

Veg Collagen from Broccoli Extract by Cold Enzymatic Digestion

Solfit Lifesciences¹

Abstract—Background: This is one of the many types of Protein developed in Solfit Lifesciences.

Objective: Highly purified and convert into a high percentage of collagen from broccoli.

Index Terms—Soy Oil Is Extracted, Then Leftover Material Is Utilized to Create Isolate Proteins.

I. INTRODUCTION

Collagen is the most abundant protein in your body, making up around 30% of your total protein mass.¹ As a structural protein, collagen plays a crucial role in the health and function of various tissues, including your skin, muscles, bones, and connective tissues.

While your body makes plenty of collagen on its own, emerging research shows that supplementing with collagen may promote various health benefits. But is there a way for plant-based eaters to get the benefits of collagen supplementation without changing their diet?

The research says yes.

This article will explore:

- The research-backed benefits of collagen supplementation
- The connection between collagen and bone health
- How vegans and vegetarians can naturally boost their collagen production without taking any animal-based products

Health Benefits of Collagen

Skin Health

If you're looking for a way to firm up your skin and enhance its elasticity, collagen may be the way to go.

Collagen makes up a significant portion of your skin's extracellular matrix (ECM) – the scaffolding that lies beneath your skin surface, which allows it to look taut and firm.²

As you age, your ECM naturally begins to break down, producing fine lines and wrinkles on the surface of your skin. Research shows, however, that when you supplement with collagen, you can enhance the integrity of your ECM and therefore create more youthful-looking skin.³

Other research shows that collagen may play a role in wound healing.⁴

A. Joint Health

As a key component of connective tissue, collagen plays an important role in the health and maintenance of your joints. Specifically, collagen supports your cartilage tissue, which wraps around the ends of your bones to protect them and prevent wear and tear.

Cartilage can break down due to excessive use (in athletes, for example), inflammation (like that seen in rheumatoid arthritis), or the natural aging process. When this happens, you start to experience joint pain that can range anywhere from inconvenient to debilitating – depending on the severity of the damage. Research shows that collagen supplementation may support joint health by providing the building blocks they need to support and protect them from damage.

In one study, participants with rheumatoid arthritis experienced significant improvement in both joint swelling and pain.⁵ In another study, athletes with activity-related joint pain significantly improved their pain measures at rest and during activity.⁶



B. Digestive Support

Although the exact mechanism is unclear, there seems to be a correlation between low serum levels of collagen and inflammatory bowel disease (IBD). One theory is that a deficiency in this structural protein makes your digestive tract more vulnerable to inflammation and therefore sets the stage for digestive issues.⁷

In one study, participants noticed improvements in bloating and other mild digestive symptoms after a 6-week trial of collagen supplementation.⁸

C. Bone Health

We've already covered the positive impact collagen can have on joint health, which indirectly relates to the health of your bones. But that's not the only connection between bone health and collagen status.

Your bones are made up of protein and minerals, with 90% of the bone matrix composed of collagen.⁹ While minerals provide strength, collagen provides structure and flexibility.¹⁰ Collagen is a vital aspect of bone health because it allows your bones to absorb impact rather than fracture, and to bend instead of break.

Studies show that taking collagen supplements can improve bone mineral density (BMD) in postmenopausal women.¹¹



Is There a Vegetarian Source of Collagen?

However, keep in mind that your body makes collagen on its own when provided with the right nutrients. This means that even for the vegan and all the vegetarian folks out there, you can enhance collagen production and gain all of the benefits discussed here; you just need to know which nutrients to incorporate.

Vegetarian Foods and Nutrients That Increase Collagen Production



E. Proline

Proline and hydroxyproline are amino acids that make up 23% of collagen and have been found to be precursors to sustaining collagen production. They play a key role in the stability of collagen.^{13,14}

Vegetarian proline sources: asparagus, beans, buckwheat, cabbage, chives, cucumbers, garbanzo beans, peanuts, soy, and watercress.

F. Silica

Silica is a mineral that assists in collagen synthesis, playing a role in the activation of enzymes responsible for collagen production.¹⁵

Vegetarian sources of silica: oats, barley, avocados, cucumbers, asparagus, horsetail, nettle, and strawberries.

G. Strontium

Strontium is a mineral that helps your body make and maintain healthy collagen levels by promoting collagen synthesis while suppressing its degradation.¹⁶

D. Vitamin C

Vitamin C adds oxygen and hydrogen to amino acids so that they can do their part in collagen production. If you don't get enough vitamin C, collagen production will slow.¹²

Vegetarian vitamin C sources: many fruits and vegetables such as broccoli, Brussels sprouts, cauliflower, citrus fruits, kale, kiwi, mango, papaya, peppers, pineapple, and strawberries.

Vegetarian sources of strontium: Brazil nuts, milk, cheese, spinach, carrots, peas, beans, and root vegetables.

Unfortunately, although the above options do contain trace amounts of strontium, it's unlikely that you'll get enough of this nutrient from diet alone, particularly if you're looking for bone health benefits. That's why you'll want to look for a high-quality strontium supplement like Strontium Boost to fill the gaps.

H. Copper

Copper increases the production or utilization of collagen and elastin; it also helps facilitate the fibril structure of these proteins.¹⁷

Vegetarian copper sources: sunflower seeds, lentils, almonds, apricots, dark chocolate, mushrooms, greens, and blackstrap molasses.



I. Lysine

Lysine helps to give collagen its stability and structure by assisting in cross-linking. It also increases intestinal calcium absorption.^{18,19}

Vegetarian lysine sources: dairy products (particularly parmesan cheese), tofu, and spirulina.

J. Arginine

Research suggests that arginine stimulates insulin-like-growth factor-1 (IGF-1) production and collagen synthesis in osteoblast-like cells. In this way, arginine makes the cells in your bone that are responsible for bone growth and mineralization more active.²⁰

Vegetarian arginine sources: sesame seeds, spirulina, coconut kernel, cultured yogurt, kefir, and raw cheeses.

K. Manganese

Manganese increases collagen and elastin production by increasing the enzyme responsible for proline formation, especially when healing wounds.²¹

Vegetarian sources of manganese: leafy vegetables, nuts, pineapple, seaweed and other sea vegetables, and whole grains.

L. Zinc

Studies show that zinc can enhance collagen synthesis while also slowing down its degradation, which in turn helps protect your current collagen stores.²²

Vegetarian zinc sources: seeds (pumpkin seeds, in particular), nuts, and beans.



Are Vegetarians Lacking Collagen?

Since your body makes collagen on its own, vegetarians may not necessarily be lacking collagen as long as they're getting enough of the right nutrients to keep their collagen stores healthy.

With that being said, as our bodies age, collagen production slows down, and the wear and tear of life starts to catch up with us. For this reason, ensuring that your collagen status is healthy as you get on in your years is important whether you're a vegetarian or not.

Takeaway

When it comes to collagen and bone health, the two big takeaways include:

1. Collagen is essential to bone health (and so much more!)
2. Your body produces collagen naturally, and will produce more if you eat foods rich in the nutrients listed above. You don't need to eat meat to maintain proper levels of collagen.

II. MATERIAL AND METHOD

Chemicals:

All chemicals used were of analytical grade this are the given below,

Sr. No.	Chemical's / Reagent	Make	Purity %	Molecular Formula	Molecular Weight
1.	Sodium Hydroxide Pellets	Qualigens	97%	NaOH	40.0
2.	Copper Sulphate	Qualigens	98.5%	CuSO ₄ 5H ₂ O	249.68
3.	Potassium sulphate	Qualigens	99.10%	K ₂ SO ₄	174.26
4.	Hydrochloric acid	Qualigens	35.37%	HCl	36.46
5.	0.1N Sulphuric acid Ampoules	Qualigens	99.90%	-	-
6.	Distilled Water	Qualigens	99.10%	H ₂ O	18.02
7.	Bromocresol green indicator	Qualigens	98%	C ₂₁ H ₁₄ Br ₄ O ₅ S	698.04
8.	Methyl red indicator	Qualigens	98.20%	C ₁₅ H ₁₅ N ₃ O ₂	269.30
9.	Boric Acid	Qualigens	96.30%	H ₃ BO ₃	61.83
10.	Proteases enzyme	-	42 %	-	-
11.	Broccoli	Local farming	-	-	-

Equipment's:

1. pH Meter
2. Kjeldahl Nitro
3. Analytical Balance
4. Muslin cloth
5. Volumetric Flasks
6. Volumetric Pipettes
7. Bukner funnel
8. Conical flask
9. Beaker
10. Timer (stopwatch)
11. Whatman 42 no. filter paper
12. Vacuum pump
13. Filtration assembly with vacuum pump
14. Homogenizer

A. Purification

1. Collect the healthy broccoli from local Market in Ahmadabad about 5 kg.
2. Washed in soft water.
3. Weighing broccoli 2 kg in glass beaker.
4. Addition 10 lit. of water (1: 5 ratio)
5. Addition Proteases enzyme 10 ml (0.5%)
6. Continually stirring with the help of glass rod in a room temp. for 6 hr.
7. Provide temp 95°C for a enzyme deactivate for a 10 min.
8. Cool down about 70°C then filtered with the help of muslin cloth.
9. Liquid filtered with the help of bukner funnel and vacuum filtration assembly.
10. Convert in to powder form with the help of spray dryer.



Broccoli



Veg Collagen

B. Assay of Protein:

Principle of the Kjeldahl Method: The principle of the Kjeldahl method is based on the digestion of the sample in concentrated sulfuric acid (H₂SO₄), which converts the nitrogen present in the sample (as organic nitrogen) into ammonium sulfate (NH₄)₂SO₄. 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 (NH₄⁺).

Procedure:

- Weigh a known amount of the sample (usually between 0.25 and 0.35 g) and place it in a Kjeldahl digestion flask.
- Add concentrated sulfuric acid (H₂SO₄) (about 20-30 mL) to the flask.
- Add a catalyst such as potassium sulfate (K₂SO₄) and copper sulfate (CuSO₄) 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

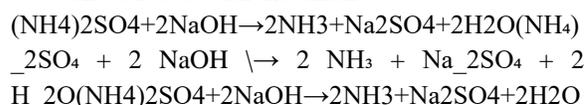
Purpose: To convert the ammonium ion (NH₄⁺) to ammonia gas (NH₃) and distill it into a receiving solution.

3. Procedure:

The formula for calculating the nitrogen content is:

- 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 (NH₃) gas is liberated when ammonium sulfate reacts with the alkali:



- The ammonia gas (NH₃) is distilled by heating the solution. The ammonia gas is passed into a receiving solution, typically a known concentration of boric acid (H₃BO₃) in water, which absorbs the ammonia.

4. Titration

Purpose:

To quantify the amount of ammonia (NH₃) 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 (H₂SO₄).
- 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.

$$\text{Nitrogen Content (g)} = 0.14 \left[\frac{(\text{Volume of Acid} \times \text{Normality of Acid}) - \text{Burette Reading}}{0.1} \right] \times \text{Sample weight (g)}$$

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 $N \times 6.25$ 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:

$$\text{- Nitrogen content} = 0.14 \left[\frac{(40 \times 0.1020 \text{ N}) - 13.2}{0.1} \right] \times 0.3 \text{ g} = 12.88 \%$$

To estimate the protein content: Protein content = 12.88 % × 6.25 = 80.50 % of protein

III. CONCLUSION

Raw broccoli contains almost 90% water, 7% carbs, 3% protein, and almost no fat. Broccoli is very low in calories, providing only 35 calories per cup, or 90 grams (g). The nutrition facts for 1 cup (90 g) of raw broccoli are (4): Calories: 35. Broccoli is a very popular vegetable that also happens to contain protein with all the essential amino acids. People can enjoy it either raw or cooked. Broccoli has the following protein content: One cup (88 g) of chopped broccoli contains 2.5 g of protein.

IV. ACKNOWLEDGMENT

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