Development and comparison of processes for the extraction of dietary protein from yellow peas

by

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ABSTRACT

The food industry is constantly on the lookout for healthier and more affordable options in place of animal-derived proteins, soy (which has taken a lead role in the replacement of animal-based proteins) and proteins that contain gluten. Pea proteins offer equal, if not superior properties to soy and thus show great promise in being used as a replacement. This is because they are non-allergenic, not genetically modified, highly nutritious and gluten-free. Yellow pea is a legume that has a high protein quantity (21 % -33%) contains a good amount of essential amino acids and is marked by its low fat content (1.5% - 2.0%), although more attention has been given to using it for animal feed rather than for human consumption. It has however been discovered that processing of the yellow pea into protein isolates improves its nutritional, functional and economic values. The yellow pea protein used in diets in South Africa is mostly imported from France and Canada, and therefore there is a huge opportunity to further explore the value of South African –grown yellow peas.

This study aimed at process optimization to maximize the concentration and yield of protein isolates from yellow peas followed by techno-economic analysis to assess the economic viability of extracting dietary protein from this crop, using two extraction strategies. Screening was conducted to obtain the most suitable cultivar for follow-up optimization using a pH of 8 using, a solid to liquid ratio of 1/5, a temperature of 35 °C, for 120 minutes. Two aqueous protein extraction methods, namely water extraction and alkaline extraction, were explored where three different cultivars, namely Slovan, Salamanca and Astranoute were screened. The screening was followed by bench-scale optimization of the preferred cultivar, selected based on protein content and extraction yield. Slovan recorded the highest protein content of 51.1% and 63.3% for water and alkaline extractions respectively , whereas values of 47.2% and 58.4% were obtained for protein yield for water and alkaline extractions respectively. Slovan, proving to be the best among the three cultivars was chosen and used in the subsequent bench-scale optimization stage of the project.

Optimization was carried out with the aid of response surface methodology (RSM) where a quadratic mathematical model was developed to determine the effects of temperature, time, pH and solid loading on protein content and protein yield of extracted isolates for both extraction methods. The highest protein content and protein yield were 88.4% and 73.4% respectively and were obtained for alkaline extractions while the highest values for water extractions were protein content of 83.3% and a protein yield of 56.2%. Desirability profiling conducted on experimental values revealed optimal values of 40℃, 60 minutes and 6.7% for temperature, time and solid loadings for water extractions. At these optimum values, the predicted values of protein content and protein yield were 83.2% and 58.2% respectively. Optimum values for pH, temperature, time and solid loadings for alkaline extractions were 10, 20 ℃, 100 minutes and 5.2% respectively with a resulting protein content and a

protein yield of 88.1%and 75.7% respectively. The protein isolates that performed best for both methods were then assessed for protein solubility, water absorption capacity and fat absorption capacity as well as amino acid profiling. Alkaline- extracted isolates had higher values for these properties as well.

A process simulation and economic model were developed using Aspen plus V8.8 software by using the experimental data from this work as input. The Aspen model generated mass and energy balances that were used to specify equipment for costing. The costing then helped evaluate the economic viability of extraction dietary protein from yellow peas. A response surface methodology (RSM) was used to determine the effects of operating parameters (pH, temperature, time and solids) on IRR. Data from this evaluation showed that alkaline extractions and lower solid loadings recording IRR values of 1.2% to 41.2% were more profitable as compared to water extractions and higher solids (4.2% to 34.7%). The most profitable method (scenario) was alkaline extraction with a solid loading of 6.7%, recording an IRR of 41.5% and an NPV of R852 255 939.

ABSTRAK

Die voedselindustrie is alewig op die uitkyk vir gesonder en meer bekostigbare opsies in plaas van dierproteïene, soja (wat ’n hoofrol geneem het in die vervanging van diergebaseerde proteïene) en proteïene wat gluten bevat. Ertjieproteïene bied gelyke, indien nie superieure, eienskappe teenoor soja en lyk dus belowend om as plaasvervanger te dien. Dit is omdat dit nie-allergies is, nie geneties aangepas is nie, hoogs voedsaam en glutenvry is. Geelertjie is ’n peulgewas wat ’n hoë proteïenkwantiteit (21% - 33%) het, bevat ’n goeie hoeveelheid essensiële aminosure en word gekenmerk deur sy lae vetinhoud (1.5% - 2.0%). Daar word egter meer aandag gegee aan die gebruik daarvan in dierevoer, eerder as vir menslike gebruik. Die geelertjieproteïen wat in Suid-Afrikaanse diëte gebruik word, word meestal uit Frankryk en Kanada ingevoer. Daarom is daar ’n groot geleentheid om die waarde van geelertjies gegroei in Suid-Afrika, verder te ondersoek.

Hierdie studie was gerig op prosesoptimalisering om die konsentrasie en opbrengs van proteïenisolate van geelertjies te maksimeer, gevolg deur tegnoëkonomiese analises om die ekonomiese lewensvatbaarheid te assesseer as die voedselproteïen uit hierdie gewas geëkstraheer word. Twee wateragtige proteïen-ekstraksie metodes, water-ekstraksie en alkaliese ekstraksie, is ondersoek waar drie verskillende kultivars, genaamd Slovan, Salamanca en Astranoute, gekeur is. Die keuring is gevolg deur proefskaal optimalisering van die gekose kultivar, gekies gebaseer op proteïeninhoud en ekstraksie-opbrengs. Slovan het die hoogste proteïeninhoud aangeteken – 51.1% en 63.3% vir water- en alkaliese ekstraksies onderskeidelik, terwyl waardes van 47.2% en 58.4% verkry is vir proteïenopbrengs vir water- en alkaliese ekstraksies onderskeidelik. Slovan, wat bewys is as die beste van die drie kultivars, is gekies en gebruik in die daaropvolgende proefskaal optimalisering-fase van die projek.

Optimalisering is uitgevoer met behulp van respons oppervlak metodologie (ROM) waar ’n kwadratiese wiskundige model ontwikkel is om die effek van temperatuur, tyd, pH, en soliede lading op proteïeninhoud en proteïenopbrengs van geëkstraheerde isolate vir beide ekstraksie metodes, te bepaal. Die hoogste proteïeninhoud en proteïenopbrengs was 88.4% en 73.4% onderskeidelik, en is verkry vir alkaliese ekstraksie, terwyl die hoogste waardes vir water-ekstraksie 88.3% vir proteïeninhoud en 56.2% vir proteïenopbrengs was. Wenslikheid profilering uitgevoer op eksperimentele waardes, het optimale waardes van 40 °C, 60 minute en 6.7% vir temperatuur, tyd en soliede lading vir water-ekstraksie bekendgemaak. By hierdie optimale waardes, was die voorspelde waardes van proteïeninhoud en proteïenopbrengs 83.2% en 58.2% onderskeidelik. Optimale waardes vir pH, temperatuur, tyd en soliede vrag vir alkaliese ekstraksies was 10, 20 °C, 100 minute en 5.2% onderskeidelik met ’n resulterende proteïeninhoud en proteïenopbrengs van 88.1% en 75.7% onderskeidelik. Die proteïenisolate wat die beste presteer het met beide metodes is toe geassesseer

vir proteïenoplosbaarheid, waterabsorpsiekapasiteit en vetabsorpsiekapasiteit, sowel as aminosuur profilering. Alkalies geëkstraheerde isolate het ook hoër waardes vir hierdie eienskappe gehad.

’n Proses simulasie en ekonomiese model is ontwikkel deur eksperimentele data van hierdie werk as inset in Aspen plus V8.8 sagteware te gebruik. Die Aspen-model het massa- en energiebalanse gegenereer wat gebruik is om toerusting vir kosteberekening te spesifiseer. Die kosteberekening het toe gehelp om die ekonomiese lewensvatbaarheid van voedselproteïene uit geelertjies te evalueer. ’n Response oppervlak metodologie (ROM) is gebruik om die effek van bedryfsparameters (pH, temperatuur, tyd en soliede lading) op interne opbrengskoers te bepaal. Data vanuit hierdie evaluasie het gewys dat alkaliese ekstraksies en laer soliede ladings interne opbrengskoerswaardes van 1.2% tot 41.2% aangeteken het, meer winsgewend in vergelyking met water-ekstraksies en hoër soliede ladings (4.2% tot 34.7%). Die mees winsgewende metode (scenario) was alkaliese ekstraksie met ’n soliede lading van 6.7%, wat ’n interne opbrengskoers van 41.5% en ’n netto huidige waarde van R852 225 593 aangeteken het.

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Nomenclature

Ake	Alkaline extraction
ANF	Anti-nutritive factor
ANOVA	Analysis of variance
BCAAs	Branch-chained amino acids
CAF	Central Analytical Facility
CAPEX	Capital expenditure
CCD	Central composite design
Da	Dalton
FAC	Fat-absorption capacity
FAO	Food and Agricultural Organization
HCl	Hydrochloric acid
IEP	Isoelectric precipitation
kDa	Kilodalton
KOH	Potassium hydroxide
N	Normality
NaOH	Sodium hydroxide
NPV	Net present value
OPEX	Operating Expenditure
PDA	Photodiode array
PPC	Pea protein concentrate
PPI	Pea protein isolate
rpm	Revolutions per minute
SE	Salt extraction
SO	Salting out

UF	Ultrafiltration

1 CHAPTER ONE: INTRODUCTION

1.1 Background

Pisum sativum L., more commonly known as the yellow field pea, is a valuable pulse providing protein to humans and animals. The Food and Agricultural Organization (FAO) defines pulses as legumes purposely cultivated for their seeds and are directly ingested (Dahl, Foster and Tyler, 2012). This group of legumes is comprised of eleven primary pulses, with the inclusion of peas, and exempts oilseed legumes as well as legumes that are ingested when immature (vegetables). With about 171 million metric tons per annum, grain legumes come fifth when grains are ranked globally in terms of yearly production rates (Ratnayake *et al.*, 2001). Legumes serve as a very suitable food source to meet the dietary requirements of animals as well as the estimated 800 to 900 million undernourished people in the world (Food And Agriculture Organization of the United and Nations, 2016; International Food Policy Research Institute, 2016; Food Security Information Network, 2018).

Legumes especially pulses, have gained interest worldwide because they have many diverse functions especially when consumed directly. Their uses include being used for food, manure, silage and fodder (Ofuya and Akhidue, 2005). Some pulses have higher protein quality, as required for human food and animal feed, especially soy beans and yellow peas. The production of soy-protein isolates has however taken the leading role in the health as well as sports and food industries because soy beans were grown and cultivated on a larger scale since they were more readily available worldwide as compared to yellow peas. Soy beans were therefore given better recognition and research development as opposed to yellow peas (Tzitzikas *et al.*, 2006).

Yellow peas are grown worldwide with the United States, Russia, Canada, China and India being the major producers (Mckay, Schatz and Endres, 2016). Yellow peas were previously mainly used as animal feed but with time, the immature vegetable part began to receive attention and was usually canned, frozen or eaten fresh. As a result, most research activities that were conducted were geared toward the improvement of the canning and freezing qualities of the vegetable while little focus was placed on pea extracts and subsequent protein products that could be sourced from the yellow pea, especially in Africa (Adebiyi and Aluko, 2011). Advancement in processing technology over the past years has shown that processing of the field pea improves its nutritional and functional as well as economic value, thereby enhancing its properties for human consumption. Hence the need the process the pea flour into protein extracts .Currently, the primary suppliers of pea protein extracts and food product formulations using these extracts are Burcon Nutriscience in Canada (http://www.burcon.ca) and Roquette Foods in France (https://www.roquette.com).

The yellow pea is a good and inexpensive source of protein, which constitutes about 22%-32% by weight of the pea. It provides fibre, vitamins, minerals, complex carbohydrates and energy (due to its starch constituent), all of which are requirements for human health. The yellow pea is also unique in that it has a low fat content (1.5% – 2.0%) as compared to other legumes such as groundnut, soybean, chickpea and cowpea (Ofuya and Akhidue, 2005; J. Boye *et al.*, 2010; Lam *et al.*, 2016). Pea proteins could be used to replace casein and whey protein in sports nutrition as well as in weight management products. Moreover, pea protein is also being recognized as an up and coming alternative for gluten-free products (Sirtori *et al.*, 2012).

In addition to their nutritional value, pea proteins also contain functional properties that aid in the processing and forming of food products. These properties include water and fat-biding capacities, foaming, colour, gelling and protein solubility (Yu, Ahmedna and Goktepe, 2007; Agboola *et al.*, 2010; Kiosseoglou and Paraskevopoulou, 2011; Barac, M. B. Pesic, *et al.*, 2015).The functional and nutritional properties of pea proteins are only retained in the extracted product if appropriate extraction and processing steps are applied. Differences in these properties could be as a result of the type of processes used during the extraction and processing of the protein isolates, the conditions for carrying out the processes, the pea genotype or cultivar as well as the tests used to determine these properties (A. K. Sumner, Nielsen and Youngs, 1981; Fernández-Quintela *et al.*, 1997; Wang *et al.*, 2010; Adebiyi and Aluko, 2011; Barac, M. B. Pesic, *et al.*, 2015; Ciabotti *et al.*, 2016). Previous research studies on production of pea protein isolates have been carried out using different wet extraction methods with the most common being a combination of alkaline-isoelectric, alkaline-ultrafiltration, salt-isoelectric and salt-ultrafiltration process (Uken and Zoe, 1992; J. Boye *et al.*, 2010; Hoang, 2012; Taherian and Mondor, 2012; Barac, M. Pesic, *et al.*, 2015) while very little information exists on data obtained from water extractions. This current research study focused on optimizing aqueous extractions process to produce protein isolates from South African yellow peas.

1.1 Motivation for study

Finding alternatives for animal proteins is a topic that has been discussed over the years due to various reasons associated with health, religious, environmental or cultural beliefs, higher costs associated with the more typical and conventional sources of protein such as those derived from animals, as well as the limited availability of animal proteins in some regions (Tzitzikas, 2005; Kirse and Karklina, 2014). A massive market opportunity has thus opened up especially because of the rising demand of plant protein as well as protein-rich dietary supplements. People have become more health conscious and as such are seeking alternative sources of protein (Vranken *et al.*, 2014; Joshi and Kumar, 2015).

The recent surge in the use of plant protein for food and feed purposes has led to yellow peas increasingly being evaluated as a nutritional and economical source of dietary proteins for human

application. Effort has been put into research work to produce yellow pea protein as well as using its starch and fibre residues as food ingredients. However, protein products from extracts containing pea protein as an alternative to animal and soy protein are not produced in South Africa. This could be attributed to limited technological expertise in efficiently extracting pea protein while maintaining its functional and nutritional properties.

Different extraction protocols for the production of pea protein isolate from locally produced yellow peas would be compared in this study. An approach using statistical design as a tool is used to maximize protein concentration and protein yield as well as economic optima of produced isolates. A techno-economic analysis will be carried out to evaluate the profitability of the production processes. Technical and economic data may lead to development of novel protein extraction processes from peas specifically designed to process South African yellow peas.

1.2 Research Aims and Objectives

1.2.1 Aim

This current research aims at developing an economically and commercially viable method for the production of locally –produced yellow pea protein by investigating extraction methods.

1.2.2 Objectives

In order to realize these aims, the research is divided into the following objectives:

1. Screening of different yellow pea cultivars by comparing their responses to wet extraction protocols to select the most suitable cultivar for subsequent optimization and extraction of yellow pea protein.
2. Investigating the efficiency of wet extraction methods for the production of protein isolate using locally produced yellow peas.
3. Developing and optimizing a laboratory process for the extraction of pea proteins.
4. Performing quality tests such as amino acid profiling and functional properties on extracted proteins.
5. Assessing the economic viability of the production of pea protein through techno-economic analysis.

1.3 Structure of Thesis

This thesis is structured into six chapters. Chapter one presents the background and motivation for the study as well as the research aims and objectives. Chapter two gives a review of literature on

pulses, yellow pea and the processes involved in the isolation of pea proteins. Parameters and indicators for assessing these pea protein isolates are also discussed and the most suitable methods for their extraction while maintaining these properties are shortlisted. In chapter three all the experimental methods used in this study are described. The results obtained from the experimental work are described and discussed in chapter four. In chapter five, data from the experiments were used to develop an economic model for the production of pea protein isolates and analysis was carried out and discussed. Chapter six is the final chapter and contains main conclusions drawn from both the economic analysis as well as the experimental work. Recommendations were also made in this chapter.

2 CHAPTER TWO: REVIEW OF LITERATURE

2.1 Pulses

The dried, edible parts of legumes are referred to as pulses and belong to the family *Leguminosae* (Faye, 2007; World Health Organization, 2007; Asgar *et al.*, 2010; Department of Agriculture Forestry Fisheries, 2011; Kirse and Karklina, 2014). Typical examples of pulses include lentils, field peas, beans, faba beans, lupin, chick peas and cow peas (Tiessen-dyck, 2014; Maskus, 2016). Pulses can be distinguished from other legumes by their relatively lower fat content (World Health Organization, 2007; Singh, 2016). The *Leguminosae* family consists of a variety of different species that are grown and consumed in many different parts of the world by both humans and animals, with the United States of America, Russia, Canada, China, and France being the major producers (Maskus, 2010). Legumes have a variety of diverse, important purposes with some such as lentils, soybeans, lupin and beans being used mostly in human diets for their various nutritional properties since they are good sources of vitamins, proteins, healthy fats and minerals. Others like faba beans, alfalfa and clover are used as green manure or fodder while groundnuts and soybeans are used in oil extraction (Ofuya and Akhidue, 2005).

The worldwide consumption rate of pulses is about 10 kg/person/year (Maredia, 2012) and has continued to rise over the years. Pulse consumption has particularly risen in countries located within Africa and Asia where there is scarcity in animal-based proteins and where these animal proteins are very expensive (Ofuya and Akhidue, 2005). Strong religious and cultural beliefs concerning the consumption of certain animals has also contributed to the rising demand for more plant-based proteins (Kumar, 2016). The surge in consuming proteins that are not derived from animals has also been attributed to fear of animal-related diseases such as mad cow disease, as well as the increasing demand for healthier protein options that have a lower fat content and lower calories (Asgar *et al.*, 2010; Kirse and Karklina, 2014; Singh, 2016). Apart from these reasons, some countries also perceive the ownership of livestock as a sign of wealth and not necessarily as a food source. In these areas, the livestock may be used for trade rather than for human consumption, whereas plant-based diets, especially legumes and cereals are rather used as food sources (Hall and Schonfeldt, 2012).

2.1.1 Advantage of pulses

Pulses have good nutritional value when incorporated into the human diet and are generally distinguished nutritionally by their high carbohydrate content, low fat content and high protein content (Berrios *et al.*, 2010). Pulses are high in starch and also have several times the amount of protein present in root tubers (less than 2% of protein) and twice of that present in cereals (Singh, 2016). The proteins help in synthesizing enzymes, muscle tissue and hormones. They also help in repairing of body tissue and the starch is a source of energy.

Different species of pulses have different fat contents, averaging at about 1%, while peanuts have 49 % and soybeans have 30 % fat content (Ofuya and Akhidue, 2005). In addition to providing energy, the fat content is also responsible for providing essential fatty acids. The major vitamins present in pulses are folic acid, vitamin E and K, B riboflavin and pyridoxine. Folic acid helps to synthesize RNA, DNA and red blood cells. It also helps in the metabolism of energy and reduces the likelihood of neural tube defects (NTDs) in babies and embryos. (Benefits and Sources, 1941; Bulletin, 2008; Food And Agriculture Organization of the United and Nations, 2016) Vitamin K aids in blood clotting while vitamin E is responsible for the maintenance of stability in cell membranes and vitamin B plays a role in biological processes as a co-enzyme. Pulses have a high fibre content, and this helps in the relief of gastrointestinal conditions like diverticulosis and constipation. It also helps in the reduction of blood cholesterol since it is capable of absorbing cholesterol in the gut.

It is also reported that pulses aid in weight loss and weight management. McCrory *et al*, 2010 reported that studies conducted on the influence of pulses on obesity indicated that they might aid in increasing satiety and weight loss due to their distinct nutritional benefits and some of the phytochemicals they contain such as phytic acid, oligosaccharides and phenolic compounds. A study carried out by *Venn et al*, 2010 also proved that incorporating whole grains and pulses into diets had an inverse effect on weight gain. (McCrory *et al.*, 2010; Venn *et al.*, 2010). An increase in the intake of plant proteins also helps decrease cholesterol levels, thereby decreasing the risk of cardiovascular diseases (Kudlackova, 2005; Chalvon-Demersay *et al.*, 2017; Padhi and Ramdath, 2017).

2.1.2 Disadvantages of pulses

Pulses are associated with undesirable flavours that are usually formed when the lipids undergo enzymatic degradation or when the pulses are treated with very severe heat. Efforts geared towards the reduction of these undesirable flavours indicate that soaking, milling, heating, processing, roasting or solvent extraction helps in their reduction (Tian, 1998; Ma *et al.*, 2011; Tiessen-dyck, 2014).

Seed-producing plants usually must compete with other plants for water, nutrients and light as well as protect themselves against viruses and animals. In order to succeed in their defense and competition with other plants, the plants have had to produce secondary metabolites such as peptides and lectins to serve as a defense mechanism. These metabolites are termed as anti-nutritional factors (ANFs) or compounds, and may be poisonous, unsavory and difficult to digest (Wink, D. Enneking, 2000; Santosh Khokhar and Richard K Owusu Apenten, 2003). The ANFs gather in the hull of the pulse seed and reduce the efficiency of nutrient utilization as well as intake of food of plants or derivatives of plants that serve as animal feed or human foods(Soetan and Oyewole, 2009a; Hall and Schonfeldt,

2012; Gemede and Ratta, 2014). The effect of these anti-nutrients is one of the main reasons why legume proteins were not used often in food products in their raw state and the reason for not maximizing the full potential of raw legumes. The most common ANFs found among pulses include phytic acid, saponins, tannins, lectins, oxalates, amylase inhibitors and protease inhibitors. Certain methods such as adequate cooking, processing, membrane separation soaking renders most of them inactive (Vidalvalverde *et al.*, 1994; Soetan and Oyewole, 2009b).

2.2 Yellow pea

The yellow pea pulse, as compared to other legumes, contain a relatively lower amount of anti-nutrients (Njoka, 2008; Barac, M. Pesic, *et al.*, 2015). Processing of pea flour using wet methods at a relatively low temperature for a prolonged retention time also helps to minimize their effects. Membrane or ultrafiltration is also another method that helps reduce anti-nutrients by allowing them to pass through the membrane while the protein is retained, since they have a larger molecular weight cut- off as compared to the pea proteins (Uken, 1991; Taherian and Mondor, 2012).

2.2.1 Composition of yellow pea

2.2.1.1 Protein properties of the yellow pea

The protein content of a typical yellow pea seed ranges from 18%-32% (Collona, Gallant and Mercier, 1980; Tian, 1998; Tulbek, 2010; Karaca, Low and Nickerson, 2011a; Taherian *et al.*, 2011; Toews and Wang, 2013; Pelgrom, Wang, Boom and M. A. I. Schutyser, 2015; Che and Lam, 2016). The protein content of the peas is affected by both environmental and genetic factors. Environmental factors include nitrogen fertilizer application, potassium and phosphorus levels of the soil, maturation and temperature (Atta, Stephanie and Cousin, 2004; Barac *et al.*, 2010; Che and Lam, 2016; Lam *et al.*, 2016). Pea proteins are categorized into two major groups, albumins and globulins, based on their solubility. Albumins are the water–soluble fraction of the protein, which make up about 34% of the protein and have a functional role in the seed such as the enzymatic and metabolic proteins, protein inhibitors, amylase inhibitors and lectins (Kiosseoglou and Paraskevopoulou, 2011). Examples of the enzymatic and metabolic proteins are proteases and glycosidases, which are responsible for germination and degradation of the proteins, while the lectins have a major role in plant defense. Their molecular masses range from 5 to 80 kDa. Albumins are also linked with the nutritional quality of pea proteins and have a higher sulphur and amino acids content (Boye, Zare and Pletch, 2010). Globulins are referred to as the salt-soluble fraction of the protein and contain a greater proportion of essential amino acids compared to the albumins. Hence, there is the need to choose extraction processes that are capable of extracting all the different classes of proteins. For most legumes, vicilin and legumin are the main globulin components, where these serve as storage proteins (S.Tian, 1998).

A third protein called convicilin is also present but in very minute quantities. The molecular weights of globulins are 60, 180 and 71 kDa, respectively (J. Boye *et al.*, 2010; Barac, M. Pesic, *et al.*, 2015).

2.2.1.2 Starch, ash and fibre

Pea starch consists of amylose and amylopectins. Amyloses are smaller, linear glucans that have few branches, while amylopectins are larger molecules with higher degrees of branching. Amylose makes up most of the starch in the legume seed and is a complex, non-digestible carbohydrate. Digestibility of starch is greatly impacted by the ratio of amylose to amylopectin and processing method (S.Tian, 1998). Pea starch in not used widely in food processing because it has limited functional properties and poor digestibility (Ratnayake *et al.*, 2001). It is mainly used to produce extruded products, noodles and snack foods. Pea starch is also known for its use in the thickening of sauces, soups and other products (Hoover *et al.*, 2010; Lam *et al.*, 2016). The cotyledon and the seed coat (hull) of the pea are responsible for its dietary fibre content. The hull comprises of polysaccharides that are mostly water-insoluble, primary cellulose, while the cotyledon fibre contains polysaccharides such as pectins, hemicellulose and cellulose that have different degrees of solubility (Dahl, Foster and Tyler, 2012).The hull however, contains some level of anti-nutritive factors although yellow peas originally have low levels of these compounds which are also reduced or removed during processing (Roquette, 2008). The ash content of the yellow pea contains major minerals which are sodium, calcium, iron, magnesium, potassium and phosphorus (Maskus, 2008). The proximate composition of typical yellow pea is compared to other protein sources and pulses in Table 1 below.

Table 1: Proximate composition of yellow pea as compared to other protein sources

g/100 g	Yellow pea[1]	Eggs[2]	Chickpea[3]	Soybeans[4]
Protein	18.0 - 32.9	10 - 12	21.9 -26.8	35.4 - 45.2
Total lipid	1.0 -2.4	11	6.25 - 6.45	18.2 - 21.2
Ash	2.3-3.0	0.4 - 3	2.67 - 3.7	3.3 - 6.4
Carbohydrates	60.3 - 71	0.5 - 0.7	55.9- 68.9	30 - 35.1
Moisture	5 - 12	74	10.4 - 11	7.10 - 13

[1]Karaca *et a*l. 2011; Collona *et al*. 1980; Toews & Wang 2013; Tian 1998; Dalgetty & Baik 2003

[2] (Hoang, 2012; Soderberg, 2013)

[3]Alajaji & El-Adawy 2006; Karaca *et al*. 2011; Withana-Gamage *et a*l. 2011; Dalgetty & Baik 2003; Xu *et al*. 2014

[4] Ciabotti *et al*. 2016; Nazareth 2009; Singh *et al*. 2008; Russin *et al*. 2007; Endres 2001; Nishinari *et al*. 2014

2.3 Benefits of Yellow pea protein

Most protein powders such as casein, soy and whey as well as some animal proteins such as eggs when taken for long periods, tend to cause an intolerance or allergy making the consumer feel nauseous, gassy or bloated. However, pea protein isolates are hypoallergenic and do not contain any allergenic ingredients. Incorporating PPI into ones diet decreases the chance of developing allergies that are associated with other protein powders (Ndiaye *et al.*, 2012; Soderberg, 2013; Bomgardner, 2015; Carbonaro, Maselli and Nucara, 2015; Hall, 2016; Bouvier, 2017).

Studies have also shown that the consumption of yellow pea proteins may have health benefits such as reduction of hypertension, cancer, diabetes, gastrointestinal disorders, osteoporosis adrenal disease and cardiovascular disease. This is as a result of the anti-oxidant, anti-bacterial and anti-inflammatory properties of pea proteins (Dahl *et al.*, 2003; J. I. Boye *et al.*, 2010; Niehues *et al.*, 2010; Pownall, Udenigwe and Aluko, 2010; Marinangeli and Jones, 2011; Ndiaye *et al.*, 2012).

Pea proteins are full of branch-chained amino acids (BCAAs). BCAAs keep one's body in a state of muscle-building throughout the day. They also decrease abdominal fat, keep one sated for a longer period of time and also provides energy for exercising. PPIs are said to help in weight loss because of these reasons (Lunde *et al.*, 2011).

Pea proteins could also be used in feed and food products due to their good functional properties especially fat and water binding properties (Kiosseoglou and Paraskevopoulou, 2011; Barac, M. B. Pesic, *et al.*, 2015; Lam *et al.*, 2016).

2.4 Processing of Pea Proteins

The method of extraction and extent of purification that is selected, determine the yield and quality of the protein extract obtained from peas (Karaca, Low and Nickerson, 2011b). Pea protein concentrates (PPC) are obtained from dry processing and contain about 47% w/w protein, whereas pea protein isolates (PPI) are obtained using wet processing methods and contain about 80% w/w protein (Soderberg, 2013). Despite large volumes of processing equipment and higher costs associated with wet processing, it is the preferred method and is more frequently used due to its higher protein yield and quality as compared to the dry method (Crispin, 1995; Kuo, 2000; Kiosseoglou and Paraskevopoulou, 2011). The dry and wet processes are applicable to most pulse proteins.

2.4.1 Dry fractionation

Fine milling followed by air classification is primarily used during dry fractionation of the pea seeds to ensure effective separation of protein (1 to 3 µm, light fine) and starch (20 µm, heavy) fractions. A high degree of precision is required during the fine milling process since the seeds have to be milled to a sufficiently small size to rupture the cotyledons to release the protein. However, excessive milling

damages the starch fraction to the extent that particle sizes become homogenous, resulting in decreased separation efficiency (Pelgrom *et al.*, 2013; Zhang, Yang and Singh, 2014).

A spiral stream of air is used to classify the flour into the light fine protein fraction and heavy coarse starch fraction. The protein particles have the tendency to attach to starch surfaces after the first separation is done. For this reason, the process could be done about two or three times to increase separation efficiency (Hoang, 2012). There is however, the disadvantage of having more starch as well as fat in the subsequent protein fractions, thereby reducing purity and yield. Also, not all the proteins can be milled off the starch granules completely and are thus retained in the coarse fraction of the PPC, reducing the efficiency of the separation process. The factors affecting efficiency of this process include particle size distribution of the flour, moisture content and the aperture size of the screen during classification (Pelgrom, Wang, Boom and M. a. I. Schutyser, 2015).

2.4.2 Wet fractionation

The unit operations involved in the wet fractionation process is carried out in four major stages as depicted in Figure 1 below (Agboola *et al.*, 2010; Boye, Zare and Pletch, 2010; Kiosseoglou and Paraskevopoulou, 2011; Taherian and Mondor, 2012; Toews and Wang, 2013; Pelgrom, Wang, Boom and M. A. I. Schutyser, 2015). The general process commences with the dry milling of pea seeds (Stage 1) and dispersion of the pea flour in a solvent that can dissolve the proteins, while the starch granules and fibrous components are retained in undissolved form; represented by Stage 2 in Figure 1.

A solid-liquid separation unit operation such as the use of a hydrocyclone or centrifuge is then used to separate the insoluble starch granules and fibre from the solubilized protein molecules. In stage 3, the protein solution then undergoes concentration where the proteins are precipitated out of solution using chemical or physical means. The concentrate obtained from stage 3 then undergoes drying to obtain the protein in a solid or powder form. The main disadvantages of these wet process lie in their demand for rather large amounts of water for the extractions and the discharge of more effluents than the dry method. The most commonly employed pea protein production technique consists of a combination of alkaline extraction and isoelectric precipitation, alkaline extraction and ultrafiltration, salting in-salting out, salt extraction and ultrafiltration.

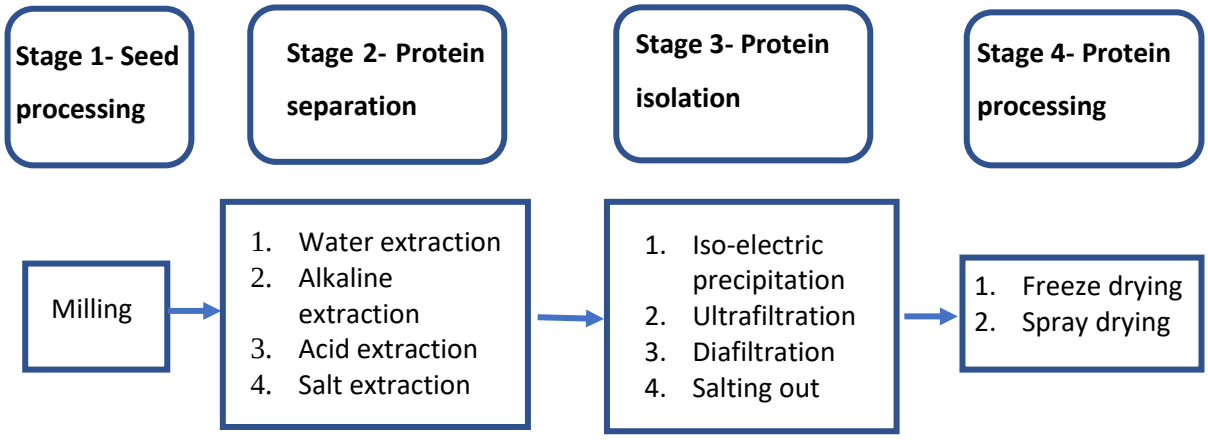


Figure 1; Major unit processes in the wet processing of yellow pea

The different stages involved in the wet extraction process are further explained below.

2.4.2.1 Milling

The aim of the seed processing stage is to increase the ratio of the surface area to volume of the pea particles in order to enhance the dissolution of protein in the extraction solvent by mechanically exposing as much proteinaceous material as possible. There are a variety of milling methods that can be used but the most common are hammer milling, roller milling and pin milling (Singh, 2003; Russin, Arcand and Boye, 2007). Previous studies on pea protein production have used particles of different sizes, however the sizes are always less than 1 mm (Kosson, Czuchajowska and Pomeranz, 1994; Kerr *et al.*, 2000; Ames, 2002; Singh *et al.*, 2005; Jarrard, 2006) with the hammer mill mostly being the equipment of choice.

A study by Hoang (2012) showed that using medium particle sizes (0.2 mm) in extraction processes produced protein isolates oh higher purity and higher yield (80% protein). Finer particle sizes lead to an increase in the energy required during the milling process and contribute to the overall production cost of the extraction process. Maskus *et al*. (2016) also conveyed in their investigations that the water absorption capacities (1.31 g/g to 1.34 g/g) as well as the level of starch damage of finely milled yellow pea flour was higher than those reported for coarsely milled flour. It was observed by Kerr *et al*. (2000) that the water absorption properties of finely milled cowpea flour were lower than coarsely milled flour while oil absorption capacities showed no significant differences with respect to particle size.

Protein extraction processes are mostly affected by solvent to flour ratio, quality of the pulse flour, temperature, pH and the strength of the salt in the extraction medium (Hoang, 2012; Che and Lam, 2016; Singhal *et al.*, 2016). Literature reports ranges of 1:5 to 1:20, 8 to 11, up to 60 °C and 60 to 180

minutes for flour to water ratio, extraction pH, temperature and time (Crispin, 1995; Tian, 1998; Roy, Boye and Simpson, 2010; Hoang, 2012; Klupšaitė and Juodeikienė, 2015; Lam *et al.*, 2016). The common methods for protein extraction are discussed further below.

2.4.2.2 Alkaline extraction/ Acid -isoelectric precipitation

Pea proteins have a high solubility in alkaline or acidic solutions. Isoelectric precipitation is a technique that is used to induce protein precipitation by addition of either a mineral acid or a base to the supernatant obtained from either the acid or the alkaline solubilization of proteins. Isoelectric precipitation takes advantage of the fact that pea proteins have low solubility at pH values between 4 and 5 (Kiosseoglou and Paraskevopoulou, 2011; Taherian and Mondor, 2012). Another centrifugation step is used to further precipitate the proteins (Taherian *et al.* 2011). The precipitation of soy and pea protein isolates at different pH levels have been studied by Cogan *et al*. (1967), Hoang (2012) and Crispin (1995) and it was discovered that pH values of 4.2 and 4.3 precipitated the most proteins. This indicates that the isoelectric point of soy and pea proteins are around 4.2 and 4.3. No significant differences were found when the effect of the type of acid or base on the precipitation of proteins was investigated using hydrochloric acid, phosphoric acid and sulphuric acid (Cogan *et al.*, 1967; Crispin, 1995). Hydrochloric acid is however more commonly used industrially because it is relatively low in cost as compared to other acids.

Studies were carried out by Kaur & Singh (2007), Ghribi *et al*. (2015) and Papalamprou *et al*. (2009) where chickpea flour was mixed with distilled water and pH was brought to 9 with 0.1 M NaOH. Proteins were precipitated out of solution at a pH level of 4.5 with 1 N HCl. Values of 90% to 94%, 91% to 92.7% and 92.5% were reported for protein content by these groups of authors respectively. Other authors have reported values of 91%, and 91.6% for the same combined extraction process of alkaline extraction-isoelectric precipitation (Tian, 1998; Kaur and Singh, 2007; Shevkani, Kaur, *et al.*, 2015). The strong bases and acids used in this combined process leads to the accumulation of salts and an increase in the ash content of the final pea isolate (Karaca, Low and Nickerson, 2011a). Also, this process produces PPIs with poorer solubility as compared to the ultrafiltration process because of the denaturing that may occur due to harsh impacts that the acids or bases used in the isoelectric process may have on the proteins (Taherian and Mondor, 2012).

Uken (1991) used 2 N HCL in the acid extraction of yellow pea flour to solubilize proteins. Protein isolates of 71.2% protein content were obtained. Reinkensmeier *et al*. (2015) precipitated yellow pea proteins out of solution after acid extraction of pea flour at a pH of 1.5 followed by acid precipitation and produced an isolate with a protein content of 81.2%. Proteins were extracted from white kidney beans after acid extraction with 0.4 N citric acid at a pH level of 4 with a follow up refrigeration process at 4 °C for 18 hours. A protein content of 95.7% was achieved in the isolate produced (Alii *et al.*, 1994).

These results were similar to protein levels of 91.9% and 91.2% obtained by Vose (1980), whom also studied the acid extraction of yellow pea protein and faba bean protein. Acid extractions are not used as often as compared to the alkaline method because the acids (especially HCl) corrode the process equipment. Proteins derived from acid extractions also tend to exhibit lower functional properties as compared to alkaline-extracted isolates (Kiosseoglou and Paraskevopoulou, 2011).

2.4.2.3 Alkaline Ultrafiltration(UF) or Diafiltration(DF)

The ultrafiltration method is more novel as compared to the isoelectric precipitation process (Klupšaitė and Juodeikienė, 2015). The supernatant obtained from the alkaline extraction undergoes ultrafiltration/diafiltration or ultrafiltration in concentrating the proteins. Ultrafiltration employs the use of membranes with carefully selected molecular weight cut-offs to concentrate proteins from the supernatant solution. The selected membrane should have a smaller pore size than the pea proteins in order to be able to retain the proteins (Taherian *et al.*, 2011). Membrane techniques are operated in mild conditions of pH and temperature and produce isolates with higher yields and better functional properties than the other methods (Kumar, Yea and Cheryan, 2003; Taherian and Mondor, 2012). In the combined concentration technique of ultrafiltration and diafiltration, the retentate obtained after ultrafiltration is diluted with water and then undergoes another ultrafiltration process (Merck Milipore, 2015; Singhal *et al.*, 2016). The combined UF/DF isolation process is however not applicable on a large scale due to the cost implications associated with the volumes of water needed for this process as well as the extra ultrafiltration step required for further concentration.

The protein content in isolates derived from alkaline extraction-IEP and alkaline UF/DF of lentil, yellow pea and chickpea were evaluated and compared by (J. Boye *et al.*, 2010). It was discovered that the UF/IEP method produced isolates with higher protein levels of 88.6%, 83.9% and 76.5% as compared to protein levels of 79.1%, 81.7% and 73.6% obtained for the isoelectric process. Studies carried out by Fuhrmeister & Meuser(2003) also showed that wrinkled pea proteins produced from ultrafiltration had a lower fat content of 2.3% and higher protein levels (70 to 80%) as compared to the proteins produced from the iso-electric process (3.8% and 68% respectively). The use of membranes for protein isolation has the advantage of reducing the quantities of most anti-nutrients in pea protein extracts, such as oligosaccharides, tannins and phytic acid (Soetrisno and Holmes, 1992; Kiosseoglou and Paraskevopoulou, 2011). The phytic acid content of pea isolates saw a 60% reduction in a study carried out by (Taherian and Mondor, 2012).

2.4.2.4 Water extraction

Proteins can be extracted directly with water at a neutral pH because they are soluble in water. The pure water extraction process is not a common technique, and this may be attributed to its inability to solubilize a lot of globulins and hence as much proteins as the other methods. The water extraction

of yellow pea proteins in not reported in literature. Martín-Cabrejas *et al*. (1995) studied the extraction of proteins from beans with subsequent isoelectric precipitation and reported values of 50% for protein content. Values of 60% to 67% were also reported for water-extracted chickpea and faba bean using $CaS0_4$ as a coagulant to isolate proteins out of solution (Cai, Klamczynska and Baik, 2001). In both of the methods employed by these authors, the extraction process was done twice to increase yield. These are the only examples of water extractions performed in literature and extractions were performed with distilled water at room temperature with vigorous agitation.

2.4.2.5 Salt extraction/Micellization

The salt extraction processes use the phenomenon of pea proteins being soluble in salt solutions at certain ionic strengths, depending on the type of salt that is used, with the most common salts being ammonium sulphate and calcium chloride (Singhal *et al.*, 2016). Salt extraction is followed by the appropriate protein concentration and desalting method. The proteins solubilized using salt extractions could be precipitated by dilution of the supernatant with cold water, forcing solubilized proteins to adapt to the lower the ionic strength of the resulting solution and causing the formation of protein aggregates (Arntfield, 2010; Klupšaitė and Juodeikienė, 2015; Lam *et al.*, 2016).

Dialysis is another method that employs semi-permeable membranes to precipitate proteins out of the supernatant, causing micelles (low molecular weight molecules) to form (Uken and Zoe, 1992; Boye, Zare and Pletch, 2010). The salt extraction-dialysis technique was employed for extraction and recovery of yellow pea proteins using potassium chloride with the supernatant being dialysed against distilled water. Protein levels and protein yields of 76.1% and 68.2% were obtained respectively (Stone *et al.*, 2014). A combination of dilution and dialysis was used to produce pea protein isolates containing 81.9%. Cold distilled water was used to precipitate proteins after solubilization in 0.3 M NaCl. Dialysis was then carried out to de-salt the protein solution (Sun and Arntfield, 2011).

Iso-electric precipitation can also be used to precipitate the proteins after salt extraction. Chickpea protein was isolated with 0.5 M sodium chloride solution and a resulting chickpea protein isolate of 87.8% purity was obtained (Paredes-Lopez, Ordorica-Falomir and Olivares-Vazquez, 1991). Similarly, Karaca *et al*. (2011) produced isolates from lentil, yellow pea, chickpea and faba bean and recorded protein levels of 81.9%, 88.8%, 85.4& and 84.1% respectively. Alternatively, Tian (1998) studied the use of UF/DF as a concentration step following salt extraction and obtained a protein content of 81.1% and a protein yield of 40%. The chemicals used for salt extractions method are expensive, rendering it unpopular in pea protein production especially on a large scale. The chemicals used in the salting process lead to the accumulation of salts and hence an increase in the ash content of the final pea isolate (Karaca, Low and Nickerson, 2011a). Protein yields obtained for salt extracted proteins were

found to be about 6% to 16% lower than those obtained from iso-electric precipitation (Uken and Zoe, 1992).

2.4.2.6 Protein processing

Protein processing or drying is the last stage in the production of PPI and involves obtaining the PPI as a dry powder. It uses either freeze drying or spray drying with the latter being the more commonly used technique for industrial scale processing of protein peas. An advantage spray drying has over freeze drying is that the texture of particles it produces is free-flowing and do not need further processing such as grinding (Kalab *et al.*, 1989; Patel, Patel and Suthar, 2009; Sloth, 2010). Spray drying employs very short drying times although the temperatures used are quite high (about 60 °C). The drying temperature is regulated carefully to prevent the protein from denaturing. The disadvantages of freeze drying lie in its high operational costs and the fact that it is not practical when drying larger volumes because it is time-consuming (Tian, 1998; Ratti, 2001).

Tian (1998) discovered no significant differences among the functional properties of isolates produced from spray and freeze drying except that the colour of spray dried isolates was lighter than that of the freeze-dried isolates. Contrary to these results, Sumner *et al*. (1981) investigated the differences in functional properties of isolates produced by different drying methods and found that isolates produced by spray drying had higher foaming and flavour properties. Gong *et al*. (2015) and *Ghribi et al*. (2015) however reported that generally protein isolates produced using the freeze drying method have better functional properties such as higher water and oil holding capacities as well as higher solubility as compared to protein isolates obtained through spray drying. With regards to the colour of the final product, spray drying produces a lighter colour as compared to freeze drying (A. K. Sumner, Nielsen and Youngs, 1981; Tian, 1998). The high temperature used during spray drying deactivates the oxidation of polyphenols which are responsible for darkening of products (Tian, 1998; Ghribi *et al.*, 2015).

2.5 Quality of Pea protein

2.5.1 Amino acid composition

The comparison of the amino acid values of a test protein with the FAO recommended values of essential amino acids is a good indicator of its nutritional quality (Fernández-Quintela *et al.*, 1997; Mune, Minka, René and Lape, 2013). The amino acid profiling of yellow peas is compared to other protein sources and recommended values in Table 2. A protein source that comprises all the essential amino acids in considered to be a complete protein while incomplete proteins contain amino acids that occur in very low quantities and therefore not capable of performing protein synthesis

(Soderberg, 2013). A study by Hoang (2012) and Fernández-Quintela *et al*. (1997) revealed that the amino acid composition of yellow pea seeds increased after processing especially when proteins have been isolated and these pea proteins possessed some of the most complete essential amino acid patterns found in plant protein sources.

The amino acid profiling found in yellow pea protein isolates can be likened to only few pulses such as cowpea, lupin, chickpea and soybeans as well as high-quality proteins derived from animals such as eggs (Endres, 2001; Hoang, 2012). Pea proteins like other pulses are however limiting in the sulphur-containing amino acids such as methionine and tryptophan (Kudlackova, 2005; Pownall, Udenigwe and Aluko, 2010; Vasconcelos *et al.*, 2010; Toews and Wang, 2013), although research by Yin *et al*. (2015) showed that these amino acids are increased with processing when the amino acid composition of raw pea flours were compared to that of pea protein isolates. Tomoskozi *et al*. (2017) investigated and compared the amino acid profiling of isolates and flours obtained from pea, soybeans and lupin. They discovered that although these pulses displayed similar amino acid profiling, the pea isolates contained a higher amount of valine, arginine and methionine but were lower in cysteine and glutamic acid as compared to soybeans and lupin.

Pea proteins could be used to enhance or correct some amino acid deficiency that may occur in some plant proteins. For example, pea proteins could be used as supplement to correct lysine deficiency as they contain quite a high level of lysine as compared to other plant proteins such as corn, lupin and wheat (Sosulski and McCurdy, 1987; Endres, 2001). Tian (1998) studied the alkaline extraction of yellow pea proteins and observed minimal variation in isolates recovered by salting out and iso-electric processes while Uken (1991) also observed similar amino acid profiling when for isolated derived from acid extractions and salt extractions.

Table 2; Amino acid composition of yellow pea protein in comparison to other protein sources

	Yellow pea protein isolate (Hoang, 2012; Babault *et al.*, 2015)	**Whole egg** (Soderberg, 2013; Joshi and Kumar, 2015)	**Soybean isolates** (Hughes *et al.*, 2011)	**Mung bean isolates** (Skylas *et al.*, 2017)	**FAO (adult requirement)**
Essential amino acids (g/100 g protein)					
Histidine	1.90 - 2.33	2.40	2.30	2.16	>1.6
Isoleucine	3.70 - 3.89	5.6	4.51	3.24	>1.3
Leucine	6.40 - 7.84	8.3	7.50	4.08	>1.9
Valine	4.00 - 5.11	7.6	5.94	4.02	>1.3
Lysine	5.70 -6.25	6.3	6.10	4.90	>1.6
Phenylalanine	4.20 - 5.17	5.1	4.86	4.99	>1.9
Threonine	2.80 - 4.46	5.1	3.56	2.14	>0.9
Tryptophan	0.61 - 0.70	1.8	1.40	0.73	>0.5
Methionine	0.80 – 1.60	3.2			
Arginine	6.60 – 7.93	6.1	5.90	5.71	
Non-essential amino acids (g/100 g protein)					
Alanine	3.30 – 4.83	5.4	4.16	2.56	
Aspartic acid	8.90 – 11.16	10.7	11.23	8.45	
Glutamic acid	13.20 – 18.46	12.0	18.50	13.18	
Glycine	3.10 – 4.82	3.0	4.66	2.17	
Proline	3.40 – 4.64	3.8	5.18	3.02	
Serine	3.90 – 5.71	7.9	4.87	3.78	

2.5.2 Functional Properties of Pea Proteins

Apart from being rich in nutrients, pea proteins also contain functional properties that aid in the processing and forming of food products and these are well documented in literature. Contradictory reports have however been reported with regards to which protein isolates have better functionality, and if pea protein could match up to other protein sources in food processing and application. Functional properties are chemical and physical properties that provide information on how a substance (in this case, pea protein) will behave in a given food structure during the stages of processing, storage, preparation and consumption (Uken & Zoe 1992). Examples of functional properties that have been investigated for pulses include solubility, gelling, foaming, emulsification and water-binding capacity.

Functional properties are assessed to determine whether the protein will be able to compete with other protein products on the market and also which field of application will suit it best (Toews and Wang, 2013; Singhal *et al.*, 2016). Many factors influence functional properties and these factors are categorized into intrinsic factors such as the amino acid composition and hydrophilicity of the protein, the molecular structure of the protein molecules , reactivity, conformation and extrinsic factors that include temperature, pH, mechanical processing, method of extraction, enzyme and ionic strength (Barac, M. Pesic, *et al.*, 2015; Che and Lam, 2016).

2.5.2.1 Solubility

Protein solubility is sometimes referred to as protein dispersibility and this indicates how easily a protein isolate disperses or is uniformly distributed in a solvent, especially water (Adebiyi and Aluko, 2011; Lam *et al.*, 2016). Other functional properties such as texture, emulsification, colour, gelation and foaming all depend on solubility (Fuhrmeister and Meuser, 2003; Kiosseoglou and Paraskevopoulou, 2011). Factors that influence the solubility of pea proteins are temperature, pH and the method of isolation used to recover the proteins (Che and Lam, 2016; Singhal *et al.*, 2016). Literature shows that soybeans, lupin, faba beans and chickpeas usually display a similar solubility profiling as shown in Figure 2. This profiling is bell-shaped (See Figure 2) and is marked by the high solubility value of 45 % to 85% being obtained at pH levels of 1 to 3 and 7 to 10 while the lowest solubility values of 2% to 10% were observed at pH levels from 4 to 6 (Sosulski and McCurdy, 1987; Fernández-Quintela *et al.*, 1997; Zhang, Yang and Singh, 2014; Tomoskozi *et al.*, 2017).

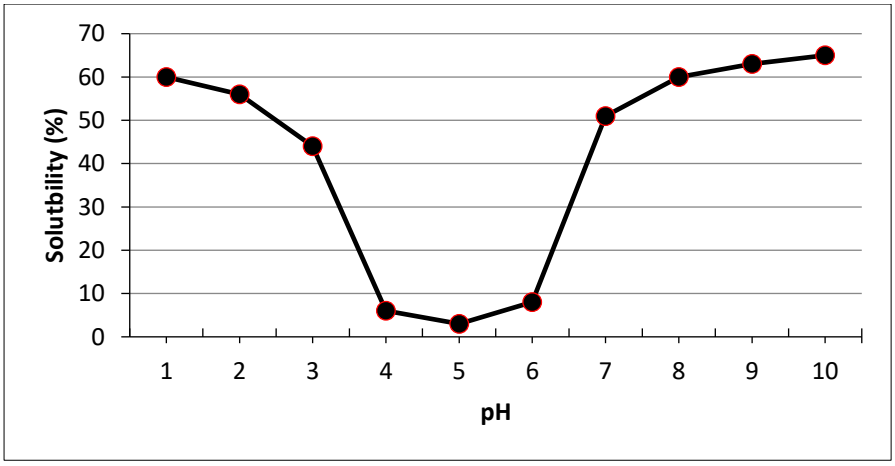


Figure 2; Effect of pH on solubility of most pulses

Studies by Kiosseoglou & Paraskevopoulou (2011), Taherian *et al*. (2011) and Karaca *et al.* (2011) showed that pea isolates produced from ultrafiltration processes exhibited better solubility than red lentil protein and yellow pea protein produced from isoelectric precipitation and salt extractions. Papalamprou *et al.* (2009) and Fuhrmeister & Meuser (2003) also recorded a 15% increase in solubility also obtained for PPI recovered through ultrafiltration as compared to IEP. Pea proteins show a reduction in solubility when they are thermally processed although a similar bell-shaped curvature is obtained over a pH range of 1 to 10 (Barac, M. Pesic, *et al.*, 2015). Generally, the solubility of protein isolates increases with increasing temperature till about 50 ℃ where protein denaturing begins to occur. Protein denaturing incites interaction between hydrophobic groups of the protein molecules, leading to precipitation and a decrease in solubility (Che and Lam, 2016).

2.5.2.2 Water-hydration capacity (WHC) and Fat hydration capacity (FHC)

Water binding capacity (WBC) or water hydration capacity (WHC) is defined as the quantity of water absorbed or retained by the protein. This increases with increasing protein content and is influenced by the amino acid composition of the protein substance. Pea isolates with too low water binding capacities might not have the ability to carry water effectively, whereas substances with too high-water binding capacities tend to make food products dry and brittle, especially during storage. Generally, pea protein products absorb between 1 to 3.3 times their weight of water (Swanson, 1990; Cousin, 1997; Ghribi *et al.*, 2015). Values of 1.5 to 2.7 g/g were obtained by Fuhrmeister & Meuser (2003) during the acid precipitation of isolates using wrinkled pea while other authors reported values of 2.5 g/g, 1.91 g/g, 1.44 g/g and 1.25 g/g for yellow pea proteins (Fernández-Quintela *et al.*, 1997; Tian, 1998; Reinkensmeier *et al.*, 2015; Tomoskozi *et al.*, 2017).

It is reported that the values for water binding capacities were higher for pea protein isolates, compared to soy protein isolates, green lentils, red lentils and chickpeas with heat treatment having a positive effect on this property (Swanson, 1990; J. Boye *et al.*, 2010). There are contradictory reports on the effects of the methods used in the protein recovery or isolation on water absorption properties. Isolates produced from salt extractions were found to produce isolates that exhibited poorer water hydration capacities than those produced through iso-electric precipitation, while protein isolates derived from isoelectric precipitation had higher water hydration capacities than those produced recovered using ultrafiltration. These differences in values were however found to be statistically insignificant (Al-Karaki and Ereifej, 1999; J. Boye *et al.*, 2010; Stone *et al.*, 2015). A contradictory report is however conveyed by Barac *et al*. (2010)and Stone *et al*. (2014), where statistical differences were found among values, indicating that water absorption values were affected by both the type of pea cultivar and method of protein isolation.

The term fat hydration capacity is used interchangeable with oil hydration capacity (OHC), oil absorption capacity (OAC), fat binding capacity (FBC) as well as fat absorption capacity (FAC). It refers to the quantity of oil or fat absorbed or retained by the protein substance. The physical structure of the protein together with the type of fat present in the raw material and extraction method may influence the fat absorption capacity of the pea protein isolate. A study by A K. Sumner *et al*. (1981) reported that the drying method used in the production of the pea isolate affected its fat absorption capacity. Drying methods such as freeze drying and drum drying increase fat absorption capacities. Soetrisno & Holmes 1992 investigated the effect of temperature on the fat absorption capacities of yellow pea protein isolates and discovered that the values of FHC decreased with a reduction in temperature. The FBC of yellow pea proteins (1.2 g/g to 2.7 g/g) were found to be lower than those of chickpeas (3.06 g/g to 5.74 g/g) but higher than those obtained for soy proteins 1.1 g/g (J. Boye *et al.*, 2010; Withana-Gamage *et al.*, 2011; Stone *et al.*, 2014). The isolates produced by ultrafiltration also had higher FHC values (1.32 g/g to 2.2 g/g) than the value of 0.87 g/g produced by isoelectric precipitation (Fernández-Quintela *et al.*, 1997; Fuhrmeister and Meuser, 2003). It was also discovered that the production method of the protein isolate significantly affected FBC values with the highest values reported for salt extraction, followed by alkaline extraction (Soetrisno and Holmes, 1992; Fuhrmeister and Meuser, 2003; Kiosseoglou and Paraskevopoulou, 2011; Stone *et al.*, 2014).

2.6 Techno-economic survey

The viability of a project can only be determined by carrying out a comprehensive economic evaluation of the project in order to determine its profitability. When performing an economic analysis, the important economic parameters to consider include:

1. Net present value (NPV): This is the difference between the value of the annual cash flow, and the investment that was initially required to start up the project. (Timmerhaus, 1991). It gives an indication pf the future value of the project when its life span ends. It is calculated using equation (1) below (Sinnot, 2013) .

$$NPV = \sum_{n=1}^{n=t} \frac{CF_n}{(1+r)^n} \quad (1)$$

Where CFn = the estimated cash flow in year n
t = the project life (years)
i = the interest or discount rate

2. Payback period or time: This is the minimum amount of time needed to recoup the initial capital investment of the project, using the cash flow. It is calculated using equation(2) (Timmerhaus, 1991)

$$\text{Payback time} = \frac{\text{depreciable fixed capital investment}}{\text{Average annual cash flow}} \quad (2)$$

3. Internal rate of return (IRR): This is the rate of discount at which the present value of the total annual cash inflow is equal to the initial investment that was initially required to start up the project, that is the NPV is equal to zero.

The economic viability is a very important factor when comparing and selecting protein production process. This is because although one process may have a technical advantage over another, it may not necessarily also have an economic advantage. These two factors (both technical and economic advantages) must be considered side by side in order to make an informed decision. Economic comparisons should include:

- Comparison of capital cost
- Comparison of operating cost
- By-product disposal
- Comparison of process yields, relative to cost.

The economic evaluation of the production of protein isolates, especially PPIs on a commercial scale is scantily reported in literature. In an economic evaluation by Crispin (1995), a comparison was made

between using IEP and UF to produce soy protein isolates from defatted soy flakes with a production scale of 11000 ton/year of defatted soy flakes to produce 3000 ton/year of soy protein isolate. Experimental data was used as input to produce economic results that served as the basis for the comparison. The economic analysis conducted by Crispin (1995) did not include further profitability evaluation parameters such as NPV, IRR, and payback period as the primary focus of the study was to determine and compare production costs between IEP and UF. It was observed the UF process had a higher plant cost of R 2 881 000 per annum as compared to that of R 2 172 000 obtained for the IEP process due to the cost of replacing membranes. However, the operating and effluent charges were lower(R 5 800 000 and R 420 000 respectively) than those of the IEP process because the IEP effluents contain salts and other unwanted substances that have to be treated before being directly discharged as waste products. (Crispin, 1995)

2.7 Conclusions drawn from literature

The following can be deduced from the literature review conducted:

1. Pea protein isolates generally contain suitable functional properties, protein content and essential amino acids when compared to other pulses. They are also unique in their low fat content and do not cause an intolerance when consumed, thus making them hypoallergenic.
2. Although there exists a range of parameters for effective extraction of pea proteins, no actual optimization has been carried out to obtain information on the significance of individual parameters or the effect of combined parameters on both protein yield and protein concentration for yellow pea protein.
3. There is no universal milling method for the particle size reduction of yellow peas, although hammer milling and roller milling are the most common.
4. Alkaline extraction is clearly the preferred and most common method for pea protein extraction while water extractions are rarely used. More frequently used methods are a combination of alkaline extraction followed by isoelectric precipitation or alkaline extraction followed by a type of membrane filtration, preferably ultrafiltration. The use of membrane filtration as a concentration process is the most effective method due to its ability to filter out unwanted substances such as anti-nutrients and salts that may be present. Membrane filtration also preserves the nutritional and functional properties of the pea isolate.
5. There is a lack of data in literature on a comparative techno-economic analysis for the production of pea protein isolates.
6. Solubility is the most important functional property as it affects other properties such as gelation and foaming. Other functional properties of interest are water hydration capacity and fat hydration capacity. There are however contradictory reports on the factors that affect these functional

properties although the effect of cultivar and method of protein recovery or isolation are the most published factors.

2.8 Research questions

For a successful process development for production pea protein isolate using wet processing methods, there is the need to address certain important questions through research as well as economic analysis. These include

1. How will the yellow peas which are produced locally respond to the selected wet extraction methods as compared to literature and to what extent will process conditions have to be modified and/or optimized?
2. How would factors such as pH, temperature, residence time and solids loading affect the selected wet extraction processes investigated in this study?
3. Will the technology developed in this study offer economically competitive market value to locally produced and locally-extracted pea proteins? Will it be comparable to other pea proteins on the market? What is the minimum scale of industrial production of pea protein to achieve an acceptable return on investment?
4. What are the nutritional and functional values of the isolates produced PPI in terms of functional properties and amino acid profiling?

3 CHAPTER THREE: MATERIALS AND METHODS

To solve the key research questions, there is the need to do extensive research work on pea protein production using wet processing methods as well as laboratory work. This proposed work has been categorized under different research activities.

3.1 Materials and Chemicals

The materials and chemicals used in this study are listed below

Table 3; Materials and chemicals used in the study

Item	Supplier
Potassium hydroxide	Scienceworld
Hydrochloric acid	Scienceworld
Catalyst tablets	Scienceworld
Sulphuric acid	Scienceworld
Sodium hydroxide	Scienceworld
Boric acid	Scienceworld
Ethanol	Scienceworld
Methyl red	Scienceworld
Bromocresol green	Scienceworld
Petroleum ether	Scienceworld

3.2 Methods of Analysis

3.2.1 Protein measurement and Protein yield

Total protein content (one of the key performance indicators) of each sample was determined by using the Kjeldahl method (AOAC, Official methods of analysis 979.09) and apparatus (Velp Scientifica). This method is divided into three stages, namely digestion, distillation and titration. The digestion stage was performed with a DK Series heating digester. Approximately 1 gram of dried, finely milled sample (to be analyzed) was placed in each Kjeldahl digestion tube (ensuring that sample materials were placed at the bottom of the flask), leaving one to be used as the blank. 2 catalyst tablets (VCM, A00000274), each containing 3.5 g of potassium sulphate and 0.1 g of copper (II) sulphate were added to the contents of the digestion tubes (including the blank) as well as 12 ml of concentrated sulphuric acid (98%) and shaken gently. The tubes were set in the appropriate holes of the Digestion Block Heaters and heated at a temperature of 420 ^{o}C for an hour and then allowed to cool for about 15 minutes. The contents of the tube turned blue after complete digestion was achieved and crystallized upon cooling.

Distillation was done with a UDK 129 Distillation unit which was automatically set to add 50 ml of distilled water and 50 ml of 32% sodium hydroxide (to neutralize and alkalinize the sample) solution to the contents of the digestion tube to be distilled. The cooled, digestion tube was placed into position in the steam distillation unit. An Erlenmeyer flask containing 4% boric acid was used to collect the distillate. Distillation was performed for 5 minutes on all the tubes, including the blank.

For preparation of the indicator used for the titrations, 0.2 g of methyl red was diluted to 100 ml in 95% ethanol and 1 g of Bromocresol green was also diluted to 500 ml in 95% ethanol. One part of methyl red was mixed with 5 parts of Bromocresol green and used as the indicator solution for all titrations. About 8 drops of indicator solution was added to the distillate (causing a colour change of blue) and titrated against 0.2 N of Hydrochloric acid. Titration was stopped once a colour change of pink was observed and the titre value was recorded. The nitrogen content of the sample was calculated as follows

$$\mathrm{N} = \frac{1.4007 \times (\text{Volume of HCl} - \text{Volume of blank}) \times 0.2}{\text{Weight of sample}} \quad (3)$$

The protein content of the sample was then obtained by multiplying the nitrogen content by a factor of 6.25.

Protein yield was calculated with the following equation

$$\text{Protein yield } (\%) = \frac{\text{protein content in isolate} - \text{weight of isolate}}{\text{protein content in flour} - \text{weight of flour}} \quad (4)$$

3.2.2 Functional Properties

The functional properties determined in this study include solubility, fat- absorption capacity and water- holding capacity.

3.2.2.1 Solubility

100 mg of pea protein isolates was dispersed in 10 ml of water and the pH was adjusted to different levels using 1 M HCL or I M KOH. The obtained solution was then stirred for 30 minutes and then centrifuged at 4000 x g for 30 more minutes. The amount of protein in the supernatant was determined at each pH level and the solubility determined with the following equation

$$\text{Solubility} = \frac{\text{Protein in supernatant}}{\text{Protein in original sample}} \quad (5)$$

3.2.2.2 Fat-absorption capacity

0.5 g of each sample was placed in a 15-ml centrifuge tube. 3 ml of canola oil was added to the sample and vortexed for 1 minute. The mixture was then centrifuged for 30 minutes at 4000 x g. The supernatant was discarded, and the tube was placed upside-down on a filter paper for the oil to drain. The tube was reweighed after one hour. Fat absorption capacity (FAC) was calculated as follows

$$\text{Fat absorption capacity} = \frac{(\text{Weight of wet sample} - \text{Weight of dry sample})}{\text{Weight of dry sample}} \quad (6)$$

3.2.2.3 Water-holding capacity

5 g of water was added to 0.5 g of pea protein extracts in a 15-ml centrifuge tube. The mixture was vortexed for 1 minute and centrifuged at 2000 x g for 30 minutes. The supernatant was then discarded, and the tube was placed upside-down on a filter paper for the water to drain. The tube was reweighed after one hour.

Water holding capacity (WHC) was then expressed as follows

$$\text{Water holding capacity} = \frac{(\text{Weight of wet sample} - \text{Weight of dry sample})}{\text{Weight of dry sample}} \quad (7)$$

3.2.3 Amino acid analysis

Pea protein isolates obtained were analyzed for essential amino acids at the Central Analytical Facility (CAF), Stellenbosch University. The protocol for the analysis is conducted in three main stages namely hydrolysis, derivatization and chromatographic analysis. For the hydrolysis stage, the sample was placed in a glass vial. 6 N HCL was added to the vial, ensuring that the sample in the vial is totally submerged in the acid. The vial was then flushed with argon or nitrogen gas to eliminate any oxygen it may contain, after which it was closed and vortexed to ensure sufficient mixing of the acid and the sample. The vial was placed in an oven (pre-set to 110 °C) for about 18 hours and taken out to cool afterwards. The hydrolysate obtained after cooling was filtered using centrifuge tube filters (Corning® Costar® Spin-X tubes). The filtrate was transferred into Eppendorf tubes, dried down and reconstituted in borate buffer. All hydrolyzed samples were treated with 6M NaOH to adjust the pH because the acid used during hydrolysis is so strong that the borate buffer does not neutralize the remaining HCl. Dilution of samples were carried out in 2 ml Eppendorf tubes and 10 µl of this was used for the follow up derivatization process. The dilution was done according to

Table 4 below

Table 4 ; Dilution strategy for hydrolysates

% protein	Dilution factor	Comment
< 7%	5x	200 µl sample + 200 µl 6M NaOH + 400 µl H_2O + 200 µl IStd
7 – 20	50 x	5x (200 µl sample + 200 µl NaOH + 600 µl H_2O) + 10x (100 µl sample + 700 µl H_2O + 200 µl IS)
20-60	100 x	5x (200 µl sample + 200 µl NaOH + 600 µl H_2O) + 20x (50 µl sample + 750 µl H_2O + 200 µl IS)
>60	250x	5x (200 µl sample + 200 µl NaOH + 600 µl H_2O) + 50x (20 µl sample + 780 µl H_2O + 200 µl IS)

The next stage which is the derivatization procedure, focuses on adding a functional group onto the amine group of the amino acid to increase the hydrophobicity of the molecule (permitting better chromatographic separation) and to facilitate easier detection of the derivatized analyte using either UV, fluorescence or mass spectrometric detection. The AccQ-Tag derivatization kit for amino acid analysis was used in this work. The AccQ-Tag Ultra amino acid kit from Waters includes the derivatization kit, AccQ-Tag Ultra C_{18} 2.1 x 100mm x 1.7 µm column, as well Eluents A and B to be used on the Waters Acquity UPLC system with photodiode array (PDA) detector. The derivatization kit contains 5 vials of each of the following: AccQ-Tag derivatizing agent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)), dry acetonitrile for preparing the AQC, and sodium borate buffer to be used in the derivatization reaction.

70 µl of borate buffer was poured into a 2ml glass vial. 10 µl diluted sample/standard solution was added, along with 20 µl AQC reagent. The vial was then capped and vortexed to ensure adequate mixing. The vials were transferred to an oven/heating mantle at 55 °C and heated for 10 minutes. After 10 minutes, the vials were deemed ready for analysis and loaded into the autosampler tray.

A Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) fitted with a photodiode array (PDA) detector was used to perform amino acid separation and detection. 1 µl of the standard solution or sample was added to and analyzed by the detector. Data acquisition and instrument control was done with the aid of MassLynx software and calibration curves were plotted for each amino acid depending on their peak responses.

3.3 Experimental Methodology

The experimental aspect of the project is divided into three main stages, namely: Screening process, Bench-scale optimization and Validation stage.

3.3.1 Acquisition of feedstock and sample preparation

Whole yellow field peas for all the different phases of the project were obtained from Agricol (Pty) Ltd (Brackenfell, Cape Town, South Africa). One batch of yellow peas was used for all laboratory experimental work to rule out errors caused by differences in seed characteristics as well as to ensure that a constant seed quality was maintained and used for all pea protein extraction processes. Pea seeds were milled to an average particle size of 150 microns using a hammer. The flour obtained was then quarter-sampled, vacuum sealed and stored at room temperature. Sampling preparation was done to make sure that selection of a representative sample was done and that samples selected varied as little as possible for the batches (Mcintosh, 2013). The moisture content of the flour upon storage was 9%.

3.3.2 Proximate analysis

AOAC official methods were used to determine the proximate composition of the yellow pea flours used in this work as well all extracts obtained after processing.

3.3.2.1 Protein and starch content

Total protein content of each sample was determined by using the Kjeldahl method (AOAC, Official methods of analysis 97.09) and apparatus (Velp Scientifica) to estimate total nitrogen present in the sample as stated above. Starch content was determined according to the Method 76-13.01, using the enzymatic starch assay kit (Megazyme, Co. Wicklow Ireland). In this method, the starch in the samples undergoes completely hydrolyzation to maltodextrins by the thermostable α-amylase enzyme on incubating at about 100 ^{o}C. Amyloglucosidase is then used to hydrolyze the maltodextrins to D-glucose after incubating at 50 ^{o}C. The D- glucose was then oxidized to D-gluconate. One mole of hydrogen peroxide (H_2O_2) was also released during this process. And this is measured quantitatively in a colorimetric reaction that uses peroxidase.

3.3.2.2 Moisture content

A crucible was weighed, and 2 g of the sample was measured and placed in it. The weight of the sample plus the crucible was recorded and this was placed into an oven which was set at 100 ^{o}C and allowed to dry for 24 hours. The sample plus crucible was then removed from the oven after 24 hours, placed in a desiccator for ten minutes to cool and then reweighed. The moisture content of the sample was then determined as follows

$$\text{Moisture content} = \frac{W_1 - W_3}{W_1 - W_0} \times 100 \quad (8)$$

Where W_0 is the weight of the crucible when empty, W_1 is the weight of crucible plus sample and W_3 is the weight of the crucible plus oven-dried sample

3.3.2.3 Ash content

A crucible was weighed, and 2 g of the sample was measured and placed in it. This was then transferred into a furnace which was set at 550 ^{o}C and the sample was left in there for about 4 hours (the sample turns to white ash after 4 hours). The crucible, together with its contents were allowed to cool to about 100 ^{o}C. When this temperature was attained, the crucible and its contents were placed in a desiccator to cool further and then reweighed. The percentage ash was calculated from the formula below:

$$\text{Ash content} = \frac{\text{Weight of ash}}{\text{Original weight of sample}} \times 100 \quad (9)$$

3.3.2.4 Crude fat determination

A 250 ml Soxhlet flask was dried in an oven, cooled in a desiccator and its weight recorded. I g of dried sample was weighed into an extraction thimble and pugged with cotton wool. The thimble was then transferred into an extractor and fitted with a reflux condenser as well as a 250 ml Soxhlet flask. The flask was then filled to ¾ of its volume with petroleum ether and transferred to a heater, together with the extractor and condenser. The heater was then left on for six hours with a constant stream of running water connected to it from a tap to ensure that the vapour from the petroleum ether being heated is properly condensed. The ether was left to siphon about 10 to 12 times until it could siphon no more. The thimble that contained the sample was then removed and dried on a bench top. The Soxhlet flask which now contains the fat was detached from the extractor and condenser and dried in an oven to a constant weight. The percentage of fat in the sample is calculated using the following formula

$$\text{Fat content} = \frac{W_1 - W_0}{\text{Original weight of sample}} \times 100 \quad (10)$$

Where W_0 is the initial weight of the dry Soxhlet flask and W_1 is the final eight of the dried Soxhlet flask plus the fat.

3.3.3 Screening Process

Three different cultivars, namely, Slovan, Salamanca and Astranoute were subjected to wet water and alkaline extractions processing extractions to obtain the most suitable cultivar for the follow-up optimization phase. The proximate composition of the flours of the different pea cultivars was determined in terms of fat, moisture, ash, crude protein and carbohydrate content. A summary of the order in which the screening stage was carried out is displayed in Figure 3 below.

Laboratory-scale experiments were carried out in 5 litre beakers to investigate water and alkaline wet extraction methods using the same processing conditions for all three cultivars. For water extractions, pea flour was mixed with water while alkaline extractions were done by adjusting the pH to a value of 8 using 1 M KOH. A solid to liquid ratio of 1/5 (600 g of pea flour dispersed in 3 litres of extraction solution) was used for the both types of extraction methods. The slurry obtained was stirred at a speed of 250 rpm and a temperature of 35 °C for 120 minutes, followed by centrifugation at 4500 x g for 20 minutes to separate the solubilized protein molecules from the insoluble starch and fibre granules. The clarified protein solution was then passed through an ultrafiltration system (Pellicon, molecular weight cut - off 5 KDa) to concentrate the proteins. This pore size is appropriate as it allows only water and low molecular weight salts as well as most anti-nutrients to be separated in the filtrate while the retentate, containing the protein is sent for drying. Ultrafiltration was performed at a pH of 7 using a volume concentration ratio of 4, that is, the protein solution was concentrated to one fourth of its original volume. The concentrated pea protein was finally dried with a freeze dryer (Virtis Bench Top 6 K) to obtain pea protein isolates and stored at 4 °C until analysis was carried out.

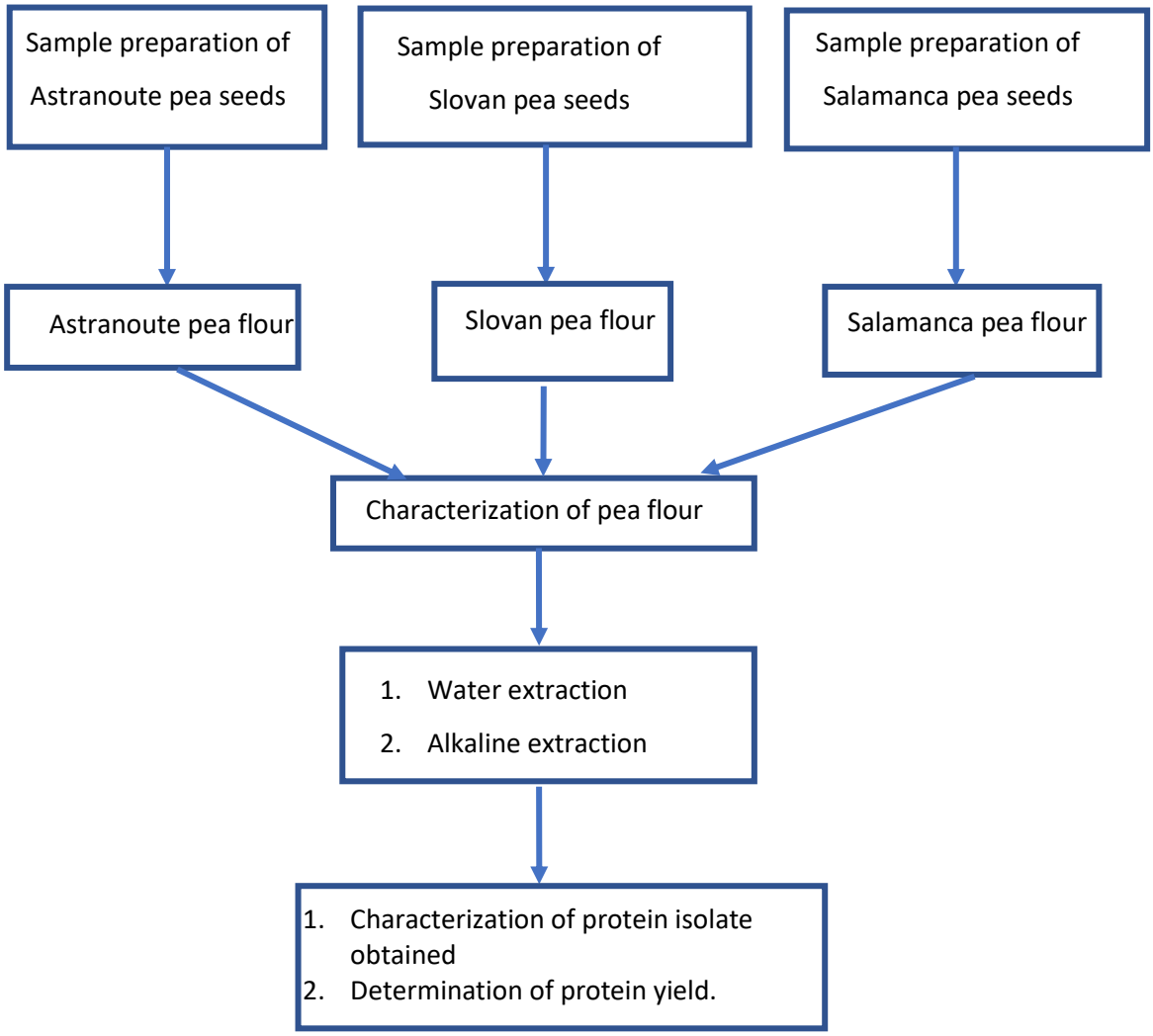


Figure 3; Screening stage of experimental work

The pea protein isolates obtained were again characterized in terms of ash content, fat content, starch content protein, moisture content. Overall protein yield was determined through comparison of the protein content of extracted with the protein content of the starting material. This was expressed as g protein obtained/ g protein added. This protein yield as well as the protein content of the isolates served as the response variables and the basis of comparison for the different cultivars. The cultivar that performed best in terms of both protein content and yield was then selected and used for the bench-scale optimization stage of the project.

3.3.3.1 Statistical evaluation of screening process

All extractions were done in triplicate for each cultivar and the means were recorded. Microsoft excel, version 2013 was used to calculate all means and standard deviations used for statistical evaluation. Statistical differences and significance in protein yield and protein content among the pea cultivars were also evaluated with a one-way analysis of variance (ANOVA) using Microsoft excel, version 2013.

3.3.4 Bench-scale Optimization and Validation Experiments

Laboratory-scale experiments carried out in 5 litre beakers were employed to investigate water and alkaline wet extraction methods using the chosen cultivar, while varying operating variables and regimes to obtain optima conditions for protein yield and protein content. Figure 4 shows the sequence in which the bench scale experiments were carried out.

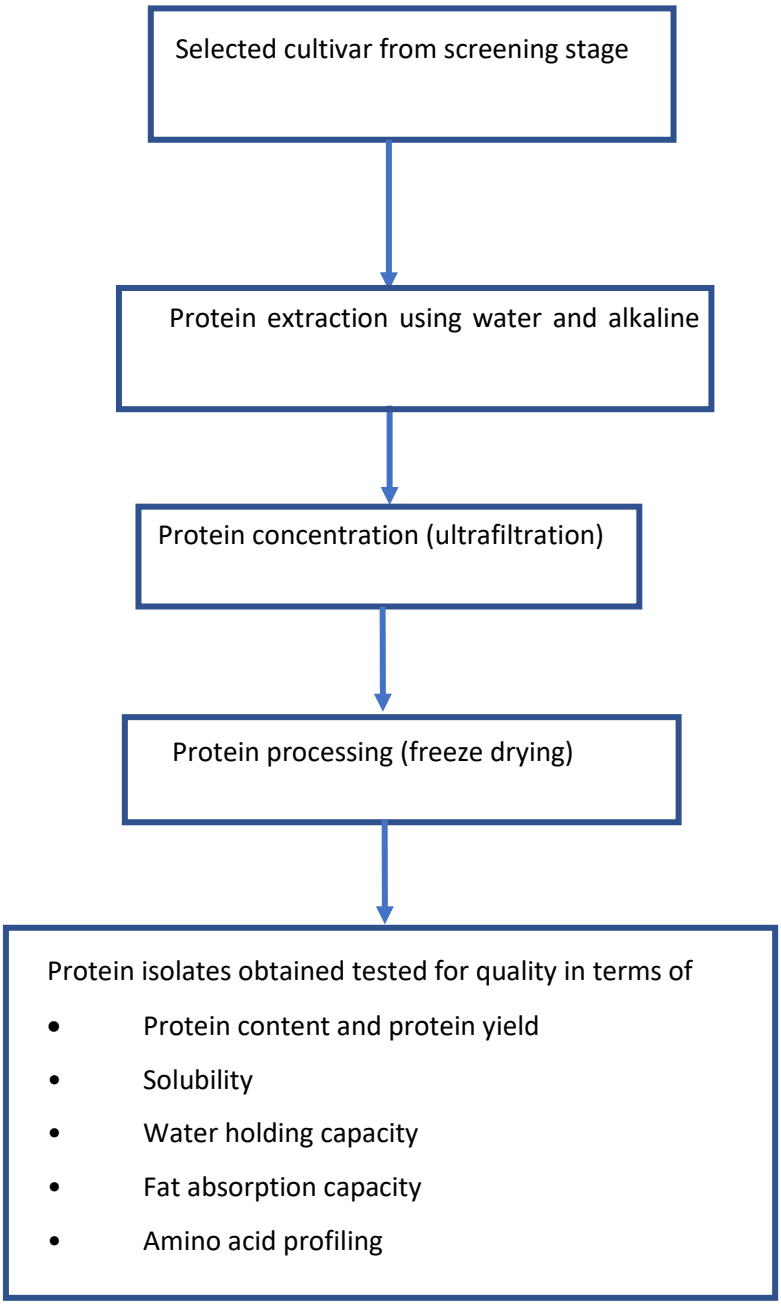


Figure 4; Summary of bench scale experiments

3.3.4.1 Statistical Design of Experiments

Statistica version 10 was used to design a central composite design (CCD) to optimize the extraction processes and determine the interactions between the independent variables and the dependent variables. The factors and levels used for the CCD for both water and alkaline extractions are presented in Table 5 and Table 6 below. Four replicates were used at the centre points to enable the pure error to be estimated. The empirical formula relating the response variables to the independent variables is represented in equation (11).

$$Y = b_o + \sum_{i=1} b_i X_i + \sum_{i=1} b_{ij} X_i X_j + \sum_{i=1} b_{ii} X_i^2 \quad (11)$$

Where Y is the response variable (protein content and protein yield), b_0 is the constant coefficient, b_i is the linear coefficient, b_{ij} is the interaction coefficient and b_{ii} is the quadratic coefficient

Table 5; Factors and levels used in the central composite design for water extractions

	Levels		
Factors	**-1**	**0**	**1**
Temperature (°C)	30	40	50
Time (minutes)	30	75	120
Solid loading (%)	6.7	13.3	20

Table 6; Factors and levels used in the central composite design for alkaline extractions

	Levels		
Factors	**-1**	**0**	**1**
Temperature (°C)	35	50	65
Time (minutes)	70	100	120
Solid loading (%)	9	12.8	16.7
pH	8.5	10	11.5

For the validation experiments, desirability analysis (using the desirability profiler in Statistica software) was used to predict the overall response desirability by identifying the optimum level of each independent parameter that will lead to a maximum protein content and yield. Water extractions were performed at a temperature of 40 °C, a residence time of 1 hour and a solid loading of 6.7 %, as predicted by the desirability analysis. Alkaline extractions were also performed at a pH of 10, a residence time of 100 minutes, a solid loading of 6.2 % and a temperature of 20 °C. These experiments were done in triplicates in 5 litre reactors. The results obtained (protein content and yield) were then compared to the predicted optimal values derived from the desirability analysis.

3.3.5 Quality tests

Other key performance indicators (quality tests) such as the functional properties described earlier (Section 3.2.2) as well as amino acid analysis were also performed on selected samples that performed best in terms of protein content and protein yield. Protein solubility, water hydration capacity and fat hydration capacity were performed three times and reported as the mean ± the standard deviation. Significant differences among these means were established at a confidence level of 5 % ($p< 0.05$) using a two -sample t- test, assuming unequal variances ($p>0.05$).

4 CHAPTER FOUR: EXPERIMENTAL RESULTS AND DISCUSSION

4.1 Screening Process

Water and alkaline wet extraction methods were used to screen the three pea cultivars to select a preferred cultivar for the follow up optimization stage of the study. The pea flours of the cultivars were characterized and the proximate composition together with comparable literature values are shown in Table 7 below. Salamanca recorded the highest protein content and this was 5% and 4.4% greater than the values recorded for Astranoute and Slovan. The total protein content of the three cultivars signifies the maximum amount of protein that can be extracted. Ash content of cultivars ranged between 2.8% (w/w) to 3.9% (w/w) and the fat content was quite low, with the highest reported as 2%, for Astranoute. The proximate composition for the three cultivars used in the screening process is comparable to that of a typical yellow pea as reported in literature (Kaack and Pedersen, 2005; Agboola *et al.*, 2010; Karaca, Low and Nickerson, 2011a; Hoang, 2012)

Table 7; Proximate composition of the different yellow pea cultivars in this study

Composition (% w/w)	**Astranoute**	**Salamanca**	**Slovan**
Protein	21.6	22.7	21.7
Ash	3.9	2.8	3.4
Moisture	7.6	8.7	4.5
Fat	2.0	0.2	0.2
Carbohydrate	64.9	65.6	70.2

Measurements were done in triplicate and the means recorded

Selection of the preferred cultivar was based on the protein concentration as well the protein yield of both extraction methods that were carried out. The compositional analysis of isolates is presented in Table 8 while the data for protein extraction and protein yield are shown in Table 9. Generally, alkaline extractions resulted in significantly higher extraction ($p < 0.05$) in terms of protein concentration and yield. The alkaline present in the mixture helps break down protein particles and reduces the viscosity of the extraction mixture (Hoang, 2012; Ruiz *et al.*, 2016). This allows for better agitation of the mixture and in effect more solubilization of protein particles, leading to higher protein concentrations

and yields. Also, at higher extraction pH (alkaline), there are a lot of negative charges in solution which causes the molecules to repel against each other and enhances protein solubility (Hoang, 2012).

The yields of pea protein isolates and the protein contents thereof were compared among the three cultivars. There were significant differences ($p< 0.05$) in protein isolates among cultivars for both water extraction and alkaline extractions. It was observed that, although the flour of the Astranoute cultivar (raw material) had the highest protein content, the PPI yield from extraction was the lowest of the three. Furthermore, PPIs from Slovan recorded the highest protein contents of 51.1% (w/w) and 63.3% (w/w) for water and alkaline extractions respectively. Slovan cultivar again recorded the highest yield for both water and alkaline extractions while Astranoute recorded the lowest. The low protein content and yield observed for the Astranoute PPI could be attributed to the higher amount of ash content as well as the fat and carbohydrate content contained in the cultivar as compared to the other two or to the extraction conditions (Piper and Boote, 1999; Stone *et al.*, 2015; Lam *et al.*, 2016). Slovan was identified as the preferred cultivar for further bench-scale optimization of the extraction processes.

It was observed that the protein content of 63.3% (Table 9) for alkaline-extracted isolates in the screening stage was lower than values of 80% , 84% and 73 obtained by Hoang (2012); A K. Sumner *et al*. (1981 and Tian 1998 although the protein yield of 58.4% also obtained by alkaline-extracted isolates (Table 9) were comparable to yields of 59% , 58.8% and 56% reported by A K. Sumner *et al.* (1981),Tian (1998) and Hoang (2012) respectively all of whom used similar operating conditions for protein extractions. Protein yield and protein content are greatly affected by operating conditions such as time, temperature, pH and solids (Hoang, 2012; Lam *et al.*, 2016). Further optimization is therefore important to identify the extent to which these parameters could affect protein content and protein yield.

Table 8; Compositional analysis of the isolates derived from the three cultivars for both extraction methods

Composition (%)	**Astranoute**		**Salamanca**		**Slovan**	
	Water extraction	Alkaline extraction	Water extraction	Alkaline extraction	Water extraction	Alkaline extraction
Protein	32	43.1	36.2	48.3	51.1	63.3
Ash	3.5	3.4	2.3	2.3	3.0	2.5
Moisture	7.0	6.9	8.0	7.2	4.3	3.0
Fat	1.2	1.0	0.1	0.8	0.1	0.6
Carbohydrate	56.3	45.6	53.4	41.4	41.5	30.6

Table 9; Protein content and protein yield of pea protein isolates for screening process

Protein content (%)	Astranoute	Salamanca	Slovan
Water extraction	32.0 ± 0.8	36.2 ± 1.1	51.1 ± 1.4
Alkaline extraction	43.1 ± 1.2	48.3 ± 0.9	63.3 ± 0.5
Protein yield (%)			
Water extraction	29.6 ± 0.7	31.9 ± 1.0	47.2 ± 0.6
Alkaline extraction	39.9 ± 0.8	42.6 ±0 .9	58.4 ± 1.1

Runs were carried out in triplicate and data reported as mean ± standard error

4.2 Bench-scale optimization and validation of protein extraction

4.2.1 Optimization using response surface methodology

Response surface methodology using a rotatable central composite design (CCD) offers an excellent approach to simultaneous optimization of two or more responses through simultaneous variation of two or more independent variables. In addition, this approach offers substantial insight onto the behaviour of a system, in this case how an increase from neutral to alkaline pH might affect protein concentration and yield during extraction. Selection of ranges for a CCD model is of critical importance to model accuracy and can be determined using either a steepest ascent approach or can be based on system limits. The latter approach was used in this study since there are certain limitations to which

you can push some of the independent variables (Myers, Montgomery Douglas and Christine, 2009; Cassettari and Mosca, 2013; Montgomery, 2013). Protein extraction techniques that occur at pH levels higher than 12 and temperatures of 60 ^{o}C are not advisable due to protein degradation and decrease in protein purity (Crispin, 1995; Xu and Chang, 2007; Hoang, 2012). The usage of solids loadings above 20% are also not worthwhile due to limitations that occur as a result of mass transfer (Crispin, 1995; Xu and Chang, 2007; Hoang, 2012). The ranges chosen for this study for both extraction methods were represented in Table 5 and Table 6. Protein concentration and protein yield in response to variation in temperature, time and solids loading are shown in Table 10 (water extraction) and Table 11 (alkaline extraction), where variation in pH was also included in the latter. Four replicates were used at the centre (C) of the CCD and the experiments were conducted in a random manner for each extraction method.

Table 10; Protein contents and protein yields obtained from water extractions at temperatures, times and solids as determined by CCD using Slovan cultivar

Experiment number	Temperature (℃)	Time (minutes)	Solids (%)	Protein content (%)	Protein yield (%)
1	30	30	6.7	82.2	46.7
2	30	30	20.0	77.2	26.8
3	30	120	6.7	81.3	43.3
4	30	120	20.0	75.9	23.3
5	50	30	6.7	81.1	50.1
6	50	30	20.0	76.8	19.8
7	50	120	6.7	77.9	34.5
8	50	120	20.0	75.6	29.1
9	23	75	13.3	78.3	31.5
10	56	75	13.3	78.8	31.4
11	40	7	13.3	80.1	34.4
12	40	150	13.3	79.5	45.1
13	40	75	2.0	81.6	56.2
14	40	75	24.0	78.1	43.7

15 (C)	40	75	13.3	83.0	59.5
16 (C)	40	75	13.3	83.3	50.8
17 (C)	40	75	13.3	81.5	48.8
18 (C)	40	75	13.3	82.2	51.8

Table 11; Protein contents and protein yields obtained from alkaline extractions at pH levels, temperatures, times and solids as determined by CCD using Slovan cultivar

Experiment number	**pH**	**Temperature (℃)**	**Time (minutes)**	**Solids (%)**	**Protein content (%)**	**Protein yield (%)**
1	8.5	35	70	9.0	86.2	46.6
2	8.5	35	70	16.7	85.2	29.2
3	8.5	35	150	9.0	86.2	66.6
4	8.5	35	150	16.7	82.7	32.6
5	8.5	65	70	9.0	85.9	41.6
6	8.5	65	70	16.7	85.2	35.8
7	8.5	65	150	9.0	84.5	51.5
8	8.5	65	150	16.7	86.7	48.9
9	11.5	35	70	9.0	86.6	52.7
10	11.5	35	70	16.7	84.9	42.7
11	11.5	35	150	9.0	84.5	48.7
12	11.5	35	150	16.7	86.3	37.0
13	11.5	65	70	9.0	86.6	71.6
14	11.5	65	70	16.7	86.8	35.4
15	11.5	65	150	9.0	86.5	51.4
16	11.5	65	150	16.7	87.5	39.3
17	7	50	100	12.9	81.7	38.9

18	13	50	100	12.9	87.3	54.7
19	10	20	100	12.9	85.4	41.1
20	10	80	100	12.9	88.4	56.2
21	10	50	180	12.9	83.3	42.8
22	10	50	180	12.9	87.7	42.8
23	10	50	100	5.2	87.2	73.4
24	10	50	100	20.6	87.2	38.5
25(C)	10	50	100	12.9	88.0	45.2
26(C)	10	50	100	12.9	85.6	45.1
27(C)	10	50	100	12.9	85.9	60.6
28(C)	10	50	100	12.9	86.6	44.3

The highest protein content of 88.4% and highest protein yield of 73.4% for isolates derived from alkaline extractions (Table 11) were 5.1% and 18.9% higher than those derived for water extractions (Table 10). These values are significantly higher than those obtained during the screening stage (Protein contents of 51.1% and 63.3% for water and alkaline extractions respectively; protein yields of 47.2% and 58.4% for water and alkaline extractions respectively as shown in Table 9) and this is because of improvement in efficiency of extractions due to the different settings of operating parameters used in the optimization stage as compared to the screening stage. The range of operating conditions (see Table 5 and Table 6) used in the optimization stage of this study are comparable to those used by (A K. Sumner, Nielsen and Youngs, 1981; Fernández-Quintela *et al.*, 1997; Barac *et al.*, 2010; J. Boye *et al.*, 2010; Hoang, 2012; Stone *et al.*, 2014; Tiessen-dyck, 2014; Zhang, Yang and Singh, 2014).

Protein contents of 81.7% to 88.4% (Table 11) obtained by alkaline extractions were similar to studies performed by Sumner *et al*, (1981), Stone *et al,* (2014), Zhang *et al*. (2014) and Boye *et al*, (2010) where protein contents of 93%, 89%, 84% and 83.9% were obtained respectively for alkaline extractions. The highest protein yield of 73.4% (Table 11) also obtained by the alkaline extraction method was similar to the value of 76.7% reported by Stone *et al*, 2014 but higher than values of 58%, 55.3% and 57.1% obtained by Sumner *et al*, (1981), Hoang (2012) and Boye *et al*, (2010) both of whom conducted their extractions using alkaline treatments. Generally, pulse proteins display maximum solubility at pH of 10 and above due to abundance of charges causing molecules to repel against each

other (Yu, Ahmedna and Goktepe, 2007; Hoang, 2012). The extractions conducted in this pH range tend to display higher protein concentrations and yield. However, using pH values greater than 12 is not advisable because despite the high amounts of protein and yields that may be obtained, unwanted alterations in the protein isolate such as discolouration and protein denaturing could occur which in turn also affects the functionality of the isolate (Yu, Ahmedna and Goktepe, 2007; Hoang, 2012).

The experimental data obtained from the runs (dependent and independent variables) were used to develop mathematical regression equations and models, which could be used to predict the protein content and protein yield for both extraction methods while also showing the correlation between dependent and independent variables within the given range used in this study. This is displayed in the regression Equations (12) and (13) for protein yield and protein content, respectively, for water extractions.

$$Y = 58.7286 + 1.1523x_1 + 0.0838x_2 + 0.0639x_3 - 0.0155x_1^2 - 0.0005x_2^2 - 0.0247x_3^2 \quad (12)$$

$$Y = -80.4269 + 6.7901x_1 + 0.2922x_2 - 0.8344x_3 - 0.0869x_1^2 - 0.0028x_2^2 - 0.0490x_3^2 \quad (13)$$

Where x_1, x_2 and x_3 represent the variables for temperature, time and solids, respectively.

Equations (14) and (15) are the regression equations for protein yield and protein content, respectively, for alkaline extractions.

$$Y = 72.1575 + 4.5692x_1 - 0.1903x_2 + 0.0106x_3 - 1.1943x_4 - 0.0085 + 0.0011 + 0.0450 + 0.00034 + 0.00789 + 0.00189 - 0.2590x_1^2 - 0.0003x_2^2 - 0.0003x_3^2 + 0.0050x_4^2 \quad (14)$$

$$Y = -34.7890 + 16.1966x_1 - 0.4658x_2 + 1.2443x_3 - 7.4146x_4 + 0.0663 \quad (15)$$
$$- 0.0881 - 0.0010 - 0.0022 + 0.0290 + 0.0003 - 0.4386x_1^2$$
$$- 0.0023x_2^2 - 0.0011x_3^2 + 0.1379x_4^2$$

Where x_1, x_2, x_3 and x_4 represent the variables for pH, temperature, time and solids, respectively.

For water extractions, the quadratic models fitted the data with R^2 values of 0.89 (Equation (12)and 0.82 (13) for protein concentration and protein yield respectively while R^2 values of 0.85 (14) and 0.84 (15) were obtained for protein concentration and protein yield for alkaline extractions. These R^2 values proved that the experimental data derived from the laboratory work were well fitted to the model predicted and that unexplained error was quite low.

4.2.2 Effects of independent parameters on response variables

Response surface plots of the quadratic models developed were constructed to illustrate the effect of the independent variables on the dependent variables for both extraction methods. An analysis of variance was also performed to show which of the independent variables had a significant impact on the response variables. The effects of these variables are depicted in the pareto charts of Figure 7 and Figure 9 for water extractions and alkaline extractions respectively.

Figure 5A shows the effects of solids and temperature on protein content for water extractions. It was observed that as the temperature and solids increased, the protein content also increased until an optimum was achieved, after which the protein content saw a decline. No significant increase in protein content was attained in excess of values above 40 °C and 7% for temperature and solids respectively. A maximum protein content of 76% was achieved at these optimum values. The influence of temperature and time on protein content for water extractions are depicted in Figure 5B. The protein content levels off at 78% and saw a reduction when temperatures beyond 40 °C and times beyond 65 minutes were employed.

From Figure 6 Figure 6B, it can be observed that a simultaneous increase in time, temperature and solids corresponded to an increase in protein yield. A threshold was however evident at a temperature of 38 °C, a time of 60 minutes and solids of 6.8% and no substantial increase in protein yield was achieved above these values with a maximum protein yield of 38% being attained (Figure 6Figure 6B)

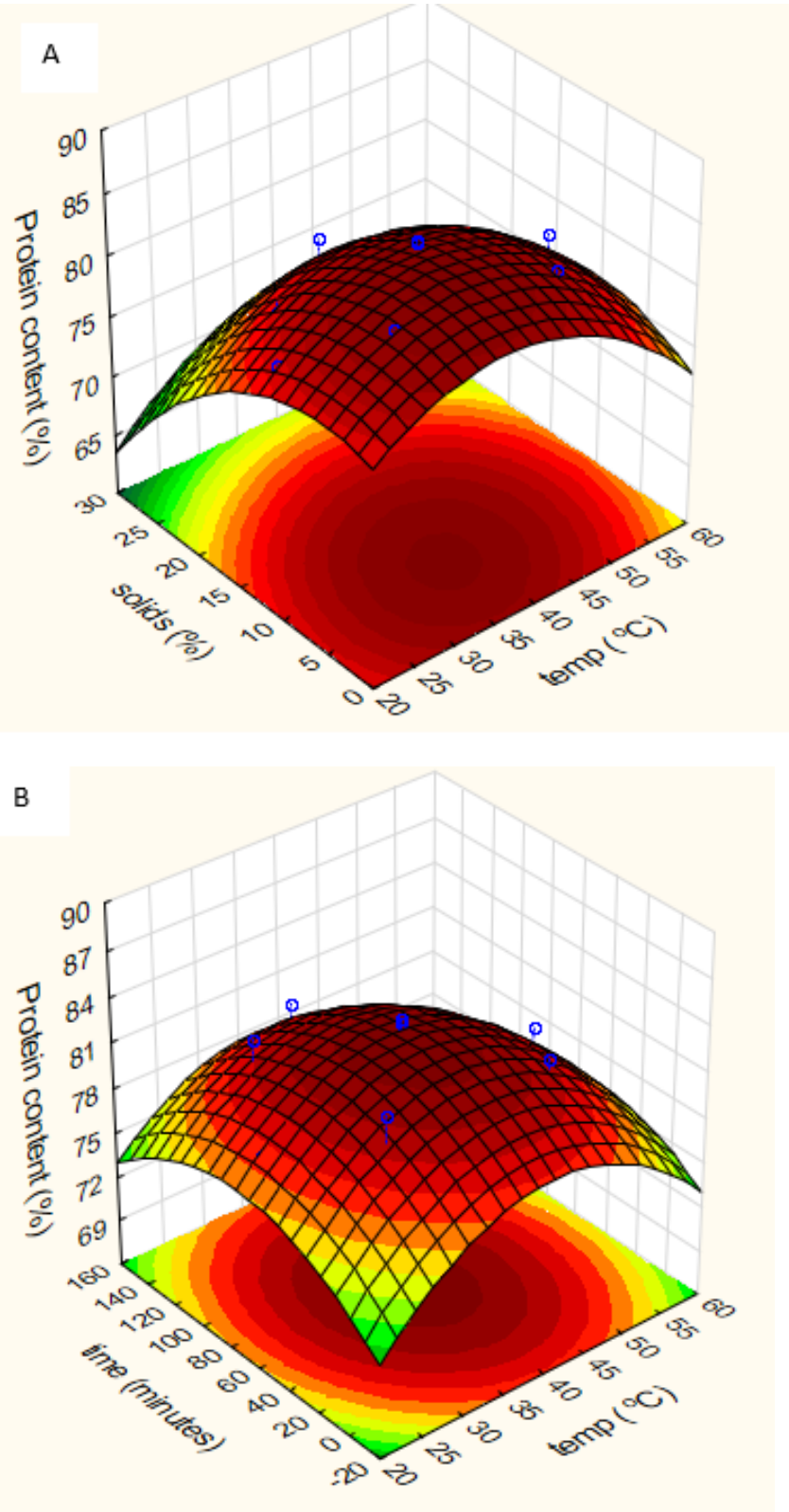


Figure 5; Response surface plots of the quadratic models predicting protein content for water extractions (A,B). A: Protein content plotted as a function of solids and temperature. B: Protein content plotted as a function of time and temperature.

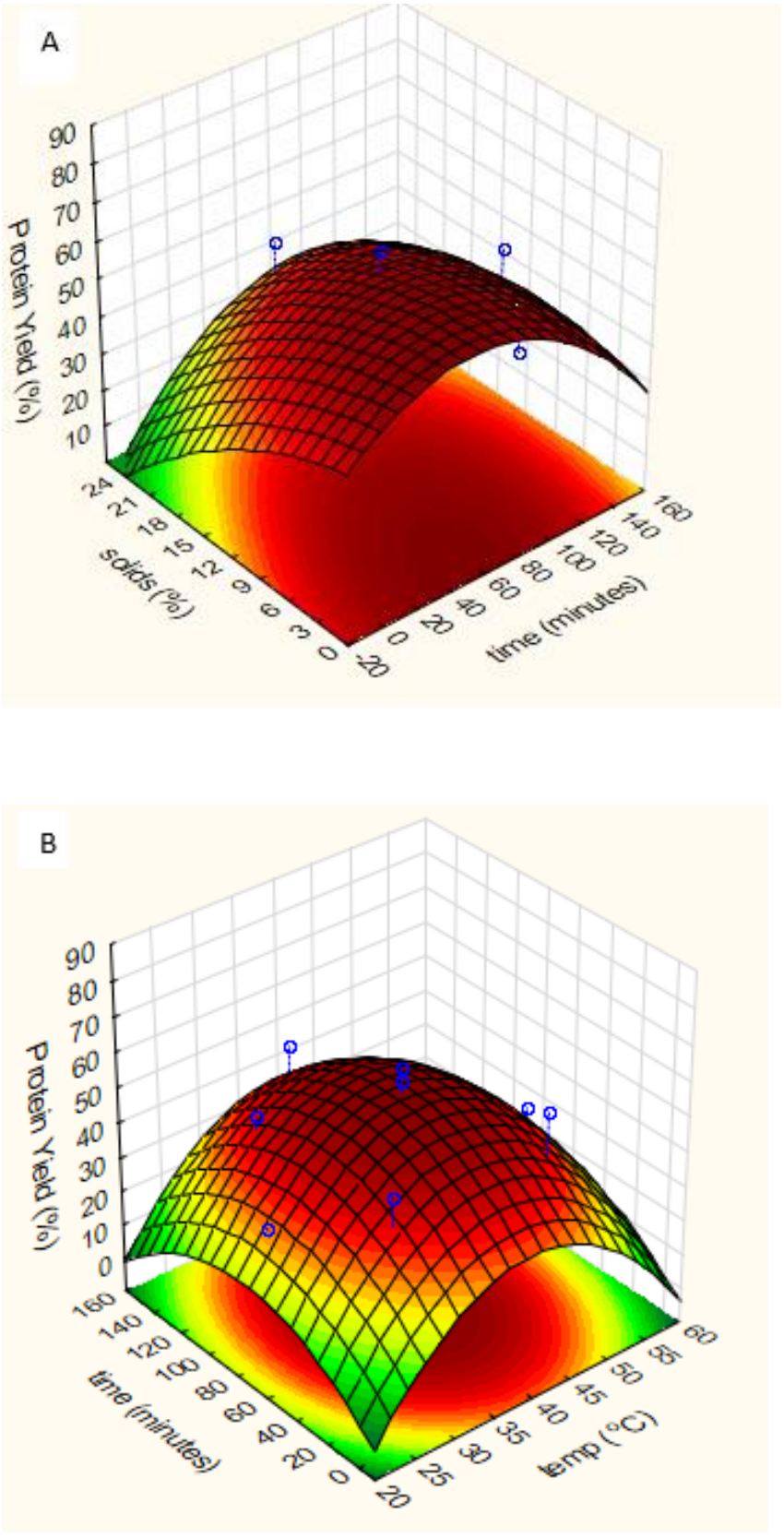
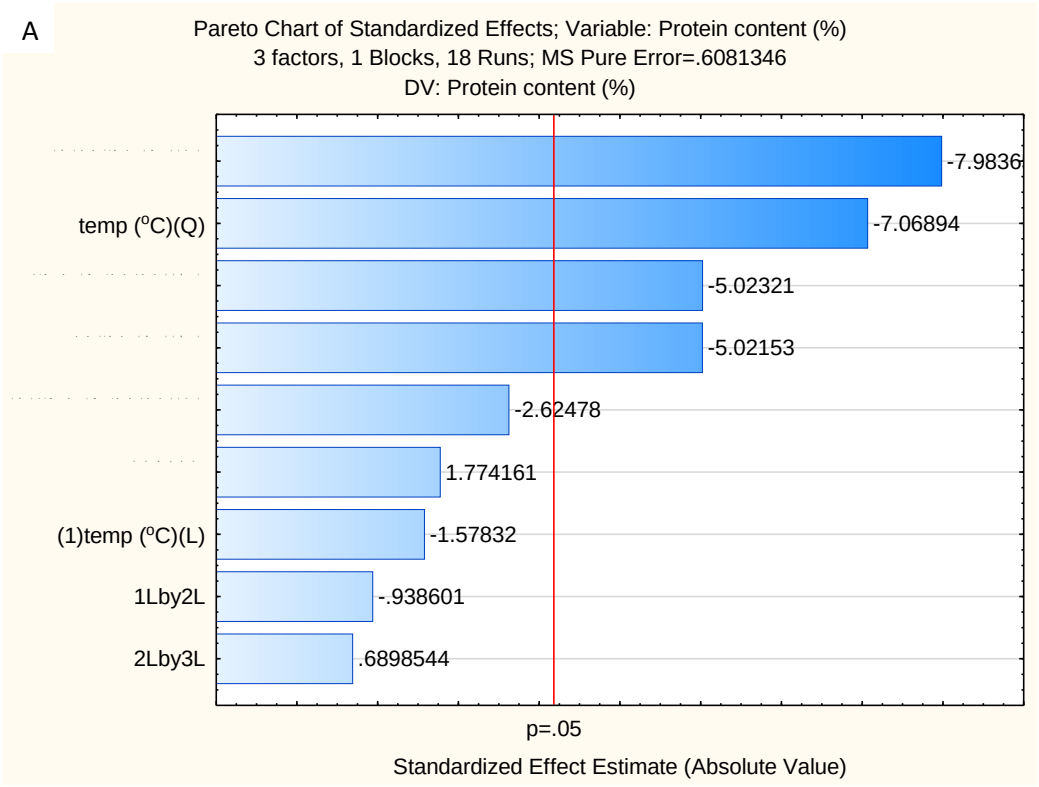


Figure 6;Response surface plots of models describing the protein yield for water extractions (A, B). A :Protein yield plotted as a function of solids and time. B: Protein yield plotted as a function of time and temperature



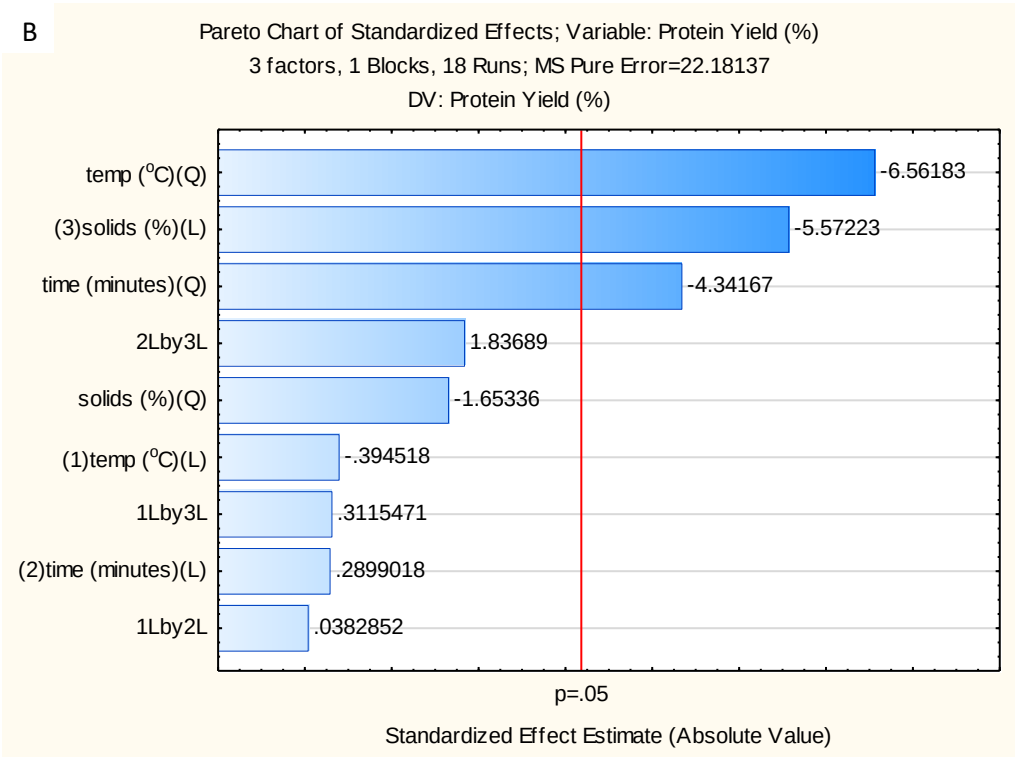
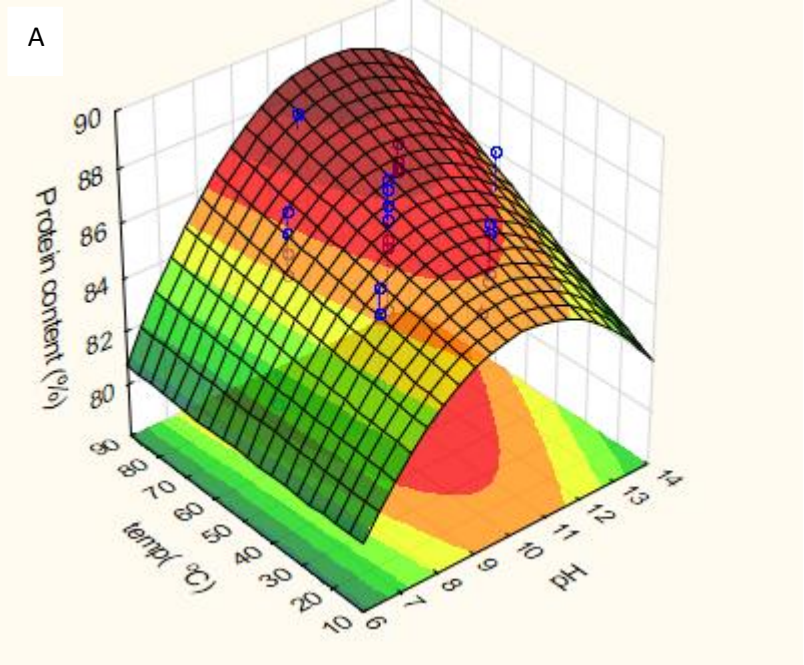


Figure 7; Pareto chart of standardized effects for water extraction – Protein content (A), Protein yield (B)

The pareto chart displaying effects of independent variables on protein content in Figure 7A shows that for water extracted isolates, temperature, time and especially solids contributed heavily within a 95 % confidence interval. It was observed that only the linear terms of the solids and the quadratic terms of temperature and time had a significant effect on protein yield (Figure 7B).

The effects of solids and temperature on protein content for alkaline extractions are depicted in Figure 8A. An increase in temperature and pH resulted in an increase in protein content until a maximum value of 88.5% was obtained for protein content. No substantial increase in protein content was achieved above values of 10 and 25 °C for pH and temperature respectively. The protein yield for alkaline extractions saw a linear increase as the solids were decreased and time was increased (Figure 8B).



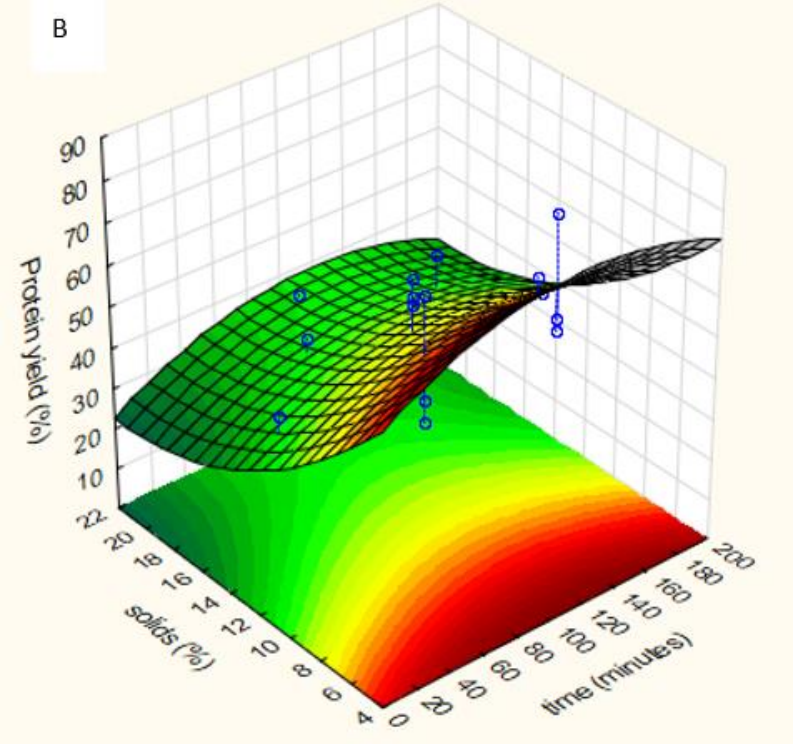


Figure 8; Response surface plots of the quadratic models predicting A) protein content plotted as a function of temperature and pH and B) protein yield plotted as a function of solids and time for alkaline extractions

For alkaline extracted isolates, only the linear term of pH a significant effect on protein content (as shown in the pareto chart of Figure 9A). As pH of the extraction solution increased, protein content of the extracted isolates also increased. However, extracting proteins at pH levels higher than 11 are not advisable due to protein degradation and decrease in protein purity (Hoang, 2012; Toldrá and Nollet, 2013) and this is evident as the protein content dropped after a pH level of 11 (as seen in Figure 8A) . Protein yield saw only the linear term for solids contributing significantly (Figure 9B). It is suggested by Hoang (2012) that the major cause of temperature and time having no significant impact on protein concentration and protein yield was the pH of the extracting solvent. Protein molecules are more easily dissociated at high pH values because the overall charge on protein molecules was boosted. The negative correlation between solids and yield in the Pareto chart indicated that a lower concentration of solids caused an increase in yield (Figure 9B). This is comparable to reports by Hoang (2012) where it was discovered that a decrease in solids from 25% to 13.3% increased the protein yield of isolates by 16.5%. As the solids to liquid ratio is increased, the concentration gradient between liquid and solid phases also increases. This causes more protein particles to dissociate from the solid into the liquid phase, leading to an eventual increase in protein yield (Hoang, 2012). None of the interaction

parameters had a significant effect on the protein yield and protein content for both methods investigated (Figure 7 and Figure 9) and this is similar to studies performed by (Guan and Yao, 2008; Hoang, 2012) who also reported no interaction among parameters and attributed this to the behavior of the system as well as the massive influence of pH and solids on protein content and protein yield.

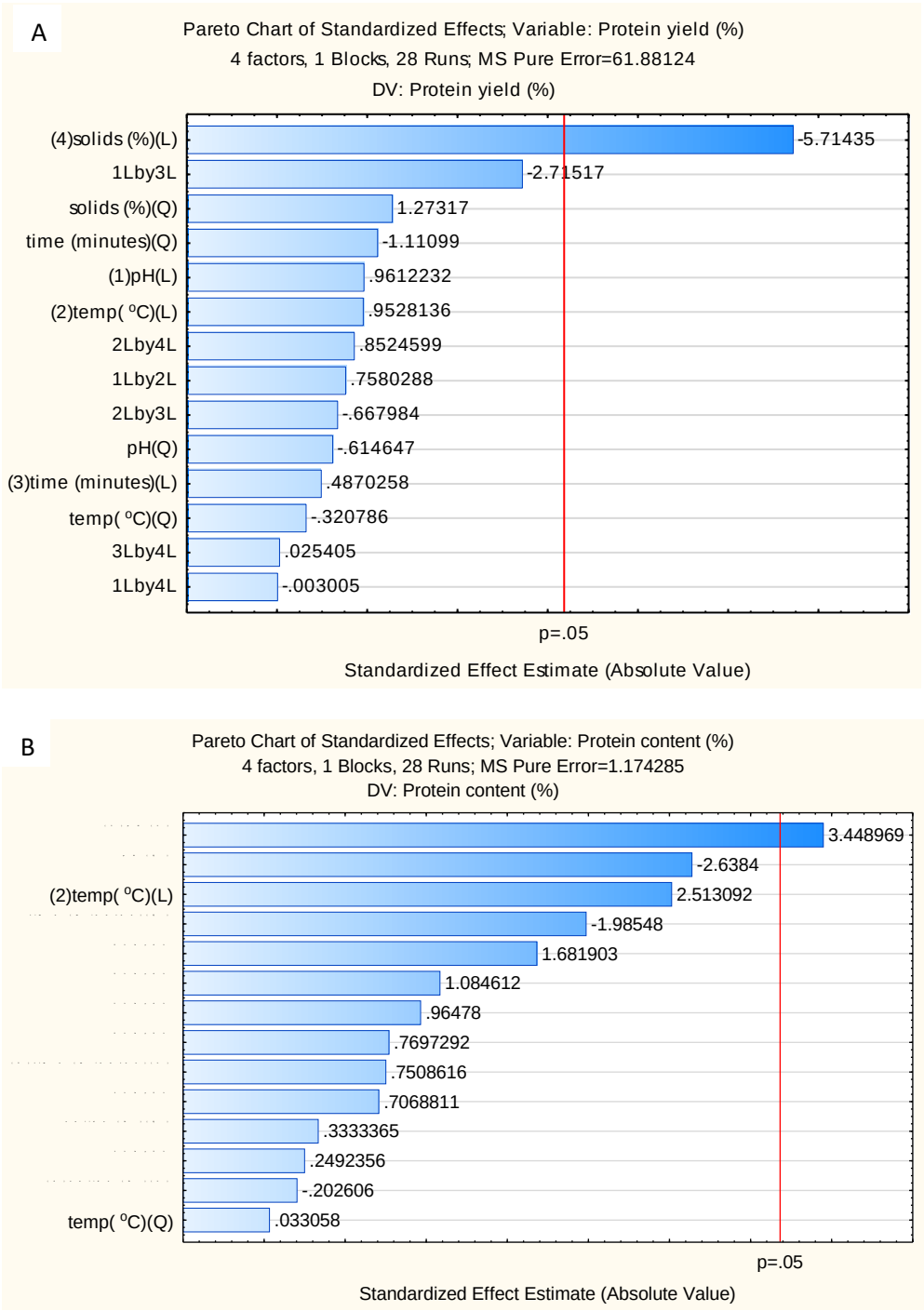


Figure 9; Pareto chart of standardized effects for alkaline extraction – Protein content (A), Protein yield (B)

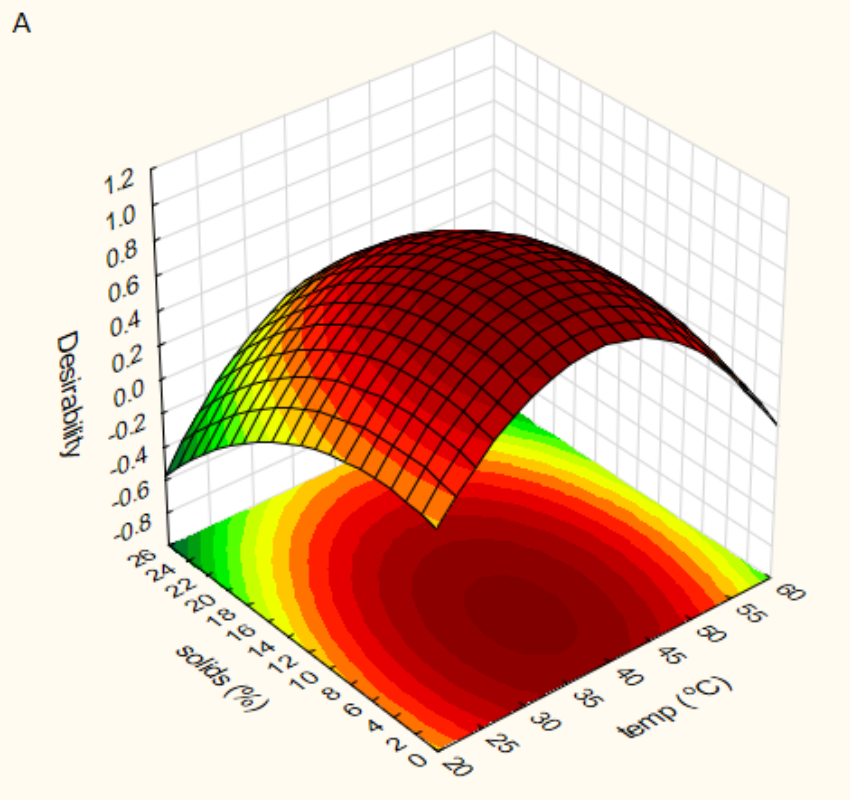
Generally, it was observed that the protein content and protein yields of isolates increased with a decrease in solids for both extraction methods. For alkaline extractions, an increase of about 6% was observed for protein content while a 47% increase was recorded for protein yield when solids were decreased from 16.7% to 9% (Table 11). Similarly, water extractions reported increments of 9% and 67% for protein content and protein yields respectively (see Table 10) when solids were decreased by 33.5%. At higher solid loadings, there is an increase in viscosity of the extraction mixture, making it difficult for proper and uniform mixing to occur. High solid loadings also reduce the ability of the water contained in the mixture to facilitate mass transfer among pea flour particles (Mizubuti *et al.*, 2000; Hoang, 2012) . This allows less of the protein contained in the mixture to solubilize, leading to production of pea protein isolates with low amounts of protein content as well as lower protein yields (Johnston and Fellers, 1971; Victor, 1980; Dua *et al.*, 2009; Hoang, 2012).

The trends in the models obtained for the response surface models and pareto charts agree with results obtained in literature (Mizubuti *et al.*, 2000; Quanhong and Caili, 2005; Guan and Yao, 2008; Shen *et al.*, 2008; Bahnasawy and Shenana, 2010; Essuman, 2013) where effects of temp, time, pH and solids on protein extractions from plants were explored. Reductions in protein content and protein yield when pH, temperature, time and solids loading are prolonged are attributed to contamination of the final protein isolate due to starch solubility and starch swelling (Shen *et al.*, 2008; Hoang, 2012). Increase in temperature was found by Shen *et al.*, (2008) and Zhang *et al.*, (2015) to favour protein yield because at higher temperatures, proteins are better hydrolysed into peptides, resulting in a decrease in the molecular size of the proteins thus accelerating protein dissolution and protein diffusion. It was discovered by Guan and Yao, (2008) and Shen *et al.*, (2008) that an increase in temperature from 25 °C till about 40 to 45 resulted in a 29% increase in protein content and protein yield after which a decline was observed. An increase in extraction time was found to be advantageous although very little increments in protein yields were observed by Shen *et al.*, (2008); Hoang, (2012); Essuman, (2013) when the extraction time was extended beyond two hours.

4.2.3 Response desirability optimization and model validation

The models for protein yield and protein content (dependent variables) were combined by the desirability profiler using response desirability optimization. This optimizes the independent variables to simultaneously maximize protein concentration and protein yield. Equal weighting was given to both protein content and protein yield although protein yield is of more importance. A value of 0 was selected for the lowest protein concentrations and protein yields obtained in the CCD while a value of 1 was selected for the highest protein concentrations and protein yields obtained. The desirability

optimization for water extractions reported optimum values of 40 ℃, 60 minutes and 6.7% for temperature, time and solid loadings respectively as shown in Figure 10. At these optimum values, the predicted values of protein content and protein yield were 83.2% and 58.2% respectively. Optimum values for pH, temperature, time and solid loadings for alkaline extractions were 10, 20 ℃, 100 minutes and 6.2% respectively (Figure 11) with a predicted protein content and a protein yield of 88.1% and 75.7% respectively. Laboratory experiments were carried out in 5 litre bioreactors using the optimum input parameters to validate the responses predicted by the response desirability. Each run was done in triplicate. The validity of the model was then determined by comparing the experimental values to the predicted values. The results obtained for the validation experiments carried out in the 5 litre reactors are summarized in Table 12 .



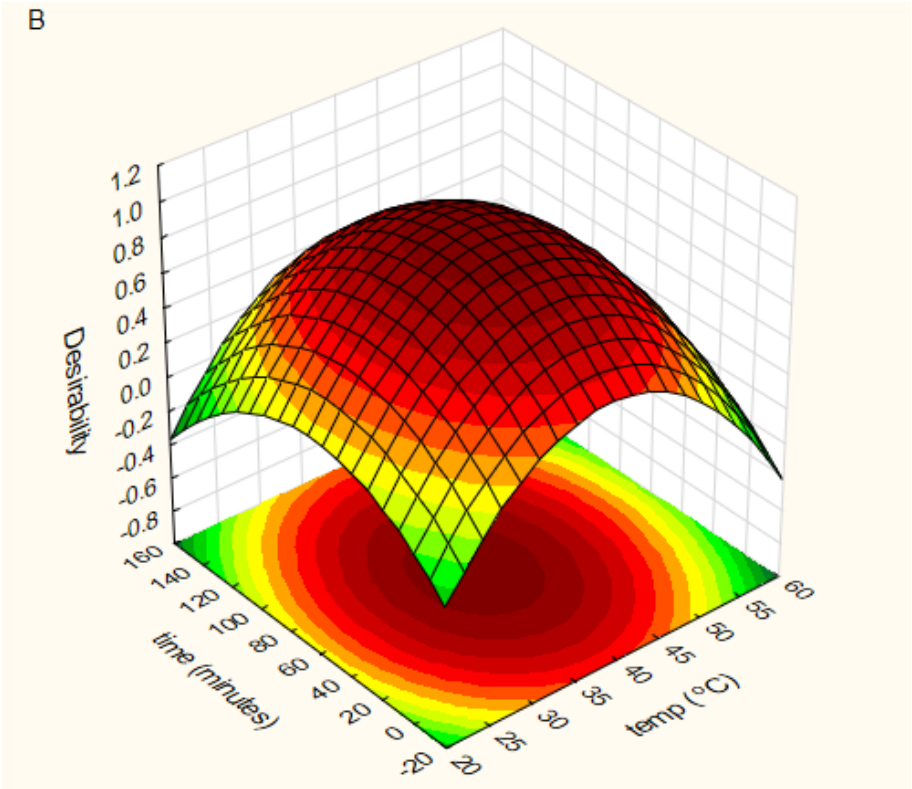
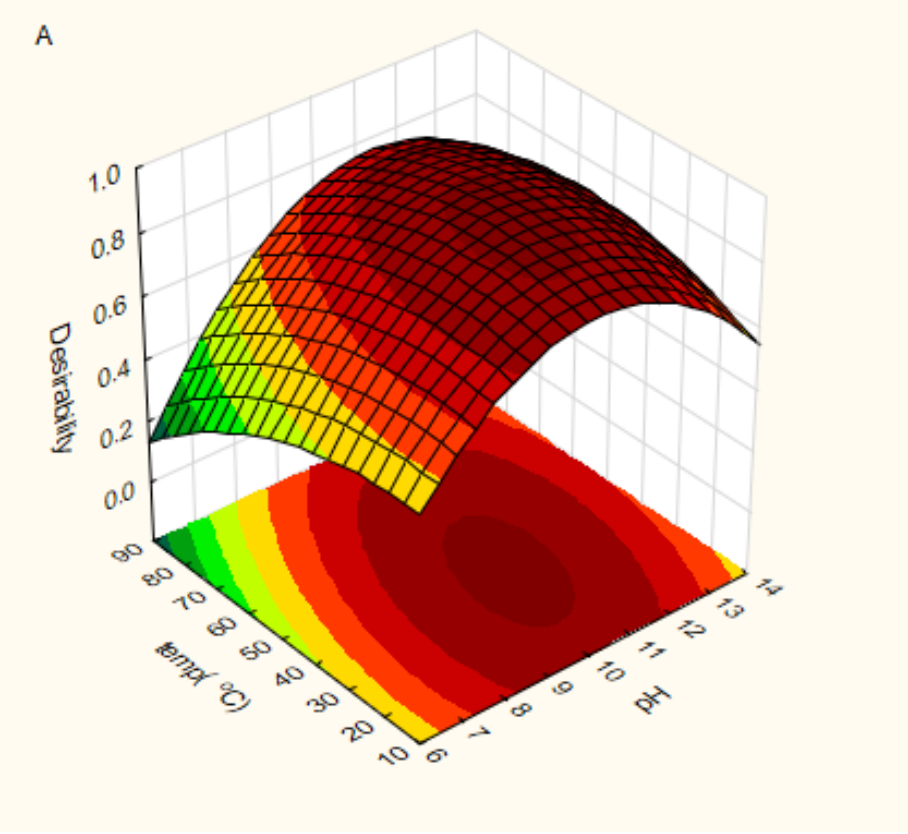
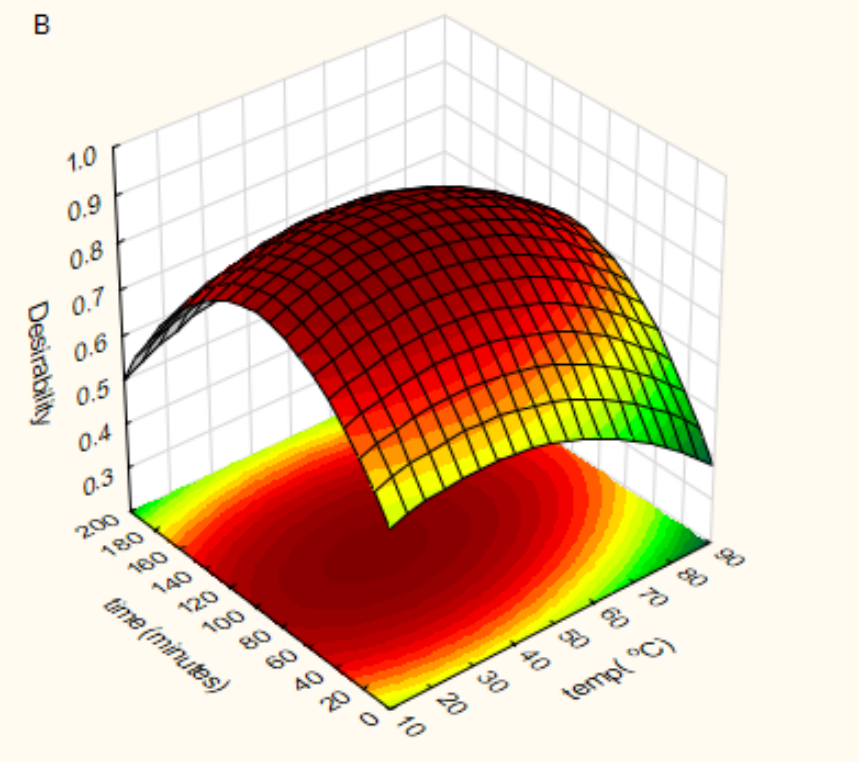


Figure 10; Multiple response optimization with desirability functions using water extractions





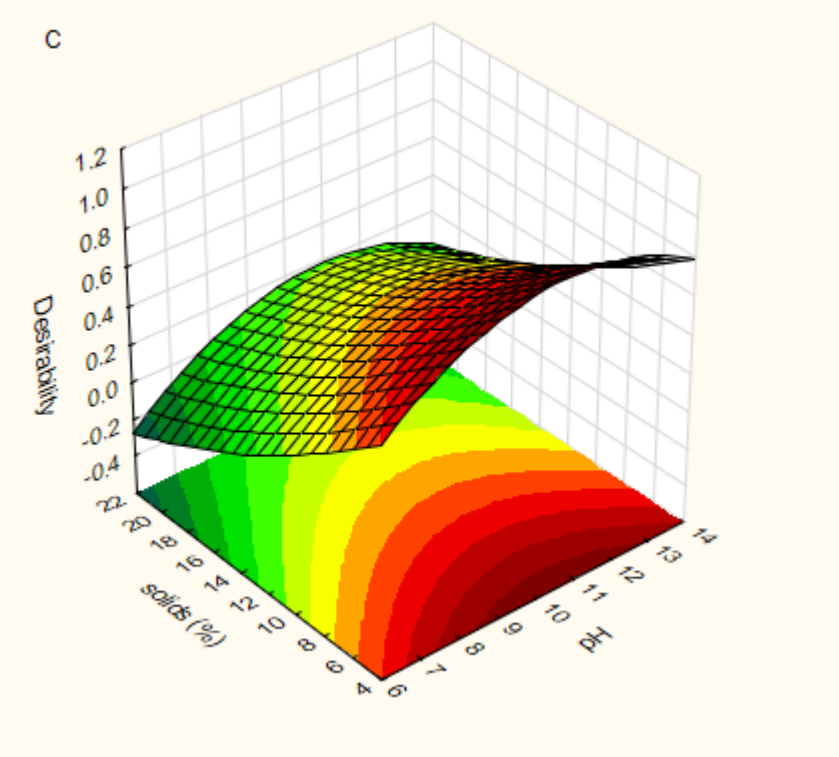


Figure 11; Multiple response optimization with desirability functions using alkaline extractions

Table 12; Summary of validation experiments

	Predicted value	Experimental value
Water extractions		
Protein content (%)	83.2	81.4
Protein yield (%)	58.2	56.8
Alkaline extractions		
Protein content (%)	88.1	85.5
Protein yield (%)	75.7	73.6

The results derived from the validation experiments showed that the difference between the experimental values and the predicted values ranged from 2.4% to 3% was not significant (Table 12). This shows that the mathematical model could competently express the relationship among parameters and proved its validity.

4.3 Quality tests

4.3.1 Amino acid profiling

The amino acid compositions showing the essential and non-essential amino acids for the different extraction methods and how they compare to recommended values are presented in Table 13 below. Generally, legumes, especially soy and pea are high in lysine but low in tryptophan as well as the sulphur-containing amino acids, which are cysteine and methionine (Sosulski and McCurdy, 1987; Sirtori *et al.*, 2012; Taherian and Mondor, 2012; Soderberg, 2013). This trend was seen in both groups of protein isolates. It was observed that alkaline extracted isolates, with higher protein levels (Table 11), displayed higher values than water extracted isolates and these values were statistically significant ($p<0.05$) as displayed in the t-test table in Table 41. The most abundant amino acids were glutamic acid, followed by aspartic acid, arginine, lysine and leucine, all of which were observed for alkaline extractions (Table 13).

Data reported by Tomoskozi *et al*. (2017) and Babault *et al*. (2015) where they investigated amino acid compositions of yellow pea isolates (Table 14) were comparable to the isolates in this study, especially alkaline-extracted isolates. Studies by Skylas *et al*. (2017) and Hughes *et al*. (2011) however, recorded higher amino acid values for mung bean isolates and soy isolates as shown in Table 14.

Table 13; Amino acid composition of isolates obtained from the two extraction methods in relation to recommended values from the FAO

	Alkaline-extracted isolates	**Water - extracted isolates**	**FAO (adult requirement)**
Essential amino acids (g/100 g protein)			
Histidine	0.90 – 1.62	0.40 – 0.80	>1.6
Isoleucine	1.44 – 2.11	0.46 – 1.19	>1.3
Leucine	2.91 – 3.88	0.74 – 1.16	>1.9
Valine	1.86 – 2.31	0.59– 1.01	>1.3
Lysine	1.68– 2.18	1.30– 1.58	>1.6
Phenylalanine	2.55– 3.71	0.56– 1.13	>1.9
Threonine	1.24– 1.71	0.59– 1.02	>0.9
Tryptophan	1.80 – 2.66	0.35– 1.10	>0.5
Methionine	0.42– 0.84	0.18– 0.22	-
Arginine	0.98– 4.00	0.94– 2.01	-
Non-essential amino acids (g/100 g protein)			
Alanine	2.43– 3.16	1.16– 2.97	
Aspartic acid	3.13– 7.58	1.80– 4.03	
Glutamic acid	3.04–12.26	1.22–8.90	
Glycine	0.99– 3.04	0.63– 2.40	
Proline	1.93–6.88	0.48– 2.85	
Serine	1.76– 2.24	0.58– 1.56	

Table 14; Amino acid composition of some pulse protein isolates

	Yellow pea protein isolate (Tomoskozi *et al.*, 2017)	**Yellow pea protein isolate** (Babault *et al.*, 2015)	**Yellow pea protein isolate** (Hoang, 2012)	**Soybean isolates** (Hughes *et al.*, 2011)	**Mung bean isolates** (Skylas *et al.*, 2017)
Essential amino acids (g/100 g protein)					
Histidine	3.40	1.90	2.33	2.30	2.16
Isoleucine	3.68	3.70	3.89	4.51	3.24
Leucine	8.16	6.40	7.84	7.50	4.08
Valine	4.81	4.00	5.11	5.94	4.02
Lysine	8.96	5.70	6.25	6.10	4.90
Phenylalanine	5.18	4.20	5.17	4.86	4.99
Threonine	3.30	2.80	4.46	3.56	2.14
Tryptophan	3.71	0.70	0.61	1.40	0.73
Methionine	0.78	0.80	1.60		
Arginine	7.15	6.60	7.93	5.90	5.71
Non-essential amino acids (g/100 g protein)					
Alanine	4.41	3.30	4.83	4.16	2.56
Aspartic acid	10.46	8.90	11.16	11.23	8.45
Glutamic acid	18.50	13.20	18.46	18.50	13.18
Glycine	4.68	3.10	4.82	4.66	2.17

Proline	5.01	3.40	4.64	5.18	3.02
Serine	6.09	3.90	5.71	4.87	3.78

4.3.2 Functional properties

Functional properties are good indicators of how well a protein substance will behave during processing and storage and the extent to which it can be used in food substances (Taherian and Mondor, 2012; Tiessen-dyck, 2014). Solubility, water absorption capacity and fat absorption capacity were investigated in this study for the optimized isolates that performed best in terms of protein content and yield as well as the protein isolates extracted under optimized conditions as per Figure 10 (water extractions) and Figure 11 (alkaline extractions).

4.3.2.1 Solubility

Solubility at a range of pH values (2 to 9) was investigated for isolates extracted at the optima values determined in the optimization stage (60 minutes, 6.7% solids and 40 °C for water extractions, and 100 minutes, pH of 10, 6.2solids and 20 °C for alkaline extractions). The solubility profiling of pea isolates was compared between the two extraction methods (Table 15). Generally, for both methods, pH below 4 and above 7 displayed the highest solubility (40% to 70%) with the lowest solubility of 4% to 11% being observed at pH of 4 to 6 (as shown in Table 15). The protein solubility displayed by the two extraction methods in this study were similar to results for other pea protein isolates reported in literature by authors such as those shown in Table 15 (S.Tian 1998; Taherian *et al*. 2011; J. Boye *et al*. 2010; Tomoskozi *et al*. 2017).

Tsumura *et al*., (2005) and Chalamaiah *et al*., (2010) discovered that the differences in protein solubility were caused by differences in protein composition of isolates. Protein solubility is also affected by the amino acid composition of protein isolates (Kiosseoglou and Paraskevopoulou, 2011). Although water-extracted isolates in this study recorded relatively good protein concentrations (83.3%) and yields (59.5%) during the optimization stage (Table 10), lower solubility values were observed (45% - 56%) as depicted in Table 15. Proteins contain carboxyl and amine groups that undergo ionization and deprotonation respectively when subjected to alkaline extractions (Valenzuela *et al.*, 2013). The ionization and deprotonation cause a surge in electrostatic repulsion between protein molecules which in turn also promotes interaction between the proteins and the alkaline extraction solvent. This causes more release of proteins, hence boosting solubility (Hamada, 2000; Valenzuela *et al.*, 2013). Alkaline extraction produced isolates that displayed better solubility profiling

(65% - 70%) and their values were more similar to literature values as compared to water extractions as indicated in Table 15. The alkaline treatment changes the structure of the proteins such that they become easier to extract and isolate which could in turn enhance the functional properties of the protein isolates obtained (Nazareth, 2009; Taherian and Mondor, 2012).

Table 15; Effect of pH on solubility of yellow pea protein isolates

	Solubility (%)					
pH	Water-extracted PPI (from this study)	Alkaline-extracted PPI (from this study)	S. Tian, 1998	Taherian *et al*. 2011	Boye *et al.* 2010	Tomoskozi *et al*. 2017
2	45	65	70	75	65	71
3	49	55	59	58	59	54
4	4	7	9	11	11	10
5	6	5	7	9	6	8
6	5	11	12	10	8	10
7	35	49	40	38	35	39
8	40	60	59	63	57	60
9	56	70	65	69	65	67

4.3.2.2 Water hydration capacity (WHC) and fat hydration capacity (FHC)

The values for water hydration and fat hydration capacities of isolates are given in Table 16. Data obtained from this study showed that the values for water hydration capacity for water-extracted isolates (0.5 - 0.63 g/g) were similar to those obtained for alkaline-extracted isolates (0.6 - 0.77 g/g). These values were not found to be significantly different from each other when tested statistically using a two-sample t-test, assuming equal variances as shown in Table 43. The values obtained from the study are lower than values of 2.5 g/g, 1.91 g/g, 2.1 g/g and 1.44 g/g reported by Reinkensmeier *et al*. (2015); Fernández-Quintela *et al*. (1997); Kaur & Singh (2007); Tian (1998), respectively, but similar to the value of 0.6 g/g obtained by J. I. Boye *et al*. (2010) and Pelgrom *et al*. (2013). The effectiveness of the protein material to absorb more water has been attributed to the structure of the protein as well as larger quantities of hydrophilic amino groups near the protein's surface (Stone *et al.*, 2014) .Studies conducted by Pelgrom *et al*. (2013) and Wang *et al*. (2010) attribute high values of water hydration capacities to high protein content. Ghribi *et al*. (2015) also observed that the water hydration capacity increased when isolates had a greater amount of polar amino acids. It is observed however that despite alkaline extractions producing isolates with higher protein content of 88.4% (Table 11) and statistically higher amino acid values (Table 13), their values obtained for WHC although being slightly higher, did not differ significantly from water-extracted isolates.

The highest value obtained for fat hydration capacity was 1.67 g/g and was observed for alkaline-extracted isolates (Table 16). This value differed significantly from and was 47% higher than the highest value of 0.88 g/g obtained by water-extracted isolates when tested statistically using a two - sample t- test, assuming unequal variances as shown in Table 42. Values from 1.1 g/g to 1.3 g/g were recorded by Stone *et al*. (2015) when the FHC of seven pea cultivars were investigated and this range was lower than the values obtained for alkaline extractions but higher than those of water extractions. Fernández-Quintela *et al.*, (1997); Fuhrmeister and Meuser, (2003) also reported values of 1.2 g/g and 0.8 g/g for fat hydration capacities of pea isolates with the latter being similar to the values (0.84 – 0.88 g/g) recorded for water-extracted isolates in this study (Table 16).

Stone *et al*. (2014) reported that the fat hydration capacity was greatly affected by the extraction method used while Stone *et al*., (2015) also attributed differences in FHC values to differences in protein composition as well as amino acid content. These reports agree with results obtained from this work where alkaline extractions producing isolates with higher protein content of 88.4% (Table 11) and statistically higher amino acid values (both essential and non-essential amino acids as seen in Table 13) also recorded higher values for fat hydration capacities (1.2 – 1.67 g/g) as shown in Table 16.

Table 16; Water and fat hydration capacities of isolates

	Water hydration capacity (g/g)	Fat hydration capacity (g/g)
Water extractions	0.5 - 0.63	0.84 - 0.88
Alkaline extractions	0.6 - 0.77	1.2 - 1.67

The functionality and nutritional value of pea isolates are good indicators of their successful utilization in the food and sports industry (Papalamprou *et al.*, 2009; Mune, Minka, René and Lape, 2013). The quality tests conducted on isolates derived from the two extractions showed that isolates derived from the alkaline extraction method were better in terms of functionality when the protein solubility (Table 15), water hydration and fat hydration capacities (Table 16) were measured for both extraction methods. Solubility profiles of isolates were correlated to water hydration and fat hydration capacities as the alkaline extraction method which produced isolates with higher solubility profiles also produced isolates with higher WHC and FHC values (Table 16). The differences observed in these functional properties are attributed to the differences in protein content (Table 10 and Table 11) and amino acid composition of isolates (Table 13) obtained by the different extraction methods. An increase in protein content increases the amount of essential amino acids contained in protein isolates which in turn positively affects the functional properties measured.

Solubility is a functional property that is important in determining how pea isolates would behave when used in subsequent food products. The greater the solubility of a protein product, the larger the range of applications that it can be used for (Prosekov *et al.*, 2018). It is also an indicator of the ease with which isolates can be added or how uniformly the proteins can be distributed within products (Crispin, 1995; Taherian and Mondor, 2012; Soderberg, 2013). Good solubility is a good determiner of sensory properties, emulsifying properties, texture, gel formations and foaming characteristics (Adebiyi and Aluko, 2011; Barac, M. Pesic, *et al.*, 2015). A high degree of solubility can also be used as an indicator of low levels of anti-nutritive factors, such as phytic acid, especially in isolates produced through ultrafiltration as was the case in this study (Taherian and Mondor, 2012). The high solubility at acidic and basic pH levels (45% - 55%) expressed by isolates from this study (Table 15) is a good indicator of the ability of the protein to be used in beverages (Boye, Zare and Pletch, 2010). The mouthfeel, retention of flavour, maintaining of quality, shelf-life and texture of products as well as the presence of water on the surface of products are determined by the WHC and FHC (Yu, Ahmedna and Goktepe, 2007; Shevkani, Singh, *et al.*, 2015; Singhal *et al.*, 2016)

The quality tests conducted on isolates derived from the two extractions showed that isolates derived from the alkaline extraction method were also better in terms of nutritional value when the amino acid profiling of isolates, especially the essential amino-acids produced by the two methods were compared in Table 13. The different values observed in the amino acid profiling of isolates from the two methods are attributed to differences in the protein content of the isolates produced by water extractions (Table 10) and alkaline extractions (Table 11). Alkaline-extracted isolates were found to have significantly (about twice the amount) higher amino-acid content for both essential and non-essential amino acids. The functional properties and amino acid profiling performed is a good indication that these proteins are acceptable for inclusion in human diets

5 CHAPTER FIVE: TECHNO-ECONOMIC ANALYSIS

5.1 Introduction

The process of protein extraction from yellow peas is an avenue that adds value to this pulse crop. This process has proven to be a technically feasible one and optimization was successfully achieved from the experimental section of this study, however there is the need to ascertain its economic feasibility. Key economic parameters as well as sensitivity analysis with regards to changes in the production process must be established. The main objective of this chapter was to evaluate and compare the economic feasibility of large-scale production of pea protein isolates using the two different extraction methods carried out in this work. The experimental work conducted showed that alkaline extractions had more superior technical results as compared to water extractions and lower solid loadings were also found to be preferable in terms of isolate purity (as observed in Section 4.3 and Section 4.2). The assumption cannot however be made that lower solid loadings and alkaline extractions also offer an economic advantage to higher solid loadings and water extractions based on the experimental results alone. This is typically because generally in biomass processing, lower solid loading comes with the cost of removal of excess water later on in the process (Bals and Dale, 2011; Razi Parjikolaei *et al.*, 2017) which may lead to higher operation costs although higher protein yields were achieved at lower solid loadings (Table 10 and Table 11). Comparison of economic data derived from experimental results therefore need to be compared.

The effect of the operating variables optimized in the experimental work on the internal rate of return (IRR) for both extraction methods used were investigated using response surface methodology. The IRR is an important economic parameter and its responses to variation in the independent variables used in chapter three (Table 5 and Table 6) was assessed by using it as a response variable in a CCD. The experimental outputs from chapter four were used as inputs for the CCD used in the techno-economic analysis to determine to what extent the optimum points predicted from the RSM approach changed. This helps to make a comparison between experimental optima and economic optima and assess to which extent these optima points shift or converge. Based on the results of the CCD, a techno-economic analysis was performed for water and alkaline extractions.

An ASPEN simulation and economic model was developed by Abdul Petersen, a postdoctoral researcher in Stellenbosch University and the author of this work then used experimental results from the laboratory work in this study to obtain economic output for the techno-economic analysis. The ASPEN plus model helps to obtain the mass and energy balances necessary to size the equipment needed in the protein extraction process. The required utilities for the process are also specified and costing is performed to determine the capital and operating costs. A cash flow analysis is carried out to establish key economic indicators after which a sensitivity analysis is performed.

5.2 Methodology

5.2.1 Description of Process model and Simulator

Firstly, a process flow sheet was developed, specifying all equipment and unit operations for the production process (Sirius Engineering). The flowsheet was implemented in ASPEN plus version 8.8 as a process model, by Abdul Petersen of Stellenbosch University and populated with experimental results obtained from this work. Aspen plus is a computer-based, process simulation software that utilizes underlying physical properties to model industrial processes. The ASPEN process model development begun with flowsheet construction with modular units and then specifying the operating conditions as well as the chemical components of each unit process. These specifications help expedite all the calculations that are vital in generating all material and energy balances necessary for equipment sizing and subsequent cost estimates of the equipment. The results from the calculations are displayed for each unit and each stream, making it easy for one to observe the outcome of the simulations on each chemical species of the process modelled by ASPEN plus (Eden, 2011, 2012). In this study the mass and energy balances as well as the cost estimates of some equipment were imported from ASPEN plus and used for the economic evaluations, which were performed with economic models developed by Abdul Petersen (Stellenbosch University) in Microsoft Excel

5.2.2 Production of pea protein isolate

The western cape of South Africa serves as a favorable location for the construction of a plant site for the production process of yellow pea protein depicted in Figure 12. In the economic model, the production process comprised of various unit operations from processing of the seed to processing (drying) of the protein. The pulse is fed into the hammer mill which reduces the yellow peas to flour (55 KW, Dijkink & Langelaan 2002). This helps to increase the surface area to volume ratio of the seeds, thereby increasing their dissolution. After the milling step is the hydration step where the milled flour is fed at a rate of 2000 kg/hr into a continued stirred tank reactor (CSTR), B2 in Figure 12. Water is added to the flour and a base (sodium hydroxide) is also used to adjust the mixture to the desired pH. The CSTR thoroughly mixes the constituents of the mixture at about 500 rpm to maximize the rate at which protein is dissolved. The resulting mixture (UP5) exits the CSTR and is sent to a centrifuge (CENT 1) where centrifugation occurs at a rate of 4000 x g to produce a clarified liquid which contains the solubilized protein (Sabbagh *et al.*, 2015). The solid particles are dried (DDGSDRY) and used as animal feed.

An ultrafiltration unit (ULTFILT) is then employed (66 KW) to concentrate the liquid protein to smaller volumes by removing excess water and other unwanted substances to reduce the energy cost of drying (Bahnasawy and Shenana, 2010). The clarified protein liquid is passed through an ultrafiltration membrane with a pore size of 5 KDa. A volume concentration ratio (VCR) of 4 is applied in the

ultrafiltration step. The protein is retained in the retentate (UP10) while the permeate (UP11A) contains the excess water and other lower molecular weight substances such as phytic acid. The permeate is recycled while the retentate is transported to a short –time evaporator (EVAP) in Figure 12. The retentate (concentrated yellow pea protein) is sent to a spray dryer (B3) to obtain a pea protein isolate (PPI) with a moisture content of about 7 %. The spray dryer uses a drying temperature of 65 °C as temperatures above 65°C tend to denature the proteins and do not preserve their quality.

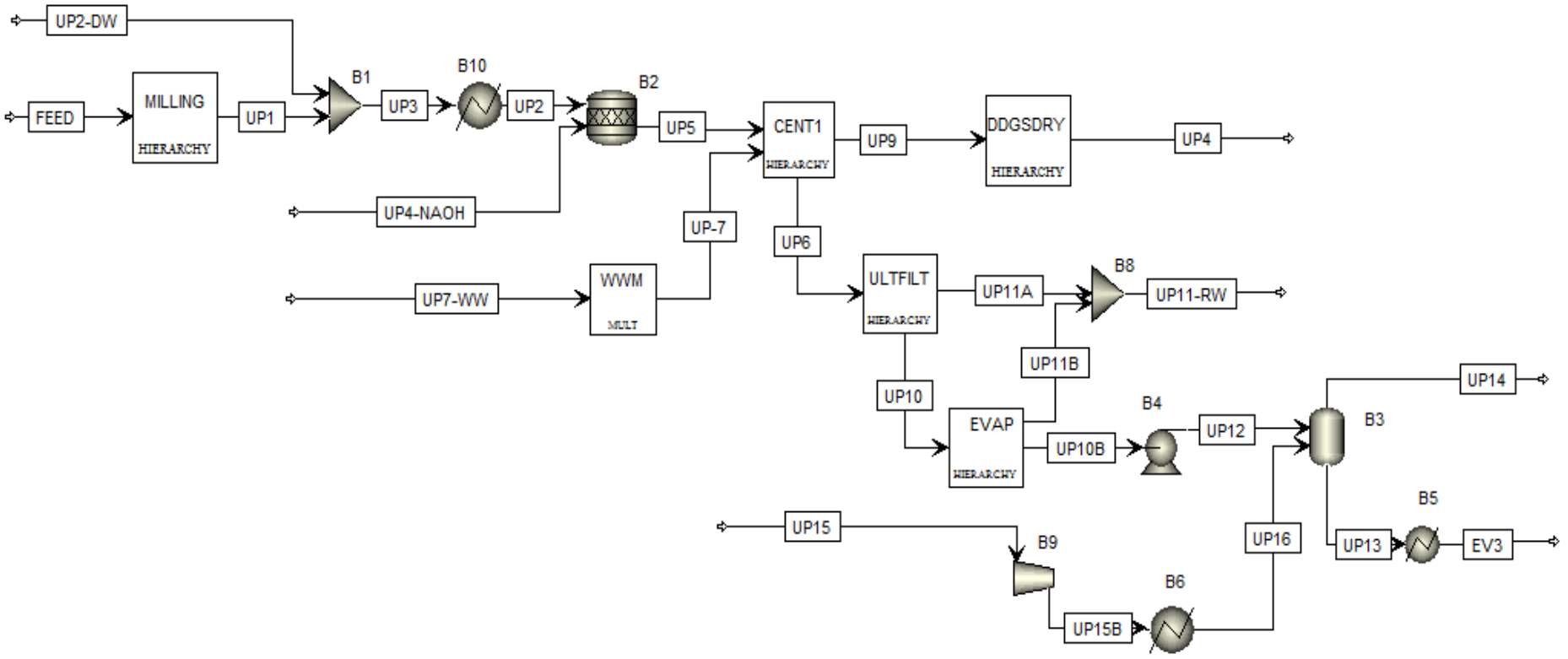


Figure 12; Pea protein production process (model developed by Abdul Petersen, Stellenbosch University)

5.2.3 Cost estimation of process

The following are the assumptions that formed the basis for the costing model for each of the scenarios modelled in this work (Abdul Petersen, Stellenbosch University). The bulk selling price of yellow pea protein on the market is about 90 to 113 rands per kg (www.alibaba.com, www.ekowarehouse.com). A cost of 100 rands per kg was therefore chosen as the cost for the pea protein isolate produced and used for all cost estimations in this study.

Table 17; Assumptions for costing model

	Assumptions
Production rate	2000 kg/h
Income tax rate	28 %
Project life	25 years
Working period	330 days per annum (7920 hours)
Working capital	5 % of the fixed capital investment
Salvage value	zero at the end of the project life
Discount rate	9.5 %

5.2.3.1 Capital cost estimation (CAPEX)

In the economic models developed, the purchase cost estimates for the different equipment were obtained from Sirius engineering while others were obtained from an in-house tool also developed by Abdul Petersen. The total plant direct cost (TPDC) as calculated in the model is the sum of all the equipment purchase costs as well as installation, piping, electricals, instrumentation. The total plant indirect cost (TPIC) consists of engineering and contingency. The TPDC and TPIC sum up to determine the fixed capital investment (FCI) while the working capital (WC) is calculated as a 5% of the FCI. The CAPEX in the economic model was then obtained by summing up the FCI and WC

5.2.3.2 Operating cost estimation

The economic model's evaluation of the operating cost, which is the cost involved in the direct or actual production of a product, is imperative in determining the viability of a project. This usually involves the cost of raw materials and labour. The operating cost is usually grouped into two as described below:

1. Fixed operating cost: They include cost of supervision, maintenance, operating labour, laboratory costs, royalty payments, insurance and plant overheads.

2. Variable operating costs: These costs depend directly on the rate or scale of production. The cost includes shipping and packaging, utilities, raw materials and other miscellaneous materials.

Some of the utility costs were extracted from ASPEN plus (Abdul Petersen) by defining the utility needed to meet the energy demand of a specific unit operation. Other utility costs were also manually evaluated using the utility prices in South Africa as well as the energy demand of the unit operation, produced by ASPEN. The cost of raw material and effluent charges were manually estimated using material stream balances that were produced in ASPEN plus. A summary of the operating costs used in the economic model is shown in Table 18.

Table 18; Cost of raw materials, utilities and waste disposal

Item	Cost	Reference
Electricity (KW/hr)	0.70	Sirius Engineering
Steam (kg/hr)	0.56	Sirius Engineering
Effluent	0.01	Dutta *et al*. 2015, Sirius Engineering
Yellow peas (R/kg)	4	www.alibaba.com
Fresh water (R/kg)	0.018	Utility service south Africa
Potassium hydroxide (R/kg)	240	www.alibaba.com
Selling price of fibre and starch residue (R/kg)	2.95	Sirius Engineering

5.2.4 Cash flow analysis

A discounted cash flow (DCF) analysis was performed for all scenarios (Table 46, Table 47, Table 48 and Table 49). This allows for all economic parameters such as IRR, NPV, payback time and minimum selling price of pea isolate to be determined. The minimum selling price is that at which the NPV was zero at an acceptable minimum IRR of 9.5% in real terms and this is achieved by iteration of the selling price of the pea isolate. The IRR is the discount rate at which the NPV value is zero and it is the maximum rate of return that an investment could acquire (Seider, Seader and Lewin, 2003; Tan *et al.*, 2015). A value of 28 %, which is the corporate tax rate in South Africa was used in the calculations for the DCF (DeloitteTouche, 2016).The total revenue was derived from the sales of the Pea protein isolate as well as the sales from the starch and fibre residue. The net profit is calculated as the total revenue minus the depreciation and the operating cost.

5.3 Results and Discussion

The results shown below were derived from the ASPEN simulation and economic models. Economic results were then obtained to compare the influence of changes in process parameters (pH, time, temperature, solids) using IRR as an economic parameter.

5.3.1 Effects of independent parameters on IRR

The effects of operating parameters (pH, temperature, time and solid loading) on IRR of both extraction methods were investigated using a rotatable central composite design (CCD) generated in Statistica version 10 for all of the laboratory runs generated in the experimental section (see Table 5 for water extractions and Table 6 for alkaline extractions). The IRR in response to variation in temperature, time and solids loading are shown in Table 19(water extraction) and Table 20 (alkaline extraction), where variation in pH was also included in the latter. Four replicates were used at the centre (C) of the CCD and the experiments were conducted in a random manner for each extraction method.

Table 19; Effects of temperature, time and solids on IRR for water extractions as determined by CCD

Experiment number	Temperature (°C)	Time (minutes)	Solids (%)	IRR (%)
1	30	30	6.7	31.2
2	30	30	20.0	15.9
3	30	120	6.7	27.9
4	30	120	20.0	11.1
5	50	30	6.7	34.7
6	50	30	20.0	4.2
7	50	120	6.7	21.3
8	50	120	20.0	20.0
9	23	75	13.3	21.5
10	56	75	13.3	19.6
11	40	7	13.3	22.9
12	40	150	13.3	33.6
13	40	75	2.0	30.1

14	40	75	24.0	31.2
15 (C)	40	75	13.3	33.0
16 (C)	40	75	13.3	33.4
17 (C)	40	75	13.3	35.7
18 (C)	40	75	13.3	33.7

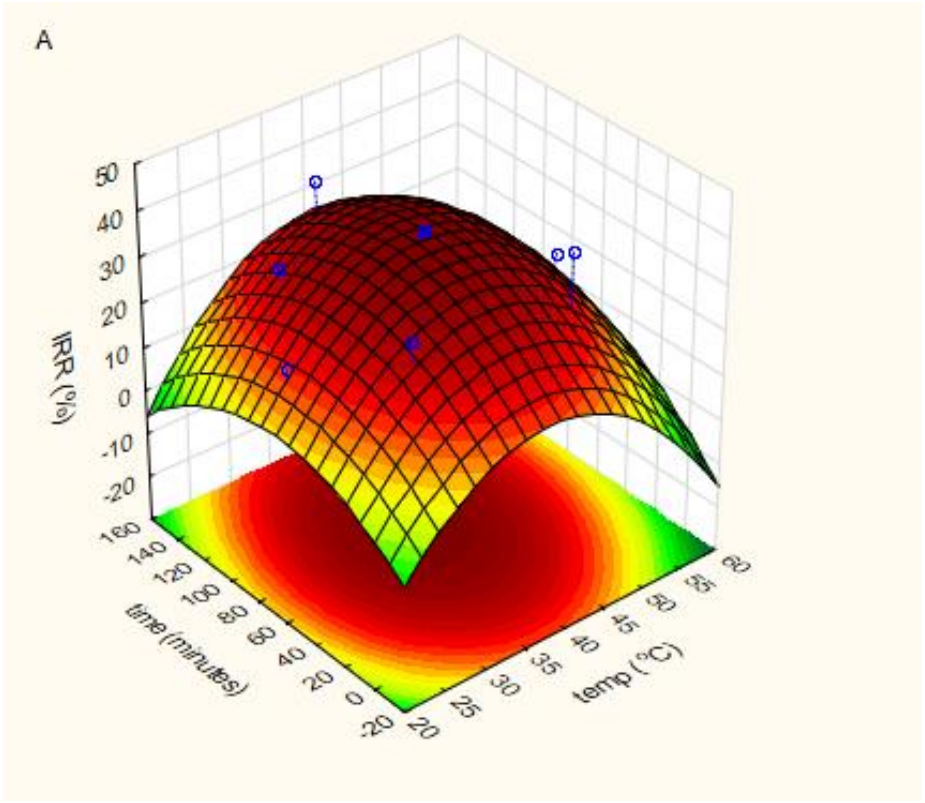
Table 20; Effects of pH, temperature, time and solids on IRR for alkaline extractions as determined by CCD

Experiment number	pH	Temperature (°C)	Time (minutes)	Solids (%)	IRR (%)
1	8.5	35	70	9.0	26.4
2	8.5	35	70	16.7	6.9
3	8.5	35	150	9.0	41.5
4	8.5	35	150	16.7	14.7
5	8.5	65	70	9.0	20.6
6	8.5	65	70	16.7	16.7
7	8.5	65	150	9.0	30.2
8	8.5	65	150	16.7	29.4
9	11.5	35	70	9.0	26.6
10	11.5	35	70	16.7	22.8
11	11.5	35	150	9.0	26.0
12	11.5	35	150	16.7	15.7
13	11.5	65	70	9.0	39.3
14	11.5	65	70	16.7	1.2
15	11.5	65	150	9.0	28.2
16	11.5	65	150	16.7	16.9
17	7	50	100	12.9	26.4

18	13	50	100	12.9	30.1
19	10	20	100	12.9	15.1
20	10	80	100	12.9	32.0
21	10	50	180	12.9	23.7
22	10	50	180	12.9	20.7
23	10	50	100	5.2	40.2
24	10	50	100	20.6	18.6
25(C)	10	50	100	12.9	25.7
26(C)	10	50	100	12.9	24.8
27(C)	10	50	100	12.9	37.6
28(C)	10	50	100	12.9	23.5

The highest IRR of 41.2% was obtained for isolates derived from alkaline extractions (Table 20) and this was 15.7% higher than that of highest IRR of 34.7% derived for water extractions (Table 19).

Response surface plots were developed to further illustrate the effects of the operatiing parameters on the IRR. Figure 13A shows the effects of time and temperature on IRR for water extractions. It was discovered that as the temperature and time increased, the IRR also increased until an optimum was achieved, after which there was a decline in their values. No significant increase in IRR was attained above values above 40 °C and 60 minutes for temperature and time respectively and a maximum IRR value of 27% was achieved at these optimum values. The effects of solids and time on protein content for water extractions are depicted in Figure 13B. The IRR levels off at 20% and saw a reduction when temperatures beyond 40 °C and solids above 6.5% were used.



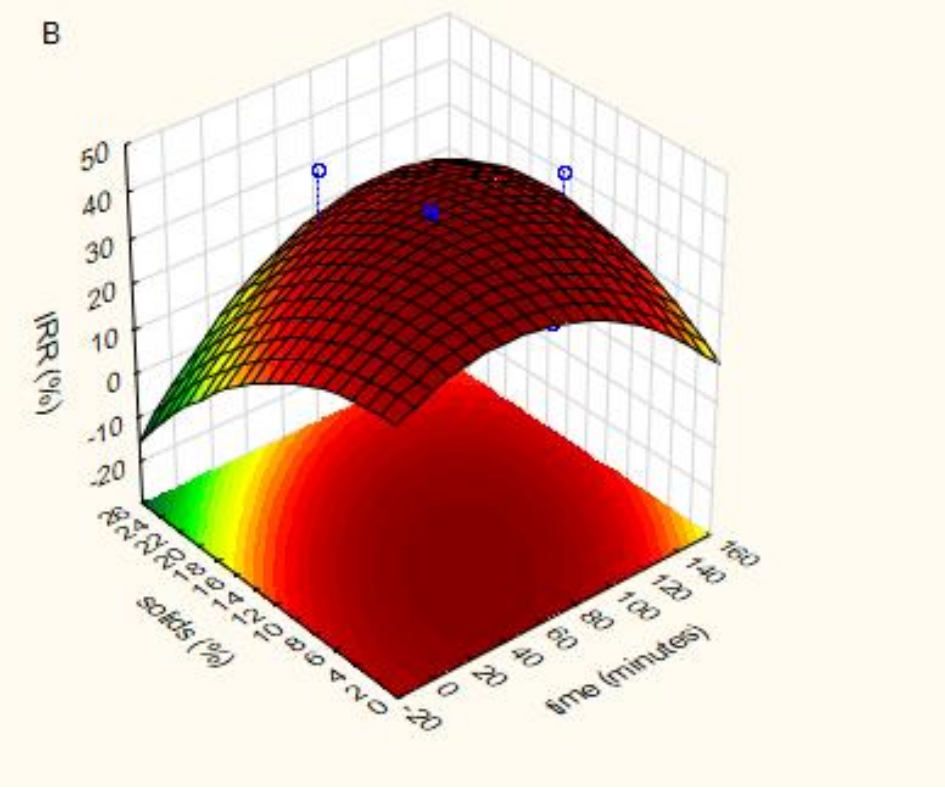
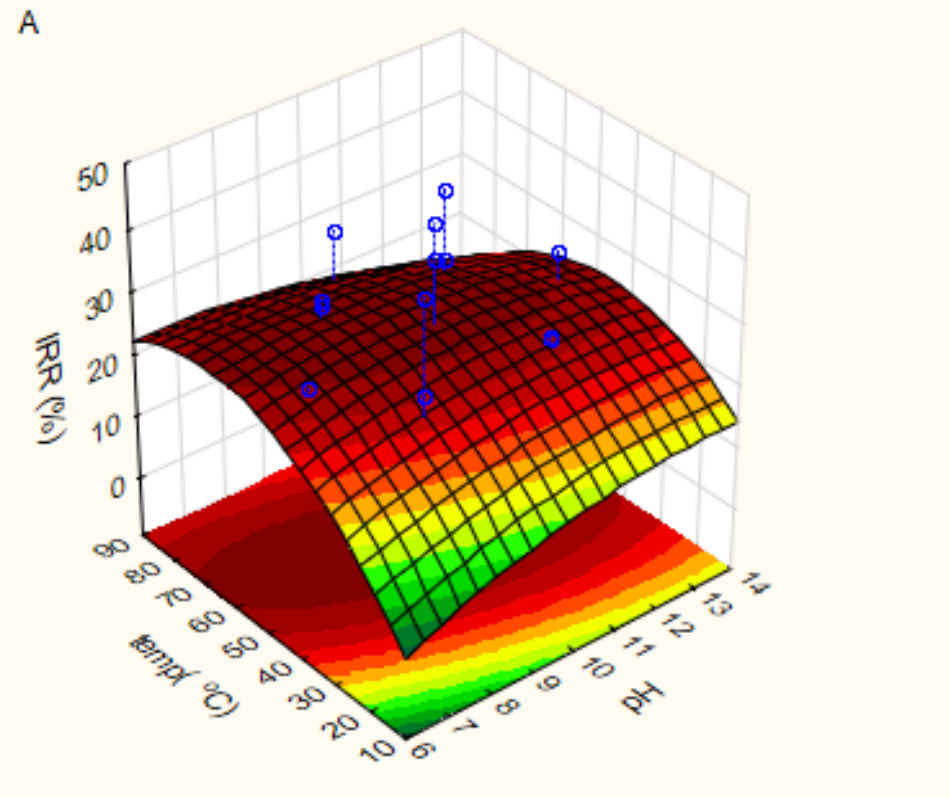


Figure 13; Response surface plots predicting IRR for water extractions

The influence of temperature and pH on IRR for alkaline extractions is depicted in the surface plot of Figure 14A. An increase in temperature and pH resulted in an increase in IRR until a maximum value of 23%. No substantial increase in IRR was achieved above values of 10 and 30°C for pH and temperature respectively. The IRR for alkaline extractions experienced a linear increase as the solids were decreased (below 7%) and time was increased (above 60 minutes) as shown in Figure 14.

The trends observed in the surface plots for IRR of both extraction methods were similar to results obtained for protein yield in the experimental section of the study. Alkaline extractions and scenarios employing lower solid loading increased the protein content and protein yield hence increasing the total revenue of the production process. This is similar to reports by Bals and Dale (2011) where a decrease in the solid loadings enhanced the yield of leaf protein, which in turn increased profitability. The levelling off experienced in IRR values beyond temperatures of 40 °C, times of 60 minutes and solid loadings of 6.5% for water extractions (Figure 13) is due to the drop in protein yield observed beyond these parameters. Similarly, for alkaline extractions, at values beyond a pH value of 10, time of 60 minutes, temperature of 30 °C and solid loading of about 7% (Figure 14), a decrease in yield is experienced which causes a subsequent drop in IRR values.



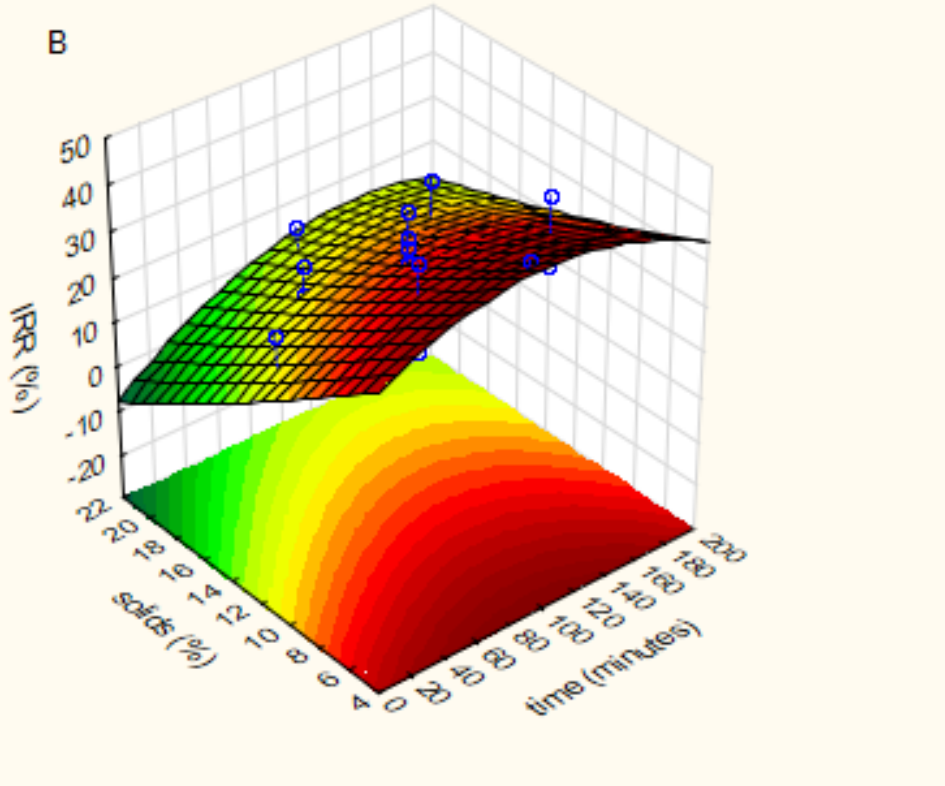


Figure 14; Response surface plots predicting IRR for alkaline extractions

An analysis of variance was also carried out to determine which of the independent variables had a significant impact on the IRR. The effects of these variables are depicted in the pareto charts of Figure 15A and Figure 15B for water and alkaline extractions respectively.The pareto chart in Figure 15A shows that for water extracted isolates, temperature, time and especially solids contributed heavily within a 95 % confidence interval. The IRR for alkaline extractions was significantly affected ($p<0.05$) by the solids (Figure 15B). Lower solid loading increased the protein content and protein yield hence increasing the total revenue of the production process which inturn influences the IRR. For water extractions, an increase of 67% was recorded for protein yield when solids were decreased by 33.5% leading to an increase in IRR from 4.2% to 34.7% (Table 19). Alkaline extractions also experienced an increase of about 47% in protein yield when solids were decreased from 16.7% to 9% (see Table 11) with a subsequent increase in IRR from 1.2% to 41.2% (Table 20).

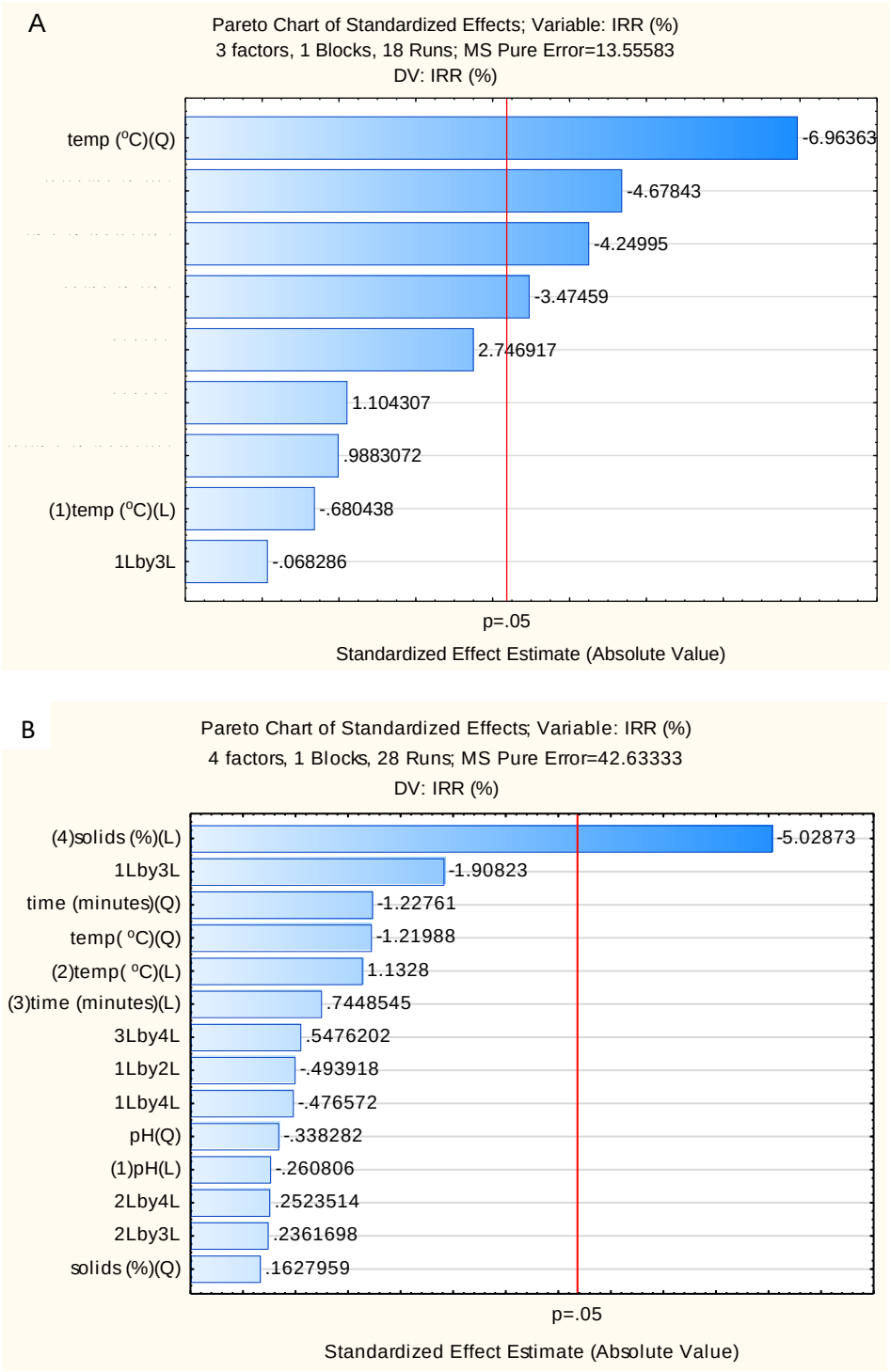


Figure 15; Pareto chart of standardized effects of IRR for (A) water extractions and (B) alkaline extractions

The values for IRR obtained in this study are more correlated with protein yield than protein content for both extraction methods as observed in the correlation coefficient vales obtained in Figure 20 - 23. Furthermore, the independent factors that significantly affected protein yield for water extractions

(temperature, time and especially solids as observed in Figure 7B) were the same factors that had a major influence on IRR values (see Figure 15A). Similarly, for alkaline extractions, both the protein yield (Figure 9B) and IRR (Figure 15B) were significantly affected by solids. This shows the massive role that solids and in effect protein yield play on economic output and profitability.

5.3.1.1 Response desirability optimization

Desirability profiler using response desirability optimization was used to optimize the independent variables to maximize IRR. A value of 0 was selected for the lowest IRR obtained in the CCD while a value of 1 was selected for the highest IRR. The desirability optimization for water extractions reported optimum values of 40 ℃, 75 minutes and 6.7% for temperature, time and solid loadings respectively. At these optimum values, the predicted for IRR was 37.7%. Optimum values for pH, temperature, time and solid loadings for alkaline extractions were 10, 35 ℃, 100 minutes and 6.2% respectively with a predicted IRR of 43.2%.

The results from the response surface optimization and statistical evaluation of the IRR showed that for both extraction methods, solids loading was a very important parameter when evaluating the economic viability of the production of pea protein isolate (Figure 15) with alkaline extractions proving to be more profitable than water extractions (Table 20). IRR values were also found to correlate with protein yield since laboratory runs that had higher protein yields (see Table 19 and Table 20) resulted in higher revenue, thus increasing profitability. A more detailed evaluation elaborating the techno-economic analysis performed for water and alkaline extractions as further elucidated below. Optimum experimental values and conditions (lower solid loading and higher protein yield) were compared to values and conditions that did not perform as well (higher solid loading and lower protein yield) for both extraction methods. The scenarios elaborated on are:

1. Water extraction (WA),6.7% solids
2. Water extraction (WA),20 % solids
3. Alkaline extraction (AE),6.7 % solids
4. Alkaline extraction (AE), 20 % solids

5.3.2 CAPEX AND OPEX

The total capital expenditure of the four different scenarios are presented in Table 21 below.

Table 21; Estimation of capital investment for 2000 kg/h of yellow peas

Item (Rand/annum)	Water extraction (6.7 % solid loading)	Water extraction (20 % solid loading)	Alkaline extraction (6.7 % solid loading)	Alkaline extraction (20 % solid loading)
Feed system	3 530 531	3 530 531	3 530 531	3 530 531
Grain cleaning system	112 083	112 083	112 083	112 083
Milling	607 013	607 013	607 013	607 013
Process vessels	1 611 846	839 408	1 612 169	839 807
Centrifuge	5 974 225	3 111 224	5 975 422	3 112 703
Ultrafiltration system	6 521 446	2 941 589	6543068	2 958 166
Evaporator	4 276 389	4 190 842	4 862 165	4 440 889
Spray dryer	12 143 909	11 900 977	13 820 867	12 645 053
Bagging	250 000	250 000	250 000	250 000
Auxiliaries	1 059 044	1 059 044	1 059 044	1 059 044
DDGS Dryer	5 819 040	5 751 680	5 644 655	5 674 005
DDGS Dryer Blower	50 663	49 757	48 329	48 719
Heat exchanger	549 464	543 104	532 998	535 769
Total Equipment purchase cost (TEPC)	**42 505 652**	**34 887 251**	**44 598 344**	**35 813 781**
Steam utilities	7 968 246	7 349 855	8 256 817	7 470 384
Water Treatment	860 516	653 666	857 823	652 440
CIP System	2 583 033	2 583 033	2 583 033	2 583 033
Cooling Towers	159 031	155 767	1 802 90	164 754
Electrical Systems	4 702 210	4 584 734	4 812 547	4 628 317
Civils	20 403 610	20 403 610	20 403 610	20 403 610
	36 676 646	**35 730 665**	**37 094 120**	**35 902 538**

Total plant direct cost (TPDC)	**79 182 299**	**70 617 917**	**81 692 464**	**71 716 319**
Engineering	8 278 320	7 421 882	8 529 336	7 531 722
Contingency	12 417 480	11 132 823	12 794 005	11 297 583
Total plant indirect cost (TPIC)	**20 695 800**	**18 554 70**	**21 323 342**	**18 829 305**
Other costs	3 600 902	3 600 902	3 600 902	3 600 902
Fixed capital investment (FCI)	**103 479 001**	**92 773 524**	**106 616 708**	**94 146 526**
Working capital	5 173 950	4 638 676	5 330 835	4 707 326
Total capital investment (CAPEX)	**108 652 951**	**97 412 200**	**111 947 543**	**98 858 852**

WA 6.7 % solids, WA 20 % solids, AE 6.7 % solids and AE 20 % solids reported CAPEX values of 109, 97, 112 and 99 million rands respectively, with the total plant direct cost being the major contributor to these values, followed by the TPIC and other costs (as depicted in Figure 16). FCI for the production processes with lower solid loadings (103 479 001 and 106 616 708 for water and alkaline extractions respectively) were about 11% greater than the production processes with the higher solid loadings (R92 773 524 and R94 146 526 for water and alkaline extractions respectively as shown in Table 21). This is because of the larger process vessels (R1 611 846 and R1 612 169) required for handling higher processing volumes of material because large quantities of water are needed for their effective extraction (see Table 21). The values of FCI for the alkaline extractions (R106 616 708 and R94 146 526) were slightly higher than those of the water extractions (R103 479 001 and R92 773 524) due to the addition of base needed in the alkaline extraction process (Table 21). The highest value for the purchased equipment cost (R11 900 977 - R13 820 867) was recorded by the spray dryer for both methods.

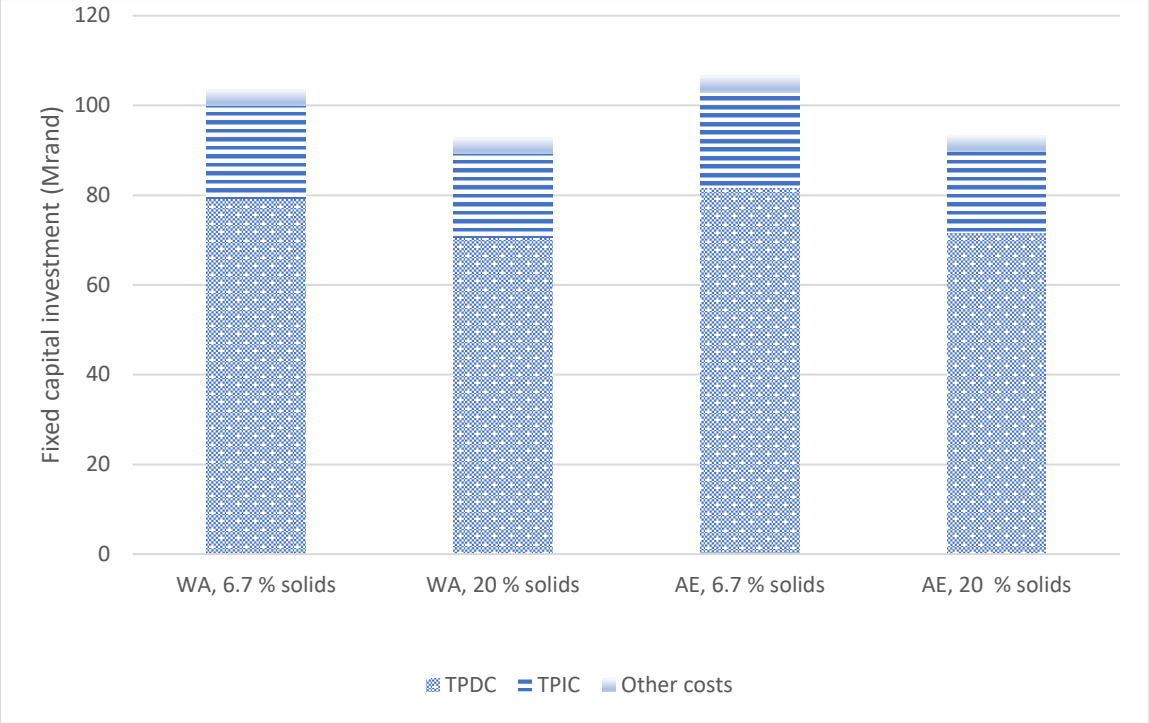


Figure 16; Comparison of the fixed capital investment for the four scenarios discussed

The mass flow of the different scenarios used to determine the OPEX as well as the constituents of the OPEX are summarized in Table 22 and

Table 23. Table 22; Mass flows for OPEX calculation

Mass flows	Water extraction (6.7 % solid loading)	Water extraction (20 % solid loading)	Alkaline extractions (6.7 % solid loading)	Alkaline extractions 20 % solid loading)
Fresh water (kg/hr)	6856	4807	6812	4782
Potassium hydroxide (kg/hr)	-	-	10	8
Steam (kg/hr)	5507	4685	5844	4946
Electricity (KW)	842	817	879	823
Protein production (kg/hr)	285	138	337	194
DDGS production (kg/hr)	1634	1781	1582	1725

Table 23; Operating cost estimation

Item (Rand/annum)	Water extraction (6.7 % solid loading)	Water extraction (20 % solid loading)	Alkaline extractions (6.7 % solid loading)	Alkaline extractions 20 % solid loading)
Yellow peas	63 360 000	63 360 000	63 360 000	63 360 000
Fresh water	760 143	532 990	755 264	530 233
Potassium hydroxide	-	-	19 008 000	15 206 400
Steam	24 426 372	21 349 401	25 918 440	21 936 094
Electricity	4 686 562	4 493 050	4 871 275	4 564 461
Waste	552 677	349 516	549 798	348 423
Total variable operating cost	**93 785 755**	**90 084 957**	**114 462 777**	**105 945 612**
Maintenance	3 104 370	2 783 206	3 198 501	2 824 396
Labour	6 244 445	6 244 445	6 244 445	6 244 445
Total fixed operating cost	**9 348 815**	**9 027 651**	**9 442 946**	**9 068 840**
Overhead	15 470 185	14 866 891	18 585 858	17 252 168
Total operating cost (OPEX)	**118 604 755**	**113 979 498**	**142 491 581**	**132 266 621**

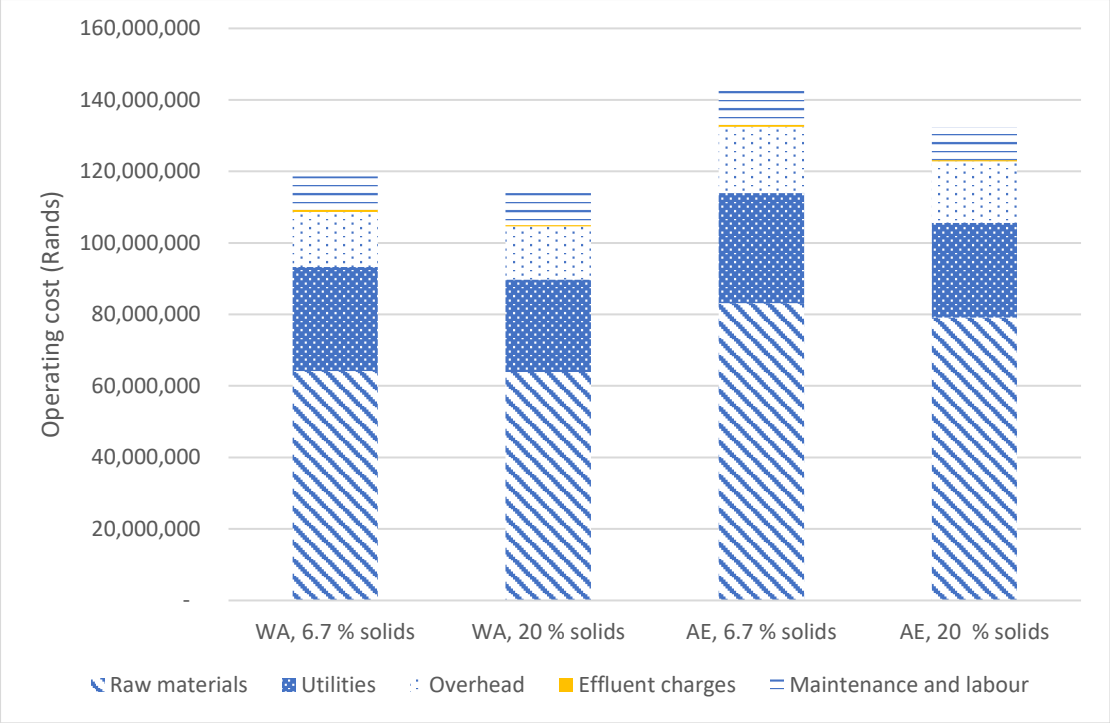


Figure 17; The contributions of utilities, effluent charges, raw materials, overhead, maintenance and labour to the total operating cost

WA 6.7 % solids, WA 20 % solids, AE 6.7 % solids and AE 20 % solids reported OPEX values of R118 604 755, R113 979 498, R142 491 581 and R132 266 621 respectively with the cost of raw material (R64 R120 143, R63 892990 and R83 123 264) as the leading contributor (> 50 %) as observed in Figure 17. The second highest contributor was the cost of utilities (R25 842 451 to R30 789 715) followed by overhead costs (R14 866 891 to R18 585 858) with lowest contributor to the OPEX being the cost of waste disposal (R348 423 to R552 677 as shown in

Table 23). The highest operating cost of R142 491 581 was recorded by Alkaline extraction, 6.7 %. This is because this scenario requires more process water (6812 kg/hr) for extractions due to the low solid loading used in the production of protein isolate (see Table 22). Also, a higher amount of base contributing a total cost of 19 008 000 to the variable operating cost (

Table 23) is needed to enable it to reach the optimum pH level required for efficient solubilization and extractions of proteins.

5.3.3 Analysis of discounted cash flow

Table 24; Economic summary of PPI production comparing scenarios at a feed rate of 15840 tonnes per annum (2000kg/hr).

	Water extraction (6.7 % solid loading)	Water extraction (20 % solid loading)	Alkaline extractions (6.7 % solid loading)	Alkaline extractions 20 % solid loading)
TCI (Rand)	108 652 951	97 412 200	111 947 542	98 853 854
OPEX (Rand)	118 604 755	113 979 498	142 491 581	132 266 621
NPV (Rand)	611 942 030	-77 468 928	852 255 939	-168 541 080
IRR (%)	34.5	4.2	41.5	1.2
Minimum selling price (Rand/kg)	88.1	275.3	73.7	255.4
Ration of Production cost per kg of yellow pea processed	0.59	0.98	0.51	0.75
Production cost (R/kg of PPI produced)	72.37	148.06	57.83	92.90
Payback period	1.58 years	6 years	1.01 years	12 years

The cash flow analysis depicted in Table 24 shows that the NPV values obtained for Water extraction, 6.7% and Alkaline extraction, 6.7% were positive, indicating that they are economically viable. IRR values higher than the acceptable minimum return rate of 25 % required by investors were also obtained for these two scenarios, further proving their economic viability (Amponsah, 2015). Alkaline extraction, 6.7 % solids had the highest IRR (41.5%) and NPV (R852 255 939) values (see Table 24), making it the most profitable although it recorded the highest OPEX (

Table 23) and CAPEX values (Table 21) of R142 491 581 and R111 947 543 respectively. At higher solids, it was observed that the utilities(R29 112 934 and R30 789 715 for water and alkaline extractions

respectively) required as well as the costs involved in water removal and waste disposal (R552 677 and R549 798 for water and alkaline extractions respectively) were higher causing an increase in production costs (as illustrated in Figure 17). There is however an increase in protein yield at lower solids which increases the revenue of the production process (Table 19 and Table 20). Comparison of production cost relative to the economic value of PPI produced, revealed that although the total OPEX for the scenarios with lower solids were higher than those with higher solids (

Table 23), the production cost per amount of PPI were higher at lower solids (Table 24). A 77% increase in the protein yield for alkaline extractions observed a 62% decrease in production when solids were decreased from 6.7% to 20% while for water extractions, a similar decrease in solids resulted in a two-fold increase in protein yield with the production cost being reduced by half (Table 24). Higher protein production yields observed when employing lower solids resulted in increases in the total revenue which led to a bigger economic advantage over the scenarios employing higher solids despite their decreased operation costs as compared to the processes employing lower solids. WA 6.7% solids and AE 6.7% solids recorded values of R88.1 and R73.7 for the minimum selling price of PPI (Table 24). These selling prices are about 2.2 % to 34.7% less than the selling price on the market (90 to 113 rands per kg) and manufacturers or investors stand to make a sizeable profit even if their prices are significantly less than existing market prices.

A sensitivity analysis was performed on the scenarios to investigate the effects of selling price of pea protein isolate (Figure 18) and price of yellow peas (Figure 19) on IRR. Identical trends were detected for all four scenarios. For influence of cost of yellow pea on IRR, a maximum cost of R5 per kg and a minimum cost of R2 per kg was used for sensitivity analysis (Figure 18). It was observed that as the cost of yellow pea increased from R2 per kg to R3 per kg and further to R4 per kg, the IRR observed a 40 to 55% decrease for the scenarios with 20% solids while those with 6.7% solids reported a 7% decrease in IRR (shown in Figure 18). At a selling price of R5 per kg, the scenarios with 20% solids had IRR values of 0% and 1.4% for alkaline and water extractions respectively while IRR values of 38.5% and 30.3% were derived for AE 6.7% solids and WE 6.7% respectively. The effect of changes in the selling price on IRR is illustrated in Figure 19. It was observed that 10% increase in the selling price of PPI resulted in a 12% increase in IRR. The lack of profitability of the scenarios employing lower solid loading were further demonstrated as it was observed that a 10% decrease in in the selling price of PPI reduced the IRR to a value of 0. This is because at lower solids, the protein yields recorded are very low and as such a substantial increase in the selling price in needed to boost profitability.

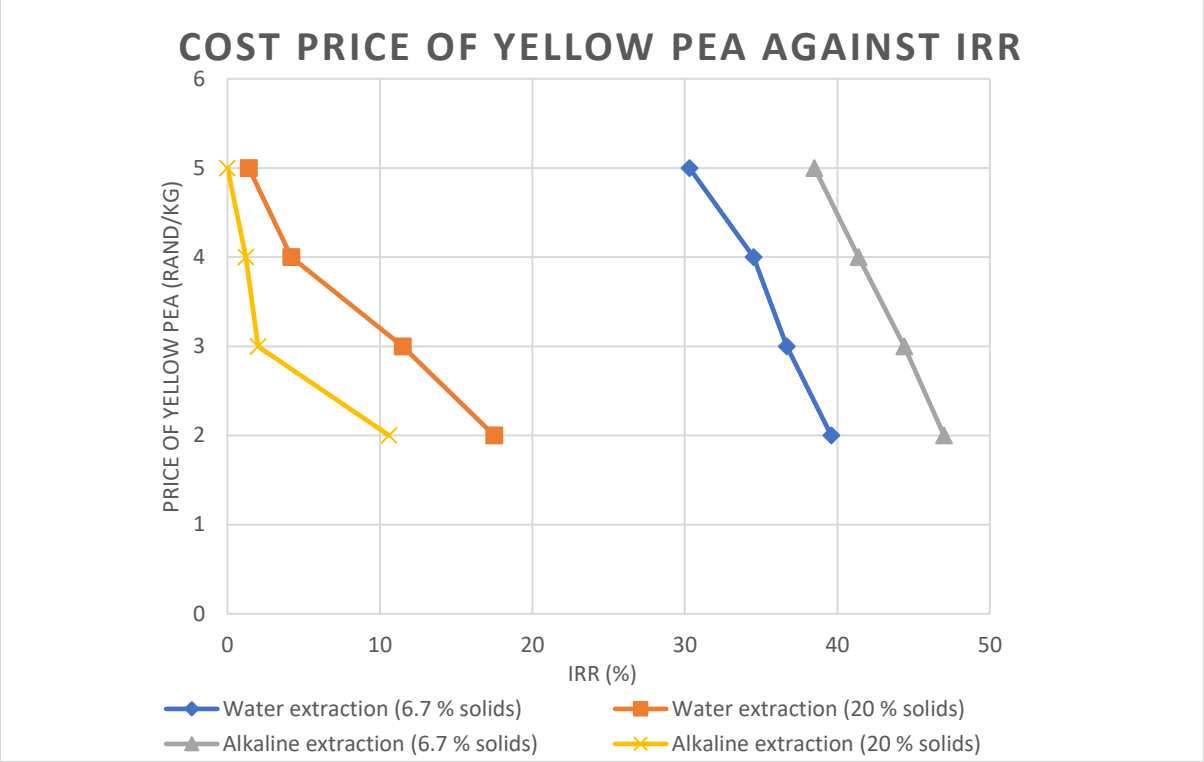


Figure 18; Effect of price of yellow pea on IRR of the scenarios

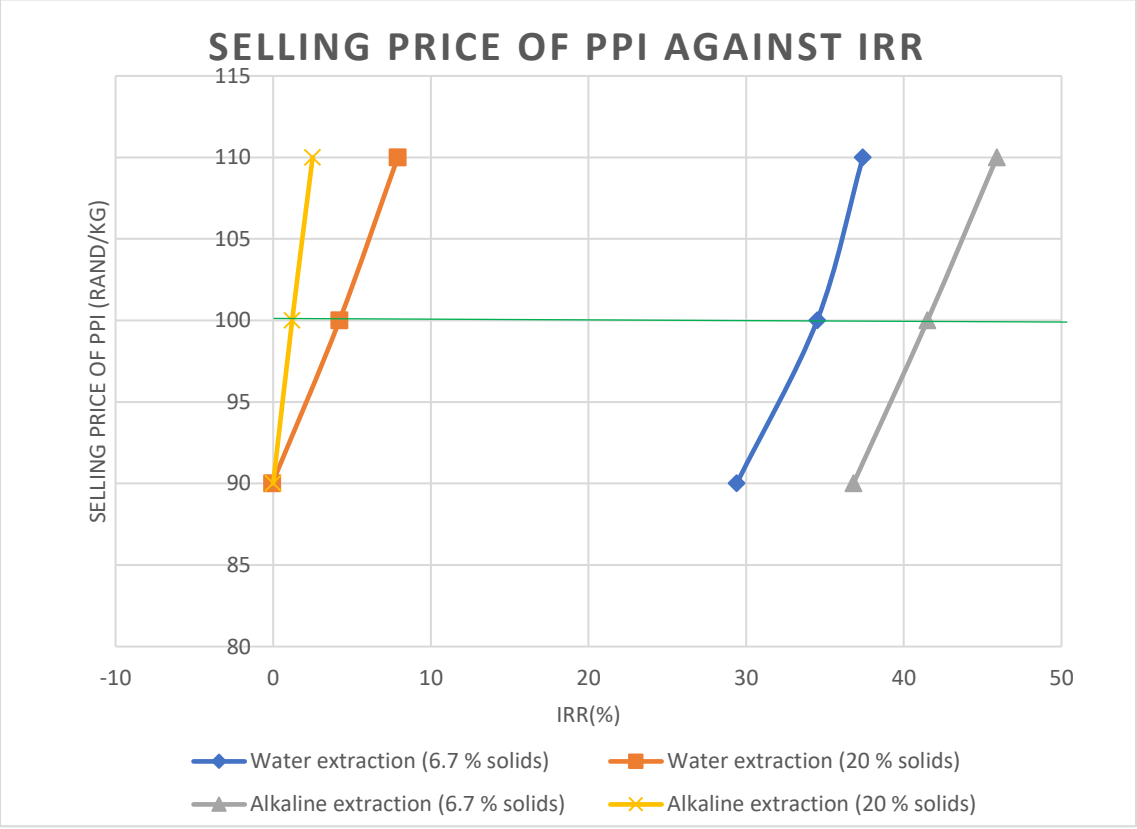


Figure 19; Effect of selling price of PPI on IRR of the scenarios

6 CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The variety of pea is important in the developing of a production process that aims at maximizing protein yields Two methods used in the wet extraction of dietary protein from yellow pes (water extractions and alkaline extractions) were selected from literature and compared. Three different cultivars (Slovan, Astranoute and Salamanca) were obtained and successfully screened using the mentioned wet extraction methods. Protein content and protein yields were compared and the best cultivar was chosen for the optimization stage of the project. Slovan had the highest values of protein content and protein yield for both water and alkaline extraction methods, with Salamanca being second highest and Astranoute with the lowest values. Slovan was therefore the best performing cultivar among the three. .Based on extraction results, South African-yellow pea cultivar performs similarly to other international yellow pea varieties reported in literature.The effect of processing parameters on protein content and protein yield were investigated on a laboratory scale and optimized. Response surface methodology was used to optimize process parameters of pH, time, temperature and solids to maximize protein content and protein yield as well as IRR. The optimum conditions for production of pea protein isolates with good economic viability were established.

The following conclusions were drawn from this study:

Alkaline extractions performed better in terms of protein content and protein yield as compared to water extractions. Proteins contain carboxyl and amine groups that undergo ionization and deprotonation respectively when subjected to alkaline extractions resulting in an abundance of charges causing molecules to repel against each other. The repulsion of protein molecules promotes interaction between the proteins and the alkaline extraction solvent, enhancing the dissolution of proteins in solution. Also, the bonds between proteins and other components of the yellow pea seed are more easily severed by hydroxyl ions contained in the alkaline.

With regards to quality, pea protein isolates produced by alkaline extractions also had better functional properties and higher values of essential amino acids. The alkaline treatment changes the structure of the proteins such that they become easier to extract and isolate. This in turn enhanced the functional properties as well as the amino acid composition of the protein isolates obtained since protein levels greatly influence the functional properties and the amino acid composition of isolates. Solubility and fat hydration capacities of isolates were affected by differences in amino acid composition and protein contents with those for alkaline-extracted isolates being significantly higher

while water hydration capacity did not differ significantly between isolates extracted using either water or alkaline extractions.

Solid loading was found to be a very important factor with regard to both technical (protein content and protein yield) and economical performances for both water and alkaline extractions. An increase in protein yield observed at lower solid loadings, increased the revenue of the production process with a subsequent increase in economic parameters (IRR, NPV). This offsets the higher production costs that are associated with using lower solids giving it an economic advantage over employing higher solids in the production process. Also, it was observed that protein yield correlated better with IRR values as compared to protein content for both methods. The independent parameters that significantly affected protein yield also significantly affected IRR, proving the massive role that protein yield plays in determination of economic profitability. The optimum conditions obtained for production of PPI for water extractions were a solid loading of 6.7%, time of 60 minutes and a temperature of 40 ℃ while those for alkaline extractions were pH of 10, time of 100 minutes, temperature of 20 ℃ and solid loading of 6.2%

6.2 Recommendations

The applications of pea protein isolates can be expanded beyond usage in human food applications by using them in animal feed. Animal feed trials should be used to determine the suitability of PPI as part of animal feed, with aquaculture being a suitable choice since large amounts of PPI are not required for trials as compared to terrestrial animals. Animal feed mostly comprising of protein are quite costly because the ingredients used in their preparation are usually imported. It would therefore be beneficial to supply locally-produced to reduce high costs associated with feed. Trials to determine thresholds of including PPI in animal diets needs to be carried out to ascertain if there are any detrimental impacts on animal health. The animal feed trials could also be used to determine the digestibility of PPI.

Majority of the yellow pea seed is composed of starch (about 45-50%) which has poor digestibility and limited functional properties and is therefore not used widely in food processing. The solid residues or by-product (mainly starch and fibre) obtained after the protein extraction processes could be further researched to explore its uses. For example, further hydrolysis and fermentation of residues would result in the production of ethanol. Pea starch also possesses some pasting and gelling properties that suit non-food productions such as papermaking, adhesives and thermoplastics. Further research could therefore be conducted to explore technologies that could exploit and convert pea starch to valuable products. A bio refinery, integrating the production of ethanol and animal feed

from the residue, alongside the protein extraction would increase the value of the yellow peas. An economic model could also be developed to assess its economic viability.

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APPENDICES

APPENDIX I: ADDITIONAL SCREENING RESULTS

Table 25; ANOVA table on protein content for water extraction

SUMMARY							
Groups	*Count*	*Sum*	*Average*	*Variance*			
Salamanca	3	2.049	0.683	0.034003		**Max**	**0.034003**
Slovan	3	3.416	1.138666667	0.024054333		**Min**	**0.023984**
Astranoute	3	1.339	0.446333333	0.023984333		**F**	**1.417717**
ANOVA							
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Between Groups	0.742968667	2	0.371484333	13.58398781	0.005919661	5.14325285	
Within Groups	0.164083333	6	0.027347222				
Total	0.907052	8					

Table 26; ANOVA table on protein content for alkaline extraction

Anova: Single Factor							
SUMMARY							
Groups	*Count*	*Sum*	*Average*	*Variance*		**Max**	**0.009187**
Salamanca	3	3.546	1.182	0.009187		**Min**	**0.007027**
Slovan	3	5.544	1.848	0.008379		**F**	**1.307386**
Astranoute	3	1.998	0.666	0.007027			
ANOVA							
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Between Groups	2.106936	2	1.053468	128.5082747	1.18715E-05	5.14325285	
Within Groups	0.049186	6	0.008197667				
Total	2.156122	8					

Table 27; ANOVA table on protein yield for water extraction

Anova: Single Factor							
SUMMARY							
Groups	*Count*	*Sum*	*Average*	*Variance*			
Astranoute (PPI)	3	88.7367	29.5789	2.133911901			**3.146965**
Salamanca (PPI)	3	95.81129	31.9371	2.616678435			**2.133912**
Slovan (PPI)	3	141.6074	47.20246	3.146965066			**1.47474**
ANOVA							
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Between Groups	549.1826	2	274.5913	104.3074501	2.19E-05	5.143253	
Within Groups	15.79511	6	2.632518				
Total	564.9777	8					

Table 28; ANOVA table on protein yield for alkaline extraction

Anova: Single Factor							
SUMMARY							
Groups	*Count*	*Sum*	*Average*	*Variance*			
Astranoute (PPI)	3	119.5743	39.85809	10.72440697			**20.62532**
Salamanca (PPI)	3	127.8571	42.61905	14.49785218			**10.72441**
Slovan (PPI)	3	175.1427	58.38091	20.62532209			**1.923213**
ANOVA							
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Between Groups	599.1539	2	299.577	19.60258023	0.002338	5.143253	
Within Groups	91.69516	6	15.28253				
Total	690.8491	8					

APPENDIX II: STATISTICA TABLES FOR BENCH-SCALE EXPERIMENTS

Table 29; ANOVA analysis on protein content for water extraction

Factor	ANOVA; Var.:Protein content (%); R-sqr=.88791; Adj:.76181 3 factors, 1 Blocks, 18 Runs; MS Pure Error=.6081346 DV: Protein content (%)				
	SS	df	MS	F	p
(1)temp (°C)(L)	1.5149	1	1.51493	2.49111	0.212602
temp (°C)(Q)	30.3885	1	30.38847	49.96998	0.005821
(2)time (minutes)(L)	4.1897	1	4.18973	6.88948	0.078677
time (minutes)(Q)	15.3449	1	15.34486	25.23267	0.015198
(3)solids (%)(L)	38.7616	1	38.76165	63.73860	0.004101
solids (%)(Q)	15.3346	1	15.33456	25.21572	0.015212
1L by 2L	0.5357	1	0.53575	0.88097	0.417177
1L by 3L	1.9142	1	1.91419	3.14765	0.174136
2L by 3L	0.2894	1	0.28941	0.47590	0.539860
Lack of Fit	9.6211	5	1.92423	3.16415	0.185979
Pure Error	1.8244	3	0.60813		
Total SS	102.1107	17			

Table 30; ANOVA table on protein yield for water extraction

Factor	ANOVA; Var.:Protein Yield (%); R-sqr=.81929; Adj:.616 (2**(3) 3 factors, 1 Blocks, 18 Runs; MS Pure Error=22.18137 DV: Protein Yield (%)				
	SS	df	MS	F	p
(1)temp (°C)(L)	3.452	1	3.4524	0.15564	0.719572
temp (°C)(Q)	955.077	1	955.0771	43.05763	0.007198
(2)time (minutes)(L)	1.864	1	1.8642	0.08404	0.790774
time (minutes)(Q)	418.121	1	418.1210	18.85010	0.022553
(3)solids (%)(L)	688.725	1	688.7251	31.04972	0.011407
solids (%)(Q)	60.635	1	60.6348	2.73359	0.196829
1L by 2L	0.033	1	0.0325	0.00147	0.971866
1L by 3L	2.153	1	2.1530	0.09706	0.775780
2L by 3L	74.844	1	74.8436	3.37416	0.163543
Lack of Fit	359.334	5	71.8667	3.23996	0.181058
Pure Error	66.544	3	22.1814		
Total SS	2356.726	17			

Table 31; Effect estimates on protein content for water extraction

Effect Estimates; Var.:Protein content (%); R-sqr=.88791; Adj:.76181 (2**(3) central composite, nc=8 ns=6 n0=2 Runs=16 ([No active dataset]
3 factors, 1 Blocks, 18 Runs; MS Pure Error=.6081346
DV: Protein content (%)

Factor	Effect	Std.Err. Pure Err	t(3)	p	-95.% Cnf.Limt	+95.% Cnf.Limt	Coeff.	Std.Err. Coeff.	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	82.52969	0.389397	211.9422	0.000000	81.29046	83.76893	82.52969	0.389397	81.29046	83.76893
(1)temp (°C)(L)	-0.66612	0.422044	-1.5783	0.212602	-2.00926	0.67701	-0.33306	0.211022	-1.00463	0.33851
temp (°C)(Q)	-3.10159	0.438763	-7.0689	0.005821	-4.49793	-1.70525	-1.55079	0.219381	-2.24896	-0.85262
(2)time (minutes)(L)	-1.10777	0.422044	-2.6248	0.078677	-2.45091	0.23536	-0.55389	0.211022	-1.22545	0.11768
time (minutes)(Q)	-2.20400	0.438763	-5.0232	0.015198	-3.60034	-0.80766	-1.10200	0.219381	-1.80017	-0.40383
(3)solids (%)(L)	-3.36145	0.421042	-7.9836	0.004101	-4.70139	-2.02150	-1.68072	0.210521	-2.35069	-1.01075
solids (%)(Q)	-2.18147	0.434424	-5.0215	0.015212	-3.56401	-0.79894	-1.09074	0.217212	-1.78200	-0.39947
1L by 2L	-0.51757	0.551423	-0.9386	0.417177	-2.27244	1.23731	-0.25878	0.275711	-1.13622	0.61865
1L by 3L	0.97830	0.551417	1.7742	0.174136	-0.77655	2.73316	0.48915	0.275708	-0.38828	1.36658
2L by 3L	0.38040	0.551417	0.6899	0.539860	-1.37446	2.13525	0.19020	0.275708	-0.68723	1.06763

Table 32; Effect estimates on protein yield for water extractions

Effect Estimates; Var.:Yield; R-sqr=.81857; Adj:.61445 (2**(3) central composite, nc=8 ns=6 n0=2 Runs=16 ([No active dataset
3 factors, 1 Blocks, 18 Runs; MS Residual=53.4489
DV: Yield

Factor	Effect	Std.Err.	t(8)	p	-95.% Cnf.Limt	+95.% Cnf.Limt	Coeff.	Std.Err. Coeff.	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	52.9788	3.650069	14.51447	0.000000	44.5617	61.39588	52.97880	3.650069	44.5617	61.39588
(1)temp (L)	-1.0088	3.956617	-0.25496	0.805183	-10.1328	8.11518	-0.50440	1.978309	-5.0664	4.05759
temp (Q)	-17.3958	4.111198	-4.23133	0.002871	-26.8763	-7.91541	-8.69792	2.055599	-13.4381	-3.95770
(2)time (L)	0.7200	3.956617	0.18198	0.860129	-8.4040	9.84398	0.36000	1.978309	-4.2020	4.92199
time (Q)	-11.5127	4.111198	-2.80033	0.023186	-20.9932	-2.03228	-5.75636	2.055599	-10.4966	-1.01614
(3)solids (L)	-14.1405	3.956617	-3.57390	0.007252	-23.2645	-5.01657	-7.07027	1.978309	-11.6323	-2.50828
solids (Q)	-4.3146	4.111198	-1.04948	0.324626	-13.7950	5.16583	-2.15730	2.055599	-6.8975	2.58292
1L by 2L	0.1275	5.169569	0.02466	0.980927	-11.7935	12.04855	0.06375	2.584785	-5.8968	6.02427
1L by 3L	1.0425	5.169569	0.20165	0.845220	-10.8786	12.96352	0.52123	2.584785	-5.4393	6.48176
2L by 3L	6.1473	5.169569	1.18913	0.268483	-5.7737	18.06835	3.07365	2.584785	-2.8869	9.03417

Table 33; Regression coefficients of protein content for water extractions

Regr. Coefficients; Var.:Protein content (%); R-sqr=.88791; Adj:.76181 (2**(3
3 factors, 1 Blocks, 18 Runs; MS Pure Error=.6081346
DV: Protein content (%)

Factor	Regressn Coeff.	Std.Err. Pure Err	t(3)	p	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	58.72862	4.877738	12.04013	0.001233	43.20548	74.25176
(1)temp (°C)(L)	1.15226	0.190822	6.03842	0.009108	0.54498	1.75954
temp (°C)(Q)	-0.01551	0.002194	-7.06894	0.005821	-0.02249	-0.00853
(2)time (minutes)(L)	0.08384	0.032210	2.60285	0.080178	-0.01867	0.18635
time (minutes)(Q)	-0.00054	0.000108	-5.02321	0.015198	-0.00089	-0.00020
(3)solids (%)(L)	0.06391	0.224427	0.28478	0.794337	-0.65031	0.77814
solids (%)(Q)	-0.02466	0.004912	-5.02153	0.015212	-0.04030	-0.00903
1L by 2L	-0.00058	0.000613	-0.93860	0.417177	-0.00252	0.00137
1L by 3L	0.00736	0.004146	1.77416	0.174136	-0.00584	0.02055
2L by 3L	0.00064	0.000921	0.68985	0.539860	-0.00230	0.00357

Table 34; Regression coefficients of protein yield for water extractions

Factor	Regr. Coefficients; Var.:Protein Yield (%); R-sqr=.81929; Adj:.616 (2**(3) cen 3 factors, 1 Blocks, 18 Runs; MS Pure Error=22.18137 DV: Protein Yield (%)					
	Regressn Coeff.	Std.Err. Pure Err	t(3)	p	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	-80.4269	29.45865	-2.73016	0.071931	-174.177	13.32371
(1)temp (°C)(L)	6.7901	1.15245	5.89192	0.009759	3.123	10.45775
temp (°C)(Q)	-0.0869	0.01325	-6.56183	0.007198	-0.129	-0.04477
(2)time (minutes)(L)	0.2922	0.19453	1.50206	0.230089	-0.327	0.91129
time (minutes)(Q)	-0.0028	0.00065	-4.34167	0.022553	-0.005	-0.00076
(3)solids (%)(L)	-0.8344	1.35541	-0.61564	0.581684	-5.148	3.47906
solids (%)(Q)	-0.0490	0.02966	-1.65336	0.196829	-0.143	0.04536
1L by 2L	0.0001	0.00370	0.03829	0.971866	-0.012	0.01192
1L by 3L	0.0078	0.02504	0.31155	0.775780	-0.072	0.08749
2L by 3L	0.0102	0.00556	1.83689	0.163543	-0.007	0.02793

Table 35; ANOVA analysis on protein content for alkaline extraction

Factor	ANOVA; Var.:Protein content (%); R-sqr=.64628; Adj:.26534 4 factors, 1 Blocks, 28 Runs; MS Pure Error=1.174285 DV: Protein content (%)				
	SS	df	MS	F	p
(1)pH (L)	13.96858	1	13.96858	11.89538	0.040967
pH (Q)	8.17436	1	8.17436	6.96113	0.077763
(2)temp(°C)(L)	7.41636	1	7.41636	6.31563	0.086701
temp(°C)(Q)	0.00128	1	0.00128	0.00109	0.975705
(3)time (minutes)(L)	0.66205	1	0.66205	0.56379	0.507266
time (minutes)(Q)	4.62919	1	4.62919	3.94213	0.141303
(4)solids (%)(L)	0.04820	1	0.04820	0.04105	0.852405
solids (%)(Q)	0.13048	1	0.13048	0.11111	0.760818
1L by 2L	0.58677	1	0.58677	0.49968	0.530600
1L by 3L	0.07294	1	0.07294	0.06212	0.819268
1L by 4L	1.09303	1	1.09303	0.93080	0.405823
2L by 3L	0.69574	1	0.69574	0.59248	0.497515
2L by 4L	3.32182	1	3.32182	2.82880	0.191178
3L by 4L	1.38141	1	1.38141	1.17638	0.357474
Lack of Fit	19.82497	10	1.98250	1.68826	0.365963
Pure Error	3.52286	3	1.17429		
Total SS	66.00562	27			

Table 36; ANOVA analysis on protein yield for alkaline extraction

Factor	ANOVA; Var.:Protein yield (%); R-sqr=.73836; Adj:.4566 4 factors, 1 Blocks, 28 Runs; MS Pure Error=61.88124 DV: Protein yield (%) SS	df	MS	F	p
(1)pH (L)	57.175	1	57.175	0.92395	0.407349
pH (Q)	23.378	1	23.378	0.37779	0.582263
(2)temp(°C)(L)	56.179	1	56.179	0.90785	0.410977
temp(°C)(Q)	6.368	1	6.368	0.10290	0.769419
(3)time (minutes)(L)	14.678	1	14.678	0.23719	0.659607
time (minutes)(Q)	76.380	1	76.380	1.23430	0.347605
(4)solids (%)(L)	2020.658	1	2020.658	32.65380	0.010633
solids (%)(Q)	100.307	1	100.307	1.62096	0.292658
1L by 2L	35.557	1	35.557	0.57461	0.503543
1L by 3L	456.197	1	456.197	7.37214	0.072846
1L by 4L	0.001	1	0.001	0.00001	0.997791
2L by 3L	27.612	1	27.612	0.44620	0.551940
2L by 4L	44.968	1	44.968	0.72669	0.456607
3L by 4L	0.040	1	0.040	0.00065	0.981327
Lack of Fit	896.956	10	89.696	1.44948	0.421343
Pure Error	185.644	3	61.881		
Total SS	4137.778	27			

Table 37; Effect estimates on protein content for alkaline extraction

Factor	Effect Estimates; Var.:Protein content (%); R-sqr=.64628; Adj:.26534 (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 ([4 factors, 1 Blocks, 28 Runs; MS Pure Error=1.174285 DV: Protein content (%) **Effect**	Std.Err. Pure Err	t(3)	p	-95.% Cnf.Limt	+95.% Cnf.Limt	Coeff.	Std.Err. Coeff.	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	86.79292	0.541822	160.1871	0.000001	85.06860	88.51724	86.79292	0.541822	85.06860	88.51724
(1)pH (L)	1.53360	0.444653	3.4490	0.040967	0.11851	2.94868	0.76680	0.222327	0.05926	1.47434
pH (Q)	-1.16722	0.442396	-2.6384	0.077763	-2.57512	0.24069	-0.58361	0.221198	-1.28756	0.12034
(2)temp(°C)(L)	1.11745	0.444653	2.5131	0.086701	-0.29763	2.53254	0.55873	0.222327	-0.14882	1.26627
temp(°C)(Q)	0.01462	0.442396	0.0331	0.975705	-1.39328	1.42253	0.00731	0.221198	-0.69664	0.71126
(3)time (minutes)(L)	0.33218	0.442402	0.7509	0.507266	-1.07574	1.74010	0.16609	0.221201	-0.53787	0.87005
time (minutes)(Q)	-0.85838	0.432329	-1.9855	0.141303	-2.23425	0.51748	-0.42919	0.216165	-1.11712	0.25874
(4)solids (%)(L)	-0.09009	0.444647	-0.2026	0.852405	-1.50515	1.32498	-0.04504	0.222324	-0.75258	0.66249
solids (%)(Q)	0.14746	0.442369	0.3333	0.760818	-1.26036	1.55527	0.07373	0.221184	-0.63018	0.77764
1L by 2L	0.38300	0.541822	0.7069	0.530600	-1.34132	2.10732	0.19150	0.270911	-0.67066	1.05366
1L by 3L	0.13366	0.536265	0.2492	0.819268	-1.57298	1.84029	0.06683	0.268133	-0.78649	0.92015
1L by 4L	0.52272	0.541807	0.9648	0.405823	-1.20155	2.24700	0.26136	0.270904	-0.60077	1.12350
2L by 3L	0.41278	0.536265	0.7697	0.497515	-1.29386	2.11941	0.20639	0.268133	-0.64693	1.05971
2L by 4L	0.91127	0.541807	1.6819	0.191178	-0.81301	2.63554	0.45563	0.270904	-0.40650	1.31777
3L by 4L	0.58162	0.536252	1.0846	0.357474	-1.12497	2.28822	0.29081	0.268126	-0.56248	1.14411

Table 38; Effect estimates on protein yield for alkaline extractions

Effect Estimates; Var.:Protein yield (%); R-sqr=.73836; Adj:.4566 (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 ([No a
4 factors, 1 Blocks, 28 Runs; MS Pure Error=61.88124
DV: Protein yield (%)

Factor	Effect	Std.Err. Pure Err	t(3)	p	-95.% Cnf.Limt	+95.% Cnf.Limt	Coeff.	Std.Err. Coeff.	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	48.7556	3.933232	12.39580	0.001131	36.2383	61.27286	48.75557	3.933232	36.2383	61.27286
(1)pH (L)	3.1027	3.227857	0.96122	0.407349	-7.1698	13.37517	1.55135	1.613928	-3.5849	6.68759
pH (Q)	-1.9739	3.211470	-0.61465	0.582263	-12.1943	8.24641	-0.98696	1.605735	-6.0971	4.12320
(2)temp(°C)(L)	3.0755	3.227857	0.95281	0.410977	-7.1969	13.34803	1.53777	1.613928	-3.5985	6.67401
temp(°C)(Q)	-1.0302	3.211470	-0.32079	0.769419	-11.2505	9.19014	-0.51510	1.605735	-5.6253	4.59507
(3)time (minutes)(L)	1.5641	3.211514	0.48703	0.659607	-8.6564	11.78456	0.78205	1.605757	-4.3282	5.89228
time (minutes)(Q)	-3.4867	3.138393	-1.11099	0.347605	-13.4745	6.50105	-1.74336	1.569196	-6.7372	3.25052
(4)solids (%)(L)	-18.4449	3.227813	-5.71435	0.010633	-28.7172	-8.17251	-9.22243	1.613906	-14.3586	-4.08626
solids (%)(Q)	4.0885	3.211272	1.27317	0.292658	-6.1312	14.30820	2.04425	1.605636	-3.0656	7.15410
1L by 2L	2.9815	3.933232	0.75803	0.503543	-9.5358	15.49880	1.49075	1.966616	-4.7679	7.74940
1L by 3L	-10.5699	3.892892	-2.71517	0.072846	-22.9588	1.81906	-5.28493	1.946446	-11.4794	0.90953
1L by 4L	-0.0118	3.933123	-0.00300	0.997791	-12.5288	12.50514	-0.00591	1.966562	-6.2644	6.25257
2L by 3L	-2.6004	3.892892	-0.66798	0.551940	-14.9893	9.78853	-1.30020	1.946446	-7.4947	4.89426
2L by 4L	3.3528	3.933123	0.85246	0.456607	-9.1641	15.86978	1.67642	1.966562	-4.5821	7.93489
3L by 4L	0.0989	3.892794	0.02540	0.981327	-12.2897	12.48750	0.04945	1.946397	-6.1449	6.24375

Table 39; Regression coefficients of protein content for alkaline extractions

Regr. Coefficients; Var.:Protein content (%); R-sqr=.64628; Adj:.26534 (2**(4)
4 factors, 1 Blocks, 28 Runs; MS Pure Error=1.174285
DV: Protein content (%)

Factor	Regressn Coeff.	Std.Err. Pure Err	t(3)	p	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	72.15750	16.10908	4.47931	0.020746	20.89123	123.4238
(1)pH (L)	4.56919	2.20052	2.07641	0.129445	-2.43384	11.5722
pH (Q)	-0.25938	0.09831	-2.63840	0.077763	-0.57225	0.0535
(2)temp(°C)(L)	-0.19033	0.17407	-1.09343	0.354146	-0.74431	0.3636
temp(°C)(Q)	0.00003	0.00098	0.03306	0.975705	-0.00310	0.0032
(3)time (minutes)(L)	0.01056	0.06224	0.16971	0.876037	-0.18752	0.2086
time (minutes)(Q)	-0.00027	0.00014	-1.98548	0.141303	-0.00070	0.0002
(4)solids (%)(L)	-1.19432	0.67860	-1.75998	0.176639	-3.35393	0.9653
solids (%)(Q)	0.00497	0.01492	0.33334	0.760818	-0.04251	0.0525
1L by 2L	0.00851	0.01204	0.70688	0.530600	-0.02981	0.0468
1L by 3L	0.00111	0.00447	0.24924	0.819268	-0.01311	0.0153
1L by 4L	0.04526	0.04691	0.96478	0.405823	-0.10403	0.1945
2L by 3L	0.00034	0.00045	0.76973	0.497515	-0.00108	0.0018
2L by 4L	0.00789	0.00469	1.68190	0.191178	-0.00704	0.0228
3L by 4L	0.00189	0.00174	1.08461	0.357474	-0.00365	0.0074

Table 40; Regression coefficients of protein yield for alkaline extractions

Factor	Regr. Coefficients; Var.:Protein yield (%); R-sqr=.73836; Adj:.4566 (2**(4) cer 4 factors, 1 Blocks, 28 Runs; MS Pure Error=61.88124 DV: Protein yield (%) Regressn Coeff.	Std.Err. Pure Err	t(3)	p	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	-34.7890	116.9401	-0.29749	0.785500	-406.944	337.3664
(1)pH (L)	16.1966	15.9741	1.01393	0.385283	-34.640	67.0335
pH (Q)	-0.4386	0.7137	-0.61465	0.582263	-2.710	1.8325
(2)temp(°C)(L)	-0.4658	1.2636	-0.36858	0.736912	-4.487	3.5557
temp(°C)(Q)	-0.0023	0.0071	-0.32079	0.769419	-0.025	0.0204
(3)time (minutes)(L)	1.2443	0.4518	2.75395	0.070509	-0.194	2.6822
time (minutes)(Q)	-0.0011	0.0010	-1.11099	0.347605	-0.004	0.0020
(4)solids (%)(L)	-7.4164	4.9261	-1.50552	0.229264	-23.094	8.2608
solids (%)(Q)	0.1379	0.1083	1.27317	0.292658	-0.207	0.4827
1L by 2L	0.0663	0.0874	0.75803	0.503543	-0.212	0.3444
1L by 3L	-0.0881	0.0324	-2.71517	0.072846	-0.191	0.0152
1L by 4L	-0.0010	0.3405	-0.00300	0.997791	-1.085	1.0827
2L by 3L	-0.0022	0.0032	-0.66798	0.551940	-0.012	0.0082
2L by 4L	0.0290	0.0341	0.85246	0.456607	-0.079	0.1374
3L by 4L	0.0003	0.0126	0.02540	0.981327	-0.040	0.0405

APPENDIX III: STATISTICA TABLES FOR QUALITY TESTS

Table 41; Two-Sample t-Test for amino acid composition of pea protein isolates

	Alk	*Water*
Mean	3.221960784	1.548627451
Variance	4.256422304	0.875490359
Observations	17	17
Hypothesized Mean Difference	0	
df	22	
t Stat	3.045560946	
P(T<=t) one-tail	0.002966262	
t Critical one-tail	1.717144374	
P(T<=t) two-tail	0.005932524	
t Critical two-tail	2.073873068	

Table 42; Two-Sample t-Test on fat hydration capacity for the two methods

	Alkaline	*Water*
Mean	1.666666667	0.836666667
Variance	0.123333333	0.002433333
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	4.053740505	
P(T<=t) one-tail	0.027904185	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.055808369	
t Critical two-tail	4.30265273	

Table 43; Two-Sample t-Test on water hydration capacity for the two methods

	Alkaline	*Water*
Mean	0.773333333	0.626666667
Variance	0.015633333	0.012933333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	1.503011369	
P(T<=t) one-tail	0.103630631	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.207261262	
t Critical two-tail	2.776445105	

APPENDIX IV STATISTICA TABLES ON IRR FOR WATER AND ALKALINE EXTRACTIONS

Table 44; ANOVA table on IRR for water extractions

Factor	ANOVA; Var.:IRR (%); R-sqr=.70013; Adj:.36278 (2**(3) 3 factors, 1 Blocks, 18 Runs; MS Pure Error=13.55583 DV: IRR (%) SS	df	MS	F	p
(1)temp (°C)(L)	6.276	1	6.2763	0.46300	0.545036
temp (°C)(Q)	657.351	1	657.3515	48.49215	0.006076
(2)time (minutes)(L)	13.241	1	13.2407	0.97675	0.395865
time (minutes)(Q)	244.846	1	244.8460	18.06204	0.023872
(3)solids (%)(L)	296.706	1	296.7064	21.88773	0.018449
solids (%)(Q)	163.657	1	163.6568	12.07279	0.040212
1L by 2L	16.531	1	16.5312	1.21949	0.350079
1L by 3L	0.063	1	0.0632	0.00466	0.949855
2L by 3L	102.286	1	102.2863	7.54555	0.070925
Lack of Fit	487.117	5	97.4235	7.18683	0.067655
Pure Error	40.668	3	13.5558		
Total SS	1760.060	17			

Table 45; ANOVA table on IRR for alkaline extractions

Factor	ANOVA; Var.:IRR (%); R-sqr=.60482; Adj:.17925 (2**(4) 4 factors, 1 Blocks, 28 Runs; MS Pure Error=42.63333 DV: IRR (%) SS	df	MS	F	p
(1)pH (L)	2.900	1	2.900	0.06802	0.811120
pH (Q)	4.879	1	4.879	0.11443	0.757441
(2)temp(°C)(L)	54.709	1	54.709	1.28324	0.339648
temp(°C)(Q)	63.443	1	63.443	1.48810	0.309663
(3)time (minutes)(L)	23.653	1	23.653	0.55481	0.510403
time (minutes)(Q)	64.250	1	64.250	1.50704	0.307132
(4)solids (%)(L)	1078.117	1	1078.117	25.28813	0.015152
solids (%)(Q)	1.130	1	1.130	0.02650	0.881027
1L by 2L	10.401	1	10.401	0.24396	0.655264
1L by 3L	155.243	1	155.243	3.64135	0.152391
1L by 4L	9.683	1	9.683	0.22712	0.666227
2L by 3L	2.378	1	2.378	0.05578	0.828507
2L by 4L	2.715	1	2.715	0.06368	0.817071
3L by 4L	12.785	1	12.785	0.29989	0.622065
Lack of Fit	861.839	10	86.184	2.02152	0.305998
Pure Error	127.900	3	42.633		
Total SS	2504.550	27			

APPENDIX V ADDITIONAL ECONOMIC RESULTS

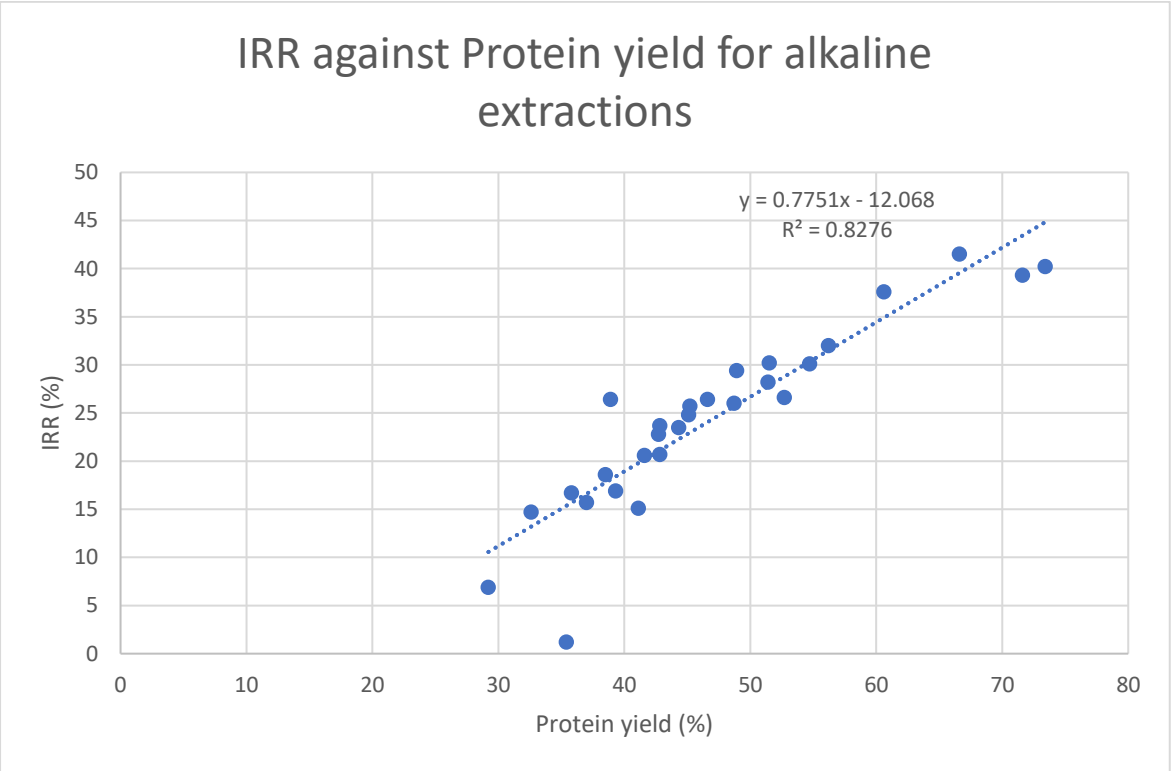

Figure 20; A graph showing the correlation between protein yield and IRR for alkaline extractions

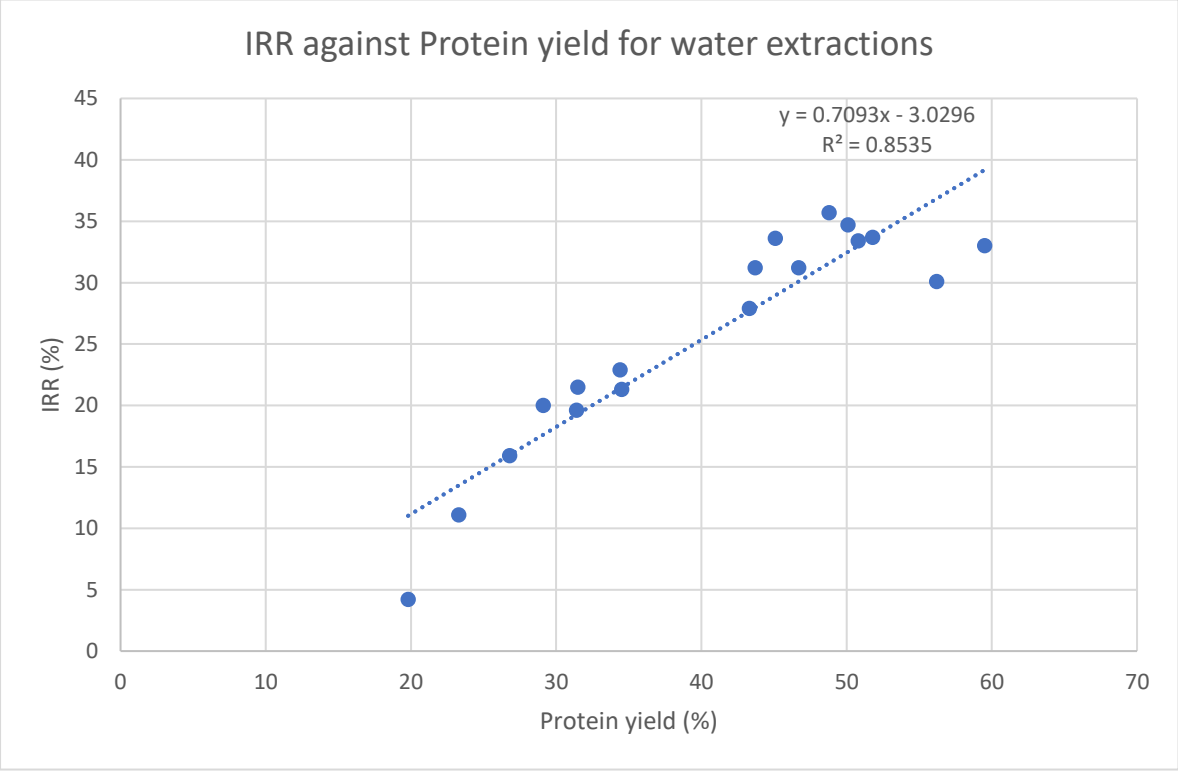

Figure 21; A graph showing the correlation between protein yield and IRR for water extractions

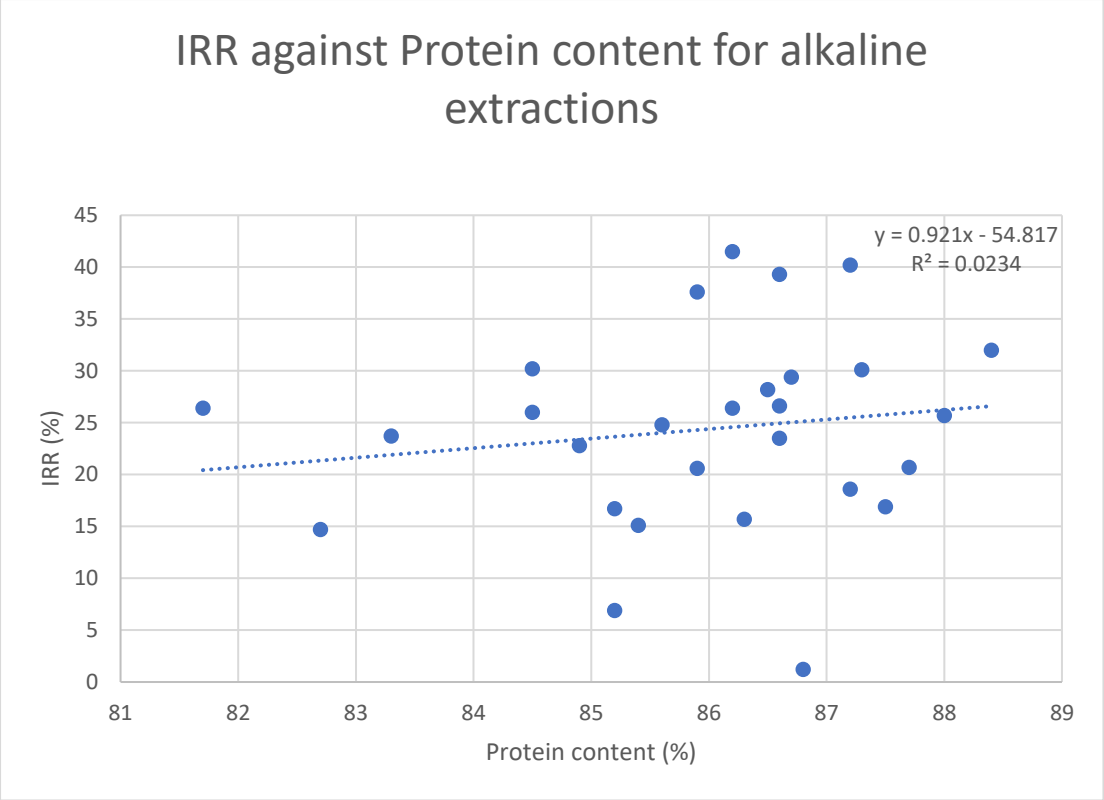

Figure 22; A graph showing the correlation between protein content and IRR for alkaline extractions

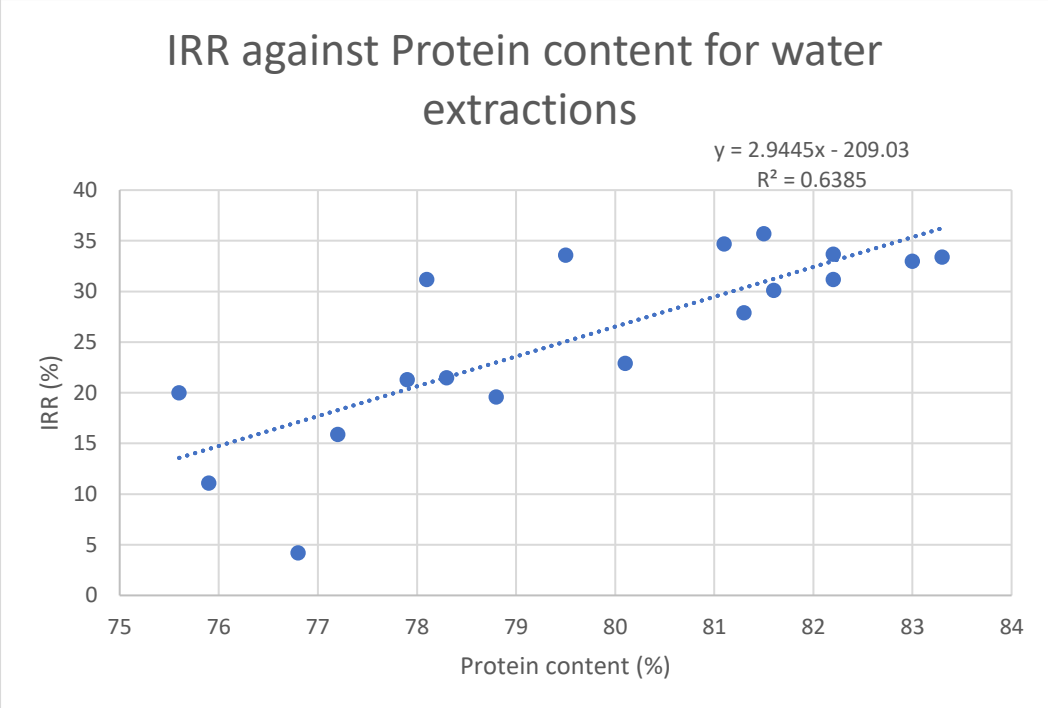

Figure 23; A graph showing the correlation between protein content and IRR for water extractions

Table 46; Discounted cash flow analysis for profitability assessment for water extraction (6.7 % solids)

DCF	Total Revenue	Total Opex	Depreciation	Net Profit	Tax	Net Cash Flow	Discounted Cash Flow
-2						-100160989	-120095529.8
-1						-52716310	-57724359.45
1	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	76422550.84
2	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	69792283.87
3	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	63737245.54
4	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	58207530.18
5	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	53157561.81
6	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	48545718.54
7	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	44333989.54
8	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	40487661.68
9	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	36975033.5
10	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	33767153.88
11	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	30837583.45
12	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	28162176.67
13	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	25718882.8
14	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	23487564.2
15	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	21449830.32
16	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	19588886.14
17	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	17889393.73
18	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	16337345.88
19	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	14919950.57

20	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	13625525.64
21	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	12443402.41
22	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	11363837.81
23	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	10377934.08
24	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	9477565.367
25	234767272.4	119853360	3373844	111540068.5	31231219	83682693.17	8655310.838

Table 47; Discounted cash flow analysis for profitability assessment for water extraction (20 % solids)

DCF	Total Revenue	Total Opex	Depreciation	Net Profit	Tax	Net Cash Flow	Discounted Cash Flow
-2						-87054663.13	-104380717.5
-1						-45818243.75	-50170976.91
1	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	7459034.747
2	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	6811903.879
3	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	6220916.784
4	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	5681202.543
5	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	5188312.824
6	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	4738185.228
7	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	4327109.797
8	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	3951698.445
9	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	3608857.027
10	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	3295759.842
11	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	3009826.34

12	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	2748699.854
13	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	2510228.177
14	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	2292445.824
15	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	2093557.83
16	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	1911924.959
17	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	1746050.191
18	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	1594566.384
19	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	1456225.008
20	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	1329885.852
21	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	1214507.628
22	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	1109139.386
23	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	1012912.681
24	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	925034.4122
25	124101103.5	113897520	2932368	7271215.9	2035940	8167643.048	844780.2851

Table 48; Discounted cash flow analysis for profitability assessment for alkaline extraction (6.7 % solids)

DCF	Total Revenue	Total Opex	Depreciation	Net Profit	Tax	Net Cash Flow	Discounted Cash Flow
-2						-94687583	-113532779.2
-1						-49835570	-54569949.15
1	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	98736606.84
2	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	90170417.21
3	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	82347412.98
4	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	75203116.87

5	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	68678645.55
6	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	62720224.24
7	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	57278743.6
8	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	52309354.89
9	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	47771100.35
10	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	43626575.66
11	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	39841621.61
12	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	36385042.57
13	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	33228349.38
14	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	30345524.55
15	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	27712807.8
16	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	25308500.28
17	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	23112785.64
18	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	21107566.8
19	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	19276316.71
20	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	17603942.2
21	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	16076659.54
22	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	14681880.86
23	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	13408110.38
24	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	12244849.66
25	282381490.9	133459920	3189476	145732094.5	40804986	108116584.5	11182511.1

Table 49; Discounted cash flow analysis for profitability assessment for alkaline extraction (20 % solids)

DCF	Total Revenue	Total Opex	Depreciation	Net Profit	Tax	Net Cash Flow	Discounted Cash Flow
-2						-470732635.6	-564420198.4
-1						-47073263.56	-51545223.6
1	453900763	132266621	3012688.868	318621453	89214006.76	232420135	212255830.9
2	453900763	132266621	3012688.868	318621453	89214006.76	232420135	193840941.4
3	453900763	132266621	3012688.868	318621453	89214006.76	232420135	177023690.8
4	453900763	132266621	3012688.868	318621453	89214006.76	232420135	161665471.1
5	453900763	132266621	3012688.868	318621453	89214006.76	232420135	147639699.6
6	453900763	132266621	3012688.868	318621453	89214006.76	232420135	134830775.9
7	453900763	132266621	3012688.868	318621453	89214006.76	232420135	123133128.7
8	453900763	132266621	3012688.868	318621453	89214006.76	232420135	112450345.8
9	453900763	132266621	3012688.868	318621453	89214006.76	232420135	102694379.7
10	453900763	132266621	3012688.868	318621453	89214006.76	232420135	93784821.68
11	453900763	132266621	3012688.868	318621453	89214006.76	232420135	85648238.98
12	453900763	132266621	3012688.868	318621453	89214006.76	232420135	78217569.84
13	453900763	132266621	3012688.868	318621453	89214006.76	232420135	71431570.63
14	453900763	132266621	3012688.868	318621453	89214006.76	232420135	65234311.08
15	453900763	132266621	3012688.868	318621453	89214006.76	232420135	59574713.31
16	453900763	132266621	3012688.868	318621453	89214006.76	232420135	54406130.88
17	453900763	132266621	3012688.868	318621453	89214006.76	232420135	49685964.27
18	453900763	132266621	3012688.868	318621453	89214006.76	232420135	45375309.84
19	453900763	132266621	3012688.868	318621453	89214006.76	232420135	41438639.12

20	453900763	132266621	3012688.868	318621453	89214006.76	232420135	37843506.05
21	453900763	132266621	3012688.868	318621453	89214006.76	232420135	34560279.5
22	453900763	132266621	3012688.868	318621453	89214006.76	232420135	31561899.08
23	453900763	132266621	3012688.868	318621453	89214006.76	232420135	28823652.13
24	453900763	132266621	3012688.868	318621453	89214006.76	232420135	26322969.98
25	453900763	132266621	3012688.868	318621453	89214006.76	232420135	24039241.99

Table 50; Stream table for yellow pea protein production

Stream name	FEED	UP1	UP2	UP2-DW	UP3	UP4	UP4-NAOH	UP5	UP6
Total flow rate [kg/hr]	2094.24	2094.24	6760.90667	4666.66667	6760.90667	1754.44317	40	6800.90666	3795.96121
Mass flow rate [kg/hr]									
WATER	94.24	94.24	4760.906671	4666.66667	4760.906671	0	0	4757.631205	3507.98926
GLUCOSE	0	0	0	0	0	0	0	32.75545294	24.15188819
AIR	0	0	0	0	0	0	0	0	0
STARCH	1474.00	1474.000001	1473.999999	0	1473.999999	1452.263231	0	1444.520001	0
PROTEIN	454.00	454.0000006	454.0000002	0	454.0000002	219.673517	0	453.9999995	234.3264825
NAOHS	0	0	0	0	0	10.50642133	40	40.00000002	29.49357866
ASH	72	72.0	72.0	0.0	72.0	72.0	0.0	72.0	0.0
Mass fraction									
WATER	0.044999618	0.044999618	0.704181688	1	0.704181688	0	0	0.699558374	0.924137278
CARBO-01	0	0	0	0	0	0	0	0	0
GLUCOSE	0	0	0	0	0	0	0	0.004816336	0.006362522
AIR	0	0	0	0	0	0	0	0	0
STARCH	0.703835282	0.703835282	0.218018096	0	0.218018096	0.827763051	0	0.212401092	0
CELLU-01	0	0	0	0	0	0	0	0	0
PROTEIN	0.216785087	0.216785087	0.067150757	0	0.067150757	0.125209822	0	0.066755805	0.061730473
NAOHS	0	0	0	0	0	0.005988465	1	0.005881569	0.007769726
ASH	0.034380014	0.034380014	0.010649459	0	0.010649459	0.041038662	0	0.010586824	0
Total	1	1	1	1	1	1	1	1	1

Table 51; Stream table for yellow pea protein production (continued)

Stream name	UP7-WW	UP9	UP10	UP10B	UP11-RW	UP11A	UP11B	UP12	UP13
Total flow rate [kg/hr]	2000	3004.94545	1422.57361	421.999989	3339.81059	2373.3876	966.422991	421.999989	265.782334
Mass flow rate [kg/hr]									
WATER	2000	1249.641949	1156.791276	156.217655	3320.036096	2353.613104	966.422991	156.2176549	0
GLUCOSE	0	8.603564731	0	0	0	0	0	0	0
AIR	0	0	0	0	0	0	0	0	0
STARCH	0	1444.519999	21.73676742	21.7367675	0	0	0	21.73676747	21.73676749
PROTEIN	0	219.6735172	234.3264829	234.326483	0	0	0	234.3264828	234.3264826
NAOHS	0	10.50642132	9.719083847	9.71908386	19.77449482	19.77449483	0	9.719083857	9.719083845
ASH	0.0	72.0	0.0	0.0	0.0	0.0	0.0	0	0
Mass fraction									
WATER	1	0.415861775	0.813167957	0.37018403	0.994079157	0.99166824	1	0.370184026	0
CARBO-01	0	0	0	0	0	0	0	0	0
GLUCOSE	0	0.002863135	0	0	0	0	0	0	0
AIR	0	0	0	0	0	0	0	0	0
STARCH	0	0.480714217	0.015279889	0.05150893	0	0	0	0.051508929	0.081784094
CELLU-01	0	0	0	0	0	0	0	0	0
PROTEIN	0	0.073103995	0.164720111	0.55527604	0	0	0	0.55527604	0.881648073
NAOHS	0	0.003496377	0.006832043	0.02303101	0.005920843	0.00833176	0	0.023031005	0.036567832
ASH	0	0.023960502	0	0	0	0	0	0	0
Total	1	1	1	1	1	1	1	1	1

Table 52; Stream table for yellow pea protein production (continued)

Stream name	UP14	UP15	UP16	CENT1.D1	CENT1.D2	CENT1.D4	CENT1.INT	CENT1.L	CENT1.SL
Total flow rate [kg/hr]	11041.3427	10885.1251	10885.1251	100000	45000	95000	6800.90666	3795.96121	3004.94545
Mass flow rate [kg/hr]									
WATER	156.2176544	0	0	100000	45000	95000	4757.631205	3507.98926	1249.641949
GLUCOSE	0	0	0	0	0	0	32.75545294	24.15188819	8.603564731
AIR	10885.12505	10885.1251	10885.1251	0	0	0	0	0	0
STARCH	0	0	0	0	0	0	1444.520001	0	1444.519999
PROTEIN	0	0	0	0	0	0	453.9999995	234.3264825	219.6735172
NAOHS	0	0	0	0	0	0	40.00000002	29.49357866	10.50642132
ASH	0	0	0	0	0	0	71.99999981	0	71.99999996
Mass fraction									
WATER	0.014148429	0	0	1	1	1	0.699558374	0.924137278	0.415861775
CARBO-01	0	0	0	0	0	0	0	0	0
GLUCOSE	0	0	0	0	0	0	0.004816336	0.006362522	0.002863135
AIR	0.985851571	1	1	0	0	0	0	0	0
STARCH	0	0	0	0	0	0	0.212401092	0	0.480714217
CELLU-01	0	0	0	0	0	0	0	0	0
PROTEIN	0	0	0	0	0	0	0.066755805	0.061730473	0.073103995
NAOHS	0	0	0	0	0	0	0.005881569	0.007769726	0.003496377
ASH	0	0	0	0	0	0	0.010586824	0	0.023960502
Total	1	1	1	1	1	1	1	1	1

Table 53; Stream table for yellow pea protein production (continued)

Stream name	CENT1.SN	.CIP.STEAM1	CIP.WATER	DDGSDRY.DUM0	DDGSDRY.DUM1	DDGSDRY.EV3	DDGSDRY.INT	DDGSDRY.UP12
Total flow rate [kg/hr]	6800.90666	63.0595498	691.442432	1	100	1754.44317	3004.94546	3004.94545
Mass flow rate [kg/hr]								
WATER	4757.631205	63.0595498	691.442432	1	100	0	1250.502287	1249.641949
GLUCOSE	32.75545294	0	0	0	0	0	0	8.603564731
AIR	0	0	0	0	0	0	0	0
STARCH	1444.520001	0	0	0	0	1452.263231	1452.263235	1444.519999
PROTEIN	453.9999995	0	0	0	0	219.673517	219.6735179	219.6735172
NAOHS	40.00000002	0	0	0	0	10.50642133	10.50642135	10.50642132
ASH	71.99999981	0	0	0	0	71.9999999	72.0000002	71.99999996
Mass fraction								
WATER	0.699558374	1	1	1	1	0	0.416148081	0.415861775
CARBO-01	0	0	0	0	0	0	0	0
GLUCOSE	0.004816336	0	0	0	0	0	0	0.002863135
AIR	0	0	0	0	0	0	0	0
STARCH	0.212401092	0	0	0	0	0.827763051	0.483291046	0.480714217
CELLU-01	0	0	0	0	0	0	0	0
PROTEIN	0.066755805	0	0	0	0	0.125209822	0.073103995	0.073103995
NAOHS	0.005881569	0	0	0	0	0.005988465	0.003496377	0.003496377
ASH	0.010586824	0	0	0	0	0.041038662	0.023960502	0.023960502
Total	1	1	1	1	1	1	1	1

Table 54; Stream table for yellow pea protein production (continued)

Stream name	DDGSDRY.UP13	DDGSDRY.UP14	DDGSDRY.UP15	DDGSDRY.UP16	EVAP.D3	EVAP.D4	EVAP.DUMIN	EVAP.EV1
Total flow rate [kg/hr]	1754.44317	98893.4492	97642.9469	97642.9469	100000	4000000	1000000	1422.57361
Mass flow rate [kg/hr]								
WATER	0	1250.502285	0	0	100000	4000000	1000000	1156.791276
GLUCOSE	0	0	0	0	0	0	0	0
AIR	0	97642.94688	97642.9469	97642.9469	0	0	0	0
STARCH	1452.263231	0	0	0	0	0	0	21.73676742
PROTEIN	219.673517	0	0	0	0	0	0	234.3264829
NAOHS	10.50642133	0	0	0	0	0	0	9.719083847
ASH	71.9999999	0	0	0	0	0	0	0
Mass fraction								
WATER	0	0.012644946	0	0	1	1	1	0.813167957
CARBO-01	0	0	0	0	0	0	0	0
GLUCOSE	0	0	0	0	0	0	0	0
AIR	0	0.987355054	1	1	0	0	0	0
STARCH	0.827763051	0	0	0	0	0	0	0.015279889
CELLU-01	0	0	0	0	0	0	0	0
PROTEIN	0.125209822	0	0	0	0	0	0	0.164720111
NAOHS	0.005988465	0	0	0	0	0	0	0.006832043
ASH	0.041038662	0	0	0	0	0	0	0
Total	1	1	1	1	1	1	1	1

Table 55; Stream table for yellow pea protein production (continued)

Stream name	EVAP.EV2	EVAP.EV3B	EVAP.EV4	EVAP.EV5	EVAP.EV6	MILLING.UP1	SERV.WATER	SPECS.DUM1
Total flow rate [kg/hr]	1000.57362	966.422991	421.999989	966.422991	34.150631	2094.24	2166.15085	0.3
Mass flow rate [kg/hr]								
WATER	1000.57362	966.422991	156.2176549	966.422991	34.150631	94.24	2166.15085	0.3
GLUCOSE	0	0	0	0	0	0	0	0
AIR	0	0	0	0	0	0	0	0
STARCH	0	0	21.73676747	0	0	1474.000001	0	0
PROTEIN	0	0	234.3264828	0	0	454.0000006	0	0
NAOHS	0	0	9.719083857	0	0	0	0	0
ASH	0	0	0	0	0	72.0000001	0	0
Mass fraction								
WATER	1	1	0.370184026	1	1	0.044999618	1	1
CARBO-01	0	0	0	0	0	0	0	0
GLUCOSE	0	0	0	0	0	0	0	0
AIR	0	0	0	0	0	0	0	0
STARCH	0	0	0.051508929	0	0	0.703835282	0	0
CELLU-01	0	0	0	0	0	0	0	0
PROTEIN	0	0	0.55527604	0	0	0.216785087	0	0
NAOHS	0	0	0.023031005	0	0	0	0	0
ASH	0	0	0	0	0	0.034380014	0	0
Total	1	1	1	1	1	1	1	1

Table 56; Stream table for yellow pea protein production (continued)

Stream name	SPECS.DUM2	ULTFILT.D2	ULTFILT.DUMO	ULTFILT.INT	ULTFILT.L	ULTFILT.SL	ULTFILT.SN
Total flow rate [kg/hr]	6	82000	1000000	3795.96121	2373.3876	1422.57361	3795.96121
Mass flow rate [kg/hr]							
WATER	6	82000	1000000	3510.40438	2353.613104	1156.791276	3507.98926
GLUCOSE	0	0	0	0	0	0	24.15188819
AIR	0	0	0	0	0	0	0
STARCH	0	0	0	21.73676747	0	21.73676742	0
PROTEIN	0	0	0	234.3264825	0	234.3264829	234.3264825
NAOHS	0	0	0	29.49357866	19.77449483	9.719083847	29.49357866
ASH	0	0	0	0	0	0	0
Mass fraction							
WATER	1	1	1	0.924773512	0.99166824	0.813167957	0.924137278
CARBO-01	0	0	0	0	0	0	0
GLUCOSE	0	0	0	0	0	0	0.006362522
AIR	0	0	0	0	0	0	0
STARCH	0	0	0	0.005726288	0	0.015279889	0
CELLU-01	0	0	0	0	0	0	0
PROTEIN	0	0	0	0.061730473	0	0.164720111	0.061730473
NAOHS	0	0	0	0.007769726	0.00833176	0.006832043	0.007769726
ASH	0	0	0	0	0	0	0
Total	1	1	1	1	1	1	1

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