THE INFLUENCE OF SOYBEAN FIBERS IN THE BISCUITS PROCESSING TECHNOLOGY - THE PRELEVATION OF AMINOACIDS IN BISCUITS BY THIN LAYER CROMATOGRAFHY

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Abstract: Due to it’s high, best quality protein content, soybean is one of the few sources of vegetal origin that provides all eight essential amino-acids, being an important source of folic acid, vitamins B3, B6, E, magnesium, potassium, iron, copper and phosphorus. It contains quality lipids as well (polinsaturated fatty acids) and it causes a drop of the “bad” cholesterol level from the body (LDL-cholesterol decreases with 12.9%). Good cholesterol level increases on an average with 9% and the triglycerid concentration from the blood drops, with approximately 10.5%. [2] The present paper points out the influences soybean fiber enrichment has on biscuits in the technological processing and also on the qualitative content of amino-acids. To achieve this, thin layer chromatography was used from biscuits samples with different soybean fiber enrichments and a witness sample of soybean fiber. Another studied aspect was the influence of extraction solvents (ethyl alcohol, isopropyl alcohol, ammonium hydroxide) upon the amino-acids spots resolution. The experiment ascertained that the rheological properties of the soybean fiber enriched dough were improved, the amino acid quantity grew exponentially with the growth of fiber content in the biscuits and the most efficient solvent was isopropyl alcohol.

INTRODUCTION

Although it is a rather simple plant, soybean is quite valuable, more so since it has earned strong appraisals from the western world. Upheld by enthusiasts, soybean managed to overcome the borders of Asia, its original cultivation ground (being more widespread than potatoes). Soybean conquered the United States of America, where it is grown on very large surfaces.

Due to their unique functional properties, soybean based products have become increasingly attractive ingredients for the majority of the food supply chains. The use of soybean protein as a raw material, supplement or as an additive, has extended to the processing of food products [3]. These ingredients fulfill one of the following roles: they contribute to water and fat absorption, extend the validity period of the product, whiten...
the product’s core and improve the nutritional value by increased quality protein and essential amino acid content.

Starting from these premises, the implications of soybean fiber enrichment on the technological fabrication process of biscuits and also on the quality content of amino acids were more elaborately studied.

The use of thin layer chromatography tried to accomplish the following:
- a quality oriented assay of the content represented by free amino acids;
- drawing up a comparative study between the three solvents used in the determination.

MATERIAL AND METHOD

Used materials:
- in the biscuit processing: white wheaten flour with different characteristics (hydration capacity 60.8%, wet gluten 26.6%, deformation index 7 mm, humidity 14%), soybean fiber, sugar, butter, cream, eggs, flavors, chemical dispersers, salt;
- in the chromatographic determinations: samples to analyze, stationary phase (chromatographic sheets, adsorbent); liquid phase (developing material- mixture of solvents), ultrasound bath, centrifuge, extraction solvents (ethylic alcohol, isopropyl alcohol, ammonium hydroxide), chromatographic tank, capillaries.

The flour was analyzed in conformity with ISO 90-88. Water absorption capacity has been determined using the dough ball method [1]. Humidity was determined on flour and on bread using the same method: mass loss by heating at 130±2°C.

For the soybean fiber the amino acids were qualitatively determined through CSS [5].

For the biscuit processing the direct method for dough preparation was used. For the biscuits the total sugar (Schoorl method), fats (Soxhlet method), humidity, amino acids through CSS with the following characteristics were determined:

S0 – soybean fiber;
W – biscuits sample without soybean fiber (witness);
S1 – biscuits sample with 6% soybean fiber;
S2 – biscuits sample with 12% soybean fiber;

The steps taken for sample preparation were:
- weighing 1 g from the S0, W, S1, S2 samples;
- amino-acids extraction (Fig 1.)

Fig.1. Amino-acids extraction
- deposition and distribution of the covering substance in 3 parts which were treated as follows:
  a) the concentrated ethyllic alcohol sample was studied without being subjected to a thermic procedure;
  b) a part of the covering substance was evaporated completely and dissolved in isopropyl alcohol;
  c) a third part of the covering substance was evaporated completely and dissolved in ammonium hydroxide.

The obtained samples were used in amino-acid assay through CSS.

The basis of thin layer chromatography is obtaining an adsorbent layer (silica gel in the present case). At about 1.5 cm from one of the sheet’s ends, one has to apply a really small quantity of the mixture of substances that are to be separated. The chromatographic sheet is inserted in a chromatographic tank, in which the atmosphere has been saturated by the vapors of the mixture of solvents used for the separation.

The separation takes place (adsorption – desorption), in which the mixture of solvents, the so-called developant, is engaging the spotted samples and moves them through the adsorbant particles. In different areas of the adsorbant, the mixture’s substances that compose the samples are adsorbed depending on the nature of the substances, on the affinity for the liquid or stationary phase.

The presence of the separated substances is shown by treating the chromatography sheets with ninhydrin. The identification of the amino acids was comparatively accomplished with standard amino acid chromatograms.

RESULTS AND DISCUSSIONS

The results of the determinations upon the samples of final products are shown in Table 1, which indicates that soybean fibre enrichment leads to fat content increase, due to lecithin supply of the fibre and sugar content decrease.

<table>
<thead>
<tr>
<th>Analyzed parameters</th>
<th>U.M.</th>
<th>Samples</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>W (witness)</td>
</tr>
<tr>
<td>Total sugar</td>
<td>%</td>
<td>20.94</td>
</tr>
<tr>
<td>Fats</td>
<td>%</td>
<td>13.64</td>
</tr>
<tr>
<td>Humidity</td>
<td>%</td>
<td>9.85</td>
</tr>
</tbody>
</table>

The humidity of the biscuits increased from 9.85% at the witness sample, to 11.56% at the sample with 12% soybean fiber supply.

During the technological process, the soybean fibre absorbs water forming a bonded, consistent dough, which assures a high saving regarding the use of eggs [4], a longer shelf life of the products (through restraining the development of propitious multiplying medium for different microorganisms) and implicitly an extension of the validity period.

The soybean fiber enrichment assured a prolongation of freshness and preservation period of the biscuits, ameliorated the structure and elasticity of the core,
and improved the color of the products (due to the sugars it contains) and gustatory qualities, giving them a specific flavor.

Through soybean fibre enrichment the capacity of water absorption increased, so that the water elimination in the baking phase was slower, extending the baking period of the biscuits. So the conclusion would be that the technological parameters should be correlated with the quantity of the fibre added to the product.

From technological point of view, biscuit fabrication with soybean fibre enrichment has the following implications: a higher level of compatibility between the dough and processing machinery; improvement of the rheological properties of the dough; growth of hydration capacity due to the high water absorption ability of the fibers; exponential increase of dough elasticity with the use of soybean fiber; decrease of kneading period and increase of baking duration of the product.

The results of the amino-acids assay using TLC (Thin Layer Chromatography) are shown in the figures below:

![Fig.2. Spots in the concentrated ethyl alcohol sample compared with Standard amino-acid spots](image)

![Fig.3. Sample dissolved in isopropyl alcohol compared with amino-acid standards](image)
By analyzing the chromatograms the following conclusions can be drawn:

a) the timeframe of the spot’s development is shorter by using ethyl alcohol with 96% concentration and the remanence of the spots is diminished due to the high volatility. The visibility of the spots is poor. Due to the fact that the sample was not thoroughly evaporated, it was too diluted, which resulted in a poor visualisation of the results.

b) by using isopropyl alcohol the visibility and remanence of the spots has increased. This solvent adds clarity to the obtained results.

c) by the use of ammonium hydroxide the visibility is improved but as disadvantage, the sheet is colored in a yellowish shade, which causes the blurring of the spots color and uncertain determination of results.

CONCLUSIONS

Proteins are essential for the evolution of the organism and also for the tissue healing process.

Even if the quantity of proteins is large enough, their nutritional value might be restricted due to the absence of a certain essential amino acid in the structure of the protein. In the case of the soybean fibre that amino acid is methionine.

The conclusions drawn from the experiments are the following:

• addition of soybean fiber in proportion of 12% increases the fat content with 5.47%;
• the humidity of the final product grows with the soybean fibre content with 1.71%;
• distinguishing the free amino-acid (some of them essential): aspartic acid, glycine, leucine, glutamic acid in all samples;
• the best solvent is isopropyl alcohol;
• free amino-acids grow exponentially with the quantity of soybean fibers in the final product.

Research will be continued through the quantified assay of amino-acids from the samples which were treated in the present paper.
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