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The effect of *Withania coagulans* as a coagulant on the quality and sensorial properties of Tofu

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Tofu is a nutritional, gel-like soy food. The present study was carried out to investigate the effects of *Withania coagulans* extract on the soymilk coagulation for producing tofu. For this purpose, soymilk was coagulated by *W. coagulans* extract, and the properties of the prepared tofu were analyzed. The results indicate that the extract of *W. coagulans* as a coagulant significantly ($p<0.05$) reduced yield and moisture content when compared with calcium sulfate tofu, but fat and protein contents of *W. coagulans* tofu and calcium sulfate tofu were the same. Results of sensory evaluation show that the sensorial properties of *W. coagulans* tofu are not different from that of calcium sulfate tofu. In this way, it was concluded that the extract of *W. coagulans* fruits could be used as a natural coagulant in coagulation processes of soymilk.

Key words: *Withania coagulans* soymilk, tofu, coagulant, syneresis.

INTRODUCTION

Soybean is a major source of protein, having a protein content of 35-40% on a dry basis, making it a relatively inexpensive source of protein for human consumption (Derbyshire et al., 1976). Soybeans are processed into different kinds of products such as tofu one of the most important and valued soy foods throughout the world (Mujoo et al., 2003), especially in East and South Eastern Asian countries due to their inexpensive and high quality protein (Birthal et al., 2010). Use of tofu and soy-products in the Western countries are in an increasing rate (Atkinson et al., 2002).

Tofu preparation generally includes soaking and grinding of soybeans in water, filtering, boiling and coagulation of soymilk. The yield, quality and texture of tofu are influenced by many factors such as variety of soybeans (Shen et al., 2006), processing methods (Kyoko, 1979; Shih et al., 2006) and type and concentration of coagulants (Lim et al., 2006; Shen et al., 2006; Sun and Breene, 1991).

Usually, calcium sulfate and glucono -δ-lactone are used more than other coagulants on an industrial scale for the tofu making (Lim et al., 2006; Sun and Breene, 1991; Tsai et al., 2006; Wang and Hesseltine, 1982). Tofu is generally known as a salt or acid coagulated soy protein gel, with soya lipids and proteins and other constituents trapped in its networks (Kohyama et al., 1995).

Most studies about tofu are available in the literature and used chemical coagulants for preparation of tofu (Beddows and Wong, 1987; DeMan et al., 1987; Schaefer and Love, 2007; Shen et al., 2006; Sun and Breene, 1991; Wang and Hesseltine, 1982). The extract of *Withania coagulans* is considered as a natural coagulant (Dastur et al., 1949).

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W. coagulans Dunal (Family: Solanaceae) is in the form of a shrub and it is usually recognized as Indian cheese maker. *W. coagulans* grows in Pakistan, Afghanistan and India as well as in southern regions of Iran. This vegetative coagulant has been used for preparation of traditional cheeses from raw cow's milk (Dastur, 1949; Roseiro et al., 2003). The main components of the berries other than the milk-coagulating enzyme are esterases, free amino acids, fatty oils (an essential oil), alkaloids and withanolides (Atta-ur-Rahman et al., 2003).

The aim of this study was to examine the effects of Vegetative coagulant from *W. coagulans* on the quality and yield of tofu making, in an attempt to use this enzyme as a new local source of coagulant in preparation of tofu.

MATERIALS AND METHODS

Soybean used for tofu making was purchased from a local market (city of Mamaghan, Iran). Calcium sulfate and other chemicals were purchased from Merck Company (Darmstadt, Germany). All chemical reagents were in analytical grade.

Dried fruits of *W. coagulans* were collected from wild plants growing in the Southeast of Iran (Sistan and Baluchestan Province).

Enzyme extraction

According to Naz et al. (2009), dried fruits of *W. coagulans* powdered by grinder (Bel-Art Products, Pequannock, NJ, USA) and enzyme extract was obtained by soaking the powder in 0.85% saline solution at 4°C for 24 h. The extract was filtered to obtain crude extract. This coagulant was kept in 4°C for future uses.

Preparation of soymilk

Soymilk was prepared according to the method of Noh et al. (2005) with a few modifications. Soybeans were soaked in water at room temperature for a period of 10 h, rinsed and drained, then ground with water at a bean: water ratio of 1:4 by using a waring blender (Bel-Art Products, Pequannock, NJ, USA) for 5 min at high speed. After grinding, the resultant slurry was cooked under stirring condition and held at 95°C for 15 min. The slurry was squeezed handy with a muslin cloth and pressed to remove the soy residue (okara), to obtain a soymilk of 12–14° Brix. A single batch of soymilk was made from the soybean and was used for the preparation of tofu by using each coagulant.

Preparation of tofu

According to Liu et al. (2004), 100 ml portion of prepared soymilk (75°C) was poured in a 250 ml beaker, and food grade 0.5% calcium sulfate solution was added to the solution with constant stirring. Stirring was stopped after complete coagulation (10 min) and content was kept without stirring for 15 min at room temperature. Tofu gel was formed and cooled to 20°C without moving from the beaker, and then kept at 4°C until the next day for analysis and yield rate measurement.

For preparation of *W. coagulans* tofu, different concentrations of prepared *W. coagulans* extract (0.25, 0.5, 1 and 1.5%) were added in a 100 ml of soymilk (37°C) with a little stirring. The content of beaker was kept for 10, 20, 30 and 40 min at 35, 37, 40 and 45°C and the same procedure was used for calcium sulfate tofu.

Analysis

Yield, moisture, protein, pH and fat analyses

Before analysis, when the tofu reached room temperature, tofu carefully was removed out from the beaker and at the same time, whey was removed and fresh tofu-gel was weighted.

Yield of tofu was calculated as fresh weight of tofu-gel obtained from a specified amount of the soybean used for its making.

Moisture content was determined by drying 5 g of fresh tofu at 105°C in an oven to constant weight (Tsai et al., 2006). Total protein was determined by the micro Kjeldahl method (AOAC, 1995). The factor N×6.25 was used to convert nitrogen to protein. The fat content of tofu was determined by the Soxhlet method (AOAC, 1995). pH of the tofu samples was measured with a digital pH meter with a glass electrode (Metrohm AG, Switzerland).

Syneresis rate

After equilibrating in the room temperature (20°C) for 1 h, the tofu-gel was carefully removed wholly from the beaker and cut cross-wise with a sharp knife to pieces with diameter of 1.5 cm.

Syneresis was evaluated by employing the modified method of Armstrong et al. (1994). After cutting, six pieces of tofu-gel samples were put on 20 mesh stainless steel grid in a plastic box. The grid was supported by four sticks. The exuded liquid was allowed to be separated from tofu-gel. The box was covered with plastic to prevent evaporating. Since the liquid was released slowly, six pieces of tofu-gel samples were stored in the box for 10 h in refrigerator. The liquid quantity exuded during the 10 h was used to calculate syneresis rate:

$$\text{Syneresis}(\%) = \frac{\text{Weight of water exuded from the sample in 10h}}{\text{Weight of sample}} \times 100$$

Sensory evaluation

Thirty-two (32) untrained panelists, composed of adult males and females, scored the sensory characteristics of tofu-gel. Tests on overall acceptability, color, flavor and texture were conducted using a 5-point hedonic scale (5 = excellent, 1 = not good) for each attribute. All samples were coded and presented in a randomized arrangement.

Statistical design and analysis

Experiments were based on a randomized complete block design. All extraction experiments and analyzes were performed seven times and values are reported as means ± SD as obtained. Analysis of variance was conducted for each data collected, using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range tests was used to determine differences between the two prepared tofus. The Statistical tests were conducted at the 5% probability level.

RESULTS AND DISCUSSION

W. coagulans tofu coagulated only when 1% prepared *W. coagulans* extract was added in a 100 ml of soymilk

Table 1. Syneresis, yield and contents of moisture, protein, fat, pH of prepared tofu with two different coagulants.

Sample	Yield					
	Soybean (kg/kg)	Moisture (%)	Protein (%, d.b.)	Fat (%, d.b.)	pH	Syneresis (%)
Calcium sulfate tofu	3.13[a]	86.75±0.16[a]	52.70±1.41[a]	28.73±0.63[a]	5.44±0.25	15.4±0.3[a]
Withania coagulans tofu	2.19[b]	85.98±0.33[b]	52.25±1.53[a]	28.44±0.81[a]	5.08±0.12	17.0±0.5[b]

Values are means ± standard deviation. Means with the same letter within columns are not significantly different from each other (P < 0.05).

Table 2. Effect of *Withania coagulans* on sensory characteristics of tofu.

Sample	Flavor	Color	Texture	Overall acceptability
Calcium sulfate tofu	2.15[a]	2.9[a]	2.84[a]	2.28[a]
*Withaniacoagulans*tofu	2.53[a]	2.87[a]	2.81[a]	2.44[a]

Means with the same letter within columns are not significantly different from each other (P < 0.05).

(37°C) with a little stirring and then it was kept for 20 min at 37°C. These results are in the line with the results of Naz et al. (2009). The main objective of their study was to purify the protease from *W. coagulans* and partially characterize it, and they concluded that 37°C and 15 μLmL^{-1} of enzyme would be the best concentration for coagulation.

Yield and composition of tofu

The composition and yield of tofu samples are shown in Table 1.

No significant differences were found between protein and lipid content of tofu coagulated by these two coagulants. The formation of tofu gels entangles proteins, fats, hydrocarbonates, and air within the gel networks. The aggregated soy protein networks which are full of air bubbles, scaffold the tofu gel. The networks form numerous boundaries among the media (Kohyama et al., 1995; Saowapark et al., 2008).The yield of *W. coagulans* tofu was lower than that of calcium sulfate tofu. The lower yield of *W. coagulans* tofu was reflected by the lower moisture content because tofu yield and moisture contents are highly correlated (Cai et al., 1997). These results are in the line with the results of Noh et al. (2005). They studied quality of tofu as affected by freezing treatment of soybeans and reported that the lower yield and moisture content of tofu from frozen soybeans may be ascribed to the denser and more compact structure, which made water easily release from the curd during pressing. The variation in the moisture content of tofu prepared with different coagulants is probably due to the differences in the gel network affected by the ionic strengths of the coagulants and/or the effect of the different anions on the water-holding capacity of the soy-protein gels (Wang and Hesseltine, 1982).The pH of *W. coagulans* tofu was lower than that of calcium sulfate tofu; these results are in line with the results of Pezeshki et al. (2011) which reported that pH was significantly (p< 0.05) lower in cheeses made with *W. coagulans*as coagulant than pH of cheeses with animal or fungi rennets. A decrease in pH was described as essential for the coagulation of soy proteins by many previous researchers (Beddows and Wong, 1987; Lu et al., 2006). The lower pH of *W. coagulans* tofu may reflect the isoelectric precipitation of soy-proteins by the release of protons from δ-lactone (Smith, 1978) because Withanolides are a group of ergostanolides, generally having a δ-lactone in the side chain (Abraham et al., 1968).

Syneresis rate

It is recognized that *W. coagulans* significantly (p<0.05) increased the tofu's syneresis rate. Probably due to an unspecific proteolitic activity of *W. coagulans* enzymes (Pezeshki et al., 2011), however, increase of syneresis from the curd could be due to increase in bonding occurring during storage, making the protein matrix more dense and compacted (Sun and Breene, 1991) and also differences between microstructures may be responsible for different physical properties between the two kinds of tofu gels (Liu et al., 2004).

Sensory evaluation

Table 2 shows the results of sensory evaluation of tofu for color, flavor, texture and overall acceptance. The results were expressed on a 5-point hedonic scale.

There were no significant differences between sensory characteristics of tofu coagulated by these two coagulants. This means that extract of *W. coagulans* had few effect on the sensory attributes of tofu.

Conclusion

This study has confirmed the possibility of replacing calcium sulfate by extract of *W. coagulans* for the coagulation of soybean milk in the preparation of tofu. Furthermore, the study has demonstrated that *W. coagulans* tofu had fat and protein content similar to calcium sulfate tofu but both yield and moisture content were lower. In addition, using *W. coagulans* could reduce heating process needed for coagulation of soymilk, without impairing the quality of tofu, and the cost of tofu preparation is reduced because of the cheaper price of *W. coagulans* than that of calcium sulfate

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