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Quality assessment of carbohydrate, protein, & energy in protein powder as claimed constituents

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Abstract

Protein powder is one of the nutritional supplements most commonly consumed, by professional and amateur athletes and also by those taking exercise for non-sporting purposes. During its manufacture, the thermal treatment involved can reduce the nutritional value of the product, an aspect that has received little research attention. In this study 15 samples of protein powder were analysed by qualitatively and quantitatively for presence of claimed components such as protein, carbohydrate and energy. After analysis it was found that the quantity of protein were less as compared to claimed, were as quantity of carbohydrate showed variation as compared to claimed and variation in the calories content was seen in samples S₂, S₄, S₆, S₇, S₈, S₁₀ and S₁₂ have high energy level (kcal) than the mentioned claimed on sachet.

Keywords: Protein powders, claimed components, nutritional supplements, quality assessment

Introduction

Proteins are important constituents of foods for a number of different reasons. Protein powder is one of the nutritional supplements most commonly consumed by professional and amateur athletes and also by those taking exercise for non-sporting purposes. Whey protein, which is soluble protein fraction in milk serum that is obtained during cheese and casein production, has been studied since the 1970 as a source of high biological value protein, as well as bioactive peptides, which might act as antimicrobial, antihypertensive, and immune response modulator agents. Protein is one of the building blocks of bone, muscle, and skin. Forensic science uses the principles of chemical techniques to aid investigation agencies and law enforcement. In one such application, food forensics is a field of study under the forensic chemistry division and deals with the identification and analysis of illegalities in relation to food products or items of local consumption. Adulteration can be defined as "an act of deliberately contaminating food material with inferior quality or cheap and inedible or toxic substances." Food fraud in terms of mislabelling and adulteration is a growing problem in India. This study was carried out to determine whether the current practice in place needs to be amended to elicit the criminal intentions of a few companies or distributors and the rules if flouted by illegally mislabelling products which finds its way into many homes across India.

Methodology

Qualitative analysis of Protein & Carbohydrate

15 protein powder samples of different brands were analysed qualitatively as well as quantitatively.

0.5 g of protein sample was dissolved in 5ml of distilled water to make suspension. Five clean test tubes were taken and 1 ml of sample was added to it. 1ml of 0.5% CuSO₄ and 0.5ml of 10% NaOH was added to each tube. The solution turns purple if protein is present. For analysis of carbohydrate 1 ml of Sample was taken and 0.5 ml of equal volume of Fehling A and Fehling B was added. After boiling, brick red precipitate of cuprous oxide was formed as carbohydrate was present in the sample.

Quantitative estimation of protein by Lowry's method:

Reagent 1: (A) - 2% sodium carbonate in 0.1 N NaOH. **(B)**- 1% NaK tartarate in 1 ml water. **(C)** - 0.5 % Copper sulphate in 1 ml water.

Reagent 2: Folin's Phenol (Folin's phenol: water in 1:2 ratio.)

Stock and Working Solution: Standard Solution – BSA Stock – 1mg/ml. Working- 100µg/ml.

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20, 40, 60, 80 and 100 µg/ml of BSA working solution was taken in four test tubes and volume was made up to 1ml using distilled water. The test tube with 1ml distilled water served as a blank. 5 ml of reagent 1 was added and incubated for 10 min. After incubation, 0.5 ml of Reagent 2 was added and incubated for 30 min in dark. The absorbance was measured at 660nm and the standard graph was plotted.

Quantitative estimation of total carbohydrate by Phenol sulphuric acid method

Reagent 1: 5% Phenol solution.

Reagent 2: 96% sulphuric acid.

Standard sugar solution: Stock- 2mg/ml. Working- 200µg/ml.

Eight tubes were taken and labeled them as Blank and 1-7. Dilutions of glucose standards were prepared with concentrations of 40, 80, 120, 160 and 200 µg per 200 µl by transferring respective amount of glucose from the standard glucose solution (1mg/ml) and adjusting it to a total volume of 200 µl by adding distilled water. 0.2 ml of 5 % phenol solution was added to all the tubes. 1 ml of concentrated Sulphuric acid was added to each tube and mixed well. After 10 minutes, the contents of the tubes was mixed and placed in a water bath set at 25-30 OC for 20 minutes. The wavelength of 490 nm was selected on spectrophotometer. Firstly the absorbance (OD) of Blank was taken and to set zero. Then the OD of all the tubes (1 – 7) was taken. The cuvette was washed each time after taking OD.

Quantitative estimation of Energy

Energy value was calculated by using following formula:-

$$\text{Energy (in Kcal)} = 4x (\text{Proteins mass in grams}) + 4x (\text{carbohydrates mass in grams}) + 9 x \text{ mass of fat in grams.}$$

Results and Discussion: 15 Protein powder sample was collected from local market of Lucknow, and stored in room temperature for further use.

Qualitative test for carbohydrate

After analysis of 15 protein powder sample, it was found that all samples from S1- S15 given +ve result for their presence.

Qualitative test for Protein

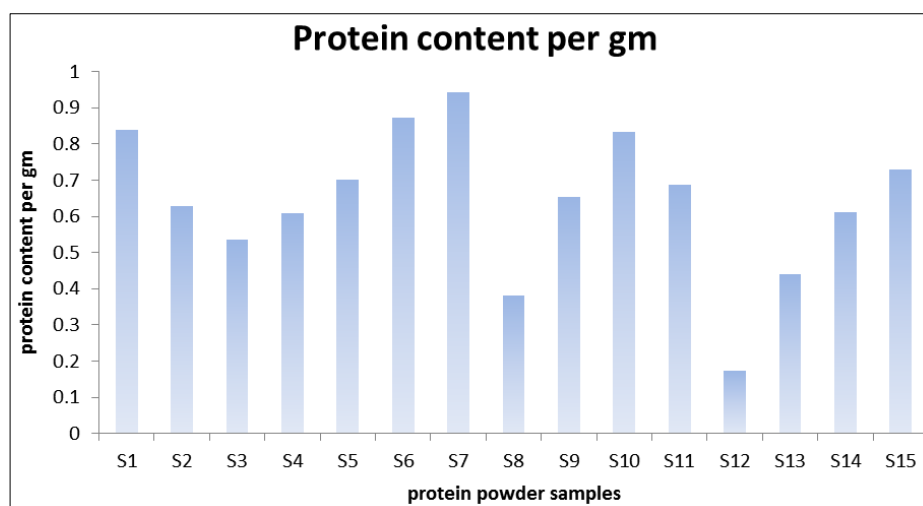
After analysis of 15 protein powder samples, it was found that all samples from S1-S15 were positive shown purple color complex.

Quantitative Estimation of Protein by Lowry's Method

After quantitative estimation of protein, in different protein powder it was found that all protein powders sold in markets of India, are having adequate protein content (< 24g/30g) as compared to those standard claimed readings on the sachet. Maximum results are in coherence with the claimed readings. Although few samples like S₃, S₄, S₇, S₉, S₁₃ and S₁₄ which have 6-10 grams less proteins than the label claims.

Table 1: Quantitative estimation of protein samples by Lowry's method.

S.N.	Sample No.	Sample (ml)	Water (ml)	Conc.	Reagent 1 (ml)	Incubation at 10 min	Reagent 2 (ml)	30 min incubation in dak	OD at 660nm	Conc./gm
1.	S ₁	0.1	0.9	10	5		0.5		1.745	0.84
2.	S ₂	0.1	0.9	10	5		0.5		2.796	0.627
3.	S ₃	0.1	0.9	10	5		0.5		2.725	0.536
4.	S ₄	0.1	0.9	10	5		0.5		2.802	0.609
5.	S ₅	0.1	0.9	10	5		0.5		2.822	0.702
6.	S ₆	0.1	0.9	10	5		0.5		1.755	0.873
7.	S ₇	0.1	0.9	10	5		0.5		2.526	0.943
8.	S ₈	0.1	0.9	10	5		0.5		1.584	0.380
9.	S ₉	0.1	0.9	10	5		0.5		1.582	0.653
10.	S ₁₀	0.1	0.9	10	5		0.5		2.903	0.833
11.	S ₁₁	0.1	0.9	10	5		0.5		1.590	0.686
12.	S ₁₂	0.1	0.9	10	5		0.5		0.835	0.174
13.	S ₁₃	0.1	0.9	10	5		0.5		2.620	0.440
14.	S ₁₄	0.1	0.9	10	5		0.5		3.00	0.610
15.	S ₁₅	0.1	0.9	10	5		0.5		2.925	0.728



Graph 1: Quantitative estimation of protein of different protein powder samples.

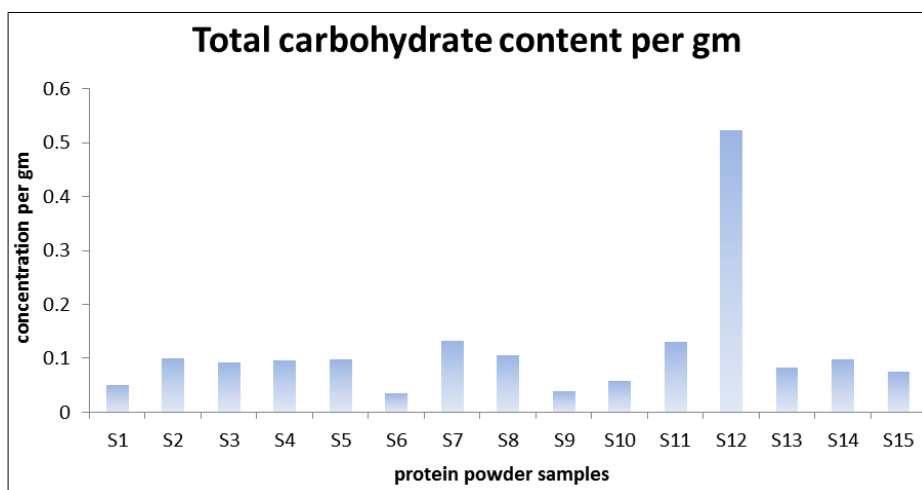
Quantitative Estimation of Total Carbohydrates by Phenol sulphuric acid Method

It is clear from Table 2 that all protein powders sold in markets of India, are having adequate carbohydrate content (2-6g/30g) as compared to those standard claimed readings on

the sachet. The results are in coherence with the claimed readings. Samples S₇ and S₁₂ was 7.6 grams less than the label claim. This is inside the accepted tolerance of +/- 8 grams (for high protein products). Although the sample S₁₁ have 1.8 g more carbohydrate as mentioned on the ingredient list.

Table 2: Quantitative estimation of total carbohydrate from different protein samples by Phenol sulphuric acid method

S. No.	Sample NO.	Sample (µl)	5% phenol	Sulphuric acid (ml)	OD at 540nm	Conc/gm
1.	S ₁	200	0.2	1	0.203	0.05
2.	S ₂	200	0.2	1	0.380	0.099
3.	S ₃	200	0.2	1	0.368	0.093
4.	S ₄	200	0.2	1	0.370	0.097
5.	S ₅	200	0.2	1	0.376	0.098
6.	S ₆	200	0.2	1	0.148	0.035
7.	S ₇	200	0.2	1	0.840	0.133
8.	S ₈	200	0.2	1	1.345	0.106
9.	S ₉	200	0.2	1	0.263	0.038
10.	S ₁₀	200	0.2	1	0.232	0.058
11.	S ₁₁	200	0.2	1	0.963	0.13
12.	S ₁₂	200	0.2	1	1.910	0.524
13.	S ₁₃	200	0.2	1	0.317	0.0823
14.	S ₁₄	200	0.2	1	0.374	0.098
15.	S ₁₅	200	0.2	1	0.298	0.076



Graph 2: Quantitative estimation of carbohydrate of different protein powder samples.

Quantitative test for Energy calculation:

Energy content is an important property of food. The total energy of one serving of the food is calculated by adding together the energy provided by the protein, total fat, and carbohydrate.

After analysis of collected protein powders are having adequate calories content (<100 kcal/30 mg) as compared to those standard claimed readings on the sachet. The results are in coherence with the claimed readings. Samples S₂, S₄, S₆, S₇, S₈, S₁₀ and S₁₂ have high energy level (/kcal) than the mentioned claim on sachet.

Table 3: Quantitative analysis of Energy from protein powder sample

S. No	Samples	Energy (Kcal)	Energy (Kcal)/gm
1.	S ₁	117.56	3.6
2.	S ₂	124.19	3.6
3.	S ₃	128.9	3.6
4.	S ₄	114	3.3
5.	S ₅	114	3.3
6.	S ₆	109.96	3.6
7.	S ₇	109	3.4
8.	S ₈	91.44	2.0
9.	S ₉	111.48	3.7
10.	S ₁₀	103.28	3.1
11.	S ₁₁	121.2	4.0
12.	S ₁₂	402.48	4.0
13.	S ₁₃	153.16	3.3
14.	S ₁₄	121.58	3.6
15.	S ₁₅	120.22	3.7

Table 4: Claimed components of protein powder

Sample No.	Protein (gms)	Carbohydrate (gms)	Energy (kcal)
S ₁ (32g)	28	1	110
S ₂ (34g)	24	5	140
S ₃ (35g)	24	4	130
S ₄ (34g)	24	3	120
S ₅ (34g)	24	5	130
S ₆ (30g)	27.3	1	112.5
S ₇ (32g)	20	8	141
S ₈ (30g)	12	4.5	103
S ₉ (30g)	23.7	2	115
S ₁₀ (33g)	24	2	114
S ₁₁ (30g)	23.7	1.5	119.5
S ₁₂ (100g)	20.6	64.4	388
S ₁₃ (46g)	30	4	160
S ₁₄ (33g)	25	3.8	128
S ₁₅ (32g)	23.56	2.5	125

Table 5: Experimental observation of the claimed components in protein powder sample

Sample No.	Protein (gms)	Carbohydrate (gms)	Energy (kcal)
S ₁ (32g)	27.12	1.6	117.56
S ₂ (34g)	21.32	3.39	124.192
S ₃ (35g)	18.77	3.28	128.9
S ₄ (34g)	20.71	3.30	114
S ₅ (34g)	23.90	3.36	114
S ₆ (30g)	26.20	1.065	109.96
S ₇ (32g)	30.2	4.264	109
S ₈ (30g)	11.42	3.2	61.36
S ₉ (30g)	19.6	1.14	111.48
S ₁₀ (33g)	27.5	1.94	103.28
S ₁₁ (30g)	20.6	3.9	121.2
S ₁₂ (100g)	17.4	52.4	402.48
S ₁₃ (46g)	20.26	3.79	153.16
S ₁₄ (33g)	20.14	3.24	121.58
S ₁₅ (32g)	23.32	2.46	120.22

Discussion

Protein powders are concentrated sources of protein from animal or plant foods, such as dairy, eggs, rice or peas. Hydrolysates appear to raise insulin levels more than other forms — at least in the case of whey protein. This can enhance your muscle growth following exercise. Whey protein is considered as an excellent protein for the choice of individuals of all ages for healthy diet and also to improve and maintain their health. Traditionally, Whey protein was only used by most of the athletes and bodybuilders to promote the muscle growth (Fry, 2003) but from past few years, whey protein is being used in some other applications. Some applications that using whey proteins are: cancer treatment, wound healing, infant health and weight loss. According to Hoffman and Falvo, additional benefits of Whey protein may include: Whey protein helps to increase the serotonin activity and helps to promote restful sleep (Mc Clements., 2010); Whey protein helps enhance energy levels; it helps to decrease the stress; it helps to keep the metabolic rate high; it helps to reduce body fat and build the lean body mass; and it helps to improve the memory loss under stress. This study deals with finding out various constituents and compositions of protein powder and analyzing the type of nutrient present in a food item helps plan a balanced. In this study to identify and estimate the quantity of the claimed components in Protein powders samples. Qualitative test performed for all claimed component in protein and carbohydrate. Some protein powder having protein concentration range content (< 24g/30g). Few samples like S₃, S₄, S₇, S₉, S₁₃ and S₁₄ which have 6-10 grams less protein than the label claims. Protein

powders sold in markets of India are having adequate reducing sugar content (0-2/30g) as compared to those standard claimed readings on the sachet. Samples S₇ and S₁₂ was 7.6 grams less than the label claim. This is inside the accepted tolerance of +/- 8 grams (for high protein products). Although the sample S₁₁ have 1.8 g more carbohydrate as mentioned on the ingredient list. There was vice versa result of total carbohydrate content which was found higher in sample S₈ and S₁₂ while low in sample S₁ and S₆. All protein powder having carbohydrate concentration ranges (2-6g/30g). Energy determined by total amount of nutrient present in protein powder and compared with shown similar results in all samples. All protein powder having calories content (<100 kcal/30 mg). Samples S₂, S₄, S₆, S₇, S₈, S₁₀ and S₁₂ have high energy level (/kcal) than the mentioned claimed on sachet.

Conclusion

All samples of protein powders used in the present study were acquired from a commercial retailer specialized on nutritional supplements. Fifteen protein powders manufactured in Indian market were investigated by qualitatively as well as quantitatively. After analysis it was found that all protein powder showed the presence of protein and carbohydrate, but in quantitative estimation it was found that the quantity claimed for protein, carbohydrate and energy showed variation as compared to claimed.

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