9 cm in dia., and were heated at 220°C for 5 min, placed to reach room temperature, and then assessed the increased dia. and shape.

Statistical analysis

All experiments were carried out in triplicate and the data were analyzed using SAS program. The data were reported as a mean value ± SEM and also subjected to ANOVA and significant differences were reported at the level of $p < 0.05$. Relationships between sensory attributes, instrumental textural properties and melting spreadability of cheese analogs were analyzed using a Pearson correlation.

RESULTS AND DISCUSSION

Moisture and crude protein contents

Moisture contents of cheese analogs and mozzarella cheese were 46.85% to 55.61% and 50.70% as low-moisture cheeses, respectively, and 6% SPI cheese analogs were very similar with mozzarella cheese. Crude protein contents of cheese analogs and mozzarella cheese were 14.62% to 23.86% and 19.80%, respectively, 6% SPI cheese analogs were very similar with mozzarella (Table 1).

Table 1. Moisture and crude protein contents of cheese analogs and mozzarella cheese. (%)

		Moisture content	Crude protein content
Untreated	U2†	51.89 ± 1.74§	15.25 ± 0.65
	U4	50.86 ± 1.55	16.46 ± 0.06
	U6	49.76 ± 2.39	19.81 ± 0.47
	U8	49.62 ± 1.69	21.11 ± 0.99
	U10	46.85 ± 1.62	23.86 ± 0.26
Treated	T2‡	55.61 ± 1.48	14.62 ± 1.32
	T4	54.10 ± 1.20	16.72 ± 1.72
	T6	50.71 ± 1.72	19.87 ± 0.61
	T8	49.36 ± 1.84	21.62 ± 0.25
	T10	47.80 ± 1.69	22.95 ± 0.86
Mozzarella cheese		50.70 ± 3.10	19.80 ± 0.05

†U2–U10: Cheese analogs containing 2%, 4%, 6%, 8% or 10% of untreated SPI.
‡T2–T10: Cheese analogs containing 2%, 4%, 6%, 8% or 10% of α-chymotrypsin treated SPI.
§Mean ± SEM.

Color

Decrease of L value indicates a loss of whiteness, more positive a' value means progressive browning and more positive b' value indicates more yellowing (Fig. 2A-C). In L, a', and b' values, cheese analog less containing SPI was whiter, cheese analog more containing SPI was more brownish and yellowish ($p < 0.01$). α-Chymotrypsin modification decreased the L and a' values and increased the b' value except 2% SPI cheese analog ($p < 0.01$). ΔE value was increased by SPI content and α-chymotrypsin modification ($p < 0.01$) (Fig. 2D). In each parameter of color, L value of T6 and T8, b' value of T2 were the most close to mozzarella cheese, whereas a' value was obviously different with mozzarella cheese. Overall, U10, T6 and T8 in combined color, ΔE value were the most similar to mozzarella cheese.

Texture profile analysis (TPA)

Textural properties of cheese analogs were considerably difference by SPI content and enzyme modification (Fig. 3). Hardness (Fig. 3A), chewiness (Fig. 3E) and gumminess (Fig. 3F) were increased by increasing SPI content, while

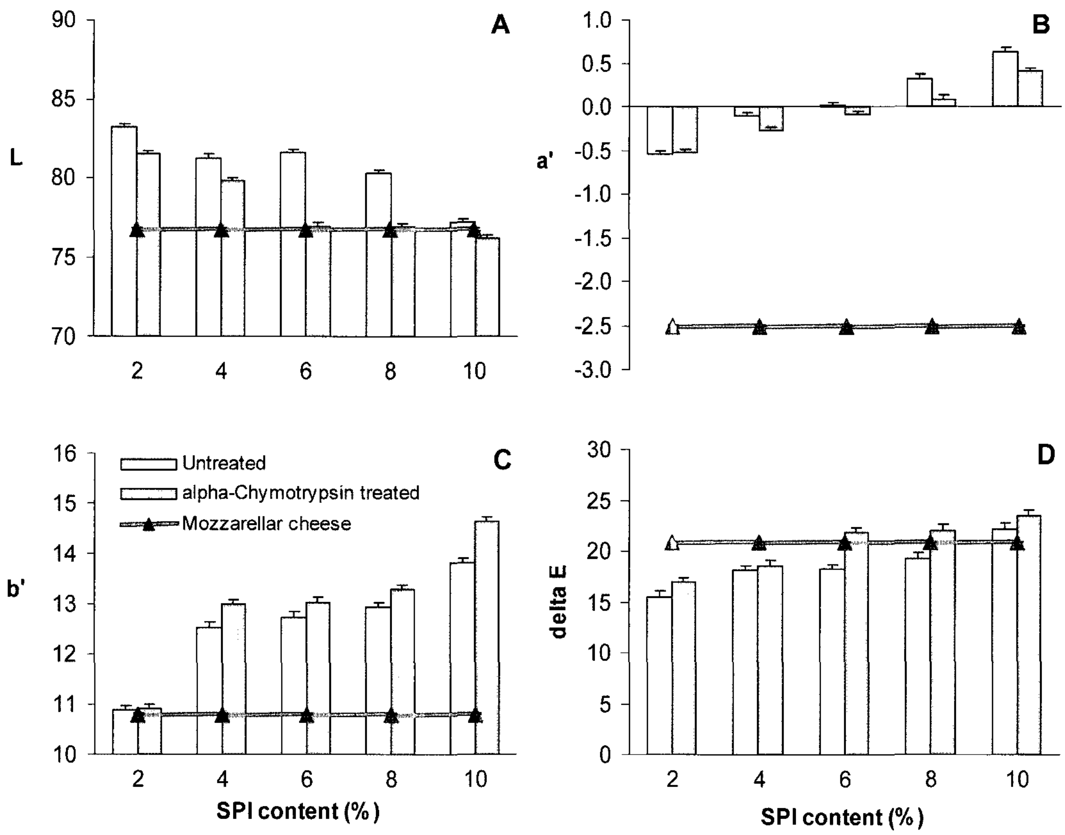

Fig. 2. Colors of cheese analogs and mozzarella cheese by color difference meter. Values are mean ± SEM. L: 100 = white, 0 = black; a': + = red, – = green; b': + = yellow, – = blue; $\Delta E = 100 - (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$.

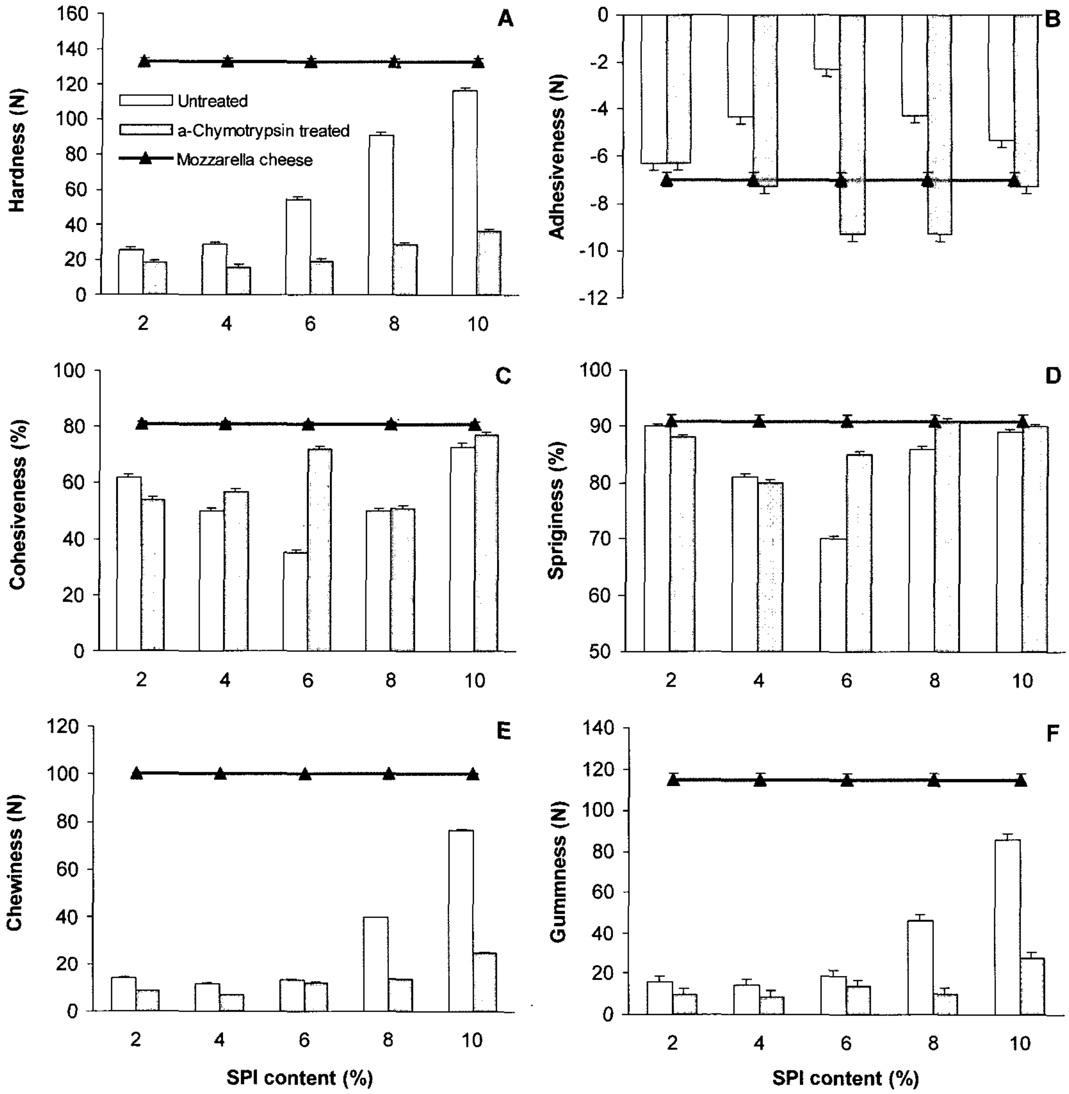

Fig. 3. Textural properties of cheese analogs and mozzarella cheese by Rheometer. Values are mean ± SEM.

were decreased by α-chymotrypsin modification ($p < 0.01$). Hardness was greatly affected α-chymotrypsin modification, and chewiness and gumminess were mainly related to hardness. Adhesiveness (Fig. 3B), cohesiveness (Fig. 3C) and springiness (Fig. 3D) were decreased up to 6% SPI cheese analog but it were increased in 8% and 10% SPI cheese analogs, while α-chymotrypsin modification increased adhesiveness, cohesiveness and springiness ($p < 0.01$) but 2% SPI cheese analog was not affected. Mozzarella cheese showed higher values of textural properties than cheese analogs. In each parameter of textural profile analysis, hardness, chewiness and gumminess of U10, adhesiveness of T4 and T10, cohesiveness of T6 and T10, springiness of U2, T8 and T10 were the most close to mozzarella cheese, respectively. Overall, 10% SPI cheese analog was the most similar to mozzarella cheese. In preparation of cheese analogs replaced 60% of casein to SPI, compared with commercial milk-based cheese, the textural properties of cheese analogs were markedly different, while SPI treated with alcalase and trypsin were more influential in modifying textural properties of cheese analogs than those with other proteases, and the use of enzyme modified SPI decreased hardness and brought adhesiveness (Kim(Lee) *et al.*, 1992). Suarez-Solis *et al.* (1998) have also shown that 10% SPI cheese analog had excellent characteristics as a melted cheese in textural, physico-chemical and microbiological properties, while Yang & Taranto (1982) have shown that in compared the textural properties of cheese analogs contained 10%, 20%, 30% or 40% SPI, respectively, and the hardness, cohesiveness, springiness, gumminess and chewiness up to 30% level of SPI were not significantly different with commercial mozzarella cheese but adhesiveness was different.

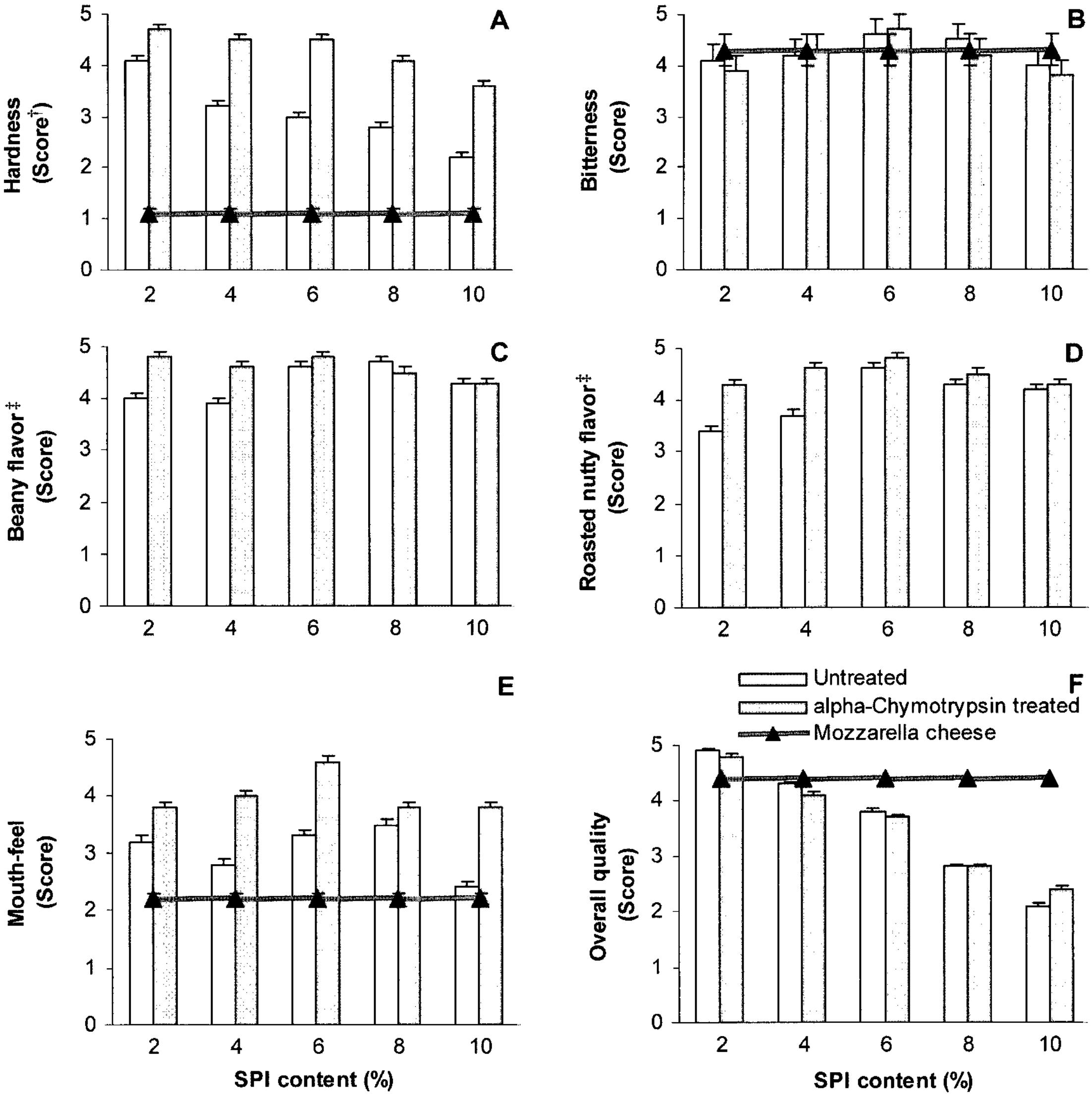

Fig. 4. Sensory attributes of cheese analogs and mozzarella cheese. Values are mean ± SEM. †Score – hardness: 1 very hard ↔ 5 very soft; mouth-feel: 1 very sandy ↔ 5 very smooth; bitterness: 1 very strong ↔ 5 very slight; beany flavor: 1 very strong ↔ 5 very slight; roasted nutty flavor: 1 very strong ↔ 5 very slight; overall quality: 1 very poor ↔ 5 very good. ‡There are no beany flavor and roasted nutty flavor in mozzarella cheese.

Sensory attributes

Sensory attributes of cheese analogs are shown in Fig. 4. Hardness of cheese analog less containing SPI was softer but it was similar between U4, U6 and U8 ($p < 0.01$), and was greatly affected and was softer by α-chymotrypsin modification ($p < 0.01$) (Fig. 4A). Bitterness did not show difference between cheese analogs and it was very highly marked, and it was not affected by α-chymotrypsin modification (Fig. 4B). α-Chymotrypsin was selected through the preliminary experiment. Supro 500E was hydrolyzed by pepsin, trypsin and α-chymotrypsin under the optimized pH and the temperature, respectively, and then α-chymotrypsin was evaluated as an enzyme which has the least bitter taste and the highest preference through sensory evaluation (data not shown), it might be affected by sorts of proteases related bitterness. Peptic proteolyzate from soy protein was known to be bitter, the bitterness is attributed to various bitter peptides including glycyl-L-leucine (Gly-Leu) and L-leucyl-Lphenylalanine (Leu-Phe), however, peptic proteolyzate treated with α-chymotrypsin had no bitterness and contained neither Gly-Leu and Leu-Phe as well as α-chymotrypsin showed the highest debittering effect (Yamashta *et al.*, 1970). Beany flavor and roasted nutty flavor, 6%, 8% and 10% SPI cheese analogs were very higher marked ($p <$ 0.05), while it were not affected by α-chymotrypsin modification except 2% and 4% cheese analogs (Fig. 4C, 4D). Mouth-feel of cheese analogs, U8 was the most smooth and U10 was the least, while mouth-feel of all cheese analogs was smoother by α-chymotrypsin modification ($p < 0.01$), on the other hand, mouth-feel of mozzarella cheese was less marked than that of cheese analogs and it was similar with 10% SPI cheese analog (Fig. 4E). In overall quality, cheese analog less containing SPI was better quality ($p < 0.05$), while overall quality of cheese analogs except 10% SPI cheese analog was not better marked by α-chymotrypsin modification (Fig. 4F). Overall quality was similar with hardness but it was not corresponding with other sensory factors. α-Chymotrypsin modification affected to improve the hardness, mouth-feel, beany flavor and roasted nutty flavor, mainly. In addition, beany flavor and roasted nutty flavor, one of sensory attributes of soy products were not mostly discriminated and highly marked.

These results indicate that lipoxygenase-defected soybean has more acceptable sensory attributes than normal soybean, and enzyme modification can be available to improve not only textural properties but also sensory attributes. The yields of volatile compounds in lipoxygenases lacking soybean were greatly less than normal soybean. (Kobayashi *et al.*, 1995), and sensory attributes of lipoxygenase-defected soybean were excellent in processing of soycurd, soymilk and soy yogurt (Kim *et al.*, 1994; Park & Lee 1997a, b; Torres-Penaranda *et al.*, 1998). When compare to mozzarella cheese, sensory attributes of cheese analogs were softer, better mouth-feel and similar bitterness. Hardness in instrumental textural properties was also softer than mozzarella cheese. However, mozzarella cheese is normally used with heating, while in this study, sensory evaluation of mozzarella cheese was performed without heating, and thus mouth-feel of mozzarella cheese might be less marked. Sensory evaluation of T6 among cheese analogs was the highest marked and the most similar to mozzarella cheese, while in instrumental textural properties, T10 was the most similar to mozzarella cheese. On the other hand, Suarez-Solis *et al.* (1998) have reported that 10% SPI cheese analog had excellent sensory attributes as a melted cheese.

Melting spreadability

Melting spreadability of cheese analog decreased by increasing the SPI content ($p < 0.05$), while it was highly affected by α-chymotrypsin modification, especially, 4%, 6% and 8% SPI cheese analogs were highly enhanced ($p < 0.05$) (Fig. 5). In milk-based cheese products also, protein size can be decreased and melting spreadability can be increased by optimized enzyme modification (Mahoney *et al.*, 1982), and the meting spreadability depends on moisture

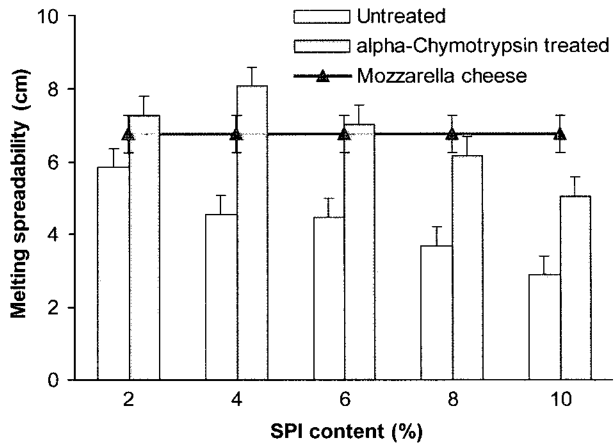

Fig. 5. Melting spreadability of cheese analogs and mozzarella cheese. Values are mean ± SEM.

content and water partition (McMahon *et al.*, 1999). Mozzarella cheese is generally used as a pizza cheese, heating is necessary and needs excellent melting spreadability, in this study, cheese analogs except 10% SPI cheese analog showed very similar melting spreadability compare with mozzarella cheese. On the other hand, Kim(Lee) *et al.* (1992) have reported that melting spreadability of cheese analog depends on protease, alcalase and α-chymotrypsin increased effectively, while liquozyme and rennet are not affected, and although melting spredability is improved by the use of enzyme modified SPI, it is inferior to natural mozzarella cheese. In addition, stretchability of cheese analogs is related to the amount of soy protein, while salts affect to improve it (Yang & Taranto, 1982).

Relationships between sensory attributes, instrumental textural properties and melting spreadability

Table 2 shows Pearson correlations between sensory attributes and instrumental or rheological parameters. Hardness in sensory attributes correlated positively with hardness and adhesiveness ($r > 0.65$ and $r > 0.56, p < 0.01$), and correlated positively and strongly with chewiness and gumminess ($r > 0.77$ and $r > 0.76, p < 0.01$), while correlated negatively and highly with melting spreadability ($r > -0.68, p < 0.01$). Mouth-feel correlated negatively with adhesiveness, chewiness and gumminess ($r > -0.51$, $r > -0.52$, and $r > -0.53$, p < 0.01), while correlated positively with melting spreadability ($r > 0.46$, $p < 0.01$), however, overall quality did not show significant correlation. Consequently, hardness in sensory attributes was considerably correlated with hardness, adhesiveness, chewiness, gumminess, and melting spreadability. Many of the sensory and instrumental analyses are

Table 2. Relationships between sensory attributes, textural properties and melting spreadability.

	Hardness	Mouth-feel	Overall quality
Instrumental parameters			
Hardness	0.65**	−0.41	−0.34
Cohesiveness	−0.06	0.08	0.10
Springiness	−0.12	0.02	0.07
Adhesiveness	0.56**	−0.51**	−0.23
Chewiness	0.77**	−0.52**	−0.39
Gumminess	0.76**	−0.53**	−0.38
Rheological parameter			
Melting spreadability	−0.68**	0.46**	0.35

** $p < 0.01$

highly correlated in cheese texture evaluation, and TPA and fundamental rheological tests work equally well or better at predicting sensory attributes of the cheese products (Drake *et al.*, 1999). In contrast, result of this study indicates that hardness in sensory attributes is obvious and important factor as a textural parameter.

CONCULUSION

Cheese analogs prepared using lipoxygenase-defected soymilk and α-chymotrypsin modified SPI were not discriminated off-flavors. Textural properties of cheese analogs were remarkably different and were highly affected by enzyme modification. Increasing SPI content increased hardness, chewiness and gumminess, while α-chymotrypsin modification decreased hardness, chewiness and gumminess, and increased adhesiveness, cohesiveness and springiness. In sensory attributes, overall quality was similar with hardness but it was not corresponding with other sensory factors. Melting spreadability was effectively enhanced by enzyme modification. Hardness in sensory attributes was considerably correlated with instrumental textural properties and melting spreadability. These results indicate that lipoxygenase-defected soybean has more acceptable sensory attributes than normal soybean and enzyme modified SPI can be available to improve not only textural properties but also sensory attributes. These results could be effectively used in manufacture of cheese analog similar to mozzarella cheese. Further studies on the other ingredients and rheological properties similar with natural mozzarella cheese would be required to improve the quality of cheese analog.

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