RESEARCH ARTICLE

Frontiers in Advanced Materials Research

DOI: 10.34256/famr2423

Influence of High-Pressure Processing on Foxtail Millet Protein Concentrate (FMPC) and its Characteristics

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Received: 01-10-2024, Revised: 05-12-2024, Accepted: 13-12-2024, Published: 24-12-2024

Abstract: The pre-treated Foxtail millet were collected with milk sample to obtain a FMPC by biochemical extraction process. The optimized milk ratio for the testing prepared sample was done with the ratio of the1:2 (Foxtail millet: Distilled water). The effect of HPP (High Pressure Processing) treatment for the millet milk was optimized at 550 MPa for 5 minutes to improve the yield of FMPC as well as improvement in Total Phenolic Content 1403.51 mg GAE/ g, dry weight for the prepared FMPC. The freeze-dried FMPC in dry powder was obtained with the optimized time period of 24 hours at -40°C. The prepared FPMC was characterized with FTIR analysis and compared with commercial protein powder. Further analysis of proximate studies revealed that the FMPC contains a higher amount of Protein content with ranging from 13-17% for the utility towards the commercial application as millet protein supplement in food products.

Keywords: Foxtail Millet Protein Concentrate, High Pressure Processing, Foxtail Millet Milk, Proximate analysis, Total phenolic content

1. Introduction

The importance of millets is focused to be a good nutritive source for maintaining the quality of human need towards utilization of high content protein, carbohydrates, mineral and micronutrients [1]. The primary concern of creating awareness in health-related aspects to promote the production of protein based by products from foxtail Millet milk in developing Indian Market for the utilization of feeding good protein content to satisfy the human and animal need [2]. The extraction of milk from foxtail millet with different methods such as soaked millet and germinated millet can be used for the production of protein-based value-added products to satisfy the nutritional constituents. The Foxtail millet is the highest yielding millet from the family of *Setaria italica* L. genus and *Poaceae* family. The harvesting period is ranging from 75-90 days. The composition contains protein, mineral, high fibre content and photochemical constituents

[3]. The millet exhibits the health beneficial properties such as hypolipidemic, antioxidant nature, low glycemic index, anti-atherosclerotic effect [4] and anticancer effect. Foxtail millet seed is providing an important necessary source of Vitamin B12, Iron supplements for infants to promote the growth development of strong bones. The nutrient composition of every 100gm of millet contains total carbohydrate 73gram, energy 378 calories, total fat 4.2 gm, saturated fat is 0.7 gm, dietary fibre 8.5 gm, protein content 11gm. The mineral composition with the content of Ca- 1%, Fe-17%, Cu- 38%, Mg- 28%, Mn- 82%, P- 28%, K- 4%, Se- 4% and Zn -11%. The micronutrient with the content of folic acid- 85 mcg, water soluble vitamin B3 (niacin)- 4.720 mg, water soluble vitamin B5 (Pantothenic acid)- 0.848 mg, water soluble vitamin B2 (Riboflavin) 0.290 mg, water soluble vitamin B1 (Thiamine) 0.421 mg, water soluble vitamin B6 (pyridoxine) 0.384 mg, Fat soluble vitamin E 0.05 mg, α-Tocopherol (type of Vitamin E) 0.05 mg and phylloquinone - vitamin K 0.9 mcg.

The water requirement condition for millet growth is less and the seed content is highly nutritious. The production of millet is obtained with less cost utilization of irrigation method and pesticide usage. The durability of millet storage is maintained for longer period by resisting the pest attack [5]. In general, the consumption of Foxtail millet seed involves with the mechanism of reduction rate of glucose release over a period and this effect which leads to the beneficial aspects in diabetes mellitus.

Foxtail millet is the unique millet seed protein with the capability of possessing high protein content containing essential amino acids useful for the promotion of health benefits as an additive in food processing and dietary supplements [6]. The biochemical composition of mature seeds contains setarin 60% of total protein content as well as proline rich constituents are also present with higher content ratio. The foxtail millets have wide range of application in the field of pharma industry, food sector and biological application. The yield of the protein concentrate is influenced by the extraction method, nutritional facts and composition [7]. The effect of bioactive agents for the FMPC is widely applied for treating the human chronic diseases. The FMPC is a good source of protein supplement with the replacement of animal protein food powder with low-cost production of new protein concentrate functional powder.

The protein concentrate is the commercial product of protein and this can be formulated with food products to manufacture health-based food products in large quantity [8], [9]. An extraction process of biochemical method is used to obtain a protein concentrate and build a desired amino acid profile from the underutilized foxtail millet seeds [10]. The FMPC is utilized widely for food application, bio sector and pharma sector [11]. The essential nutritional fact is inclined with fortifying process for complementary protein to satisfy the protein feed for humans [12].Usually the less fractions of Millet protein exhibit an excellent property of nutritional availability and palatability nature [13], [14]. The ingestion of nutrient source present in the food diet is helpful for the human growth metabolism. The essential amino acids are the indispensable nutrient components with different degree of nutritional quality, bioavailability and

digestibility. The plant protein is obtained from various sources containing balanced nutrition quality for application toward human demand of value added agro-industrial products.

The isolated FMPCs is useful for the sustainment of protein nutrient supplement in blending with other food substances. The Foxtail millet is the recommendable millet protein concentrate as compared with all other millet protein concentrates. This research work is carried out to create an awareness to improve the quality of human life and development of protein concentrate millet -based products.

2. Materials and Methods

2.1 Material collection and Preparation

Foxtail millets of about 5 kg were collected from the Centre for Excellence in Grain Science, NIFTEM-T, Thanjavur, India. After the collection of the sample were de-husked by traditional method for the processing of dehulling mechanism. The pre-treatment methods such as soaking and germination method. Then, the remaining samples were stored with air-tight pouches or polypropylene bags.

2.2. Reagents collection

The reagents such as Sodium hydroxide, Sulphuric acid, Folin Ciocalteu Reagent, Gallic acid, Sodium carbonate, Sodium sulphate, Copper sulphate, Boric acid. Whatman filter paper, Ninhydrin reagent and ethanol were collected from HIMEDIA, India. Hexane was supplied from LOBA Chemie Pvt. Ltd, Mumbai, India.

2.3. Pre- treatment methods

2.3.1. Soaking of Foxtail-millet

250 gm of foxtail millet were collected and cleaned with distilled water to obtain a desired quality after de-husking treatment. The addition of water in soaking process is in the ratio of 1:2 (Foxtail-millet: Distilled water -250:500). The ideal soaking period is maintained for 6-8 hours at normal room temperature.

2.3.2. Germination of Foxtail-millet

The ratio of foxtail millet of about 1:2 (Foxtail-millet: Distilled water -250:500). The millet seeds were soaked in distilled water over a period of 12 hours and drain the water from the seeds before placing them in the bag. Damp a paper towel and sealed it tightly for the process of sprouting in the foxtail millet seeds. The complete sprouting was observed for the period of 2 days.

2.4. Extraction of the Foxtail millet milk

The foxtail- millet were soaked in water ratio of 1:2 for a time duration of 6-8 hours. Then, the soaked millets were processed further for grinding in a mixer up to 20 minutes. The millet milk is completely extracted by using a cloth mesh and the remaining residues were stored separately for further analysis. The same procedure was done for the germinated foxtail-millet for the extraction of foxtail-millet milk.

2.5. Preparation of FMPC

The extraction procedure is followed for the preparation of FMPC from the foxtail millet milk by using the ratio of 1:2 (w/v). The pH was adjusted with Sodium hydroxide solution (1 mol L^{-1}) to obtain a various pH range of about (7.5 up to 11.5). The solid suspension was collected and stirred with magnetic stirrer for the different extraction time period of (10 to 60 minutes) at different temperature (30 to 70 C). The extracted suspension were centrifuged at 2000 rpm for the period of 10 minutes and the resulted supernatants were removed to determine the (FMPC) by using the standard method of Kjeldhal, AOAC, 2000 method. The foxtail millet milk was taken at the ratio of 1:2 (W/V) and pH was adjusted with 9.5 using 1.0molL^{-1}NaOH. Then again, the prepared sample was stirred in the magnetic stirrer plate for about 10 minutes at 30 C. The prepared extracts were centrifuged again stirred for a constant speed 2000 rpm, 10 minutes. Then finally isolated supernatant was adjusted with pH 4 of iso-electric point with hydrochloric acid (1molL^{-1}) the formation of a solid precipitate at room temperature for 1hour and the FMPC was recovered by centrifugation process and washed with deionized water at neutral pH to obtain a FMPC. Wash the protein concentrate with the distilled water for a period of routine of two times and re-suspended in water (pH 7.0) with NaOH. The FMPC was freeze dried at (-30 C to -150 C) and followed by subsequent freeze-drying process for 24 hours to obtain a fine powder of FMPC. Four batches of different FMPC were prepared with S1- Soaked -FMPC, S2- Germinated- FMPC, S3- HPP treated Soaked -FMPC and S4- HPP treated Germinated -FMPC for further analysis.

2.6 HPP treated Foxtail-millet milk

The pre-treated Foxtail millet milk solution of about 175 ml was taken in sealed PET bottles. The HPP treatment was carried out with the instrument HPP 600/5L model, KK life sciences, model number KK-HPP-TE. The operation procedure of maintaining the water temperature control unit around 10°C, 415 V, 50 Hz, oil temperature constant 45°C and power 2.2 kw. The prepared sample of about different batches of 175 ml soaked and germinated foxtail millet milk were loaded in the PET bottles and treated with 550 MPa for 300 seconds. The optimization of high-pressure flow range within the HPP pressuring vessel was processed to improve the extraction of FMPC and optimized for further characterization as well as proximate analysis for the prepared samples of S3 and S4.

2.7. Proximal analysis of FMPC

2.7.1. Determination of Moisture content

The moisture content of the prepared sample FMPC was measured in triplicates as loss in weight of the sample on heating at 105 ºC for 3 hours. 2 gm of sample were taken in weighed stainless-steel dish having lids. Sample in the dishes were placed in a hot air oven (Ever flow, Chennai, India). After drying, the dishes were transferred to a desiccator. The final weight of the dish with sample was recorded with method of AOAC, 2000. Moisture content was calculated by using the formula given below

$$\text{Moisture content (\%)} = \frac{Initial\ weight\ of\ dish\ with\ sample(g) - Final\ weight\ of\ dish\ with\ sample\ (g)}{weight\ of\ sample\ taken(g)}\ X\ 100 \quad (1)$$

2.7.2. Determination of FMPC content

The total FMPC content was estimated by the standard AOAC, 2000 method. 0.2 gm of the prepared was hydrolysed with the composition of 10 ml concentrated Sulphuric acid, 5 gm Sodium sulphate and 1 gm Copper sulphate. The chemical composition along with test sample were used in digestion flask at 420 ºC for the time period of 2 hours. The acid tank contains 4% of Boric acid 4% added and 40 % of sodium hydroxide solution in alkali tank. The test samples were digested in the Kjeldhal tubes and distilled with the presence of acid and base. Finally, the distillate was collected in the conical flask (250ml). The distilled sample was taken in the conical flask and titrated against the mixed indicator solution (two drops). The 0.1 N HCl was used as titrant with distillate until the solution colour changes to pale pink as an indicator for the process completion. The conversion factor of about 6.25 was used for the calculation of the total nitrogen value. The measurement was carried out with triplicates.

The total nitrogen and crude protein in percentage of FMPC were calculated by using the given the formula as mentioned below,

$$\%\ N = \frac{\text{Titre value} - \text{Blank value} \times \text{Normality of acid} \times 14.01 \times 100}{W \times 1000} \quad (2)$$

Where, TV= Titre value (ml), BV= Blank value (ml), W = Weight of the prepared sample (g), N = Nitrogen (percent '27').

2.7.3. Determination of Fat content

The FMPC of about 3gm of test portion was taken in a thimble. Without previous drying, the sample was Soxhlet extraction, with hexane for 6 hours. The fat content in the prepared sample was measured using soxhlet extraction, a modified AOAC method, 1995. The dissolved fat was weighed after the solvents had been evaporated to dryness in a hot air oven at 105 ºC for 3 hours. The fat content was determined in triplicates by using 2gm of defatted sample by using the formula given below,

$$Fat\ Content, \text{DM basis } (\%) = \frac{\text{AWres} - \text{Wta}}{\text{Weight of FMPC sample (g)}} \times \text{DM } (\%) \quad (3)$$

Where Wta is tare weight of beaker (gm); AWres is Total weight of the beaker and fat residue (gm)

2.7.4. Determination of Ash content for FMPC

The Ash content of FMPC was calculated in triplicates by the method of AOAC, 1990. 2gm of prepared FMPC sample was taken and weighed previously after dried form and also the weight of the porcelain crucible. The test sample FMPC was holded in the crucible and kept in a furnace with heated temperature 550 °C for 6 hours. Then, the crucibles were cooled in a desiccator till it reached room temperature and reweighed. The weight of the ash content calculated using by using the formula

$$\text{Ash of FMPC } (\%) = \frac{(\text{Weight of Crucible (after ash process)} - \text{Weight of empty crucible})}{\text{Weight of FMPC sample}} \times 100 \quad (4)$$

2.7.5. Determination of FMPC Crude fibre

The prepared Sample Crude fibre was analysed by Weende's method, AOAC 1995. 2gm of prepared sample FMPC was treated with 100 ml of 1.25% of H_2SO_4 and the mixture was heated till the sample is reduced to half of its original volume. This was filtered using muslin cloth and digested with by 100 ml of 1.25% NaOH solution. Heating and filtration were repeated. Finally, the prepared mixtures were filtered with filter paper (Whatman) and dried in hot air oven. % crude fibre was calculated by using the formula

$$Crude Fibre\ (\%) = \frac{\text{Weight of residue}}{-\text{Weight of sample}} \times 100 \quad (5)$$

2.7.6. Estimation of carbohydrate content

Carbohydrate content of the prepared sample was estimated by difference method. It is evaluated by deducting the sum of all contents (moisture, protein, fat and ash) with 100 by using the formula,

$$\text{Carbohydrate } (\%) = 100 - (\text{Moisture} + \text{Protein} + \text{Fat} + \text{Ash content}) \quad (6)$$

2.7.7. Estimation of Energy value

Energy value of the prepared FMPC sample was determined by Theoretical method. It is expressed in Kcal/g, by using the formula given below.

$$\text{Energy value (Kcal/g)} = (9 * \text{fat value}) + (4 * \text{carbohydrate value}) + (4 * \text{protein value}) \quad (7)$$

2.8 Determination of Colour parameters

The colour parameter values (L*, a*, b*) were determined by using Hunter Lab Chroma meter. The prepared test samples of protein concentrate were placed on the white standard plate (Calibration plate values: L*=97.39, a*=0.03, b*=1.77)

The total color difference (ΔE*) was calculated by using following Equation (8)

$$\Delta E^* = \sqrt{(L^* \text{ control} - L^* \text{ sample})^2 + (a^* \text{ control} - a^* \text{ sample})^2 + (b^* \text{ control} - b^* \text{ sample})^2} \quad (8)$$

$$\text{Yellowness index (YI)} = 142.86\frac{b^*}{L^*} \quad (9)$$

$$\text{Whiteness Index (WI)} = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2} \quad (10)$$

2.8.1. Determination of pH

The pH of the sample was identified by using Hanna instrument model as the analysis of protein concentrate was done by using pH Meter.

2.9. Qualitative Determination of Amino acids in FMPC prepared sample

The sample preparation was examined with dissolution of 0.2gm of ninhydrin in 10 ml of ethanol or acetone to make a ninhydrin solution. The prepared sample of 1% FMPC solution containing amino acid (analyte) with distillessd water must be used for further analysis. The lesser drops of about 2% prepared ninhydrin solution were added to tested FMPC solution. The test sample was placed in the tube for keeping it in water bath (hot) for 5 minutes. The observation of deep blue or violet colour denoted the amino acids presence in the prepared FMPC and record the readings with UV- spectrometer 1900-i Shimadzu model.

2.10. Identification of Total Phenolic content (TPC) in FMPC

The total phenolic content was determined using Folin-Ciocalteu's reagent according to the method. The reaction mixture was composed of (100 µl) of FMPC sample (100 µg/ml). The composition of about Folin-Ciocalteu's reagent (0.5ml) along with sodium carbonate (20%) were used in TPC Assay. The mixture was mixed completely with prepared sample and the volume of 10ml makeup with distilled water. The prepared sample was allowed to rest for 2 hours. The absorbance of the prepared sample (FMPC) was recorded at 500-800nm and gallic acid as standard. The readings were taken in triplicate to determine the total phenolic content value was expressed in mg (GAE) / g extract. The readings are measured by using UV-spectrometer 1900i-Shimadzu model.

2.11. Functional properties of foxtail-millet protein concentrate

2.11.1. Estimation of Water Absorption Property:

The water absorption is determined by the method [15] FMPC powered sample (1gm) mixed with 10 ml of distilled water for 1minute by hand shaking and allowed to stand at 25º C for 30 minutes. Then, the content was centrifuges at 1500rpm for 20 minutes, then the supernatant was transferred to 10ml measuring cylinder and its volume was noted.

The amount of water absorbed by the sample were absorbed by the samples were expressed by,

$$\text{Water absorbed } \left(\frac{\text{ml}}{\text{g}}\right) = \text{ vol. of the water added (Initial)} - \text{vol. of the supernatant (prepared)} \quad (11)$$

Where volume of the water added to the sample (Initial); Volume of the supernatant (prepared sample)

2.11.2. Estimation of Oil Absorption Property

The oil absorption was estimated by the method [15]. FMPC Powered sample (1gm) was taken and mixed with 10 ml of refined palm or vegetable oil for 1 minute by hand shaking and allowed to stand at 25 °C for 30 minutes. Then, the content was centrifuged at 1500rpm for 20 minutes, then the supernatant was transferred to 10ml measuring cylinder and its volume was noted. The amount of oil absorbed by the sample were absorbed by the samples were expressed by,

$$\text{Oil absorbed } \left(\frac{\text{ml}}{\text{g}}\right) = \text{ Vol. of the oil added to sample (Initial)} - \text{Vol. of the supernatant} \quad (12)$$

2.11.3. Estimation of Foam absorption Property

The foaming behaviour and stability property were estimated with the method [16]. The prepared FMPC sample (2gm) were dissolved in 100 ml of the distilled water. The resulting solution were homogenized for 5 minutes at high speed. The mixture was poured quickly into a 250 ml graduated measuring cylinder. Finally, the foaming absorption readings were noted.

The amount of foam formed by the samples were expressed as,

$$\text{Foam absorbed (\%)} = \frac{\text{Volume after homogenization} - \text{Volume before homogenization}}{\text{Volume before homogenization}} \times 100 \quad (13)$$

2.12. FTIR spectrum of Foxtail-millet protein concentrate

The FTIR spectrum was analysed for determining the modification in the functional groups after HPP treatment with the prepared FMPC sample. The spectral analysis was recorded

over the wavelength 4500-400 cm^{-1} and resolution of 4 cm^{-1} by using the FT/IR-4000 Instrument Model.

3. Result and Discussion

3.1 Functional properties of FMPC

The FMPC is primarily responsible to stabilize the structural and texture characteristics of processed food products [17]. The foaming property is generated by the dispersion of protein concentrate by the production of cohesive layer that is surrounded over the air bubble formed in the foaming process. The foxtail millet protein contains the non- polar residue which increases the stability of the foaming property. The foaming capacity is getting increased due to the higher degree solubility factor of FMPC and stability. The figure 1(a) results are obtained with the value of 80.313±0.191 (S1), 78.386±0.305 (S2), 77.45±0.240 (S3) and 78.156±0.0083 (S4).

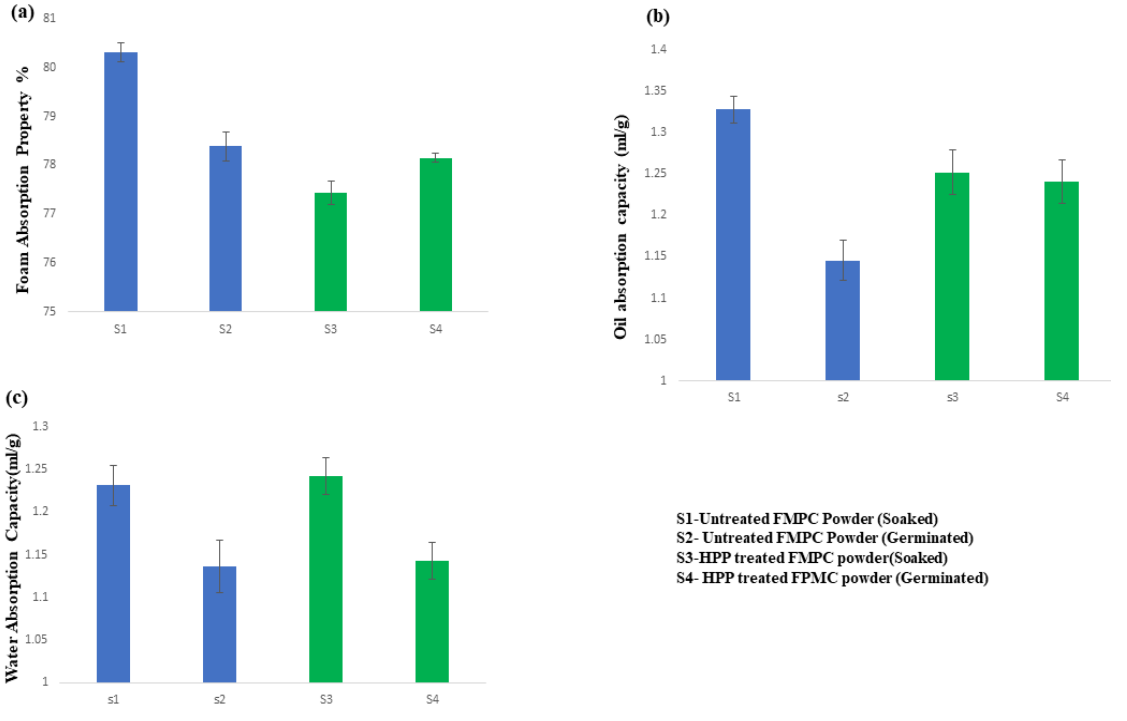

Figure 1. A schematic representation of Foxtail Millet Protein Concentrate (FMPC) (a) Foam Absorption Property (%) (b) Oil Absorption Capacity (ml/g) (c) Water Absorption Capacity (ml/g)

There are several parameters to improve the functional property of the millet protein concentrate such as amino acid profile, surface polarity nature and structural confirmation. The improvement in functional property leads to the maintaining the flavour, total fat content and moisture content [18]. The oil absorption capacity of FMPC is recorded with the value of

1.327±0.016 (S1), 1.145±0.023 (S2), 1.252±0.026 (S3) and 1.241±0.026 (S4) for the prepared four samples as represented in Figure 1(b) and Table 1. The water absorption property of the prepared FMPC Samples S1, S2, S3 and S4 is indicated with the value of about 1.231±0.023, 1.136±0.030, 1.242±0.021 & 1.142± 0.021 as denoted in Figure 1 (C). Similar results were observed with highest value of water absorption capacity for foxtail millet flour sample with the value of 1.26 ml/g [19]. The water absorption property is an important functional parameter in the food dough formulated especially with protein concentrate. From the results, it is suggested that the water absorption capacity is exhibited with higher content of hydrophilic polysaccharides and protein concentrate containing polar amino acid residues from foxtail millet milk[20].

3.2 Estimation of pH and color parameter value for the prepared FMPC

The pH value is determined for the prepared FMPC of about 4.7 (S1) to 5.5 (S2) and HPP treated samples the range is between 5.6 (S3) to 6.1 (S4) with slight modification in pH range for the protein concentrate from Foxtail Millet Milk. The color variation parameters (L^*, a^*, b^*, total difference color (ΔE^*), yellowness index (YI), whiteness index (WI)) and characteristics of the prepared FMPC are represented in the Table 1. From the Figure 2 (a) L^* value slightly decreases upon treatment of HPP for the prepared soaked FMPC which displays the value in between 82.09 ±0.005 (S1) to 71.49± 0.008 (S3) and also untreated germinated FMPC 83.57 ± 0.004 (S2) is getting reduced to 73.01 ± 0.004 (S4). The figure 2 (b) a^* value shows the positive value and increases slowly after the HPP treatment from 1.47 ± 0.003 (S1) to 1.99 ±0.007(S3); 1.06±0.004 (S2) to 2.29±0.006 (S4) due to the presence of the red-coloured amino acid components.The figure 2 (c) b^* value expresses a presence of proline content in the prepared FMPC which is occurred with a slight gain of the yellow color intensity as well as significantly increases from 14.64±0.012(S1) to 15.67±0.045(S3); 12.65±0.007 (S2) to 15.67±0.055(S4) [24]. The total color difference (ΔE^*) in Figure 2(d) measures the increase value from 20.04±0.127 (S1) to 29.46 ±0.779 (S3); 17.62±0.395 (S2) to 28.151±0.549 (S4) after the HPP treatment which influences a brightness in color appearance for both soaked FMPC and germinated FMPC. The whiteness index (WI) for the prepared FMPC is represented in Figure 2 (e) with depletion in the value of 76.82± 0.604 (S1) to 67.41 ± 0.635(S3), 79.24 ± 1.018 (S2) to 68.71 ± 0.861 (S4). In the present study, the yellowness index value is increased normally for the HPP treated FMPC (Fig 2(f)) from 24.87±0.931 (S1) to 31.31± 0.615 (S3); 21.62±0.657 (S2) to 30.66 ±0.825 (S4) which is reflecting higher impact on the change of colour parameters for the prepared FMPC.

3.3 Proximal analysis of FMPC

The proximate analysis data for the prepared FMPC are represented in the Table 2. The moisture content (Fig 3(a)) for all the samples is noted as 12.610±0.002 (S1), 11.638±0.011(S2), 14.025±0.073(S3) and 14.025±0.073(S4) whereas the moisture content for foxtail millet flour were reported around 9.4± 0.3 (per 100g) [21].

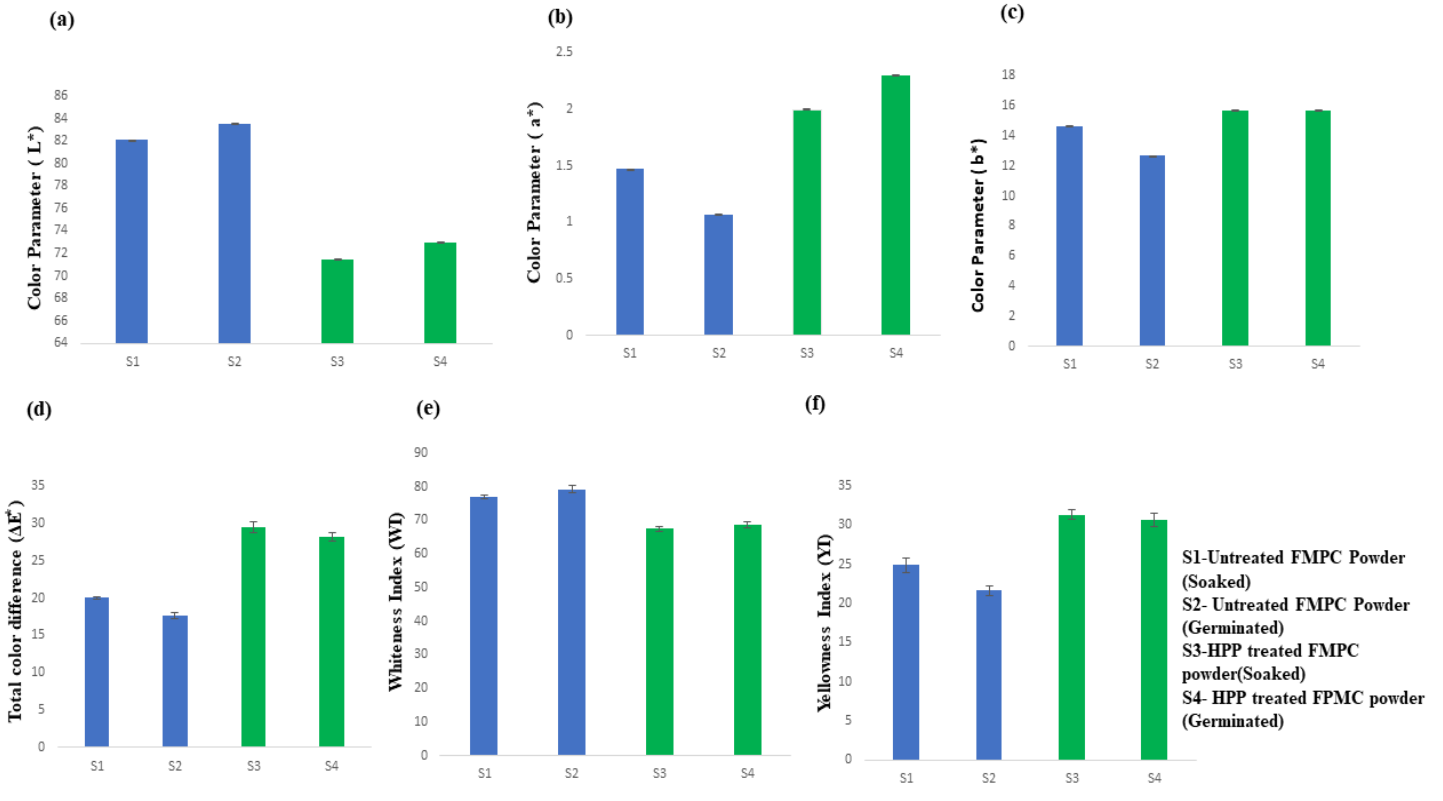

Figure 2. A schematic representation of Color parameter for Foxtail Millet Protein Concentrate (FMPC) (a) Color Parameter (L*) (b) Color Parameter (a*) (c) Color Parameter (b*) (d) Total color difference (ΔE*) (e) Whiteness Index (WI) (f) Yellowness Index (YI)

Table 1. Functional properties and Color parameters L^*, a^*, b^*, total difference color (ΔE^*), yellowness index (YI),whiteness index (WI) for the Prepared Foxtail- Millet Protein Concentrate (FMPC)

Samples	Foam Absorption Property (%)	Oil absorption capacity (ml/g)	Water Absorption capacity (ml/g)	L^*	a^*	b^*	ΔE^*	WI	YI
S1	80.313±0.191	1.327±0.016	1.231±0.023	82.09±0.005	1.47±0.003	14.64±0.012	20.04±0.127	76.82±0.604	24.87±0.931
S2	78.386±0.305	1.145±0.023	1.136±0.030	83.57±0.004	1.06±0.004	12.65±0.007	17.62±0.395	79.24±1.018	21.62±0.657
S3	77.45±0.240	1.252±0.026	1.242±0.021	71.49±0.008	1.99±0.007	15.67±0.045	29.46±0.779	67.41±0.635	31.31±0.615
S4	78.156±0.082	1.241±0.026	1.142± 0.021	73.01±0.004	2.29±0.006	15.67±0.055	28.15±0.549	68.71±0.861	30.66±0.825

Note:Values are expressed as Mean ± Standard deviation (n=3)

Table 2. Proximate Analysis of Foxtail Millet Protein Concentrate (FMPC)

Samples	Moisture content %	Protein Content %	Fat Content%	Ash content%	Crude Fibre	Carbohydrate Content (%)	Energy Value (Kcal/g)
S1	12.610±0.002	14.078±0.06	7.056±0.034	3.116±0.060	0.520±0.015	63.356±0.038	373.48±0.565
S2	11.638±0.011	17.066±0.003	7.656±0.007	1.063±0.034	0.519±0.010	62.639±0.015	387.92±0.134
S3	14.0253±0.073	16.182±0.001	10.337±0.004	1.05±0.0337	1.089±0.045	58.488±0.004	391.82±0.349
S4	10.151±0.001	13.564±0.002	13.336±0.003	1.152±0.081	0.519±0.014	61.991±0.002	421.810±0.502

Note:Values are expressed as Mean ± Standard deviation (n=3)

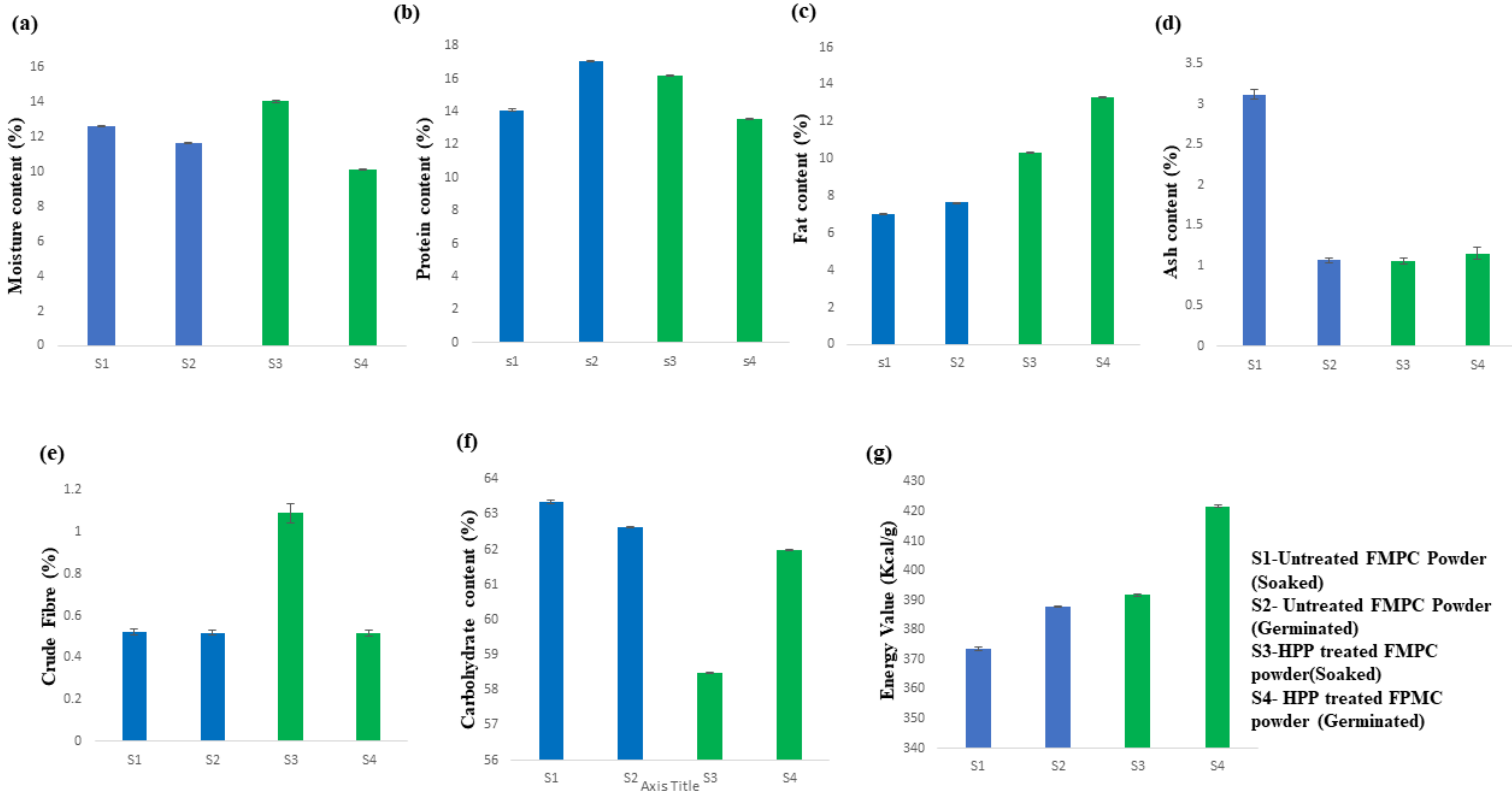

Figure 3a. schematic representation of Proximate analysis for Foxtail Millet Protein Concentrate (FMPC) (a) Moisture content (%) (b) Protein content (%) (c) Fat content (%) (d) Ash content (%) (e) Crude Fibre (f) Carbohydrate content (g) Energy value (Kcal/g)

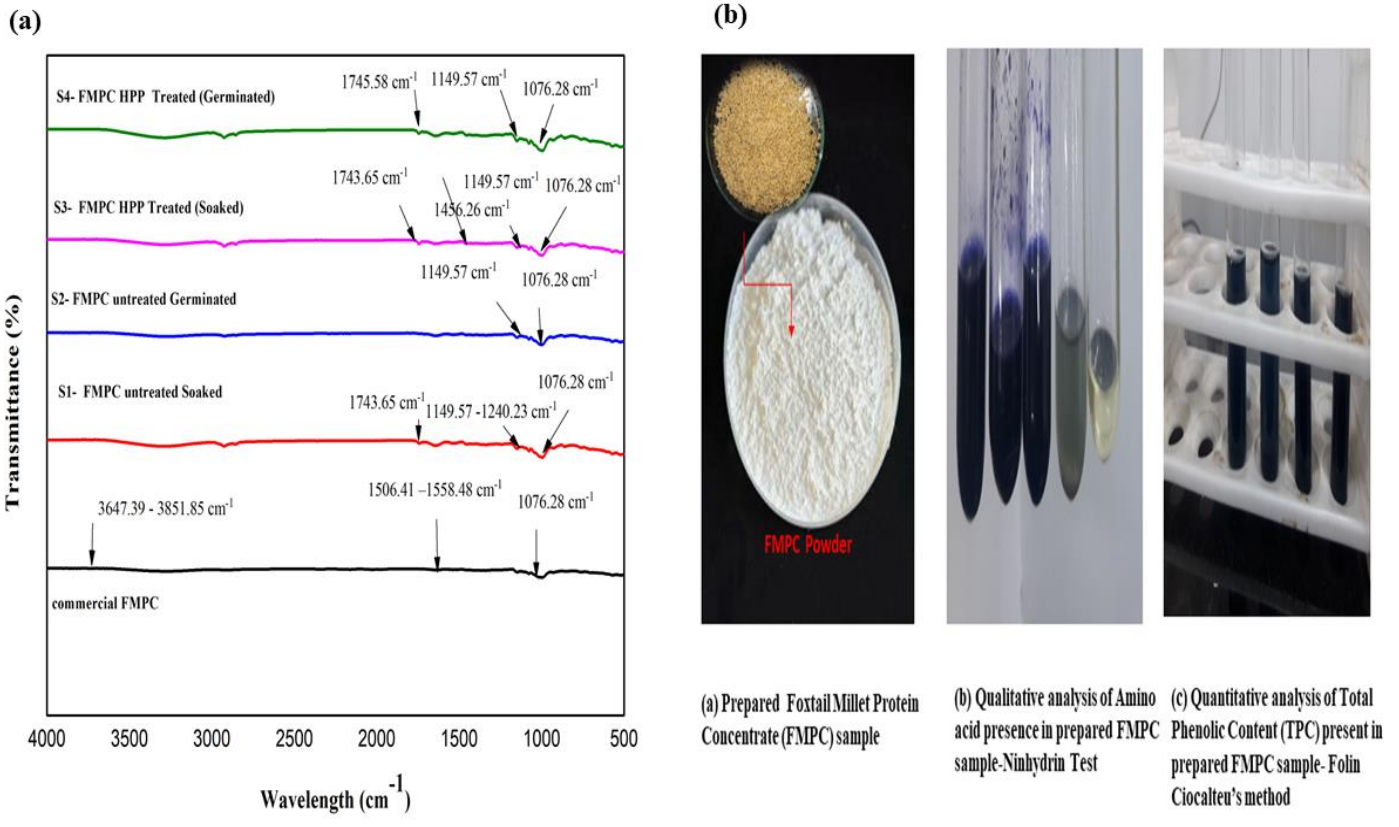

Figure 4. (a). FTIR spectrum of the Foxtail Millet Protein Concentrate (FMPC) for the prepared samples (b). A visual observation for the presence of amino acid and Total Phenolic Content (TPC) in the prepared FMPC (a) Prepared Foxtail Millet Protein Concentrate (FMPC) powder (b) Qualitative analysis of Amino acid presence in prepared FMPC sample-Ninhydrin Test (c) Quantitative analysis of Total Phenolic Content (TPC) present in prepared FMPC sample- Folin Ciocalteu's method.

The protein content (Fig 3(b)) of the FMPC is recorded with the value of 14.078±0.066 (S1), 17.066±0.003 (S2), 16.182±0.001 (S3) and 13.564±0.002 (S4). The value of protein content is increased as compared with the results of Foxtail millet flour protein 10.28g/100 g[22], 12.3 g/100 g, 11.0 ± 0.2 g/100g . The fat content profile (Fig 3(c)) for the prepared FMPC is expressed with increased value in the range of 7.056±0.034 (S1), 7.656±0.007 (S2), 10.337±0.004 (S3) 13.336±0.003 (S4). For other foxtail millet flour sample the fat content 4.3 g/100 g[3], 3.40 g/100 g, 4.4–7.3 g/100 [23], The ash content (Fig 3(d)) of the FMPC is evaluated as 3.116±0.060 (S1), 1.063±0.034(S2), 1.058±0.033 (S3) and 1.152±0.081 (S4). The results of the soluble crude fibre content (Fig 3(e))for FMPC is represented with the value as 0.520±0.015 (S1), 0.519±0.010 (S2), 1.089±0.045(S3) and 0.519±0.014 (S4) which is compared with the value of soluble crude fibre 1.1± 0.1g/100g[21], 1.92 g/100[10]. The carbohydrate content (fig 3(f)) of the FMPC of the samples is represented with the value 63.356±0.038 (S1), 62.639±0.015 (S2), 58.488±0.004 (S3) and 61.991±0.002 (S4). The energy value (Fig. 3(g)) of the FMPC is found to be higher as 373.48±0.565 (S1), 387.92±0.134 (S2), 391.82±0.349 (S3) a nd 421.8103±0.502 (S4) and it is compared with foxtail millet protein flour 349 kcal per100g.

3.4 FTIR spectral analysis of the prepared

The FT-IR spectrum of the four different samples of FMPC are observed at 4cm^{-1} resolution between the range of 500 to 4500cm^{-1} by SHIMADZU & IR Affinity -1S Instrument. The Figure 4(a). is represented with the detailed spectrum analysis for the developed FMPC of four different samples with pre- treatment and High pressure treated FMPC. In the spectrum of commercial FMPC, the presence of the characteristic absorption peak with stretching vibration of the-OH bonds (3647.39 cm^{-1} and 3851.85 cm^{-1}), amide II (1506.41 cm^{-1}, 1539.20 cm^{-1}, 1558.48 cm^{-1}), C-O stretching vibration (1076.28 cm^{-1}) and CH_2 bending (1147.65 cm^{-1}) have been identified. The similar observation is found with the intensities of amide I (1743.65-1745.58 cm^{-1}), amide III (1456.26 cm^{-1}), CH_2 bending (1149.57 cm^{-1}) and C-O stretching vibration (1076.28 cm^{-1}) for the prepared FMPC samples.

3.5 Identification of the Total phenolic content and amino acids in the Prepared FMPC

The presence of amino acids is identified by the colour indication of deep purple colour after addition of Ninhydrin reagent to the prepared FMPC powder (Figure 4 (b)-(1)). The visual image (Figure 4(b)-(2)) is observed with the confirmation of the presence of amino acids in the FMPC. The TPC assay is experimentally done for the prepared to measure the value quantitatively by the method of Folin Ciocalteu's method (Figure 4 (b)-(3)). The total phenolic content is expressed as 358.442 mg GAE/ g, dry weight (S1), 357.978 mg GAE/g, dry weight (S2), 1403.51 mg GAE/ g, dry weight (S3) and 937.75 mg GAE/g, dry weight (S4). From the results, it is inferred that the Total phenolic content is getting increased due to the impact of

High-Pressure Processing (HPP) method which is processed for minimal time 5 minutes at optimized pressure 550 MPa for improving the yield of FMPC for sample S3 and S4.

4. Conclusion

The present study results are concluded with the isolation of FMPC from the millet seed milk and the pre-treated was done by both soaking and germination method. The total phenolic content yield and proximate profile efficiency is increased with the influence of HPP treated was expressed as a good source of millet protein supplement for commercial utilization in food processing industry. From FTIR analysis, it is confirmed that the prepared FMPC from the HPP-treated Foxtail millet milk is exhibited with similar structural composition as compared with Commercial protein concentrate dry powder. The color parameter is getting increased after treated with High Pressure Processing for FMPC prepared sample. From this obtained data it is indicated that FMPC could be a promising protein replacement in the formulation of Milk based protein products for improving the quality and standard of human health.

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Conflict of interest: The Authors have no conflicts of interest to declare that they are relevant to the content of this article.