

## **COEI-1-PROVEG Protein plant origin from wheat, peas and potatoes**

### **1. Object, origin and field of application**

The plant protein matter described in this monograph is extracted from wheat (*Triticum vulgare*), peas (*Pisum sativum*), or potatoes (*Solanum tuberosum*). It is mainly made up of proteins and may contain, as minority constituents, carbohydrates (fibres, starch, sugars), fats and minerals. It is intended for human consumption.

The plant protein matter is used for the fining of musts and wines.

It comes in the form of a whitish, beige or yellowish powder. It is totally or partially soluble in water depending on the pH. It can also be in liquid form with content more than or equal to 50 g/l. The solutions are stabilised with sulphur dioxide.

### **2. Labelling**

The following indications must appear on the label of the package: plant origin of the protein, minimal protein content, safety and storage conditions and expiry date. Without prejudice to the provisions in force in the countries where these products are marketed to be used, GMO origin of the raw material is indicated on the package label.

### **3. Test trials**

#### **3.1. Loss from desiccation**

In a silica capsule with a 70 mm diameter with a lid, place 2 g of proteins. Dry in incubator at 105°C for 6 hours. Allow to cool in open capsule and desiccator. Weigh.

Weight loss must not be more than 12% of the powder preparation.

**All limits set below concern dry weight.**

#### **3.2. Determination of total nitrogen**

On a 0.2 g test sample proceed as indicated in chapter II of the Oenological Codex.

The total nitrogen must be more than 10% of the powder weight (corresponding to about 65% in protein).

#### **3.3. Ashes**

Incinerate the residue left from the determination of the loss from desiccation (3.1) by progressively heating at 600°C in a muffle oven until a white residue is obtained and after having sprinkled it with 0.2 to 0.3 g of ashes paraffin in order to avoid mass overflow.

Total ashes must be less than 8%.

#### **3.4. Preparation of the test trial solution**

After weighing, dissolve the ashes in 2 ml of concentrated hydrochloric acid (R) and 10 ml of water. Heat in order to activate the dissolving and add distilled water until a volume equal to 25 times the weight of dry protein is obtained. 1 ml of this solution contains mineral substance of 0.04 g of dry protein.

### 3.5. Iron

1 ml of concentrated hydrochloric acid (R), a drop of potassium permanganate at 1% (R) and 2 ml of potassium thiocyanate at 5% (R) were added to 10 ml of the test solution prepared according to 3.4.

If a red colouration appears, it must be lighter than the control prepared with 6 ml of iron solution (III) at 0.010 g per litre (R), 4 ml of water and the same quantities of concentrated hydrochloric acid (R) and potassium thiocyanate at 5% (R).

The iron content must be less than 150 mg/kg, with the exception of proteins from peas and potatoes, whose content should be less than 300 mg/kg.

It is also possible to proceed with the determination of iron by spectrophotometric atomic absorption according to the method described in chapter II of the International Oenological Codex.

### 3.6. Chromium

In a 50 ml conical flask, place 10 ml of solution prepared according to 3.4, 1 ml of 15% (R) ammonium persulphate solution at, 0.5 ml of a 1% (R) silver nitrate solution at. Heat and add drop by drop until a persistent pink colouration appears of the 3% (R) potassium permanganate solution at. Put a few drops in excess and maintain a gentle boil for 10 minutes. If during boiling, the solution becomes discoloured, add potassium permanganate. After 10 minutes, introduce drop by drop diluted hydrochloric acid at 1/10 (R) until the solution is once again colourless.

After cooling, transfer to a 20 ml graduated flask and add 2 ml of 0.05% diphenylcarbazide in solution at in freshly prepared alcohol (R). Bring to 20 ml.

If a red purplish colouration appears, it must be lighter than that obtained by treating 4 ml of 0.001 g of chromium per litre (R) potassium dichromate solution at by 2 ml sulphuric acid at 5% (R), 5 ml of distilled water, by adding after mixing 2 ml of diphenylcarbazide solution at 0.05% in alcohol (R) and by bringing to 20 ml.

Chromium content must be less than 10 mg/kg.

It is also possible to proceed with the determination of chromium by atomic absorption according to the method described in chapter II of the International Oenological Codex.

### 3.7. Copper

2.5 ml of the test trial solution prepared according to 3.4, are placed in a test tube with

7.5 ml of water, 0.5 ml of hydrochloric citric solution (R), 1 ml of ammonium hydroxide 5 M (R), 0.5 ml of sodium diethyldithiocarbamate reagent (R). If a yellow colouration appears, it must not be darker than that obtained by adding the same quantities of the same reagents to 4.7 ml of a copper solution at 1 mg per litre (R) brought to 10 ml.

*Copper content must be less than 35 mg/kg.*

It is also possible to proceed with the determination of copper by atomic absorption according to the method described in chapter II of the International Oenological Codex.

### 3.8. Zinc

To 1.25 ml of the test solution prepared according 3.4, add 3.75 ml of distilled water, 5 ml of acetate buffer solution (R), 1 ml of sodium thiosulphate solution at 25% (m/v) (R), 5 ml of dithizone solution at 25 mg per litre in chloroform or dichloromethane (R). Shake for 2 minutes. Separate the organic phase; its colouration must be lighter than that obtained by treating 2 ml of zinc solution at 1 mg per litre (R) with the same quantities of the same reagents.

Zinc content must The zinc content must be less than 50 mg/kg, with the exception of proteins from peas, whose content should be less than or equal to 150 mg/kg.

It is also possible to proceed with the determination of zinc by atomic absorption according to the method described in chapter II of the International Oenological Codex

### 3.9. Lead

Using the test trial solution (3.4), perform the determination using the method described in chapter II of the International Oenological Codex.

Lead content should be less than 5 mg/kg.

### 3.10. Mercury

Perform the determination of mercury using the method described in chapter II of the International oenological Codex.

Mercury content should be less than 1 mg/kg

### 3.11. Arsenic

Perform the determination of arsenic using the method described in chapter II of the International oenological Codex.

Arsenic content should be less than 3 mg/kg.

### 3.12. Cadmium

Perform the determination of cadmium using the method described in chapter II of

the International Oenological Codex.

Cadmium content should be less than 1 mg/kg.

#### 4. Microbiological control

##### 4.1. Total viable micro-organisms

Proceed as described in Chapter II of the International Oenological Codex.

Content less than  $5 \cdot 10^4$  CFU/g.

##### 4.2. Escherichia coli

Proceed with counting as described in Chapter II of the International Oenological Codex.

Absence checked on a 1 g sample.

##### 4.3. Salmonella

Proceed with counting as described in Chapter II of the International Oenological Codex.

Absence checked on a 25 g sample.

##### 4.4. Coliforms

Process with counting as described in Chapter II of the International Oenological Codex.

Content less than  $10^2$  CFU/g.

##### 4.5. Yeasts

Proceed with counting as described in Chapter II of the International Oenological Codex.

Content less than  $10^3$  CFU/g.

##### 4.6. Moulds

Proceed with counting as described in Chapter II of the International Oenological Codex.

Content less than  $10^3$  CFU/g.

#### 5. Search for mycotoxins and pesticide residues

##### 5.1. Aflatoxins B<sub>1</sub>

Proceed with analysis according to ISO method 16050

Content less than 4 µg/kg.

5.2. Aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>

Proceed with analysis according to ISO method 16050

Content less than 4 µg/kg in total.

5.3. Organophosphorous pesticide residues <sup>[1]</sup>

Content less than 10 mg/kg.

5.4. Organochlorine pesticide residues <sup>[2]</sup>

Content less than 0.1 mg/kg.

5.5. Ochratoxine A

Using an aqueous solution of 5 g/l of plant protein, perform the determination using the method described in the Compendium of methods of analysis of musts and wines.

Content less than 5 µg/kg.

**6. Reasearch specific for solanum teberosum**

6.1. Glycoalkaloids (- solanine & - chaconine)

Proceed with analysis according to AOAC method 997.13

Maximum content less than 300 mg/kg of potatoes protein

**7. Storage**

The plant proteins should be stored in closed containers or in watertight bags impervious to humidity under temperate conditions.

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<sup>[1]</sup> Method to be determined at a later date

<sup>[2]</sup> Method to be determined at a later date