# Morphological, Microbiological and Proximate Analysis of Cookies Prepared from Defatted Soy Flour 

Deepika ${ }^{1}$, Priyanka Shankar ${ }^{2}$

${ }^{1}$ Student, Department of food and nutrition, School of Home Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India.
${ }^{2}$ Assistant Professor, Department of Food and Nutrition, School of Home Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India.

Corresponding Author: dipikaarchu@gmail.com


#### Abstract

The defatted soy flour contains a lot of essential amino acids and proteins of high biological value and, therefore, it is suitable for the development of protein rich food products. The aim of this study was the development of cookies with the use of defatted soy flour to increase the protein content of cookies. The proximate, physical, microbial and morphological analysis were done. The result of proximate analysis show that there is increase in moisture, ash, protein and fat content of the fortified cookies than that of control cookies. Microbial analysis show that there were few microbial growths which indicates that the fortified cookies are safe to consume. Morphological structure of the soy flour was found to be of consistent oval shaped. Interconnecting fibrils like strands is present on the particles of soy flour.


Key Words: - Defatted soy flour cookies, high protein, Microbial, morphological, proximate analysis.

## I. INTRODUCTION

In recent years, consumer perceptions of food have undergone major changes due to lifestyle changes, modernization, increased female employment, rising per capita income, and new marketing strategies served by major food manufacturers. global industry experts have revealed in a recent major access market study that the global bakery industry faces both opportunities and challenges brought about by the current economic crisis. The demand for bakery product continues to grow worldwide. Cookies are ready-to-eat, convenient and affordable and are one of the most popular and consumed processed foods in India. 1

[^0]In recent years, as consumers have become more nutritionally aware, interest in food fortification has increased. Legumes are one way to satisfy our need for protein-rich foods, especially baked foods. Cookies with relatively long shelf-life and edible properties are widely used. This property of food makes it possible to produce and distribute it on a large scale in a short period of time. Cookies can be easily fortified with high-protein flour to provide a convenient snack to supplement protein in the diet. Cookies are the most popular bakery product in the world, it has been a human food for a long time. 2 It is high in carbohydrate, fat and calorie but low in protein, vitamin and minerals, making it unhealthy for daily consumption. The main ingredients of the cookies are wheat, which is deficient in the essential amino acid lysine, and soybeans are rich in lysine, making it possible to supplement wheat in cookies. 3
soybean is an excellent source of protein and contain 35-45\% of all the essential amino acids needed for proper growth and maintenance of the body. It also contains many vitamins, minerals, and antioxidants such as isoflavones, which help to lower cholesterol, prevent cancer and regulate menopause. 4 Soy flour was therefore defatted to reduce the fat content of the cookies. Protein-rich cookies can be made with two ingredients: wheat flour and defatted soy flour.

Protein sources for cookies should have a pleasant or mild taste, low water absorption, and a high protein efficiency ratio that does not adversely affect texture, leave unwanted color or alter dough rheology. For protein enrichment, defatted soyabean meal is the best option to increase protein efficiency. 5

The defatted soy meal can be used to produce protein isolates and concentrates. Nutritionally, soy protein is more similar to animal protein than other plant proteins. 6 In terms of protein yield per hectare, soyabean have the highest yield at the lowest cost ( 800 kg ) and have one of the highest amino acid contents of any plant protein. Soya protein is called complete protein because it provides a variety of amino acids needed to build and repair body tissues.
The specific purpose of the present study was to development high protein and low-calorie cookies using defatted soy flour, to evaluate physical properties, proximate analysis and shelf life of developed cookies.

## II. Material And Methods

### 2.1 Materials

The uncooked substances such as wheat flour, sucralose, baking powder, butter, toned milk, and many others have been bought from neighbourhood marked.

### 2.1.1 Preparation of cookies

Mix the butter and sucralose. In a huge bowl, beat the butter and sucralose on excessive velocity till fluffy. Now, in a separate bowl, add measured quantity of the wheat flour, defatted soy flour, baking powder, and salt. Beat them together. You also can use a sifter in case you want.

Next, upload the components to the butter aggregate and beat with a hand mixer till nicely combined. Mix simply till the flour has been incorporated. Do not blend too much. After that make dough by adding adequate amount of milk, now refrigerate the dough for 2 h . After refrigeration of dough make cookies Now you can take the dough with a spoon and place it on a baking tray or tray lined with baking paper. Bake withinside the oven for 15-20 minutes. When it comes out of the oven, permit it cool on a baking sheet for approximately five minutes, then switch it to a wire rack to chill completely. Serve the cookies.

### 2.1.2 Flow chart of development of recipe



Fig.1. schematic representation of preparation soy flour cookies

### 2.2 Proximate Analysis

### 2.2.1 Moisture

About 10 g of sample was weighed and transferred to dish. Weighed sample was then dried in hot air oven at 105 degree C for 5 hrs. The dish dried sample was transferred to the desiccators and cooled to room temperature. The dish with dried sample was then weighed. Moisture content material in percentage were calculated from loss in weight.

$$
\text { Moisture }(\%)=\frac{\mathrm{W} 1-\mathrm{W} 2 \mathrm{X}}{\mathrm{~W} 1} 100
$$

Where;
$\mathrm{W} 1=$ Weight ( g ) of the material before drying $\mathrm{W} 2=$ Weight $(\mathrm{g})$ of the material after drying

### 2.2.2 Ash

About 3 g of sample was weighed and transferred to crucible after that place crucible covered with lid in muffle furnace. Heat at $550^{\circ} \mathrm{C}$ for 5 hr after heating place the crucible in desiccator to room temperature for cooling. When the sample turns grey weigh the sample with crucible.

$$
\text { Ash }(\%)=\underset{\text { Weight of sample }}{\text { Weight of ash }} \mathrm{X} 100
$$

### 2.2.3 Fat

Weigh about 3 g of sample to filter paper and wrap. Take the sample into extraction thimble and transfer into Soxhlet, Fill the petroleum ether about 250 ml into the thimble. Connect the Soxhlet apparatus and turn on the water to cool them. Heat the sample for about 5 hrs . after that weigh the beaker, take the solvent into the beaker and heat it on a hot plate until solvent is completely evaporate and then weigh the beaker and its dried content.

$$
\text { Fat }(\%)=\frac{\text { Weight of fat }}{\text { Weight of sample }} \times 100
$$

### 2.2.4 Protein

About 1 g of sample was weighed and places in digestion flask. The Kjeldahl catalyst mixture ( 1 g ) and 5 ml each of H 2 O 2 and conc. H2SO4 were added carefully. After that the digestion sample were carried out until it became colourless by frequent rotating the flask. The flask was allowed to cool and a 5 ml portion of water was slowly added with mixing. After cooling, the content was transferred to 50 ml volumetric flask with 2-3 rinsing, and the volume was made up with distilled water and mixed thoroughly. ${ }^{8}$ Blank digestion was carried out simultaneously. The distillation unit was cleaned by starting and sucking back the water. A beaker of 10 ml capacity containing 10 ml boric acid was taken. four drops of indicator were added and located beneath the condenser with its tip dipped in solution. The digest $(5 \mathrm{ml})$ with rinsing was transferred to distillation flask, 5 ml NaOH was added and closed with stop cork. The digest changed into allowed to boil and approximately 50 ml distillate of ammonia liberated in boric acid changed into collected. The distillate was titrated with hydrochloric acid until blue colour disappeared. Blank titration was carried out simultaneously. Protein content was calculated by the usage of following formula.


Where,
$\mathrm{A}=$ volume (ml) of 0.2 N HCL used sample titration
$\mathrm{B}=$ volume $(\mathrm{ml})$ of 0.2 N HCL used in blank titration
$\mathrm{N}=$ Normality of HCL
$\mathrm{W}=$ weight $(\mathrm{g})$ of sample
$14.007=$ atomic weight of nitrogen
$6.25=$ the protein nitrogen conversation factor for fish and its by-product.

### 2.3 Physical Parameters

The physical parameters of soy flour cookies such as diameter, thickness and spread ratio were determined according to American association of cereal chemists (AACC;2000).

### 2.3.1 Diameter

The Diameter of six cookies were determined by using vernier calliper. Cookies were turned $90^{\circ}$, the diameter was measured as a check determinate. The common of diameter of six cookies had been determined.

### 2.3.2 Thickness

The thickness of six cookies were determined by keeping the cookies horizontally by using vernier calliper. After that the average of six cookies were determined.

### 2.3.3 Spread ratio

Spread ratio of cookies was determined by the ratio of average value of diameter to the average value of thickness according to AACC (2000).

$$
\text { Spread factor }=\text { spread ratio of sample } \times 100
$$

Spread ratio of control sample

### 2.4 Microbiological Analysis

### 2.4.1 Nutrient agar media

Media preparation was done by Suspending 28 g of nutrient agar powder in 250 ml of distilled water. Dissolve them in distilled water then Autoclave the dissolved mixture at $121^{\circ} \mathrm{C}$ at 15 psi pressure for 15 minutes. permit it to chill however now no longer solidify. Pour the prepared media into petri plate and leave plates on the sterile surface of laminar air flow and switch on Ultraviolet lights of laminar until the agar has solidified. After that serial dilution was done. I have selected the T-1 and T-2 dilution factor for inoculation. Spreading was done equally all over the plate. Cover the spreaded plate with their lid and
put it by inverting them in the incubator at 37 degree C for incubation. Incubation was done for 24-48 hours. After the incubation time is completed, colonies were counted manually by making quadrant and CFU was calculated with the help of formula which is given below-

$$
\mathrm{CFU} / \mathrm{ml}=\frac{\text { No. of colonies (whole plate) X Dilution Factor }}{\text { Volume of culture plate }}
$$

Where;
CFU = Colony forming unit

### 2.5 Morphological Study of High Protein Cookies Through Sem (Scanning Electron Microscopy)

The SEM is an instrument that produces high magnification images using electrons instead of light to form images. The electron beam is emitted by an electron gun located at the top of the microscope. The electron beam travels vertically through the vacuum microscope. The beam passes through a lens and an electromagnetic field that directs the beam to the sample. After the beam hits the sample, electrons and x-rays are emitted from the sample. A detector collects these x-rays, backscattered electrons, and secondary electrons and coverts them into signals that are transmitted to a screen similar to a television screen. This will create the final image. ${ }^{7}$

### 2.6 Energy Dispersive X-Ray Spectroscopy (EDX)

Energy-dispersive X-ray spectroscopy is a powerful technique that enables the user to analyse the elemental composition of a desired sample. The major operating principle that allows EDS to function is the capacity of high energy electromagnetic radiation (X-rays) to eject 'core' electrons (electrons that are not in the outermost shell) from an atom. This principle is known as Moseley's Law, which determined that there was a direct correlation between the frequency of light released and the atomic number of the atom.

## III. Result And Discussion

### 3.1 Proximate analysis

The moisture content of cookie sample was $9.8 \%$ as compared to the control with moisture content of $7.40 \%$. The gradual increase in moisture content may be due to an increase in protein content by the addition of defatted soya flour in cookies as protein exhibits hygroscopic nature.

The ash content of cookie samples of defatted soy flour was 2.19 \% was higher than the control (1.45\%) which may be attributed to the presence of more minerals in fortified cookies than control. The increase in the ash content may be due to an incorporation of defatted soya flour containing higher proportion of ash.
fat content was found to be $3.1 \%$ in fortified cookies containing defatted soy flour compared to $5.6 \%$ fat in control. Increase in fat content in fortified cookies may be due to an increased proportion of defatted soya flour as soya flour contains higher percentage of fat. Higher fat content improves the mouth feel and retains the flavor of cookies.
The protein content of fortified cookies was found to be $16.90 \%$ higher than the control with $10.02 \%$ protein content. The significant increase in protein content may be due to the addition of defatted soya flour in the product as defatted soy flour is rich in proteins.
Table 1: proximate analysis of cookies

| Treatment | Moisture \% | Ash \% | Fat \% | Protein \% | Carbohydrate\% |
| :---: | :---: | :---: | :---: | :---: | :---: |
| T0 | $7.4 \%$ | $1.45 \%$ | 5.6 | $10.02 \%$ | $75.53 \%$ |
| T1 | $9.8 \%$ | $2.19 \%$ | 3.1 | $16.90 \%$ | $68.01 \%$ |
| T2 | $10 \%$ | $2.08 \%$ | 3.5 | $17.50 \%$ | $66.92 \%$ |



Fig.2. graphical representation of proximate analysis of prepared cookies

### 3.2 Physical parameter

Table.2. Mean for effects of treatments on physical characteristics of cookies

| S. No. | Parameters | T0 (control) | T1 | T2 |
| :---: | :---: | :---: | :---: | :---: |
| 1. | Diameter(mm) | 41.2 | 44.6 | 43.1 |
| 2. | Thickness(mm) | 14 | 15 | 15 |
| 3. | Spread ratio | 29.4 | 29.7 | 29.9 |

### 3.3 Microbiological analysis

Microbial studies conducted for total plate count revealed that $120 \mathrm{CFU} / \mathrm{ml}$ was detected in fortified cookies and for control sample it was $202 \mathrm{CFU} / \mathrm{ml}$ which is lower than the permissible limits. On the basis of these findings, it could be contended that the product is safe to consume due to the high heat treatment and proper hygienic considerations during preparation of high protein, low calorie cookies.

### 3.4 Scanning electron microscope (SEM)

The morphologies of the flour samples were determined using SEM at different level of magnification. The size of the particles varies, according to their magnifying power. Increase in magnification showed that the particles of soy flour samples are of consistent oval shaped. Interconnecting fibrils like strands is present on the particles of soy flour cookies is due to unfermented dehydrated wheat flour.

### 3.5 Energy dispersive $X$-ray Spectroscopy (EDX)



Fig.3. DX Spectrum for functional soy flour cookies

## IV. Conclusion

The biscuit fortified with defatted soy flour were nutritionally superior to that of whole wheat flour cookies. The results of this study strongly indicate that quality and acceptable cookies can be produced from defatted soy flour. It is evident from the experiment that the cookies can be made with substitution defatted soy flour upto $20 \%$ without adversely affecting the sensory characteristics of cookies. The cookies were developed from artificial sweetener; therefore, the cookies may be recommended for diabetic patients. Cookies were also microbiologically stable since there were very few microbial growths was found. It can also be served as a vehicle for delivery of important nutrients.

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