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Effect of Ultrasound Assisted Extraction upon the Protein Content and Rheological Properties of the Resultant Soymilk

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Abstract: The purpose of this study was to determine the effect of ultrasound treatment on the protein content and rheological properties of soymilk extracted from soybeans. Soybean slurry was exposed to ultrasound treatment then filtered. In this work, an ultrasound cleaning bath set with 35 kHz frequency and different treatment temperatures (20 and 40ºC) and times (20, 40, and 60 min) was used. Results indicated that ultrasound treatment could significantly ($p<0.05$) increased the protein content of extracted soymilk by nearly 6.3%. These results were attributed to induced cavitation, which increased the permeability of plant tissues. The flow behaviour of soymilk was observed to be Newtonian for all samples. Viscosity (μ) data calculated with a power-law equation ($R^2 = 0.99$) increased slightly but not significantly ($p>0.05$) after ultrasound treatment. The consistency coefficient (K) did not change after ultrasound treatment. On the other hand, a significant increase in flow-behaviour indices (n) was observed.

Key words: Rheological properties, Soymilk, total solid, ultrasound treatment, protein, viscosity

INTRODUCTION

Lately, soy-based foods have attracted much attention for their preventive effects against chronic diseases like arteriosclerosis, cancer, osteoporosis, and menopausal disorders. Among different kind of soy products, soymilk has gained much popularity as an excellent source of high-quality protein which is commonly recommended as a less-expensive substitute for cow's milk (Amigo-Benavent *et al*., 2008). This traditional oriental drink is considered to have been invented in China between 179 B.C. to 122B.C (Min *et al*., 2005).

Ultrasound technology is growing rapidly in the field of research and development of non-thermal food-processing methods. The beneficial use of sound is realized through its chemical, mechanical, or physical effects on the process or product (Suslick, 1988). Ultrasound is defined as mechanical waves with a frequency above the threshold of human hearing (16 to 20 kHz). It can be divided into two frequency ranges. High-frequency ultrasound (low-power ultrasound) uses frequencies in the 5-10 MHz range with low intensity levels, typically less than 1 W/cm^2. It is mainly used in diagnostic analysis of food materials. Low-frequency ultrasound (high-power ultrasound) uses frequencies in the 20-100 KHz range with much high intensity levels, usually in the range of 10 to 1000 W/cm^2 (Mason, 1998; Barbosa-Cánovas *et al*., 2005; Jambrak *et al*., 2009). Some known applications of high-power ultrasound in the food industry include extraction (release of plant material), inactivation of microorganisms, enzyme inhibition, homogenization, emulsification, filtration, crystallization, and deforming (Brennan, 2006; Vilkhu *et al*., 2008).

Generally, high-power ultrasound can improve the extraction of intracellular compounds from plant material. It is supposed that this positive effect of ultrasound in the liquid/solid extraction is mainly caused by a phenomenon known as cavitation (Mason *et al*., 1996; Vinatoru, 2001; Knorr *et al*., 2002; Sun, 2005). A pilot-scale study of continuous ultrasonic extraction of protein from soybeans was reported by Moulton and Wang (1982). Li *et al*. (2004) applied ultrasound treatment to enhance oil extraction from soybeans. Karki *et al*. (2010) showed that the use of ultrasound technology can significantly improve protein release from defatted soy flakes. These studies conclude that applying power ultrasound may affect the protein content of soymilk extracted from soybeans.

Ultrasound application may also change the viscosity. Viscosity affects sensorial properties and consumer acceptability of soymilk and it is considered an important factor in quality control, and in the design and operation

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of processing equipment. About half of the solid in soymilk consists of soybean protein. Thus, the main contribution to soymilk viscosity might be friction caused by protein molecules rubbing against each other (Liu and Chang, 2007). Possible effects of ultrasound treatment (shear forces generated due to the collapse of cavitation bubbles, as well shock waves) on the concentration, structure, molecular weight, hydrogen bonding, and other intra-and intermolecular forces of the protein may affect the viscosity of soymilk; more investigation in this field seems essential.

The present study is the first to examine the effect of ultrasound treatment on the protein content and rheological properties of soymilk.

MATERIALS AND METHODS

Preparation of soymilk: Soybeans (Clark variety) were obtained from local growers and stored at room temperature under dark conditions until they were processed for soymilk. Soymilk was prepared according to the procedure of Min *et al*. (2005). Twenty grams of soybeans were washed and soaked in distilled water for 16 h at room temperature. Hydrated beans were drained, rinsed, and ground with boiling water (ratio of soybeans to water was 1:10 on a weight basis) using a Waring blender for 3 min at high speed. Then, produced slurry was filtered through four layers of cheesecloth to separate the soymilk from the residue. Figure 1 shows the flow diagram for the preparation of ultrasound-treated soymilk.

Ultrasound treatment with 35 kHz ultrasound cleaning bath: Ultrasound treatment was carried out in an ultrasonic cleaning bath (Elma, Model: D-78224 Singen/Htw, Germany; overall dimensions: 340×300×370 mm; internal dimesions: 240×130×150 mm) with temperature and time control. The bath was operated at frequency of 35 kHz with maximum input power of 100 W to investigate the effect of ultrasound treatment on tested parameters. One hundred mL of produced soybean slurry was placed in a 250 mL Erlenmeyer conical flask, which was then immersed into the ultrasound bath. Samples were treated for 20, 40 and 60 min at 20 and 40ºC. An ultrasonic transducer was attached to the bottom of the outer surface of the liquid container and the liquid was irradiated with ultrasonic waves from the surface of the liquid container. The suspension in the conical flask was kept at the level of the water in the bath, which was circulated and regulated at constant temperatures to avoid water-temperature increase from ultrasonic exposure. Frequency sweeping is often used to produce a more uniform cavitation field and reduce standing wave zones.

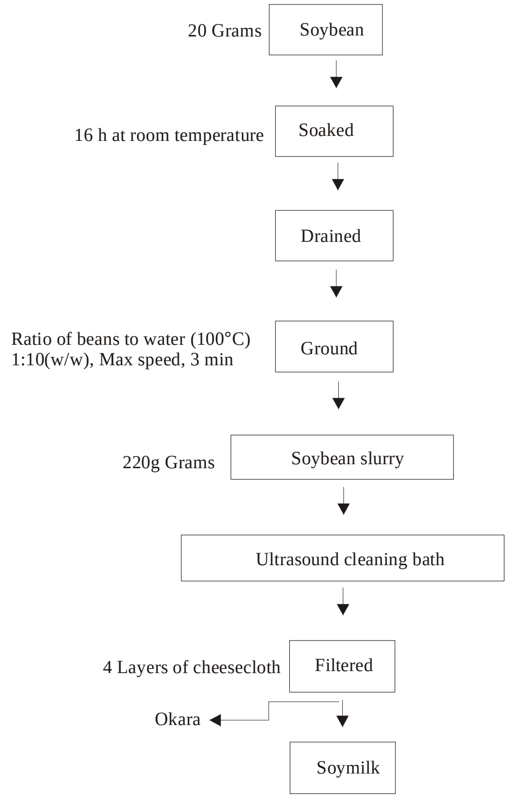

Fig. 1: Flow diagram of preparation of ultrasound treated soymilk

Determination of total solid and protein contents of soymilk: The total solid content of sonicated soymilk was determined by drying soymilk to constant weight at 110ºC in an air oven. Protein contents of sonicated soymilk were determined by the Kjeldahl method according to AOAC (2005). The conversion factor from nitrogen % to protein % was 6.25.

Determination of rheological properties: The viscosity of the soymilk samples was measured immediately after ultrasound treatment at room temperature using a Brookfield rotational viscometer (Model LVDV-11+P, Brookfield Engineering Laboratories, Middleboro, MA.2346, USA) equipped with spindle 60 at a speed of 30 to 140 rpm. The beaker volume was 20 mL. Flow curves were obtained at shear rates between 36.69 and 171.22 s^{-1}. Shear rate versus shear stress was interpreted on plots using a rheometric computer program (Rheocalk Demo, USA) according to the power-law expression:

$$\tau = K(\gamma)^n \quad (1)$$

where; τ is the shear stress (Pa), K is the consistency index ($Pa.s^n$), γ is the shear rate (s^{-1}), and n is the power-

Table 1: Effect of ultrasound treatment on protein and total solid contents of soymilk

Treatment	Protein (%)	Total solid (%)
Untreated	2.86±0.11 [a]	3.18±0.09[c]
35 kHz /20ºC / 20 min	2.93±0.05[ab]	5.54±0.20 [a]
35 kHz / 20ºC / 40 min	2.99±0.12[ab]	5.69±0.02[ab]
35 kHz / 20ºC / 60 min	3.06±0.11[bc]	5.86 ±0.21[bc]
35 kHz / 40ºC / 20 min	2.98±0.09[ab]	5.86 ±0.16[bc]
35 kHz / 40ºC / 40 min	3.19±0.02[c]	5.72±0.05[ab]
35 kHz / 40ºC / 60 min	6.09 ± 0.08 [c]	6.11 ± 0.25[c]

Values are expressed as mean±SD from three independent experiments. Means with different letters within the same column are significantly different at p<0.05

law index, which expresses the flow behaviour. When the magnitude of n<1 the fluid is shear-thinning (pseudoplastic), when n>1 the fluid is shear-thickening (dilatants) in nature. For the Newtonian fluid n = 1 (Steffe, 1996).

Statistical analysis: All the experiments were conducted in triplicate independent batch of soy slurry. Data were statically analyzed by one-way analysis of variance (ANOVA). The mean comparison was carried out with Duncan's multiple range tests using SPSS for Windows version 18.0 (2010). Significant levels were defined using the value p<0.05.

RESULTS AND DISCUSSION

Effect of ultrasound treatment on protein and total solid contents of soymilk: Table 1 gives protein and total solid contents of soymilk samples. The results showed that ultrasound treatment of soybean slurry significantly (p<0.05) increases the release of protein (nearly by 6.3%) and other compounds into the extracted soymilk. The most probable governing mechanism for ultrasonic enhancement of extraction was cavitation. According to previous studies, when a sonic wave propagates in a liquid medium as a longitudinal wave, it creates alternating compression and expansion cycles. When negative pressure in the liquid, created by the expansion cycle, is low enough to overcome intermolecular forces (tensile strength), small bubbles are formed. Due to surface tension, presence of other bubbles, foreign bodies, and gradients in the pressure waves, each bubble becomes increasingly unstable beyond a critical size and eventually collapses violently (Suslick, 1988; Barbosa-Cánovas *et al.*, 2005). In the case of soybean slurry, it seems that Shear forces generated by the ultrasound treatment due to the collapse of cavitation bubbles, as well shock waves, physically disrupt the plant cell walls and intensify the penetration of the solvent into the soybean cells, leading to a better mass transfer of intracellular proteines into the extractable soymilk (Maricela *et al.*, 2001; Vinatoru, 2001; Karki *et al.*, 2010).

The influence of two sonication temperatures (20 and 40ºC) on protein and total solid contents of soymilk

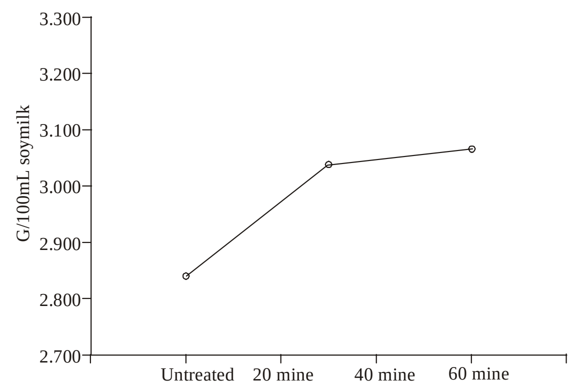

Fig. 2: Effect of ultrasonic temperature on protein and total solid contents of soymilk

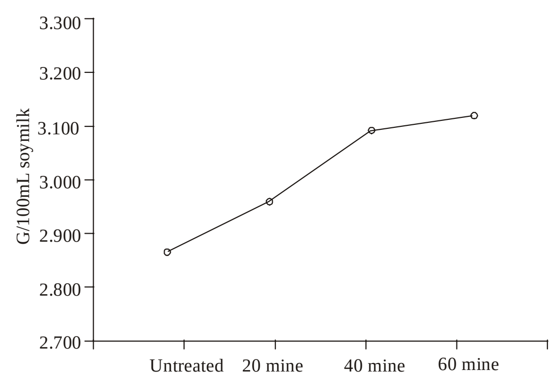

Fig. 3: Effect of ultrasonic time on protein and total solid contents of soymilk

samples were investigated (Fig. 2). The contents were found to increase slightly when the temperature was raised from 20 to 40ºC. possibly because: As the liquid temperature become higher, vapour pressure increases and viscosity and tensile strength decreases, which reduce the cavitation threshold, making the formation and growth of bubbles easier. However, this increase was not statistically significant (Patist and Bates, 2008; Zhang *et al.*, 2004).

Figure 3 shows the influence of sonication time on the protein and total solid contents of soymilk samples.

Table 2: Effect of ultrasound treatment on rheological parameters of soymilk

Treatment	Viscosity (μ) cp	Consistency coefficient (k)Pa.s	Flow index (n)	Determination coefficient®²)
Untreated	2.82±0.21[a]	3.16±0.34[a]	0.93±0.04[a]	0.99
35 kHz /20ºC / 20 min	3.39±0.36[a]	0.38±0.04[a]	0.98±0.02[ab]	0.99
35 kHz / 20ºC / 40 min	3.28±0.27 [a]	0.35±0.05[a]	0.99±0.01[ab]	0.99
35 kHz / 20ºC / 60 min	2.85±0.30[a]	0.34±0.01[a]	0.98±0.05[ab]	0.99
35 kHz / 40ºC / 20 min	3.44±0.72[a]	0.30±0.04[a]	1.00±0.05[ab]	0.99
35 kHz / 40ºC / 40 min	3.38±0.16[a]	0.35±0.16[a]	1.02±0.03[b]	0.99
35 kHz / 40ºC / 60 min	0.29±0.02[a]	0.26±0.06[a]	1.03±0.03[b]	0.99

Values are expressed as mean±SD from three independent experiments. Means with different letters within the same column are significantly different at p<0.05

Results indicated that total solid and protein contents of treated soymilk samples gradually rose for the first 20 min, while there was a significant ($p<0.05$) increase at the 40 min. On the other hand, the contents increased moderately but not statistically significant when the time was raised from 40 to 60 min, possibly because most of the extractable materials had already been released within the first 40 min.

Effect of ultrasound treatment on rheological properties of soymilk: In the food industry, determining the exact rheological properties of foods is essential for equipment design, sensorial assessment, consumer acceptance, and process and quality control of the product. Changes in rheological properties of soymilk after different ultrasound treatments were investigated; Table 2 shows the results. Rheological properties of all samples can be best explained by a power-law model (Eq. 1) with a high regression coefficient (0.99). The data shows that flow-behaviour index (n) values ranged from 0.93 to 1.03 (which is closer to 1) for all soymilk samples, which indicates a Newtonian fluid behaviour. This finding was in close agreement with results obtained by Son and Singh (1998) and Oguntunde and Akintoye (1991). In contrast, some researchers reported that soymilk made from whole soybeans showed non-Newtonian pseudoplastic flow behaviour (Forster and Ferrier, 1979). Newtonian fluids, by definition, have a straight-line relationship between the shear stress and the shear rate with a zero intercept (Steffe, 1996). Figure 4 plots shear stress against shear rate for untreated and ultrasonically treated soymilk samples.

In addition to increasing protein and total solid contents, ultrasound treatment causes changes in the flow behaviour of soymilk. This effect has been shown by statistically significant ($p<0.05$) increases in flow-behaviour indices (n). According to Phillips and Williams (1995), the changes in flow behaviour after ultrasound treatment are a consequence of changes in the binding capacity for water: the hydrophilic parts of amino acids are opened toward water surroundings, leading to higher binding of water molecules.

Table 2 gives the viscosity (μ) of different soymilk samples. Results indicated that ultrasound treatment caused a slight but not statistically significant increase in the viscosity of soymilk. It is understandable that the soymilk viscosity increased slightly with the increase of protein concentration, because more protein molecular interactions would occur (Fig. 4). However, at 2.8 and 3.1% protein concentrations, the differences in soymilk viscosity between the untreated (control) and the sonicated soymilk were not significant. Further, the results in Table 2 showed that ultrasound treatment had no significant effect on the consistency coefficient (K) of sonicated samples compared to the control. This was close to the trend shown by the viscosity.

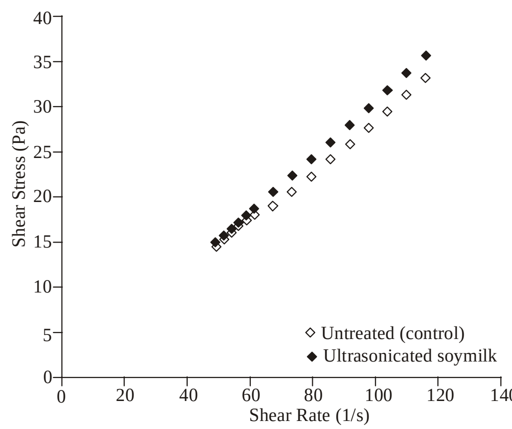

Fig. 4: Relationship between shear stress and shear strain of untreated (control) and ultrasonicated soymilk

CONCLUSION

The present study is the first to examine the effect of ultrasound treatment on the protein content and rheological properties of soymilk. Our preliminary results presented in this report showed that ultrasound treatment of soybean slurry by 35 kHz cleaning bath can significantly ($p<0.05$) increase the protein contents (by nearly 6.3%) into the extracted soymilk. These results were attributed to mechanical effects of induced cavitation, which increased the permeability of plant tissues. The flow behaviour of soymilk was observed to be Newtonian for all samples. Soymilk viscosity (μ) was not changed significantly by ultrasound treatment. On the other hand, a significant increase in flow-behaviour indices (n) was observed. However, further research work, from both an industrial and an academic viewpoint, is needed in order to expand the findings for a number of food extraction and processing applications. A wider range of ultrasound frequency, ultrasound power, treatment temperature, treatment time, and other parameters should be investigated.

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