

# Product Development with Wheat Grass and Nutrient Analysis

Suneetha Runjala<sup>1</sup>, Dr. YLN. Murthy<sup>2</sup>

<sup>1</sup>Research Scholar, Department of Home Science, Acharya Nagarjuna University

<sup>2</sup>Professor, Department of Organic Chemistry Andhra University, Visakhapatnam, Andhra Pradesh

**Abstract:** Shoot of *Triticum aestivum* Linn. is called as a wheat grass, belonging to family: Gramineae, which possesses high chlorophyll content and essential vitamins, minerals, vital enzymes, amino acids, dietary fibre. Wheat grass has been shown to possess anti-cancer activity, anti-ulcer activity, antioxidant activity, anti-arthritis activity, and blood building activity in Thalassemia Major. It has been argued that wheat grass helps blood flow, digestion and general detoxification of the body. The major clinical utility of wheat grass in diseased conditions might be due to the presence of biologically active compounds and minerals in it and due to its antioxidant potential which is derived from its high content of bioflavonoid. The presence of 70% chlorophyll, which is almost chemically identical to haemoglobin, in wheat grass makes it more useful in various clinical conditions involving haemoglobin deficiency. A product development with wheat grass and nutrient analysis is an attempt to find out the nutrient composition of wheat grass powder even under processing of baking at the temperature of 100 to 150°C biscuits were prepared in various percentages of 20 gm, 30gm and 40 gm wheatgrass powder which is rich in energy, protein, fat, calcium, iron, and vitamin A, E and C essential for immune system of human body. These elements were analysed in nutrition laboratory and also conducted palatability test by nutrition experts and tested shelf life of the product developed for four months in mean time of 30 days. There is no rancidity, microbial or fungal growth at room temperature in airtight packing.

**Keywords:** Product development, Nutrient Analysis, Vitamins and minerals, Hormones, anti carcinogenic, amino acids

## 1. Introduction

### Source and Chemical Constituents

#### Classification of *Triticum aestivum*

Kingdom : Plantae  
Division : Magnoliophyta  
Class : Liliopsida  
Order : Cyperales  
Family : Gramineae  
Genus : *Triticum*  
Species : *aestivum*

Shoot of *Triticum aestivum* Linn. is called as a wheat grass, belonging to family: Gramineae. *Triticum* is a genus of annual and biennial grasses, yielding various types of wheat, native to south west Asia and the Mediterranean region. *T.aestivum* Linn. common or bread wheat, is widely cultivated almost all over the world. Generally, 15-20 species are recognized, of which 8 have been reported to occur in India. Wheat grass is a good source of mineral nutrients. It contains significant amount of iron, phosphorus, magnesium, manganese, copper & zinc. Wheatgrass is a rich source of Tocopherols with high vitamin E potency. Wheatgrass stimulates metabolism, restores alkalinity to the blood, its abundance of alkaline minerals helps reduce over acidity in the blood. Wheatgrass is also a de-toxicant and helps restore healthy cells. Wheat grass, young grass of common wheat plant, is freshly juiced or dried into powder for animal and human consumption- both the forms provide chlorophyll, amino acid, minerals, vitamins and enzymes. Many active compounds of wheat grass can cleanse the blood and neutralize and digest toxins in cells. Japanese scientist Dr. Yoshihide Hagiware M.D., found that the enzymes and amino acids in grass plants deactivated the carcinogenic and mutagenic effects of 3,4 benzpyrene, a

substance found in smoked fish and charcoal-broiled meats. Wheatgrass is a good source of 'B' vitamins, which facilitate the use of carbohydrates for energy and aid the nervous and digestive system. Steadily supply of B vitamins is also essential for normal brain and body development.

Lysine improves immune system and potential anti-aging factor. Isoleucine is also needed for growth, especially in infants, and for protein balance in adults. A deficiency of isoleucine could end in mental retardation, as it affects the production of other amino acids. Lucine, Is an amino acid that keeps us alert and awake. Cryptophane, Is essential for building rich red blood, healthy skin, and hair. It also helps to calm the nerves & stimulate better digestion. Phenylalanine, which aids the thyroid gland in its production of thyroxine hormone necessary for mental balance & emotional calm. Threonine, which stimulates smooth digestion, assimilation of foods and overall body metabolism. Valine, which activates the brain, aid muscle co-ordination and calms the nerves. Methionine, which helps, cleanses & regenerates kidney and liver cells. Alanine, blood builder, Arginine, which is essential to men and it present large amount in seminal fluids. Aspartic acid, It helps in the conversion of food into energy. Glutamic acid, which improves mental balance and provides for smooth metabolic function. Glycine, helps in the process whereby cells use oxygen to make energy. Histidine, which seems to affect hearing and nervous function. Proline, which becomes Glutamic acid & performs the same tasks. Serine, a stimulator of the brain and nerve functions. Tyrosine, which aids the formation of hair & skin & prevents cellular aging.

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## 2. Objectives

- 1) To select ingredients which are suitable for the developing Macro-Micronutrient rich products using wheatgrass powder.
- 2) To develop the product using appropriate processing condition.
- 3) To study the nutrient composition of the developed product.
- 4) To conduct the acceptability studies by consumers on the developed product.
- 5) To study the shelf life of the developed product.

## 3. Materials and Methods

### 3.1 Germination of Wheat Grains

Superior good quality wheat was procured, and cleaned properly. The wheat grains were soaked in cold water for 12 hours. The process of soaking helps the wheat grains to become tender. It also reduces the phytin content of wheat. After 12 hours of soaking the water was strained and the soaked grains were tied in wet woven cotton cloth and hung for a period of 12 hours. Water was sprinkled over the cotton cloth at least thrice during germination period. Moisture and warm temperature are needed during the germination period.



The proximate principles, amino acid composition, vitamin and mineral content of wheat germ, bran and grass were estimated. The proximate principles namely moisture, total ash, total fibre, carbohydrate, total fat and protein were analyzed by the standard procedures of National Institute of Nutrition (NIN, 1999). The amino acids from wheat grass were analyzed using High Performance Liquid Chromatography (HPLC) (Heinrikson and Meridith, 1984). The minerals viz; calcium, phosphorus, iron, sodium, zinc, copper and selenium and vitamins viz; vitamin A, vitamin C, vitamin D, vitamin E, thiamine, riboflavin, niacin, folate and vitamin B<sub>12</sub> were analyzed using standard procedures of NIN (1999)

Below nutrients were analysed in food and nutrition lab and HPLC conducted in RV labs guntur

- a) Moisture
- b) Fat
- c) Ash
- d) Iron
- e) Protein
- f) Vitamin – A
- g) Vitamin – E

### 3.4 Research Design

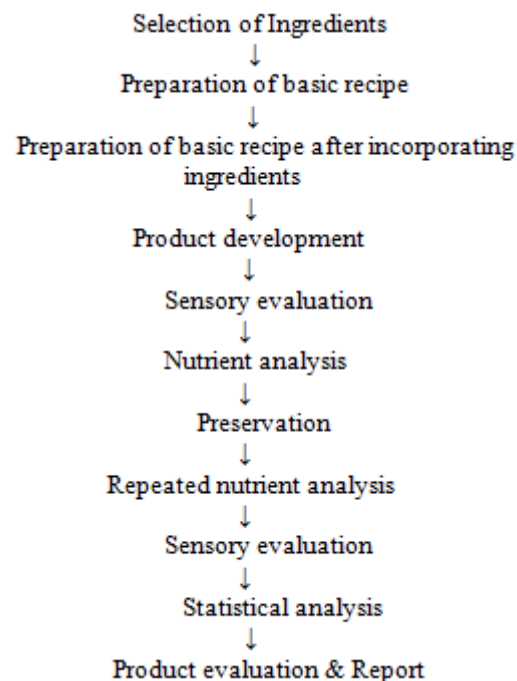
During this process, enzymes get activated, thus increasing the availability of nutrients and digestibility. It also increases the ration of non essential amino acids and content of vitamins like riboflavin, niacin and biotin. It also increases the action of cytases and pectinases. It releases minerals like calcium, zinc and iron from their bound form. This process also reduces trypsin inhibitor factors (Jensem et al., 2005). Wheat sprouts contain four times more folic acid and six times more vitamin C than un sprouted wheat or ordinary grass (Davis et al, 1999)

### 3.2. Cultivation of Wheat Grass

After 12 hours of germination, the germinated wheat was sowed in a shady place. Since wheat can grow in all temperatures, shady place is preferred to avoid excess nutrient loss due to exposure to direct sunlight. The sowed seeds started to grow and on the seventh day, the grass reached the length of 15 to 18cm which was then harvested (Ben and Goldin, 2002). 150g of wheat was required to cultivate 100g of wheat grass

### 3.3 Analysis of wheat grass

Nutrient content of wheat grass grown in trays



The below methods were used to analyse the nutrients in wheatgrass biscuits.

**3.3(A) Moisture:** About 10 gm of the material is weighed into a weighed moisture box and dried in oven at 100 to

105°C and cooled desiccators. The process of heating and cooling is repeated till a constant weight is achieved.

$$\text{Moisture \%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of the sample}} \times 100$$

**a) Basic plain biscuits:**

$$\begin{aligned} W_1 &= 58.47 \\ W_2 &= 68.47 \\ W_3 &= 68.19 \\ &\Rightarrow \frac{W_2 - W_3}{\text{sample wt}} \times 100 \\ &\Rightarrow \frac{68.47 - 68.19}{10} \times 100 \\ &\Rightarrow \frac{0.28}{10} \times 100 = 2.8 \text{ for 100gm of sample} \end{aligned}$$

**3.3(A) Wheat grass plain biscuits 20gm:**

$$\begin{aligned} W_1 &= 47.20 \\ W_2 &= 57.20 \\ W_3 &= 57.08 \\ &\Rightarrow \frac{W_2 - W_3}{10} \times 100 \\ &\Rightarrow \frac{57.20 - 57.08}{10} \times 100 \\ &\Rightarrow \frac{0.12}{10} \times 100 = 1.2 \text{ for 100gm of sample} \end{aligned}$$

**3.3(A) Wheat grass 20gm with cashew:**

$$\begin{aligned} W_1 &= 53.92 \\ W_2 &= 63.92 \\ W_3 &= 63.75 \\ &\Rightarrow \frac{W_2 - W_3}{\text{sample}} \times 100 \\ &\Rightarrow \frac{63.92 - 63.75}{10} \times 100 \\ &\Rightarrow \frac{0.17}{10} \times 100 = 1.7 \text{ for 100gm} \end{aligned}$$

**3.3(A) Wheat grass 30gm with cashew:**

$$\begin{aligned} W_1 &= 55.98 \\ W_2 &= 65.98 \\ W_3 &= 65.73 \\ &\Rightarrow \frac{W_2 - W_3}{\text{sample}} \times 100 \\ &\Rightarrow \frac{65.98 - 65.73}{10} \times 100 \\ &\Rightarrow \frac{0.25}{10} \times 100 = 2.5 \text{ for 100gm} \end{aligned}$$

**3.3(A) Wheat grass 40gm with cashew:**

$$\begin{aligned} W_1 &= 51.10 \\ W_2 &= 61.10 \\ W_3 &= 60.79 \\ &\Rightarrow \frac{W_2 - W_3}{\text{sample}} \times 100 \\ &\Rightarrow \frac{61.10 - 60.79}{10} \times 100 \\ &\Rightarrow \frac{0.31}{10} \times 100 = 3.1 \text{ for 100gm of sample} \end{aligned}$$

**3.3(B) Fat estimation**

Fat is estimated from crude ether extract of the dry material. The dry sample (5 – 10g) is weighed accurately into thimble & plugged with cotton. The thimble is then placed in soxhlet

apparatus and extracted with anhydrous ether for about 16hrs. The ether extract is filtered into a weighed conical flask. The flask containing the ether extract is washed 4 to 5 times with small quantities of ether in the washings are also transferred. The ether is then removed by evaporation and the flask with the residue dried in an oven at 80 – 100°C cooled in a desiccator and weighed.

$$\text{Fat content (gm /100 sample)} = \frac{\text{wt. of ether extract}}{\text{wt. of the sample (equivalent to fresh sample taken)}} \times 100$$

$$\begin{aligned} W_1 [\text{Flask weight}] &= 240 \text{ gm} \\ W_2 [\text{Flask c sample}] &= 260 \text{ gm} \end{aligned}$$

After extraction of fat weight = 8 gm

Residue weight = 20 gm

$$\Rightarrow \frac{8 \times 100}{20} = 40 \text{ gm}$$

⇒ 40% of fat was present in all samples

**3.3(C) Ash estimation**

About 5 – 10 gm of the sample is weighed accurately into a tared platinum (or porcelain) crucible [with has previously been heated to about 600°C and cooled]. The crucible is placed on a clay pipe triangle and heated first over a low flame till all the material is completely charred, followed by heating in a muffle furnace for about 3 – 5 hrs at about 600°C. It is then cooled in desicator and weighed. To ensure completion of ashing, the crucible is again heated in the muffle furnace for 1 hr, cooled and weighed. This is repeated till two consecutive weights are the same and the ash is almost white greyish white in colour.

$$\text{Ash content (gm/100 sample)} = \frac{\text{wt. of the ash}}{\text{wt. of the sample taken}} \times 100$$

**Wheat grass 20 gm plain:**

$$\begin{aligned} W_1 &= 44.08 \\ W_2 &= 49.08 \\ W_3 &= 44.11 \\ &\Rightarrow W_3 - W_1 = 44.11 - 44.08 \\ &\Rightarrow \frac{0.03}{5} \times 100 \\ &\Rightarrow 0.6 \text{ in 100 gm of sample} \end{aligned}$$

**Wheat grass 20 gm cashew:**

$$\begin{aligned} W_1 &= 40.05 \\ W_2 &= 45.05 \\ W_3 &= 40.10 \\ &\Rightarrow W_3 - W_1 = 40.10 - 40.05 \\ &\Rightarrow \frac{0.05}{5} \times 100 \\ &\Rightarrow 1 \text{ gm in 100 gm of sample} \end{aligned}$$

**w heat grass 30 gm cashew:**

$$\begin{aligned} W_1 &= 43.75 \\ W_2 &= 48.05 \\ W_3 &= 43.84 \\ &\Rightarrow W_3 - W_1 = 43.84 - 43.75 \end{aligned}$$

$$\Rightarrow \frac{0.09}{5} \times 100$$

⇒ 1.8 gm in 100 gm of sample

**Wheat grass 40 gm cashew:**

$$W_1 = 40.15$$

$$W_2 = 45.30$$

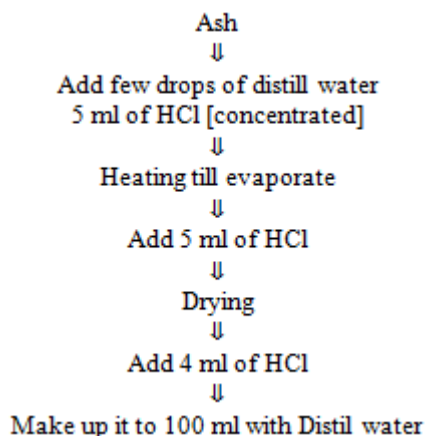
$$W_3 = 40.30$$

$$\Rightarrow W_3 - W_1 = 40.30 - 40.15$$

$$\Rightarrow \frac{0.14}{5} \times 100$$

⇒ 2.9 gm in 100 gm of sample

**3.3(D) Iron estimation in flow chart:**



**Iron estimation Procedure:** Iron in food is determined by converting the iron to ferric form. Using oxidizing agents like potassium per sulphate or hydrogen peroxide treating thereafter with potassium thiocyanate to form the red ferric thiocyanate which is measured calorometrical at 480 nm.

**Reagents:**

**1) Concentrated H<sub>2</sub>SO<sub>4</sub> [Iron free]**

Saturated Potassium per sulphate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) solution. Shake 7 to 8 gm of reagent grade solution. Shake 7 to 8gm of reagent grade iron free. K<sub>2</sub>O<sub>8</sub> with 100 ml of water in a glass stoppered bottle. The un dissolved excess settles to the bottom and compensates for loss by decomposition. Shake briefly before using, keep the reagent in the refrigerator.

**2) 3N Potassium thiocyanate [KSCN] solution:**

Dissolve 146gm of reagent grade KSCN in water and dilute to 500ml, filter if it turbid. Add 20ml of pure acetone to improve the keeping quality.

**3) Standard iron solution:**

Dissolve 0.72g (0.7gm) of reagent grade crystalline, ferrous ammonium sulphate [Fe So<sub>4</sub> (NH<sub>4</sub>)<sub>2</sub> So<sub>4</sub>. 6H<sub>2</sub>O] in 100 ml D.W. Add 5ml of concentrated Potassium Permanganate solution drop by drop until one drop produces a permanent colour, to transfer to a one litre volumetric flask, rinse with water to make up to volume. This solution contains 0.1mg of ferric iron per ml and is stable indefinitely.

**4) Procedure:** Use the ash solution of the sample prepared by dry ashing for colour development into three separate stopper measuring cylinders pipette the solutions as given below.

**Standard iron Blank ml Standard Sample Solution**

Standard iron	Blank ml	Standard	Sample Solution
1 ml = 0.1 mg Fe	0.0	1.0	0.0
Sample ash solution	0.0	0.0	5.0
Water	5.0	4.0	0.0
Conc. H <sub>2</sub> SO <sub>4</sub>	0.5	0.5	0.5
Potassium per Sulphate	1.0	1.0	1.0
KSCN	2.0	2.0	2.0

In each of the above cases make up the volume to 15 ml with water; measure the colour at 480nm, setting the blank at 100% transmission.

**Calculation:**

Iron mg/100gm =

$$\Rightarrow \text{O.D sample} \times 0.1 \times \text{Total volume ash solution} \times 100$$

**Wheat grass 20 gm**

$$\Rightarrow 0.1 \times 0.1 \times 0.6 \times 100$$

= 0.6 mg in 100gm of sample

**Wheat grass 20 gm with cashew**

$$\Rightarrow 0.1 \times 0.1 \times 1 \times 100$$

= 1.1 mg is 100gm of sample

**Wheat grass 30 gm with cashew**

$$\Rightarrow 0.23 \times 0.1 \times 1.8 \times 100$$

= 4.14 mg in 100gm of sample

**Wheat grass 40 gm with cashew**

$$\Rightarrow 0.30 \times 0.1 \times 2.0 \times 100$$

= 6 mg in 100gm of sample

**3.3(E) Protein estimation by micro-kjeldahl method:**

Nitrogen is the major element next to carbon, hydrogen and oxygen found in living things. Nitrogen occurs in amino acids, purine and pyrimidine bases, vitamins, amino sugars, alkaloids, compound lipids etc. However the major nitrogen source is proteins. In most proteins, nitrogen constitutes 16% of the total make up and hence, the total nitrogen content of a sample is multiplied by 6.25 to arrive at the value of the crude protein. By and large macro-kjeldahl technique is adopted to estimate the total nitrogen content in variety of sample ranging from microbial cells to meat. The procedure described here is highly suitable for food samples such as cereals and pulses flour. In addition, procedures for non-protein nitrogen, protein nitrogen and amino nitrogen are also included.

**Principle:** The nitrogen in protein or any other organic material is converted to ammonium sulphate by H<sub>2</sub>SO<sub>4</sub> during digestion. This slat, on steam distillation, liberates ammonia which is collected in beaker acid solution and titrated against standard acid. Since 1 ml of 0.1N acid is equalent to 1.401 mg N, calculation is made to arrive at the nitrogen content of the sample.

**Materials used**

Kjeldahlasks: 30ml hard glass flasks [ 10ml size for micro sample]



Digestion Rack: Commercial heating apparatus  
 Distillation apparatus: Glass distillation apparatus assembly  
 Sulphuric acid sp.gr. 1.84  
 Mercuric oxide  
 Potassium sulphate  
 Sodium hydroxide – Sodium thiosulphate solution  
 Dissolve 600g NaOH and 50g Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O in distilled water and make on one litre.

- Indicator solution: Methyl red 0.2g/100ml ethanol, methylene blue 0.2g/100ml ethanol. For mixed indicator two parts of methyl red solution are added to one part of Methylene blue solution.
- Boric acid 4% solution
- Standard Hcl (or) H<sub>2</sub>SO<sub>4</sub>, 0.02N
- Boiling chips (or) glass beads

### Procedure

- 1) Weigh 100 mg of the sample [containing 1-3 mg nitrogen] and transfer to a 30ml digestion flask.
- 2) Add 1.9 ± 0.1g potassium sulphate and 80 ± 10 mg mercuric oxide and 2ml concentrated H<sub>2</sub>SO<sub>4</sub> to the digestion flask. If sample size is larger than 20mg dry weight, 0.1ml H<sub>2</sub>SO<sub>4</sub> should be added for each 10mg dry material.
- 3) Add boiling chips and digest the sample till the solution becomes color less [ the time of digestion will vary with regard to the size of the sample, temperature and the mode of digestion]
- 4) After cooling the digest, dilute it with a small quantity of distilled ammonia – free water and transfer to the distillation apparatus [when the nitrogen content of the sample is high, the digest can be made up of know volume and an aliquot may be transferred to the distillation flask. The kjeldahal flask should be rinsed with successive small quantities of water.
- 5) Place a 100ml conical flask containing 5ml of boric acid solution with a few drips of mixed indicator with the tip of the condenser dipping below the surface of the solution.
- 6) Ass 10ml of sodium hydroxide & sodium thiosulphate solution to the test solution in the apparatus.
- 7) Distill and collect the ammonia on boric acid [at least 15-20ml of distillate should be collected]
- 8) Rinse the tip of the condenser, and titrate the solution against the standard acid until the first appearance of violet colour, the end point.
- 9) Run a reagent blank with an equal volume of distilled water and subtract the titration volume from that of sample litre volume.

### Protein Nitrogen:

Multiplying total nitrogen value with 6.25 will give the crude protein content which also includes non-protein nitrogen. To get true protein content deduct the non-protein nitrogen from the total nitrogen and then multiply with the factor.

### Calculation

The nitrogen content of the sample can be calculated based on any one of the following formulae as the case may be

$$\text{Ng/Kg} = \frac{(\text{mlHcl} - \text{mlblank}) \times \text{Normality} \times 14.01}{\text{weight (g)}}$$

### Plain biscuits:

$$\Rightarrow \frac{(3.1 - 0.3) \times 0.02 \times 14.01}{1}$$

$$\Rightarrow 0.78456 \times 6.25$$

$$\Rightarrow 4.9 \text{ gm in 100gm of sample}$$

### 3.5(b) Wheat grass 20gm

$$\Rightarrow \frac{(3.19 - 0.3) \times 0.02 \times 14.01}{1}$$

$$\Rightarrow 0.80977 \times 6.25$$

$$\Rightarrow 5.06 \text{ gm in 100gm of sample}$$

### Wheat grass 20gm with cashew:

$$\Rightarrow \frac{(4.8 - 0.3) \times 0.02 \times 14.01}{1}$$

$$\Rightarrow 1.2609 \times 6.25$$

$$\Rightarrow 7.8 \text{ gm in 100gm of sample}$$

### Wheat grass 30gm with cashew:

$$\Rightarrow \frac{(9.0 - 0.3) \times 0.02 \times 14.01}{1}$$

$$\Rightarrow 2.43774 \times 6.25$$

$$\Rightarrow 15.23 \text{ gm in 100gm of sample}$$

### Wheat grass 40gm with cashew:

$$\Rightarrow \frac{(12.1 - 0.3) \times 0.02 \times 14.01}{1}$$

$$\Rightarrow 3.30636 \times 6.25$$

$$\Rightarrow 20.6 \text{ gm in 100gm of sample}$$

By sensory evaluation, based on nutritive values and wheatgrass with cashew 30gm biscuits were accepted as better to supplementation:

### 3.4 Wheat grass 30gm with cashew:

#### 3.4(a) Moisture :

$$W_1 = 55.88$$

$$W_2 = 65.88$$

$$W_3 = 65.63$$

$$\Rightarrow \frac{w_3 - w_1}{10} \times 100$$

$$\Rightarrow \frac{0.25}{10} \times 100 = 2.5 \text{ mg in 100 gm of sample}$$

3.4(b) Fat: There is no change in total fat of the biscuits.

#### 3.4(c) Ash:

$$W_1 = 43.85$$

$$W_2 = 48.85$$

$$W_3 = 43.90$$

$$\Rightarrow \frac{w_3 - w_1}{5} \times 100$$

$$\Rightarrow \frac{43.90 - 43.85}{5} \times 100$$

$$= 0.8 \text{ gm of ash in 100 gm of sample}$$

#### 3.4(d) Iron

$$\text{Iron mg/100gm}$$

$$\Rightarrow \text{O.D of sample} \times 0.1 \times \text{Total volume} \times 100 \text{ of ash solution}$$

$$\Rightarrow 0.28 \times 0.1 \times 1.9 \times 100$$

⇒ 5.32mg in 100gm of sample

**3.4(e) Protein:**

$$\text{Ng/Kg} = \frac{(\text{ml Hcl} - \text{ml blank}) \times \text{Normality} \times 14.01}{\text{weight}}$$

⇒ Nitrogen value X 6.25

$$\Rightarrow \frac{(8.9 - 0.3) \times 0.02 \times 14.01}{1}$$

⇒ 2.4097 X 6.25

⇒ 15.06 gm in 100gm of sample

**3.5(a) Moisture:**

$$W_1 = 55.87$$

$$W_2 = 65.86$$

$$W_3 = 65.82$$

$$\Rightarrow \frac{W_2 - W_3}{W_2} \times 100$$

$$\Rightarrow \frac{10}{65.86} \times 100$$

⇒ 0.4mg in 100gm of sample

**3.5(b) Fat:** There is no change in fat. 40% of fat is appeared.

**3.5(c) Ash:**

$$W_1 = 43.71$$

$$W_2 = 48.71$$

$$W_3 = 43.85$$

$$\Rightarrow \frac{W_2 - W_3}{50} \times 100$$

$$\Rightarrow \frac{48.71 - 43.85}{50} \times 100$$

$$\Rightarrow \frac{0.14}{5} \times 100 = 2.8$$

**3.5(d) Iron:**

$$\Rightarrow 0.29 \times 0.1 \times 1.8 \times 100$$

⇒ 5.22 mg in 100gm of sample

**3.5(e) Protein:**

$$\Rightarrow \frac{(8.8 - 0.3) \times 0.02 \times 14.01}{1}$$

⇒ 2.3817 X 6.25

⇒ 14.88 gm in 100gm of sample

**3.5 Estimation of Vitamin A in selected biscuits wheat grass@30gm**

These studies were carried at R.V labs and those results were presented in fig :1 – 7

**1) Principle :**

Vitamin A (Retinol) is determined by high performance liquid chromatography with UV-detection after saponification and extraction. The method is in particular usable for products containing a little amount of vitamin A.

**2) Apparatus:**

- High Pressure Liquid Chromatograph
- UV-V is detector SPD 10A
- H.P.L.C Pump
- UV/VIS Spectrophotometer

**3) Reagents**

- Potassium hydroxide
- Ascorbic acid
- Sodium sulphate
- Butylhydroxytoluen (BHT)
- Ether
- Ethanol (99%)
- Nitrogen
- I-pentanol = n-amylalcohol
- Heptane
- Milli – Q Water
- Isopropanol = 2-propanol
- Vitamin A
- Diluted Sodium Hydroxide solution (2M) e.g. Baker art.7067
- Phenolphthalein solution
- Potassium hydroxide solution (17M/90%): Dissolve 180g of potassium hydroxide in 126 ml of water.
- Sodium sulphate solution (3%). Dissolve 1.0 g of butylhydroxytoluen in ethanol and dilute with ethanol to 1000 ml.
- Sodium ascorbate solution (2%): Dissolve 3.5g of ascorbic acid in 10ml of diluted sodium hydroxide solution and dilute with water to 200 ml.

**4) Chromatographic conditions:**

- Column : C18, 250mm, 4.6 mm chromosil
- Mobile phase : Heptane : 1-propanol (99:1)
- Flow rate : 2 ml/minute
- Detection : UV – absorption 325 nm
- Injection volume : 100ml
- Chart Speed : App.3 mm/minute
- Run time : 12 minutes
- Retention time: App.7 minutes for Retinol

**Method**

**Solutions**

**Standard Solution**

Weigh out, in duplicate, about 0.30g of the standard in a conical flask. Use A-acetate concentration,. Add 10ml of sodium ascorbate solution (2%) and heat in a steam bath for 5 minutes. Add 30 ml of BTH – solution (0.1%) and 3 ml of potassium hydroxide (17M). Connect the flask of an air condenser and reflux for 30 minutes on a steam bath (shake frequently). After cooling, transfer the solution by 30 ml of sodium sulphate 3% and 100 ml of ether to at separating funnel containing 100 ml of ether.

**Test Solution**

**Anhydrous solutions:**

Mix the sample and weigh out accurately the sample into an Erlenmeyer flask. Dilute to 10 ml with sodium ascorbate solution (2%)

**Saponification**

Add 30 ml of alcoholic BTH-solution (0.1%) and 3 ml of potassium hydroxide (17M). Connect the flask of an air condenser and reflux for 30 minutes on a steam bath (shake frequently). Cool and transfer with 30ml of sodium sulphate

3% and 100ml of ether to a 500 ml separating funnel containing 100 ml of ether.

#### Extraction

Shake for 2 minutes. Let stand until the layers are clearly separated (about 30 minutes), and discharge the lower aqueous layer (if an emulsion is formed, add some drops of ethanol 99%). Wash the ether extract with 4 X 50 ml of water; shake carefully in the beginning in order to avoid emulsification. Afterwards pour a couple of ml of water transfer into a centrifuge tube containing a few drops of phenolphthalein – R1. If it is red – continue washing until the washings are no longer coloured red. Afterwards, transfer the ether layer to a 250ml volumetric flask or a round-bottom flask.

#### Final preparation

#### Tablets and solutions

A + B: Transfer through a cotton plug covered with anhydrous sodium sulphate by ether to a round – bottom flask. Evaporate in vacuum and dilute with n-heptane until a final concentration of 3-4 µg/ml is found. C: Transfer to a 250 ml volumetric flask and fill up to volume with ether. 20.00 ml in a 50 ml flask is evaporated under a steam of nitrogen until a rest of 2 ml is left. Dilute

#### Standard assay concentration determination

Dilute 2.00 ml of STD A with 2-propanol to a final volume of 100 ml. Measure the absorbances at 310, 325 and 334 nm.

#### Standard to HPLC

Dilute 2.00 ml of STD A with n-heptane to a final volume of 100 ml.

#### Chromatography

Transfer sample and standard to vials and inject 100 ml of the solutions (double). Record the area of the A – vitamin peaks

### 3.5 Estimation of Vitamin E in wheatgrass@30 gm:

#### Principle

Sample is treated with a methanol solution to denature lipoproteins. Alkaline saponification of the test material eliminates fats and liberated vitamin E from test material as an saponifiable material that is successively extracted with petroleum ether. The extract is dried, solubilized with methanol and injected in HPLC (C 18 column, reversed-phase). The quantitative determination of vitamin E was carried out by UV detector set at 294 nm.

#### Equipment

- 1) Vortex
- 2) Thermostatic multiple water-bath (GFL 101) equipped with round bottom flask (100ml) fitted with ground stopper, cooling columns and gas-tube;
- 3) Separating funnels (100 ml)
- 4) Volumetric flasks and (100 ml)
- 5) Rotary Evaporator
- 6) Centrifuge
- 7) HPLC Shimadzu
- 8) Detector UV – V s

- 9) Column CHROMOSIL C 18, 250 mm

#### Reagents

- 1) Extra pure petroleum ether
- 2) 0,5% ascorbic acid daily prepared solution (Dissolve 0,5g of pure crystallized ascorbic acid in 4ml distilled water, mix with 20ml ethanol and dilute with methanol to 100ml)
- 3) 50% potassium hydroxide (1 kg potassium hydroxide pellets in 1 L Distilled water).
- 4) Pure DL- $\alpha$  tocopherol for calibration CURVE
- 5) Methyl alcohol
- 6) Nitrium or helium gas (oxygen-free)

#### Standard solution

Stock solution (1000 mg/l) was obtained dissolving 100mg of DL- $\alpha$ -tocopherol in 100ml methanol.

**Work solutions** (1ppm, 25ppm, 50ppm, 75ppm 100ppm) were obtained progressively diluting stock solution in methanol

#### Samples

After homogenization, WB sample was divided in 10g aliquots, conserved in polyethylene tube and frozen at -20°C until analysis

#### Saponification

WB Sample, at room temperature, was mixed and 10g were put into a round-bottom flask, 10ml of ascorbic acid solution were added and brought to 80°C in water-bath while purging with helium gas. At boiling point (after about twenty minutes) 2ml of KOH solution were added. After 20 minutes the flask was removed from water-bath and kept in the dark until cooling.

#### Extraction

After cooling, test material was put into a separating funnel, rinsed two times with 5ml water and successively with 30ml ether. The funnel was closed and mixed several times. Aqueous phase was recovered in the round-bottom flask and ether phase was put into a flask. Extraction procedure was repeated 2 times with 30 ml ether. Ether phases were combined and transferred in the separating funnel, rinsed 6 times with 50ml water, and recovered in a round – bottom flask. Then, the test material was evaporate to dryness in a rotary evaporator under partial vacuum at water-bath temperature of 45°C (5 minutes). After cooling, test material was recovered with 5ml methanol, well mixed and transferred in a glass tube, centrifuged at 4000 rpm for 5 minutes. To prepare four samples about four hours were needed.

Condition of H.P.L.C

Column : Stainless steel, length 25cm, diameter 4,6mm

Stationary phase : ODS

Mobile phase: Methyl alcohol

Flow rate: 1 ml/min

Injection volume: 20 µl

Detection: UV Detector

Wave length: 294 nm

Retention time: 5 min

Column temperature: ambient

#### 4. Results and Discussion



#### 4(a) Ist phase

Moisture per 100gm of the sample is presented in basic plain biscuits is 2.8 gm. In wheat grass plain biscuits with 20gm is 1.2 gm. In wheatgrass 20 gm with cashew is 1.7 gm, In wheatgrass 30gm with cashew is 2.5 gm and in wheat grass 40 gm with cashew is 3.1 gm. There is 40 gm of total fat is occurred in all samples of biscuits because there is no replacement of butter. Ash content in basic plain biscuits is 0.5 gm, wheat grass 20gm plain biscuits – 0.6 gm, wheat grass 20gm with cashew is 1 gm, wheat grass 30 gm with cashew is 1.8 gm, and wheat grass 40 gm cashew is 2 gm.

Iron content in plain wheat grass 20gm is 0.6 mg, wheat grass 20 gm with cashew 1.1 mg, wheat grass 30 gm with cashew is 4.14 mg and wheat grass 40gm with cashew is 6 mg. Protein in plain biscuits is 4.9 gm, plain wheatgrass 20 gm is 5.06 gm, wheat grass 20 gm with cashew 7.8 gm, wheat grass 30 gm with cashew is 15.23 gm and wheat grass 40 gm with cashew is 20.6 gm shown in table no.1

**Table 1:** Nutrient Analysis

Nutrient	Plain biscuits	Plain Wheat grass 20gms	Wheat grass 20gm with cashew	Wheat grass 30 gms with cashew	Wheat grass 40gms with cashew
Moisture	2.8	1.2	1.7	2.5	3.1
Fat	40	40	40	40	40
Ash	0.5	0.6	1	1.8	2
Iron	-	0.6	1.1	4.14	6
Protein	4.9	5.06	7.8	15.23	20.6

#### 4(b) II phase analysis

Based on organoleptic evaluation the investigator selected one sample to made further analysis of vitamin A and E. i.e, wheatgrass 30 gm with cashew was selected and analysed. In that moisture is 2.5 mg, fat is 40gm ash is 0.8 gm, iron is 5.32 mg, protein is 15.06 gm vitamin A is 31.175 µg/g and vitamin E is 17.58 µg/g.

Total shelf life period the investigator analysed the sample in every 30 days gap. In III phase analysis – moisture is 2.4 mg, ash is 2.8, Iron is 2.4 mg, fat is 40gm, protein 14.88. Vitamin A is 31.185 µg/g and vitamin E is 17.68 µg/g

In IV phase of analysis moisture is 2.3 mg, fat is 40 gm, ash is 2.7 gm, Iron is 5.23 gm, protein is 14.88, vitamin A is 31.165 µg/g and vitamin E is 17.67 µg/g shown in table no.2

**Table 2:** Nutrient Analysis during shelf life period

Nutrient	Phase - II	Phase - III	Phase - IV
Moisture	2.5	2.4	2.3
Fat	40	40	40
Ash	0.8	2.8	2.7
Iron	5.32	5.22	5.23
Protein	15.06	14.88	14.88
Vitamin A	31.175	31.185	31.165
Vitamin E	17.58	17.68	17.67

#### 4. (c) Mean sensory scores of wheatgrass biscuits

By observing all these mean values variation III wheat grass 30 gm with cashew is comparatively higher that the other 4



variations. The appearance, colour, texture, taste, flavor and overall acceptability's mean values is higher than other four variations. So variation III is selected as a standardized.

**Organoleptic evaluation of wheatgrass biscuits:**

Four variations of wheatgrass biscuits were prepared by incorporating of wheatgrass at a level of 10 gm, 20 gm, 30 gm and 40 gm with [variation I, variation II, variation III and variation IV respectively]. It was evaluated by 15 trained panel members who were given score cards for various sensory attributes. This mean scores are presented in Table no:

**Table 3:** Organoleptic evaluation of wheatgrass biscuits in different variations:

Attributes	Basic recipe	Variations			
		V - I	V - II	V - III	V - IV
Appearance	4.1	3.5	3.6	4.5	3.4
Colour	3.8	4.2	4.4	4.8	3.8
Texture	3.8	4.1	3.2	4	3.3
Taste	3.5	3.6	3.6	4.5	3.4
Flavour	4.3	4.2	4.3	4.6	4
Overall acceptance	4	3.8	3.8	4	3.6

**Sensory evaluation of the developed product during storage**

Shelf life of any product plays a very important role in increasing it's acceptability on storage. Food undergo number of changes in flavour, colour, appearance, texture and taste.

The fresh product was prepared and stored in airtight containers to see the effect of storage on the organoleptic characteristics of the developed product.

**Appearance:** Appearance of the cooked influences the palatability and therefore the acceptance by the consumer.

**Colour:** Colour is used as an index to the quality of a number of foods. Colour give pleasure and it increases the visual appeal of the product.

**Texture:** Texture is the product indentified by the characteristic attributes because it cannot, be explained by a single attribute.

**Flavour:** Flavour is alfactory sense which perctrates beyond the visual range.

**4.5(e) Overall acceptability:** The overall acceptability of product includes every aspect of the product is visual, guestatory, alfactory and tactile sense.

**Table 4:** Sensory evaluation shelf life assessments of wheatgrass 30gm with cashew biscuits:

Name of the product	Sensory attributes	Storage period			
		I month	II months	III month	IV month
Wheat grass 30g with cashew biscuits	Appearance	5	5	4.5	4
	Colour	5	4.5	4.5	4.5
	Texture	5	4.5	4.5	4
	Taste	5	5	4.5	4.5
	Flavour	5	4.5	4	4
Overall acceptability		5	4.5	4	4

The above table shows the sensory evaluation shelf life assessment of wheatgrass 30gm with cashew biscuits. The shelf life studies and sensory evaluation was for four months and Each month of the sensory attributes were noted.

The wheatgrass (30gm with cashew) biscuits is evaluated by using trained panel members. The result show the mean sensory score of appearance of the wheatgrass 30gm with cashew biscuits for the first month is : 5 and later changed. But it is stable upto 2<sup>nd</sup> month and changed in 3<sup>rd</sup> and 4<sup>th</sup> month.

Regarding the colour of wheatgrass biscuits, shows there is no change from first month storage period to fourth month of the storage period.

The result obtained from the data, the texture of the wheatgrass biscuits is evaluated and identified. Little difference from first month of shelf life period to final month of the shelf life.

The values observed for the taste of the wheatgrass biscuits from the storage period of initial to final stage values were changed. i.e (0.5)

The wheatgrass biscuits flavor is slightly changed from Ist month to last month of the storage period. The four months sensory scores get some value (4).

The overall acceptability of wheatgrass biscuits sensory score is 5 to 4.5, 4 from initial month to final month during storage. So the results indicates wheatgrass 3 gm with cashew biscuits overall acceptability is slightly changed during storage

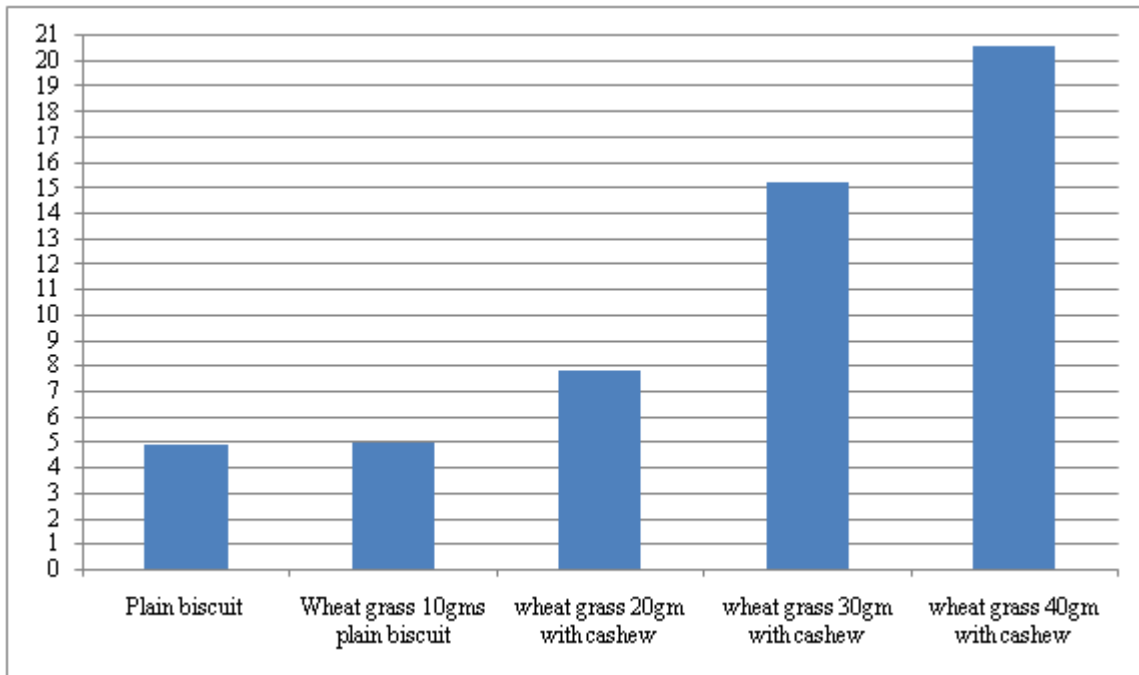
**5. Conclusion**

From the study it can be concluded that the development, standardization and evaluation of the developed wheatgrass biscuits are rich in protein, Amino acids, antioxidants and micronutrients and trace elements. This product is preferable for any age group who are deficient of protein energy malnutrition, micronutrient deficiency and in chronic disease like diabetes, cardiovascular, and cancer of any organ.

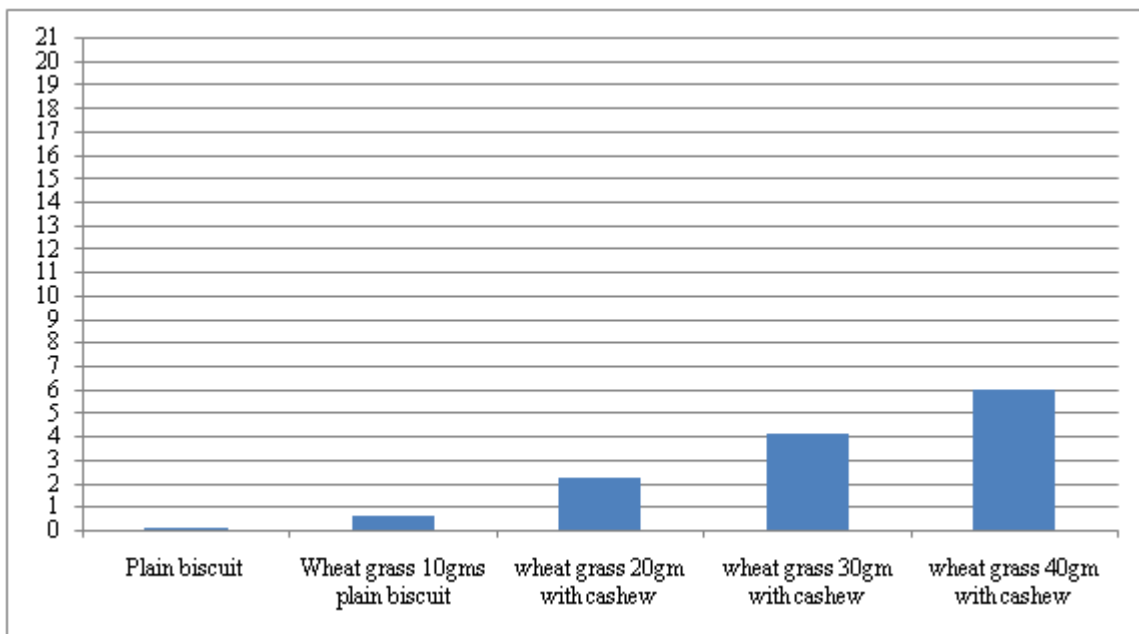
This product is really nutritious and it's preparation is easy and low expensive and it has good shelf life to stay up to 4 or 5 months in good and air tight packing.

**Table 5:** Comparison of different nutrients of developed products with basic recipe in 100 gms

Name of the Product	Analyzed Values							
	Energy (k.cal)	Moisture (mg)	Fat (g)	Ash (mg)	Protein (g)	Vitamin A (µg)	Vitamin E (µg)	Iron (mg)
Plain biscuits	1455	2.8	40	0.4	4.9	960	4.5	0.4
Wheat grass 30gm with cashew biscuits	1753	0.4	40	2.8	14.88	31.175	17.58	5.2



**Figure 1:** Comparative bar diagram showing the protein levels of developed products with basic recipe



**Figure 2:** Comparative bar diagram showing the iron levels of developed products with basic recipe

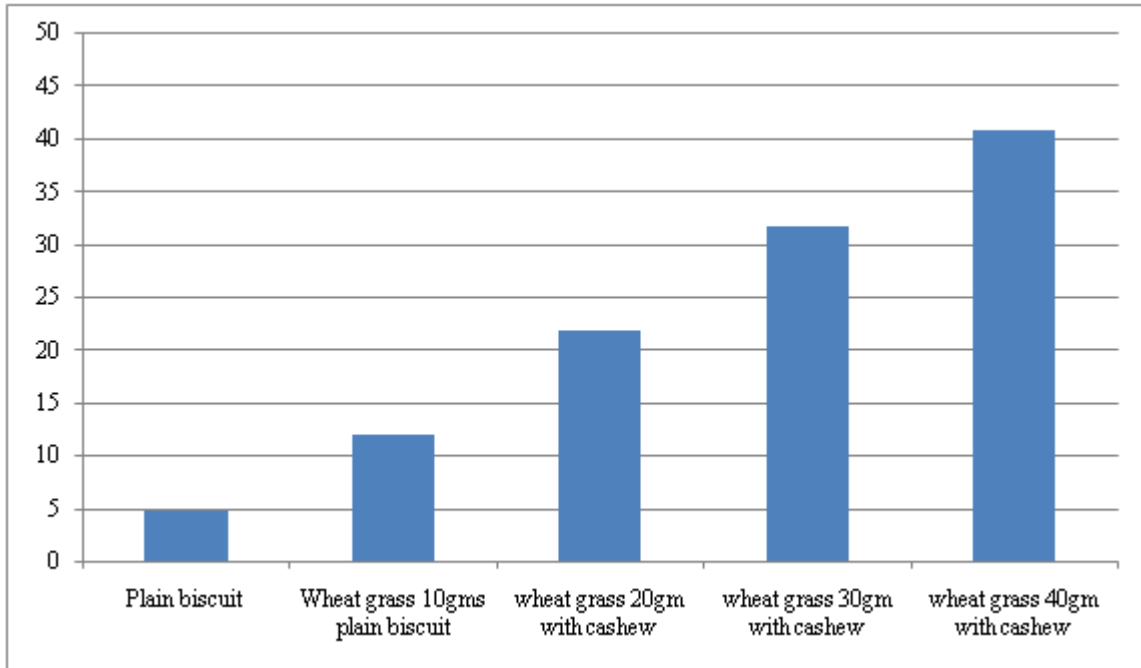


Figure 3: Comparative bar diagram showing the Vitamin A levels of developed products with basic recipe:

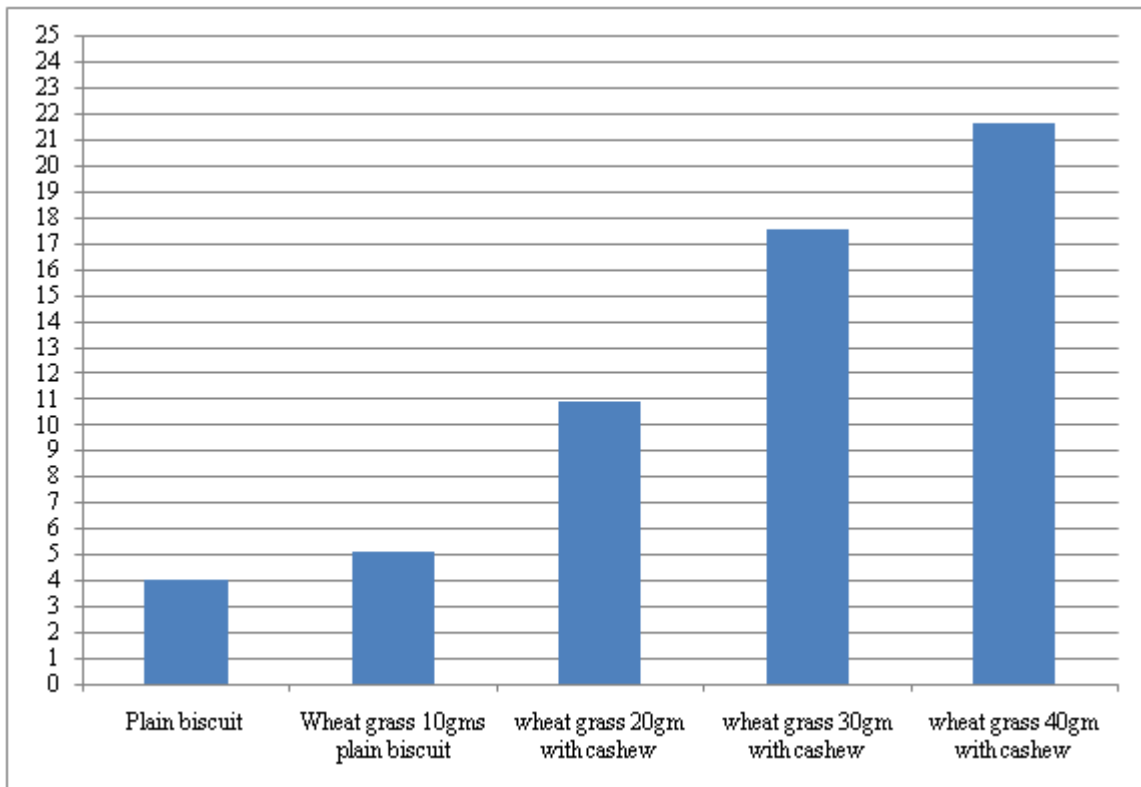
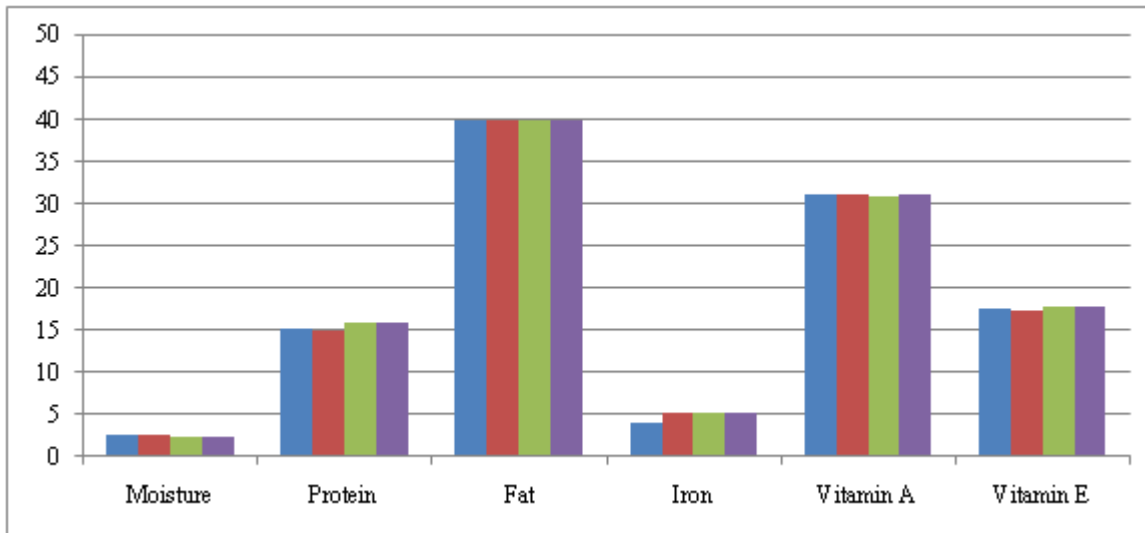


Figure 4: Comparative bar diagram showing the Vitamin E levels of developed products with basic recipe



**Figure 5:** Comparison of shelf life quality of developed product during storage i.e., wheat grass 30gms with cashew phase I to IV

Wheatgrass 30gm with cashew biscuits nutritive values throughout the shelf life period, moisture is little decreased 2.5gm at initial and 2.3gm at final stage protein is slightly decreased from initial 15.23 to 15.88 of final stage. Total fat of the biscuits were no change in value it is 40gm from initial to final stage. Iron is increased slightly from initial 4.14 and 5.23 at final stage. Vitamin A is 31.17 at initial stage and 31.1 at final stage and Vitamin E is 17.5 at initial stage and 17.9 at final stage.

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