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Total Storage Protein and Protein Fractions in Different Sunflower Lines

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ABSTRACT: Sunflower, though major source of vegetable oil, has been lately recognised for the importance of its seed proteins in human and animal foods. Although, there are reports regarding protein content and globulin content in sunflower seeds, but these are generally limited to single sunflower line. In present studies, the experiments were conducted by using 8 lines of Sunflower. The protein content in dehulled and defatted seed meal ranges from 29-47.3% and the seed meal is emerging as an alternative source to the conventional food sources. The distribution of four protein fractions shows the same trend as legumes, having salt soluble globulin fraction as major component.

KEYWORDS: Sunflower, protein, protein fraction, nutritive value

I. INTRODUCTION

Helianthus annuus is grown all over the world and is a native crop of North America. Approximately, 16 million tonnes of sunflower oil is produced all over the world. Ukraine and Russia are the main producers of Sunflower. Sunflower in bread-and-butter production (Martins *et al.*, 2017) is a composite ingredient. It is globally the fourth most profitable and economic oil seed crop after soybean, rapeseed and safflower. Sunflower is nutritionally and economically important crop. Sunflower oil has long shelf life and high temperature stability, and they are good substitute to trans fats (Cheng *et al.*, 2019).

Due to presence of large amount of oleic acid it is known as good vegetable oil in human diet. Its seeds have high nutritive value and are rich in antioxidants, phenolic acids, dietary fibres, minerals, essential amino acids and MUFA and PUFA (Alagawary *et al.*, 2016). As sunflower is rich in proteins, it is used as a substitute of Soybean. Aspartic acid, cysteine, lysine, valine, tyrosine, methionine, phenylalanine, glycine etc. are present in seeds of sunflower (Karangwa *et al.*, 2015). The sunflower seeds are processed into flour, roasted, baked or boiled and this processed form have low carbohydrates and high protein, fibres, vitamins and minerals (Shahbaz *et al.*, 2018) and are effective in controlling cancer, heart disease, diabetes and hypertension (Katsarou *et al.*, 2015). Natural functional oil from plants is used as phyto-medicines and is used to cure many human diseases (Maqsood *et al.*, 2020).

Sunflower is also used in paints, lubricants, biodiesel and drugs production. At commercial level sunflower seeds are primarily used for oil content, but as its seeds have high protein content and other nutrients, sunflower may be used as human dietary protein (Dadalt *et al.*, 2016).

Sustainability and circular economy is the need of today and we have to develop technologies to minimize the waste during supply chain and use the by-products produced during process. Sunflower oil cakes are the by-products obtained after oil extraction and can be used for human use. Around 21.9 million tonnes sunflower meal is produced in 2019 all over the world. (Rahoveanu *et al.*, 2018).

To increase the biomass of animals, sunflower meal is used as a source of proteins (Serrapica *et al.*, 2019). People interested in healthy lifestyles are recommended to consume sunflower seeds as they are rich in nutrients and have high nutritive profile. The sunflower meal has high protein content (Maliket *et al.*, 2017; Malik & Saini, 2018). essential amino acids, (Hussain *et al.*, 2020; Nevara *et al.*, 2021; Dhingra *et al.*, 2022), vitamin B, minerals (Wanjari & Waghmare, 2015; Islam *et al.*, 2016; Gupta *et al.*, 2018; Adeleke & Babalola, 2020), dietary fibres, high antioxidant property, large amount of oleic acid and high ratio of linoleic/ linolenic fatty acids. Antinutritional content such as Protease and Arginase inhibitor saponins, insoluble fibres etc, present in the sunflower meal (Grasso *et al.*, 2019) can be removed by adequate processing (Adesina, 2018).

II. MATERIALS AND METHODS

Following 8 lines of *Helianthus annuus* are used:

APSH-11, P271 R, 7-1A, CMS- 234A, LDMRSH-3, MRHA-1, KBSH-1, CMS-207A

Methods

Preparation of Defatted Seed Meal: To analyse seed meal, it was first defatted. The kernels were dehulled and pulverized to a fine powder in the grinder. One gram of seed meal and 10 ml of hexane were stirred at 40°C for two hours and then centrifuged in a bench centrifuge for 10 min. Hexane in the supernatant was decanted. The process is repeated twice with hexane. The pellet which has defatted seed meal was spread in a Petridish and dried under vacuum.

Protein Estimation: Protein estimation was carried out by two different methods as follows:

1) Semi-micro Kjeldahl Method

Protein in sunflower seed meal was estimated by Semi- micro Kjeldahl Method. 100 mg of the dried seed meal was digested in 5ml of concentrated H₂SO₄. 100mg of the catalyst mixture (20 parts of K₂SO₄, 1 part of CuSO₄, 1 part of SeO₂) was added for rapid digestion. Digestion was carried out until the solution became clear and the colour changed to apple green. This solution was then distilled by adding 40% NaOH in Markham's distillation unit. Ammonia so evolved was collected in a conical flask containing 4% Boric acid. Its titration against N/40 HCl gave its concentration and hence of nitrogen in the seed meal. This was calculated as per the following formula:

$$\%N = v \times n \times 14 \times 100/w$$

Where,

n = Normality of HCl

v= Volume of HCl used

w= Weight of sample

The nitrogen so obtained was multiplied by 5.3 (multiplication factor) to get protein content of the seed.

Separation of seed Protein fraction:

Protein fractions were separated by solubility differences method. Extraction of 1gram defatted sunflower seed meal with 10 ml Borate buffer (50mM, pH 8.0) was done by stirring the solution on magnetic stirrer for 2 hours at 4°C. After this the solution was centrifuged at 23,000g at 4°C in a refrigerated centrifuge for 40 minutes. The extraction is done twice, and the supernatant was collected, this supernatant '1' contained two protein fractions i.e. albumins and globulins

10ml of 0.1 N NaOH was used for the extraction of pellet and this solution was then centrifuged for 20 min at 14,000g at 4°C. This process was also repeated twice. The supernatant '2' represented glutelins. The residue left after this was further extracted for prolamins, the fourth protein fraction with 70% ethanol and was centrifuged at 9,000 g for 10 min at 4°C in the supernatant '3'.

To separate albumins and globulins from supernatant '1', it was dialysed against the acetate buffer (33mM & pH 4.8). Supernatant '1' get precipitated and then centrifuged for 40 minutes at 23,000 g. The supernatant thus obtained was collected (Supernatant '4') and it contained albumins while, pellet had globulins which was dissolved in minimum amount of borate buffer.

2) Estimation of protein fractions by Lowry's Method

Lowry's method with few modifications was used to estimate the protein concentration in all the four protein fractions. 0.1 ml of protein fraction was added to 5ml of Lowry's mixture (1% CuSO₄, 2% K-Na- tartarate and 2% Na₂CO₃ in 0.1N NaOH in the ratio 1:1:48) and then left it for 10 minutes. After this 0.2 ml of Folin and Ciocalteu's phenol reagent (1N) was added. To develop the colour, allowed it to rest for 30 min. The intensity of the colour so developed was read at 540 nm using a spectrophotometer.

Protein concentration in the respective protein fractions was determined from a standard curve prepared by using different concentrations of Bovine Serum Albumin in the range of 100 µg to 800 µg.

RESULTS AND DISCUSSION

Seed Protein Content:

Total Protein in dehulled sunflower seed meal as estimated by semi-micro Kjeldahl method is given in Table 1. Percentage of protein in dehulled seeds varied over a range of 29.0 to 47.3. Whereas, it was highest (47.3%) in line LDMRSH-3 and line P-271 R had the lowest (29.0%) protein content.

Seed Protein Content of Various Sunflower Lines

Sr. No.	Sunflower Line	Protein Percent (% N x 5.3)
1	APSH-11	45.61
2	7-1-A	42.60
3	P-271-R	29.0
4	KBSH-1	33.1
5	CMS-234A	46.0
6	LDMRSH-3	47.3
7	MRHA-1	43.6
8	CMS-207A	43.5

Table 1

Seed Protein Fractions Ratio

Protein concentration in four protein fractions was determined by using a BSA (Bovine-Serum-Albumin) standard curve prepared by Lowry’s method (Fig.1) The protein content present in each fraction was used to analyse the proportion of four protein fractions with respect to each other. The proportion of these four protein fractions i.e., albumins, globulins, glutelins and prolamins in different lines of sunflower seeds can be seen in Table 2. The salt soluble globulins represented the maximum proportion in all the eight lines. This varied from 63.0 in line CMS 207A to 68.6 in line KBSH-1. These were followed by glutelins, albumins and prolamins. Whereas, line CMS-234A had the highest proportion (17.3%) of glutelins, line LDMRSH-3 has the lowest (14.7%) glutelin proportion. The third ranking fraction, albumins was the highest (14.9%) in line CMS 207A and lowest (11.5%) in line KBSH-1. Relative concentration of prolamins was the lowest of all protein fractions and varied from 4.0% in line LDMRSH-3 to 6.0 % in CMS 207A.

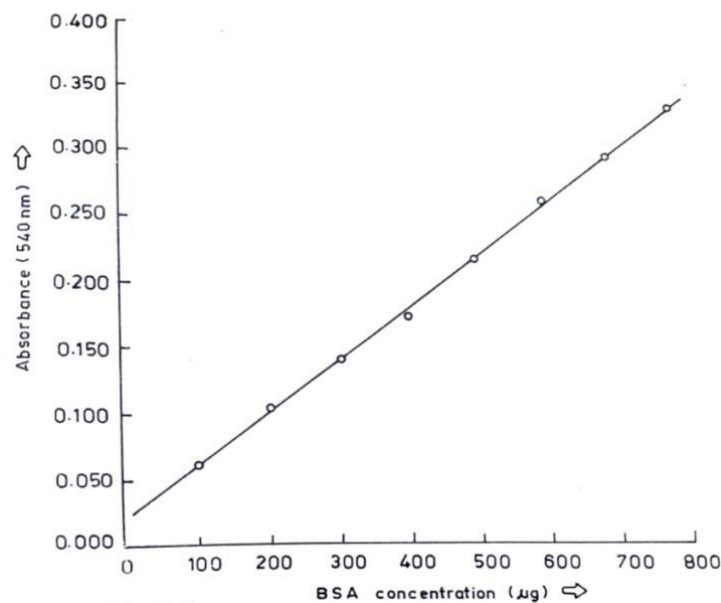


Fig. 1 (Standard curve of BSA for protein estimation by Lowry’s Method)

Sr. No.	Sunflower Lines	Albumins (g/100g)	Globulins (g/100g)	Glutelins (g/100g)	Prolamins (g/100g)
1	APSH-1	13.4	63.8	17.0	5.8
2	7-1-A	13.6	64.2	16.7	5.5
3	P-271 R	11.5	63.3	17.1	5.8
4	KBSH-1	11.5	68.6	14.9	5.0
5	CMS-234A	13.2	64.0	17.3	5.5
6	LDMRSH-3	13.3	68.0	14.7	4.0
7	MRHA-1	14.6	64.0	16.0	5.4
8	CMS-207 A	14.9	63.0	16.3	6.0

Table-2 (Proportion of Four Seed Protein Fractions in Sunflower)

Storage proteins are the most important indicators of the quality of grains. Cereals are a basic foodstuff all over the world and contain around 75% saccharides, 10 to 15% proteins and 2% fats. Cereals are rich in prolamins and may cause autoimmune diseases in some individuals.

Pseudocereals are also rich in starch, but unlike cereals, they are dicotyledonous and crops like Amaranth and Buckwheat are good alternatives for patients suffering from coeliac disease caused due to cereals, as these are gluten free crops.

Legumes seeds are rich in protein content and globulin fraction is the major protein fraction and constitutes around 50-60% of total protein content. Legumes are characterised by a higher content of proteins as compared to cereals and pseudocereals.

The amount of protein in defatted sunflower seed meal varied from 29 to 47 percent. All the four protein fractions were present in varying proportions. Globulin represented the dominating fraction (65 to 70%) while prolamin was the lowest (4 to 6%). The proportion of glutelin and albumin varied between 14 to 17% and 12 to 15% respectively. The albumin and globulin fractions of proteins have large amount of essential amino acids and have positive influence on the nutritional quality of products. Thus, sunflower proteins have the proportion of these fractions in a trend similar to that of legumes. As, sunflower seed meal or cake has high protein content, it can be used in human diet and animal feed. It also has many applications in agriculture, pharmaceuticals and food processing.

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