QUANTITATIVE ANALYSIS OF CHOLESTEROL IN PROTEIN POWDER

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Abstract: Protein supplements are frequently consumed by athletes and recreationally active adults to achieve greater gains in muscle mass and strength and improve physical performance. All protein powders sold in markets of India, are claimining that they have adequate cholesterol content (5-50 mg/30g). In this study 15 samples of protein powder were analysed and the quantity of cholesterol was calculated and compared with the claimed. After analysis of 15 samples of protein powder that is S_1-S_{15}. Out of 15 protein powder samples, 3 samples that is S_1, S_2 and S_{12} were detected with the lower quantity of cholesterol as compared to claimed. The variation in the cholesterol was seen in samples S_3, S_4, S_6, S_9, S_{10} and S_{15} which have 1.6 grams more than the label claim of 70 grams.

***Index Terms*: Protein powders, claimed components, cholesterol content.**

1. INTRODUCTION:-

Cholesterol is a fat-like, waxy substance found in the cells of the body. Cholesterol travels through the bloodstream in small packages referred to as lipoproteins; these packages are made out lipid (fat) on the inside and proteins on the outside. The two lipoproteins that carry cholesterol throughout the body are low-density lipoproteins and the high-density lipoproteins. Our body needs to have a healthy level of both types of proteins to maintain and sustain healthy functioning. Everybody needs some amount of cholesterol to make important substances such as vitamin D, hormones and other substances that help in the digestion of food. Our body produces all the cholesterol that it needs, but we can also consume cholesterol via high doses of certain foods. The tricky part is that while we do need some level of cholesterol in our body, our risk of getting heart disease increases if our cholesterol levels are too high. Different types of protein shakes affect your cholesterol in different ways, but studies show that whey protein and soy protein may lower cholesterol levels, optimize their particle size and reduce your risk of heart disease and metabolic syndrome. There are also other benefits of protein shakes, like weight loss and lower blood pressure. When discussing cholesterol, many people focus on the different types, like LDL, HDL and VLDL, but emerging research shows these may not be the right things to look at. Since the topic has significance in the forensic field as well, 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 distributers and the rules if flouted by illegally mislabelling products which finds its way into many homes across India. Food forensic is an emerging branch of forensic chemistry which comes to rescue when protein powder is mislabelled or adulterated. It helps to check food safety and quality amongst others. Food fraud in terms of mislabelling and adulteration is a growing problem in India. Cholesterol is both good and bad. At normal levels, it is an essential substance for the body. However, if concentrations in the blood get too high, it becomes a silent danger that puts people at risk of heart attack. Cholesterol determination essential for food quality.

2. METHODOLOGY: - The amount of cholesterol was determined by Zak's method.

Before analysis of cholesterol in protein powder required reagent were prepared as:

1. Stock ferric chloride 10g of pure dry ferric chloride was weighed and dissolved in 100 ml glacial acetic acid.
2. Ferric chloride precipitating reagent 1 ml of stock ferric chloride reagent was taken in a 100 ml of standard flask and made up to the mark with pure glacial acetic acid. .
3. Concentrated Sulphuric acid.
4. Cholesterol Solution.
(i) Stock Standard – 100 mg of cholesterol was dissolved in 100 ml of glacial acetic acid.
(ii) Working standard – 10 ml of stock was dissolved in 0.85 ml of stock ferric chloride reagent and made up to 100 ml with glacial acetic acid. The concentration of working standard is 100µg / ml.
Cholesterol reacts with ferric chloride in the presence of concentrated sulphuric acid to give a pink colour. The intensity of colour developed is directly proportional to the amount of steroids present and it is read at 540 nm in a spectrophotometer.

Procedure:-

Saponification conditions of milk fat: Milk fat (0.1-0.15 g) was taken in test tube with Teflon lined screw cap. 5 ml of (2.5%, 5%, and 7.5%) methanolic KOH was added and mixed thoroughly. Incubated the capped tubes in water bath for 80°, 85° & 90°C/ 20 min with intermittent shaking after every 5 min. cooled the contents under tap water.

Extraction of unsaponifiable matter: After saponification, 1 ml of distilled water was added to the contents followed by the addition of 5 ml of hexane. The final extract had the hexane removed by heating in a water bath (45°C).

Preparation of coloring reagent: The stock reagent was prepared by dissolving 10g of $FeCl_3.6H_2O$ in glacial acetic acid using a 100ml volumetric flask.

Working: - Prior to use, the 1.0ml of the stock reagent was transferred into a 100ml flask and make up the volume to 100 ml with glacial acetic acid.

Colour Reaction: The dried extracts from were resuspended in 3mL glacial acetic acid, 2mL of $FeCl_3$ coloring solution was added and the resultant colour was noted at 565nm (Pye Unicam UV1 Double Beam Scanning Spectrophotometer). The absorption was compared against an external cholesterol standard and the cholesterol content was calculated using the following equation.

$$\text{Cholesterol} = (\text{mg}/100\text{gm}) \frac{C \times DF \times 20 \times W}{4 \times 100}$$

Where: C = concentration of cholesterol (from standard curve); DF = dilution factor; W = weight of sample.

3. RESULT AND DISCUSSION:-

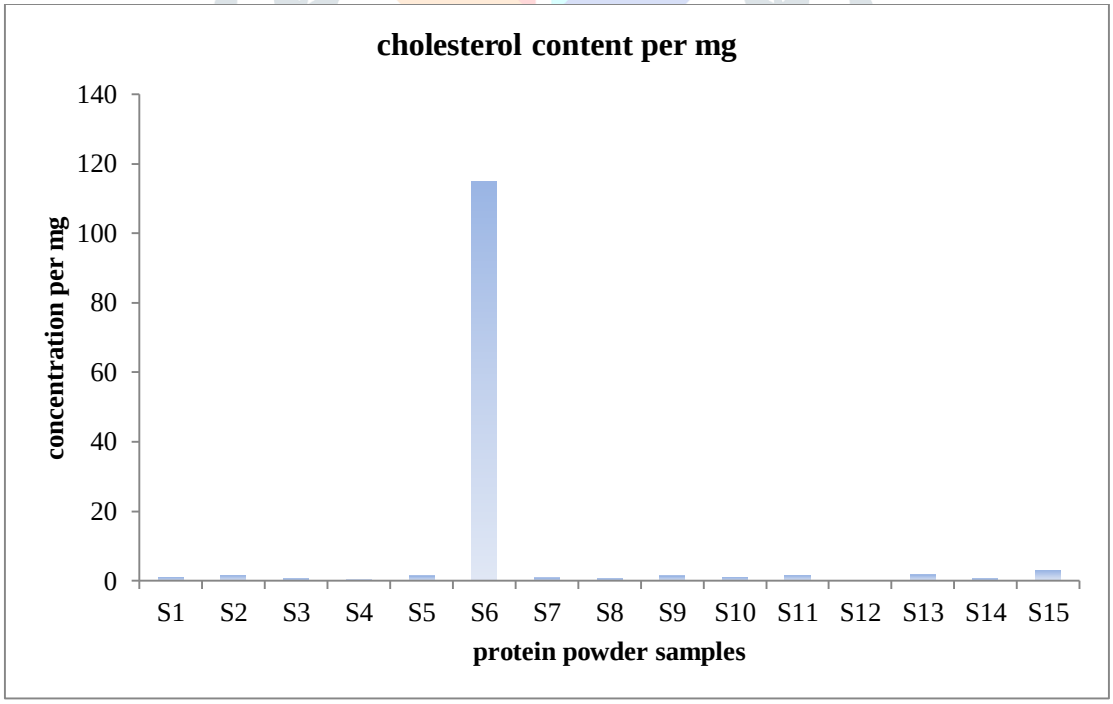
15 Protein powder sample was collected from local market of Lucknow, and stored in room temperature for further use.

In order to use a rapid analytical method for cholesterol determination in protein powder by UV-VIS Spectrophotometric method. All protein powder was analysed for cholesterol and average amount of cholesterol concentration was found.

All protein powders sold in markets of India, are having adequate cholesterol content as compared to those standard claimed readings on the sachet. Maximum results are in coherence with the claimed readings. After analyzing the table no. 1 which was shown the relation between amount of samples and OD (565nm) value, the quantity of cholesterol were estimated. The quantity of cholesterol in milligram were 1.08, 1.6, 0.6, 0.3, 1.4, 115, 0.9, 0.74, 1.44, 1.14, 1.6, 0.06, 1.8, 0.76, 3 for S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_9, S_{10}, S_{11}, S_{12}, S_{13}, S_{14}, S_{15} respectively.

Table 1: - Quantitative estimation of total Cholesterol from different protein samples by Zak's method

S.No	Sample Name	Sample (ml)	Hexane	Ferric chloride soln	OD at 565 nm	Cholesterol (mg)
1.	Sample S_1	5	5	2	0.902	1.08
2.	Sample S_2	5	5	2	1.755	1.6
3.	Sample S_3	5	5	2	1.756	0.6
4.	Sample S_4	5	5	2	1.276	0.3
5.	Sample S_5	5	5	2	0.986	1.4
6.	Sample S_6	5	5	2	0.952	115
7.	Sample S_7	5	5	2	1.849	0.9
8.	Sample S_8	5	5	2	1.726	0.74
9.	Sample S_9	5	5	2	1.075	1.44
10.	Sample S_{10}	5	5	2	1.094	1.14
11.	Sample S_{11}	5	5	2	1.519	1.6
12.	Sample S_{12}	5	5	2	1.881	0.06
13.	Sample S_{13}	5	5	2	1.152	1.8
14.	Sample S_{14}	5	5	2	2.190	0.76
15.	Sample S_{15}	5	5	2	1.859	3



Graph: - Quantitative estimation of cholesterol of different protein powder samples

Table 2: - Claimed components observation and experimental observation of cholesterol in protein powder:-

Sample No.	Claimed component of cholesterol(mg)	Experimental observation of cholesterol(mg)
S_1(32g)	30	32.4
S_2(34g)	70	54.6
S_3(35g)	15	**23.12**
S_4(34g)	5	**11.88**
S_5(34g)	55	49
S_6(30g)	3600	**3450**
S_7(32g)	21	28.8
S_8(30g)	22	22.2
S_9(30g)	58.02	**43.2**
S_{10}(33g)	48	**37.62**
S_{11}(30g)	-	48.
S_{12} (100g)	-	6.6
S_{13}(46g)	80	82.8
S_{14}(33g)	33	25.08
S_{15}(32g)	92.4	**96**

After analysis of 15 samples of protein powder that is from S_1-S_{15}, it found that the low quantity in sample S_1, S_2, and S_{12}. The variation in the cholesterol was seen in samples S_3, S_4, S_6, S_9, S_{10} and S_{15} which have 1.6 grams more than the label claim of 70 grams.

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. Some powders are also fortified with vitamins and minerals, especially calcium. As cholesterol also present in protein powder as one of its component and intake of this cholesterol should be in limited amount. Food fraud in terms of mislabeling and adulteration is a growing problem in India. Cholesterol is both good and bad. At normal levels, it is an essential substance for the body. However, if concentrations in the blood get too high, it becomes a silent danger that puts people at risk of heart attack. Cholesterol determination essential for food quality, this type of analysis has a disadvantage is that they require dedicated laboratory equipment and skilled staff which makes them expensive and time-consuming to carry out. So, in this we used Zak's method for Cholesterol analysis was also determined by rapid method. The quantitative analysis of cholesterol was performed by using spectrophotometric method. The result was found low quantity in sample S_1,S_2 and S_{12}.All protein powders sold in markets of India, are having adequate cholesterol content (5-50 mg/30g) as compared to those standard claimed readings on the sachet. Variation in the cholesterol was seen in samples S_3, S_4, S_6, S_9, S_{10} and S_{15} which have 1.6 grams more than the label claim of 70 grams. The WPC as it carries excellent nutritional and functional properties, find great potential in various food formulations. The largest potential use of whey solids as a replacement for non-fat dry milk (NFDM) in the food industry **(Mann., 1987)**. WPC can also be seen competing with casein, egg albumin and soy proteins with the existing markets **(Melachouris *et al.*, 1984)**. More product formulation work specially in food industry is needed for WPC. Because of the unique inherent properties of WPC, there lies a strong potential of its utilization in acid foods, beverages, meat products, bakery products, infant foods, humanized milks, cheese, fermented products and dietetic and therapeutic applications **(Guptha and Thapa *et al.*, 1991)**.

4. SUMMARY AND 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. In this study, we have measured the cholesterol concentration of protein powders. All protein powders sold in markets of India, are having adequate cholesterol content (5-50 mg/30g) as compared to those standard claimed readings on the sachet. After analysis of 15 samples of protein powder that is from S_1-S_{15}, it found that the low quantity in sample S_1, S_2, and S_{12}. The variation in the cholesterol was seen in samples S_3, S_4, S_6, S_9, S_{10} and S_{15} which have 1.6 grams more than the label claim of 70 grams.

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