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## Effect of ohmic heating on protein characteristics and beany flavor of soymilk

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### Abstract

The functional and nutraceutical food market is growing at the fastest rate in all segments of food. Plant-based milk substitutes' popularity is increasing worldwide as these beverages are rich in functional and nutraceutical compounds. Soymilk is a highly consuming plant-based milk substitute rich in protein, vitamins, minerals and cholesterol-free. The novel ohmic heating technology was potentially applicable in improving protein characteristics with a reduction in beany, off-flavor of soymilk. Ohmic heating is rapid and uniform heating technology. Effect of ohmic heating voltage (160V, 180V, 200V) and target temperature (70 °C, 80 °C, 90 °C) on extraction yield, protein characteristics and the beany flavor were investigated. Maximum yield (83.25±0.17%) of soymilk was found at 200V and 90 °C. Ohmic heating significantly increased ( $p < 0.05$ ) in crude protein content and soymilk's protein digestibility. The highest amount of protein content (8.14±0.08%) found at 180V and 80 °C. The digestibility of soymilk increased with an increase in temperature and voltage and found a maximum of 75.55±0.55% at 200V and 90 °C. The increase in voltage protein solubility decreased to 8.03±0.29%. The hydroperoxides responsible for beany flavor were significantly decreased ( $p < 0.05$ ) with increasing temperature and found a minimum 0.47±0.02 meq/kg at 200V and 90 °C target temperature.

**Keywords:** Ohmic heating, voltage, temperature, protein characteristics, hydroperoxides

### 1. Introduction

Soymilk is a beverage extracted by the grinding of soybeans. Production of soybean is increasing day-by-day because of its high nutritional quality and health benefits. Soybean is a cheap source of good quality protein (37-42%). Soybeans have useful properties such as an anticarcinogenic, prevent calcium loss, cholesterol-lowering property and a source of phytochemicals (Messina, 1995) [10]. In 1999, the US Food and Drug Administration (FDA) had approved the claim that soybean protein has a role in reducing cardiovascular diseases. Consumption of soybean protein reduces the cholesterol level.

The protein quality of soybeans is equivalent to animal protein quality. However, the soybean protein lacks sulfur-containing amino acid methionine (Mateos *et al.*, 2008) [8]. Ohmic heating is a novel thermal technology that utilizes the electrical resistance of food material for heat production. When an electric current is passed through the food material, it creates high impedance resulting in electrical energy conversion to heat energy. So, there is rapid and uniform heating of products causes retention of nutritional value. There is a degradation of protein under high voltage causes protein denaturation may occur. This will make protein fragmentation related to amino acid content (Li *et al.*, 2018) [7]. Denaturation of protein may occur at higher voltage and temperature (Sun *et al.*, 2008) [19]. Also, the protein digestibility increases with an increase in processing temperature because of changes in the structural arrangement (Vanga & Raghavan, 2018) [22]. *In-vitro* protein digestibility (IVPD) can be done by using inoculum from the animal digestive system or using digestive enzymes. This is because the particular bonds of a substrate require specific enzymes for its breakdown (Caprita *et al.*, 2013) [3]. Traditionally, soymilk was extracted by soaking soybeans overnight, then grinding, filtration and finally cooking. However, this traditionally produced soymilk does not have a smooth texture like a cow's milk and bland flavor. However, soymilk has a beany flavor, which end consumers do not accept. The odor and off-flavor in soymilk are characterized as a beany flavor.

This beany flavor is due to some volatile compounds produced by oxidation of unsaturated lipids by lipoxygenase enzyme during wet grinding of soybeans (Kwok & Niranjana, 1995)<sup>[6]</sup>.

During the grinding process, when cotyledons get broken and come in contact with water, unsaturated lipids, and oxygen, the lipoxygenase enzyme gets activated and produces off-flavor compounds n-hexanal, n-hexanol, acetaldehyde, pentanol, methanol, etc. Hydroperoxides are the intermediate compounds formed by the oxidation of fatty acids by lipoxygenase (Tripathi *et al.*, 2015)<sup>[20]</sup>. Soybean oil contains about 85% of unsaturated fatty acids. So, it got quickly auto oxidized and hydroperoxides formed by lipoxygenase. These hydroperoxides are not stable and can be readily cleaved by enzymes or non-enzymatic processes resulting in the generation of off-flavour compounds such as n-hexanol and n-hexanal. This cleavage of hydroperoxide lipids is due to the hydroperoxylyase enzyme. N-hexanol causes bean-like flavor formation (Arai *et al.*, 1967)<sup>[2]</sup>. According to some reports, n-hexanal converts to n-hexanol during processing (Matoba, 1989)<sup>[9]</sup>. The threshold value of the n-hexanol is more than the n-hexanal. So, this n-hexanol is considered a principle compound responsible for off-flavor formation (Mizutani & Hashimoto, 2004)<sup>[11]</sup>. There is a linear relation between hydroperoxides and beany flavor of soymilk (Tripathi *et al.*, 2015)<sup>[20]</sup>.

In this experiment, ohmic heating was used to extract soymilk from the plant matrix. This study's objective was to

investigate ohmic heating's effect on protein characteristics, yield, and beany flavor of soymilk.

## 2. Materials and Methods

### 2.1 Sample preparation

Good quality soybean (*Glycine max* L) was procured and stored at 4-8 °C until use. Soybeans were soaked 12 h before use in freshwater. Then grinding was done in a grinding unit for 15 min with 6 L water for every 1 kg of soybean. This prepared soymilk slurry was then used for ohmic heating.

### 2.2 Ohmic heating treatment to soymilk

Ohmic heat treatment to soymilk was given at three different voltage levels (160V, 180V, 200V) and different target temperatures (70 °C, 80 °C, 90 °C). The laboratory-scale ohmic heating apparatus comprises a square shaped acrylic chamber of 15×15 cm dimensions. Apparatus consisted of two stainless steel electrodes with 1 mm thickness, and the distance between them was maintained at 15 cm. The temperature of soymilk was measured by using an Optic fiber temperature sensor (Opticon optical sensors). The current was measured by ammeter (A) and the voltage using a digital voltmeter (V) fixed on the apparatus. The continuous temperature was measured at different points. After ohmic heat treatment, the slurry was filtered through a muslin cloth to separate Okara, and the soymilk was stored at refrigeration temperature for further analysis.

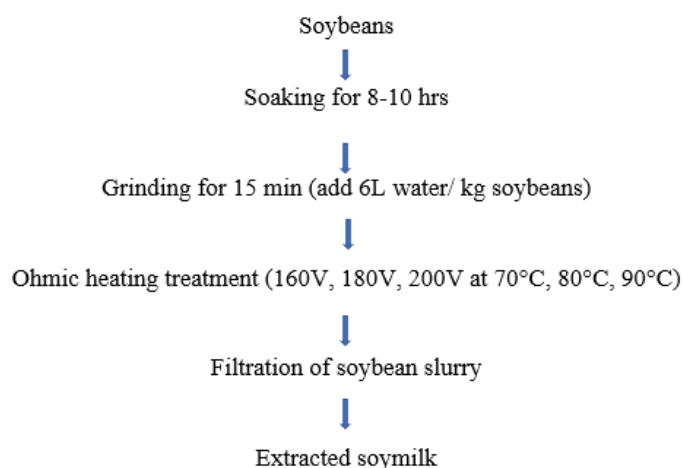


Fig 1: Flow chart for extraction of soymilk

### 2.3 Yield of soymilk

The extraction efficiency of ohmic heating was estimated by calculating the percent yield of soymilk. The yield was calculated by measuring the weight of the final soymilk obtained and the weight of soybean slurry produced after grinding of soaked soybeans using equation (1).

$$\% \text{ Yield} = \frac{\text{final weight of soymilk obtained}}{\text{weight of soybean slurry produced}} \times 100 \quad (1)$$

### 2.4 Protein characteristics of soymilk

Soybean products contain two major proteins, glycinin and  $\beta$ -conglycinin, namely 11S and 7S, respectively. Proteins are high molecular weight compounds made up of different amino acids linked together by a peptide chain. Protein characteristics of ohmic-treated soymilk such as total protein content, protein solubility and *In-vitro* protein digestibility were investigated.

#### 2.4.1 Total protein content

The crude protein content of ohmic heating treated soymilk was determined by the Kjeldahl apparatus (Abdulrazak *et al.*, 2014)<sup>[1]</sup>.

#### 2.4.2 Protein solubility

Protein solubility of ohmic heat-treated soymilk was estimated according to the procedure described by (Morr *et al.*, 1985)<sup>[12]</sup>. 25 ml of soymilk was taken, and its pH was adjusted to 4.5 with HCl (0.1 N). Then centrifugation was done at 12000 RPM for 20 minutes in the microcentrifuge. The collected supernatant was filtered through Whatman filter paper to remove suspended solid particles. Then the protein content of both whole soymilk and supernatant was estimated using equation (2).

$$\text{PS} (\%) = \frac{P_s}{P_{\text{SOY}}} \times 100 \quad (2)$$

Where  $P_s$  is protein content in collected supernatant,  $P_{soy}$  is the protein content in whole soymilk.

### 2.4.3 In-vitro protein digestibility (IVPD)

IVPD of soymilk was determined by the two-step pepsin-pancreatin enzymatic method described by (Caprita *et al.*, 2013) [3]. 3 ml of soymilk was added with 4 ml of phosphate buffer (pH 6.0, 0.1M) and 0.2 ml of HCl (2M). Then 1 ml of pepsin solution (3,000 NFU/mg, from porcine gastric mucosa) of 4% concentration was added. 0.5 ml of 0.5% chloramphenicol was added to avoid microbial growth. Then gastric digestion was initiated by incubating the whole mixture in the shaking incubator at 37 °C, 120 rpm, and 2 h. Then add 2 ml phosphate buffer of pH 6.8 (0.2 M) and 2 ml of 0.6N NaOH in solution. Also, add 2 ml pancreatin solution (2%) and again keep for incubation for 4 h with the same conditions.

Then centrifugation of this digested sample was done at 5000 rpm for 10 min to extract the supernatant. The protein content of both supernatant and undigested soymilk was estimated. Protein digestibility was calculated by the given equation (3).

$$\text{IVPD (\%)} = \frac{P_{\text{digested}}}{P_{\text{whole}}} \times 100 \quad (3)$$

Where  $P_{\text{digested}}$  is the protein content in the supernatant of digested soymilk, and  $P_{\text{whole}}$  is the protein content of whole undigested soymilk.

### 2.5 Estimation of hydroperoxides

Hydroperoxides are the intermediate components of lipoxygenase reaction on polyunsaturated fatty acids (PUFA), which produces beany flavour compounds. The peroxide value (PV) of the sample was determined using the procedure given by (Naik *et al.*, 2020) [14]. 5.0±0.02 g of soymilk was taken in 250 ml of conical flask.

Followed by add acetic acid: chloroform solution in 3:2 proportion. Then add 0.5 ml of freshly prepared potassium iodide, 30 ml distilled water and 0.5 ml of starch as an indicator. Keep the mixture in the dark for 1 min and then vigorously for a yellow color alteration of the final solution. Then titration was done with 0.5 M sodium thiosulphate up to the blue color get disappeared. Peroxide value was expressed in meq/kg of soymilk and calculated using equation (4).

$$\text{PV} = \frac{S \times M}{G} \times 100 \quad (4)$$

Where S is the volume of sodium thiosulphate (ml), M is the molarity of sodium thiosulphate, and G is the weight of soymilk (g).

### 2.6 Statistical analysis

Statistical analysis was completed by the analysis of variance (ANOVA) at 5% level of significance ( $p < 0.05$ ). The experimental data were analyzed by using Minitab 17. All the experiments were completed in triplicates, and their average values were taken for statistical analysis.

## 3. Results and discussion

Effect of ohmic heating on yield, total protein content, protein solubility, and soymilk digestibility have been investigated. Also, the effect of ohmic heating on beany flavor has been discussed.

### 3.1 Effect of ohmic heating on the yield of soymilk

The soymilk was extracted using the novel ohmic heating technology. The extraction efficiency of ohmic heating was investigated with different voltage and temperature. The yield of soymilk was significantly increased ( $p < 0.05$ ) with ohmic heating treatment. The maximum amount of yield (83.25±0.25%) was obtained at 200V and 90 °C temperature. The soymilk yield was minimum (75.75±0.35%) at 160V and 70°C, as depicted in Figure 2. The increase in extraction yield may be due to the leaching of solutes from okara. There might be more rupture of the plant cell matrix because of electric field strength. So, the electric field may have increased the membrane's diffusivity and permeability when an alternating electric current is passed through it. 'Electroporation effect' occurred during ohmic heating of soymilk causes pores formation on the cell membrane which increased the soymilk yield (Saberian Hamed *et al.*, 2018) [17].

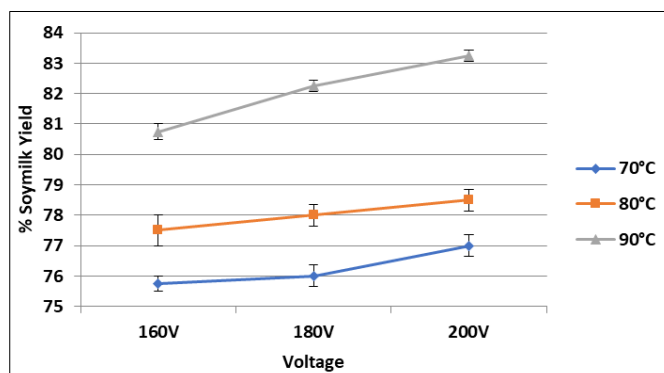


Fig 2: Effect of ohmic heating with different voltage and temperature on percent yield of soymilk.

### 3.2 Effect of ohmic heating on protein characteristics

Soymilk is the protein-rich plant-based milk substitute consumed globally. The protein characteristics of soymilk such as protein content, protein solubility and digestibility were investigated.

#### 3.2.1 Effect of ohmic heating on total protein content

The effect of different voltage and target temperature on protein content was investigated. Both the ohmic heating voltage and target temperature were found to be significant ( $p < 0.05$ ). The effect of ohmic heating on protein content is depicted in Table 1. The protein content of the ohmic heated soymilk sample was ranged between 5.08±0.017% to 8.14±0.08%. The highest protein content was found at 180V voltage and 80 °C temperature. Whereas lower protein content was found at 200V and 90 °C temperature. As the voltage increased from 160V to 180V, there was an increase in total protein content. This increase in protein content may occur due to plant matrix components' permeability and superheating of plant matrix water (Tsubaki *et al.*, 2009) [21]. When the voltage increased from 180V to 200V, protein content reduced to 5.08±0.0175%. This may happen due to the denaturation of proteins. Thermal protein denaturation occurs when hydrogen bonds and other non-covalent bonds get ruptured during thermal processing. Most of the protein denaturation reactions are irreversible as many structural changes occur in protein molecule causes impossible to retain its native state of the protein. These unfolded molecules of protein associate to form accretion of different irreversibly denatured protein molecules as these free molecules interact with other sites of proteins (Mulvihill & Donovan, 1987) [13].

### 3.2.2 Effect of ohmic heating on protein solubility

The effect of ohmic heating with varying voltage and temperature on protein solubility was studied. The voltage and temperature were found significant ( $p < 0.05$ ) to soymilk protein solubility. Protein solubility was found in the range of  $8.03 \pm 0.29\%$  to  $18.11 \pm 0.63\%$  depicted in Table 1. The maximum protein solubility was found at 160V and 70 °C target temperature, i.e., with minimum voltage and temperature. There is more loss of solubility at 90 °C and 200V.

**Table 1:** Effect of different voltage and temperature on protein characteristics of soymilk.

Run	Voltage (V)	Target temperature (°C)	Protein Content (%) <sup>a</sup>	Protein solubility (%) <sup>a</sup>	Protein digestibility (%) <sup>a</sup>
1	160	70	$7.09 \pm 0.087$	$18.11 \pm 0.63$	$66.26 \pm 0.40$
2	160	80	$7.44 \pm 0.174$	$12.94 \pm 0.54$	$67.07 \pm 1.18$
3	160	90	$5.78 \pm 0.088$	$9.84 \pm 0.04$	$72.41 \pm 1.08$
4	180	70	$7.35 \pm 0.087$	$17.05 \pm 0.38$	$67.84 \pm 0.36$
5	180	80	$8.14 \pm 0.086$	$11.64 \pm 0.41$	$68.99 \pm 0.51$
6	180	90	$5.95 \pm 0.174$	$8.32 \pm 0.24$	$74.26 \pm 0.02$
7	200	70	$6.74 \pm 0.175$	$16.01 \pm 0.22$	$69.91 \pm 0.25$
8	200	80	$6.48 \pm 0.176$	$10.34 \pm 0.62$	$70.29 \pm 0.99$
9	200	90	$5.08 \pm 0.017$	$8.03 \pm 0.29$	$75.55 \pm 0.55$

<sup>a</sup> values are means of triplicates  $\pm$  SD.

There is a significant difference in all the treatments as  $p < 0.05$ .

The principal factor responsible for the decrease in solubility at elevated temperature is protein denaturation. The increased temperature affects the bonds involved in stabilizing the secondary and tertiary structures of the protein. Also, at high temperatures, more hydrophobic amino acids may have produced causes reduction in protein solubility observed by several authors. There may be a decrease in solubility as the voltage increased may have increased the heating rate. So, a higher heating rate causes fast heating of soybean slurry increased denaturation of proteins resulting in decreased protein solubility (Varghese *et al.*, 2019) [24]; (Pelegrine & Gasparetto, 2005) [15].

### 3.2.3 Effect of ohmic heating on protein digestibility

The *In-vitro* digestion process investigated the effect of ohmic heating on protein digestibility. Protein digestibility indicates the number of proteins hydrolyzed and the absorption of peptides and amino acids. The target temperature and voltage were found significant to protein digestibility ( $p < 0.05$ ). Protein digestibility was found in a range of  $66.26 \pm 0.40\%$  to  $75.55 \pm 0.55\%$ . The highest digestibility was found at 200V and 90 °C temperature and lowest at 175V and 70°C temperature as depicted in Table 1.

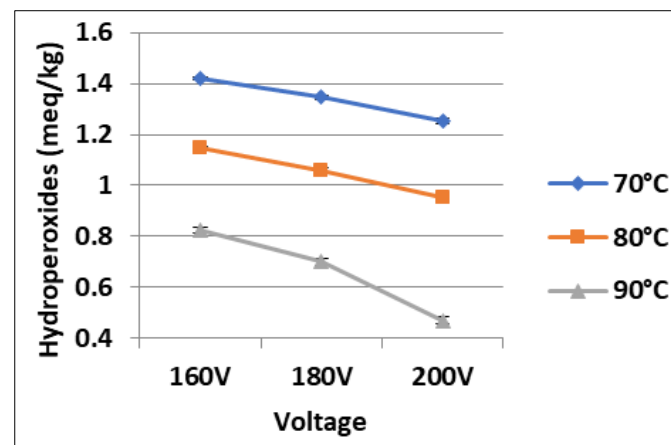
The digestibility of protein depends on external and internal factors. The external factors like temperature, pH, ion strength and internal factors like amino acid profile, protein crosslinking and folding (Joye, 2019) [5]. Protein digestibility was increased with increasing temperature and voltage level. This change is due to the structural rearrangement in protein molecules causes changes in the functional properties of the protein (Singh *et al.*, 2012) [18]. Changes in secondary protein structure cause an increase in protein digestibility (Vanga *et al.*, 2016) [23]. (Wallace *et al.*, 1971) [25] found the same trend of increase in protein digestibility with an increase in temperature and decrease in trypsin inhibitory activity (TIA). Trypsin inhibitors are proteinaceous in nature, and heat treatments can quickly inactivate them. So, as temperature

increased, there might be a decrease in trypsin inhibitory activity increasing protein digestibility (Joye, 2019) [5].

### 3.3 Effect of ohmic heating on beany flavor

The effect of ohmic heating with varying voltage and target temperature on beany flavor was studied. The voltage and target temperature were found significant to hydroperoxide reduction ( $p < 0.05$ ). Hydroperoxides are the intermediate components of lipoxygenase reaction on polyunsaturated fatty acids (PUFA) (Jiang *et al.*, 2016) [4]. Lipoxygenase is the principal enzyme responsible for beany flavor formation. When lipoxygenase acts on PUFA, such as linolenic and linoleic acids, oxidation of PUFA occurs, producing odourless hydroperoxides (Naik *et al.*, 2020) [14]. Later, degradation compounds of hydroperoxides such as 3-cis-hexenal, n-pentylfuran, hexanal and 2-(1-pentenyl) furan forms (Roland *et al.*, 2017) [16].

Hydroperoxides were found in the range of  $0.47 \pm 0.02$  meq/kg to  $1.42 \pm 0.01$  meq/kg. The highest amount of hydroperoxides ( $1.42 \pm 0.01$  meq/kg) were found at 160V and 70 °C, i.e., at the lowest voltage and target temperature. There was a linear decrease in hydroperoxides content with an increase in temperature, as shown in Figure 3. The same trend for decreasing hydroperoxides was observed by (Mizutani & Hashimoto, 2004) [11]. They found that hydroperoxides content at 80 °C was half of that 30 °C grinding temperature. Grinding of soybeans above 80 °C prevents off-flavor formation (Wilkens *et al.*, 1967) [26]. n-hexanal is the principle component formed after the degradation of hydroperoxides. The decrease in hydroperoxides may be due to the evaporation of off-flavour compounds in the air, or they might have combined with the denatured protein molecules. Soymilk processing at high temperatures is useful to reduce beany flavor, but high-temperature causes a decrease in nitrogen content (Mizutani & Hashimoto, 2004) [11].



**Fig 3:** Effect of different voltage and temperatures on hydro peroxides.

## 4. Conclusion

The present study with ohmic heating technology on soymilk was proved to be the better option for improving protein content, overall protein solubility, and digestibility and extraction yield of soymilk. 'Electroporation effect' creates holes in the cell which causes an increased diffusivity and permeability of the membrane when an alternating electric current is passed through it. There was a significant ( $p < 0.05$ ) increase in protein content and protein digestibility, and the highest amount was  $8.14 \pm 0.08\%$  and  $75.55 \pm 0.55\%$ , respectively. Increased voltage and temperature resulted in

decreased protein solubility as there may have structural changes in the protein. The principal factor responsible for the change in protein characteristics is the protein denaturation and antinutrients present in soymilk, such as trypsin, Bowman-Birk inhibitors, etc. A significant reduction ( $p < 0.05$ ) in hydroperoxides amount resulted in the reduction of the beany flavor of soymilk. Hence, the adopted ohmic heating technology was feasible and the better alternative for enhancing soymilk quality parameters.

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