JAP&T
TPA-Timişoara

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Journal of Agroalimentary Processes and Technologies 2009, *15*(3), 449-451

Journal of Agroalimentary Processes and Technologies

Determination of the quality parameters in order to validate the method of determining the protein from the vegetable matrix

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Abstract
The determination of crude protein content of vegetable matrix was achieved by Kjeldahl, direct method. To validate this method has worked in terms of repeatability and reproducibility and were calculated the uncertainty type A and B. Expanded Uncertainty for k=2 and P=95% was U=0,29.

Keywords: protein, quality parameters, repeatability, reproducibility

1. Introduction

Proteins are macromolecular organic substances composed of simple or complex chains of amino acids. They are present in all cells of living organisms in the proportion of over 50% of dry matter. All proteins are polymers of amino acids, in which the sequence is coded by a gene. Each protein has its unique sequence of amino acids, determined by the nucleotidic sequence of the gene.

The proteins enter in the composition of all cells, its take part in the formation of some yeast and intervene in developing all vital processes from the body: enters in the structure of many hormones; participates in the formation of antibodies with the role of defenders of the body against microbes and their toxins; enters in the chemical combination with the toxic chemicals, transforming them into non-toxic substance; fulfilling the carrier functions and forming different complexes (proteino-lipidic, proteino-glucidic, proteino-mineral, proteino-vitaminic, proteino-hidric and with some medications), it participates in maintaining osmotic balance, at the division of water and dissolved substances in it in different parts of the body.

The validation of a method by definition, is the process of determining the performance characteristics and the limits of a method and identifying the influences that may change these characteristics and how much.

2. Materials and Method

Vegetable matrix for that were made the determinations, was made of 6 samples of soybeans *(Glycine hispida L.*) and a sample witness.

The samples were analyzed in terms of repeatability and reproducibility, with a protein determination equipment for Kjeldhal method, that has a Selecta digest bloc for the mineralization and a Selecta Pro-nitro I for the distillation.

To determine crude protein was used Kjeldahl direct method [1]. The coefficient for total conversion of nitrogen in protein is 5.7.

Mineralization was achieved in the presence of concentrated sulfuric acid and of catalysts of potassium sulphate and copper.

Working and mineralization parameters: *Step 1*: 125°C, 15 minutes; *Step 2*: 300°C, 15 minutes; *Step 3*: 400°C, 90 minutes.

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The release of total nitrogen, during the distillation was realized in strong alkaline environment and the capture was made in the solution of 4% boric acid.

The titration phase was performed with 0.1n hydrochloric acid until the color of Tashiro indicator turns from green to gray with blue tint.

3. Results and Discussion

During the first day of analysing 8 samples of soybean, the obtained values of protein were between 32,6% and 32,8% (Table 1):

Table 1. The analysis in the first day

Soybean Samples	Sample Mass (g)	Total Protein (%)
1	1.506	32.831
2	1.507	32.688
3	1.505	32.762
4	1.500	32.780
5	1.504	32.754
6	1.506	32.688
7	1,506	32,820
8	1,503	32,800
M	0	0

Table 2. The analysis in the second day

Soybean Samples	Sample Mass (g)	Total Protein (%)
1	1.502	32.918
2	1.505	32.852
3	1.504	32.875
4	1.504	32.814
5	1.503	32.897
6	1.501	32,815
7	1,504	32,864
8	1,502	32,890
M	0	0

In the second day of analysing the 8 samples, were registrated a content of crude protein between 32,8 and 32,9% (Table 2):

a) The type A uncertainty

Average: $\bar{x}$ = 32.812%

Standard deviation: S = 0,00466

Calculation of relative uncertainty of type A: $u_{rA}^2 = 1.96 \times 10^{-10}$

b) The uncertainty of type B

b_1) Analytical balance

Expanded Uncertainty:

U=0,22x10^{-3}g for k=2, P=95%.

Calculation of total uncertainty:

$$u_{cB1} = 0{,}155 \times 10^{-3} g$$

Calculation of relative uncertainty:

$$u_{rB1} = 0.103 \times 10^{-3};$$

$$u_{rB1}^2 = 1{,}06 \times 10^{-9}$$

b_2) pipeta

Expanded Uncertainty:

U=0,25x10^{-2} ml for k=2, P=95%.

Calculation of total uncertainty:

$$u_{cB2} = 0{,}125 \times 10^{-1} ml$$

Calculation of relative uncertainty:

$$u_{rB2} = 0{,}5 \times 10^{-3},$$

$$u_{rB2}^2 = 2{,}5 \times 10^{-9}$$

b_3) graduated cylinder

Expanded Uncertainty:

U=0,5ml for k=2, P=95%.

Calculation of total uncertainty:

$$u_{cB3} = 0{,}25 ml$$

Calculation of relative uncertainty:

$$u_{rB3} = 5 \times 10^{-3};$$

$$u_{rB3}^2 = 2{,}5 \times 10^{-5}$$

b_4) burette

Expanded Uncertainty:

U=0,024 ml for k=2, P=95%.

Calculation of total uncertainty:

$$u_{cB4} = 0{,}012 ml$$

Calculation of relative uncertainty:

$$u_{rB}^2 = 2.435 \times 10^{-7}$$

Combined Uncertainty:

$$u_{c(y)} = 0{,}146$$

Expanded uncertainty for k=2, P=95%

$$U = k \times u_{c(y)} = 2 \times 0{,}146 = 0{,}29$$

Results:

$$Y = 32.812 \pm 0{,}29 \text{ (g \%)}$$

4. Conclusion

We managed to achieve in terms of repeatability and reproductibility of total protein using the direct method of determination. The average of the protein from soybean is 32,812%.

We performed the calculation of uncertainty type A and B and of expanded uncertainty for soya beans:

- The type A uncertainty:

$$u_{rA}^2 = 1.96 \times 10^{-10}$$

- The uncertainty of type B:

$$u_{rB}^2 = 2.435 \times 10^{-7}$$

- Expanded Uncertainty for k=2 and P=95%: U = 0.29

It is necessary to determine the recovery rate, to quantify the losses induced by sample preparation.

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