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Original Research Article

Evaluation of proximate, determination of minerals and chromatographic quantification of water soluble vitamin in newly developed soy protein isolate

Md. Nazim Uddin*, Dr. KanikaMitra[1], Md. MahfuzurRahman[2], A T M Abdullah[2], Dr. Md. Zahurul Haque [3]
*Scientific officer, Baby food, Supplementery food and legume product section, Institute of food science and technology (IFST), Bangladesh council of scientific and industrial research (BCSIR), Dhanmondi, Dhaka-1205
[1]Senior Scientific officer, Baby food, Supplementery food and legume product section, Institute of food science and, technology (IFST), Bangladesh council of scientific and industrial research (BCSIR), Dhanmondi, Dhaka-1205
[2]Senior Scientific officer, Food safety Laboratory, Meat, poultry & Slaughter house Waste, Institute of food science and technology (IFST), Bangladesh council of scientific and industrial research (BCSIR), Dhanmondi, Dhaka-1205
[3]Director and Chief Scientific officer, Institute of food science and technology (IFST), Bangladesh council of scientific and industrial research (BCSIR), Dhanmondi, Dhaka-1205

***Corresponding author**
Md. NazimUddin
Email:nazimbiochem@gmail.com

Abstract: The aim of this study was to evaluate of proximate, determination of minerals and chromatographic quantification of water soluble vitamin in newly developed soya protein isolate. Fat, moisture, ash, protein, crude fibre, total carbohydrate, total energy, lactose and total solids were determined and it is showed that (91.04±0.4) % of protein was found in this soy protein isolate. Minerals were analyzed by atomic absorption spectrophotometer and showed that 219.84±6.07 mg calcium, 96.48±11.62 mg magnesium; 22.19±1.04 mg iron, 1081.84±16.64 mg potassium, 9.08±2.02 mg zinc, 2.76±0.66 mg copper, 1030.02±5.40 mg sodium and 1.41±0.23 mg Manganese are present in 100 g soya protein isolate. Water soluble vitamins were also analyzed by UPLC-MS-MS and micro levels of vitamins are found in SPI. Besides with other nutrients, this soya product contains high amount of protein, minerals, B vitamin, low fat, no lactose and low carbohydrate that are valuable for human health.
Keywords: Soy proteins isolate atomic absorption spectrophotometer, UPLC-MS-MS, vitamins, minerals, and Health

INTRODUCTION

Soya bean contain approximately 38% protein which is an important source of vegetable protein and contains no cholesterol [1].These proteins contain all amino acids essential to human nutrition, which makes soy products almost equivalent to animal sources in protein quality but with less saturated fat and no cholesterol [2]. Soy foods can be best described as uniformly high in protein but low in calories, carbohydrates, and fats, entirely devoid of cholesterol, high in vitamins, easy to digest, tasty, and wonderfully versatile in the kitchen, which positions them as irresistible new food staples for the evolving spectrum of health diets [3].

The number of population increase is continuous process and inadequate supply of protein has inadvertently increased the occurrence of malnutrition in developing countries [4]. Thus, considerable focus has been given to protein from plants especially soya bean but raw soy must processed to edible product. So we developed soya protein isolate (SPI) as a source of natural protein and our present objectives are to evaluate of proximate, determination of minerals and chromatographic quantification of water soluble vitamin in newly developed soya protein isolate.

MATERIALS AND METHODS

Samples

Soya was collected and cleaned. Soy protein isolate was developed in our laboratory by many trials. After developing as a finished product, soy protein isolate was preserved in refrigerator for analysis.

Proximate and other chemical analysis

Determination of moisture content

The method described by Pearson's was used [5]. Moisture content was determined as the loss in weight due to evaporation from sample at a temperature of 105°C.

Determination of ash

This was determined according to the method described by Pearson's [5]. The crucible with sample was gently heated on the Bunsen flame until smoke ceased, and then transferred into a muffle furnace where it was burnt at 600°C to white ashes. The crucible and its contents were then removed and placed in a desiccator to cool after which it was weighed to a constant weight and calculated the amount of ash content.

Determination of crude protein by Kjeldahl Method

The method described by Pearson's was used [5]. The nitrogen content was multiplied by 6.25 (conversion factor) to obtain the percentage protein for soya protein isolate. The procedure was carried out in three stages: digestion, distillation and titration.

Determination of fat

Determination of fat was carried out by Werner-Schmid process [5]. Proteins are digested with conc. hydrochloric acid. Liberated fat is extracted with alcohol, ethyl ether and petroleum ether. Ethers are evaporated and residue left behind is weighed to calculate the fat content.

Determination of crude fibre content

The crude fibre content was carried out using the method described by Pearson's [5]. 2-4 g of sample was defatted. The defatted sample was boiled under reflux for 30 min with 200 ml (1.25%) H2SO4. It was further filtered and washed with boiling water until the washing was no longer acidic. The residue was boiled in a round bottom flask with 200 ml (1.25%) NaOH for another 30 min filtered and washed with boiling water until the washing was no longer alkaline. The residue was scraped into a previously weighed crucible and dried at 100°C. It was left in a desiccators to cool and weighed. It was thereafter incinerated in a muffle furnace at about 600°C, left in a desiccator to cool and then weighed and calculated the crude fibre.

Carbohydrate estimation

Carbohydrate content was calculated by subtraction of the sum of moisture, protein, and fat, crude fibre and ash contents [5].

Total Energy (Calorific Value) Determination

Energy was calculated as described by Osborne and Voogt[6] using the Atwater factors: 1g of carbohydrates (C.) provides (4Kcalories), 1g of protein (P.) provides (4Kcalories) and 1g fat (f.) provides (9Kcalories).

Total Solid Content

Total solid content of the SPI were determined gravimetrically by drying a sample to constant weight in an oven at 105ºC according to the AOAC (2006) [7]. Soy protein isolate sample (3 g) was crushed with 20 g sea sand and glass stick in pre dried weighing dish. The difference in weight before and after drying for 4-5 hours at 105ºC gives the results of total solid content.

Determination of lactose

Lactose was determined by the copper reduction method [5]. Weight the sample into 250ml volumetric flask, dilute with hot water and allow standing for 30 minute. Cool and add 4 ml carrez I solution, mix and 4 ml carrez II solution. Finally dilute to mark, filter and determine the lactose by Lane and Eynone's method using standard Fehling solution.

Minerals and heavy metal determination

Minerals were determining according to Pearson's[5, 8].Weight the sample and ash was prepared in muffle furnace. The stock solution was prepared by using hydrochloric acid and then minerals and heavy metals were determined by using the Atomic Absorption Spectrophotometer (AAS), model: Thermo scientific, ICE 3000 series.

Determination of B vitamins by UPLC-MS-MS

Water soluble vitamins were determined according to Nazim, M. U *et al.*[8], ShanazPerveen*et al.*[9] and Evelyn Goh[10]by UPLC-MS-MS (model: Thermo scientific, ultimate 3000) with some modification.

Materials

Formic acid (BDH, Anala R) and methanol (BDH, Anala R) were used. Vitamin standard were purchased from Sigma-Aldrich. Acetic acid (BDH, Anal R), HCl (reagent grade) and water (deionized) were used.

LC Condition

LC System: Acquity UPLC system, Column: Acquity UPLC C 18, 2.1X50 mm, 1.8µm, Column temperature: 40° c, Sample temperature: 4°c, Flow rate: 0.4 ml/min. Mobile Phase A: 0.1% formic acid in water, Mobile Phase B: 0.1% formic acid in ACN, Total runtime: 5.0 min, Injection Volume is 10.0 micro liters, full loop.

MS Condition

MS System: Xevo TQ MS, Ionization: ESI Positive, Capillary Voltage: 1.0 KV, Source Temperature: 130° c, Desolvation Temperature:450° c, Desolvation gas :900L/hr, Acquisition: Multiple reaction monitoring (MRM) with RADAR full scan, Collision gas: Argon at $3.5x10^{-3}$mhb.

Preparation of Standard Solution

The standards (each) were freshly prepared by dissolving into acetic acid and methanol. The contents were diluted to volume with water. Prior to injecting into the liquid chromatograph, the solution was filtered through 0.45 micrometer membrane filter.

Preparation of Sample Solution

The sample was refluxed for 15 minutes on boiling water bath using 0.1 N hydrochloric acid and water. The content was centrifuged and the supernatant was filtered through filter paper followed by 0.45 µm membrane filter before inject into LC system.

Acquisition and processing method:

Data were acquired using MassLynx software, v.4.1 and processed using TargetLynx Application manager. IntelliStart Technology was used to automatically develop fully optimized MRM acquisition methods for the vitamin compounds targated in this analysis.

Two MRM transitions were optimized for each vitamin compound. The dwell times for the transitions were automatically optimized to give a minimum of 12 point across each chromatographic peak for reproducible quantitation. The MRM transitions, cone voltages, and collision energies for the analyzed compounds, along with expected retention times, are shown in table1.

STATISTICAL ANALYSIS

The mean and standard deviation was determined by using MS Excel software.

RESULTS AND DISCUSSION

Proximate and other chemical analysis

Results proximate and other chemical analysis are shown in table 2. This soya product contains (91.04±0.4) % protein and soy protein dramatically reduces serum concentrations of total cholesterol, low-density lipoproteins (LDLs), and trigylcerides [11-12]. Soy protein also helps to the control of hyperglycemia and reduced body weight, hyperlipidemia, and hyperinsulinemia [13]. (91.04±0.4) % of protein in this SPI may provide physiological activities for human body.

Minerals and heavy metals

Minerals play an essential role in the body and heavy metals are toxic to the body. Many vitamins and enzymes need a mineral cofactor for proper function.

Minerals and heavy metals are analyzed by atomic absorption spectrophotometer and content of the minerals are 219.84±6.07 mg calcium, 96.48±11.62 mg magnesium, 22.19±1.04 mg iron, 1081.84±16.64 mg potassium, 9.08±2.02 mg zinc, 2.76±0.66 mg copper, 1030.02±5.40 mg sodium and 1.41±0.23 mg Manganese are present in 100 gm fresh SPI (table 3). Heavy metals (cadmium, chromium, lead and arsenic) were not detected in the sample by atomic absorption spectrophotometer.

SPI contain remarkable amount of calcium, magnesium and iron and the role of calcium and magnesium in bone and teeth health is well documented and recognized [14-15]. Other minerals such as sodium, zinc, copper, manganese and potassium are present in the SPI. These are involved in the metabolism of carbohydrate, fat, and protein, as well as DNA and RNA replication. These minerals functions as an antioxidant, aids in maintaining healthy bone structure development, maintains healthy immune functions, maintains healthy vision, supports normal foetal growth and play an important role in neuromuscular activity [16]. These minerals are also an activator of hundreds of enzymes those are essential to active life.

Water soluble vitamin

Results of water soluble vitamin are shown in Table 4. Many of the water soluble vitamins are acts as a coenzyme. These coenzymes are responsible for many of the physiological activities, function, growth, and development. Deficiency of these vitamins leads to pathologic, dermatologic, and neurocutaneous manifestations. These vitamins are involved in important metabolic pathways such as gluconeogenesis, fatty acid synthesis, and amino acid catabolism. Biotin regulates the catabolic enzyme propionyl-CoA carboxylase at the posttranscriptional level whereas the holo-carboxylase synthetase is regulated at the transcriptional level [8, 17]. Folic acid, thiamin, riboflavin pantothenic acid and its derivatives are beneficial in the maintenance of healthy skin and for cellular wound healing processes [19].

Table 1: The MRM transitions, cone voltages, and collision energies for the analyzed compounds, along with expected retention times

Analyte	Parent (m/z)	Dau 1/Dau 2 (m/z)	CV (V)	CE 1/ CE 2 (eV)	RT (min)
Ascorbic acid,C	177.0	141.0	16	8	0.37
Thiamine, B1	265.2	122.0	18	16	0.41
Pyridoxal, B6	168.0	150.0	14	14	0.64
Nicotonic acid, B3	124.0	80.2	34	20	0.51
Pantothenic acid, B5	220.1	90.0	20	14	2.73
Folic acid, B9	442.2	295.1	18	16	2.99
Biotin, B7	245.1	227.0	20	14	3.10
Riboflavin, B2	377.2	243.1	36	24	3.15

Table 2:Proximate and other chemical analysis (means ± SD, n=3)

Parameters	Contents in 100 g SPI
Moisture %	1.01±0.02
Ash %	5.00±0.15
Protein %	91.04±0.4
Fat %	0.16±0.03
Crude fibre %	0.01±0.005
Total carbohydrate %	2.77±0.46
Energy (kcal)/100 gm	376.74±0.73
Total Solid %	98.99±0.02
Lactose %	Not detected

Table 3: The concentrations of Minerals and heavy metals in SPI in mg/100 g (means ± SD, n=3).

Minerals / Heavy metals	Contents in 100 g SPI
Calcium	219.84±6.07
Magnesium	96.48±11.62
Iron	22.19±1.04
Potassium	1081.84±16.64
Zinc	9.08±2.02
Copper	2.76±0.66
Sodium	1030.02±5.40
Manganese	1.41±0.23
Cadmium	ND
Arsenic	ND
Chromium	ND
Lead	ND

ND= Not detected

Table 4: The concentrations of B vitamin in SPI in μg/100 g (means ± SD, n=3).

Vitamins	Contents in 100 g SPI
Vitamin B1	1.15±0.122882
Vitamin B2	0.496667±0.053125
Vitamin B6	1.83±0.052915
Pantothenic acid	10.52666667±1.406852278
Folic acid	0.206667±0.020817
D Biotin	26.94333333±0.090737717
Vitamin C	193.2867±20.12855

CONCLUSION

Human's body needs a multiple nutrients to lead a healthy and active life. SPI is one of the good sources of high quality protein can play an important role in solving the malnutrition problem of Bangladesh. Soybean is a crop which can produce high quality and highest quantity of protein. Low income people of our country can easily fill up their protein demand by consuming this soya product. As per we know, SPI industry is not present in Bangladesh. So this project may help the industrialist to develop this new soya product by using our cultivated soya. We concluded that lactose free, low fat and low carbohydrate containing this soya product may provide the high amount of protein, minerals and vitamin for running the healthy life.

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