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Dietary Fiber Extraction from Soybean and Chickpea Hull Using Acid-Alkali Digestion and Enzymatic Digestion Methods

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ABSTRACT

In order to promote economic efficiency and reduce waste, there is a growing interest in increasing the value of by-products from agro-processing industries. According to the findings of this study, chickpea and soybean hulls are viable by-products as a source of dietary fiber (DF) with high value addition potential. Two DF extraction methods, acid-alkali digestion and enzymatic digestion, were effective in extracting dietary fiber from soybean and chickpea hulls. The DF extraction from the chickpea hulls required an additional bleaching process to remove the pigments for both extraction methods. The analysis of the isolated DFs revealed that the enzymatic technique was more effective in DF extraction. However, the acid-alkali technique also produced DF with the necessary characteristics for food application. Nevertheless, when the cost of the procedure is taken into account, the acid-alkali method is preferred for economically extracting DF from both sources.

Introduction

Dietary fiber, a group of compounds containing carbohydrate polymers and non-carbohydrate components, is generally derived from certain cereals, legumes, fruits, and vegetables. It is usually categorized as either water-soluble or insoluble. Dietary fiber has gained considerable attention in recent years due to its potential role in improving human health. For instance, it has been shown to prevent heart disease, obesity, and cancers (Huang *et al.*, 2013). In addition, it can be incorporated into other food products for enhancing textural properties and promoting good mouth-feel because the addition of dietary fiber is associated with specific food properties, including water and oil retention capacity (Elleuch *et al.*, 2011).

Dietary fiber can include soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). SDF refers to fibers that cannot be digested or absorbed by human bodies but are partly soluble in water. Examples of SDF are some gums, such as pectin, gum Arabic, guar gum, and glucan, including some biological polysaccharides and synthetic polysaccharides. IDF is a fiber that cannot be digested or absorbed by human bodies and is insoluble in water. IDF includes some components of the structure of cell walls, such as cellulose, hemicellulose, and lignin. The dietary fibers obtained from different sources differ in total dietary fiber content, SDF content, physicochemical properties, and physiological properties (Zhang *et al.,* 2017)

Consumer demand for added benefits beyond essential nutrition is catered by health supplements and functional foods that contain dietary fibers. Consumer awareness regarding healthy foods has intensified over the years. They have also become increasingly conscious about the quality of ingredients, which has resulted in the growing demand for dietary fibers. Changing industry trends, product introductions, and changing preferences of the end-users are some of the factors currently impacting the dietary fibers market. Based on the source, the market has been segmented into whole grains and fruits and vegetables, among others. The former has been classified into wheat, rice, and bran, while the latter has been classified into pear, raspberries, apple, and carrot. Global dietary supplements markets are expected to grow at a CAGR of 14% until 2022. The global dietary fibers market size was valued at $ 3.31 billion in 2015 and is estimated to grow 8.2 billion by 2022. The insoluble fiber segment accounts for more than 55% of the market share. DF market is split into Europe, North America, Asia Pacific, Middle East, Africa, and Latin America. The Asia Pacific is anticipated to exhibit the fastest growth, with a significant contribution from India and China.

Soybean contains many components with health benefits, such as proteins, isoflavones, and dietary fiber. In many studies, soy protein is considered as possible source of these to prevent cardiovascular disease. However, the dietary fiber present in soybean plays a vital role in reducing cholesterol levels in some hyperlipidemic individuals. In diabetes, it can also be used to improve glucose tolerance.

Dietary fiber also seems to affect diarrhea and constipation positively and as a treatment for irritable bowel. Chickpea is a highly nutritious pulse that ranks third on the significant list of food legumes grown worldwide. Chickpea output in the globe averages roughly 8.5 million metric tons per year and is cultivated on around 10 million hectares of land. India is the world's largest producer of chickpeas, accounting for around 70% of global production (Vasishtha *et al.,* 2014). The chickpea hull is the primary byproduct obtained in chickpea processing.

Soybean hull and chickpea hull are presently discarded or used as animal feed. DF extraction from these can generate income and reduce the waste disposal problem.

This also provides high-value addition to the low-value agricultural byproducts like husk, straw, etc. The objectives of this work were to extract the dietary fiber from soybean and chickpea using different extraction methods like acid-alkali extraction and enzymatic extraction from soybean hulls and chickpea hull, quantify fiber content based on proximate analysis and characterize specific physicochemical properties

Materials and Methods

Sample

Raw soybean and chickpea hulls were procured from the local market. The hulls were cleaned manually and rinsed with water, further dry in sunlight up to 40% moisture content. The soybean hull used to extract the dietary fiber was named SH, while the chickpea hull used to extract the dietary fiber was named CH.

Procedure for dietary fiber extraction

Acid- alkali extraction

Soybean and chickpea hulls with 40% (w.b) moisture content were ground into 1.5-2.0 mm particle size. The ground sample dried in sunlight for two hours and further digested with acid (2N HCl pH 2.0) and kept for 2hr at 65 °C. The hulls were rinsed with normal water to remove the HCl from the sample and remove subsequent water using a centrifuge. The dewatered hulls were hydrolyzed with alkali (2N KOH pH 10.0) for 2 h and again followed the same procedure for neutralizing and dewatering the sample with centrifuge techniques. After sequentially hydrolyze, the hulls were autoclaved for 10 min and dried through hot air until moisture content reached up to 10%. The dried hulls were ground further and finally got the dietary fiber in powder form (Fig.1 & Fig.2). Dietary fiber extracted from soybean and chickpea hulls using acid-alkali method was denoted as SDF-A and CDF-A respectively

Enzymatic extraction

The effective extraction of DF from the hulls (soy/chickpea) was achieved by sequentially applying enzymes such α-amylase and cellulase under optimal conditions. An α-amylase aids in the removal of starch, while cellulase aids in the digestion of dietary fiber. The enzyme penetrates the liposome membrane, and its decomposition function to lipopolysaccharide and lipoprotein is beneficial for removing lipids. The substrate-specific catalytic ability of enzymes improves the efficiency of the enzymatic process and results in a high output of DFs from soy hull and chickpea hulls (Qi *et al.*, 2011). Dietary fiber extracted from soybean and chickpea hulls using the enzymatic method was denoted as SDF-E and CDF-E, respectively (Fig.3 & Fig.4).

Determination of color

The color of extracted fiber was determined using a Hunter Lab spectrocolorimeter with the CIE L*, a*, and b* color scale (Hunter Lab Inc., Reston, VA, USA). In this coordinate system, the L* value is a measurement of lightness, ranging from 0 (darkness) to 100 (brightness), the a* value ranges from −100 (greenness) to +100 (redness) and the b* value ranges from −100 (blueness) to +100 (yellowness). The ground samples were packed evenly and smoothly up to the lid of the three small petri dishes before being placed in the spectrocolorimeter one at a time. The L*, a*, and b* values were recorded each time.

Determination of water absorption index (WAI)

Two centrifuge tubes were weighed individually using independent weights. After that, 2.5 g of sample was placed in each tube. To avoid lumps, 30 mL of distilled water was added and mixed with the sample. The tubes were closed and placed in a 25 ˚C water bath for 20 minutes. These tubes were centrifuged at 5000 rpm for 10 minutes. The water was then decanted from the tube with no solids loss. The tube and residues were then weighed. The WAI was determined using the following equation:

$$WAI\% = \frac{\text{Weight of centrifuge tube with sample} - \text{Weight of centrifuge tube}}{\text{Weight of sample}} \times 100$$

Determination of water solubility index (WSI)

2.5 g of material was put in a centrifuge tube, followed by 30 mL of distilled water. The mixture was agitated for 5 minutes with intermittent agitation on a vortex mixer, then centrifuged for 10 minutes at 5000 rpm. The supernatant was placed into a known-weight moisture tray. The sample was dehydrated overnight in a 40°C oven. The dish was taken out of the oven and placed in the desiccators to equilibrate. Finally, the dish containing the dried sample and the lid was weighed. The WSI was calculated as follows:

$$WSI\% = \frac{Weight\ of\ petriplate - Weight\ of\ petriplate\ with\ dried\ sample}{Weight\ of\ sample} \times 100$$

Proximate analysis

Measurement of moisture content

The moisture content of the sample was determined by keeping the 5g sample at 70°C in a hot air oven up to a constant weight of the sample, and measured the moisture content by using the formula given below:

$$\%\ \text{Moisture content} = \frac{Initial\ weight\ of\ the\ sample\ (5g) - final\ weight\ of\ the\ sample}{Initial\ weight\ of\ the\ sample\ (5g)} \times 100$$

Fat content determination

The fat content in the extracted fiber was determined by the Soxhlet method. Five grams of the sample placed in the thimble was kept in the soxhlet extractor. 90 mL of isopropanol was used as the solvent, placed the whole set on the heating mantle, and allowed the boiling of isopropanol for 5to 6 hr. After the extraction of the fats from the sample collected, the solvent and dried in a desiccator. Took the weight of the sample and calculated the total fat using the formula given below:

$$Total\ fat\ \% = \frac{Weight\ of\ thimble - Weight\ of\ thimble\ with\ dreid\ sample}{Weight\ of\ sample} \times 100$$

Protein content

Protein content in the extracted dietary fiber was determined using the Kjeldahl method. Five grams of the sample were placed in a Kjeldahl flask and digested with concentrated H_2SO_4 followed by distillation and titration. The percentage nitrogen was measured, and the protein percentage was estimated by multiplying with 6.25.

$$\%\ Nitrogen = \frac{(ml\ standerd\ acid - ml\ blank) \times N\ of\ acid \times 1.4007}{Weight\ of\ sample\ in\ grams}$$

$$Protein\ \% = \%\ Nitrogen \times 6.25$$

Total and crude fiber content

The total dietary fibre content of samples was determined according to the AOAC method 985.29.

Ash content

Ash content was determined by the use of muffle furnace. 1gm of dry sample were placed in muffle furnace at 600°C for 4 hr. and calculate the amount of ash content by using the following formula-

$$\%\ Ash = \frac{wieght\ of\ ash\ (in\ gm)}{weight\ of\ sample\ (in\ gm)} \times 100$$

Statistical analysis

The results were all given as mean values of three samples ± standard deviation. Statistical significance among samples was determined using analysis of variance (ANOVA) followed by Tukey's test using SAS software (SAS 9.3, USA). Statistical significance was determined at a level of probability of $p < 0.05$ (5 percent).

Results and Discussion

In terms of proximate composition, there were significant differences ($p<0.05$) between soybean hulls, and chickpea hulls with the former having the highest levels of TDF, protein, and fat and the latter having the highest levels of ash and total carbohydrates(Table 2). Dietary fiber is mostly made of cellulose, hemicelluloses, pectin, and lignin. Fiber consumption benefits human health by lowering cholesterol levels and decreasing the incidence of colon cancer (Tharanathan & Mahadevamma, 2003). The dietary fiber content of chickpea and soybean hulls was greater than 70%, while crude fiber content was around 30%, with the highest content in soybean hull indicating significant differences ($p<0.05$) when compared to chickpea hull (Table 2).

Color

The color difference in soybean and chickpea hull powders and dietary fiber extracted were given in Fig.5. Table 2show the color parameters (L*, a* and b*) of raw material used and the dietary fiber extracted using different extraction methods. The L* reading indicates the brightness of the sample, with values closer to 100 indicating greater brightness. The L* value was maximum for SDF-E (75.90), whereas CDF-A had the minimum (72.02) value indicating a significant difference. The b* represents the color range from yellow to blue, with positive values indicating yellow and negative ones representing blue. The b* value also had significant difference among soybean and chickpea hull based DFs. The higher b* value obtained for soybean hull based DF indicated its more yellowish nature of the DF. Nevertheless, the difference between the extraction methods for same source of DF was not significant. The DF obtained from chickpea hulls were darker in color due the dark pigments present in it. The bleaching operation involved in the dietary fiber extraction from chickpea hull had enhanced its color parameters to acceptable level. However, the color parameters were significantly different for both the source. Whereas the extraction process, acid-alkali digestion and enzymatic method, had no significant effect on the color parameters.

Water solubility index and water absorption index

WAI is a measure of the water-holding capacity (WHC) of extracted DFs. The water solubility index (WSI) is used to calculate the number of water-soluble components in the DF samples prepared.WAI value of soybean hull-based DF, both acid-alkali and enzymatic method, was significantly higher due to higher water-soluble molecules in soybean hull than chickpea hull. This higher WAI values of SDF-A can be attributed to the lower total fiber content (TDF) (SDF-A- 85%) than DF enzymatic method (SDF-E 89%). It was previously stated that samples containing more total dietary fiber would have lower WAI, but DF from the enzymatic revealed higher WAI (Moczkowska *et al.,* 2019).

The word WSI refers to the amount of water-soluble materials in a product. Because of the decreased insoluble to soluble fibre ratio, the WSI values obtained for all the DF in this investigation were comparatively lower than those derived from sources such as fruit and vegetable peels, plant extracts, and so on(Henríquez *et al.,* 2010). WSI values were more for soy hull-based samples than the chickpea hull-based DFs by the existence of more insoluble dietary fiber in chickpea hull than in soybean husk. The higher values of WSI for the enzymatic extraction method indicate that the more soluble matters had been removed in enzymatic treatment than the acid-alkali treatment.

Fig.1 Extraction of dietary fiber from soy hulls using acid-alkali digestion

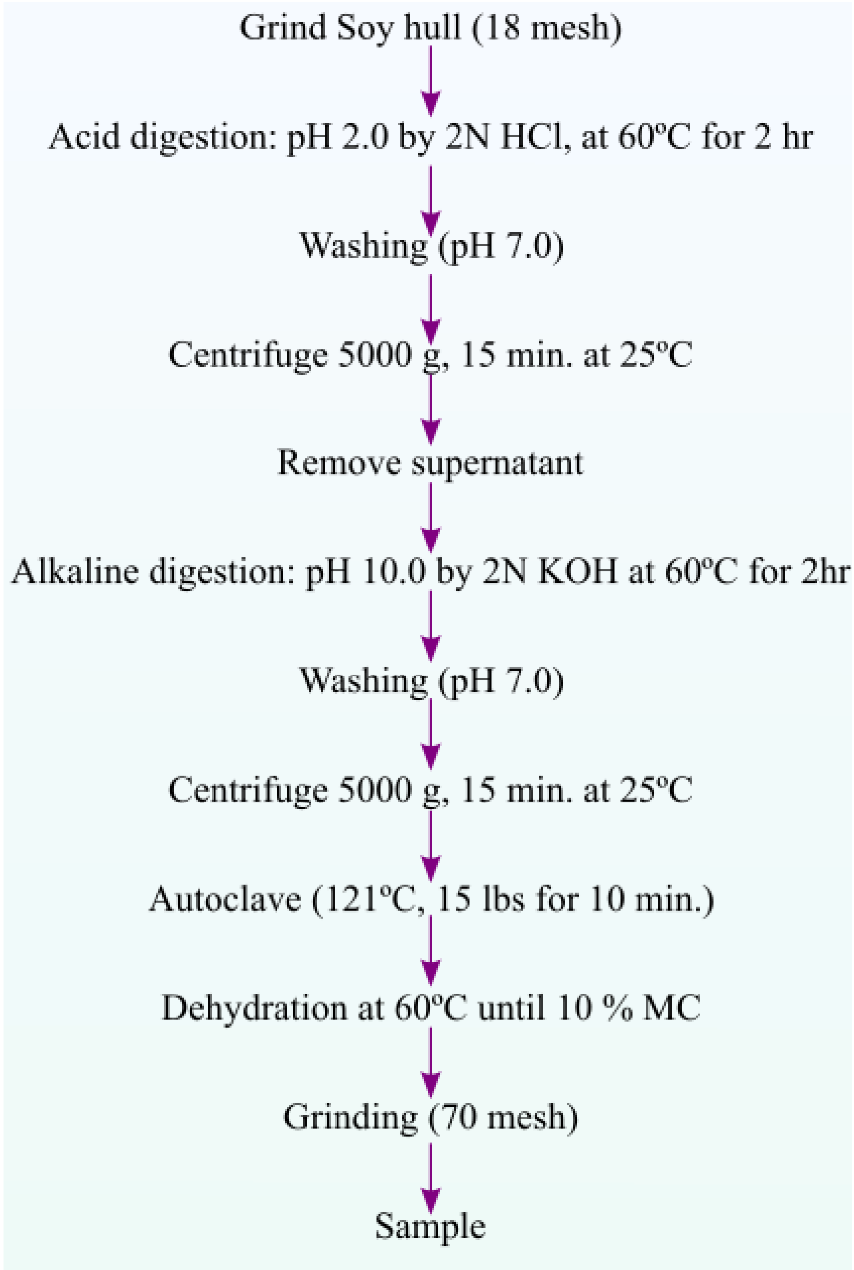


Table.1 Proximate analysis of the different samples

Sample	TDF %	Protein%	Fat %	Ash%	Moisture %
SH	57.20±0.50^{e}	11.12±0.50^{a}	2.50±0.50^{a}	3.90±0.70^{b}	8.51
CH	52.15±0.65^{f}	5.0±0.80^{b}	0.70±0.30^{b}	4.50±0.15^{a}	9.50
SDF-A	85.20±0.40^{b}	1.6±0.25^{c}	0.30±0.50^{b}	2.84±0.50^{b}	9.41
CDF-A	78.35±0.50^{d}	0.85±0.20^{d}	0.23±0.50^{b}	4.73±0.30^{a}	9.25
SDF-E	89.10±0.85^{a}	1.70±0.40^{c}	0.55±0.30^{b}	2.98±0.35^{b}	9.10
CDF-E	82.40±0.30^{c}	0.75±0.50^{d}	0.21±0.60^{b}	4.62±0.40^{a}	9.23

Table.2 Physio-chemical properties DF extracted

Sample	WAI (%)	WSI (%)	L*	a*	b*
CDF-A	310±14.50^{b}	2.65±0.20^{b}	72.02	5.97	7.05
SDF-A	380±10.0^{a}	3.50±0.15^{a}	75.5	1.75	12.85
CDF-E	330±12.0^{b}	2.73±0.30^{b}	73.10	6.25	6.75
SDF-E	400±18.0^{a}	3.40±0.25^{a}	75.90	1.40	13.05

Fig.2 Extraction of dietary fiber from chickpea hulls using acid-alkali digestion

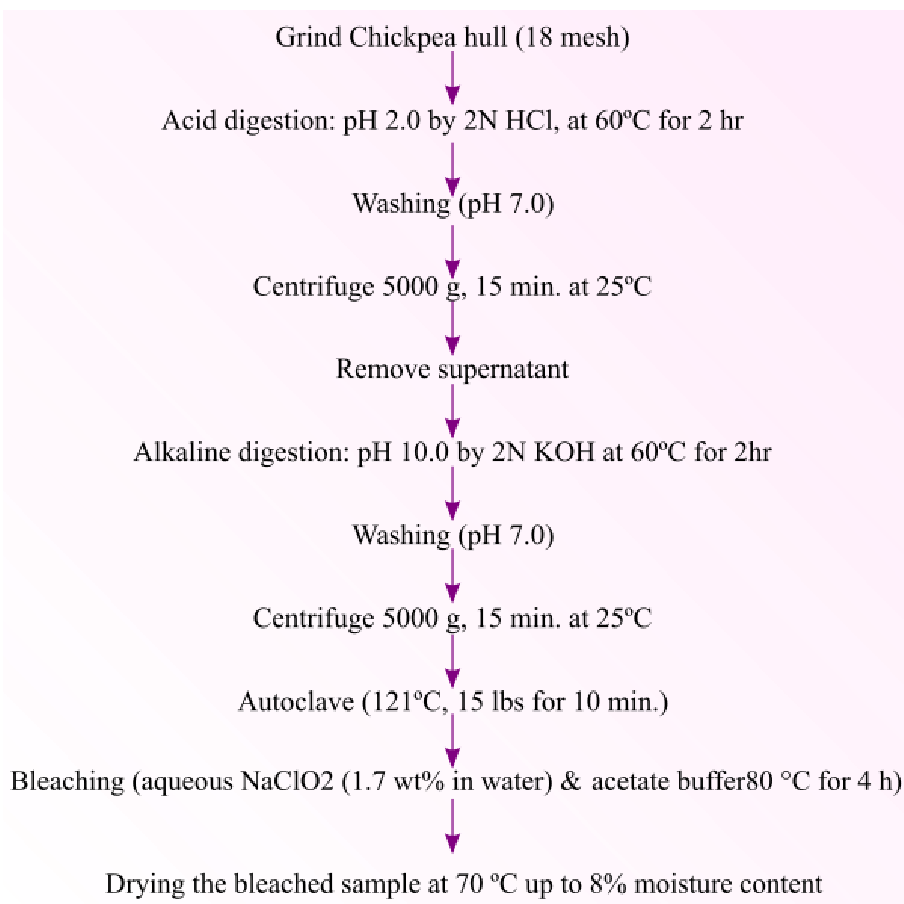


Fig.3 Extraction of dietary fiber from soy hulls using enzymatic digestion

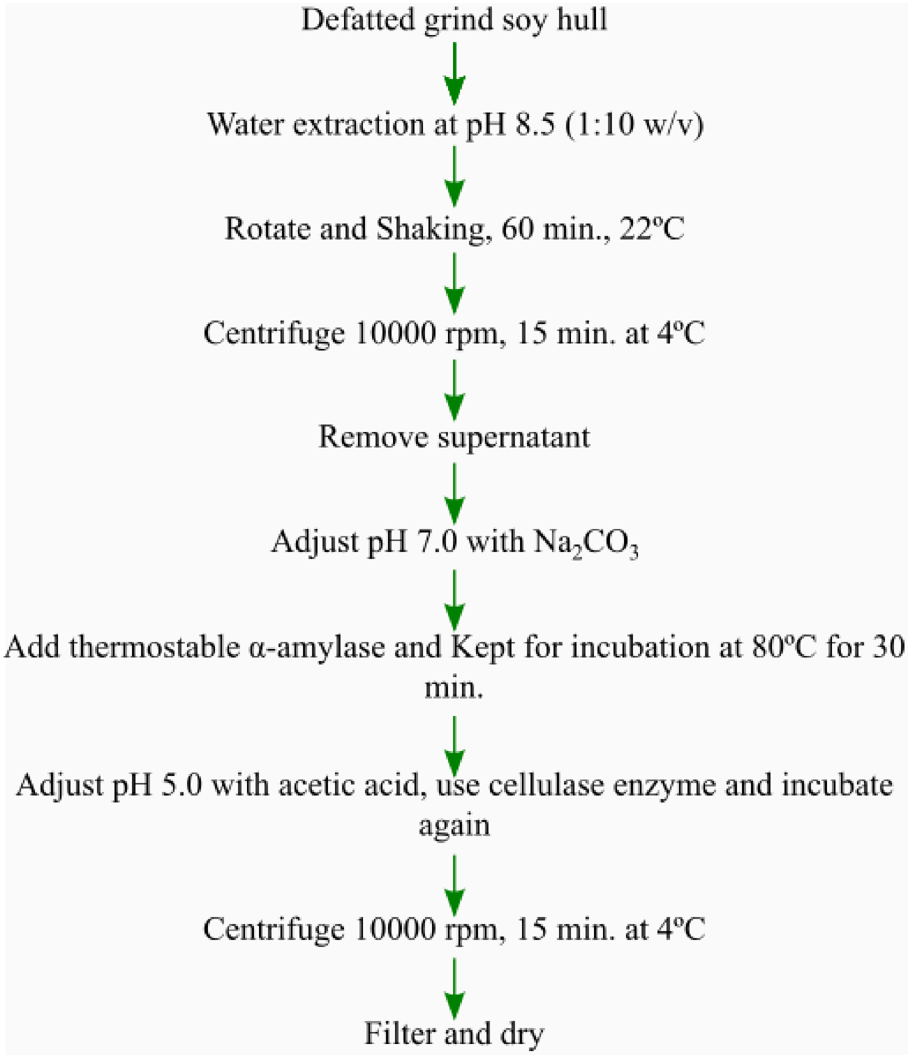
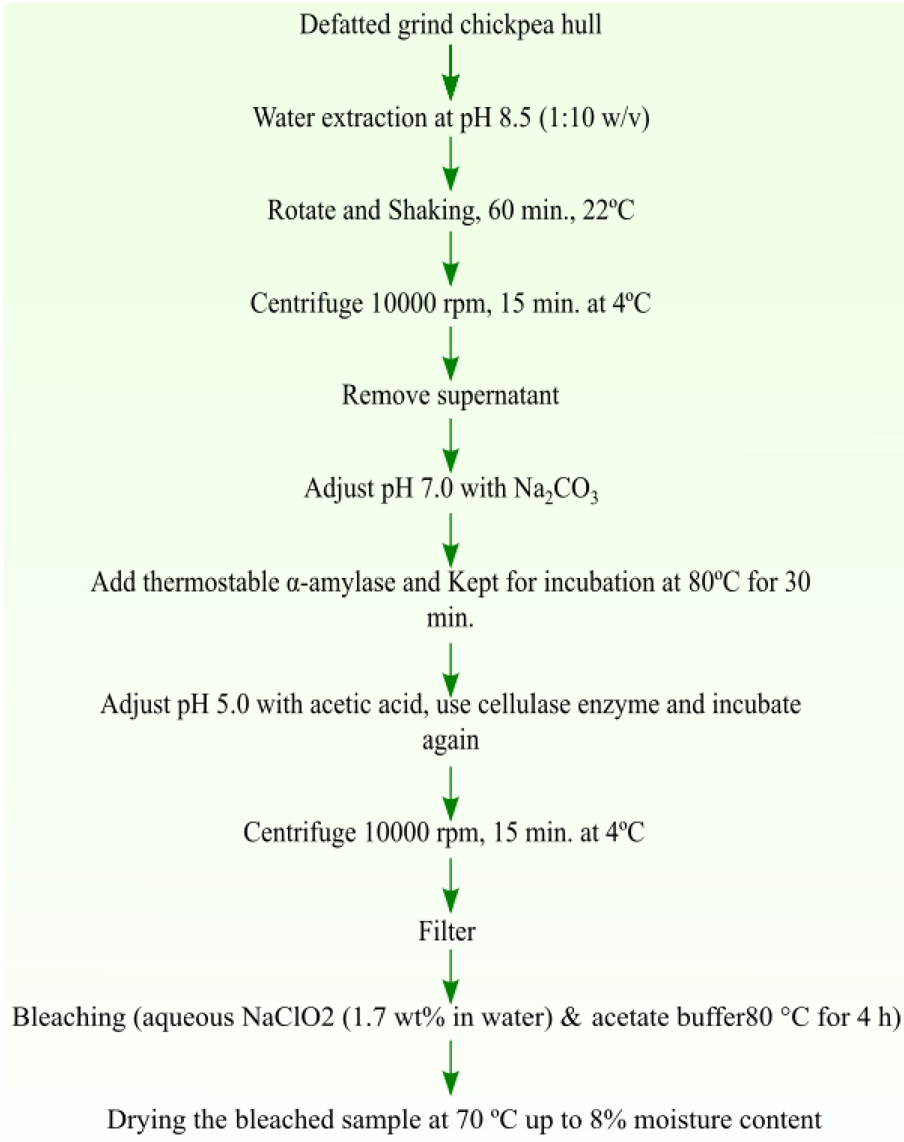


Fig.4 Extraction of dietary fiber from Chickpea hulls using enzymatic digestion



Dietary fiber analysis

Both the extraction methods have significantly increased the TDF. From Table 1, it was evident that among the different extraction methods used, the enzymatic method yielded DF with maximum total dietary fiber content. It shows the higher efficacy of the enzyme in removing protein and other carbohydrates than acid-alkali digestion. Enzyme assisted DF extraction from soybean yielded maximum TDF (89%) while acid-alkali treated chickpea obtained minimum value (78%). These results are in consistent with the WAI and WSI value obtained. Although the extraction efficiency of the enzymatic method is higher than the acid-alkali treatment, the results of the latter yielded DF with sufficient properties that meet the requirement as DF. However, while considering the cost of the process, the acid-alkali method is more suitable for the economic extraction of DF from both sources. There is a growing interest in boosting the value of waste streams from processed fruits and vegetables in order to increase economic efficiency and reduce waste. According to the findings of this study, chickpea and soybean hulls are viable byproducts as a source of dietary fiber with great potential for valorization. Two DF extraction methods, acid-alkali digestion and enzymatic digestion methods were found effective for the successful extraction of the DF from soybean and chickpea hulls. The characterization of the extracted DFs showed higher efficacy for the enzymatic method. However, the acid-alkali method also yielded DF with sufficient properties required of DF in different applications. However, when the cost of the procedure is considered, the acid-alkali approach is preferable for the economical extraction of DF from both sources.

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