PEA PROTEIN ISOLATE PRODUCTION

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ABSTRACT

PEA PROTEIN ISOLATE PRODUCTION

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Pea seeds were tempered at moisture contents of 12.0±0.1, 13.0±0.1, 14.0±0.1 and 15.0±0.3%. The seeds with different moisture contents were then milled and fractioned according to the particle size of 53, 106, 212, 425 and 850 μm. Tempering the pea seeds (12.0±0.1, 13.0±0.1, 14.0±0.1 and 15.0±0.3%) did not significantly affect the mass and protein fraction in comparison with the pea seeds that are not tempered (11.45±0.05%).

For the production of pea protein isolate, aqueous-solvent extraction method was used. The protein was extracted with an alkali solution from the ground pea-seeds and precipitated from the extract by bringing the pH down to isoelectric point (pH=4.5). The precipitated protein was separated from the supernatant by centrifugation.

The effects of extraction parameters on the yield of extraction such as pH, particle size, temperature, solvent to solid ratio, and salt were studied. The maximum yields were obtained at these conditions; pH: 12.0 for the alkalinity of the extraction medium, 53 μm for the particle size, 40°C for the extraction temperature, 5.0 for the solvent to solid ratio and 0.0 M for the saline concentration. At these extraction conditions, the maximum protein recovery was 72.75% resulting in a product containing 93.29% protein on a dry basis.

Key words: Pea, Pea Protein Isolate, Aqueous-solvent Extraction

ÖZ

BEZELYE PROTEİN İZOLATININ ÜRETİMİ

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Bezelye taneleri yüzde 12.0±0.1, 13.0±0.1, 14.0±0.1, %15.0±0.3 nem içeriğine ulaşması için koşullandırılmış, öğütülmüş ve 53, 106, 212, 425 ve 850 μm tanecik büyüklüklerinde sınıflandırılmıştır. Koşullandırma işleminin (12.0±0.1, 13.0±0.1, 14.0±0.1, %15.0±0.3) farklı tanecik büyüklüklerine sahip koşullandırılmamış (%11.45±0.05) bezelye tanelerine kıyasla, kütle ve protein dağılımı oranlarında önemli bir fark olmadığı gözlemlenmiştir.

Bu çalışmada bezelye protein izolatı üretimi için sulu-çözücü özütlemesi yöntemi kullanılmıştır. Protein özütlemesi için öğütülmüş bezelye taneleri alkali solüsyon ile işleme tabi tutulmuş ve sonrasında çökelmenin gerçekleşmesi için çözeltinin pH' sı protein izoelektrik noktasına (pH=4.5) kadar düşürülmüştür. Santifurüj yöntemi ile çöken protein kısmı geriye kalan sıvı kısımdan ayrılmıştır.

Bu çalışmada kullanılan sulu-çözücü özütlemesi yönteminin değişkenleri olarak özütleme yapılan ortamın pH'sı, tanecik büyüklüğü, özütleme yapılan ortamın sıcaklığı, katı maddenin çözücüye oranı ve özütleme yapılan ortamın tuz yoğunluğu çalışılmıştır. Buna göre en yüksek verim şu koşullarda elde edilmiştir; özütleme yapılan ortamın pH' sı: 12.0, bezelyenin tanecik büyüklüğü: 53 μm, özütleme yapılan ortamın sıcaklığı: 40 °C, özütleme çözeltisinin bezelye ununa oranı: 5.0 ve özütleme ortamında tuz bulunmamasıdır. Yukarıda belirtilen özütleme değişkenleri ile elde edilen en yüksek verim 72.75% olup elde edilen ürün kuru bazda 93.29% protein içermiştir.

Anahtar kelimeler: Bezelye, Bezelye Protein İzolatı, Sulu-çözücü Özütleme

To My Parents

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INTRODUCTION

In relation to population growth, researches focus on the present and potential protein sources. It is estimated that larger supplies of protein will be needed in the future [1]. This fact stimulates research on developing new sources. Legumes and seeds are high in protein content and have been used as an inexpensive protein source where animal proteins are either unaffordable or are considered detrimental to the health. Legume and seed proteins are also used in the formulation of new products, or in conventional foods.

1.1 Recent Developments in Food Processing and Plant Protein

Meat, besides dairy products, is the most important source of proteins in the diet. Another and the most important source of protein is the plants. Animal proteins are superior to plant proteins, as a food supply to man. Many plant proteins are deficient in certain essential amino acids such as lysine and methionine. However, there are also plant proteins, which have essential amino acids. Based on amino acid composition alone, such proteins are superior to animal proteins [1,2].

Among, pulses are the important group used by the man as a food source. They are rich in protein and nutrient content. They can also be easily handled and stored. Due to these facts, not only in Turkey, but also worldwide demands have increased [3].

The pulses consumed by man are lentils, chickpea, bean and peas. They are sufficient for average protein requirements of healthy individuals. Thus, replacing a part of products of animal origin with a low cost high protein pulses can lower the cost of the food products and in a way makes them more acceptable to man. Also, in a modern society, health and nutrition awareness is in an increasing trend. Thus, people choose plant protein sources that are low in fat, cholesterol and sodium [4].

It is estimated that half of all human environmental impact is food-related. Production of food has negative effects on the environment because of the fact that, using fertilizers, crop protectants, energy and water results in the generation of waste streams. From the environmental point of view, in many developing countries, consumption of meat shows a decrease. This is because of the fact that, conversion of feed by animals into meat is an inefficient process. At the end of the conversion process, huge waste streams such as manure, emissions of ammonia are produced which causes soil, water and air pollution. Also, the space required for the production of raw material, which would be converted into meat by the animals, causes loss of the species. Despite these disadvantages of the meat products, pulses proteins can be introduced into animal protein food systems. In this way the inefficient conversion step from plant to animal can be decreased. Among the plant protein sources, their ease of production and relatively cheaper price causes an increase in the demands on pulses [5].

1.1.1 Production of Protein Rich Crops in Turkey

Turkey has an important place among the world pulses production. Developments in Turkey's pulses production are shown in Table 1.1. In Turkey, no significant increase or decrease in pulses production was observed between 1995 and 2003 in comparison to world pulses production [3].

Soybean is a very popular legume due to the fact that it is an inexpensive source of protein and high quality oil. Soybean is mainly used in these two areas, it is mostly processed and then used in the industry and it is consumed as meal. In Turkey, due to the wrong price policies, production area and production quantity of the soybean shows a significant decrease (Table 1.2) [1, 3].

Table 1.1 Pulses production in Turkey.

Years	Production Area (Ha)	Production (Tons)	Efficiency (Tons/Ha)
1995	1,871,034	1,849,434	0.9885
1996	1,875,571	1,832,221	0.9769
1997	1,750,155	1,699,960	0.9713
1998	1,657,770	1,599,360	0.9648
1999	1,582,795	1,360,300	0.8594
2000	1,542,107	1,316,487	0.8537
2001	1,560,875	1,454,525	0.9319
2002	1,606,700	1,647,500	1.0254
2003	1,602,500	1,577,000	0.9841

(From References 3)

Table 1.2 Soybean production, consumption and developments in importation of soybean in Turkey.

Turkey	1995/ 1996	1996/ 1997	1997/ 1998	1998/ 1999	1999/ 2000	2000/ 2001	2001/ 2002	2002/ 2003
Production Area (1000 ha)	45	40	30	23	28	30	20	35
Production (1000 tons)	75	65	40	60	60	40	45	95
Efficiency	1.67	1.63	1.33	2.61	2.14	1.33	2.25	2.71
Importation (1000 tons)	180	225	278	335	313	382	545	720
Processed (1000 tons)	255	220	170	150	70	80	160	250
Animal feed and others	0	65	153	235	293	332	430	550
Total consumption	255	285	323	385	363	412	590	800

(From References 3)

An increase in the production and consumption of soybean is observed in the world market [3]. However, the amount of production is still between 75,000 and 95,000 tons in Turkey. Against to that limited amount of production, Turkey's demand on soybean increases from 255,000 tons to 800,000 tons [3].

In Turkey, about 250,000 tons of soybeans are processed (Table 1.2). As a result, the amount of production does not support the amount that Turkey needs. Therefore, Turkey has to import soybean from the world market. Indeed, the importation of soybean was about 180,000 tons in 1995. Nowadays, this number reaches about 750,000 tons a year. Thus, if Turkey continues to process soybean as a protein source, the country will depend on the imported soybean. This also means that Turkey uses some of its budget for the importation of soybean [3, 6]. From now on, the disadvantages of the soybean are examined and studies focuses on peas, which is an alternative source of protein produced in Turkey. The production data for the green peas is shown in Table 1.3.

Table 1.3 green peas' production in Turkey.

Year	Production Area (Ha)	Production (tons)	Efficiency (Tons/Ha)
1995	8,900	49,000	5.5056
1996	7,800	43,000	5.5128
1997	8,700	50,000	5.7471
1998	9,000	52,000	5.7778
1999	9,600	55,000	5.7292
2000	8,500	48,000	5.6471
2001	8,700	60,000	6.8966
2002	9,000	69,000	7.6667
2003	9,000	55,000	6.1111

(From References 3)

From Table 1.3, it is seen that the amount of production changes with respect to year. However, it could be concluded that production is about 60,000 tons a year for the green peas. There are few data about the consumption areas of peas in Turkey. The production amount of peas totally meets the amount of consumption in Turkey. For this reason, Turkey would not be dependent for the importation of peas. In conclusion, pea could be an alternative protein source with respect to soybean due to its sufficient quantity of production.

1.1.2 Comparison of Pea and Soybean

In recent years, much of the researches focus on the use of soybean proteins in the formulation of food products. Nowadays in addition to soybean, pea proteins are being introduced into the food systems [4]. The price and the nutritional value of the protein are taken into consideration for the comparison of the pea and soybean protein. The price of the two important protein sources is compared in Table 1.4 [1-7].

Table 1.4 Real prices of Peas and Soy in Turkey (* with respect to 2002 prices)

Years	Price of Peas (TL/kg)	Price of Soy (TL/kg)
1995	798,947	410,509
1996	911,803	475,989
1997	665,359	487,054
1998	756,047	457,389
1999	614,424	454,868
2000	541,570	368,713
2001	459,773	344,186
2002	496,000	337,000

(From References 3) (*) Wholesale price index, 1994=100

According to Table 1.4, between 1995 and 2002, it could be realized that soybean had always-lower price than the peas. Thus, it can be concluded that, peas would not be advantageous against soybean with respect to its price.

Nutritional value of the proteins is determined by its amino acid composition. Many plant proteins are deficient in certain essential amino acids such as lysine and methionine. These amino acids are the ones that are inferior to animal proteins [6, 7, 21]. Therefore, for the comparison of the nutritional value of the soy and pea protein, essential amino acid content of these two important protein sources can be considered (Table 1.5).

Table 1.5 Amino acid composition (g AA/100 g protein) of pea and soybean seeds

	Pea Seed	Soybean Seed
Alanine	3.3	3.7
Arginine	6.9	5.3
Aspartate	8.4	7.9
Glutamate	13.7	15.8
Glycine	3.0	2.5
Histidine	2.6	3.4
Isoleucine	3.9	4.7
Leucine	5.5	6.5
Lysine	2.4	1.6
Methionine	0.5	0.9
Phenylalanine	3.8	4.3
Serine	3.3	3.2
Threonine	2.8	2.7
Tyrosine	3.5	3.9

(From References 16)

From Table 1.5, it could be concluded that pea protein profiles showed higher levels in some amino acids such as arginine, aspartate, glycine, lysine, serine, and threonine. Among all, lysine is the most important essential amino acid. Pea has the advantage of higher levels of lysine in its protein profile. In conclusion, against its higher price than the soybean, pea can still be an alternative protein source due to its high nutritional value protein.

As a result, pea and soybean can be used for replacing a part of the products of animal origin. However, consuming the pea or soybean alone would not be sufficient for the protein requirements of people. Therefore, using pea and soybean in a combination would increase the total protein availability for people.

1.2 Peas (Pisum Sativum L.)

The pea has been known since decades. Peas are probably originated in Abyssinia and Afghanistan and later colonized in the Mediterranean areas. From these areas the pea spread to other parts of Europe and Asia [7].

Several thousand varieties of peas exist throughout the world. They can be classified into the following categories:

1. *Field peas*, providing forage for animal feed;
2. *Market peas*, from which pods are harvested for human consumption as a fresh vegetable;
3. *Vining peas*, for canning or freezing;
4. *Dried peas*, partly for consumed as food but mostly for animal feed

The two best-known members are *Pisum sativum,* var. *Arvense Poir,* field or smooth pea, and *P. sativum L.,* or wrinkled pea. Duke (1981) [8] reported that garden peas are treated as *P. Sativum* ssp. *hortense* Asch. & Graebn, field peas as *P. Sativum* ssp. *Arvense* (L.) Poir, and edible podded peas as *P. Sativum* ssp. Macrocarpon; early dwarf pea as *P. Sativum* var *humile*. Later, Smart (1990) [8], based on studies undertaken by Ben-Zeiev and Zohary (1973), and Polhill and van der Maesen et al., [8] reported that pea comprises only two species, viz; *Pisum sativum* and *P. fulvum* (Sibeth. & Smith) [1].

Pea is a self-pollinated annual herb and they are generally 30-150 cm long. The leaves alternate with 1-3 pairs of leaflets. The leaves are generally 1.5-6 cm long. Davies et al., [8] mentioned that the leaf type could be conventional, semi-leafless and leafless. Leaf size in most cases increases up to the first node bearing the first flower. Stipules are large, leaf like and up to 10 cm long. The inflorescence of pea is a raceme arising from the axils of the leaf. The corolla is generally white, pink, or purple. The pods are swollen or compressed, short-stalked, straight curved and 4-15 cm long. On a whole plant basis, flowering is

sequential and upward from node to node. The color of the seeds is changing from white to gray and from green to brown. Duke [8] also said that, 100 seeds weigh from 10 to 36 g [7, 8].

Peas require a cool and relatively humid climate, and are grown at higher altitudes in tropics with temperatures from 7 to 30 °C. As reported by Slinkard et al. [8], the optimum temperature levels for the vegetative and reproductive periods of peas are on a day and night basis 21 and 16 °C, and 16 and 10 °C, respectively. Temperatures over 27 °C shorten the growing period and adversely affect pollination. A hot spell is mostly damaging the peas rather than the light frost. Peas can be grown successfully during midsummer and early fall in those areas having relatively low temperatures and good rainfall [7, 8, 9].

1.2.1 Composition of the Pea Seed

A relatively high protein content (25%) and even greater proportion of carbohydrates (45%) generally characterize peas. The oil content of these legume seeds is usually low [1].

The growing pea contains two major components. These are the hull and the dehulled seed. The composition and the proportion of these two differ according to the type of the peas. As Daveby at al. [10] stated that, in the mature seed, the hull (seed coat, or testa) weighs 70 to 140 g kg^{-1} and consists mainly of non-starch polysaccharides. However the major components in the dehulled seed (kernel, or cotyledons) are starch (~ 450 g kg^{-1}) and protein (~250 g kg^{-1}). The dehulled seeds contain fewer amounts of ash, crude fat, fiber, and low molecular weight carbohydrates such as glucose, fructose, sucrose, and oligosaccharides [10]. The gross chemical composition of dry flours, concentrates, and isolate are shown in Table 1.6

Table 1.6 Gross chemical compositions of pea seed and protein products.

	Seed	Pea Conc.	Isol.
% Protein	26.0	48.5	89.6
% Lipid	1.4	0.9	1.6
% Ash	3.0	3.0	2.6
% Moisture	13.0	8.6	5.3

(From References 15)

1.2.1.1 Pea Carbohydrates

Dry peas contain large amounts of oligosaccharides and polysaccharides. For the polysaccharide part, dry peas include starches and the fiber components such as cell wall non-starch polysaccharides. Carbohydrate components of various pea types are shown in Table 1.7.

Table 1.7 Carbohydrate components of various pea types. (From Castell and Guenter).

Components ($g\ kg^{-1}$ on dry basis)	Seed shape[a]		Cultivar[b]	
	Round	Wrinkled	Spring	Winter
Nitrogen-free extract	670	600	639	601
Starch	479	329	500	475
Total sugars	80	102	70	70
Sucrose	34	42	-	-
Total oligosaccharides	61	114	-	-

[a] From Cerning-Beroard and Filiatre-Verel (1977). [b] From Grosjean and Gatel (1986).

(From References 11)

Pea starch consists of two polymers of glucose. These are the amylose (linear) and the amylopectin (branched). The proportions of each vary among seeds, different species, and even between different strains of the same species. The proportion of the fiber decreases during the development of the seed.

Saini et al. [11] stated that α-galactosides (raffinose, stachyose and verbascose) represent usually less than 5% of the pea on dry basis. The increase in concentration of α-galactosides during seed maturation occurs as the content of other low molecular weight sugars declines. According to Gatel and Grosjean [11], sucrose may still represent 38% of all the sugars in smooth-seeded peas. On the other hand, it is likely to be less than 50 g kg^{-1} in dry pea [11].

1.2.1.2 Pea Lipids

The Pea lipids are predominantly unsaturated fatty acids (69.04%) with low content of saturated fatty acids (30.69%) and a total absence of linolenic acid. Davis at al. [12] found that the dried peas contain 1.4% fats [11, 13].

1.2.1.3 Pea Minerals

Pea has a very high content of potassium and phosphorus, moderate content of calcium and magnesium, and low content of iron, zinc, copper, and manganese. Most of the minerals are evenly distributed between the testa and the kernel. However, the kernel tends to be relatively rich in calcium and potassium. In general, the average availability of pea minerals is 58.09% [11, 13]

1.2.1.4 Pea Proteins

According to the studies performed in 1983 by Monti [18] crude protein analyses of the pea seeds ranged from 155 to 397 g kg^{-1} on dry basis. This high variability of pea protein composition is generally related not only to genetic characteristics, but also to environmental factors. In another study in 1990 by Gatel and Grosjean [14], it was found that the crude protein was higher in wrinkled than the smooth seed [11, 14].

The two major soluble protein fractions of pea are globulins and albumins. Globulins, principally legumins and vicilins, make up 65-80% of the extractable proteins of the pea. Albumin fraction, constituted of two major albumins, PA_1 and PA_2 contributes to 20-35% of the extractable proteins. In every case, the globulin fraction represents about 50-60% of the total protein, whereas albumins ranges generally between 15 and 25 %. It has been suggested that the nutritional value of whole pea protein decreases as the proportion of globulins especially vicilin increases. This is due to the fact that, vicilin has low sulphur amino acid content [11, 15].

The nutritive value of peas will reflect the differences in amino acid composition of the globulin fractions. Although peas are highly variable in protein content, the amounts of individual amino acids were linearly correlated to the crude protein content. The nutritive value of a seed is usually based on the content of essential amino acids and their availabilities. Table 1.8 lists the amino acid composition of the pea proteins [11, 14]. The below results for the amino acid composition of the pea protein is different from the ones obtained in Table 1.5. In the study of Fernandez et al. [16], the peas were supplied from Spain whereas in the study of Tömösközi et al. [15], peas were supplied from Hungary. This difference in protein content is due to both genetic and enviromental effects where the peas are grown [21].

Table 1.8 Amino acid composition [g AA/100 g protein] of pea protein products.

Amino acid (in percent)	Flour	Pea Conc.	Isol.
Asp	10.46	11.58	11.52
Tre	3.66	3.12	3.69
Ser	4.37	4.96	6.09
Glu	16.60	16.39	17.03
Pro	5.56	4.30	5.01
Gly	4.43	4.50	4.68
Ala	4.53	4.13	4.41
½ Cys	0.34	0.35	0.73
Val	5.20	5.13	4.81
Met	0.86	0.85	0.78
Ile	3.80	3.48	3.68
Leu	6.36	6.94	8.16
Tyr	3.05	3.35	3.79
Phe	4.54	4.67	5.18
Lys	8.58	8.12	8.96
His	3.40	3.39	3.81
Trp	0.50	0.51	0.51
Arg	13.76	14.22	7.15

(From References 15)

The determined essential amino acids were His, Ile, Leu, Lys, Met, Phe, Tyr and Val. Among all, the ones of primary interest are lysine and methionine contents. Pea is rich in lysine, so it could be an alternative supplement to the food system. However, methionine is the limiting amino acid in pea proteins. This limitation must be taken into consideration for the nutritive value of the pea proteins [10, 16, 17].

1.2.2 Forms of Pea Proteins

The pea proteins are classified into three major groups based on protein content. These forms are described below.

1.2.2.1 Flours

Flours are the least refined form of plant proteins. They have varying fat content, particle size, texture and degree of heat treatment. Flours are prepared by first cleaning and pulverizing peas; with the bran (hull) being removed and ground. According to Turkish Standard Institute, the seeds are ground to 180 micro meter or finer and the maximum moisture content is 14.5%. In the commercial applications, protein contents of these materials are more than 25% with the fat content of about 2%. By using turbo milling and air classification, the protein content of pea flour can be obtained at a range of 40-50 % [17, 18, 21]. As the physical characteristics, the pea flour has a mild aroma and light cream color.

1.2.2.2 Concentrates

The pea protein concentrates are prepared from the defatted pea flour by removing the oligosaccharides. In here, the mechanism is simply based on the solubility of sugar in aqueous alcohol. The sugar dissolves in the alcohol and then the mixture is removed. The remaining part is dried to obtain the pea protein concentrate. They are more refined than the pea flours, containing 50 to 90% protein on dry basis. Some of the concentrates are specially processed and mechanically refined to increase their protein content for food and industrial usage. They are also immediate raw materials for the pea protein isolates [19].

1.2.2.3 Isolates

As a simple definition, a pea protein isolate is the pea protein with the highest content of protein. The pea protein isolate is also made of defatted pea. They are prepared by removing the water-insoluble polysaccharides, as well as the oligosaccharides and other low molecular weight components that are not separated during manufacture of protein concentrates. At the end of the process, a product with 90% or higher pea protein is obtained.

The production of protein isolate by using laboratory methods usually involves the dispersion of the protein in neutral salts and precipitation by dilution, dialysis, salt dehydration, and sometimes by solution or precipitation with organic solvents. Such procedures require large amounts of water and chemicals to be suitable for economic commercial operations. The high yields essential for production of commercial isolates from concentrates are obtained only by extraction of the protein concentrates with an alkali solution and precipitation with acids [4].

1.2.3 Brief History of the General Uses of Pea Protein

The pea seeds have been used as a source of food for a long time. For the simplest way, the bitter seeds are soaked in water to remove most of the alkaloids and then cooked. In recent years, the pea proteins are used to provide nutritional quality. They impart desirable structure, texture, flavor, and color characteristics to food products such as meat analog, meat or cheese extenders, or they are incorporated in baked and confectionary goods, being used as flours in products such as baby formula or supplemental diet for preschool children. They are also essential to complement cereals for the feeding of the animals, especially for the poultry [6].

On the other hand, the traditional means of the manufacture of the pea protein products are either by removal of water- or alcohol-soluble components of meals, that is the manufacture of protein concentrates, or by the manufacture of isolates through the extraction of globulins from the meals. The pea protein concentrates and the isolates play an important role in many new food formulations. Generally the pea proteins are increasingly used as food ingredients due to their key functional properties, e.g. texture stabilization and optimum consumer costs [21, 25].

The pea proteins can also be used in the non-food applications. By some modification, the pea proteins can be used in the industry. Such applications involve; enzymatically hydrolyzing the pea proteins to improve surface activity to be used as a surfactant, using the pea proteins as coating for extruded starch. Also, the pea proteins have good emulsifying properties for preparing oil in water emulsions [19].

1.3 General Principle and Mechanism of Protein Extraction

Among the various methods, the aqueous phase method is used for the extraction of the proteins from peas. This is due to the facts that, a product with better functional and nutritional properties can be obtained by using an aqueous extraction method. For the extraction of the pea protein using a solvent, the following general steps occur in the overall process. First of all, the solvent must be transferred from the bulk solvent solution to the surface of the solid. Next, the solvent must penetrate or diffuse into the solid. The solid dissolves in the solvent. The solute diffuses through the solid solvent mixture to the surface of the particle. Finally, the solute is transferred to the bulk solution [22, 26, 27].

For the commercial pea protein extraction, Belter et al. [1] described an operation in more details. Extraction of the protein from the meal is carried out at a meal-to-water ratio of 5-20 with an alkaline solution at pH values of 9-12. After the

extraction step, the protein solution is separated from the insoluble residue by a continuous-discharge type of centrifuge. After that, the protein solution is pumped to another tank. The protein is then precipitated from the clarified solution by adjusting pH to 4.5. In the tank, protein curd was kept to settle to the bottom. Then, the supernatant solution or the whey is discharged. The last step is the drying of the protein curd [17, 27].

1.3.1 Extraction Equipment and Operation

The simplest type of extractor used is the single-stage extractor. It consists of a tank that contains the solid to be extracted. The liquid phase flows over the solid and is drained from the bottom of the tank. This type of extractor operates as a batch system. After the extraction process, the tank has to be emptied and refilled. Several such batch extractors may be connected in series, with the solvent being pumped from one stage to the next, forming an extraction battery [27, 28].

1.3.2 Effect of Extraction and Fractioning on Protein

Several types of unit operations are performed during the protein extraction. These are briefly, extraction, isoelectric precipitation, and salt precipitation. During these operations, some of the proteins in the crude extract might be lost. For example during isoelectric precipitation, some sulfur-rich albumin-type proteins, which are usually soluble at isoelectric pH, might be lost in the supernatant fluid. This fact causes the alteration of the amino acid composition and nutritional value of protein isolates [22, 29].

When the proteins are exposured to alkaline pH at elevated temperatures (above 75 °C) irreversible conformational changes are observed. This is partly because of deamidation of Asn and Gln residues, and β-elimination of cystine residues. Also, the gross structural changes in proteins are observed when they are

exposed to alkali. This is because of an increase in the electro negativity and breakage of disulfide bonds. Moderate heat treatment has a beneficial effect that includes inactivation of proteinaceous toxins. Cutting the peptide bonds during severe heat treatment of protein solutions forms low-molecular weight-peptides. Severe heating under alkaline and acid pH conditions also causes partial hydrolysis of proteins. The amount of low molecular weight peptides in protein isolates can affect their functional properties [25].

1.3.3 Production Process for High Quality Pea-Protein Isolate

An important aspect of the production of high quality pea protein isolate is to minimize the growth of microorganisms during the process. Control of the microbiological quality can be obtained by minimizing the processing time, thereby restricting the growth of microorganisms [17, 30]. The steps for the production of pea-protein isolate are described below.

1.3.3.1 Dehulling and Milling

The pea seeds are dehulled after soaking in water for 10 hours, and dried in a forced air oven at 60 °C. The milling process has often been applied to feed because of its nutritional and zoo technical impact. Brennan et al [27] explained this effect by an increase of the ratio of surface/volume of the substrate and by the breakdown of cell wall structures, leading to an increased accessibility to cellular contents. According to their idea, milling has been demonstrated to produce individual heterogeneous particles in size and chemical composition [31, 32].

In another study, the effect of milling on extraction is explained as follows; the proteins are cellular in structure and the soluble constituents are generally found inside the cells. The cell wall provides resistance to diffusion. As a result of this fact, the extraction rate is comparatively slow.

To avoid this disadvantage, the cell walls of many plant seeds are largely ruptured and the original materials are reduced in size to about 0.1 mm to 0.5 mm by rolling or flaking [29].

Francisco, Varriano-Marston, & Hoseney [29] suggested that the milling time and the energy expenditure are determined by the hardness of the seeds. The properties and appearance of the final ground product are also determined by the hardness of the seeds. The seed hardness also affects the dry fractionation process, where milling is used as pre-treatment. The pre-dried dehulled green peas were brought to desired moisture contents. The harder pea results in the finer flour. The overall result is a lower total energy uptake for milling peas with high moisture content [30].

1.3.3.2 Processing Meal for Protein Isolation

The dehulled seeds are first milled. The hulls (seed coats) should be finely milled for use in high fiber bread. Studies showed that dehulled pea flour gave lower crude fiber and higher protein contents. Also seed hulls contain dark-colored pigments and impart unpleasant flavors to food products. Thus, they are undesirable in isolates [15, 17, 34, 35].

In the next step, the resultant flours are dispersed in water at a (w/v) ratio of 5-20, and the suspension was adjusted to basic pH of 9.0-12.0. The mixture was stirred at room temperature. The insoluble matrices, which contain the water insoluble polysaccharides plus residual protein, are separated by centrifugation and discarded. The supernatant containing the bulk of the proteins plus sugar was adjusted to acidic pH of 4.5-6.0 and stirred at room temperature [15]. This treatment precipitates the proteins. The precipitated protein was separated, washed, and dried to give the isoelectric protein [16]. The steps of the pea protein extraction process are shown in Figure 1.1.

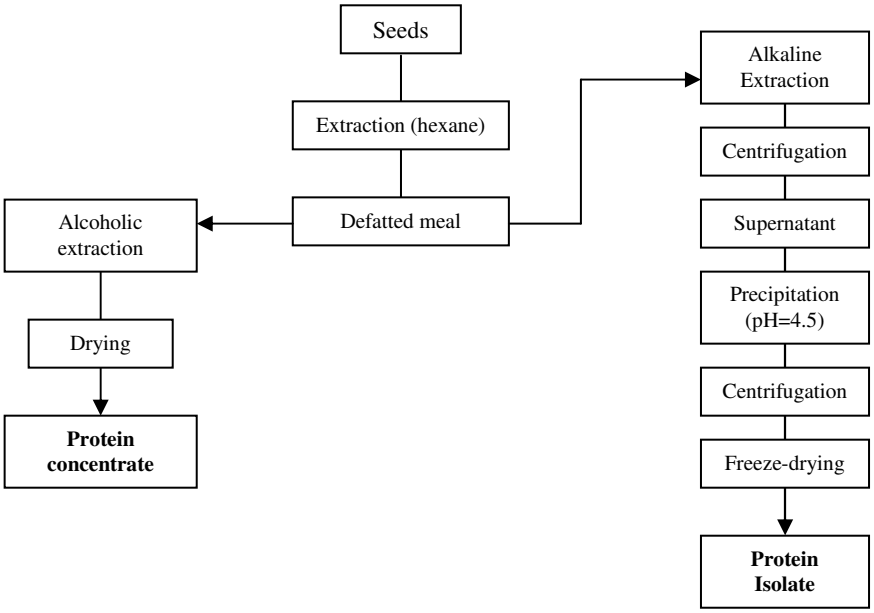

Figure 1.1. Flow diagram for production of pea protein concentrates and isolates. (From Reference 15)

In a study performed in 1979 by Kinsella [30], it was shown that many factors affected the extractability of protein. These are basically the particle size, quality of flour, solvent to flour ratio, pH, the temperature during extraction, ionic strength, and addition of the salts into extract. It is important to recover as much protein as possible during extraction, to get maximum protein content in the concentrate or isolate products [20, 35].

The effect of size and treatment of the particles on an extraction yield was studied in 1989 by Aguilera and Garcia [30]. It observed that the smaller particle size increases the protein yield, while flaked or exploded and dehulled material has higher diffusion coefficients, which gave higher yield. Sumner et al. [30] studied the extraction yield of whole and dehulled pea flour in 1981. Results showed that dehulled pea flour gave lower crude fiber and higher protein contents [15, 35].

According to a study in 1983 by Wang et al. [32], there existed a positive relationship between protein curd yield with protein recovery during solvent extraction. Ulloa et al. [33] found that alkali extraction at pH 7.0 – 12.0, followed by precipitation at pH 4.5 gave protein yield of 60 – 92 %. The yield was highest at pH 12.0, but this required large amounts of NaOH that may cause extreme changes in the environment. The NaOH can also be detrimental to protein quality. Water and other solvent extractions at pH 7.0 could produce 80% or more protein yield [36, 38, 39].

Low temperature (0 – 10 °C) during extraction is important if the protein is to be evaluated for its biological activity or its intact storage protein. Most reported extractions are done at room temperature (20 – 25 °C) where the quality of the protein is the important criteria rather than the yield of extraction [30]. On the other hand, the extractions are done in the temperature range of 40 to 60 °C where the extraction yield is the important criteria [22].

Most extractions used flour to solvent ratios of 1:5 to 1:30, with 1 to 4 times repetitive extractions. A study by Rhee et al. [30] showed that 2 extractions were generally adequate since the third and fourth did not significantly increase the yield. Rhee et al. reported the optimum ratio as 1:20. This made the process easier to handle the total amount of solvent and reduced water volume [16, 34, 38].

The effects of drying methods on chemical and functional properties of pea proteins were evaluated by Sumner et al. [29]. Drying of the proteins would be performed in three different ways. These are either in a vacuum oven; freeze drier or a spray drier. Drying in a vacuum oven, compared to freeze-drying and spray drying, decreases the nitrogen solubility index and increases the water absorption. This fact is due to protein denaturation caused by the elevated temperatures. Freeze dried and spray dried isolates had the highest emulsification and water absorption values. Also, the spray dried protein content may have had less Maillard reaction or polyphenol oxidation.

On the other hand, despite these advantages the recovery of the protein by spray drying was the lowest. The highest recovery of the protein is obtained by drying the pea protein isolates in a vacuum oven [22, 34].

1.4 Summary of Literature Review

During the literature survey, only the study of Mizubuti et al. [34] has similarities when compared with this study. Mizubuti et al. [34] studied the optimization for the pea protein extraction by using response surface methodology. They design their experiments by using three variables as, salt concentration, pH, and liquid to solid ratio. Within this study, alternative protein sources for the protein isolate production is investigated, the advantages and the disadvantages of using pea as a protein source is examined. When compared with the study of Mizubuti et al. [34], the experimental design for the extraction is formed by using five variables, pH, particle size, temperature, distribution ratio, and salt concentration. The main difference of this study from the ones in the literature is that the two related subjects are investigated and combined in one study. Within the first subject, effect of moisture content on dry fractioning during milling is studied. The second subject includes the effect of extraction parameters on extraction yield.

1.5 Objectives of the Study

As few data are reported about the effect of extraction parameters on the yield of pea protein isolation, two folds have been formed:

1) To investigate the effects of seed hardness with respect to moisture content on dry fractioning and selecting the best moisture content for the pea seeds to obtain the highest extraction rates of the protein during milling.

2) To study the effects of extraction parameters on the yield of the pea protein isolate production. The extraction parameters studied are pH, particle size, temperature, solid to solvent ratio, and salt concentration.

CHAPTER 2

MATERIALS AND METHODS

2.1 Pea Samples

The dried peas grown in Adana region were used in the experiments. The composition of these dry pea seeds were 11.5% moisture, 1.6% fat, 21.9 % protein, 2.2 % ash and the remaining 62.9 % was the carbohydrates. The flour obtained was stored in polyethylene bags and kept at 10 °C until use.

2.2 Grinding Treatments

For the demonstration of the effect of moisture content on the extraction yield, 500 ± 2.5 g of pea seeds were taken at five portions respectively. The four portions of pea seeds were tempered for the desired moisture content and stored at 45 °C for three days. To prevent the moisture loss, leakage was hindered. The remaining portion was held as crude pea seeds, which was not tempered.

The pea seeds were milled with Brook Crompton series 2000 mill having a screen size of 1.0 mm. Each group of sample was weighed after milling and the amount of flour was recorded. The sieves were placed one above the other as in the order of 53, 106, 212, 425 and 850 μm. The one with the largest particle size (850 μm) was on the top and the one with the smallest particle size (53 μm) was on the bottom. The milled pea seeds were placed on the top sieve and weighed. The sieves

were placed on the shaker and the shaker was operated for 30 minutes. Each sieve was weighed after the period of 30 minutes shaking and the data were recorded.

The data obtained were used for the differential and cumulative analysis. After differential analysis, the graph of mass fraction vs. particle size was sketched for each moisture contents. From the cumulative analysis, the extraction percentages of the protein were calculated.

Mass fraction was calculated as;

$$\Phi n = \frac{W_{flour}}{W_{totalflour}}$$

Where, W is the weight and Φn is the mass fraction in differential analysis.

Extraction percentage of protein was calculated as;

$$EP = (1 - \Phi n) \times 100$$

Where, EP is % Extraction Percentage of protein.

The pea seed moisture content was found as 11.45±0.05%. For the tempering operation, the amount of water that must be added had to be calculated. A simple mass balance was performed for the determination of the water content.

$$\%\ \text{desired moisture} = \frac{\%\ \text{moisture in pea} + \text{g water added}}{100\ \text{g} + \text{g water added}}$$

At the end of the tempering stage, the moisture content of the four portions of pea seeds were, 12.0±0.1, 13.0±0.1, 14.0±0.1 and 15.0±0.3% respectively.

2.3 Extraction of Pea Samples

Extraction of the pea samples is summarized in Figure 2.1.

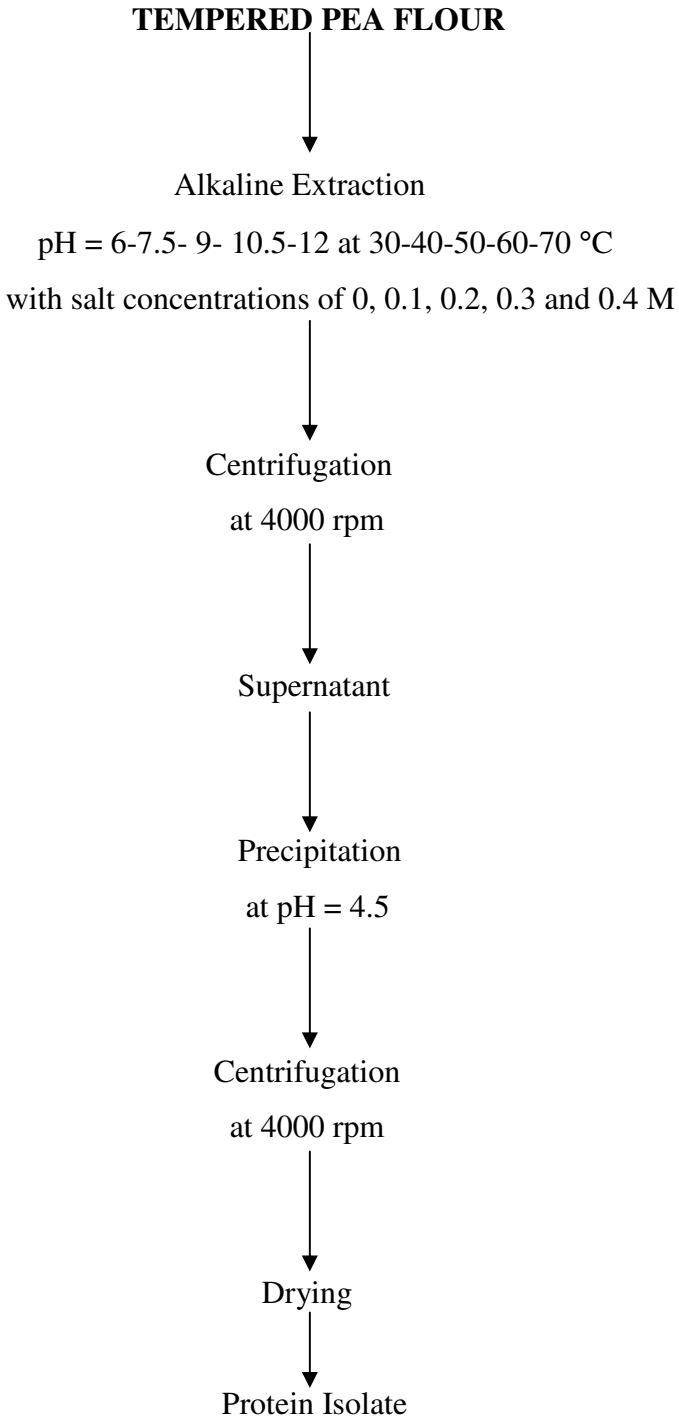

Figure 2.1. Experimental procedure

The pea meals having diameters of 53, 106, 212, 425 and 850 μm were added to distilled water at flour to water ratio of 1:2.5, 1:5, 1:7.5, 1:10, and 1:12.5. The salt concentrations of 0.1, 0.2, 0.3 and 0.4 M were adjusted by adding pure NaCl. The pH adjustments were done with 0.1 N NaOH and 0.5 N HCl and a Metrohm 691 pH meter was used to record pH value. The pea proteins were extracted at pH 6, 7.5, 9, 10.5 and 12. Extractions were carried out in a 100 ml Erlenmeyer immerged in a water bath at 30, 40, 50, 60 and 70 °C for 30 minutes. The Erlenmeyer flask was continuously stirred during extraction to keep the sample dispersed and to improve heat transfer.

The resulting suspension was then centrifuged to separate the insoluble matrices. Centrifugation was done at 4000 rpm for 20 minutes at room temperature. Sorvall Instruments RC5C Model Centrifuge Equipment was used for the centrifugation.

After centrifugation, the insoluble matrices were discharged. The supernatant (aqueous phase) was adjusted to pH 4.5 with 0.5 N HCl and the mixture was stirred at room temperature for 10 minutes. The precipitated protein was separated by centrifugation at 4000 rpm for 20 minutes. After separation of proteins by centrifugation, the precipitate was washed with distilled water (at a solid to solvent ratio of 1:2) and then dried. Drying was performed at 40 °C under 20 in. Hg vacuum in a ST Model Vacuum Oven produced by Georgia Oven Compant Inc. At the end of these steps the protein isolate was obtained.

As a short summary, this study was divided into two main parts:

1. *Dry fractioning by milling of pea seeds* (11.45±0.05, 12.0±0.1, 13.0±0.1, 14.0±0.1, 15.0±0.3%) on extraction yield was investigated. One portion of pea seeds was not tempered and the other four portions were tempered (45°C for 3 days). The comparisons were made among these five (11.45±0.05, 12.0±0.1, 13.0±0.1, 14.0±0.1 and 15.0±0.3%).

2. *Yield Analysis for pea protein isolation*; pH (6.0, 7.5, 9.0, 10.5, 12.0), particle size (53, 106, 212, 425 and 850 μm), temperature (30, 40, 50, 60 and 70 °C), weight of pea flour to volume of solvent (1:2.5, 1:5, 1:7.5, 1:10, 1:12.5), salt concentration (0, 0.1, 0.2, 0.3, 0.4 M). The yields for each parameter were compared.

2.4 Chemical and Physical Analyses

The analysis performed within this study were; moisture, ash, fat, protein analysis and also the measurement of pH

2.4.1 Moisture Analysis

Five grams of the pea sample was ground to obtain a homogeneous mixture and dried at 130 °C for 2 h. The sample was then transferred to desiccators, cooled and weighed.

2.4.2 Ash Analysis

Two and half grams of sample were weighed into a porcelain crucible and 1 ml of ethyl alcohol was added into the sample. The porcelain crucible with sample was placed in a temperature-controlled furnace at 800 °C for 2h. After 2 h, the crucible was transferred to desiccators, cooled, and weighed.

2.4.3 Fat Analysis

For the determination of fat content, Gerhardt Soxtherm 2000 automatic Soxhlet apparatus was used: 3.5 grams of ground sample was wrapped in a filter paper. The paper containing the sample was rewrapped in a second filter paper. As the extraction liquid, diethyl ether was used. The parameters of the device were set

as 150 °C boiling temperature and 30 minutes boiling time, 3.5 hours for the extraction time and 5 minutes for the solution reduction. After the solution reduction step, the oil plus diethyl ether mixture was kept at 60 °C oven until diethyl ether evaporated. Next, the sample was weighed and the fat content was calculated.

2.4.4 pH Measurement

The pH values of samples were measured by a pH-meter (Metrohm 691 pH meter).

2.4.5 Protein Analysis

For the determination of the protein content, Foss 2300 Kjeltec Analyzer Unit Foss Tecator was used. One gram of ground sample was placed into a glass tube. 15 ml of 95-98% sulfuric acid and 2 Kjeltabs Cu/3.5 tablets containing 3.5 grams of K_2SO_4, 0.4 gram of $CuSO_4$ x $5H_2O$ was also added into the glass tube. The glass tube was then placed into specially designed oven at 410 °C. It was kept for two hours untill the color of the solution turned into bright green. The bright green color was the indication for that the protein in the sample that it was burned and ready to evaluated for the nitrogen content. After the burning step, the tube was placed into the Foss 2300 Kjeltec Analyzer Unit. The device calculates the nitrogen content of the sample with the accuracy of point four decimals. For the determination of protein content, nitrogen content calculated by the device was multiplied by the nitrogen factor of 6.25.

2.5 Analysis of Results

The results of the treatments were submitted to a one-way ANOVA. Significant differences between means were tested using a Duncan's Multiple Range test with a probability level fixed at $p<0.05$. The statistical treatments were carried out with SPSS 10.0 for Windows.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Grinding Treatments

Dijkink et al [29] stated that the yield and the composition of starch and protein concentrates were found to be related to the hardness of the pea seeds. At lower moisture contents, the brittleness of the pea seeds increased. At higher moisture contents, finer flour was obtained.

Only five different moisture contents of pea seeds (11.45±0.05, 12.0±0.1, 13.0±0.1, 14.0±0.1 and 15.0±0.3%) were studied in the grinding treatments. After differential analysis, the graph of mass fraction vs. particle size was sketched for each moisture contents. (Figures 3.1, 3.2). At the end of the differential analysis, different mass fractions were obtained for the particle size of 53, 106 and 850 μm. Pea flour having particle size of 212 μm has the highest mass fraction. However, by the statistical explanation pea flour having particle size of 212 and 425 μm has the same mass fraction (Figure 3.1). It has been claimed that, the data for the mass fraction analysis, obtained from 1mm. screen size, fitted the mass fraction analysis curve like an inverse V-shape, which was the expected behavior.

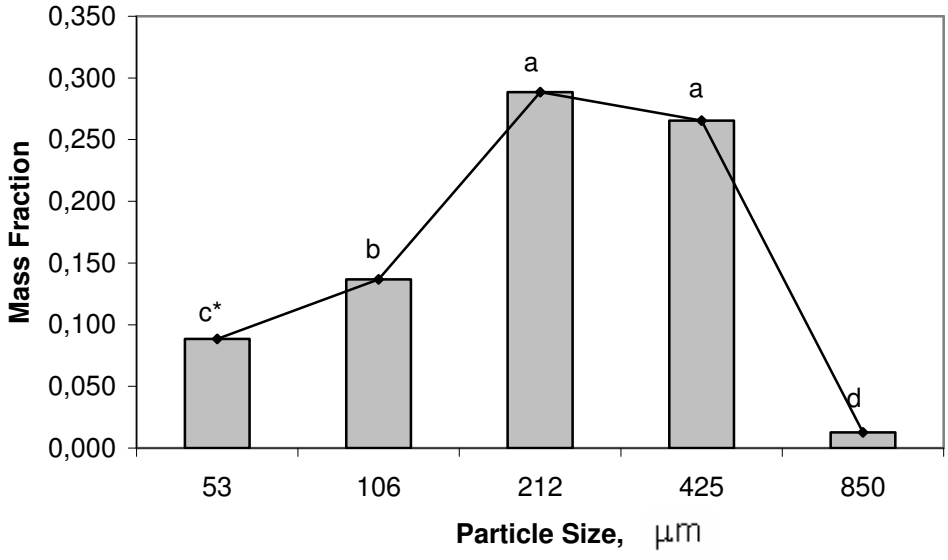

Figure 3.1. Mass fraction vs. particle size
*Different letters indicate means separation at p<0.05

Mass fraction of 53 and 106 µm flour was increased as the initial moisture content of the pea seeds increased. On the other hand, the mass fraction of 212, 425 and 850 µm flour was decreased (Figure 3.2). In Figures 3.2, as Dijkink et al [29] stated, it can be seen that finer flour was obtained at higher moisture contents of pea seeds. However, mass fraction was not significantly different (p<0.05) for the 11.45±0.05, 12.0±0.1, 13.0±0.1, 14.0±0.1 and 15.0±0.3% moisture contents.

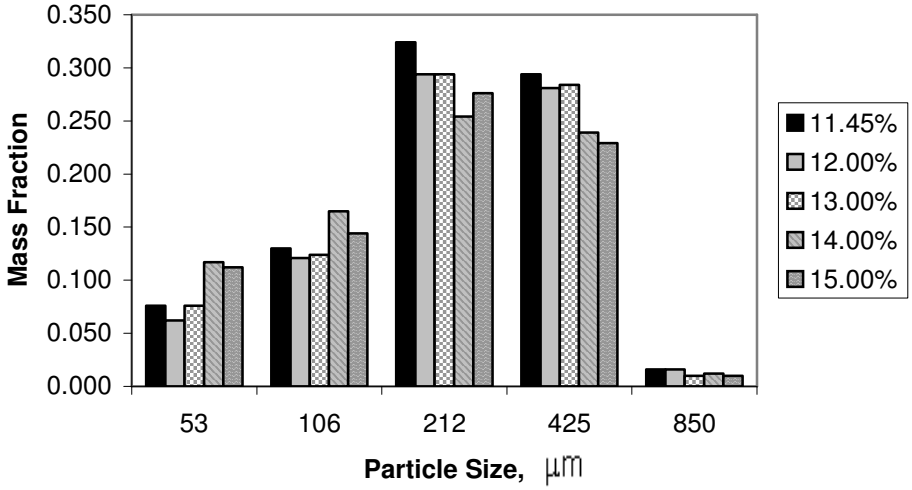

Figure 3.2. Variation of mass fraction according to the moisture content

Maaroufi et al. [27] claimed that the smaller the size of classified particles, the higher the content of crude protein. In Figure 3.3, it can be seen that the protein content of the 53 μm pea flour was the highest within the parameters studied. Also increasing the particle size resulted in the decrease of the protein content. According to Maaroufi et al. [27], this decrease in protein content was accompanied by a change in the proportion of kernel and hulls. Increasing the particle size caused an increase in the amount of hulls and decrease in the amount of kernels. Pea hulls contained 3.3% crude protein. On the other hand, pea kernels contained 23.9% crude protein.

The protein contents of pea flour at different moisture contents (11.45±0.05, 12.0±0.1, 13.0±0.1, 14.0±0.1 and 15.0±0.3%) were also studied. The protein analysis of the fractions at different moisture contents showed that increasing the moisture of the pea seeds up to 14±0.1% slightly increases the protein fraction of the 53, 106, 212, 425 and 850 μm pea flour (Figure 3.4 and Figure 3.5). This result may due to effect of milling at differing moisture contents. During milling at different moisture contents, the protein to starch ratio changes, and does so the extraction of protein. As mentioned before, at higher moisture contents finer flour would be obtained. It was stated that finer flour has the higher protein content. With all these statements and results, moisture content seems to have slight effects on the extraction of protein during milling. However, protein content was not significantly different ($p<0.05$) for the 11.45±0.05, 12.0±0.1, 13.0±0.1, 14.0±0.1 and 15.0±0.3% moisture contents.

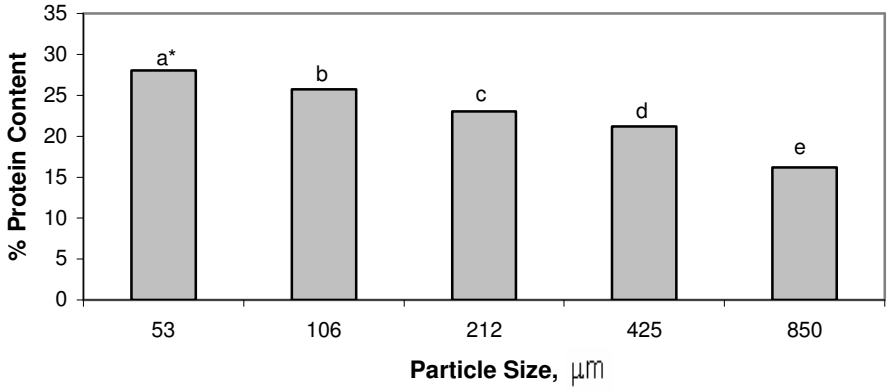

Figure 3.3. Variation of protein content according to particle size

*Different letters indicate means separation at p<0.05

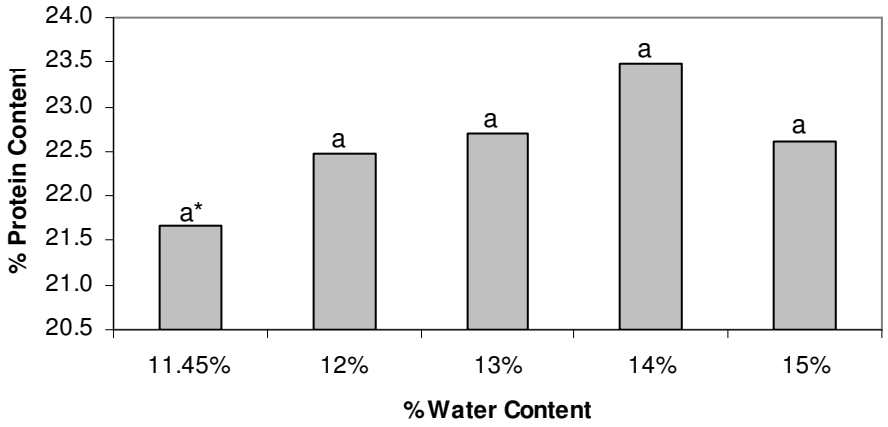

Figure 3.4. Variation of mean protein content according to moisture content for the particle sizes of 53, 106, 212, 425 and 850 µm.

*Different letters indicate means separation at p<0.05

In Figure 3.4, the mean values of the protein contents for the particle sizes of 53, 106, 212, 425 and 850 µm are presented. The more detailed protein analysis of the fractions with different initial moisture contents is shown in Figure 3.5. The optimum moisture content was 14±0.1% for obtaining flour with the highest protein content. This result is obtained for 53, 106, 212, 425 and 850 µm particle sizes. Increasing the moisture content of the pea seeds above 14±0.1% results in

the change of the protein to starch ratio. At 14±0.1% moisture content, protein to starch ratio is found as 0.400. However, at 15±0.3% moisture content this ratio decreased to 0.386.

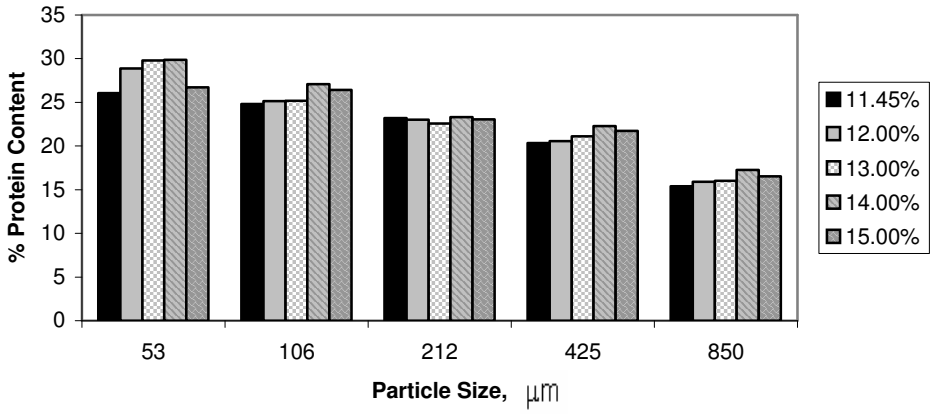

Figure 3.5. Variation of protein content according to moisture content and particle size

3.2 Effect of pH on Extraction

Sant'Anna et al. [34] studied the solubility of pea protein and found that the maximum extraction took place at pH below 3.0 and above 7.0. On the other hand, Uken Sukaeni [30] reported that the protein yield was highest at pH 12.0. However, this requires large amounts of NaOH that may cause extreme changes in environment. The NaOH can also be detrimental to protein quality. In the light of these explanations, the extractions were performed at pH of 6.0, 7.5, 9.0, 10.5 and 12.0.

While performing the experiments, some parameters were held constant to observe the effect of pH of the solution on extraction. The extractions were performed with the pea flour of 53 µm, 212 µm and 425 µm at a solvent to solid ratio of 5.0 with no salt, for one step of 30 minutes within a temperature of 30 °C.

In Figure 3.6, it can be seen that the protein yield was affected by pH. As the pH of solution during extraction increased, the protein yield increased. This may be explained by the effect of pH on protein solubility. During the experiments, an expected behavior observed was that until the pH of 9.0 suitable amounts of NaOH was used. However, above the pH of 9.0 large amounts of NaOH was used.

Uken Sukaeni [30] stated that protein yield was highest at pH 12.0, but this requires large amounts of NaOH. This statement was truly observed while performing the experiments. Mizubuti et al. [34] found that the optimum condition for the protein extraction would be at pH 8.5. On the other hand, Fernandez et al [21] found the optimum condition as 9.0 and Lopez-Leiva [34] found the optimum condition as 8.0, respectively.

While performing the experiments, the protein yield was taken into account rather than the protein quality. It was known that high amounts of alkali have detrimental effects on the protein quality. Fernandez et al. [16], Mizubuti et al. [34], and Lopez-Leiva [34] may take this situation into consideration and found different results. Also, the temperature of the extraction medium was totally different from the one of Mizubuti et al. [34]. In their study, Mizubuti et al. [34] performed the extraction at room temperature. Due to this fact, different result was obtained.

When comparing the results of the experiments with the ones in literature, the maximum yield for the extraction of pea protein was found at pH 12.0 within the parameters studied. This result is in agreement with that of Sukaeni. But, the results are totally different from those of Mizubuti et al., Fernandez et al and Lopez-Leiva [34]. In conclusion, within the parameters studied, maximum protein yield apart from the protein quality is obtained with an extraction solution having pH of 12.0.

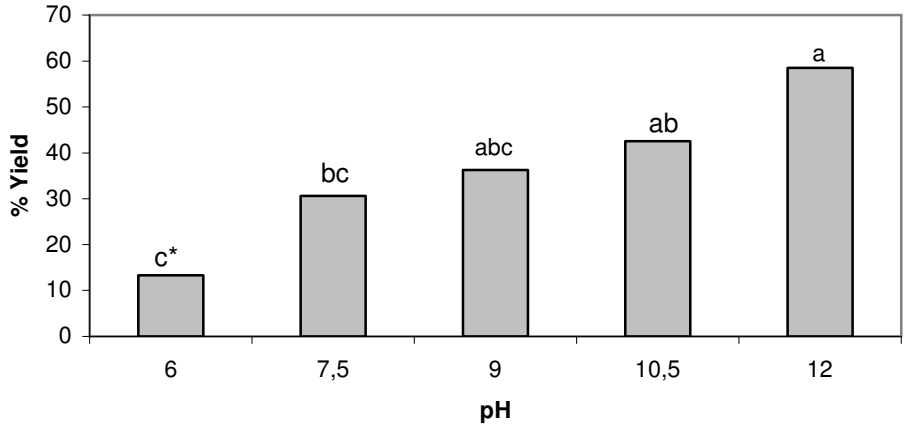

Figure 3.6. Dependence of protein yield on the extraction Ph (T=30°C, t=30 min., solvent to solid ratio of 5.0 and 0.0 M salt)
*Different letters indicate means separation at p<0.05

3.3 Effect of Particle Size during Extraction

Early investigations on the effect of size and treatment of particles on protein yield establish that the smaller particle size increased the protein yield. Brennan et al. [27] explained this effect by an increase of the ratio surface/volume of the substrate and by the breakdown of cell wall structures, leading to an increased accessibility to cellular contents. Mizubuti et al. [34] used 250 μm (60 mesh) screen for the meal that would be used for the extraction. With respect to these statements particle sizes of 53, 106, 212, 425 and 850 μm were used during the experiment

While performing the experiments, some parameters were held constant to observe the effect of particle size on extraction. The extractions were performed at pH of 7.5 and 9.0 respectively at a solvent to solid ratio of 5.0 with no salt, for one step of 30 minutes at 30 °C.

According to the literature, optimum extraction time was found as 30 minutes. After 30 minutes, protein yield remained almost constant [10, 12, 20, 30, 34]. For this reason, during the experiments, the extraction time was held constant as 30 minutes for each experiment. There is a risk that, if the extraction time were minimized there would not be enough time for the extraction of the protein from the pea flour having large particle sizes. As an example, more time is needed when protein is extracted from 850 μm flour rather than 53 μm flour. In spite of this fact, extraction time was held constant for each particle size. Also, separation of the supernatant from the whey is much harder when 53 μm flour is used instead of 850 μm flour.

In Figure 3.7, it can be seen as the particle size is reduced, the protein yield increased. Maaroufi et al [27] stated that the smaller the size of classified particles, the higher the contents of crude protein. At the end of the experiments, Maaroufi's statement was successfully observed. When combining the results, protein yield of 53 μm flour was the highest. As a result, within the parameters studied, maximum yield for pea protein extraction was found to be at a particle size of 53 μm.

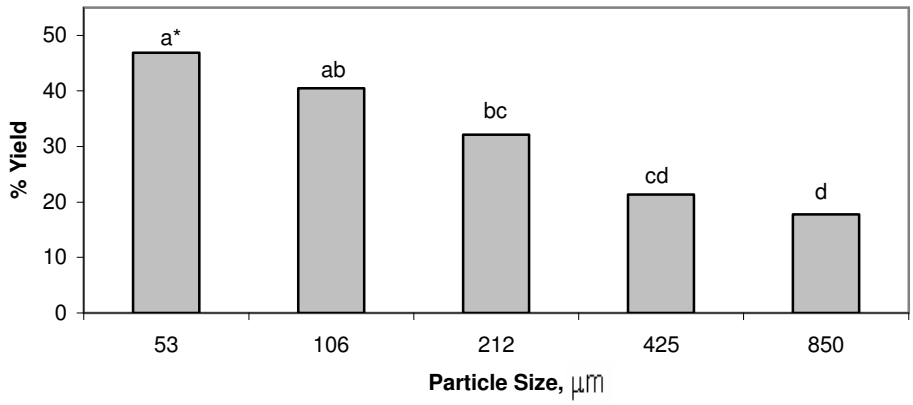

Figure 3.7. Dependence of protein yield on particle size
(T=30°C, t=30 min., solvent to solid ratio of 5.0 and 0.0 M salt)
*Different letters indicate means separation at p<0.05

3.4. Effect of Temperature on Extraction

During the experiments, to observe the effect of temperature on extraction, some parameters were held constant. The extractions were performed at pH of 7.5 and 9.0 respectively at a solvent to solid ratio of 5.0 with no salt, for one step of 30 minutes within a temperature of 30, 40, 50, 60 and 70 °C.

Uken et al. [30] stated that low temperature (0-10 °C) during extraction would be important for the biological activity of the protein. For this reason, most extractions were done at room temperature (20-25 °C). Above 55 °C, there would be a risk for the denaturation of protein. Also it was known that above 75 °C, protein denaturation would be rapid and irreversible. The thermal denaturation of proteins has been associated with the loss of solubility. As a result, the protein yield decreases.

In Figure 3.8, it can be seen that as the temperature is increased to 50 °C, the protein yield increased. However, above 60 °C decrease in the protein yield could be seen from the Figure 3.8. This situation is due to cleavage of protein chains occurred at high temperature alkali treatment. On the other hand, above 50 °C solubility of the proteins meaning the protein yield decreased due to the protein denaturation. Lopez-Leiva [34] found the optimum temperature for the extraction as 50 °C. At the end of the experiments, Lopez-Leiva's statement was successfully observed. When combining the results, maximum yield for the extraction of pea protein was found to be at 50 °C. However, when the results of the experiments were statistically explained, there was not a significant difference between 40 and 50 °C ($p<0.05$).

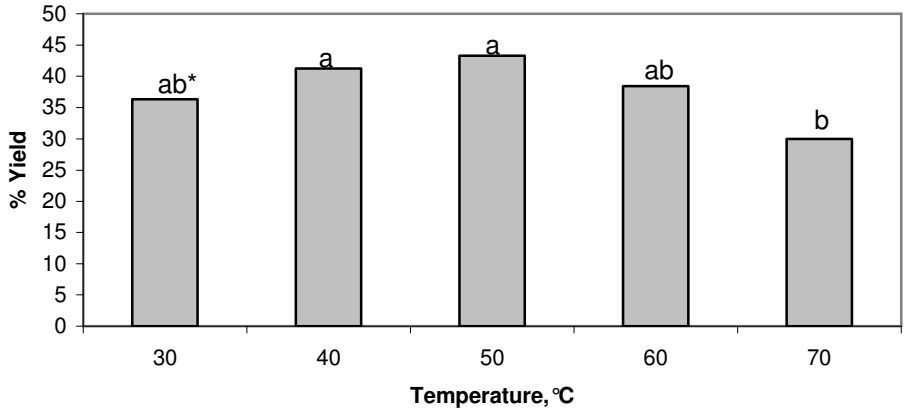

Figure 3.8. Dependence of protein yield on temperature
(t=30 min., solvent to solid ratio of 5.0 and 0.0 M salt)
*Different letters indicate means separation at p<0.05

3.5 Effect of Solvent to Solid Ratio on Extraction

The distribution ratio is an important parameter for the protein extraction. It is the ratio of the protein concentration in solvent to solid. Reducing the amount of solvent causes viscosity and gelation problems during extraction. On the other side, large amounts of usage of solvent cause pollution problems. Also it is not economical to use large amounts of solvent during extraction. In the light of these explanations, the extractions were performed at a solvent to solid ratio of 2.5, 5.0, 7.5, 10.0, and 12.5.

While performing the experiments, some parameters were held constant to observe the effect of solvent to solid ratio on extraction. The extractions were performed with the pea flour of 106 and 212 μm at pH 9.0, with no salt, for one step of 30 minutes within a temperature of 50 °C.

In Figure 3.9, it can be seen that as the amount of solvent increased the protein yield also increased. This happened up to a certain point. In the experiments up to a solvent to solid ratio of 10.0 there was a significant increase in the protein yield. However at a solvent to solid ratio of 12.5, there was a negligible amount (% 1) of increase in the protein yield.

Mizubuti et al. [34] found that the 5.0 were the optimum solvent to solid ratio. Also Lopez-Leiva [34] found that ratio as 8.0. In another study, Bello and Okezie [30] found optimum condition for solvent to solid ratio as 20.0. When combining the results of the experiments and the literature surveys, the maximum yield for the extraction of pea protein was found to be at a solvent to solid ratio of 10.0. However, when the results of the experiments were statistically explained, above the solvent to solid ratio of 5.0 the yield for the extraction of pea protein was not significantly affected ($p<0.05$). From the economical point of view, extraction at a solvent to solid ratio of 5.0 is acceptable.

The results are in agreement with those of Mizubuti et al. and Lopez-Leiva. It is totally different from the Okezie et al. The different result of Okenzi could be due to the initial moisture content of the pea meal used during the study. Some part of the liquid would be encapsulated by pea meal as used for tempering. Increasing the solvent to solid ratio more than 10.0 caused a negligible amount of increase in the protein yield.

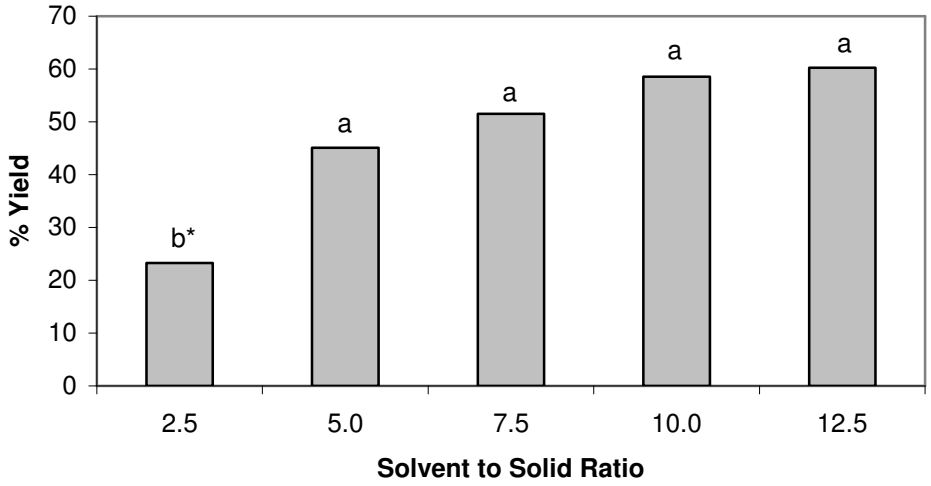

Figure 3.9. Dependence of protein yield on solvent to solid ratio (T=30°C, t=30 min., pH=9.0 and 0.0 M salt)
*Different letters indicate means separation at $p<0.05$

3.6 Effect of Salt on Extraction

Akintayo et al [35] stated that NaCl has effects on the functional properties of the proteins. They found that the gelation capacity of the flour improved in the presence of moderate NaCl concentration. Also foams were observed to be more stable as the concentration of NaCl increased. From this point of view, addition of salt could be helpful for the protein quality. On the other hand, Mizubuti et al [34] found that addition of salt decreased the protein yield. They stated that the optimum condition for protein extraction was reached with no NaCl. In the light of these explanations, the extractions were performed with NaCl concentrations of 0, 0.1, 0.2, 0.3 and 0.4 M.

While performing the experiments, some parameters were held constant to observe the effect of salt concentration on extraction. The extractions were performed with the pea flour of 106 and 212 μm with a solvent to solid ratio of 10.0 at pH 9.0, for one step of 30 minutes within a temperature of 50 °C.

In Figure 3.10, it can be seen that as the salt concentration increased, the protein yield decreased. Extension of high salt concentration resulted in the rapid decrease in protein yield. This decrease would be due to the fact that, presence of salt causes linkage with the protein and so decreases the solubility of the protein in the solvent. From the study of Mizubuti and the results of the experiments, it could be concluded that the maximum protein extraction was obtained when the saline concentration was close to 0.0 M.

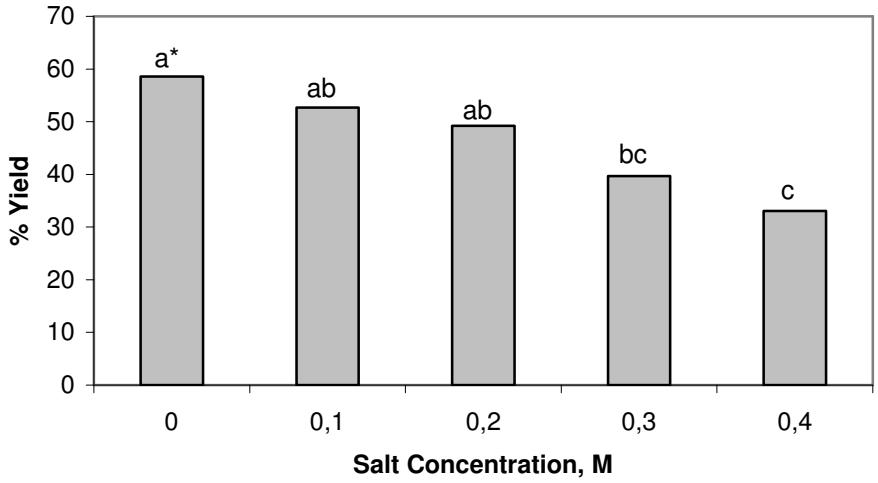

Figure 3.10. Dependence of protein yield on salt concentration (T=50°C, t=30 min., solvent to solid ratio of 10.0 and pH=9.0)
*Different letters indicate means separation at $p<0.05$

3.7 Material Balance For The Pea Protein Isolation

A flow diagram of pilot scale production for the pea protein isolation was prepared based on the experimental procedure and shown in Figure 3.11.

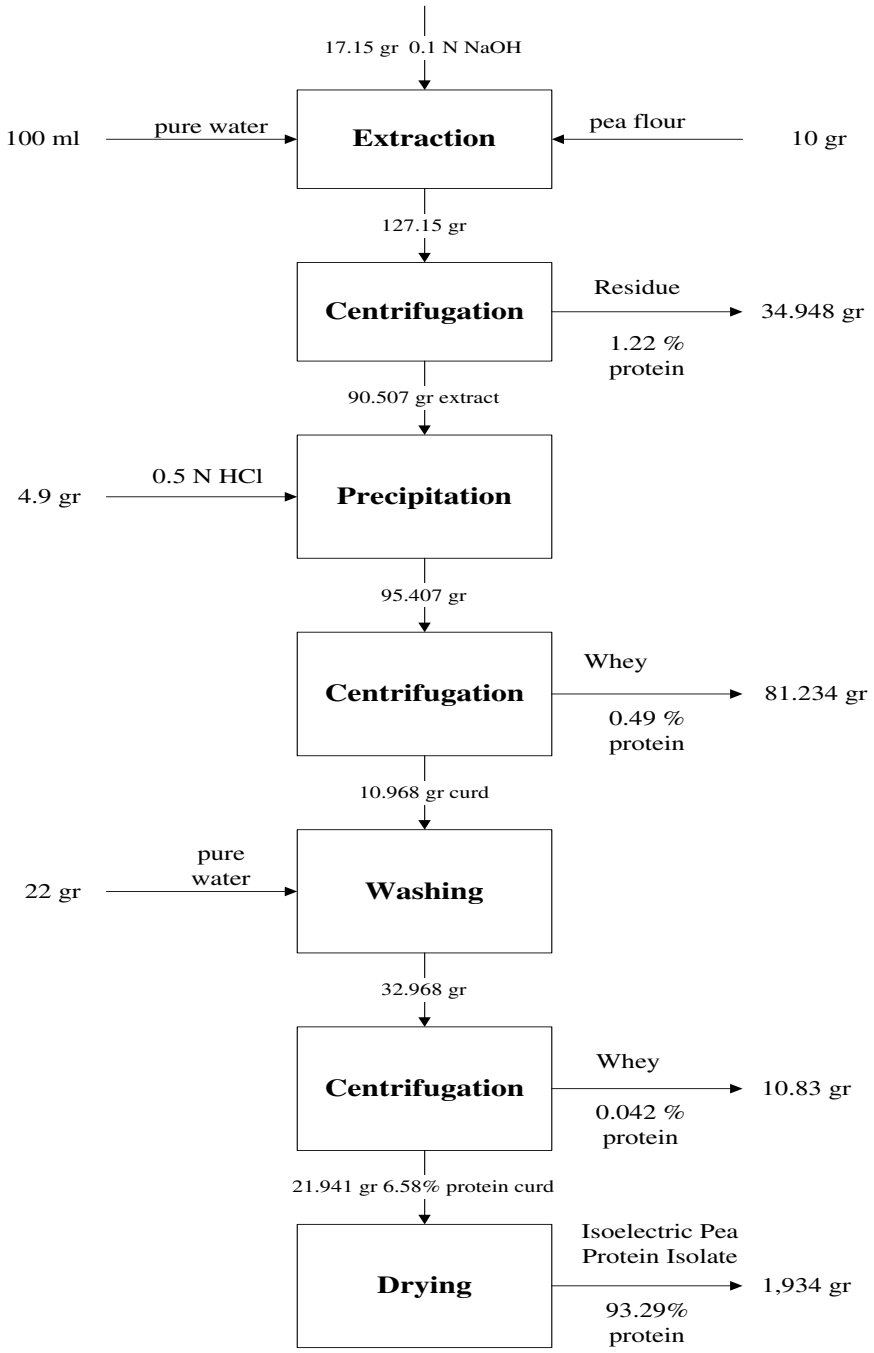

Figure 3.11. Material balance for the pea protein isolation

While performing the material balance for the pea protein isolation, it was seen that 17.19 % of the total protein was left in the residue after extraction step. During isolation process, the yield for the extraction step was 82.81 %.

After the precipitation step, 9.49 % of the protein was left in the whey. For the purification, the protein isolate was washed with pure water and then centrifuged. Only 0.18 % of the protein was left in the waste washing water.

At the end of the washing step, 72.75 % of the protein was recovered as the protein isolate. The simple mass balance equation was used for the whole process to control the results. From the mass balance equation, the amount of protein that had to be recovered at the end of the washing step was calculated as 73.14 %. However, the result obtained was 72.75 %. The difference of 0.39 % less protein could be due to the experimental errors.

As a result, the yield for the pea protein isolation was 72.75 % and the chemical composition of the isolate was 93.29 % protein, 1.2 % fat, 1.73 % ash and 3.53 % moisture. According to literature, Tömösközi et al. [15] found the yield for the pea protein isolation as ranging from 70 to 80%. Mizubuti et al. [34] found the yield for the pea protein isolation as about 75%. Sumner et al. [19] found the yield as 79.8% and the isolate contains 90.0% protein. The differences in the could be due to the differences in the experimental procedure.

CHAPTER 4

CONCLUSIONS

The production of pea protein isolate was aimed in this study. For the production of pea protein isolate, aqueous extraction process was applied. The unit operations performed during the process were grinding, extraction, centrifugation, mixing and drying. The effects of process variables and grinding treatments were also studied.

For the grinding treatments, mass fraction of flour was highest at 212 μm particle size. Moisture content of pea seeds was found not to affect the mass fraction ($p<0.05$). Highest protein content was observed in 53 μm pea flour. As the particle size increased, the protein content decreased. Moisture content of pea seeds was also found not to affect the protein content ($p<0.05$).

The process variables for the extraction were pH, particle size, temperature, solid to solvent ratio and salt. The effects of these variables on the extraction yield were also studied.

Maximum yield was obtained at pH 12.0. It was seen that when the pH of extraction increased, the protein recovered also increased.

Particle size had an important effect on the protein extraction. Maximum protein recovery was observed at 53 μm particle size. As the particle size increased,

the yield of extraction decreased. On the other hand, the mass fraction of the flour was highest at 212 and 425 μm particle size. Only the small amount of pea was used for the extraction. The remaining part that was 212 and 425 μm could be sold as pea flour.

Maximum yield was found at 50 °C. Above 50 °C, decrease in protein yield was observed. However the yield of extraction was not significantly different at 40 and 50 °C ($p<0.05$)

Optimum protein recovery was observed at a solid to solvent ratio of 1:10. However above the solid to solvent ratio of 1:5, the yield of extraction was not significantly affected ($p<0.05$).

Salt had a negative effect on the protein recovery. Increasing the salt concentration caused a decrease in the protein recovery. Maximum yield of extraction was observed when the saline concentration was close to 0.0 M.

With the conditions mentioned above, the maximum yield for the pea protein isolation process was 72.75 % with the product containing 93.29 % protein on a dry basis.

CHAPTER 5

RECOMMENDATIONS

Further research is needed to evaluate the functional properties of the pea protein isolate. As the functional properties, especially emulsifying activity, stability, foaming properties, water, and oil binding capacities could be taken into consideration for the production of high quality pea protein isolate.

Further research is also needed to evaluate the nutritive values and the amino acid composition of the pea protein isolate. Protein digestibility and completeness could be one of the nutritional criteria to be considered.

The PER (Protein Efficiency Ratio) in humans of the pea protein isolate should be considered and evaluated. This is important for the evaluation of the protein nutritive value.

The presence of impurities such as antitrypsin, oligosaccharides, phytate, or lipoxydases could be evaluated. If present, ultra filtration following precipitation could be applied to remove oligosaccharides. Also exogenous phytase enzyme could be added to the pea protein solution for the degradation of phytate. These impurities influence the quality of the pea proteins negatively.

For a more stable pea protein isolate, the fat content could be reduced below 0.2 %. Above this limit, off-flavor develops during the shelf life of the isolate due to the oxidation of fat.

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APPENDIX A

TABLES OF RESULTS OF THE EXPERIMENTS

Table A.1 Dependence of Mass Fraction and Protein Content on Particle Size and Moisture Content

% Moisture Content	Particle Size (μm)	Mass Fraction	% Protein Content
11.45	53	0.076	26.07
11.45	106	0.130	24.81
11.45	212	0.324	23.19
11.45	425	0.294	20.35
11.45	850	0.016	15.38
12.0	53	0.062	28.87
12.0	106	0.121	25.13
12.0	212	0.294	23.03
12.0	425	0.281	20.57
12.0	850	0.016	15.89
13.0	53	0.076	29.79
13.0	106	0.124	25.18
13.0	212	0.294	22.57
13.0	425	0.284	21.12
13.0	850	0.010	16.01

Table A.1 Dependence of Mass Fraction and Protein Content on Particle Size and Moisture Content Continued

% Moisture Content	Particle Size (μm)	Mass Fraction	% Protein Content
14.0	53	0.117	28.86
14.0	106	0.165	27.10
14.0	212	0.254	23.30
14.0	425	0.239	22.28
14.0	850	0.012	17.26
15.0	53	0.112	26.73
15.0	106	0.144	26.44
15.0	212	0.276	23.06
15.0	425	0.229	21.73
15.0	850	0.010	16.53

Table A.2 Dependence of Protein Yield on Extraction pH

Particle Size (μm)	pH	% Yield
53	6.0	24.17
53	7.5	46.03
53	9.0	47.76
53	10.5	58.88
53	12.0	71.73

Table A.2 Dependence of Protein Yield on Extraction pH Continued

Particle Size (μm)	pH	% Yield
212	6.0	8.84
212	7.5	24.80
212	9.0	39.46
212	10.5	42.26
212	12.0	61.23
425	6.0	6.93
425	7.5	21.13
425	9.0	21.62
425	10.5	26.49
425	12.0	42.57

Table A.3 Dependence of Protein Yield on Particle Size

pH	Particle Size (μm)	% Yield
7.5	53	46.03
7.5	106	37.89
7.5	212	24.80
7.5	425	21.13
7.5	850	15.61
9.0	53	47.76
9.0	106	43.13
9.0	212	39.46
9.0	425	21.62
9.0	850	19.83

Table A.4 Dependence of Protein Yield on Temperature

pH	Particle Size (μm)	Temperature (°C)	% Yield
7.5	106	30	37.89
7.5	106	40	41.72
7.5	106	50	46.96
7.5	106	60	42.72
7.5	106	70	31.84
7.5	212	30	24.80
7.5	212	40	35.79
7.5	212	50	36.00
7.5	212	60	31.80
7.5	212	70	24.99
9.0	106	30	43.13
9.0	106	40	47.16
9.0	106	50	48.57
9.0	106	60	41.32
9.0	106	70	32.56
9.0	212	30	39.46
9.0	212	40	40.32
9.0	212	50	41.61
9.0	212	60	37.95
9.0	212	70	30.62

Table A.5 Dependence of Protein Yield on Solvent to Solid Ratio

Particle Size (μm)	Solvent to Solid Ratio	% Yield
106	2.5	24.99
106	5.0	48.57
106	7.5	56.43
106	10.0	63.89
106	12.5	64.89
212	2.5	21.56
212	5.0	41.61
212	7.5	46.57
212	10.0	53.26
212	12.5	55.62

Table A.6 Dependence of Protein Yield on Salt Concentration

Particle Size (μm)	Salt Concentration (M)	% Yield
106	0	63.89
106	0.1	57.03
106	0.2	54.01
106	0.3	43.53
106	0.4	34.46
212	0	53.26
212	0.1	48.3
212	0.2	44.42
212	0.3	35.79
212	0.4	31.69

APPENDIX B

ANOVA and DUNCAN TABLES

Table B.1 ANOVA Table for pH treatments

ANOVA

Yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3277.582	4	819.396	4.411	.026
Within Groups	1857.767	10	185.777		
Total	5135.350	14			

Post Hoc Tests

Homogeneous Subsets

Table B.2 Duncan's Multiple Range Table for pH

Yield

Duncan[a]

		Subset for alpha = .05		
pH	N	1	2	3
6.0	3	13.3133		
7.5	3	30.6533	30.6533	
9.0	3	36.2800	36.2800	36.2800
10.5	3		42.5433	42.5433
12.0	3			58.5100
Sig.		.077	.332	.085

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table B.3 ANOVA Table for particle size treatments

ANOVA

Yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1221.463	4	305.366	11.593	.010
Within Groups	131.707	5	26.341		
Total	1353.171	9			

Post Hoc Tests

Homogeneous Subsets

Table B.4 Duncan's Multiple Range Table for particle size

Yield

Duncan[a]

		Subset for alpha = .05			
Particle Size	N	1	2	3	4
850	2	17.7200			
425	2	21.3750	21.3750		
212	2		32.1300	32.1300	
106	2			40.5100	40.5100
53	2				46.8950
Sig.		.508	.090	.163	.269

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table B.5 ANOVA Table for temperature treatments

ANOVA

Yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	421.451	4	105.363	3.429	.035
Within Groups	460.916	15	30.728		
Total	882.367	19			

Post Hoc Tests

Homogeneous Subsets

Table B.6 Duncan's Multiple Range Table for temperature

Yield

Duncan[a]

Temperature	N	Subset for alpha = .05	
		1	2
70	4	30.0025	
30	4	36.3200	36.3200
60	4	38.4475	38.4475
40	4		41.2475
50	4		43.2850
Sig.		.058	.121

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Table B.7 ANOVA Table for solid to solvent ratio treatments

ANOVA

Yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1787.438	4	446.859	12.540	.008
Within Groups	178.178	5	35.636		
Total	1965.615	9			

Post Hoc Tests

Homogeneous Subsets

Table B.8 Duncan's Multiple Range Table for solid to solvent ratio

Yield

Duncan[a]

Solid : Solvent	N	Subset for alpha = .05	
		1	2
2.5	2	23.2750	
5.0	2		45.0900
7.5	2		51.5000
10.0	2		58.5750
12.5	2		60.2550
Sig.		1.000	.059

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table B.9 ANOVA Table for salt treatments

ANOVA

Yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	836.210	4	209.053	5.994	.038
Within Groups	174.379	5	34.876		
Total	1010.589	9			

Post Hoc Tests

Homogeneous Subsets

Table B.10 Duncan's Multiple Range Table for salt concentration

Yield

Duncan[a]

Salt Concentration (M)	N	Subset for alpha = .05		
		1	2	3
.4	2	33.0750		
.3	2	39.6600	39.6600	
.2	2		49.2150	49.2150
.1	2		52.6650	52.6650
.0	2			58.5750
Sig.		.316	.086	.184

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table B.11 ANOVA Table for mass fraction at different particle size treatments

ANOVA

Mass Fraction

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.285	5	5.694E-02	106.547	.000
Within Groups	1.283E-02	24	5.344E-04		
Total	.298	29			

Post Hoc Tests

Homogeneous Subsets

Table B.12 Duncan's Multiple Range Table for mass fraction at different particle size

Mass Fraction

Duncan[a]

Particle Size (micron	N	Subset for alpha = .05				
		1	2	3	4	5
850	5	1.28E-02				
53	5		8.86E-02			
106	5			.13680		
0	5				.20800	
425	5					.26540
212	5					.28840
Sig.		1.000	1.000	1.000	1.000	.129

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Table B.13 ANOVA Table for mass fraction at different moisture content treatments

ANOVA

Mass Fraction

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.333E-07	4	8.333E-08	.000	1.000
Within Groups	.298	25	1.190E-02		
Total	.298	29			

Post Hoc Tests

Homogeneous Subsets

Table B.14 Duncan's Multiple Range Table for mass fraction at different moisture content

Mass Fraction

Duncan[a]

Water Content (%)	N	Subset for alpha = .05 1
14.00	6	.16650
12.00	6	.16667
13.00	6	.16667
15.00	6	.16667
11.45	6	.16683
Sig.		.996

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table B.15 ANOVA Table for protein content at different particle size treatments

ANOVA

Protein Content (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	421.653	5	84.331	97.186	.000
Within Groups	20.825	24	.868		
Total	442.478	29			

Post Hoc Tests

Homogeneous Subsets

Table B.16 Duncan's Multiple Range Table for protein content at different particle size

Protein Content (%)

Duncan[a]

Particle Size (micron)	N	Subset for alpha = .05				
		1	2	3	4	5
850	5	16.2140				
425	5		21.2100			
0	5		21.2700			
212	5			23.0300		
106	5				25.7320	
53	5					28.0640
Sig.		1.000	.920	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Table B.17 ANOVA Table for protein content at different moisture content treatments

ANOVA

Protein Content (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.162	4	2.540	.147	.963
Within Groups	432.316	25	17.293		
Total	442.478	29			

Post Hoc Tests

Homogeneous Subsets

Table B.18 Duncan's Multiple Range Table for protein content at different moisture content

Protein Content (%)

Duncan[a]

Water Content (%)	N	Subset for alpha = .05
		1
11.45	6	21.6667
12.00	6	22.4650
15.00	6	22.6100
13.00	6	22.7000
14.00	6	23.4917
Sig.		.504

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

APPENDIX C

FIGURES

Figure C.1 Protein Analysis Equipment (Foss 2300 Kjeltec Analyzer Unit, Foss Tecator)

Figure C.2 Extraction Equipment

Figure C.3 Fat Analysis Equipment (Gerhardt Soxtherm 2000 Automatic)

Figure C.4 Moisture Analysis Equipment

Figure C.5 Ash Analysis Equipment

Figure C.6 pH Measurement Equipment