Surveillance of Nutritional composition, Urease activity, and Aflatoxin contamination in processed Soya Chunks or Textured Vegetable Protein (TVP)

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Abstract

***Background**: Soybean protein is an important nutrient in the human diet. Soya chunks are the best protein sources and also contain good sources of minerals, fibers, and vitamins. Due to good fiber content, it helps to fight diabetes and control obesity. Minerals help to strengthen bones and fight insomnia. Vitamins play an important role in blood clotting and pregnancy.*
***Methods**: A total of 4 different soya chunks (TVP) samples were collected from different companies for analysis of total plate count, moisture content, water activity, yeast, and mold count.*
***Results:** The study revealed no microbial contamination in soya chunks (TVP) however moisture content and water activity were 6 to 10 % and 0.5 – 0.6 respectively because soya chunks are highly processed and high-temperature extrusion products. Further study, 20 different samples taken for quantification analysis of urease activity, and contamination of aflatoxins. Urease activity aims to study the grade of the soya chunks processing treatment. This test estimate increase in the pH outcomes of the liberation of ammonia into the media transpires from the breakdown of urea by the urease present in soybean products. The degree of processing of urease activity (ΔpH) is under processing is above 0.20, adequately processed is ranges from 0.05 to 0.20, and over-processed is below 0.05. It was found that the urease activity of soya chunks was under process.(0.2 -0.4) The 20 different samples of soya chunks tested for the contamination of aflatoxin and the result shows that all 20 different samples are not detected below the limit of quantification of aflatoxinB1 is 5 microgram/Kg*

Keywords - *Protein, Soybean, Soya chunks, Extrusion product, TVP.*

I. Introduction

Soya chunks (TVP) are the very low-fat food from extracting oil as a by-product of the soybean. Extrusion of soya flours and concentrated soy protein are blended with water, flavors, colors, and supplementary nutrients, and then pass to process mixture in the extruder. Pressure and temperature leave from the extruder and change wet soy protein into a soft texture product that is pressed out with different shapes of dyes. Then coarsely formation because pressure and fall of temperature cause extension. Then dried the extended product. The extrusion process made the soybean protein product. At the time of hydration, the product gets crispy texture. It is a substitutional use of meat analog and extender. Soybean is a widely consumed legume that is cheap and nutritionally healthy (Steinke, 1992). Soya chunks also have a low budget origin of proteins, minerals, and vitamins. Soybean provides health benefits that suggest humans cure obesity, cancer, diabetes, etc. Therefore the new products made from soybean such as soy chunks, soy- milk, soy-bread, tofu, etc.

TVP is easy and quick to cook, and has a high content of protein comparable to other meats. The aim of these projects has to determine the quality and microbial safety of the products for humans. Evaluate the nutritional values of soya chunks i.e. protein, fat, fiber, carbohydrate. Aflatoxin is the harmful carcinogenic mycotoxin present in all seeds, thus to check the contamination of aflatoxin in soya chunks. Ureases that contain various numbers of bacteria, fungi, etc. that harm to the human. Therefore to study the urease activity in the soya chunks

II. Sampling

A. Sample collection

The project was done in two phases. For Phase-I 4 different samples of soya chunks were taken and for phase-II 20 different samples of soya chunks were taken from various supermarkets and local markets for quantification analysis.

B. Sample preparation

In phase-I was about analysis of the Nutritional factors of soya chunks, moisture content, and microbial safety, so 4 different samples of 100g were taken from various markets. In phase-II was about Analysis of Aflatoxins, Urease activity, and Trypsin Inhibitors, therefore total 20 different samples of soya chunks of 100g (textured soy protein) was collected from various supermarkets and local markets and then both phase samples finely powered by the electric mixer and stored in sealed plastic pouches.

III. Methodology

Protein analysis

The Kjeldahl Method: A digestion unit that digests temperatures in the range of 360 –380°C for periods up to 3 hours, Kjeldahl flasks (500 – 800 ml). A distillation unit that air-tight distillation from the flask with the digested sample into 500 ml Erlenmeyer flasks (distillation receiving flask). The burette to calculate exactly the acid that requires to be titrated in the receiving flask to neutralize the collected ammonia hydroxide. All Kjeldahl installations require acid-vapor removal devices. This may be by a fume removal or exhaust-fan system, water recirculation, or a fume cupboard.

Chemicals: Kjeldahl catalyst: contains 10 g of K2SO4 (potassium sulfate) + 0.30 g of CuSO4 (copper sulfate). Reagent: concentrated H2SO4 (sulfuric acid). Mixed indicator solution: 3125g methyl red and 0.2062 g methylene blue in 250 ml of 95% ethanol (mix for 24 hours). Boric Acid Solution: 522 g U.S.P. boric acid in 18 l of deionized water. Add 50 ml of mixed indicator solution and allow overnight. Zinc: powdered or granular, 10 mesh. Sodium hydroxide: 50% wt/vol. aqueous (saturated)
Standardized 0.1 N HCl (hydrochloric acid) solution.

Procedure: 1g of sample weight and transfer into an ash-free filter paper, and fold it. Put one catalyst in the Kjeldahl flask. Adding 25 ml of reagent, concentrated H2SO4 (sulfuric acid) to each Kjeldahl flask. Switch on the digestion by preheating the digester block to 370°C, and place Kjeldahl flask on it for 3 hours. Remove flasks from the digester, and let it cool, then add up 400 ml of deionized water. Make ready the receiving flask for steam distillation by putting in 75 ml of boric acid solution to a clean 500-ml Erlenmeyer flask and place on a distillation rack shelf. Place a delivery tube from the condenser into the flask. Turn the water on the distillation system and all the burners on. Ready the sample for distillation by adding up approximately 0.5 g of powdered zinc to the flask, stir vigorously and allow to clear. After digest has settled, take 100 ml of saturated, aqueous NaOH (sodium hydroxide) (50% wt/vol) into a graduated cylinder. Incline Kjeldahl flask containing prepared digest solution about 45° from a vertical position. Run NaOH gradually into the flask so that a layer forms at the bottom. Join flask to distillation-condenser assembly. Do not stir flask contents until strongly attached. Holding the flask strongly, checks cork well in place, agitating contents to stir completely. Instantly set flask on a heater. Take out receiving flask from the distillation condenser delivery tube briefly to permit pressure to equalize and prevent back suction. Continue distillation until approximately 250 ml of distillate was collected in receiving flask. Turn off the heater. Disconnect receiving flask partially and wash the delivery tube with deionized water, collecting the washout water into receiving flask. Replace receiving flask with a beaker containing 400 ml of deionized water. This water will be sip back into the Kjeldahl flask as it cools, clean with water from the condenser tube. Titrate green distillate back to original purple using 0.1 N HCl (hydrochloric acid) and record the volume of acid used in the titration. It is suggested to use a couple of blanks and controls or standards on every run. Blanks - Kjeldahl reagents normally contain small amounts of nitrogen, which must be estimated and rectified in calculations. Make ready blanks for dry samples by folding one ash-free filter paper and put it into the Kjeldahl flask. Treat blanks absolutely like samples to be examined. Standards: weigh two 0.1 g samples of urea, put into an ash-free filter paper, and use just like the rest of the samples. Calculate the percent recovery of nitrogen from urea and make confident and get the result is the one expected.

$$\text{Crude protein, \%} = \frac{(\text{ml of acid} - \text{ml of blank}) \times \text{Normality} \times 0.014 \times 6.25 \times 100}{\text{Original weight}} \times 100$$

Fiber Analysis

Chemicals: Sulfuric acid solution, 0.255N, 1.25 g of H2SO4/100 ml, Sodium hydroxide solution, 0.313N, 1.25 g of NaOH/100 ml, ree of Na2CO3. Alcohol - Methanol, isopropyl alcohol, 95%ethanol, reagent ethanol Antifoam agent (n-octanol).

Equipment: Digestion apparatus, Ashing dishes, Desiccator, Filtering device (Buchner filter) Suction filter: filtering devices. join suction flask to catch in line to the vacuum source with a valve to interrupt or control the vacuum.

Procedure (AOAC 2005)**:** 2 g of sample weight, eliminate moisture and fat using ether (removing fat is not important if the sample has less than 1% fat), take 600 ml beaker, and put in it. Add approximately l g of prepared asbestos, 200 ml of boiling 1.25% H2SO4, and 1 drop of diluted antifoam. Don't add excessive antifoam, as it may overvalue fiber content. Put the beaker on a digestion apparatus with a pre-adjusted hot plate and simmer for 30 minutes, spin the beaker regularly to prevent solids from sticking to sides. Filter through Buchner filter and wash beaker with 50 to 75 ml of boiling water. Repeat and apply a vacuum until the sample is dried. Separate mat and residue from the bottom of Buchner against a top, while covering the stem with the thumb and put it in a beaker. Add 200 ml of boiling 1.25% NaOH, and boil for 30 more minutes. Take out the beaker and filter through the Buchner filter and Wash with 25 ml of boiling 1.25% H2SO4, three 50 ml part of H2O, and 25 ml of alcohol. Dry mat and residue for 2 h at 130°C. And remove, put in a desiccator, cool, weigh and record. Separate mat and residue, and collect to a dashing dish. Burn for 30 minutes at 600°C. Cool in a desiccator and reweigh. Calculate crude fiber content on a dry matter basis as:

$$\text{Crude fiber, \%} = \frac{\text{(Weight after acid and base extraction),(B- weight after ashing, C)}}{\text{(Original weight, A)} \times \text{\% Dry matter}} \times 100$$

Fat analysis (Ether Extraction)

***Chemicals*:** HCl- hydrochloric acid (3N), and anhydrous diethyl ether

Equipment: A Soxhlet extraction system, funnels, filter paper.

Procedure: Weigh approximately 2 g of sample ground through 1 mm-mesh into an Erlenmeyer. Pour 100 ml of 3 N HCl (hydrochloric acid) and heat for 1 hr. Chill at room temperature. Filter directly in a filter paper and wash with distilled water to eliminate all HCl. (hydrochloric acid). Separate the moisture of the sample by drying it in an oven at 105°C for 24 hours. (If the sample were not dried the ether would have difficulties penetrating all the areas of the ingredient). Put the sample with anhydrous diethyl ether in a Soxhlet extractor. Switch on the heater coil high enough to evaporate 2-3 drops of ether per second in the condenser. Extract for 24 hours. The ether should be separated, and put back with clean ether, place the samples in the soxhlet for 8 hours. Take out the sample from soxhlet, air-dry it for about 2 hours, and oven-dry at 105°C for 12 hours. The calculation of crude fat is as follows:

$$\text{Crude fat, \%} = \frac{\text{(Final weight after extraction,(g)}}{\text{(Original weight, g)}} \times 100$$

Moisture content (AOAC method, 1999)

Procedure: Take an empty dish put in the oven at 105°C for 3 hours and place it into the desiccator to cool and measure the empty dish. Weigh the 3g of the sample, pour the sample into the dish, and put the dish with the sample into the oven-dry for 3 hours at 105°C. Take out after drying and put it into a desiccator for cool and again weigh that dried sample. The Calculation is done by the following formula:

$$\text{Moisture \%} = \frac{(W1 - W2) \times 100}{W1}$$

Where,
W1- Weight (g) of the sample before drying
W2- Weight (g) of the sample after drying

Aflatoxin Analysis

***Chemicals*:** Acetone, Sodium chloride, Cupric carbonate**,** Chloroform, Sodium hydroxide, Sulfuric acid, Potassium chloride, Potassium oxide, Ferrous chloride

***Procedure*:** 25 gram of the samples was weigh-in a beaker, adding 106 ml acetone, 19 ml of distilled water, and 1 scoop of sodium chloride (NaCl). The mixture was then filtered through a filter paper. 75 ml was collected in the fresh beaker along with 0.3 gm cupric carbonate powder in another beaker, 85 ml of 0.2 N sodium hydroxide (NaOH) and 15 ml of 0.4M ferrous chloride ($fecl_3$) was taken and content of the two beakers was mixed by quickly exchanging in the two beakers The mixture was then allowed to settle for 10 min before filtering through Whatman paper (No.1) and 100 ml was collected. 100 ml filtrate was transferred into a 500 ml separating funnel and added 100 ml of 0.03 % sulfuric acid (H_2SO_4) and 25 ml of chloroform. The mixture was shaken vigorously releasing the fumes or gases and allowed to settle for 10 minutes. The contents get divided into 2 distinct layers with the lower layer containing chloroform bound to aflatoxin. The lower layer was collected into a 100 ml separating funnel and mixed slowly with 40 ml of 1 % potassium chloride (KCl) and 0.02M potassium chloride (KOH) solution by gentle shaking and allowing for separation. The lower layer containing aflatoxin bound to chloroform was then filtered through a bed of 10 gm anhydrous sodium sulfate and collected in a beaker. The content of the beaker was then evaporated by placing them on the hot plate with a constant temperature of 120° C. The content was transferred to amber colored vials before complete drying. The extract was evaporated on a hot plate under the fume hood. Finally, the dried extract was re-dissolved in 0.2 ml of chloroform and used for TLC and HPTLC spotting.

Urease activity

Chemicals: Hydrochloric acid solution, 0.1N, Sodium hydroxide solution, 0.1N, Phosphate buffer solution, 0.05M: dissolve 4.45g disodium hydrogen phosphate (Na2HPO4.2H20) and 3.40g potassium dihydrogen phosphate (KH2PO4) in water and dilute to 1,000ml. Urea releasing agent: dissolve 30.0g urea, in 1.000ml phosphate buffer solution (3.4.3.3). Prepare immediately before use.

***Equipment*:** Potentiometric titration apparatus or high-sensitivity pH-meter (0.02pH), Water bath controlled at 30°C, Test-tubes, 150x18mm, provided with ground-glass stoppers.

***Procedure*:** Weigh 0.2g of the sample and put it into a test-tube provided with a ground-glass stopper. Pour 10ml urea releasing agent, and close by stopper quickly and shake roughly. Keep the test tube in a water bath at 30°C for 30 minutes. Quickly pour 10ml 0.1N hydrochloric acid solution by pipette and cool immediately to 20°C. Pour the contents of the tube to a titration vessel, wash out twice with 5ml of water. Titrate rapidly using the potentiometric apparatus to pH 4.7, using a 0.1N sodium hydroxide solution.

For Blank test- Weigh 0.2g of sample and put it into a test-tube of a ground-glass stopper. Adding 10ml 0.1N hydrochloric acid solution by pipette and 10ml of urea solution. Rapidly cool the test tube in chilled water for 30 minutes. Pour the sample of a tube into the titration vessel, wash out two times with 5ml of water. Titrate rapidly using the potentiometric apparatus till pH 4.7, using a 0.1N sodium hydroxide (NaOH) solution.

The urease activity is calculated by using for the formula:

$$\text{mgN/g/minute at } 30°C = \frac{1.4\,(b - a)}{30 \times E}$$

Where,

a - ml 0.1N sodium hydroxide solution used in the titration of the sample;

b - ml 0.1N sodium hydroxide solution used in the titration of the blank test; and

E - Weight of sample in grams

IV. Results

Protein

Protein estimation by Kjeldahl method (AOAC method). Soya chunks are the best source of plant-based protein. The protein content of soya chunks ranges from 36-56% of dry weight.

Fat

The fat estimation by Ether Extraction (AOAC method) with the soxhlet apparatus. Soya chunks are the defatted product so that fat content is very low. It ranges from 0.5 to 1%.

Carbohydrate

Soya chunks have low carbohydrates and low in the glycemic index suitable for diabetes people. The carbohydrate in soya chunks ranges 30-33%.

Fibers

Fiber estimation by the procedure of (AOAC method, 2005). Soya chunks have a fair amount of fibers**,** good fiber content helps to fight diabetes and control obesity. The fiber content in soya chunks ranges from 8-12%.

Table I: Nutritional Composition Values in Soya Chunks.

Nutrients*	Sample A	Sample A	Sample B	Sample C
Carbohydrates	33.05	32.36	34.36	32.05
Proteins	54.64	56.09	56.48	54.48
Fats	0.54	1.07	0.54	1.06
Fibers	10.92	10.46	8.54	12.39

* All values are given in % dry basis.

Moisture content

The moisture content is determined by (AOAC method, 1999). The moisture affects the freshness and firmness for the storage of food for a longer period and it also determines the actual quality of the food before utilization. The moisture content in soybeans is 13-15%. But in soya chunks, the moisture content is 6-10%.

Table II: Moisture Content in Soya Chunks

Parameter	**Sample A**	**Sample B**	**Sample C**	**Sample D**
Moisture	8.5	7.3	7.6	6.4

Water activity

Water activity determines the water activity device. It is the prediction of the possible growth of microorganisms that causes spoilage of food products. It ranges from 0.2 for very dry foods products to 0.99 for moist fresh food products. In this paper, the result shows water activity is 0.5-0.6, therefore there is no possible growth of microorganisms in soya chunks.

Table III: Water Activity in Soya Chunks.

Parameter	Sample A	Sample B	Sample C	Sample D
Water Activity (Tempt 30.1°C)	0.583	0.546	0.522	0.529

Table IV: Microbial Contamination in Soya Chunks

Parameter	Sample A	Sample B	Sample C	Sample D
Total plate count/ Yeast and Mold	Nil	Nil	Nil	Nil

Urease activity
This is the test used for the checking of the soybean quality, and the method evaluates the unused urease activity to check the trypsin inhibitors present in the soybean that is killed by the processing of heat. Urease test estimates increase in pH outcomes of the liberation of ammonia into media transpires that split urea into the urease existing in the soybean. The degree of processing of urease activity (ΔpH) is under processing is above 0.20, adequately processed is ranges from 0.05 to 0.20, and over-processed is below 0.05.

Table V: Urease Activity in Soya Chunks.

Soya samples	Urease activity (MgN/g/min)
A1	0.23
A2	0.25
A3	0.42
A4	0.26
A5	0.21

Soya samples	Urease activity (MgN/g/min)
B1	0.28
B2	0.26
B3	0.30
B4	0.24
B5	0.30

Soya samples	Urease activity (MgN/g/min)
C1	0.35
C2	0.21
C3	0.37
C4	0.32
C5	0.25

Soya samples	Urease activity (MgN/g/min)
D1	0.35
D2	0.44
D3	0.39
D4	0.32
D5	0.21

Aflatoxin
The Aflatoxin contamination in soya chunks is determined by (AOAC method). Aflatoxin is a common mycotoxin in soybean. The existence of aflatoxin in soy products is comparatively rare. The limit of quantification of aflatoxin in soya chunks is 5 ppb. There are 20 different samples tested and the result revealed that the aflatoxin contamination of all 20 samples was negative in soya chunk.

V. CONCLUSION

Soybean protein is a major important nutrient in human food. Soya chunks are perfect protein sources and also contain good sources of minerals, fibers, and vitamins. The nutritional parameter results conclude that the soya chunks are protein-rich, low carbs, a defatted, good source of fiber soybean by-product. The moisture content in soybean is 13-15%, and water activity ranges from 0.2 for dry food products to 0.99 for moist fresh food products. In this paper, the result shows water activity is 0.5-0.6, and moisture content is 6-10%, therefore there is no microbial contamination due to low moisture and low water activity and high-temperature extrusion process. The degree of processing of urease activity (ΔpH) is under processing is above 0.20, adequately processed is ranges from 0.05 to 0.20, and over-processed is below 0.05. It was found that the urease activity of soya chunks was under processing (i.e. 0.2 -0.4). Aflatoxins are poisonous carcinogens and mutagens produced by molds. But the occurrence of aflatoxin in soy products is relatively rare. Aflatoxin is produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The limit of quantification of aflatoxin is 5 ppb. There are 20 different samples tested and the result revealed that the aflatoxin contamination of all 20 samples was negative in soya chunks. The usage of soybean products may be increased in the future.

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