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Starch-Protein Separation from Chickpea Flour Using a Hydrocyclone

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Abstract

Whole chickpea flour and defatted chickpea flour were slurried in the distilled water at the initial pH and pH of 9.0. The slurry was subjected to double-pass hydrocyclone process to get overflows and underflows. The effect of defatting and increasing pH on starch-protein separation was evaluated. Application of defatted flour resulted in higher total solid in the underflows. In terms of starch separation, the use of defatted flour at a pH of 9.0 resulted in highest starch content in the underflow with separation efficiency of 99.83%. Using defatted flour at an initial pH of 9.0 resulted in 88.31% (d.b.) protein content in the sediment of the overflows with a separation efficiency from 62.50 to 67.40%. The first-pass process of defatted flour at a pH of 9.0 resulted in protein separation efficiency of 83.11. Defatting of the whole chickpea flour and increasing the pH to 9.0 improved starch and protein separation efficiencies. Starch content of the underflow and protein content of the overflow sediment were enriched to 3.2 and 1.8 times of those of the defatted flour, respectively.

Keywords: Fractionation, liquid cyclone separation, chickpea, flour, separation efficiency.

INTRODUCTION

Legumes such as chickpea grains are good sources of starch and protein. Researchers have tried different methods to separate starch and protein from legumes and improve starch and protein purification. The protein and starch fractions of legumes can be used as ingredients in food processing (Tian, et al., 1999; Neves and Lourenco, 1995) and non-food products (Sánchez-Vioque et al., 1999).

The isolation of starch fraction from legume seeds is difficult because of the presence of insoluble flocculent proteins and fine fiber which diminishes sedimentation, co-settling with the starch fraction and resulting in a brownish deposit (Hoover and Sosulski, 1991; Ratnauake, et al., 2002). Research work on starch separation from legume seeds which involves the separation of starch from protein fraction using the isoelectric method, has been undertaken by Anderson and Romo (1976), Vose (1980), Colonna et al. (1980), Colonna et al. (1981), Hoover and Sosulski (1990), and Liu and Hung (1998). Colonna et al. (1981) reported that the protein of smooth pea and broad bean was dissolved using an aqueous medium at pH 9.0 followed by starch extraction using sieving and washing. The yield of starch extraction was high ranging between 93.8 and 96.7%. The starch was contaminated by cell-wall polysacchrides and protein that was less than 0.4%. Tian et al. (1999) used an aqueous media with a pH 9.0 to dissolve field pea protein followed by centrifugation and filtration. According to Anderson and Romo (1976), the pH of extraction ranging between 5.5 and 7.5 had high effect on the contamination of lentil and field pea starch with protein. The effect of higher pH values, up to 9.5, was less, although higher pH increased protein solubility. However, pH of the medium did not affect starch yield.

Plant protein isolates enhance the nutritional quality of final products (Sánchez-Vioque et al., 1999). Particle size, purity of protein, and processing conditions, such as the method of isolation and pH extraction, affect the physico-chemical properties of protein fraction (Tian, et al., 1999). The most used method in isolating proteins is alkaline extraction of proteins in an aqueous medium followed by precipitation at the isoelectric point. The fundamental principle of this method of separation is the Osborne fractionation (Osborne, 1924). This method was discussed by Colonna et al. (1980), Vose (1980), Gebre-Egziabher and Sumner (1983), Onuma Okezie and Bello (1988), Swanson (1990), Swusu-Ansah and McCurdy (1991), Sánchez-Vioque et al. (1998), Liu and Hung (1998), and Tian et al. (1999). The advantage of this method is the low cost of chemical used for processing (Sánchez-Vioque et al., 1999). Tian et al. (1999) adjusted the filtrate resulting from field pea starch precipitation, at pH 4.5 followed by centrifugation at 8000 × g for 20 minutes to recover the protein fraction. However, Liu, and Hung (1998) used pH 4.2 using 3 *M* HCl. Vose (1980) applied the same method using hydrocyclone to separate the starch and protein from field pea and horsebean. He reported that the overflow had 3 to 8% solids containing 60 to 70% protein. Protein recovery was performed by reducing pH from 8.5 to an isoelectric point of 4.4 to 4.6 using 2 *N* HCl followed by centrifugation at 1500 × g; the protein cake contained 85% protein. The protein content of the precipitate could increased by re-suspending the protein cake in water maintained at pH 4.4 followed by centrifugation.

Sosulski and Sosulski (1986) studied the separation of protein, starch, and fiber from field pea and faba bean using dry and wet processing. A combination of pin milling and double-pass air classification was used in dry processing while alkali extraction and centrifugation were applied in wet processing. The study showed that the recovery efficiencies of protein and starch using dry processing ranged from 75 to 80% and from 88 to 93%, respectively. However, the recovery percentages of protein and starch using wet processing were between 73 and 79%, respectively. In terms of protein purity, wet processing resulted in higher purity (88 and 94%,

respectively for field pea and faba bean) than dry processing (53 and 73%, respectively for field pea and faba bean). Starch fraction resulting from wet processing of field peas and faba bean showed higher starch content (94% for both field peas and faba bean) than dry processing (83 and 77%, respectively for field peas and faba bean).

The objective of this study was to determine the effect of pH of the media and defatting on starch-protein separation form chickpea flour. Moreover, the proportion of protein particles transferred to the sediment and supernatant of each flow was of interest.

MATERIALS AND METHODS

Sample Preparation

Commercial dehulled split desi chickpea (dhal) from the crop harvested in fall of 2003 were obtained from Canadian Select Grains, Eston, Saskatchewan, Canada. The split chickpea grain was stored in a walk-in cooler maintained at a temperature of 2 ± 2°C. It was then milled using a pin mill (GM 280/S-D, Condux werk, Hannau, Wolfgang, Germany). The pin mill had two discs: the first one had 86 pins rotating at 8034 rpm; and the second one had 108 pins and was stationary. A portion of the whole chickpea flour was defatted using ACS grade isopropyl alcohol (EMD, Gibbstown, NJ). The whole chickpea flour and defatted chickpea flour were used as two feed materials in the trials.

Processing

The hydrocyclone system (Figure 1) included a 10-mm hydrocyclone (Dorrclone, GL&V Canada Inc., Orillia, ON) which was connected to a positive displacement pump (Model 4100C, Hypro Inc., St. Paul, MI) supplied by a feed tank. The pump was running at 1950 rpm. The Dorrclone unit consisted of four 10-mm hydrocyclones operating in parallel. Three of these hydrocyclones were removed and the vacant ports were plugged using rubber stoppers. Using only one hydrocyclone reduced the feed requirement and increased operating pressure, making the unit suitable for lab-scale operation. A by-pass valve, located between the pump and feeding tank, was employed to circulate the solution, keep flour particles in suspension form, and help control pressure. A valve was located before cyclone to control inlet pressure. The overflow and underflow valves were kept fully-opened during the test. A slurry was made using distilled water and flour (whole chickpea flour or defatted chickpea flour) at a concentration of 1.5% (w/w). The slurry was fed at two pH values (initial slurry pH (pH 6.6) and at pH of 9.0). The solution of 10 *N* NaOH was used to adjust the pH. The slurry which was left overnight was stirred for one hour using a mixer (OMINI-MIXER 17105, Sorvall Inc., Newtown, CT) employing shear force.

The hydrocyclone process is shown schematically in Figure 2. The slurry was subjected to the hydrocyclone at an inlet pressure of 690 kPa resulting in overflow and underflow of the first-pass products. The first-pass overflow and underflow were separately subjected passed to the hydrocyclone to obtain the overflow and underflow of the second-pass products.

Analytical and Physical Methods

Protein content was measured by AACC method 46-30 (AACC 1995) using LECO Model FP-528 (LECO Corporation, St. Joseph, MI). The factor of 6.25 was used to convert nitrogen to protein content. Starch content was assessed using the method described by Holm et al. (1986). The moisture content was measured using the method described by Egan et al. (1990) for determining moisture content of syrup and condensed milk. For this purpose, the vacuum oven was set at 70˚C and a vacuum gage pressure of 3.33 kPa was applied. All measurements

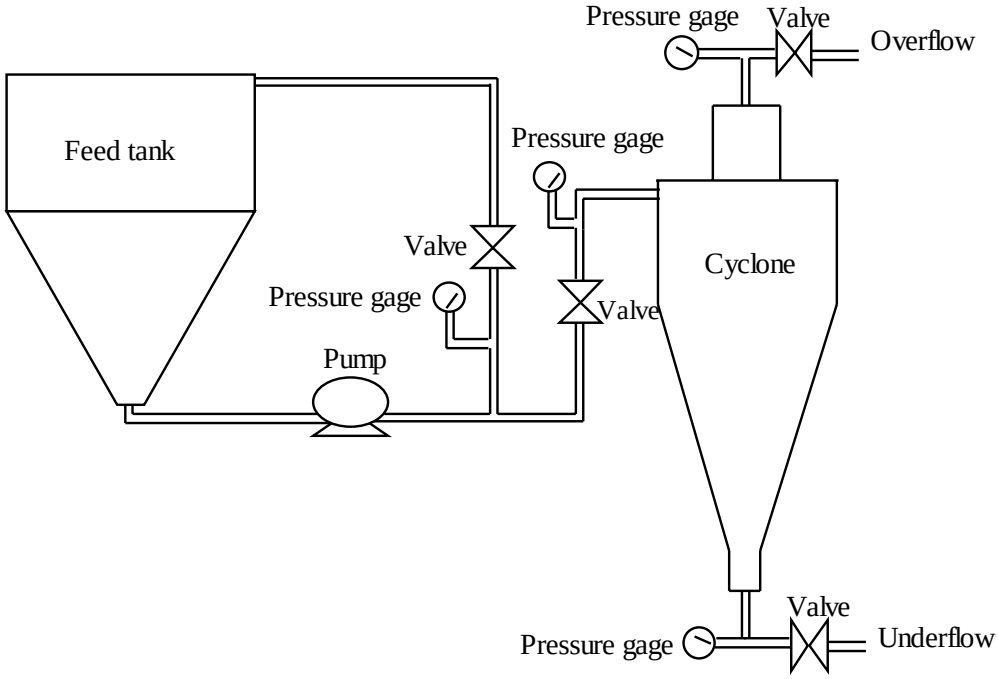

Figure 1. Schematic of the hydrocyclone system

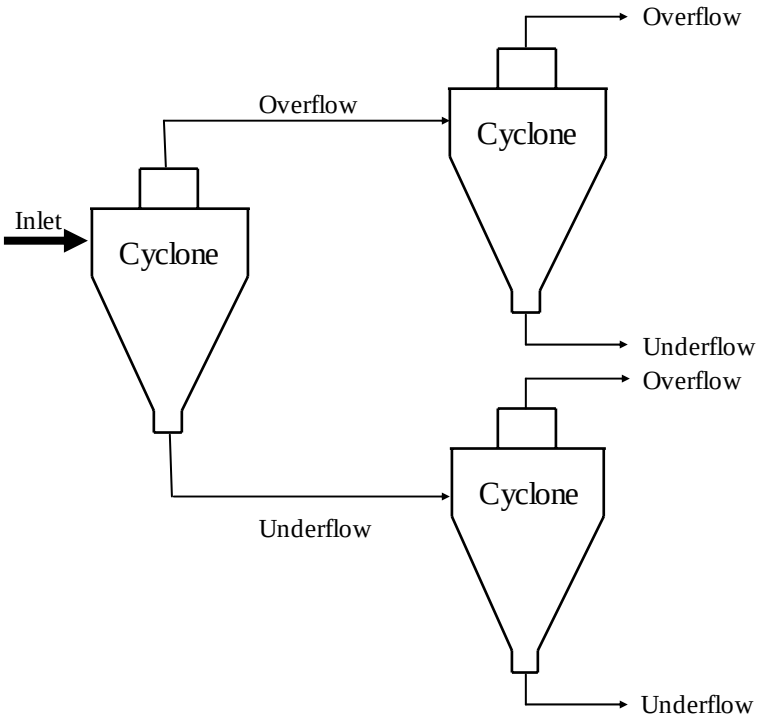

Figure 2. Schematic of the process

were conducted in triplicate. The product yield of each flow was calculated using the following equation:

$$\text{Product yield}\,\% = \frac{\text{Product mass}}{\text{Intitial feed mass}} \times 100 \quad (1)$$

Starch and protein separation efficiency were calculated according to the method used by Tyler et al. (1981) with some modification for dilute materials. The following equation was used to calculate starch or protein separation efficiency:

$$SE = \frac{TS_P \times C_P}{TS_F \times C_F} \times 100 \quad (2)$$

where: SE = separation efficiency (%);

TS_P = total solid of product (kg);

C_P = component content in the product (%d.b.);

TS_F = total solid of inlet material (kg);

C_F = component content in the inlet material (%d.b.).

Statistical Analysis

The Statistical Analysis System (SAS, 2001) was used to perform statistical analysis and mean values were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

Table 1 shows the fraction yield and total solids of the overflow and underflow. Because some material (slurry) remained in the hydrocyclone, the sum of the overflow and underflow in some runs were less that 100%. In all runs, the fraction yield of the underflow was greater than the overflow, except the first-pass of whole flour at initial pH and pH of 9.0. In addition, the total solids of the underflow was more than that of the overflow. It shows that large solid particles, such as starch granules, are collected in the underflow. Adjusting the pH to 9.0 did not make marked difference in the fraction yield although it increased the total solid of the overflow which was not of interest in this study.

Starch Separation

The starch content of the overflow and underflow sediments is presented in Table 2. In the overflow of the whole flour, the effect of the double-pass process was significant in starch content at both pH levels. In the underflows, there was significant difference in starch content of the first- and double-pass process. The second-pass underflow products were high in starch compared to the whole flour (47.98%) and defatted flour (55.85%). The starch contents of the second-pass process were 1.3 times those of the first-pass process, ranging from 89.80 to 90.85% at the initial pH and pH of 9.0, respectively for whole flour feed material. It showed that the double-pass process enriched the underflow starch content effectively. The first-pass and the second-pass underflows showed that there was no marked difference in the starch content at the initial pH and at pH 9.0. The small amount of the starch content in the overflow is associated with damaged starch granules resulting from pin milling.

Table 1. Fraction yield and total solid* of the overflow and underflow using different feed materials and pH values.

Feed material	Initial pH (pH 6.6)				pH 9.0			
	Fraction yield (%)		Total solids (%w.b.)		Fraction yield (%)		Total solids (%w.b.)	
	OF	UF	OF	UF	OF	UF	OF	UF
Whole flour								
First-pass	50.19	49.81	0.54	1.94	49.28†	47.14	0.60	2.00
Second-pass overflow	48.35	51.65	0.46	0.45	46.10	53.90	0.61	0.83
Second-pass underflow	47.88	52.11	0.50	2.73	47.16	52.84	0.58	2.80
Defatted flour								
First-pass	48.97	48.97	0.60	1.96	47.32	49.11	0.55	1.96
Second-pass overflow	49.00	51.00	0.40	0.67	47.35	52.65	0.46	0.50
Second-pass underflow	46.48	53.52	0.47	3.02	46.30	53.70	0.50	2.92

* Values are an average of three determinations.
OF = overflow
UF = underflow
† Because of unavoidable residual material in the system, the summation of the overflow and underflow yield is less than 100%.

Table 2. Starch content* (%d.b.) the overflow and underflow sediments using different feed materials and pH values.

Feed material	Initial pH (pH 6.6)		pH 9.0	
	Overflow	Underflow	Overflow	Underflow
Whole flour†				
First-pass	3.29 b	66.33 b	3.46 b	68.04 b
Second-pass overflow	1.87 c	5.80 c	2.48 c	3.817 c
Second-pass underflow	5.16 a	89.80 a	5.54 a	90.85 a
Defatted flour‡				
First-pass	8.58 a	77.78 b	0.65 a	79.93 b
Second-pass overflow	1.13 b	14.67 c	0.62 a	0.84 c
Second-pass underflow	1.13 b	94.12 a	1.13 a	99.71 a

* Values are an average of three determinations.
† Whole flour starch content = 47.98% d.b.
‡ Defatted flour starch content = 55.85% d.b.
a-c values in the same column in each group of the flour followed by common letter are not significantly different at 5% level.

In the defatted flour, the effect of the double-pass process was significant on starch content of the overflow at the initial pH, but there was no significant difference on the overflow at pH 9.0. The overflow resulting from the second-pass underflow at the initial pH showed lower starch content than that of the first-pass. Therefore, the double-pass process for defatted flour at initial pH (pH 6.6) was more effective than the double-pass process for the whole flour in starch separation. The starch content resulting of the underflows confirmed this. Like whole flour, the underflow resulting from the second-pass underflow at initial pH and pH of 9.0 resulted in more starch enrichment than the first-pass process. Among all the applied conditions, the defatted flour at pH 9.0 resulted in the highest starch content (containing 99.71%), followed by the defatted flour at the initial pH (containing 94.12% starch). This result shows that defatting and the double-pass hydrocyclone process enriched the starch content of the underflows effectively. These results were similar to those reported by Vose (1980) for field peas and horsebeans and by Tyler et al. (1981) in the air classification of legumes.

Table 3 shows the starch separation efficiency values. The values resulting from the first- and second-pass underflow ranged between 98 and 99% and these magnitudes were similar to those reported by Tyler et al. (1981) in air classification of legumes. It showed that the starch content is enriched in the underflow of hydrocyclone process similar to the coarse fraction in air classification. The lowest starch separation efficiency belonged to underflow from second-pass overflow. Since the second-pass overflow was obtained from the overflow of the first-pass, it had higher protein content than starch content compared to the inlet of the first-pass and the second-pass underflow. Therefore, it presumably has more agglomerates of starch granules and proteinaceous material, resulting in lower starch separation efficiency. Seeds with hard cotyledons have higher agglomerates of starch-protein material. Effective pin milling improves the separation of starch and protein from the agglomerates.

Table 3. Starch separation efficiency (%) achieved in the underflows.

Feed material	Initial pH (pH 6.6)	pH 9.0
Whole flour		
First-pass	98.63	98.42
Second-pass overflow	76.54	71.05
Second-pass underflow	99.03	98.89
Defatted flour		
First-pass	96.75	99.78
Second-pass overflow	98.29	62.64
Second-pass underflow	99.84	99.83

Protein Separation

Table 4 shows the protein content of supernatant and sediment of the overflow and underflow. In the whole flour, the first-pass process resulted in higher protein content in the overflows than the underflows using the initial pH or pH 9.0. This difference was obvious between the overflow and underflow sediments. It shows that overflow is rich in protein. Adjustment of the pH to 9.0 did not make marked a difference in protein content of the overflow sediments. They were enriched 2.4 and 2.5 times the protein content of the whole flour using the initial pH and pH of 9.0, respectively. The second-pass overflow did not result in marked difference between the

Table 4. Protein content* (%d.b.) of sediment and supernatant of the overflow and underflow using different feed materials and pH values.

	Initial pH (pH 6.6)				pH 9.0			
	Overflow		Underflow		Overflow		Underflow	
Feed material	SE	SU	SE	SU	SE	SU	SE	SU
Whole flour†								
First-pass	66.91 a	69.62 a	8.03 b	34.10 c	68.36 b	66.99 a	7.01 b	22.40 c
Second-pass overflow	68.51 a	64.08 a	52.34 a	67.06 a	70.31 a	68.29 a	53.20 a	55.31 a
Second-pass underflow	64.02 b	72.13 a	7.04 b	41.61 b	65.68 c	65.16 a	5.42 c	26.56 b
Defatted flour‡								
First-pass	72.78 c	68.09 a	18.51 b	67.12 a	92.50 b	63.53 ab	16.64 b	61.11 a
Second-pass overflow	97.81 a	82.40 a	58.13 a	69.64 a	98.92 a	66.06 a	90.07 a	64.08 a
Second-pass underflow	88.31 b	72.61 a	12.02 c	71.74 a	88.31 c	59.09 b	11.06 c	61.43 a

* Values are an average of three determinations.
† Whole flour starch content = 26.26% d.b.
‡ Defatted flour starch content = 27.70% d.b.
a-c values in the same column in each group of the flour followed by common letter are not significantly different at 5% level.
SE = Sediment
SU = Supernatant

overflow and underflow using the initial pH and pH of 9.0. It is due to agglomeration starch granules and porteinaceous material. However, the second-pass underflow showed noticeable difference between the overflow and underflow for both media pH values. The supernatant of underflows contained higher protein than the sediment. Therefore, the majority chickpea flour proteins, legumin and vicilin, are accumulated in the supernatant rather than in the sediment.

In the defatted flour, the first-pass process, similar to the whole flour, resulted in higher protein content in the overflow than the underflow for both media pH values employed. The overflow sediment especially was much higher in protein than the underflow sediment. Therefore, the sediment of overflow was enriched in protein to 72.78 and 92.50% using initial pH and pH 9.0 media, respectively. The protein content of the overflow sediment was enriched 2.6 and 3.3 times that of the defatted flour using the initial pH and pH 9.0, respectively. Increasing the pH to 9.0 improved the protein enrichment of the overflow sediment. The second-pass overflow did not result in marked difference between the overflow and underflow for both pH. This result confirms that some of the starch granules and proteinaceous material agglomerate together. Additionally, since the alkaline medium (pH 9.0) increases the solubility of the chickpea flour protein, the liquid media in the underflow carries proteinaceous material in soluble form. Thus, it cannot be separated by the hydrocyclone. However, the second-pass underflow showed marked difference between overflow and underflow in the both applied pH values, particularly in the overflow sediments. The ratio of the protein content of the overflow sediment to the underflow sediment was 7.3 and 8.0 at initial pH and pH 9.0 media, respectively. The protein content in the overflow sediment was enriched 3.2 times that of the defatted flour.

Table 5 shows values of protein separation efficiency. The first-pass process resulted in higher protein separation. For both whole flour and defatted flour, adjustment of the pH to 9.0 improved the protein separation. This is due to the higher solubility of the chickpea flour protein in the alkaline medium than at the initial pH (pH 6.6). The first-pass process using defatted flour using pH 9.0 medium resulted in the highest protein separation. Similar result was reported by Tyler et al. (1981) in air classification of field pea and lima bean. The second-pass overflow had low protein separation efficiency. It is due to the high agglomeration of starch granules and proteinaceous material.

Table 5. Protein separation efficiency (%) achieved in the overflows.

Feed material	Initial pH (pH 6.6)	pH 9.0
Whole flour		
First-pass	65.69	70.04
Second-pass overflow	48.61	50.06
Second-pass underflow	51.17	55.70
Defatted flour		
First-pass	67.53	83.11
Second-pass overflow	74.28	51.88
Second-pass underflow	62.50	67.40

CONCLUSION

The double-pass hydrocyclone process was able to enrich starch and protein in the underflow and overflow, respectively. The underflow resulting form the second-pass underflow contained high starch ranging from 89.80 to 99.71% with the starch separation efficiency was between 99.03 and 99.84%. The overflow resulting from the second-pass underflow contained high protein ranging from 64.02 to 88.31% in the overflow sediment. The highest protein separation efficiency was achieved from the overflow of the first-pass process of the defatted flour using pH 9.0 media. Defatting of the whole chickpea flour and using alkaline medium (pH 9.0) improved starch and protein separation efficiencies. Since the second-pass overflow resulted in low starch and protein separation efficiency, it is suggested that the overflow from the first-pass process is returned to the feed tank as mother liquid. This technique was mentioned by Li and Lin (2004) to improve protein separation efficiency.

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