Effect of chemically modified soy proteins and ficin-tenderized meat on the quality attributes of sausage

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Abstract: The purpose of this investigation was to use ficin-tenderized meat and cysteine modified-soy proteins in the production of bologna and to evaluate the effect of these modifications on water holding capacity (WHC), emulsion stability (ES), texture and protein solubility. The effect of ficin on meat protein was also evaluated by SDS-PAGE. Results indicated that both ficin-tenderized meat and modified soy proteins substantially improved WHC, ES and other quality factors such as appearance and texture of the product. SDS-PAGE results showed disappearance of several protein bands in ficin-treated meat. Solubility of meat proteins increased when ficin was used for meat tenderization. Taken together, the results of this study indicated that some quality attributes of meat products can be improved by enzymatic and chemical modification of protein sources in the manufacture of meat products.

Key words: Ficin, tenderized meat, modified soy proteins, bologna.

INTRODUCTION

Soy products such as soy flour, soy protein isolates, and concentrates are commonly used in meat-based products for their functional properties. One important physical characteristics of soy proteins is their water solubility. This property significantly contribute to superior functional properties of soy products [1]. Physical, chemical and enzymatic methods have been used to improve the protein solubility of soy products such as ultrafiltration [1,2,3]. Chemically modified soy proteins significantly improve functional properties such as water holding capacity, foaming properties, appearance and texture of finished meat products. Proteolytic enzymes of plant origin have been extensively studied and used for

meat**.** Ficin, the sulfhydryl protease of the fig tree (*Ficus carica)* is reported to
hydrolyze and increase solubility of muscle proteins and its application for meat tenderization has been suggested [4,5]. In the present paper we describe the effect of application of chemically modified soy protein isolate and enzyme ficin on the quality of an emulsion-type meat product.

MATERIALS AND METHODS

Beef sausage was manufactured in the pilot plant of the Department of Food Science. The procedure for making sausage was adapted from Serbanek and others (1989)[6] with some modification. Soy proteins were modified by cysteine as described by Abtahi and Aminlari (1997). The enzyme ficin was partially purified according to the procedure of Englund and others (1968) and the proteolytic activity of ficin was determined using casein as substrate, according to the method of Englund and others (1968)[7]. Different volumes of ficin solution containing 0.0, 0.75, 1.5 or 2.25 units ficin activity were added to 5 g meat and samples were kept at 25 ^{o}C for 1h for NSI determination or 1, 2 or 3 h for SDS-PAGE (see below). Distilled water (5mL) was added to each meat sample and the mixtures were homogenized in a meat blender set at fixed speed for one min.The NSI was determined as described by Inklaar and Fortin (1969)[8] with some modification. The slurry was centrifuged at 1250 Xg for 5 min and the nitrogen content of the supernatant determined by the standard microKjeldahl procedure. The NSI was calculated as follows:

NSI = (water soluble nitrogen/ total nitrogen in meat sample) X 100.

The meat used for NSI experiments was obtained from the same sample used for sausage preparations. The supernatants that were used for NSI determination were also used for SDS-PAGE studies. Slab for SDS-PAGE were formed according to the discontinuous buffer system of Laemmli (1970)[9] on a 5-20% (w/v) gradient polyacrylamide gel. Tensile strength was calculated from the maximum load during a tension test carried to rupture the specimen (Mohsenin, 1996)[10] by using an Instron Universal testing machine (Instron Co, Model 1140, England). 3mm thick slices were hooked to the testing machine and the resistance to tearing (tensile stress) was determined at tensile velocity of 0.5 cm/min. Water holding capacity (WHC) was determined as described by Hung and Zayas (1992)[11] was used for determination of WHC. Emulsion stability was measured by the method of Hung and Zayas (1991)[12]. Meat to be used for preparation of sausage was treated with ficin. Ground beef (3 kg) was mixed with 4 mL enzyme solution containg 30 units ficin. The mixture was incubated at 37 ^{o}C for 2 h and was used for sausage production. An untrained panel of 18 members was used for evaluation of color, texture, and taste preferences on the finished sausage product**.**

RESULTS AND DISCUSSION

The effect of different formulations in manufacturing sausage is shown in Table 1. The control group was sausage made using untenderized meat and unmodified soy protein isolate with or without caseinate. Application of chemically modified soy protein isolate, tenderized meat, in combination or individually, significantly improved WHC of the emulsion. Sodium caseinate slightly increased WHC of the control group, but its absence was overcome by inclusion of chemically modified soy protein and/or tenderized meat in the emulsion formula. Similar results appeared in the emulsion stability and texture data. CMS and tenderized meat improved emulsion stability and texture of meat emulsion in the presence or absence of sodium caseinate. The chemical modification of soy protein and tenderization of meat did not have a significant effect on color of the finished product as judged by the members of the panel. Table 1 shows that meat tenderization improved the taste of the sausage but chemical modification of soy protein had no significant effect. When used in combination, CMS and TM had significant effect on TS ($P<0.05$). In the absence of sodium caseinate, this difference was still statistically significant ($P<0.05$). Sodium caseinate did not have significant effect on TS in all formulations ($P>0.05$). Table 2 show the effects of different levels of ficin on NSI and electrophoretic behavior of meat proteins. An increase in NSI occurred when meat was treated with different concentrations of ficin. However, ficin, especially at high concentrations, caused disappearance of many protein bands in SDS-PAGE (data not shown).

Studies on functional properties of proteins and the factors that contribute to these properties in food systems are essential to determine their potential for use in food products. Protein solubility is a key functional property since proteins generally have to be in solution to exert desirable functional characteristics. Improvement in the solubility of proteins can be achieved by chemical and enzymatic modifications [13]. In manufacturing comminuted meat products, the emulsion stability of the product and the emulsion capacity of meat proteins is of paramount importance [12]. These characteristics are strongly influenced by the concentration of water-soluble proteins in the emulsion [4,14,15]. In the present study we used chemical modification of soy protein isolate and the meat tenderizing enzyme ficin to improve the quality of the sausage. As shown in Table 1, both procedures significantly increased water-soluble-proteins, water holding capacity, and emulsion stability. The positive effects of modification of soy proteins by reducing agents on the protein solubility in different soy products have been reported by other investigators [1,16,17]. These effects are believed to involve reduction of disulfide bonds and decrease in the polymerization of soy proteins [1,18]. Cleavage of intermolecular disulfide bonds by reducing agents has resulted in the disruption of the structure of 11S glycinin and appearance of the cleavage products in the supernatant of the centrifuged soy protein slurry, hence increased NSI. Similar phenomena have been reported by other investigators [1,19]. The interaction of these proteins with other constituents of the sausage emulsion, including meat proteins, might have contributed to the improved quality of the sausage product.

Previous studies have shown that a number of proteolytic enzymes can solubilize the proteins of muscle [4,20,21] Proteolytic enzymes are known to increase the tenderness of meat when properly used. Ficin, the proteolytic enzyme from fig trees, is a sulfhydryl protease. The results of the present study showed that ficin significantly affects meat proteins. An increase in solubility of proteins was observed when ficin was used even at a low concentration (Table 2). An increase in the solubility of proteins has resulted in an increase in the water holding capacity, increased rupture resistance (improved texture), and emulsion capacity and stability. Although the concentration of ficin used for NSI measurement was significantly higher than that used for meat tenderization in te sausage product, the SDS-PAGE and data of Table 1 suggest that the ficin-solubilized proteins improved emulsion stability while those proteins that were precipitated by ficin had significant effect on texture of the sausage. It has been reported that proteolysis of meat proteins increases the viscosity of meat emulsions due to the increase in the number of degraded protein molecules with the axial ratio in the range of 100:1-200:1 [22,23]. These proteins may associate along their lengths giving rise to a vast number of total molecular weight permutation. The aggregation of these molecules then increases the viscosity of the emulsion and improves the texture of the final product. SDS-PAGE results indicated that, when used at low concentration, ficin degraded meat proteins (data not shown). The proteins that are mostly affected are heavy chain of myosin (180-200 kDa), C-protein (about 140 kDa), and α-actinin (105 kda) [24]. At higher enzyme concentrations, these proteins were totally degraded and disappeared. SDS-PAGE was performed on the supernatant of ficin-treated meat after centrifugation.

CONCLUSION

Taken together, the results of NSI analysis and SDS-PAGE suggested that ficin might increase the solubility of meat protein by degrading the proteins into smaller molecular weight units which when aggregated form a 3- dimensional network. This high molecular weight aggregate can precipitate during centrifugation and hence was not detectable in SDS-PAGE. When used in combination, chemically-modified soy proteins and tenderized meat augmented the effect of each other and the quality of the finished product was superior to that of either one used alone. Also, the data from the present study show that these modifications might allow the manufacture of sausage without sodium caseinate while retaining water holding capacity, emulsion stability, and the organoleptic properties of the product. In some parts of the world milk products, including caseinate, might not be readily available and too expensive. The procedure described in this paper might be useful for the manufacture of a more economical product.

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Table1: Physical and organoleptic characteristics of sausage formulation

Characteristics	Control	CMS	TM	CMS+ TM	Control	CMS	TM	CMS + TM
Sodium Caseinate	-	-	-	-	+	+	+	+
WHC	0.42^{a}	0.44^{b}	0.45^{c}	0.47^{d}	0.41^{e}	0.42^{a}	0.44^{b}	0.46^{c}
ES (%)	44.1^{a,b}	47.5^{b}	52.3^{c,d}	55.4^{c}	42.8^{a}	46.3^{a,b}	51.0^{c}	54.0^{c,d}
Texture stability (g/cm^2)	390^{a}	410^{a,b}	435^{b,c}	450^{c}	385^{a}	405^{a,b}	425^{b,c}	445^{c}
Organoleptic Scores								
Texture	8.0^{a}	8.2^{a}	8.5^{b}	8.9^{c}	8.0^{a}	8.2^{a}	8.5^{b}	8.8^{c}
Color	7.3^{a}	7.3^{a}	7.4^{a}	7.4^{a}	7.3^{a}	7.7^{a}	7.4^{a}	7.4^{a}
Taste	7.1^{a}	7.1^{a}	7.5^{b}	7.5^{b}	7.0^{a}	7.0^{a}	7.5^{b}	7.5^{b}

Each value is the average of three replications. In each row the numbers bearing different superscripts are statistically different ($P < 0.05$). CMS: chemically modified soy protein isolate, TM: tenderized meat, WHC: water holding capacity, ES:emulsion stability.

Table 2: Effect of ficin on nitrogen solubility index (NSI)*

Units ficin/g meat	NSI
0	8^{a}
0.15	17^{b}
0.30	25^{c}
0.45	30^{d}

* Each NSI value is the average of 3 replicates. a-d:NSI values are significantly different ($P < 0.05$).