IMPROVEMENT OF "WHOLE" SOYMILK QUALITY BY ENZIMATIC AID PROCESS

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ABSTRACT: Several hydrolytic enzymes - Celluclast® 1.5L, Pectinex® ultra sp, Rohalases® (SEP, F. 7069 e 7118) - were independently studied, regarding their effect on the sensory quality of the whole soymilk. The whole soymilk processing included the following operations: dehulling, boiling, draining, grinding, homogenisation, enzymatic treatment, and heat treatment. The sensory attributes bodiness, beany flavour, chalkiness, and flavour global impression were evaluated by a selected and trained sensory panel from the Embrapa Food Technology, using a 9-point non-structured linear scale.

The treatment with Celluclast 1.5L resulted in a considerable reduction of the particle size suspended in the soymilk. Such reduction originated products with low level of chalkiness, in comparison to the control, submitted to one homogenisation. The enzymatic treated soymilk resulted in higher global impression of flavour, followed by the products submitted to one homogenisation.

KEYWORDS: Soymilk; enzymatic treatment; chalkiness; sensory evaluation.

Introduction

Soybean has been regarded as an important protein source for Eastern people. However, the recent knowledge of soy nutritional and functional properties has considerably increased the interest and consumption of soy products also in Western World. The consumer is looking for more nutritious and healthy products, concerning with the consequences of his/her food choices and life styles may have in the health. Several studies have demonstrated the associated advantages of the use of soy products in preventing heart disease, osteoporosis, blood pressure regulation, and menopause symptoms release.

Regarding the nutritional quality of protein, Smith and Circle demonstrated the absence of limiting essential amino acids in soy taking into account that all elements are present in a higher level than protein standard. In the same study, soymilk protein resulted in a NPU (net protein utilisation) of 76, which was considered about the same quality level of cow milk, which presented a NPU of 79.

The composition of soymilk depends on different factors, such as the bean composition and parameters that depend on the type of process being used, such as the maceration time, the squeezing and dilution level during the process. According to Mello, soymilk produced by the traditional process presents the following composition, in average: 3.4% of protein, 1.8% of lipids, 1.5% of carbohydrate and 0.4% of ash.

There are several different methods for soymilk processing. The traditional method involves soaking the beans, followed by wet grinding (cold water grinding), filtering and cooking. The traditional process results in a typical oriental soymilk with an intense characteristic of "beany" flavour, which is not generally accepted by the westerns consumers. Such off-flavour characteristic results from the reaction catalysed by soybean enzyme lypoxigenase that acts almost immediately upon wet grinding.

The use of hot water (80-100°C) to inactivate the lypoxigenase either previously or during the grinding is the basis of other methods of the soymilk processing. The referred methods avoid the reaction of the enzyme and the formation of the attribute "beany" flavour. Some of those methods involve, like the traditional one, the grinding of the beans and the discharge of the solid residue retained in the filtration.

In other methods the bean is ground with hot water and the remaining slurry is not filtered nor centrifuged. These
methods have the advantage of avoiding generating solid residue. In one of these methods the slurry is homogenised in two stages. One negative aspect of the methods that use the whole grain to produce soymilk, without generating solid residue by filtration or centrifugation, is the presence of another flavour attribute in the product named "chalkiness". The resulting products are also, sometimes, physically unstable, i.e., phase separation occurs shortly during the product storage, depending on the pressure of homogenisation.

The use of enzyme to degrade soybean cell wall components has been used mainly with different aims of extracting soybean components. Such use is mainly aimed at decreasing or replacing the use of organic solvents during the oil extraction. The use of cell wall degrading enzymes could also be potentially applied to produce other soybean products. The aim of this study was to evaluate the use of enzymes to improve the sensory characteristics and physical stability of "whole" soymilk by reducing the soymilk particle size.

Materials and methods

Raw material: Soybean variety BR-16 specially developed for human feeding by EMBRAPA Soybean (Brazilian Corporation for Agricultural Research - Soybean Research Centre) was used in the study.

Soymilk processing: the following steps were carried out for soymilk processing, adapted from Nelson et al.:

- Dehulling: Soybeans were dehulled after maintaining the grain at 100°C for 5 min., to facilitate husk removal.
- Blanching: carried out by heat treating the beans in boiling sodium carbonate solution (0.25%) for 30 min (1:3: dehulled beans: solution v/v)
- Grinding: blanched and drained grains were ground together with boiled water in a warring blender (15,000 rpm for 2 min.) in order to result in a suspension with about 6 % of total solids;
- Homogenisation: The suspension originated from the milling operation was homogenised in an APV Gaulin homogeniser at a pressure level of 351.5 kgf/cm² (60°C). The material enzymatically treated was singly double homogenised;
- Enzymatic treatment: carried out in the homogenised sample (single run), using enzyme concentration of 1.2% (v/v) and 3h of enzymatic treatment. The enzymes evaluated in the study were:

1. Celluclast® 1.5L: Novo Nordisk, EC-No. 3.2.1.4, having a declared activity of 5.1 unit/mg of solids, determined at pH 5.0 and 37°C. Cellulase activity unit is the enzymatic activity that can release 1µ-mol of D-glucose from cellulose per hour;
2. Pectinex® ultra sp: Novo Nordisk, EC-No. 3.2.1.15, having a declared activity of 26,000 galacturonic acid/ml, measured at pH 3.5 and 20°C;
3. Rohalase® SEP: Rohm Enzymes, EC-No. 3.2.1.8, an enzyme which contains xylanase and β-glucanase, having a declared minimum activity of 225.000 BXU/g and 255.000 BU/g;
4. Rohalase® F: Rohm Enzymes, EC-No. 3.1.1.5, an enzyme which contains lyso-phospholipase, having a minimum activity of 2000 PLU/g;
5. Rohalase® 7069: Rohm Enzymes, EC-No. 3.2.1.4, an enzymes which contains cellulase, having a minimum activity of 15.000 ECU/g;
6. Rohalase® 7118: Rohm Enzymes, EC-No. 3.2.1.32, an enzyme which contains xylanase.
- Supplementary heat treatment: carried out in the homogenised soymilk (either enzymatic treated or not) by enmeshing the soymilk container in boiling water for 5 min.

Proximate composition analyses (moisture, protein, oil, ash and total solids) of whole soymilk were determined by standard methods of AOAC. The particle size in the soymilk was determined using a laser particle analyser (Fritsch GmbH model "analysette 22").

The Sensory evaluation was carried out using the evaluation of the attributes by a selected and trained 8-people sensory panel. The attributes bodiness, beany flavour, chalkiness, and flavour global impression were evaluated using a 9-point non-structured scale, with three determinations for each sample, following a balanced design. Samples were coded with three digit numbers and offered to participants monadically in 50-ml plastic cups, at room temperature. All sensory tests were conducted in individual sensory booths at EMBRAPA Food Technology, using the red light to mask the appearance of samples. Chalkiness and beany flavour were anchored and labelled as 1 = weak, and 9 = strong. Bodiness scale ranged from 1 = a few (thin) to 9 = too much (thick). Flavour global Impression rated from 1 = very poor to 9 = very good. The subject's task was to make a vertical line across the scale at the point that best described the intensity of the attribute perceived for each soymilk sample. Mineral water at room temperature and unsalted cream cracker were provided to rinse the mouth between samples.

The suspension stability was determined according to Priepke et al. by checking the occurrence of phase separation in a portion of the beverage after 5 days of quiescent storage. If a demarcation line between upper and lower portion of the beverages contained in a flask was observed, its height was measured and calculated as a ratio to the total height of the beverage.

Analysis of variance (ANOVA) was used to verify the existence of significant differences among the data, and Tukey test to check the differences among the values (means) at the 5% level of significance.

Results and discussion

Proximate composition of whole soymilk is shown in Table 1.
Table 1 - Proximate composition of whole soymilk.

<table>
<thead>
<tr>
<th>Component</th>
<th>Whole Soymilk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>6.19</td>
</tr>
<tr>
<td>Protein</td>
<td>2.86</td>
</tr>
<tr>
<td>Fat</td>
<td>1.53</td>
</tr>
<tr>
<td>Ash</td>
<td>0.27</td>
</tr>
<tr>
<td>Moisture</td>
<td>93.81</td>
</tr>
<tr>
<td>Carbohydrate*</td>
<td>1.53</td>
</tr>
</tbody>
</table>

*Calculated as the difference to 100%. *Mean of three replications.

The effect of different enzymatic treatments on the mean diameter of soymilk particles is shown in Table 2. The particle size distribution in the soymilk treated with different enzymes is shown in Figure 1. The higher effectiveness of Celluclast 1.5 L resulted in a higher proportion of lower size particles and, as a consequence, in a lower mean particle diameter, in comparison to the other enzymes used in this study. The enzymes Pectinex Ultra sp, Rohalases SEP, Rohalases F, and Rohalases 7118 had a mean particle diameter significantly higher than the Celluclast (p<0.05), revealing their inappropriateness to be used in further evaluations. No significant difference (p>0.05) was found between the control (no enzymes) and the soymilk added of Rohalases F and 7118. Those two enzymes presented the worst performance considering the particle diameter in this study. The higher efficiency of Celluclast acting on the soymilk was also demonstrated in other studies, which aimed, either at extracting oil and protein from soybean in an aqueous medium\cite{11,12} or to assist soybean oil extraction with hexane.\cite{2} Although there was no mean particle diameter difference (p<0.05) between the soybean milk added of Celluclast and Rohalases 7069 (Table 2), one can see from Figure 1 higher frequency of small particle size for the Celluclast than for Rohalases 7069. As a consequence, further analyses in this study focused only on Celluclast.

The effect of Celluclast concentration on the mean particle diameter and suspension stability, can be observed in Table 3. The decreasing of the mean particle size and the increasing of the index of stability with the increase of Celluclast concentration is evident from Table 3. However, Celluclast concentration higher than 0.8% (v/v) didn’t result in significant alteration (p <0.05) of the mean diameter particle in the suspension neither on the stability index. Figure 2 presents the particle size distribution profile with the enzymatic concentration variation. The enzyme concentration increasing clearly contributed to the increase the lower mean diameter particle frequency, and decreased the frequency on the higher mean diameters particles.

The effect of the enzymatic reaction time (Celluclast 1.5L) on soymilk particle size distribution profile is shown in Figure 3. It is clear the reduction on the higher particle size frequency and the increase on the lower one with reaction time. No further significant reduction was verified after three hours reaction time, as it can be seen from Table 4. Similarly, a significant increase in the stability index happened in the first two hours of reaction, without any further significant change in the index.

Results of the soymilk sensory evaluation resulted from the different enzyme concentration treatments are shown in Figure 4. The results of sensory analysis demonstrated that the use of the enzyme affected significantly (p<0.05) all the evaluated flavour attributes. There was an evident reduction on the chalkiness and the beany flavour sensory attribute scores, as the enzyme concentration increased. However, no significant changes happened by increasing enzyme concentration above 1.2%. On the other hand, the attributes bodiness and global quality reached the highest values with the increasing of the enzyme concentration, up to the level of 1.2%. In spite of the mean particle diameter has not suffered further reductions above the enzyme concentration of 0.8%, significant differences in flavour attributes were still verified up to the 1.2% of enzyme concentration, resulting in a product with better sensory characteristics.

Table 2 - Effect of different enzymes on the soymilk mean particle diameters (enzyme concentration = 1.2%)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Mean particle diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No enzyme)</td>
<td>58.74 ef</td>
</tr>
<tr>
<td>Celluclast 1.5L</td>
<td>42.95 a</td>
</tr>
<tr>
<td>Rohalases 7069</td>
<td>46.45 ab</td>
</tr>
<tr>
<td>Pectinex Ultra sp</td>
<td>49.23 bc</td>
</tr>
<tr>
<td>Rohalases SEP</td>
<td>51.71 cd</td>
</tr>
<tr>
<td>Rohalases F</td>
<td>57.53 f</td>
</tr>
<tr>
<td>Rohalases 7118</td>
<td>58.33 f</td>
</tr>
</tbody>
</table>

Means with different letters in the same column present significant differences (p<0.05).
FIGURE 1 - Effect of several enzymes (at 1.2% v/v and 3h treatment) on the particle size distribution of soymilk.

<table>
<thead>
<tr>
<th>Enzyme concentration (% v/v)</th>
<th>Mean particle diameter (µm)</th>
<th>Suspension stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>58.90 c</td>
<td>0.69 a</td>
</tr>
<tr>
<td>0.2</td>
<td>48.86 b</td>
<td>0.68 a</td>
</tr>
<tr>
<td>0.4</td>
<td>48.41 b</td>
<td>0.69 a</td>
</tr>
<tr>
<td>0.8</td>
<td>42.26 a</td>
<td>1.00 b</td>
</tr>
<tr>
<td>1.0</td>
<td>42.81 a</td>
<td>1.00 b</td>
</tr>
<tr>
<td>1.2</td>
<td>41.89 a</td>
<td>0.99 b</td>
</tr>
<tr>
<td>1.6</td>
<td>42.49 a</td>
<td>1.00 b</td>
</tr>
</tbody>
</table>

Means with different letters in the same column present significant differences (p<0.05).

FIGURE 2 - Effect of several concentration (0 to 1.6%) of Celluclast 1.5L on the particle size distribution of 1X homogenised soymilk.
Table 4 - Effect of treatment time with Celluclast 1.5L (enzyme concentration - 1.2% (v/v)) on the mean particle diameter and stability index of 1X homogenised soymilk

<table>
<thead>
<tr>
<th>Time</th>
<th>Mean particle diameter (µm)</th>
<th>Suspension stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0h</td>
<td>59.35 a</td>
<td>0.67a</td>
</tr>
<tr>
<td>1h</td>
<td>47.85 b</td>
<td>0.85 b</td>
</tr>
<tr>
<td>2h</td>
<td>46.38 b</td>
<td>0.98 c</td>
</tr>
<tr>
<td>3h</td>
<td>42.95 c</td>
<td>1.00 c</td>
</tr>
<tr>
<td>4h</td>
<td>40.41 c</td>
<td>1.00 c</td>
</tr>
<tr>
<td>5h</td>
<td>40.24 c</td>
<td>0.99 c</td>
</tr>
</tbody>
</table>

Means with different letters in the same column present significant differences (p<0.05)

FIGURE 3 - Effect of treatment time with Celluclast 1.5L (enzyme concentration=1.2%) on the particle size distribution of 1X homogenised soymilk.

FIGURE 4 - Sensory evaluation of the treated soymilks with different enzyme concentrations.
Conclusions

Enzymatic treatment with Celluclast 1.5L has shown to be efficient in the reduction of particle mean size and in the whole soymilk stability, as well as in the improvement of the sensory characteristics of the product. Increase in the enzyme concentration levels and enzymatic reaction time reduced the mean particle size and increased the product stability up to level of 1.2% (v/v), and 3 hr, respectively.

Besides the reduction on the sensory attribute chalkiness, enzymatic treatment also reduced beany flavour and resulted in a higher global quality soymilk. It is possible to conclude that the enzymatic process can be applied with success in the development of whole soymilk with better physical and sensory properties.

References


