Veg Collagen from Kalonji Extract by Unique Enzymatic Hydrolysis

INTRODUCTION :

About Kalonji Seeds

The Nigella Sativa or Kalonji plant grows 20-30 cm tall, consisting of linear leaves that are finely divided. The fruit of this plant looks like a large capsule consisting of several united follicles with numerous seeds, known as black seeds or kalonji seeds. It is also known as black cumin or black caraway and is a popular ingredient in many cuisines.

Besides being a popular culinary ingredient, kalonji is used in various other forms. It is revered in traditional medicinal systems of Ayurveda, Unani, Tibb and Siddha. It is famously known as the 'seed of blessing' and is used extensively to treat disorders of the stomach, eyes, heart and neurological conditions.

Nutritional Value of Kalonji (Per 100 gm)

Content	**Value Per 100 gm**
Calories	375 kCal
Total Fat	22.27 g
Saturated Fat	1.5g
Carbohydrates	44.24g
Protein	17.81g
Monounsaturated fat	14.04g
Polyunsaturated fat	3.27g
Sodium	168 mg
Iron	66.36mg
Potassium	1788 mg
Calcium	931 mg
Sugar	2.25g
Fibre	10.5g
Vitamin A	64IU
Vitamin E	3.33mg
Vitamin K	5.4mcg
Vitamin C	7.7mg
Phosphorus	499 mg

Kalonji (or Nigella) seeds contain a significant amount of protein, with nutritional data showing approximately 16-18 grams of protein per 100 grams of seeds. These plant-based seeds are a good source of essential amino acids and are also rich in other nutrients, including

healthy fats, fiber, vitamins, and minerals, making them a valuable addition to a diet, especially for vegetarians and vegans.

Kalonji, also known as black seeds or *Nigella sativa*, contains a notable amount of protein, making it a valuable addition to plant-based diets. While it is not a primary source of protein for most people, its protein content is part of its rich overall nutritional profile.

Kalonji's protein content

- Approximate value: Kalonji seeds contain about 17.81 grams of protein per 100 grams. Other sources place the protein content slightly higher, at around 21% by weight.

- Amino acids: The protein in kalonji contains essential amino acids, which are vital building blocks for the body and must be obtained from food.

Other nutritional benefits

Beyond its protein content, kalonji is also recognized for many other beneficial components:

- Vitamins: It is rich in vitamins, including A, C, E, and B-complex vitamins.
- Minerals: It is a good source of important minerals such as calcium, iron, potassium, and zinc.
- Fats and fatty acids: It contains essential fatty acids, including linoleic acid and oleic acid.
- Antioxidants: Kalonji seeds are known for their potent antioxidant properties, with compounds like thymoquinone that help protect the body's cells from damage.

How to use kalonji

Due to its robust nutritional makeup, kalonji can be used in various ways to boost your intake of protein and other nutrients:

- Add to food: The seeds can be used whole or ground to add a distinct, aromatic flavor to curries, vegetable dishes, bread, and pickles.
- Sprinkle on salads: Lightly toasted kalonji seeds can be sprinkled over salads for extra crunch and nutrients.
- Add to smoothies: For a protein and nutrient boost, add a small amount of ground kalonji to smoothies.

Hydrolysis of Collagen in Kalonji :

1. Defatted Kalonjipowder enzymatic hydrolysis temp. 60^{o} C around 4 hr. and deactivate enzyme with the help of rise of temperature.
2. Charcoal treatment 30 min.
3. Filtration process.
4. Spray Drying & collect the powder.
5. Granulation Process.

Result / Analysis

Principle of the Kjeldahl Method: The principle of the Kjeldahl method is based on the digestion of the sample in concentrated sulfuric acid (H_2SO_4), which converts the nitrogen present in the sample (as organic nitrogen) into ammonium sulfate $(NH_4)_2SO_4$. After digestion, ammonia is distilled from the solution and quantified by titration with a standard acid solution. Steps Involved in the Old Kjeldahl Method

1. Digestion

• Purpose: To convert organic nitrogen in the sample into ammonium ion (NH_4^+).

Procedure:

• Weigh a known amount of the sample (usually between 0.25 and 035 g) and place it in a Kjeldahl digestion flask.

• Add concentrated sulfuric acid (H_2SO_4) (about 20-30 mL) to the flask.

• Add a catalyst such as potassium sulfate (K_2SO_4) and copper sulfate ($CuSO_4$)) to speed up the reaction.

• Heat the flask gently. The sulfuric acid will break down the organic material, and nitrogen will be converted into ammonium sulfate.

• This digestion process can take 1 to 2 hours. The mixture turns clear, which indicates that the organic matter has been successfully digested.

2. Neutralization and Distillation

Purpose: To convert the ammonium ion (NH_4^+) to ammonia gas (NH_3) and distill it into a receiving solution.

3. Procedure:

➢After digestion is complete, cool the flask and dilute the solution with water.

➢Add a strong base, usually sodium hydroxide (NaOH), to make the solution

alkaline. The ammonia (NH_3) gas is liberated when ammonium sulfate reacts with

the alkali: (NH4)2SO4+2NaOH→2NH3+Na2SO4+2H2O$(NH_4)_2SO_4$ + 2 NaOH

→ 2 NH_3 + Na_2SO_4 + 2 H_2O(NH4)2SO4+2NaOH→2NH3+Na2SO4+2H2O

➢The ammonia gas (NH_3) is distilled by heating the solution. The ammonia gas is passed into a receiving solution, typically a known concentration of boric acid (H_3BO_3) in water, which absorbs the ammonia.

4.Titration

Purpose:

To quantify the amount of ammonia (NH_3) captured in the receiving solution, which corresponds to the nitrogen content of the sample.

Procedure:

• The ammonia solution (from step 2) is then titrated with a standard solution of a strong acid, typically hydrochloric acid (HCl) or sulfuric acid (H_2SO_4).

• A few drops of an appropriate pH indicator, such as methyl red or bromocresol green, are used to monitor the endpoint.

• The amount of acid required to neutralize the ammonia solution is directly related to the amount of nitrogen present in the sample.

Method of Protein content calculation the nitrogen content is determined by the volume of acid used in the titration.

The formula for calculating the nitrogen content is:

$$0.14 \left(\frac{\text{(Volume of Acid X Normality of Acid) - Burette Reading}}{\frac{0.1}{\text{Wt. of Sample}}} \right)$$

Where:

• Equivalency factor: This factor depends on the type of acid used for titration. For HCl, it is typically 1, but it may vary depending on the acid and its concentration.

• Normality of acid: The normality of the titrant (HCl or other acid) is typically expressed in equivalents per liter. Once the nitrogen content is determined, you can estimate the protein content by using the N × 6.25 factor, assuming that protein contains approximately 16% nitrogen. The conversion factor may vary depending on the type of protein being measured.

Result of isolate proteins: • If 0.3 grams of sample were used, and the titration required 40 mL of 0.1 N HCl for neutralization, the nitrogen content can be calculated as:

$$\frac{0.14\ (40 \times 0.1020\ N) - 16.20}{0.1}$$

$$0.3028$$

- Nitrogen content = 11.37 %.

To estimate the protein content: Protein content = 11.37 % × 6.25 = 71.08 % of protein.

Conclusion :

Kalonji (nigella seeds) are rich in fiber, protein, and healthy fats, providing a good source of minerals like iron and calcium, and containing various vitamins, including B vitamins, though specific amounts vary by source. For every 100 grams, kalonji typically contains approximately 375 calories, around 18-22 grams of protein, 22-26 grams of fat, and 44-52 grams of carbohydrates, with high dietary fiber content.

Hydrolysis of Kalonji powder protein content is 71.08 %