



Determination Protein Content of Protein Supplements and Determining Genuine or Counterfeit Protein Supplements with a Novel Consumer Friendly Kit

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KEYWORDS	ABSTRACT
<p><i>PPR (Protein Precipitation Reagents), Protein Supplements Development, Hydrophobic Aggregation, Nutritional Quality</i></p>	<p><i>The consumption of protein supplements by athletes and fitness enthusiasts to improve exercise performance has increased over the last few years. However, the supplements available in the market does not guarantee the authenticity of products as there has been also an increase in the take protein supplement market, which can pose a serious health risk to the customers. The present invention is to provide a kit for the determination of protein content in a protein supplement by the consumer in a home setting and to provide a method that reduces the requirement of complex laboratory apparatus and chemical reagents and the consumer can carry out the method in a simple setup such as a home. The method works on the principle of hydrophobic aggregation in which the protein precipitation solution precipitate out the protein present in the protein supplement such as whey protein. The kit comprises a graduated tube with markings to measure the volume of precipitate, small vials containing the protein precipitation solution, and a protein measurement chart indicating precipitate volume in milliliters and its corresponding amount of protein as percentage. The protein precipitation solution is a ready to use solution packed in a suitable container for use by the consumer to test the whey protein supplements in an environment that does not require expensive instruments and controlled conditions of an analytical laboratory. This kit is cost effective and easy to perform test, which gives accurate results making the whole niche of fake whey supplements easier to detect and causing awareness about fake products available in the market. The same test kit can also be used to determine if the protein supplement sample the kit method were confirmed in a NABL approved is genuine or a counterfeit supplement All results of Laboratory through quantitative analysis of the same samples using standard Kjeldahl method for testing content. The recovery for the entire sample was found to be in acceptable range as per the label claim of different samples used in the analysis.</i></p>



• **INTRODUCTION:**

Many active individuals, including athletes, often turn to protein supplements to meet their protein needs. Experts generally recommend consuming 0.8-0.9 grams of protein per kilogram of body weight for adults. However, recent research has focused on protein recommendations specifically for active individuals (Khan et al., 2021). It's commonly understood that active individuals require more protein than their sedentary counterparts. Endurance athletes are advised to consume 1.2-1.4 grams of protein per kilogram of body weight, while power athletes should aim for 1.2-1.7 grams. Whey protein is a popular ingredient in protein supplements due to its high nutritional value compared to other protein sources like caseinates, egg, soy, and wheat proteins. Whey protein is a complex mixture of globular protein molecules, primarily composed of α -lactalbumin (α -La) and β -lactoglobulin (β -Lg), which together make up about 70% of the proteins in whey (Khan, et al., 2021). The nutritional benefits of whey protein supplements can be influenced by their physical-chemical composition. Various factors such as the amino acid composition, bio-availability of essential amino acids, protein digestibility, and the physiological utilization of specific amino acids following absorption need to be taken into account to assess the nutritional quality of whey protein supplements (Ramos-Garcia, et al., 2023). Essential amino acids such as leucine, isoleucine, and valine, found in whey protein, have been associated with increased stimulation of skeletal muscle protein synthesis (Ionescu, et al., 2023), making it an important source of nutrients.

In recent times, athletes and fitness enthusiasts have increasingly turned to supplements to enhance their exercise performance. While some use them to supplement their diets, others rely on them to boost their sports performance. However, the medical and training communities are divided on the advantages of these supplements due to the widespread prevalence of contaminated, counterfeit, or ineffective products. Such supplements can pose serious health risks to athletes and even result in evidence of doping during drug tests. Whey protein, a soluble protein derived from milk serum during cheese and casein production, has been extensively researched since the 1970s for its high biological-value proteins and bioactive peptides. These peptides have the potential to function as agents that regulate immune response, possess anti-hypertensive properties, and even exhibit antimicrobial effects (Kua et al., 2022). In a recent U.K. study involving 874 athletes, approximately 60% (520 individuals) reported taking nutritional supplements. Of those supplement users, the most popular performance-related options were creatine (used by 36.1%) and whey protein-based supplements (used by 30.6%). However, the study also revealed that some athletes may be using supplements without fully



understanding their benefits or relying on unsupported claims about their effects (Kua, et al., 2022). Additionally, it is unfortunate that powdered milk-derived products are frequently contaminated, often with added compounds.

- Easy, do it yourself kit to check against misleading and fake whey supplements.
- Directionally accurate and fairly indicative results within 24 hours.
- No expensive or time-consuming external lab testing required.
- Applicable to all whey protein supplements available around the world.
- Environmentally friendly, no gaseous fumes released.

• MATERIAL AND METHOD:

The present invention provides a method and a kit for quantitative determination of protein content in a protein supplement such as a protein supplement. The method works on the principle of hydrophobic aggregation in which the protein precipitation solution precipitates out the protein present in the protein supplement such as a whey protein (van Vliet, et al., 2021). The protein precipitation solution is a ready-to-use solution packed in a suitable container for use by the consumer to test the protein supplement in an environment that does not require sophisticated instruments and costly reagents in an analytical laboratory.

The protein precipitation solution is prepared by dissolving a weighted quantity of protein precipitation reagents in an amount of water and making up the desired volume with water for example, to make 100ml of protein precipitation solution, about 30gm of PPR is dissolved in 50 ml of water (van Vliet, et al., 2021). Optionally, to this solution 10 ml of ethanol is added and the final volume of 100ml is made with water.

Ethanol if used can be present in an amount of about 5% to 20%, preferably about 10%. The solution can then be packed in the form of a kit. The kit may comprise of a container containing the protein precipitation solution, a graduated container such as a centrifuge tube or a graduated tube (van Vliet, et al., 2021). The kit can optionally also contain a shaker for preparing the solution suspension of the protein supplement in water. Alternatively, the consumer can use any suitable container readily available to prepare the solution (Bapita, et al., 2021).

The protein precipitation solution is effective at lower amounts than the other precipitation reagents. ~30% for PPR helps to denature the protein. Further, it does not affect total protein content in the



sample and the precipitate can be further analyzed by standard methods (McWhite, et al., 2020). The method precipitates the proteins independently of their physio-chemical properties.

The method is further made easy to perform with the help of the kit. The kit comprises a graduated tube with markings to measure the volume of precipitate; a non-graduated tube or a vial containing the protein precipitation solution; and a protein measurement chart indicating precipitate volume in milliliters and its corresponding amount of protein as percentage. The graduated tube may have a length of about 120 mm and a diameter of about 25 mm with 1 ml least count. The non-graduated glass tube or vial may contain about 30 mL to 40 ml of protein precipitation solution, preferably 35 ml (Roberts, et al., 2022).

Further, the method can also be used to test whether the protein supplement is a genuine protein supplement or a counterfeit supplement. For example, after measuring the precipitate volume, the graduated tube can be inverted to observe if the precipitate remains intact and does not fall down or the whole precipitate does not mix with the solution (Roberts, et al., 2022). If so observed then it can be concluded that the protein supplement sample is a genuine protein supplement. If it is observed that the precipitate mixes in a few seconds or the whole precipitate mixes with the contents of the tube then it can be concluded that the protein supplement sample is a counterfeit protein supplement (Bapita, et al., 2021). Alternatively, the same method can be followed by using a spatula or a spoon like device. The precipitate is scooped out of the graduated tube by a spatula and if a thick and uniform precipitate comes out, then it can be concluded that the protein supplement sample contains genuine protein supplement (Nehra, et al., 2019). If the precipitate comes out as a watery precipitate rather than a thick uniform paste, then it can be concluded that the protein supplement sample is a counterfeit protein supplement.

✧ **The method can be performed by steps as follows:**

- Take 30g of protein supplement powder in 200ml of drinking water (8-15°C) in a Shaker bottle and mix the protein supplement powder with manual shaking for 15 to 30 seconds;
- In a graduated tube add 35 ml of protein precipitation solution.
- To the above graduated tube add 10 ml of protein powder solution /suspension of step one.
- Shake the graduated tube for 60 seconds and keep it in vertical position overnight at room temperature (Nehra, et al., 2019).



- Measuring the precipitate volume in the graduated tube and tallying the corresponding volume from the protein measurement chart indicating corresponding amount of protein as percentage.

- ◆ **Note - While taking the scoop take precaution that it should not be heaped. Scoop should be leveled, with some flat edge (back of a knife, etc).**

Table 1: Protein Measurement Chart

S. No.	Precipitation (in ml)	Corresponding Protein Content (%)
1	6 ml - 7 ml	35-45 %
2	7 ml - 8 ml	45-55%
3	8 ml - 9 ml	55-65 %
4	9 ml - 10 ml	65-75%
5	10 ml - 11 ml	75-85 %
6	>11 ml	More than 85 %

● **RESULTS:**

The same test kit can also be used to determine if the protein supplement sample is genuine or a counterfeit supplement. The method can be performed after the protein precipitation solution and the protein powder solution/suspension have been mixed in the graduated tube as that in Step 4 above. If about one minute of mixing, some precipitate will float at the top of the solution, while some precipitate will start settling at the bottom of the tube as a coagulate i.e. the supplement tested contains protein such as a whey protein. If mixture is uniformly spread out in the same colour as of the initial dispersion that was poured, without any separation or precipitation at all, it is the first indicator that the tested protein supplement could actually be a counterfeit (McWhite, et al., 2020). It could be a flour with



maltodextrin - some kind of carbohydrate or even a non-food item. After five minutes, if there is even more separation of the precipitate settling at the base of the tube as a coagulate and the precipitate floating at the top of the tube becomes thinner and the solution in the middle starts to become clearer it can be concluded that supplement tested does contain protein and is a genuine protein supplement (McWhite, et al., 2020). In another scenario, after five minutes, the mixture is still uniformly spread out in the same color with not much difference from the first observation five minutes earlier and is still without any separation or precipitation, it can be concluded that the supplement tested is counterfeit protein supplement.

The test method of the invention can check the amount of protein in any protein sample and several marketed whey protein supplement products were tested by this novel method. Table 2 provides the results of testing the protein content of some commercially available protein supplements.

All the results were confirmed in the Laboratory through quantitative analysis of the same samples using standard Kjeldahl method for protein content. The recovery for the entire sample was found to be approximately 95 % as per the label claim.

This method is fast, reliable and consumer friendly which can be done at home using the kit. The purpose of this study was to transfer the complex laboratory method to the consumer's home where anyone can test the quality and genuineness of the protein supplements. This way consumer can become more aware and confident of the quality of the protein supplement such as whey protein they have purchased from the source.

Table 2: Protein Content of Some Commercial Protein Supplements Determined by the Protein Precipitation Method Using the Kit.

Products	(80%) Raw Whey®	Beginner's Protein®	Pro Burst®	Biozyme Whey®	Whey Gold®
Approximate Protein Content by standard method	80%	40%	73%	76%	83%
Over night results	>11 mL	6-7 mL	9-10 mL	10-11 mL	10-11 mL



Determined Content (Kjeldahl method)	80%+	40%	73%	75%+	80%+
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Table 3: Protein Content of Some Commercial Protein Supplements Determined by the Protein Precipitation Method Using the Kit (FAKE/REAL) Products.

Standard Products (Real)	Product Name	Protein % by		After 1 min	After 5 min	Inversion Test	Spatula test	Final Observation (Pass/Fail)
		Kjeldahl Method (AOAC 991.20)	Protein Testing Kit method (ml)					
	On Nutrition	79%	10	√	√	√	√	Pass
	Fast & Up	79.80%	11	√	√	√	√	Pass
	Asitis WPC 80	73.32%	9	√	√	√	√	Pass
Sub Standard Products	Big Muscle Crude Whey	38.57%	6	√	√	√	√	Fail
Fake Products	On optimum nutrition - 0001094013	10.12%	<5	X	X	X	X	Fail
	ISO-100-2241280	11.15%	<5	X	X	X	X	Fail

• **CONCLUSION:**

22 Different whey protein brands over the market were tested and it is converted that the results obtained by the convention kjeldahl method for protein estimation and the results obtained using the kit method coincide the proves that kit gives accurate precise and correct results for protein estimation for whey supplements.

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