Textural, Physico-chemical, Micro-structural and Antioxidant Properties of Soy Paneer prepared from admixtures of Skim cow milk and Soymilk.

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ABSTRACT

Milk is considered to be a wholesome food. Any food product made out of milk will obviously be highly nutritious. However, milk has certain limitations like lactose intolerance, cholesterol, saturated fat content, etc. The aim of this research is to produce soy paneer that has improved chemical composition, antioxidant property and better texture. Skimmed cow milk and Soy milk were mixed in different proportions to produce paneer blends and their chemical composition, structural arrangement, color, antioxidants and textural properties were determined. The scanning electron microscopic observation shows that the particulate network structure is composed of protein aggregates held together. Total antioxidant capacity of various blends of soy paneer made from combination of soy milk and skimmed cow milk was determined by Ferric Reducing Antioxidant Power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) which increased as the percentage of soy milk increased. Texture was analyzed in terms of hardness, cohesiveness, gumminess, chewiness, springiness and adhesiveness. As the soy milk proportion in the blend increased from 0% to 50%, hardness, cohesiveness and chewiness of the sample decreased initially, but further increase of soy milk in the blend from 50% to 100% caused these values to increase slightly, whereas springiness did not show much effect.

Key words: Antioxidant activity, Coagulation, Microstructure, Texture, Soy paneer.

I. INTRODUCTION

Soy paneer (Tofu) is a traditional and popular food item in oriental diet, and is one of gel products relating to the aggregation and gelation of soy proteins. Although the highest quality protein is found in animal products (meat, milk, eggs and fish) these products are expensive and often exceed the financial capacity of people in the developing world. To prevent this people are now relying on plant protein and one of them is soybean protein. Soybean is the most important legume in relation to total world grain production and the most frequently used because of its high protein content and relatively low price. Soybean is high in nutritional value and provide satisfactory alternative to animal products. Soybean protein molecules are relatively simple and can be easily coagulated by acids like acetic acid (vinegar) and salts like calcium sulphate, magnesium sulphate or glucono-delta-lactone (GDL). Soybean is often called the “golden miracle bean” and is the world foremost provider of protein and oil, used for healthy food, feed sources and industrial products. It contains about 20% oil and 40%

high quality protein. Soybean or soymilk has always been a rich source of protein which is inexpensive, and abundantly available. Tofu has been reported as low-calorie food and rich source of iron, calcium, low in saturated fat and as a source of isoflavones which can mimic human estrogens and can have beneficial effects on human health. Several potential health benefits have been attributed to the consumption of soy foods [9, 18]. Soybean products also have protective properties against breast, prostrate, colon and lung cancer because of isoflavone content. Soy products are also used to lower cholesterol levels and blood pressure and to relieve symptoms of the menopause and osteoporosis. There are many soy products available in the market and soy paneer (tofu) is one of them. Soy paneer (tofu) is a high protein soy product widely consumed in the Asia. It was first introduced to Japan in the year 1183, then to the other Asian countries. Soy paneer is sold in the form of wet cake of creamy white color with soft texture and bland taste [23]. Soy paneer has been a very popular food in Eastern Asia since ancient times. After soybean soaking, grinding and filtering of the slurry, the resultant raw soy milk is heated to 90^{o}C [2, 20] which is then coagulated. Heating Soymilk achieves multiple objectives [14]. One of them is to denature proteins to enable soymilk to coagulate thereafter. Soy paneer is especially rich in protein, fat and minerals as reflected in the Chinese proverb "tofu is meat without bones". It is prepared using soybean curd, obtained by precipitating proteins from soy milk either with an acid or an alkali salt, which appears like soft white cheese and very firm yoghurt. Soy paneer is a versatile meat or cheese substitute with bland taste and porous texture.

Because the cost of buffalo and cow milk is increasing considerably making paneer from these milks costlier. There is thus need for research to develop technology for the production of low cost nutritional paneer like products to be made affordable for middle class and low income groups [21, 22]. Thus Soy paneer is the main product that could serve as a substitute for milk paneer. Because tofu is a soy protein gel, the amount of soy protein used to make the soymilk is critical for tofu yield and quality. Many studies have evaluated tofu at a given water to bean ratio, such that the amount of protein in the soymilk is related to the crude protein content in the seed [3, 7]. The physical appearance, texture, composition and the basic processing technology of soy cheese are quite similar to the milk cheese in many aspects. Unfortunately, unlike milk cheese, comprehensive scientific studies of soy cheese leading to further developments and improvements were not carried out in the past. The aim of this work was to determine the chemical composition, microstructure, color, antioxidant property and instrumental texture of soy paneer and soy paneer blended with different concentration of skimmed cow milk.

III.MATERIALS AND METHODS

2.1 Materials

Soybeans were procured from the local market and were cleaned by removing split, undersized, wrinkled, damaged beans and foreign matter. It was then stored under ambient conditions in an air tight container until use. The coagulant used in the experiment was food grade 4% Acetic acid (Vinegar).

2.1.1 Preparation of Soymilk

Soy bean seeds were soaked in water (1:3 w/v) for 14-16 h. The soaked water was decanted and the seeds were washed with fresh water. Hundred grams of soaked soybean seeds per liter of water was used for grinding i.e. 1:10 (w/v). The resulting suspension was filtered through a double layered muslin cloth and the filtrate was boiled for 10 min with continuous stirring to prevent sticking of solids and scorching. The soymilk thus prepared, was analyzed for total solids content and used for blending with skimmed milk while preparing soy paneer.

2.1.2 Preparation of Soy paneer

Soy paneer was prepared in the laboratory using 5L of soymilk for each experiment (Fig. 1). A 4% acetic acid was used to coagulate soymilk. Soymilk samples were heated to a temperature of 90^{o}C. The coagulant was added into soymilk slowly with gentle and continuous stirring. After complete coagulation, stirring was stopped and contents were left undisturbed at room temperature for 10 min. Whey was then removed by straining through a muslin cloth. The coagulum (soy paneer) thus obtained was pressed for 45 min in a small sized wooden hoop. Soy paneer was removed from the hoop and soaked in chilled water for 20 min. Then it was taken out and the free water on the surface was removed by wrapping paneer blocks on a clean muslin cloth.

The skimmed cow milk and soya milk were mixed in various proportions (25% soy milk and 75% skimmed milk; 50% soy milk and 50% skimmed milk; 75% soy milk and 25% skimmed milk; and 25% soy milk, 75% skimmed milk . These different soy paneer blends were subjected to different tests.

2.2 Physico-chemical analysis

The soy milk and soy paneer were initially subjected to chemical analysis – including pH, titratable acidity, total solid content, moisture content, as well as fat and protein content. The pH was measured by using 1213 microprocessor pH meter , total solid content were measured by oven method , moisture content by Sartorius moisture analyzer , fat by Soxtec 2043 apparatus and protein by Kjeldhal apparatus (classic Dx).

2.3 Microstructural analysis

Scanning electron microscopy (SEM) was performed on soy paneer samples .The soy paneer (tofu-gel) was cut into small pieces (<2 mm cube) with a razor blade and were then dried. Each sample was fragmented before mounting on SEM stubs to reveal their internal microstructure and coated with a thin layer of gold. A high vacuum scanning electron microscope were used to view each sample at magnification of 500x and 3000x.

2.4 Color Analysis

A portable Hunter Lab Spectrocolorimeter (Mini Scan XETM, Hunter Associates Laboratory Inc., Reston, Virginia, USA) was used to measure the color of the samples. Hunter Lab L*, a* and b* values were read from the samples. The L*value ranges between 0 and 100 and was used as a measure of lightness. Positive or negative increases of a* value correspond to increases in red (+) or green (−) color proportions. The b*value represent color ranging from yellow (+) to blue (−). Measurements were taken on different points of the surface of cheeses cut in two halves. Three consecutive measures were taken for every paneer samples.

2.5 Antioxidants Determination

2.5.1 DPPH Scavenging Analysis

Antioxidant activity was determined by using DPPH as a free radical. For each antioxidant different concentration were tested. The antioxidant solution in methanol was added to DPPH solution and absorbance was measured at 515 nm. DPPH free radical reacts with antioxidant (AH) or a radical species (R) according to following equation:

$$DPPH\cdot + AH \rightarrow DPPH - H + A\cdot$$

$$DPPH\cdot + R\cdot \rightarrow DPPH - R\cdot$$

Any substance that can donate a hydrogen atom (antioxidant) to the solution of DPPH· can reduce the stable free radical and change the color of solution from violet to pale yellow. Non reacted radical form of DPPH absorb in the visible range, and spectroscopic method is based on the measurement of color intensity at 515nm [4, 6]. The stock solution was prepared by dissolving 24mg DPPH with 100ml methanol. The extract was then allowed to react with 200µm DPPH solution for 30 minutes and absorbance were then measured at 515nm using spectrometer. The radical scavenging capacity of antioxidants was expressed in terms of % inhibition. The inhibition percentage of DPPH• was calculated according to the following equation:

$$\%Inhibition = \frac{A_0 - A}{A_0} X100$$

where A_0 is the absorbance of DPPH• in micelle solution without an antioxidant, and A is the absorbance of DPPH• in the presence of an antioxidant.

2.5.2 FRAP (Ferric-ion reducing antioxidant power) Analysis

The total antioxidant capacity of the samples was determined using a modification of the FRAP assay [11]. Briefly FRAP reagent was prepared from 300mM acetate and glacial acetic acid buffer (pH 3.6), 20mM ferric chloride and 10 mM 4,6-tripryridyl-s-triazine (TPTZ) made up in 40 mM HCl. All three solutions were mixed together in the ratio 10:1:1. The FRAP assay was performed by adding 25µl of sample and 1ml of reagent and incubating at 37 °C for 4 min. Absorbance at 593nm was determined relative to a reagent blank also incubated at 37 °C. The total antioxidant capacity of samples was determined against a standard (Ascorbic acid) of known FRAP value. FRAP value can be determined by following formula:

FRAP value= Change in absorbance from 0 to 4 minutes X FRAP value of standards

0.022

2.6 Determination of Textural properties

The textural properties were evaluated using the TAXT-2 Plus Texture Analyzer of Stable Micro System equipped with 5 × 10^4 N load cell [5]. The analyzer is linked to a computer that recorded the data *via* a software programme XT.RA Dimension, version 3.7H (Texture Technologies Corp., New York, USA). Samples of length 80 mm, width 50 mm and height 40 mm pieces were cut from the central portion of tofu cake with a stainless steel cutter. A stainless steel probe of 5 mm diameter with a flat end was used. In the first stage, the probe traveled 75% depth of the soy paneer chunk. The speed of the probe was fixed at 1 mm/s during the pre-test compression and relaxation of the samples. Experiments were carried out by compression test that generated plots of force (g) vs distance (mm) from which the texture values were obtained. During the testing, the samples were held manually against the base plate. All the tests were replicated and the average values of the textural properties were recorded. The following textural parameters were determined.

2.8 Statistical analysis

Analysis of variance (ANOVA) using the general linear models were conducted and average and reported along with the standard deviation (±SD).The differences in mean were calculated using the Duncan's multiple range tests for means with 95% confidence limit ($p \leq 0.05$). Statistical analysis of the data was done using the SPSS software package (SPSS inc, Chicago, U.S.A).

III. RESULTS AND DISCUSSION

3.1 Physico−Chemical Analysis

The average pH of the soy milk was in the range of 6.2 to 6.4 and the titratable acidity was found to be from 0.11 to 0.18 for all the samples used for the preparation of paneer. The composition of the soy paneer is given in the Table 1. It was found that with increase in soy milk concentration, the protein percentage increased from 29.20% to 36.87%. This may be because of quantity of soy bean present which are rich in protein (40%). However reverse were found in case of fat i.e. with increase in soy milk concentration fat percentage decreased. The moisture remained in the range from 50% to 62%.

3.2Texture Analysis

The textural properties of soy paneer play an important role in terms of quality and consumer acceptability. Since microscopic examination revealed a clear difference in the structures of soy paneer samples, a mechanical test was carried out to reveal any link between the structural characteristics and the textural properties. As shown in Table 2, Soy paneer texture varied with soy milk concentration. From the texture profile analysis (TPA) following textural properties were evaluated.

3.2.1 Hardness

Hardness is the most commonly evaluated characteristic in determining texture of soy paneer. The effect of blend constitution on the hardness of paneer is presented. In general it can be seen that as the proportion of soy milk increases in the sample the hardness decreases initially and then starts increasing. When the proportion of soy milk increased from 25% to 50% in the sample, hardness value decreased. However, when soy milk content increased further from 50% to 75% and from 75% to 100%, the hardness value increased. The hardness values of soy paneer so obtained were in the range as already reported [22, 19]. This decrease in the hardness of soy paneer can be explained by increase in the moisture content of the product and because of increase in the soy milk proportion in the blend. Also reduction may be because of a reduction in fat content in the sample, which increased the compact nature of the protein network and thus decreased the number of milk globules dispersed within the network.

3.2.2 Cohesiveness

Cohesiveness is an important textural property of soy paneer. It depends on the nature of the protein matrix. In general it can be seen that as the proportion of soy milk increases in the sample the cohesiveness goes on decreasing initially as in case of hardness. The cohesiveness values of soy paneer samples made from varying soy milk proportions in the blend ranged from 0.37 to 0.55. When the proportion of soy milk increased from 25% to 50%, cohesiveness value decreased. However, when soy milk content increased further from 50% to 75% and from 75% to 100%, the cohesiveness value increased. The cohesiveness values of Soy paneer so obtained were in the range as already reported [22, 19]. As the nature of the protein matrix and the extent of fat dispersion contribute to the cohesiveness, so this decrease in cohesiveness with increase in the blend may be because of the globular nature of soy protein as it does not allow the formation of the compact network structure generally observed in whole milk coagulated products because of their fibrous structure.

3.2.3 Adhesiveness

Adhesiveness is the force necessary to remove the material that adheres to the teeth during eating and it is a negative force. It was observed that as the soy milk in the blend increased, the adhesiveness increased. When the proportion of soy milk increased from 25% to 50% in the sample, adhesiveness value increased i.e. from -0.36 to -0.18 as shown in the Table 2. However, when soy milk content increased further from 50% to 75% and from 75% to 100%, the adhesiveness valued decreased i.e. from -0.22 to -0.11. The adhesiveness values of soy paneer so obtained were in the range as already reported [22, 19]. This slight increase or decrease in the adhesiveness is because of fibrous structure formed by coagulated products like milk fat.

3.2.4 Springiness

Springiness is the rate and extent to which a deformed material returns to its original condition after the force is removed. Springiness depends on factors such as heat treatment, protein interaction, flexibility and degree of unfolding of protein. From the experiment conducted it can be seen that with increase in soy milk percentage in the blend there is no significant changes in the springiness as there is slight variation ranging from 0.68 to 0.91.

The springiness values of Soy paneer so obtained were in the range as already reported [22, 19]. Therefore it can be said that springiness is the property that cannot be much affected by blend proportion.

3.2.5 Chewiness

Chewiness is the energy required to masticate a solid food product to make it ready for swallowing. It was observed that as the soy milk in the blend increases, the chewiness went on decreasing. When the proportion of soy milk increased from 25% to 50% in the sample, chewiness value decreased i.e. from 17.93 to 9.89. However, when soy milk content increased further from 50% to 75% and from 75% to 100%, the chewiness value increased slightly i.e., from 13.53 to 17.99. The decrease in the chewiness values with increase in fat percentage of the paneer samples has also been reported [22]. This may be because of the compact protein matrix as chewiness is the energy required during compression of the material.

3.2.6 Gumminess

When the proportion of soy milk increased from 25% to 50% in the sample, gumminess value decreased. However, when soy milk content increased further from 50% to 75% and from 75% to 100%, the gumminess value increased slightly. The decrease in the gumminess values with increase in fat percentage of the paneer samples has also been reported [22]. This may be because of the compact protein matrix.

3.3 Microstructure Analysis

The scanning electron microscopic (SEM) observation was carried out to explore whether the SEM image would differentiate the network structure of soy paneer prepared under different conditions. Fig. 2. shows the SEM of soy paneer samples, a typical view of a particulate protein gel. It appears that the particulate network image structure is composed of protein aggregates held together (forming the threads of the network). In addition, there were differences in size and density of the particles among the soy paneer samples. As described earlier, it is likely that enhanced hydrophobic interactions [17] and intermolecular disulphide linkages [10] are responsible for the more orderly and denser network of Soy paneer. It has been reported [13, 16] that heat treatment makes the molecules of globular proteins unfold, exposing hydrophobic residues to the surface. In soy paneer making, therefore, with a longer heating time, more hydrophobic residues of the protein molecules would be exposed, resulting in enhanced hydrophobic interactions among the protein molecules. Water trapped in the gel network formed under these circumstances can easily be removed out by pressing. This may cause the gel structure to be more spacious. The SEM image taken [15] revealed well defined three dimensional honeycomb structures with larger holes. The result [20] showed a sponge-like structure with holes of different sizes. Different heating times may cause these differences. For globular protein gels, two types of network, random aggregation of molecules and aggregation of 'string of beads' polymers, had been reported [8]. As shown in Fig. 2, the network of soy paneer prepared with soymilk was considered to belong to the random aggregation type and consistent with those observed in tofu-gels prepared with isolated soybean proteins [12].

3.4 Color profile analysis

Good quality soy paneer is creamy white or light yellow in color. All of the soy paneer samples prepared in this experiment were light yellow in color. From the analysis conducted it was found that there was a decreasing trend for L^* value as L^* value is directly proportional to fat content of paneer, and which may be due to light scattering of fat particles (Table 3). Also the a^* and b^* values of soy paneer blends were higher than pure soy paneer. This may be because pure soy paneer would have higher amine compounds that react with aldehydes during Maillard reaction to form dark pigments (melanoidins) [1]. Values for a^*, which signify red (+) and green (−) and b^*, which signify yellow (+) and blue (−), increased with increasing levels of soy milk concentration, demonstrating that soy paneer blends were more green and yellow colored. This could be attributed to the color difference between soy paneer blends and pure soy paneer.

3.5 Antioxidant analysis

3.5.1 DPPH-radical scavenging activity

DPPH• radical scavenging activity was determined in terms of percentage inhibition of free radical by antioxidants in each sample. There was a significant variation in the percentage inhibition of the DPPH• radical by the soy paneer samples (Table 4). It was found that with increase in concentration of antioxidant extract the free radical scavenging activity increases as is found in Table 4.

3.5.2 FRAP Analysis of Soy Paneer

FRAP analysis was done in terms of percentage inhibition of free radical by antioxidants in each sample. Absorbance at 593nm was determined relative to a reagent blank. Absorbance was measured initially at zero minute and after four minute. From the experiment conducted it was found that the reducing power was higher in case of pure soy paneer and decreased with decrease in soy milk percentage as in case of soy paneer blended with skimmed milk (Table 5). The change in absorbance is directly proportional to the reducing power of electron donating antioxidants in the reaction mix.

IV.CONCLUSION

Soy paneer making has close resemblance to that of milk cheese production in terms of biochemistry, microbiology and processing technology. Even the final products have great similarities in appearance, texture and chemical constituents. The main difference between animal milk and soy milk is that, soy milk does not contain lactose and casein, and its proteins have the same role like that of milk proteins. The texture of food is one of the most challenging areas of food characteristics and a main quality parameter affecting food preference. Hardness, cohesiveness, adhesiveness and chewiness are important textural properties affecting the quality of paneer. This study revealed that as the fat percentage decreased in the milk, hardness, cohesiveness and chewiness of the prepared paneer decreased initially, but when fat content decreased further these properties increased slightly for the all coagulation temperatures. The reduction in fat percentage in the paneer significantly affected hardness, cohesiveness, adhesiveness, chewiness and yield. The scanning electron microscopic (SEM) observation revealed a typical view of a particulate protein gel were observed. It appears that the particulate network image structure is composed of protein aggregates held together (forming the threads of the network). Good quality soy paneer is creamy white or light yellow in color. From the analysis conducted, it was found that there was a decreasing trend for L^* value as L^* value is directly proportional to fat content of paneer. Also the a^* and b^* values of Soy paneer blends were higher than pure soy paneer. DPPH• radical scavenging activity revealed a significant variation in the percentage inhibition of the DPPH• radical by the soy paneer samples. It was found that with increase in concentration of antioxidant extract the free radical scavenging activity increased. Soy paneer (tofu) is highly nutritious and it acts as a vegan substitute for meat.

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Table 1: Physico−Chemical Analysis of Soy paneer.

Sample	Protein (%)	Fat (%)	Moisture (%)	Ash (%)
SP 25%	29.20±0.04^{a}	11.79±0.03^{d}	50.17±0.025^{a}	3.30±0.3^{a}
SP 50%	30.39±0.05^{b}	10.43±0.03^{c}	60.71±0.04^{b}	3.33±0.2^{a}
SP 75%	33.38±0.04^{c}	8.55±0.03^{b}	61.05±0.03^{c}	3.70±0.3^{ab}
SP Pure	36.87±0.02^{d}	4.60±0.03^{a}	62.71±0.03^{d}	3.93±0.2^{b}

Values reported are meant ± standard deviation.

Mean in the row with different superscripts are significantly ($p \le 0.05$) different.

Table 2: Texture Analysis (TPA) of Soy paneer.

Sample	Hardness/ Peak force(g)	Cohesiveness	Adhesiveness(gm/s)	Gumminess	Springiness	Chewiness
SP 25%	62.41 ± 0.03^{d}	0.45 ± 0.04^{b}	-0.36 ± 0.05^{b}	26.28 ± 0.03^{d}	0.69 ± 0.02^{d}	17.93 ± 0.03^{a}
SP 50%	31.99 ± 0.02^{a}	0.37 ± 0.04^{a}	-0.18 ± 0.03^{a}	11.11 ± 0.03^{a}	0.91 ± 0.03^{a}	9.89 ± 0.03^{b}
SP 75%	46.18 ± 0.04^{b}	0.45 ± 0.03^{b}	-0.22 ± 0.02^{b}	20.29 ± 0.03^{b}	0.68 ± 0.03^{b}	13.53 ± 0.04^{a}
SP pure	46.30 ± 0.02^{c}	0.55 ± 0.04^{c}	-0.11 ± 0.03^{c}	24.19 ± 0.15^{c}	0.73 ± 0.04^{c}	17.99 ± 0.04^{a}

Values reported are meant ± standard deviation.

Mean in the row with different superscripts are significantly ($p \le 0.05$) different.

Table 3: Color Analysis of Soy paneer.

	Hunter values		
Sample	L^*	a^*	b^*
SP 25%	77.33 ± 0.05^{c}	0.25 ± 0.03^{d}	19.39 ± 0.03^{c}
SP 50%	76.49 ± 0.04^{a}	0.17 ± 0.025^{c}	21.61 ± 0.04^{b}
SP 75%	74.55 ± 0.04^{b}	0.14 ± 0.025^{b}	$23.17 \pm 0.04a^{b}$
SP Pure	70.04 ± 0.03^{c}	0.09 ± 0.02^{a}	23.94 ± 0.04^{a}

Values reported are meant ± standard deviation.

Mean in the row with different superscripts are significantly ($p \le 0.05$) different.

Table 4: DPPH Scavenging Analysis.

Sample	% Inhibition at 40µl	% Inhibition at 80µl	% Inhibition at 120µl
SP 25%	46.73±0.05[a]	56.23±0.05[b]	58.13±0.04[c]
SP 50%	58.91±0.02[a]	65.13±0.04[b]	66.05±0.08[c]
SP 75%	55.34±0.06[a]	58.93±0.04[b]	62.33±0.05[c]
SP Pure	26.73±0.04[a]	55.93±0.05[b]	58.53±0.05[c]

Values reported are meant ± standard deviation.

Mean in the row with different superscripts are significantly ($p \leq 0.05$) different.

Table 5: FRAP Analysis of Soy Paneer.

Sample	Absorbance		FRAP Value
	0 minute	4 minute	
Standard	1.138	1.116	2.00
SP 25%	2.434	2.339	8.64
SP 50%	2.532	2.433	9.00
SP 75%	2.679	2.575	9.45
SP Pure	2.673	2.562	10.09

Values reported are meant ± standard deviation.

Soy milk + Skimmed milk

↓

Blending

↓

Heating

↓

Cooling

↓

Coagulation (By addition of acid at 90°C)

↓

Whey drainage → Whey

↓

Coagulum

↓

Pressing

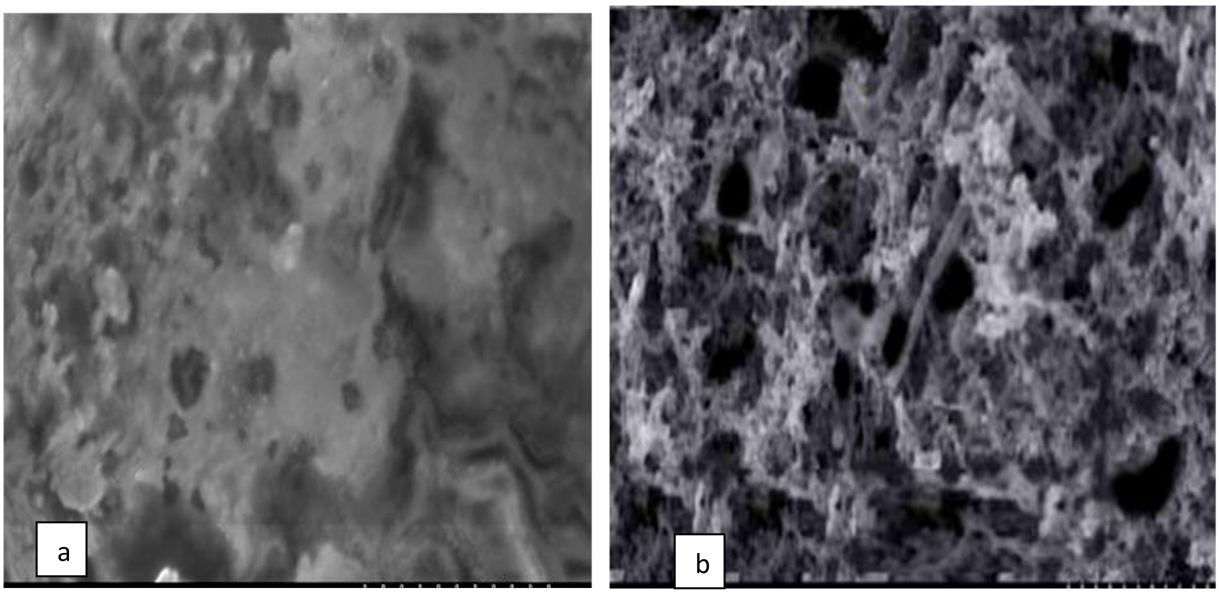
↓

Dipping in chilled water

↓

Soy fortified Paneer

Figure. 1. Process flow chart for the preparation of soy fortified paneer.



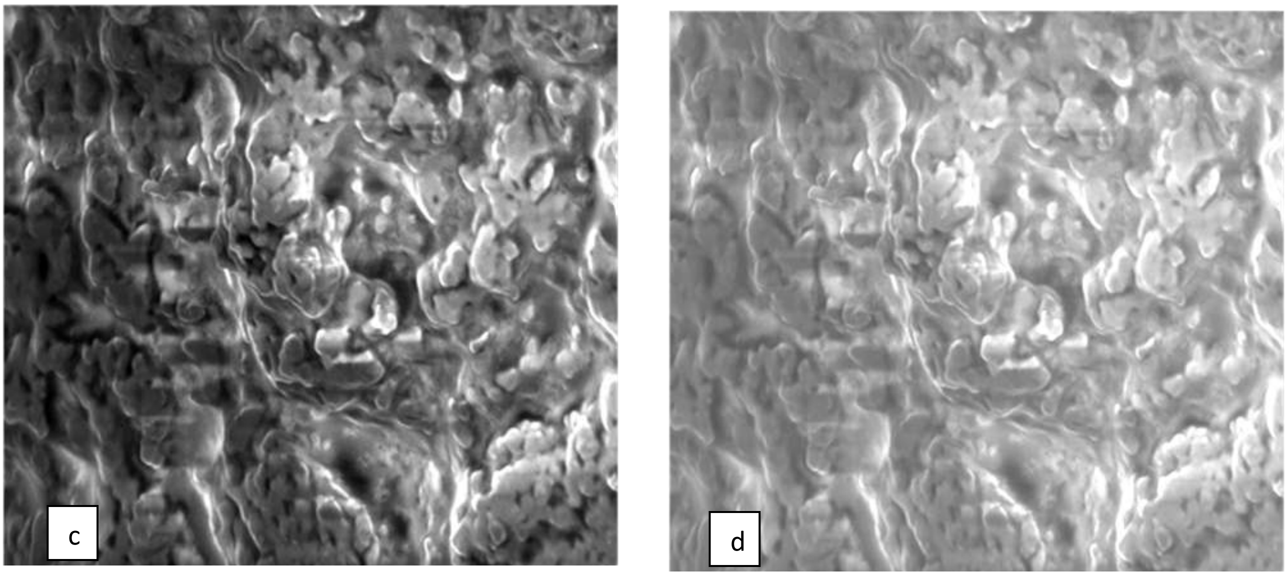


Figure. 2. Microstructural analysis of soy paneer: