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RESEARCH ARTICLE

BIOCHEMICAL EVALUATION OF “*Spinaciaoleracea*” L. (SPINACH).

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Key words:-

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Abstract

Through biochemical compositions we can study many important nutritional aspects essential for human body. The study was held to determine the seasonal variation of fats and carbohydrates from leaf, stem and root of *Spinaciaoleracea* L. which is one of the most enrich vegetable used all over the world by people. Comparative account of carbohydrate in summer was higher compared to winter and monsoon but at the same time it was highest in stem during summer and highest in root during winter and monsoon. Fats content were high during summer compared to winter and monsoon, but roots showed high concentration of fats compared to stem and leaves.

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Introduction:-

S. oleracea, commonly called spinach, was long considered to be belonging to the family Chenopodiaceae, but in 2003, that family was integrated into the family Amaranthaceae in the order Caryophyllales. Spinach belongs to subfamily Chenopodioideae in family Amaranthaceae. Variation is observed in the older and more modern varieties of the spinach plant. During warm conditions older varieties are more liable to bolt early. Newer varieties tend to grow more rapidly, but have less of an incline to run up to seed. The older species tend to have a stronger and bitterer taste and narrower leaves. Broader leaves and round seeds are mostly observed in the new varieties.

In the ranking list for nutrient richness, among the World's Healthiest vegetables, Spinach tops the chart. Not just rich in vitamins and minerals, but is also concentrated in health-promoting phytonutrients like the carotenoids (beta-carotene, zeaxanthin, and lutein) and flavonoids which produce the powerful antioxidant protection. Spinach is of much importance to skin, bone, hair as it provides proteins, minerals, iron and vitamins to us. It acts as a major precaution for blood glucose control in diabetics, low blood pressure, cancer risk, weak bones, asthma and many more.

Variation is observed in the older and more modern varieties of the spinach plant (Barzegar M, Erfani F, Jabbari A, Hassandokit MR, 2007). During warm conditions older varieties are more liable to bolt early. Newer varieties tend to grow more rapidly, but have less of an incline to run up to seed. The older species tend to have a stronger and bitterer taste and narrower leaves. Broader leaves and round seeds are mostly observed in the new varieties.

The research work regarding the chemical processes within or relating to living organisms is said to be biochemistry, also known as biological chemistry. The complication of life can occur during biochemical process, if we control the information flow through biochemical signaling and through metabolism chemical energy flow. Various study fields like botany, medicines, genetics, etc. (Voet 2005). which are areas of life science, are engrossed in research related to biochemical because biochemistry has made it easy to understand the living processes, this

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made biochemistry more successful since last few decades of the 20th century. Now-a-days, studying and understanding the biological molecular processes that takes place within a living cell is greatly to the whole organisms due to the main focus on pure biochemistry.

Materials And Methods:-

The required species of Spinach was collected from the local market and was used for preparing the dry powder. Before sun drying it was separated into leaf, stem & root. The fresh material was used for chlorophyll estimation. The remaining was dried & converted into powder form. This particular procedure was carried out in seasonal format i.e., summer, monsoon & winter respectively.

Quantitative estimation of total Carbohydrate:

Carbohydrates were estimated by methods suggested by McGready (1950), and Nelson (1941).

Reagents:-

- Somogy's reagent (4 gm. CuSO_4 +24 gm. anhydrous Na_2CO_3 +16 gm. Na-K tartarate (Rocheette salt) + 180gm Anhydrous Na_2SO_4 .
- Nelson arsenomolybdate reagent :- (24gm $(\text{NH}_4)_6\text{MO}_7\text{O}_{24}$, $4\text{H}_2\text{O}$ Ammonium molybdate) + (3gm Na_2SO_4 , $7\text{H}_2\text{O}$).
- Both solutions were mixed and incubated at 37°C for 24 hours before use and they were stored in brown bottle.
- Standard sugar solution was prepared by dissolving 10 mg glucose in 100 ml distilled water.

Procedure:-

1. 1gm. of sample was crushed with 10ml 80% ethanol in mortar and pestle by adding acid free sand, and then filtered through Whatman filter paper. The filter and residue were collected separately.
2. The alcohol residue was taken in 250 ml in conical flask. 150ml distilled water and 5ml conc. HCL were added in it. Hydrolysed for 30 minutes and cooled to room temperature. Na_2CO_3 was added bit-by bit until the extract became neutral (pH=7). The extract was filtered. Residue was discarded. Total volume of filtered was served as a sample for starch. First filtrate was taken in conical flask and condensed on water bath up to 2-3 minutes then distilled water was added to the filtrate, and then filtered, after mixing residue was discarded and the volume of filtrate was served for reducing sugar.
3. 20 ml of this filtrate was taken in 150 ml conical flask, 2ml of conical flask; 2ml of conc. HCl was added to it and corked. It was then hydrolysed for 30 minutes and cooled at room temperature. Na_2CO_3 was added bit-by bit until the extract became neutral (pH=7). Then this extract filtered and residue discarded. The final volume of the filtrate was measured. It was served as a sample for total sugar.
4. 0.5 ml of aliquot sample was taken in each test tube and 1 ml of Somogy's reagent was added in it. All test tubes were placed in boiling water bath for 30 minutes, cooled the tubes to room temperature and 1ml of arsenomolybdate reagent which is poisonous was added to it. The content was mixed thoroughly. Then the content was diluted to a volume of 10ml and its absorbance measured OD at 560 nm in spectrophotometer.

Estimation of fats:-

A small quantity of free acids is usually present in oils along with the triglycerides (Cox H.E. and Pearson. D, 1962). The free fatty acid content is known as acid number/acid value. It increases during storage. The keeping quality of oil therefore relies upon the free fatty acid content.

Reagents:-

- 1% phenolphthalein in 95% ethanol
- 0.1N potassium hydroxide
- Neutral solvent: Mix 25ml 95% alcohol and 1ml of 1% phenolphthalein solution and neutralize with N/10 alkali.

Procedure:-

Dissolve 1-10g of oil or melted fat in 50ml of the neutral solvent in a 250ml conical flask. Add a few drops of phenolphthalein. Titrate the content against 0.1N potassium hydroxide. Shake constantly until pink colour which persists for fifteen seconds is obtained.

Result And Discussion:-

Total Carbohydrates:-

The total carbohydrate content of leaves, stem & root were usually higher in summer as compared to winter and monsoon.

The range of total carbohydrate content of leaves was 3.75 mg/g dry wt. to 3.49 mg/g dry wt. where in summer accumulation of total carbohydrates was (3.75 mg/g) than in winter (3.66 mg/g) and in monsoon it was found lowest (3.49 mg/g).

Where as in stem it ranged from 6.13 mg/g to 3.55 mg/g dry wt., in Monsoon it was recorded lowest (3.55 mg/g) & highest in summer (6.13 mg/g) whereas modest in winter (4.37mg/g).

The range of total carbohydrate in root was ranged from 6.06mg/g to 4.67mg/g dry wt., in summer it was highest (6.06 mg/g) compared to monsoon (4.67 mg/g) & winter (5.06 mg/g). The comparison can be studied in the following table-1 below.

Fats:-

The fats content of leaves was found in the range of 0.033 to 0.01 mg/g dry wt., in summer it was highest (0.033mg/g) compared to winter (0.015 mg/g) and monsoon (0.010 mg/g).

Where as in stem it ranged from 0.014 to 0.005 mg/g dry wt. in monsoon it was lowest (0.005 mg/g), in winter was modest (0.011 mg/g) & in summer was highest (0.014 mg/g)

The concentration of fats was highest in root as compared to leaf & stem. It ranged from 0.038 to 0.009 mg/g dry wt., in summer it was highest (0.038 mg/g), in winter it was modest (0.028 mg/g) & in monsoon was lowest (0.009 mg/g). The comparison can be studied in following table no.2.

Conclusion:-

Carbohydrates can be observed from table no.1 and seen that their production was high in summer, to be precise in the stem region during summer and in root region during winter and monsoon. Fats content observed in table no.2 was high in summer in the root region while in monsoon and winter it was high in leaf region. This result indicates that the particular season shows high amount which is beneficial for that particular product harvesting.

Table No.1: Seasonal variation in total carbohydrate.

Sr. No	Plant Part	Seasons (Total Carbohydrate) (Mg/g dry wt.)		
		Summer	Monsoon	Winter
1	Leaf	3.75	3.49	3.66
2	Stem	6.13	3.55	4.37
3	Root	6.06	4.67	5.06

Table No. 2: Seasonal variation in fats.

Sr. No	Plant Part	Seasons (Fats) (Mg/g dry wt.)		
		Summer	Monsoon	Winter
1	Leaf	0.03	0.01	0.015
2	Stem	0.014	0.005	0.011
3	Root	0.038	0.009	0.028

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