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# Nutritional analyses for proteins and amino acids in beans (*Phaseolus* sp.)

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The chemical index is a good estimator of seed protein quality of *Phaseolus* beans. In order to estimate this value, a protein hydrolysis and amino acid quantification are realised. The problems inherent to these techniques are presented.
**Keywords.** *Phaseolus*, amino acid, hydrolysis, chemical index.

**Analyses nutritionnelles des protéines et des acides aminés chez le haricot (*Phaseolus* sp.).** L'index chimique est un bon paramètre d'estimation de la qualité protéique des graines chez les haricots. Pour estimer cette valeur, une hydrolyse des protéines suivie d'une quantification des acides aminés est effectuée. Les problèmes inhérents à ces techniques sont également présentés.
**Mots-clés.** *Phaseolus*, acide aminé, hydrolyse, index chimique.

## 1. INTRODUCTION

Protein analysis is of great importance in the nutritive value determination of food. In this context, crude protein and chemical index determination, with the essential amino acid composition, give a good estimation of protein quality. To determine the chemical index, proteins are first hydrolysed in amino acids and then these amino acids are analysed. Nonetheless, several problems are linked to these operations.

## 2. DRY MATTER AND CRUDE PROTEIN DETERMINATION

In order to compare amino acid data from one laboratory to another, results have to be expressed on the basis of either a dry matter percentage or a protein content. Dehydration is often made on the flour in a oven 24 h at 105 °C. Nitrogen is quantified by the accurate Kjeldahl method and an estimation of the crude protein content is calculated multiplying the nitrogen content by an approximate factor of 6.25.

## 3. HYDROLYSIS OF PROTEINS AND AMINO ACID QUANTIFICATION

The analysis of amino acids present several problems, i.e. standardization and control of the protein hydrolysis steps and selection of the most accurate amino acid analysis method.

### 3.1. Hydrolysis of proteins

In order to evaluate the quality of proteins, a hydrolysis method which does not destroy the essential amino acids is needed. The classical acid hydrolysis (HCl 6N) destroys completely tryptophan and partially cystine, and somewhat methionine. For sulfur amino acids quantification, the sample must be oxidized before acid hydrolysis. For tryptophan, alkaline hydrolysis is a good method. None of these methods give alone the entire amino acids composition with precision. For example, if the sample is oxidized before acid hydrolysis step, tyrosine content estimation will not be accurate (Bech-Andersen *et al.*, 1990).

For all these methods, different parameters must be standardised to obtain comparable results: flour particle size, ratio of the sample/hydrolysis mixture or the reagent (for oxidation), additives to HCl solution, nitrogen atmosphere of the reaction vial, hydrolysis time and temperature (classical oven or microwave eating), and treatment of hydrolysate before injection (evaporation or adjustment of pH).

The flour particle size can influence the results. A size smaller than 0.6 mm is generally prepared in order to avoid heterogeneity and incomplete hydrolysis.

A too high ratio of sample/hydrolysis mixture can lead to an incomplete hydrolysis and an underestimation of some amino acid contents. In the oxidation procedure, a minimum ratio of sample:oxidation mixture must also be respected in order to recover all

cysteic acid and methionine sulfone. In **table 1**, the methionine content is underestimated when the nitrogen content of the sample is significantly above 10 mg.

Additives are useful to avoid degradation of some amino acids. An additive such as thioglycolic acid is readily oxidized and minimizes degradation of tyrosine, methionine, threonine and serine. Phenol which is readily halogenated, serves as a scavenger to prevent degradation of tyrosine (in 3-chlorotyrosine), threonine and serine (Pickering, Newton, 1990). Higher losses of threonine and serine (up to 80%) were observed by Weiss *et al.* (1998) in absence of phenol.

A nitrogen atmosphere avoids oxidation and degradation of amino acids which is important for the estimation of sulfur amino acid contents in acid hydrolysis without previous oxidation (**Table 2**).

When the hydrolysis time increases, threonine and serine contents decrease, and hydrophobic amino acids (isoleucine, leucine, valine, etc.) increase. A 24 h hydrolysis treatment destroys about 5% threonine and 10% serine. Peptide bonds involving Leucine-Leucine, Isoleucine-Leucine, Leucine-Isoleucine are difficult to hydrolyse. An hydrolysis time between 48 h and 72 h is necessary to approach the real value of these hydrophobic amino acids (**Table 3**). For all these amino acids, extrapolation with different hydrolysis time is necessary to obtain the real values. But this is time and money consuming. Hydrolysis temperature is also critical but the general temperature adopted in oven is 110 °C. Hydrolysis with microwave technology seems to give an underestimation of threonine, serine, isoleucine and leucine (Weiss *et al.*, 1998; Chen *et al.*, 1987).

After hydrolysis, the hydrolysate is evaporated by heating under vacuum but sometimes loosing sensible amino acids. Otherwise, the pH of the sample is adjusted by a NaOH solution. This method is recommended by the European Community (EC, 1998) and seems adequate if sufficient raw matter is available.

This method dedicated to the quantification of all the amino acids except tryptophan presents the following main characteristics:

– the oxidation of sulfur amino acids before acid hydrolysis is made with a hydrogen peroxide/formic acid/phenol solution for 16 h at 0 °C. The excess of reagent is destroyed by a sodium disulfite solution.

– The acid hydrolysis step is carried out with the utilization of HCl 6N. The open flask is heated during 1 h at 110 °C; after it is closed and heated during 23 h at the same temperature. For analysis by ion exchange chromatography, the pH of the hydrolysate is adjusted to pH 2.2 with NaOH and aminohexanoic acid (norleucine) added as an internal standard.

For tryptophan determination in protein hydrolysates different techniques are available using alkali. However none of the alkaline hydrolysis procedures recover 100% tryptophan. The barium hydroxide digestion is now associated with HPLC especially with fluorescence detection (Molnár-Perl, 1997). The latter technique is recommended by the European Union (Fontaine *et al.*, 1998).

**Table 1.** Variation of the methionine content in relation to the nitrogen value of the sample (*Parkia biglobosa* L.) — *Variation de la teneur en méthionine en fonction de la concentration en azote de l'échantillon (*Parkia biglobosa *L.)*.

| **N in sample** | | | |
|---|---|---|---|
| (mg) | 7.9 | 11.96 | 16.14 |
| **Methionine** | | | |
| (g/100g DM) (**1**) | 0.562 | 0.560 | 0.385 |

(**1**) DM = Dry Matter.

**Table 2.** Influence of the hydrolysis flask atmosphere and of the hydrolysate post-treatment on the estimation of amino acid contents of a commercial bean princess Pieterpikzonen ref. 3668 — *Influence de l'atmosphère du flacon d'hydrolyse et du traitement de l'hydrolysat sur l'estimation de la teneur en acides aminés d'une variété commerciale de haricot princesse Pieterpikzonen ref. 3668.*

| | **Treatments** | | | |
|---|---|---|---|---|
| **%** | **a**(**1**) | **b** | **c** | **d** |
| **Cys-Cys** | 0,85 | 0,71 | 0,70 | 0,92 |
| **Met** | 1,00 | 0,28 | 0,63 | 0,97 |

(**1**) Hydrolysis with [a, d] or without [b, c] nitrogen. Hydrolysis 24 h closed flask [a, b, d] or 1 h open flask and 23 h closed flask [c]. Hydrolysate adjusted to pH 2.2 [a, b, c] or evaporated before analysis [d].

**Table 3.** Variation of amino acid contents in commercial bean princess Pieterpikzonen ref. 3668 in relation to the hydrolysis time (hydrolysis under nitrogen atmosphere and pH adjustment after hydrolysis) — *Variation de la teneur en acides aminés dans une variété commerciale de haricot princesse Pieterpikzonen ref. 3668 en fonction du temps d'hydrolyse (hydrolyse sous atmosphère azotée et ajustement du pH après l'hydrolyse)*.

| | **Hydrolysis time** | | | |
|---|---|---|---|---|
| % | 24 h | 48 h | 72 h | 96 h |
| **Thr** | 5,1 | 5,2 | 4,9 | 4,7 |
| **Ser** | 6,3 | 6,1 | 5,6 | 5,1 |
| **Val** | 5,9 | 6,6 | 6,5 | 6,7 |
| **Ile** | 4,8 | 5,2 | 5,1 | 5,1 |

### 3.2. Amino acid analysis

Different methods can be used like HPLC, gas chromatography (Duncan, Poljak, 1998), or capillary electrophoresis (Smith, 1997). For foodstuffs, HPLC is often chosen but most of the amino acids are not easily detected by UV or fluorescence. To make them detectable, amino acids are derivatized. In HPLC, two techniques can be selected:

– a pre-column derivatization with a separation of amino acids on a reversed phase column (Gratsfeld-Huesgen, 1998; Bruton, 1986; Haynes *et al.*, 1991; Simmaco *et al.*, 1990; Ou *et al.*, 1996; Fürst *et al.*, 1990), and

– a post-column derivatization with a separation on a cation exchange resin. The last method corresponds to the classical Stein and Moore's method with a ninhydrin coloration (Moore, Stein, 1951).

Many pre-column derivatization methods have been developed. The advantage is their simplicity, sensitivity, and speed of separation. The drawbacks is a lesser reproducibility for foodstuffs, due partly to the matrix interaction. Secondary amines and cystine are sometimes not quantified. For example, cystine determination is not feasible when using o-phthaldialdehyde (OPA). The combination of two pre-column derivatizations (OPA and 9-fluorenylmethyl chloroformate - FMOC-Cl) overcomes this difficulty (Gratsfeld-Huesgen, 1998). With phenyl isothiocyanate (PITC) the reproducibility and linearity are poor, whereas the 1-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl-Cl) method allows reliable quantification (Molnár-Perl, 1997).

Post column derivatization is interesting for food and complex samples. Derivatization occurs on pure compounds, eliminating the matrix effect. The drawbacks of the method are the higher cost of the analyser apparatus and a longer analysis time (1–2 h).

Many problems affect the results using the chromatography method: the stability of the derivatives, the presence or absence of an internal standard, the precision of the standard mixture for calibration, the reproducibility of injections of an identical sample, the reproducibility of hydrolysis replications, and the correlation among different methods.

## 4. TREATMENT OF THE RESULTS

With a standardized hydrolysis method, variations among injections of an identical sample are generally very low; variations should be below 4%, except for very small peaks. To overcome this bias, integration must be well controlled.

Results should include the dry matter, crude protein in % fresh matter, amino acids in gAA/100 g dry matter, the sum of grAA/100 g dry matter, amino acids in grAA/16 g nitrogen, and the sum of gAA/16 g nitrogen. For each sample, the chemical index may be calculated with FAO/WHO (1990) data.

It is also essential to replicate the analysis and to calculate the mean between replicates and the coefficient of variation. An analysis of variance is performed to indicate eventual significative differences among samples.

## 5. CONCLUSION

Amino acid results are sensible to many parameters and it is difficult to control simultaneously all of them. Hydrolysis and chromatography conditions can affect drastically the results impeding inter-laboratory comparisons. Different results could be obtained using the same method with identical apparatus or applied by one technician. Nevertheless, the methods must be standardized in order to minimize these problems.

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