A dissertation

entitled

Effect of enzymatic modification on *in vitro* digestibility and selected functional properties of chickpea hydrolysates

submitted

by

Matheba Ndivho

(63286939)

in accordance with

the requirements for

the degree of

MASTER OF CONSUMER SCIENCE

in the

DEPARTMENT OF LIFE AND CONSUMER SCIENCES

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

of the

UNIVERSITY OF SOUTH AFRICA

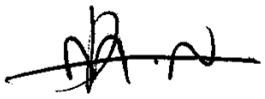
SUPERVISOR: DR D BESWA

February 2022

DECLARATION

I, Matheba Ndivho of student number 63286939, hereby declare that the dissertation titled **"**Effect of enzymatic modification on *in-vitro* digestibility and selected functional properties of chickpea hydrolysates, which I submit for the degree of MSc in Consumer Science at the university of South Africa, is my own work and has not previously been submitted for a degree at this or any other University. I declare that sources, materials, and results that are not original to this thesis are fully cited and referenced. I declare that during my study I have adhered to the Research Ethics Policy of the University of South Africa and have not acted out of the guidelines (2019/CAES-HRE C/116) (Appendix 1).

I declare that the content of my dissertation has been submitted through "Turn-it-in" (Appendix 2) and edited for language before the final submission for examination (Appendix 3).

Student signature: 

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Date: 28 February 2022

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ACKNOWLEDGEMENTS

I would like to thank the almighty who has been giving me strength throughout my studies. I appreciate the University of South Africa and the National Research Foundation for funding my MSC studies.

I sincerely appreciate my supervisor, Dr Daniso Beswa for his full support and attention to my studies, the effort he has made towards my studies, his constructive criticism, his input and suggestions and his motivation. It would be very difficult for me to complete this study without your wisdom and knowledge. Thank you for always been available for me whenever I have research questions and suggestions.

I would also like to thank Mr Hosana Mkoyi for his laboratory assistance during my experiment trial at the lab. He sacrificed his time just to assist me whenever I need lab assistance, he made sure that the required chemicals for my experiment are available in the lab, he made sure that the lab instruments required for my experiment were up and running and this helped a lot as my experiment were conducted smoothly.

I appreciate Mary from the Biochemistry Laboratory who allowed me to use their centrifuge, incubator, chemicals, and water bath during my lab work. Thank you for always been available for me.

I would like to extend my thanks to Prof John Dewar for assisting me with editing my thesis and making input on my research document. I'm glad I had the opportunity to meet and learn from you and benefited from your wisdom and knowledge.

I thank Prof E Madala from Department of Biochemistry, University of Venda, for assisting me with protein determination analysis using the Bradford method.

I would also like to thank Mr E M Nyathi from the Animal Science Department, University of Venda, for giving me an audience and accommodating me in their lab when I wanted to do protein determination analysis using the Kjeldahl method.

I want to acknowledge my husband and cheerleader Mr F.B. Mutshaeni for his constant support, for allowing me to be the best that I can, his Patience, his love. Thank you for always praying for me and for encouraging me to focus on my studies.

I sincerely appreciate my grandmother (Mrs. Elsie Nyadzani), my mother (Mrs. Sarah Magoloi) and my father (Mr. Nelson Moyahabo Matheba) for your prayers and support. Thanking you for believing in me.

DEDICATION

This research is dedicated to my mother Mrs S. Magoloi, my husband Mr. F.B. Mutshaeni, my daughter Vhugala and my son Mashuvho as well as my late mother in-law Prof. N.H. Mutshaeni who passed on when I was still busy with this study.

ABSTRACT

Chickpea (*Cicer arietinum* L.) is a good source of protein (16% to 30%); it also contains appreciable concentrations of other nutrients, including glycaemic carbohydrate (59% to 70%), fat (4.5%), fibre (5% to 8%) and minerals (3%). In this study, pre-treated chickpea flour was obtained from seeds of two chickpea varieties (*Kabuli* and *Desi*), separately, through two methods- isoelectric and micellized precipitation, respectively. The effect of enzymatic modification on proximate composition, in vitro digestibility and selected functional properties (solubility, water absorption capacity, oil absorption capacity, and syneresis) of the pre-treated chickpea flour were investigated. The isoelectric precipitate of *Desi* had a moisture content of 6.7% which is approximately 2% lower than the control sample (8.2%) while a mean value of moisture content was 7.0% for the micellized precipitate. Crude fat content had a mean value of 9.78% which is approximately 2% lower than its control sample (11.9%). Isoelectric precipitates showed high total mineral (ash) content (*Kabuli*: 4.2%, *Desi*: 3.9%) compared to the micellized precipitates (*Kabuli*: 1.9%; *Desi*: 1.8%). The degree of hydrolysis of pre-treated chickpea flour was over 80% by the start of the experiment and the figures decreased over time. The isoelectric precipitate for *Kabuli* exhibited the highest *in vitro* digestibility (77.79%) at T_0 followed by the micellized precipitate of *Desi* (76.2%). The isoelectric precipitated *Desi* exhibited high syneretic properties (174.25%) compared to micellized *Desi* (117.6%) at 0 min. Higher oil absorption capacity (micellized *Desi*: 228.39% to isoelectric *Desi*: 225.92%) was recorded for *Desi* while *Kabuli* showed the lowest mean values (micellized *Kabuli*: 90.88% to isoelectric *Kabuli*: 69.89%) of oil absorption capacity. The isoelectric and micellized precipitated *Desi* had similar (191.46% and 191.52%) water absorption capacity compared to the control sample (191.52%). Isoelectric precipitated *Desi* (4.94%) and *Kabuli* (4.46) recorded high percentage protein solubility compared to the micellized *Kabuli* (2.60%) and *Desi* (4.19%). The findings of this study indicate that chickpea seed protein hydrolysates are highly digestible, which implies that they are suitable for use in the production of legume-based products, such as health shakes and instant porridge that would be beneficial to old people who usually experience digestion problems and cardiovascular diseases. In addition, the highly digestible protein hydrolysates can be incorporated in cereal-based products, which would assist those with stomach digestion problems as well as those with protein deficiency.

Keywords: Chickpea protein, Precipitates, *Desi* and *Kabuli*, functional properties, *in vitro* protein digestibility.

MANWELEDZO

Nawa ndi tshiliwa tshandeme tshine tshavha ntha kha proteini (16% uya kha 30%). Nawa dzi dovha hafhu dzavha na mushumo wa nthesa kha u thusa nga uvha na dzinwe pfushi dzi nonga glycaemic carbohydrates (59% uya kha 70%), mapfura (4.5%), fibre (5% uya kha 8%) na mi mineral (3%). Kha hetshi sitadisi, pre-treated chickpea flour dzo wanala kha mbeu dza mifuda mivhili ya nawa (*Kabuli* na *Desi*) dzo khethekanywaho nga kha mi methodo mivhili (isoelectric na micellized precipitation). Masiandaitwa a enzymatic modification kha proximate composition, in vitro digestibility na dzi functional properties dzo vhalwaho (solubility, water absorption capacity, oil absorption capacity na syneresis) dza dzi pre-treated chickpea flour dzo senguluswa. Dzi isoelectric precipitate dza *Desi* dzovha na moisture concentration ya 6.7% zwine zwavha fhasi nga 2% zwi tshi vhambedzwa na control sample (8.2%) ngeno thanganyelo ya moisture content yovha i 7.0% kha micellized precipitation. Mapfura a isoelectric precipitated *Desi* o sumbedza thanganyelo ya (9.78%) zwine zwavha fhasi nga percent mbili zwitshi vhambedzwa na percent ya controlo ya 11.9%. Isoelectric precipitate yo bveledza pre-treated chickpea flour dzine dzavha ntha kha ash content (*Kabuli*: 4.2%; *Desi*: 3.9%) zwi tshi vhambedzwa na micellized (*Kabuli*: 1.9%; *Desi*: 1.8%). Mveledzo dzo sumbedza uri degree ya hydrolysis ya protein ya nawa yo vha I ntha ha 80% nga lwa u tou thoma ha experimente. Hone ha, Figara edzi dzodo tsela fhasi musi tshifhinga tsha tshitshiya phanda. Isoelectric precipitated protein hydrolysates ino khou bva kha *Kabuli* yo bveledza tshivhalo tsha ntha tsha tsukanyo (77.79%) ha tovhela *Desi* hydrolysates ino khou bva kha micellized precipitation (76.2%) zwine zwavha ntha u fhirisa micellized *Desi* (117.6%). *Desi* hydrolysate yo sumbedza pecente ya ntha ya oil absorption capacity (micellized *Desi:* 228.39% uya kha isoelectric *Desi:* 225.92%) ufhirisa *Kabuli* hydrolysate (micellized *Kabuli*: 90.88% uya kha isoelectric *Kabuli*: 69.89%). Isoelectric kana micellized precipitated *Desi* yo vha na water absorption capacity ya ntha zwi tshi vhambedzwa na sampulu ya control (191.52%). Isoelectric *Desi* na *Kabuli* yo ripota pecente ya ntha ya protein solubility zwi tshi vhambedzwa na micellized *Kabuli (2.60%)* na *Desi (4.19%)*. Mveledzo dza sitadisi etshi dzi sumbedza uri dzi proteini hydrolysate dza nawa dzina tsukanyo ya nthesa, zwine zwa amba uri dzia kona u shumiswa kha dzi legume products dzinonga dzi shakes dza mutakalo na mikapu wa vhaaluwa vhunga vhanzhi vhana thaidzo ya tsukanyo na malwadze a mbilu. Zwinwe hafho dzi hydrolysates dzine dzavha na proteini ya tsukanyo ire ntha dzi dovha hafho dza thusa musi huchico itiwa zwiliwa zwina dzi cereals zwine zwa do thusa kha vhathu vhana thaidzo ya tsukanyo thumbuni na protein deficiency.

Maipfi a ndeme: Chickpea protein, precipitate, *Desi* na *Kabuli*, functional properties, *in vitro* protein digestibility.

USHWANKATHELO

I-Chickpea (i-Cicer arietinum L.) ngumthombo olungileyo weprotheyini (16% ukuya kwi-30%); iqulethe i-concentrations exabisayo yezinye izondlo, kubandakanywa i-glycemic carbohydrate (59% ukuya kwi-70%), i-fat (4.5%), i-fiber (5% ukuya kwi-8%) kunye neemaminerali (3%). Kolu phononongo, iiproteni zodwa zifunyenwe kwiimbewu zeentlobo ezimbini zechickpea (i*Kabuli* kunye ne*Desi*), ngokwahlukeneyo, ngeendlela ezimbini- imvula ye-isoelectric kunye ne-micellized, ngokulandelanayo. Umphumo wokuguqulwa kwe-enzymatic ekubunjweni okusondeleyo, i-in vitro digestibility kunye neempawu ezikhethiweyo zokusebenza (i-solubility, umthamo wokufunxa amanzi, amandla okufunxa ioli, kunye ne-syneresis) ye- pre-treated chickpea flour yaphandwa. I-isoelectric precipitate ye-*Desi* yayinomswakama we-6.7% omalunga ne-2% ngaphantsi kwesampuli yokulawula (8.2%) ngelixa ixabiso eliqhelekileyo lokufuma laliyi-7.0% kwi-precipitate ye-micellized. Isiqulatho samafutha akrwada sinexabiso elilinganiselweyo le-9.78% elimalunga ne-2% ngaphantsi kunesampulu yolawulo (11.9%). I-Isoelectric precipitates ibonise umxholo ophezulu weeminerali (uthuthu) (i-*Kabuli:* 4.2%, i-*Desi*: 3.9%) xa kuthelekiswa ne-micellized precipitates (i-*Kabuli:* 1.9%; i-*Desi*: 1.8%). Iqondo le-hydrolysis ye- pre-treated chickpea flour ibingaphezulu kwe-80% ekuqaleni kovavanyo kwaye amanani ehlile ngokuhamba kwexesha. Imvula ye-isoelectric precipitate yase*Kabuli* ibonise owona mgangatho wokugaya uphezulu kwi-in vitro digestibility (77.79%) kwi-T0 ilandelwa yimvula eyenziwe nge-micellized ye-*Desi* (76.2%). I-isoelectric precipitated *Desi* ibonise iipropati eziphezulu ze-syneretic (174.25%) xa kuthelekiswa ne-micellized *Desi* (117.6%) kwi-0 min. Umthamo ophezulu wokufunxa i-oyile (i-micellized *Desi:* 228.39% ukuya kwi-isoelectric *Desi:* 225.92%) yarekhodwa kwi-*Desi* ngelixa i-*Kabuli* ibonise amaxabiso aphantsi kakhulu (i-micellized *Kabuli:* 90.88% ukuya kwi-isoelectric *Kabuli*: 69.89%) yomthamo wokufunxa i-oyile. I-isoelectric kunye ne-micellized precipitated *Desi* yayine-*Desi* efanayo (191.46% kunye ne-191.52%) yokufunxa amanzi xa kuthelekiswa nesampuli yokulawula (191.52%). I-Isoelectric precipitated *Desi* (4.94%) kunye ne*Kabuli* (4.46) zirekhode ipesenti ephezulu yokunyibilika kweprotheyini xa kuthelekiswa ne-micellized *Kabuli* (2.60%) kunye ne*Desi* (4.19%). Iziphumo zolu phononongo zibonisa ukuba i-chickpea seed protein hydrolysates igaywa kakhulu, nto leyo ethetha ukuba ikulungele ukusetyenziswa kwimveliso ye-legume-based, njenge-shakes yezempilo kunye ne-porridge ekhawulezayo enokuba luncedo kubantu abadala abadla ngokufumana ukugaya. iingxaki kunye nezifo zentliziyo. Ukongeza, iprotheyini ye-hydrolysates egaywa kakhulu inokudityaniswa kwiimveliso ezisekwe kwi-cereal, ezinokuthi zincede abo baneengxaki zokwetyisa kwesisu kunye nabo banqongopheleyo.

Amagama angundoqo: Iprotheni ye-Chickpea, i-Precipitates, i-Desi kunye ne-Kabuli, iipropati ezisebenzayo, i-in vitro protein digestibility.

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%	Percent
ANOVA	Analysis of variance
AOAC	Association of Agricultural Chemists
BSA	Bovine Serum Albumin
CAES	College of Agriculture and Environmental Sciences
CO_2	Carbon dioxide
Corp	Corporate
DH	Degree of hydrolysis
DW	Dry weight
FAO	Food and Agriculture Organisation
FFA	Free fatty acids
g	Gram
GI	Glycaemic index
H_0	Null hypothesis
H_1	Hypothesis
HCl	Hydrochloric acid
hr	Hour
IAAs	Indispensable amino acids
Isoel	Isoelectric precipitation
IVPD	*in vitro* protein digestibility
M	Mole
MCS	Masters in Consumer Science
mg	Milligram
Micel	Micellization precipitation
min	Minute
ml	Millilitre
N_2	Nitrogen
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
Nr	Number

OAC	Oil absorption capacity
^{o}C	Degrees celsius
PEM	Protein energy malnutrition
RO	Reverse osmosis
SD	Standard deviation
Sec	Second
SPSS	Statistical Package for Social Science
SW	Sample weight
TCA	Trichloroacetic acid
Unisa	University of South Africa
USA	United States of America
VWR	Vortex mixer
w/v	Weight/volume
WAC	Water absorption capacity
WHO	World health organisation

CHAPTER 1 INTRODUCTION

1.1 Background

Chickpea (*Cicer arietinum* L) plays a significant role in sustaining the lives of communities in most Asian and North African countries where it is considered as a food security crop due to its resilience to various climatic and soil conditions (Rachwa-Rosiak *et al.,* 2015). This legume has two main commercially available varieties (Roy *et al.,* 2010) - *Desi* which is grown in semi-arid regions and *Kabuli* in temperate climatic conditions (Miao *et al*., 2009). According to culinary practices, chickpea seeds can be eaten as is or de-shelled (Simsek *et al.,* 2016) while crushed chickpeas can be used in soups, salads, and snacks (Boye *et al.,* 2010).

The major components of chickpea which are exploited during processing and food preparations are protein and starch (Vassilis *et al.,* 2011; Foegeding and Davis, 2011). The nutritional components of chickpeas are mainly glycaemic carbohydrates (70%) (Bashir *et al.,* 2016), protein (17-30%) (Boye *et al.,* 2010), dietary fibre (9.0%) (Meng *et al.,* 2010) and are also high in iron and other minerals such as molybdenum, manganese (Aguilar *et al.,* 2015) and is, therefore, considered as a healthy vegetarian food (Simsek *et al.,* 2016).

Consumers are becoming aware of the potential benefits of chickpeas in their diet and there is an increase in the interest of using its derivative ingredients in the development of novel foods (Boye *et al.,* 2010). In chickpea flour, the molecular interactions of chickpea proteins play a significant role on their physicochemical, textural, rheological, and thermal properties that allow for the development of new products (Milan-Noris *et al*., 2019; Mohamed *et al.,* 2009). Among cereal grains, corn, wheat, and rice are the most used cereals worldwide but white maize is the preferred source of starch and protein in sub-Saharan African countries while chickpea as a food is unpopular in South Africa, especially among native people (Lone *et al*., 2021; Jukanti *et al*., 2012).

Starch is a plant storage polysaccharide that consists mainly of two monosaccharides amylose and amylopectin. It is a major energy source in the human diet (Choi *et al.,* 2018) and the starch content in rice ranges from 70-90%, in corn from 70-73% and in wheat from 65-67% (Halal *et al.,* 2019). While cereal grains are the most popular raw materials used to isolate and extract starch, this process is made more difficult by the low moisture content in cereal grains (Giuberti *et al.,* (2018). The endosperm of the cereal grains contains a matrix of protein bodies infused around starch granules together with other cellular structure (Shewry *et al*., 2002) and so

milling of the endosperm inevitably physically damages the granules (Halal *et al*., 2019}. The protein-starch interaction in cereal grains occurs due to the electrostatic attraction between the negatively charged protein molecules and positively charged starch granules (Wang *et al.,* 2017). The protein–starch interactions can occur in two ways: the proteins associated with the granule surface and proteins present within the granule (intrinsic proteins). A weak protein–starch interaction prevents the adhesion of starch molecules to the protein matrix and facilitates separation during milling (Halal *et al.,* 2019). The degree of protein binding on the starch granule surface varies considerably among cereal grains. Interaction between protein and starch have been shown to be (at least in part) responsible for variation among cereal grains in the case of ruminal degradation. Crude protein sub-fractions and molecular structures of protein play important roles in its potential of gastrointestinal degradation rate (Ying *et al*., 2019) and might have relation with starch degradation potential in cereal grains (Yu *et al*., 2004).

An advantage to chickpeas is that they are well-balanced nutritionally and are a good source of carbohydrates and protein with a low cholesterol content and glycaemic index (Tan *et al.,* 2021). Unlike in cereal grains, starch in chickpea ranges between 29.1-46.0% on a dry weight basis which is lower compared to the figures for rice, wheat, and corn listed above (Hoover *et al*., 2010).

Pellegrini *et al*. (2020) reported that after World War 2, people switched from their accustomed diet and began to consume food rich in refined flour and gelatinized starch, cereal-based products, maize porridge, and white bread. These foods are major contributors to a high calorie intake and the so-called 'obesogenic environment' of modern societies. The addition of chickpea grains or flour in the human diet can help address the problem of obesity and cardiovascular disease as chickpea grains are associated with a reduced risk of various health medical conditions including cardiovascular disease, different types of cancer, type 2 diabetes, osteoporosis, hypertension, digestive disorders, and adrenal disease (Jukanti *et al*., 2012; Mpai *et al.,* 2018).

It is well-known that the essential amino acid lysine is generally deficient in cereal grains, including maize grain (Butts *et al.,* 2012). Lysine deficiency that is associated with the prevalence of protein-energy malnutrition (PEM) in populations where maize is a major staple food (Nyakurwa *et al.,* 2017).The groups most vulnerable to PEM are infants and young children in developing countries (Dettwyler, 2011). Thus, diversifying diets with affordable sources of proteins such as chickpeas seeds that, compared to maize, contain a high lysine

content, could significantly reduce the risks of PEM. Currently, there are on-going efforts to familiarize native African communities regarding the uses and advantages of chickpea and to encourage its use as a source of protein. However, chickpeas have low protein and starch digestibility (Xu *et al.,* 2016; Ma *et al.,* 2011; Wang *et al.,* 2010; Roy *et al.,* 2010; Khattab *et al.,* 2009). According to Ren *et al*. (2015) and Hawkins and Johnson (2005), foods associated with a slow rate of starch digestion are helpful in human diet because they evoke a low post meal blood glucose response and contain a low glycaemic index. On the other hand, these authors continued, the digestibility of protein is an important element of protein quality in humans as this provides an approximation of the bioavailability of individual amino acids. Therefore, its slow digestion in addition to its binding anti-nutritional factors have a negative effect on human nutrition (Bar-EL-Dadon *et al.,* 2017).

Modification of chickpea protein may influence its structure and functional properties; thus, the aim of this study was to evaluate whether enzymatic modification of chickpea protein improved its digestibility and functional properties and thus facilitate the use of the protein in staple diets.

1.2 Research problem

Protein is one of the essential nutrients required by humans and is critical for health and development, particularly by infants and children (Elmadfa and Meyer, 2017). However, due to its expense, the availability and accessibility of animal protein sources is often low in low-income communities where cereal grain remains the major source of protein contributing towards a relatively high prevalence of protein-energy malnutrition (Khalid *et al.*, 2012). High morbidity and mortality of infants and children are a major concern to health authorities in developing countries (WHO, 2002) and a possible sustainable strategy to alleviate protein-energy malnutrition is to increase dietary intake of chickpea grain. This is relatively high in protein compared to cereal grains but, like other plant proteins, chickpea protein has relatively low digestibility (Xu *et al.,* 2016). In addition, the low digestibility of chickpea protein is compounded by the existence of anti-nutritional elements that have an adverse effect to one's health (Bar-EL-Dadon *et al.,* 2017; Jukanti *et al*., 2012).

1.3 Relevance of study

The affordability of animal protein is a challenge to low-income households and so finding alternative sources of protein that are affordable and locally available may offer a sustainable strategy to address protein deficiency. Chickpea is highly nutritious and resilient to harsh climatic and soil conditions and its flour has a wide range of potential applications in household food preparations. Thus, scientific study of chickpea and its derived ingredients may contribute at the household level towards a novel dietary use of chickpea that will contribute towards nutrition security and reduction in protein-related deficiencies in communities where there is currently a heavy dependence on cereal staple diets.

1.4 Research aim and objectives

1.4.1 Study aim

To determine the effects of enzymatic modification on *in vitro* digestibility of chickpea protein and its suitability as a functional food ingredient. In addressing this aim, the following study objectives were formulated**:**

1.4.2 Study objectives

1. To determine the proximate composition of pre-treated chickpea flour
2. To determine the effect of enzymatic modification on selected functional properties of chickpea protein hydrolysates.
3. To determine the effect of enzymatic modification of *in vitro* digestibility of chickpea protein hydrolysates.

1.5 Research hypotheses

Hypothesis (H_1): The use of enzymes to modify chickpea protein will improve its digestibility and its functional properties.

Null hypothesis (H_0): The use of enzymes to modify chickpea protein will not influence its digestibility or its functional properties.

1.6 Ethical considerations

The reported research work was conducted in the Eureka Laboratories of the UNISA Science Campus under the supervision of Dr D Beswa. Ethics approval was obtained from the UNISA Ethics (Ethic nr: 2019/CAES-HREC/116) (Appendix 1). To avoid any injuries to other students and staff members, the laboratory code of conduct and the safety rules were meticulously followed. The COVID-19 rules such as maintaining a 2 m social distance from other laboratory occupants, wearing a mask and sanitising were adhered to all the time. Plagiarism was avoided, and all published research were suitably cited. All research work was kept strictly confidential until published as a completed MCS dissertation.

1.7 Outline of the dissertation

This dissertation consists of five chapters:

Chapter 1: This chapter presents a brief overview of the study with background information on chickpea protein and its suitability as a functional dietary ingredient. This follows with the problem statement, the relevance of the study followed by the aim and objectives of the study. It concludes with a layout of the dissertation as well as the ethical features for the study.

Chapter 2: This chapter provides a review of the literature of surrounding chickpea protein and its functional properties. Firstly, the background information of chickpea is provided followed by a review of the chemical composition of chickpea. The protein composition of chickpea and its behaviour under different processing conditions is reviewed to highlight the functional properties of protein. Finally, the review of interaction of chickpea protein with other food components in a food matrix, it's *in vitro* digestibility as well as its use in various food products.

Chapter 3: This chapter describes the methodology applied to address the research objectives of the study. The chapter describes the materials used, the milling of the chickpea seeds, isolation of chickpea protein, the proximate composition of pre-treated chickpea flour, enzymatic hydrolysis of pre-treated chickpea flour and determination of degree of protein hydrolysis. Furthermore, *in vitro* protein digestibility of chickpea protein hydrolysates as well as a simulated gastric and intestinal digestion methods are detailed. Methods used to analyse selected functional properties of chickpea protein hydrolysates; statistical analysis used in the study followed by ethical considerations are also described.

Chapter 4: Study results are analysed and presented and then described in relation to the study objectives and hypotheses. These results are presented in the form of tables and figures. In the same chapter, the results are interpreted in relation to the study objectives and hypotheses.

Chapter 5: Research conclusions are presented in this chapter in relation to each study objective. Furthermore, this chapter discusses the contributions and limitations of the study and, based on the findings of the study and the literature reviewed, suggests recommendations for future research on chickpea protein as well as its applications in food and pharmaceutical industry. Chapter 5 is followed by a list of references and appendices.

CHAPTER TWO: LITERATURE REVIEW

This chapter provides a brief overview of legumes and background information on two varieties of chickpea (*Desi* and *Kabuli*), their food components, behaviour of protein under different processing conditions, functional properties of their protein, interaction of their protein with other components in the food matrix, in vitro protein digestibility, food applications of chickpea protein, and conclusion.

2.1 An overview of legumes

Legumes are among the most grown crops in the world, and they are an excellent low-fat source of vegetable protein, amino acids, and fibre (Gu *et al*., 2021). Globally, legumes play an important part in promoting healthy eating habits as they also assist in addressing nutritional needs in addition to their prominent position in many plant-based diet by providing a rich supply of dietary fibre (Didinger and Thompson, 2021). The five most common pulses are chickpea, dry bean, dry pea, lentils and cowpea and the pulses shown in Figure 1 are edible seeds that grow within a pod (Gharibzahedi *et al*., 2021).

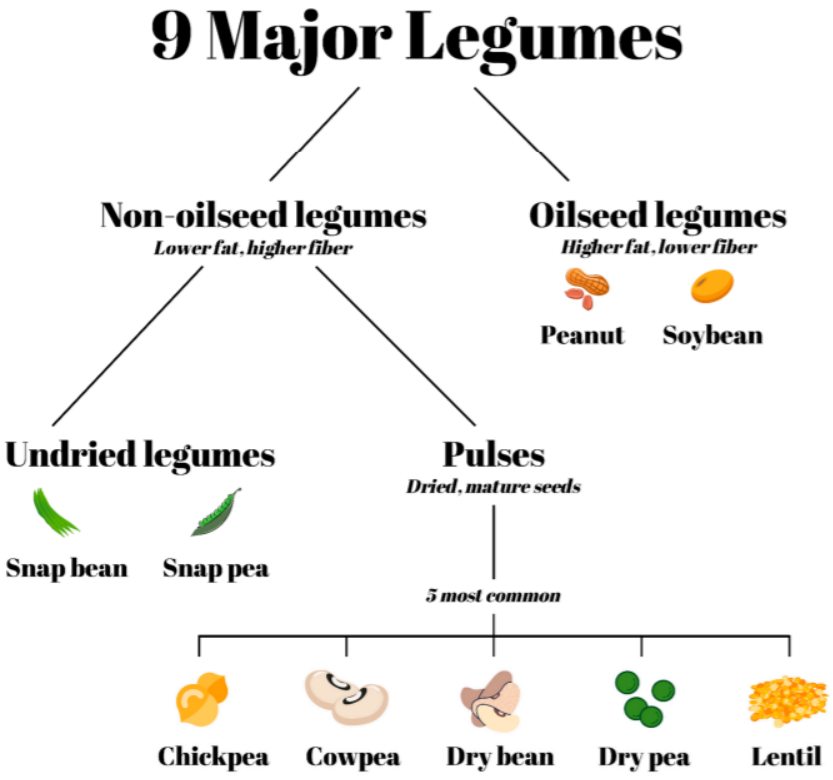

Figure 1. Commonly consumed types of legumes (Didinger and Thompson, 2021).

The terms legume and pulse are frequently utilized interchangeably but there are clear distinctions between them (Didinger and Thompson, 2021). Pulses are a yearly leguminous crop yielding between 1 and 12 grains or seeds of varying size, shape, and colour within the pod (FAO, 2016). Pulses are crops that are harvested solely for dry grain and this excludes those that are harvested green for foods which are categorised as vegetables crops including those used for oil extraction and leguminous crops that are utilized specifically for sowing purpose (FAO, 2016).

Pulses are known to boost the nutritional value of cereal-based foods by enhancing protein content as well as the availability of lysine (Arab *et al.,* 2010). Legumes, on the other hand, are regarded as an important source of protein, starch, dietary fibre, vitamin, and mineral. Legumes can be incorporated with other food to improve the nutritional value of extruded foods, and this has been reported to reduce malnutrition in developing countries (Pasqualone *et al.,* 2020).

Cowpea is a grain legume crop that provides protein and generates income to many smallholder farmers in developing countries (Opoku *et al.,* 2021). It is a good source of carbohydrate and protein making them a viable non-traditional source of these nutrients (Segura-Campos *et al.,* 2012. Lentils are widely grown and provide a considerable amount of carbohydrate intake in many Middle Eastern and Southern Asian countries (Thavarajah *et al.,* 2011). Lentils, as a result, may play a key role in supplying low digested carbohydrates, often known as prebiotic carbohydrates (Johnson *et al.,* 2013). Dry peas are the second most important food legume grown worldwide (Wang and Daun 2004) as they have a high nutritional value due to their well-balanced amino acid composition and minimal antinutrient content (Walia *et al*., 2017). In southern Africa and central America, the dry bean is a popular legume for human consumption, and it is consumed by all sectors of the population, along with rice (Fageria, 2002). Dry bean is eaten as a seed as well as a green vegetable in Africa and Asia. It is high in protein content making it a good source of calories especially for people with a low-income and its consumption is increasing in emerging economies countries (Fageria *et al.,* 2012). This study focuses on the chickpea pulse which is said to be an annual herbage plant and the third most important grain legume in the world based on total grain production (Li *et al*., 2008).

2.2 Background information on chickpeas

The chickpea (*Cicer arietinum* L.) is an old-world pulse, or edible seed, within the legume family (Wallace *et al*., 2016) and is the only cultivated species in the genus *Cicer* (de Camargo *et al*., 2019). Chickpeas are a common staple food in some Asian, Mediterranean, and African countries with India being the world's leading producer of chickpeas (Wallace *et al*., 2016; Mohamed, 2014). Chickpeas are amongst the most important legumes and are considered the third-most produced pulse crop after beans and peas (Aviles-Gaxiola *et al.,* 2018). Based on colour and geographical distribution, chickpea seeds are normally divided into the light seeded *Kabuli* type which is mostly found in the Middle Eastern region and Mediterranean and the smaller, dark *Desi* variety that is commonly found in the Indian region (Wallace *et al*., 2016; Yegrem, 2021). Some of these morphological characteristics are shown in Figure 2.

Desi *Desi*

Kabuli *Kabuli*

Figure 2: Photographs showing differences between *Desi* and *Kabuli* chickpea flowers and seeds. Adopted from Pulse Australia (2016).

The *Desi* chickpea plant has purplish or bluish flowers while the *Kabuli* plants have larger leaflets with white flowers (Hughes *et al.,* 2009; Yadav *et al.,* 2005). Their seeds vary as to shape, size, and colour (Wood *et al.,* 2011) - the *Desi* chickpea seed has small seeds, usually with 2-3 seeds per pod and a thick coat that are angular in shape with a prominent, characteristic ‘‘beak’’ that houses the embryonic axis (Wood *et al*., 2011). In contrast, *Kabuli* seeds are round, cream in colour and the pod usually contains 1 or 2 seeds and have a thin, white seed coat (Yadav *et al.,* 2005).

As with other legumes such as peas, beans, soybeans and lentils, chickpea seeds are an excellent source of proteins, calories, minerals, and vitamins and are low in lipids (Ribeiro *et al*., 2017). They contain carbohydrates (mainly starch) (63%), protein (22%) crude fibre (8%), fat present in low amounts (4.5%) and minerals (ash) (2.7%) (Zhang *et al.,* 2016) and are high in nutritionally important unsaturated fatty acids such as linoleic acid and oleic acid (Yegrem, 2021). These figures for chickpea are comparably higher than those of white maize grain crude protein (7.28%), crude fibre (4.69%) and mineral ash content (1.23%) (Oluba *et al.*, 2018). However, maize remains an excellent source of carbohydrates (72.89%), at a level comparable to chickpea seeds (60-72%) (Rahimi Jahangirlou *et al*., 2021; Oluba *et al*., 2018). Chickpea proteins are known to be low in anti-nutritional factors such as protease inhibitors, tannins, phytic acid, and saponins (Xu *et al*., 2016) and high in bioavailable essential amino acids (Zhang *et al.,* 2011).

Anti-nutritional factors are molecules produced in natural plant foods by normal metabolism and by interfere with metabolism in animals, including human beings- metabolic interference includes inactivation of some nutrients, and a reduction in digestive process (Soetan *et al.,* 2019). The presence of anti-nutritional factors, such as phytic acid, polyphenols, and trypsin inhibitors, limits the bioavailability of chickpea nutrients (Lasse *et al.,* 2015). Anti-nutritional factors have a negative effect on chickpea protein - trypsin inhibitors interfere with the digestion of chickpea proteins in the digestive tract of humans (Bar-EL Dadon *et al.,* 2017). Other anti-nutritional factors in chickpea seed are tannins (0.4-0.8%) (Zia-ul-hag, 2017) which are known to bind proteins through non-covalent interactions, thereby reducing their nutritional bioavailability. Tannins also affect the nutritive value of legumes as they bind to enzyme and non-enzyme proteins to form tannin-protein complexes that inactivate digestive enzymes and

reduce protein digestion to then form more complex bonds with starch, cellulose and minerals that reduce their digestion (Lasse *et al.,* 2015).

2.3 Chemical composition of chickpea

2.3.1 Composition of major food components

Chemical compounds in chickpeas consist of glycaemic carbohydrates (59 - 70%), protein (16 - 30%), fat (4.5%), crude fibre (5 - 8%) and ash (3%) (Lucero *et al.,* 2018), while Zhang *et al.* (2016) analysed these chickpea components as carbohydrates (63%), protein (22%), fat (4.5%), crude fibre (2.9%) and ash (2.7%). Raw chickpeas have a relatively high fibre content compared to other pulses (Aquilera *et al*., 2009) such as cowpea (3.16%), pigeon pea (3.13%), green pea (7.47%), sugar pea (2.23%), yellow pea (14.84%), fava bean (13.80%) (Samaila *et al.,* 2020; Pastell *et al*., 2019; Millar *et al*., 2019). Pulses have shown several health benefits such as lower glycaemic index, cancer prevention and protection against cardiovascular disease (Arab *et at.,* 2010). Pulses are known to boost the nutritional value of cereal-based foods by enhancing protein content as well as the availability of lysine (Arab *et al.,* 2010). Legumes, on the other hand, are regarded as an important source of protein, starch, dietary fibre, vitamin, and mineral. Legumes can be incorporated with other food to improve the nutritional value of extruded foods, and this has been reported to reduce malnutrition in developing countries (Pasqualone *et al.,* 2020).

Table 1 shows the proximate composition of *Desi* and the *Kabuli* chickpea cultivars. According to Khan *et al.* (1995), Rincon *et al.* (1998) and Singh *et al.* (2004), the *Desi* chickpea contains protein ranging between 16.1% and 26.7%, carbohydrates between 47.4% and 66.9%, a fat content ranging between 3.10% and 4.93% and an ash content between 2.7% and 3.6% (dry weight basis).

Table 1. Proximate composition of chickpea seed (% dry matter) (Khan *et al.* (1995); Rincon *et al.* (1998); Singh *et al*. (2004); Miao *et al*., (2009); Khalil *et al.* (2007).

Chickpea variety	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fibre (%)	Crude Fat (%)	Carbohydrates (%)
Desi	8.0-8.3	2.70–3.60	16.1–26.7	5.5-6.0	3.10–4.93	47.4–66.9
Kabuli	8.0-8.5	2.80–3.42	19.9–25.5	4.8- 5.5	4.60–5.67	47.6–66.9

This contrast with results from analysis of *Kabuli* chickpeas that contain protein ranging from 9% to 25.5%, a carbohydrate content ranging between 47.6 and 66.9%, a fat content of between 4.60 and 5.67%, and an ash content ranging from 0.80% to 3.42%. Khalil *et al*. (2007) reported that the moisture content of *Desi* chickpeas ranges between 8.0% to 8.3% while that for *Kabuli* chickpeas ranges between 8.0% and 8.5%. The fibre content of *Kabuli* chickpeas ranges between 4.8% and 5.5% while in *Desi* chickpea fibre content ranges between 5.5 % and 6.5% (Miao *et al*., 2009).

Glycaemic/available Carbohydrate: At over 60%, carbohydrates are the major component of chickpeas, and this compares to the total carbohydrate content of other pulses ranging between 57% - 67% for Bambara ground nuts), 50% to 60% for cowpeas and 66.6% for horse gram (Sreerama *et al.,* 2012; Segura-Campos *et al.,* 2012).

Protein: Chickpea proteins are usually classified into two major fractions, globulins, and albumins (Singh *et al.,* 2008) with smaller amounts of glutelins and prolamines (Wallace *et al*., 2016).

Fat: Chickpeas are considered to have a relatively high fat content compared to grains, but such fats are high in essential unsaturated fatty acids such as linoleic acid, oleic acid, and linolenic acid (Raza *et al*., 2019). The fatty acids composition of chickpea depends on the season in which they were planted, all chickpeas planted in autumn have 35 - 63% oleic acid, 18 - 47% of linoleic acid (Bar-EL Dadon *et al.,* 2017).

Fibre: Raw chickpea seed is high in dietary fibre content compared to other pulses (Aguilera *et al.,* 2009) with soluble dietary fibre ranging from 4% to 8% while insoluble dietary fibre ranges from 10% to 18% dry weight (Rachwa-Rosiak *et al.,* 2015). Chickpeas also have unavailable carbohydrates such as oligosaccharides and resistant starch (Bar-EL Dadon et al., 2017).

Ash: The raw chickpea seeds seem to have low ash content compared to other legume; chickpea (Chickpea (2.2%), cowpea (2.9%), Horse gram (2.7%) (Sreerama *et al.,* 2012).

As this study focussed on an analysis of chickpea proteins, the following sections will detail relevant features of chickpea proteins.

2.4 Protein composition of chickpea

Chickpea has a relatively high protein content (14.9% to 24.6%) which can be separated into 56% salt-soluble globulin, 12% water-soluble albumin, 2.8% alcohol-soluble prolamin, 18.1% acid/alkali-soluble glutelin, and residual proteins (Rachwa-Rosiak *et al.,* 2015). Globulins, the major seed proteins, contain mainly legumin and vicilin, which represent 60% to 80% of the extractable proteins while the albumin fraction represents 15% to 25% of the total cotyledonary proteins. According to Clemente *et al.* (2000), albumins play a crucial part in seeds since they contain most of metabolic proteins and possess a large amount of nutrients due to their high lysine content and sulphur amino acids. Their lysine content ranges between 6.83% and 7.2% while sulphur amino acids range between 2.11 g/110 g and 2.20 gm/110 gm (Kaur *et al* 2021). Furthermore, chickpea protein displays good functional properties such as solubility, water, and oil absorption capacity, emulsifying properties, foaming, and gelling which are highly dependent on amino acid composition and protein structure and, from a research perspective, these properties are affected by the choice of extraction approach and processing parameters such as pH and temperature) (Boye, *et al.*, 2010; Day *et al*., 2013). Thus, chickpea protein can be explored in various food applications.

Dissimilarities in protein content and the amount of protein observed in chickpeas and other legumes could be attributable to variety, environmental factors, geographical location, plant growth season and analytical methods (Maheri-Sis *et al.,* 2008). Chickpea flour consist of 39.89 g/100 g protein content of essential amino acids and 58.64 g/100 g protein content of endogenous amino acids. Methionine and cysteine are deficient amino acids in chickpea seeds while aspartic acid and arginine are substantially high in the seeds of chickpea (Rachwa-Rosiak *et al.,* 2015; Boye *et al.*, 2010; Chiaiese *et al*., 2004).

Chickpea protein isolates are characterised by a looser structure which makes them more bio-accessible to the human digestive system. The nutritiousness of chickpea plays a crucial role when chickpea is incorporated as a supplementary ingredient in other food formulations. In a study by Rachwa-Rosiak *et al.* (2015), where sorghum flour was supplemented with chickpea flour, a significant increase in the content of some essential amino acids (lysine, methionine, cysteine, and tyrosine) in the resultant composite flour was observed. However, when such composite flours were subjected to heat processing, their amino acid content decreased slightly due to thermal degradation (Omima *et al.*, 2010; Simons *et al.,* 2015; Nosworthy *et al.,* 2020). Chickpeas were analysed and found limited in the sulphur-containing amino acids, cysteine, and methionine. However, after cooking (0.86%), extrusion (0.97% and baking (0.95%) the

amino acid scores were higher than expected and this is because cooking increases protein digestibility (Jukanti *et al.,* 2012; Wang *et al.,* 2004). Differences in amino acid composition as well as the score of the processed chickpea can result from different chickpea varieties, geographical location of the crop as well as environmental factors (Nosworthy *et al.,* 2020).

2.5 Behaviour of chickpea protein under different processing conditions

As with other food components, chickpea protein behaves differently under various processing methods and conditions. One of the processing methods that is known to influence the behaviour of protein is extrusion cooking that has a positive effect on the nutritional characteristics of the end-product since it induces essential modifications of both starch and proteins, improving their digestibility and reducing the tannin content, trypsin inhibitors, lectins and phytic acid (Pasqualone *et al.,* 2020). When subjected to extrusion cooking, the protein undergoes denaturation due to high temperature caused by cooking heat and friction and heat as well as shear forces that change it's 3-dimensional structure to expose sites making their protein accessible to enzymes (Alam *et al*., 2015) and, consequently, it's digestibility increases (Patil *et al*., 2016; Ghumman *et al*., 2016; Zarzycki *et al*., 2015) by 13 - 18% (Arribas *et al.,* 2019). The study by Nosworthy et al. (2020) revealed that extrusion cooking is the optimal method for producing high quality chickpea protein and this results in changes in the physical, chemical, and nutritional properties if the food, while increasing protein digestibility (Arribas *et al.,* 2017).

Extrusion cooking is a technique that is widely used to produce several ready-to-eat products such as crisp expanded snacks, breakfast cereals, instant soup meat analoques and sports food (Offiah *et al*., 2018). The amount of anti-nutrients such as tannins, trypsin inhibitors, lectin and phytic acid which are found in legumes can be decreased by Extrusion cooking (Pasqualone *et al.,* 2020). Furthermore, extrusion cooking can increase the digestibility of starch and protein (Patil *et al.,* 2016). As indicated above, extrusion cooking significantly improves *in vitro* protein digestibility (IVPD) which is known to have an impact on protein quality (Aguirre *et al.,* 2000; Ojokoh *et al.,* 2011; Boye *et al.,* 2012; Giacomino *et al*., 2013; Arribas *et al*., 2017; Zhang *et al.*, 2017). At the molecular level, changes in the chickpea protein including denaturation, chain dissociation or aggregation or changes in covalent crosslinking bonds influence the degree of protein hydrolysis and the extent and the type of changes to chickpea proteins (Zhang *et al.,* 2017).

Fermentation is another processing method where protein undergoes denaturation. This process degrades legume proteins to release small peptide fragments and amino acids (Maleki *et al.,* 2021). Fermentation increases the presence of protein because of the limited denaturation of the storage protein along with the decrease in unpleasant compounds resulting from microbial enzymic activity (Coda *et al.,* 2017; Giami 2004; Espinosa-Paez *et al.,* 2017; Fawale *et al.,* 2017). Reduction in the protein content by fermentation may be due to proteolysis resulting from the formation of the ammonia which is a component of such processing of protein in wholesome foods (Beaumont, 2002). Insoluble protein undergoes structural changes during the fermentation process (Giami, 2014) and this can improve *in vitro* protein digestibility of chickpea protein particularly in association with proteolysis caused by fermentation microorganisms (Sakandar *et al*., 2021). Hydrolysis of protein throughout the fermentation process contributes towards the release of ACE-inhibiting bioactive peptides by the action of microbial proteases from specialized protein bodies found in the organelles of chickpea seeds (Khattab *et al.,* 2009).

Food irradiation is a technology where food is subjected to ionization in a certain environment for a specific time and under process-controlled conditions (Sa *et al.,* 2020). This process may eliminate or enhance the safety and shelf life of products by eliminating microorganisms (Byanju *et al*., 2021). While food irradiation is prohibited from being used to increase the nutritional value of foods, some researchers have assessed its impact on protein quality (Boye *et al.,* 2012). Bhat *et al.* (2008) reported the influence of electron beam radiation on nutritional elements as well as the anti-nutritional factors of lotus seeds and increased concentration of indispensable amino acids (IAAs) such as threonine, valine, leucine, phenylalanine, lysine, tryptophan following irradiation.

Protein-rich legumes are also pre-treated prior to further processing. Soaking is one of the common pre-treatment methods used for legume or pulse proteins. Soaking can be used to decorticate various legumes before cooking. The endosperm contains high amount of protein, so when removing the hull portion, it increases the protein concentration of chickpeas (Prakash *et al*., 2016) and decreases the tannins and phytate which bind protein and enzyme needed for protein digestibility. However, Khattab et *al.* (2009) reported that soaking has a slight effect on protein while cooking and boiling to improve the protein quality of legumes by inactivating anti-nutritional factors and protease inhibitor which are known to decrease digestibility of protein (Alberta *et al*., 2016).

Autoclaving was found to be the most effective treatment for improving protein quality parameters followed by micronization, microwaving, cooking, and fermentation (Khattab *et al*., 2009).

2.6 Functional properties of proteins

Functional properties of food protein that are important in food processing include solubility, water retention capacity and fat-binding capacity, foaming, emulsifying properties, thickening and gel formation (Awuchi *et al*., 2019). These properties play a crucial role in the physical qualities of food during their preparation, processing and storage and are characterized by the structure, quality, texture, nutritional value, organoleptic characteristics of food, acceptability, and/or appearance of the food product (Ghribi *et al.,* 2015). They are essential in processing of products such as confectioneries, beverages, salad dressing and meat production (Boye *et al.,* 2010).

Most researchers reported that suspended *Desi* and *Kabuli* chickpea flours displayed viscosifying ability during heating (Noordraven *et al*., 2020). In a study by Aydermir *et al*. (2013) which involved the use of Turkish *Kabuli*-type chickpea and red and green lentil cultivars as a source of soy and animal origin functional properties, chickpea protein exhibited outstanding gelling capacity. Chickpea protein was also reported to have the highest emulsifying capacity, stability and gelling performance, high oil absorption capacity as well as high foaming capacity and foam stability (Aydemir *et al.,* 2013).

2.7 Interaction between chickpea protein and other food components in a food matrix

In a food system, protein is usually part of a matrix with other food components such as starch, lipids, fibre, and fatty acids (Parada and Santos, 2016). Interaction between protein and lipids takes place during the processing of food products such as cheese, bakery products, dough, and meat products, all leading to the formation of the induced protein-lipids complexes (Alzagtat *et al.,* 2002).

Some researchers have reported improvement in the functional and physicochemical properties of protein resulting in their interaction with lipids, including improvement in the properties of soy film (Bates & Wu *et al*., 1975; Farum *et al.,* 1976) and enhancement in breadmaking quality

(Fraizer 1983). Interaction between protein and lipids can also affect organoleptic qualities of the various foods (Alzagtat *et al.,* 2002).

The interaction between protein and starch is mainly electrostatic in nature, involving the anionic groups of the starch and positively charged groups of the protein. This interaction in bulk solutions and at interfaces has a great effect on the stability properties of food dispersions (Jamilah *et al*., 2009). Miscibility, thermodynamic incompatibility and complex coacervation or complexation are three possible equilibrium states for protein and starch in aqueous solution (Martinez *et al*., 2005). For instance, the matrix in bread responsible for the textural properties of the porous crumbs are the protein-starch wall lining of air cells (Aguilera *et al.,* 2019).

Starch and proteins are two key biopolymers with inseparably complex interactions. However, the availability of free amino acids (FFA) has been reported to favour the development of three complexes. Where conceivably three various structural factors function-development of starch-FFA complexes, creation of protein-FFA complexes and production of disulphide bond-linked protein aggregate. Parada *et al.,* (2016) and Zhang *et al.,* (2003), reported interaction amongst sorghum starch, serum protein and free fatty acids using a rapid viscosity analyser and observed the existence of a peak in viscosity during the cooling time different from that shown by binary systems composed only by starch and protein/free fatty acids, where there was absence of the viscosity peak. Here, protein concentration is essential because it can result in high ionic strength leading to an improvement in protein-protein interactions and aggregations (Sun-Waterhouse *et al.,* 2014).

These types of adjustments were due to the development of three complexes amongst starch, free fatty acids, and protein, the variation of basic structural factors of the compound amylose-FFA would be controlled, the development of more ordered complexes would be aided and the likelihood of the new complexes between amylose and free fatty acids would be reduced (Parada *et al.,* 2016). However, Zhang *et al.* (2010) found that FFAs can operate as a thermodynamically incompatible connection between amylose and protein molecules

Therefore, there is still insufficient research as to the protein-starch interaction mechanism. Javier Parade *et al.* (2016) also studied the interaction between starch, lipids, and proteins in foods by focusing on the microstructure control for glycaemic response modulation.

2.8 *In vitro* protein digestibility

Protein digestibility is linked to the nutritional quality of protein and is a crucial food attribute as it provides an estimate of protein proteolysis that relies on the structure of protein, thermal processing intensity and availability of some compounds that are disadvantageous to protein digestibility (Amanda *et al.,* 2019). The digestibility of protein measurements indicates the quantity of the protein that is hydrolysed by the digestive enzymes relative to the consumed protein amount (Lopez *et al.,* 2018). Undigested and less digestive foods have a reduced absorptive potential and intact proteins can potentially inflammatory as well as being harmful (Alberta, 2016).

In vitro protein digestibility (IVPD) measurements techniques are based on the digestion of samples with proteolytic enzymes under standardised conditions and are useful in rapidly screening the nutritive value of new protein foods and the impact of processing methods (Monsoor, 2002) on the nutritional value of protein. Bioassays with animals to investigate a correct protein digestibility are costly and requires too much time while *in vitro* digestibility methodologies are quite affordable, necessitate less man ability and does not need too much workspace and require smaller quantities of protein (Giami 2004; Boye *et al.,* 2012; López *et al*., 2018).

In vitro protein digestibility techniques include the pH drop method, pH-static method, simulated gastric and intestinal (pepsin-trypsin-α-chymotrypsin) digestion, one-step-single-enzyme *in vitro* protein digestion (pre-hydrolysis) and sequential (pepsin-pancreatin) digestion (Alberta, 2016). In attempting to improve digestibility of lentil flour protein using one-step-multi-enzyme, two-step-sequential multi-enzyme or pre-hydrolysis with one-step-single-enzyme systems, Alberta *et al*. (2016) reported that the IVPD of raw and green lentil concentrate hydrolysed by pepsin-tripsin-α-chymotrypsin and papain were 27.1%, 29,1% and 27.2% and 37.9%, respectively, and that all the digestibility methods were influenced by the cooking process as an increase of 10% was observed after cooking the lentil flour.

In a study by Ghribi *et al*. (2015), chickpea protein was hydrolysed using Alcalase to determine the effect of hydrolysis on conformational and functional properties of isolated protein. These researchers reported an improvement in protein recovery and solubility which resulted in a decrease in molecular weight bands while increasing the intensity and appearance of protein bands consist of apparent low molecular mass below 20 kDa.

Protein digestion begins in the stomach through the action of pepsin at low pH then continues in the small intestine by multiple actions of different proteases secreted within pancreatic fluid (trypsin, chymotrypsin, and carboxypeptidases) (Rieder *et al*., 2021). In *in vitro* protein digestibility (IVPD), on the other hand, gives information on the stability of protein and how their digestion process is conducted (Coda *et al*., 2017). The IVPD is a crucial factor in determining protein nutritional abilities. However, it can exaggerate the correct nutritional value since it ignores the biological inaccessible amino acid (Aguilar *et al*., 2015).

2.9 Chickpea protein as an ingredient in various food products

Like other pulses, chickpeas are naturally gluten-free, and their protein composition (essential amino acids) are complementary to that of cereals (Lopes *et al*., 2020). This is beneficial to the health of consumers who are intolerant or to gluten. Therefore, chickpea seeds can be milled into flour that can be utilised as an ingredient in a wide variety of food formulations including snack foods, cereal-based, infant food, meat products, beverages, and baked products (Figure 3) (Jukanti *et al.,* 2012). In many countries, chickpea has been incorporated into various food formulations including puree, soups, pasta, and health breads (Milan-Noris *et al.,* 2019). When incorporated in cereal-based foods, it was reported to improve protein quality and content as well as enhancing nutritional value and other organoleptic characteristics of the food products (Grasso *et al.,* 2021).

Chickpea protein is also a vital ingredient in the production of nutraceuticals (Boye *et al*., 2010; Shevkani *et al.,* 2019). There is also an emerging technology with great potential where chickpea proteins are used in the encapsulation of micronutrients (Ariyarathna *et al.,* 2015).

Some commercial chickpea-based products are shown in Figure 3.

A. **B.** **C.**

Figure 3. Some chickpea-based food products: A. Chickpea and onion crackers (https://www.krippu.com/e-shop/); B. Chickpea beverage (https://www.godairyfree.org/news/dairy-free-friday-bites-1) and C. Chickpea flour beer bread (https://www.powerhungry.com/2020/04/chickpea- flour-beer-bread-3-ingredients-vegan/)

Consumers wish for a premium experience from consuming a snack and it was reported that using healthy ingredients in the snack may achieve this objective (Martinez *et al.,* 2021). Ingredients such as quinoa, chickpea and spelt wheat do not affect the taste and other sensory properties nor the texture of the product (Euromonitor, 2019). Incorporation of legume flours in bakery products such as biscuits, pasta and bread were found to reduce the *in vitro* glycaemic response of such products, Thus, providing them with the potential for new product development/innovative ideas for people who require low glycaemic index-type foods (Monnet *et al*., 2019). Ghribi *et al.* (2018) reported that chickpea protein concentrate was utilized to improve the organoleptic profile of sausages with chickpea protein concentrate added at 1.5%, 2.5% or 5% protein into cooked sausages. Developing formulated products that incorporate chickpea protein and more proteins (lentils, cowpea, pea) can assist in meeting recommended daily protein consumption (Day, 2013).

In addition, chickpea protein has been incorporated into various foods such as bread, pasta, and cakes where it was shown to improve the quality of cereal-based product more especially in terms of protein content, nutritional value, and sensory properties (Dandachy *et al*., 2019). Chickpea protein ingredients can be used to produce chickpea pasta and is commercially available worldwide. The addition of chickpea flour in pasta was revealed to significantly slow sugar release into blood (Valentin-Gamazo, 2003). Chickpea flour is utilized together with

other legume flours to develop puff snacks and crisps currently available in the retail sector (Grasso *et al.,* 2021).

Malunga *et al.* (2014) conducted a study investigating the use of chickpea protein from *Desi* and *Kabuli* varieties in the formulation of follow-on infant formulae. The resulting formula was reported to meet the nutritional requirements set by the World Health Organisation (WHO) with respect to protein and carbohydrate content, amino acid profile and most micronutrients with minimal addition of oils, minerals, and vitamins (Malunga *et al.,* 2014).

In a study by Noordraven *et al*. (2021) on the ability of various processed chickpea flours as the other thickening element in an instant soup recipe, chickpea flours showed good potential as alternative thickening ingredients. It improved the protein, mineral and vitamin content, and the powder flowability of the soups. Chickpea has also been studied for its potential as an ingredient in legume beverage (Lopes *et al.,* 2020). In this study, novel pulse-based beverages with several appealing features were developed and were thought to be highly competitive in the current commercial non-dairy beverages.

2.10 Theory behind analysis of chickpea protein

Zhang *et al.* (2011) reported on the high protein content in chickpea but that the proteins were not easily digestible due to the presence of anti-nutritional factors. Protein hydrolysis can be achieved using microbes, chemicals, or pure enzymes (Molnar *et al.*, 2013). Along with several anti-nutritional factors, legumes contain trypsin inhibitors which decrease the digestibility of protein (Wang *et al*., 1998). Therefore, as the total nutritive value of a protein relies on its complete digestion. As stated earlier, *in vitro* methods of protein evaluation are useful in screening nutritive value of new protein foods and, also, the impact of processing methods because of their rapidity compared with *in vivo* methods. In the current study, enzymatic modification was used to improve digestibility of chickpea proteins.

2.10.1 Isolation of chickpea protein

Aqueous alkaline extraction, isoelectric precipitation and salt extraction methods are commonly used techniques to obtain legume protein isolates (Karaca *et al*., 2011). It has been reported that the extraction methods for isolates have a significant effect on the protein functionality as it influences both the globulin/albumin and the physico-chemical

characteristics of the protein (Papalamprou *et al*., 2010). Selection of a suitable technology and conditions for protein extraction is important in food processing as it influence the nutritional properties of a finished product (Paredes-lopez *et al*., 1991). In this study, the techniques of isoelectric precipitation and micellization were used for chickpea protein isolation. Isoelectric precipitation has been explored as a processing technique to produce higher purity protein fractions with improved digestibility (Alberta *et al.,* 2016). Ordorica-Falomir *et al.* (1989) reported that the mainly utilized method to extract legume and oil seed protein isolate is by isoelectric precipitation. Proteins are precipitated by adding acid until their isoelectric point is attained and this happens after alkaline (pH 8-10) protein micellization precipitation is the second procedure that includes dilution of the neutral salt extracted in cold water to form protein that has a micellar structure before drying and as a result hydrophobic interactions may play a part in stability of such isolates (Murray *et al.,* 1981).

2.10.2 Hydrolysis of chickpea protein

Protein hydrolysis is the process of using single or more catalysts (chemicals, microbes, or pure enzymes) to speed up the breakage of peptide bonds resulting in the production of smaller protein/peptides molecules as a result there will be major structural changes of the protein. Because the intestinal tissues absorb small peptides and amino acids, hydrolysis facilitates protein digestion and improve bioavailability (Molnar & Gair, 2013). In this study, enzymatic hydrolysis was used, and this method holds promise for the plant protein industry as these catalyst lead to little or no effect to the protein content while accommodating a considerable degree of control over the type and quantity of the generated hydrolysate (Goertzen *et al.,* 2020). In the current study, two enzymes were used for hydrolysis: alcalase and flavourzyme. Alcalase is a non-specific serine-type protease from *Bacillus licheniformis* and its optimum pH for catalysis ranges from pH 6.5 to pH 8.5. Alcalase has been mostly used to produce protein hydrolysates that have improved nutritional or functional properties compared to the intact protein (Yust *et al.,* 2010). The second protease used was Flavourzyme which is used to enhance the nutritional value of the products by modifying protein structure (Patto *et al.,* 2015). This is an exopeptidase-endoprotease complex enzyme generated by *Aspergillus orizae*, which can generate very high degrees of hydrolysis and diminish the bitter off-taste produced in some products (Segura-Campos *et al.,* 2012).

2.10.3 Analysis of digested protein

Legume proteins are reported to have lower digestibility when compared to animal protein resulting from their large size and the presence of anti-nutritional factors such as trypsin inhibitors (Potier *et al.,* 2008). Digestibility of legume protein can be improved by several pre-treatment techniques such as soaking, germination, cooking/boiling roasting, and fractionation (e.g., isoelectric precipitation). In the current study, *in vitro* protein digestibility was used to improve the digestibility of chickpea protein and its suitability as a functional property. Digestibility is a tool used to evaluate the nutritional quality of protein (Tinus *et al.,* 2012). Researchers prefer *in vivo* digestibility, but *in vitro* digestibility is useful as there is lower cost, shorter analysis time, greater ease of analysis and limited to no ethical concerns. Digestion can be mediated by secretions and enzymes found in the digestive tract which break down food macromolecules into smaller building blocks to facilitate absorption into the body (Clemente 2000). Alberta *et al.* (2016) reported that producing hydrolysates using exogenous endo- and exopeptidases could be used to provide pre-hydrolysed pulses and peptides with biological activity. The current digestibility study used pancreatin as a digesting enzyme together with pepsin. Pancreatin is a combination of enzymes containing trypsin-like and chymotrypsin-like proteinases in addition to amylases and lipase (Kaur. 2010).

In vitro protein digestibility techniques include the pH drop method, pH-static method, simulated gastric and intestinal (pepsin-trypsin-α-chymotrypsin) digestion, one-step-single-enzyme *in vitro* protein digestion (pre-hydrolysis) and sequential (pepsin-pancreatin) digestion (Aryee and Boye, 2016. The current study also used simulation gastric and intestinal digestion method to determine protein digestibility. Protein digestibility begins in the stomach through the action of pepsin at low pH before continuing in the small intestine by multiple actions of different proteases secreted by the pancreas (trypsin, chymotrypsin, and carboxypeptidases) (Rieder *et al*., 2021). It is also well known that during gastrointestinal digestion, proteins are hydrolysed to small peptides and amino acids so that these can be absorbed. Chymotrypsin is also reported to be a non-specific protease that partially cleaves adjacent to amino acids like leucine, tyrosine, phenylalanine, and tryptophan, and to a few amounts of glutamine, serine, and threonine (Sanchez-Velazquez *et al.,* 2021).

In conclusion, literature revealed that chickpea contain high protein content than cereal grains and this implies that chickpea can be used to assist the developing countries with challenges of protein -energy-malnutrition (PEM) which led to high morbidity and mortality of infants and children. Chickpeas can be used as a source of protein like cereal grains. However, the only

challenge with chickpea is that the available protein is not easily digestible which implies that they have low digestibility. Literature revealed that chickpea has low digestibility due to the antinutritional factors which are said to be health threatening to human beings. Digestibility of chickpea protein can be improved by chemical and biological modification. The use of enzymatic modification for improving digestibility of chickpea protein is not known. literature did not display if enzymatic modification can improve digestibility of the chickpea protein and the suitability of chickpea proteins as functional ingredients.

CHAPTER 3: MATERIALS AND METHODS

3.1. Materials

Two chickpea seed varietals, i.e., *Desi* and *Kabuli*, were obtained from the Department of Plants and Crop Production at the University of Venda, Limpopo Province, South Africa. Random sampling was used to collect samples and a non-probability sampling technique was used to sample chickpea seeds, before the samples were transported to the Department of Life and Consumer Sciences, University of South Africa, Florida Campus, Johannesburg, Gauteng, South Africa and stored at room temperature (25˚C) until they were analysed.

The enzymes alcalase and flavourzyme were generously supplied by Novozymes (Switzerland AG, Greek Office). Pepsin, pancreatin from porcine pancreas, α-chymotrypsin type ll from *bovine pancreas*, protease from *Bacillus licheniforms* and from *Aspergillus oryzae* were purchased from Sigma Aldrich, Modderfontein, Johannesburg, South Africa.

The Bradford protein assay kit was generously provided by the Department of Biochemistry, University of Venda, South Africa.

3.2 Methods

3.2.1 Milling

About 1 kg chickpea seeds were milled using a coffee grinder and a blender and the resulting flour was screened through 250 μm sieve. The flour was then stored at 5˚C for further analysis.

3.2.2 Chickpea protein isolation

pre-treated chickpea flour was prepared according to a method described by Khalid *et al*. (2012) using isoelectric precipitation and micellization precipitation methods. These methods are described in detail in the following sections.

3.2.2.1 Preparation of pre-treated chickpea flour by isoelectric precipitation (Protein isolate A)

The flour obtained as described above (Section 3.2.1) was defatted with hexane, the ratio was 1g flour:5 ml hexane. This involved adding 10 g of chickpea flour to 50 ml of hexane before the mixture was stirred on an IKA orbital shaker (KS 130 Basic shaker, Lasec laboratories) for 3 hr at 320 rpm. The mixture was then placed in a fume hood overnight to remove excess

hexane (Ruzengwe *et al.,* 2020). The defatted chickpea flour (10 g) was dispersed in 50 ml distilled water. Then the pH of the suspension was adjusted to pH 9.0 with approximately 5 ml of 0.1 M NaOH using a plastic pipette/dropper, followed by shaking at 320 rpm on the orbital shaker at room temperature for 20 min. Insoluble matrices were sedimented by centrifugation at 4 000 g for 20 min at room temperature(25°C) using a refrigerated centrifuge (Neya 8 bench top centrifuge) before the supernatant was discarded. The extraction, shaking and centrifugation procedures were repeated on the residue. The pH of the supernatant was adjusted to 4 with 1.0 HCl using a 5 ml plastic dropper) and shaken at room temperature for 20 min. The precipitate was centrifuged at 4 000 g for 20 min followed by washing. This involved gently pouring 10 ml of distilled water over the precipitate in the centrifuge tubes, centrifuging as described and pouring off the supernatant. This washing step was repeated four times. Then, 50 ml of distilled water was added to the precipitate and the solution was then neutralised by adding 5 ml of 1.0 N NaOH to pH 7.0 and left overnight at 4°C. pre-treated chickpea flour was then freeze-dried before it was ground into a powder using a clean, sterile ceramic mortar and pestle. The protein powder (9.82 g) was distributed into plastic centrifuge tubes and stored in a desiccator at room temperature until analysis (Khalid *et al*., 2012).

3.2.2.2 Preparation of protein isolate using micellization precipitation (protein isolate B)

The defatted chickpea flour (10 g) was suspended in 5 ml of 1.0 M NaCl in a 1:10 (w/v) ratio. The suspension was stirred for 2 h at room temperature and centrifuged at 300 g for 30 min. The residue was extracted as described in the isoelectric precipitation method (above). The combined supernatant was diluted ten-fold in distilled water and stored at 4°C for 18 h. The supernatant was then discarded, and the precipitate centrifuged at 3 000 g for 30 min in refrigerated centrifuge. The precipitated pre-treated chickpea flour was freeze-dried and processed as described for the isoelectric pre-treated chickpea flour (Khalid *et al*., 2012).

3.2.3 Proximate composition

3.2.3.1 Protein concentration

The protein content of pre-treated chickpea flour and that of raw chickpea flour was determined according to the method described by Xie *et al*. (2019). The protein content was analysed using a Leco® FP-428 protein analyser (Leco Corp., St.-Joseph, MI). The Leco® FP-428 incinerates protein samples at 1000°C in the presence of oxygen, resulting in the conversion of carbon and

nitrogen to CO_2 and N2 respectively. A Dumas method (AOAC 46-30.01) was followed whereby pre-treated chickpea flour were incinerated at high temperature in an oxygen atmosphere through subsequent oxidation and reduction tubes, nitrogen was quantitively converted to N_2 and other volatile combustion products were either trapped or separated. A thermal conduction detector measured nitrogen gas and results were measured as % or mg and a nitrogen conversion factor of 6.25 were used to convert the nitrogen values into percentage protein. Samples were analysed in triplicate and the calculated average mean was recorded.

3.2.3.2 Fat extraction

Fat content was determined according to an official method of analysis, method 7.0 [Association of Agricultural Chemists (AOAC), 2000]. A clean empty 100 ml beaker was oven dried for 30 min and then cooled in a desiccator for a further 30 min. Then empty Soxhlet extraction paper thimbles were oven-dried at 105 degrees for 30 minutes and placed in a desiccator for 30 min to cool. An empty beaker was weighed, and 2 g of sample was also weighed. the extraction was carried out in an automated Soxhlet apparatus for 5 h with petroleum ether (40-60°C (2:1, v/v) as a solvent with boiling stones inside. The Soxhlet apparatus was programmed to three settings extraction, rinsing, and drying. 70 ml of solvent were poured in the extraction vessel and the samples were suspended in the boiling solvent for 2 hr and then subjected to reflux washing for another 2 hr and 1 hr recovery and any ether left in the collection beaker was dried in an oven-drying (at 103°C) overnight. Data were collected in triplicate and reported as mean ± SD. Fat content was calculated using the equation:

$$Crude\ fat\ content\ (\%) = \frac{W2 - W1}{SW} X\ 100$$

where W_1 = the weight of the beaker before extraction; W_2 = the weight of the beaker after extraction; SW = the weight of the sample

3.2.3.3 Moisture concentration

The moisture content of pre-treated chickpea flour was determined using a modification of an official method of analysis, method: 925.10 (AOAC, 2006. Exactly 2 g sample of pre-treated chickpea flour was weighed and placed in an oven dryer at a temperature of 105°C for 5 hr. After drying, samples were cooled in a desiccator for 30 min. Measurements were taken in triplicate and results were reported as mean ± SD. Moisture content was calculated by the equation:

$$Moisture\ content(\%) = \frac{W1 - W3}{W1 - W0}\ X\ 100$$

Where W1 = weight of the crucible + sample; W0 = weight of the empty crucible; W3 = weight of the crucible + oven dried sample

3.2.3.4 Ash concentration

The ash content of pre-treated chickpea flour was determined using an official method of analysis, method 923.03 (AOAC, 2006) with slight modification. Exactly 2 g samples of each of pre-treated chickpea flour was weighed and placed in an oven dryer at a temperature of 105°C for 5 hr. After drying, the samples were cooled in a desiccator for 30 min. Crucibles were then taken to the muffle furnace and ignited at 550°C for about 5 hr. At the end of ashing, the furnace was allowed to cool below 200°C before transferring crucibles to a desiccator. The samples were further cooled to room temperature in desiccator with stoppered lids for 45 min. The cooled samples weights were measured and recorded in triplicate. The ash content was calculated using the following equation:

$$Ash\ content\ (\%) = \frac{W3 - W2}{W1}\ X\ 100$$

Where W_3 = weight of the crucible + ashed sample; W_2 = weight of the empty crucible; W1 = the original weight of the sample

3.2.3.5 Crude fiber

The sample was prepared by weighing the filter bag (W_1), 0.5 g (± 0.05 g) of air-dried sample (W_2) using a tared weighing balance (details of weighing balance), the powdered sample passed through a 1 mm screen, directly into a filter bag. One blank bag was weighed and included in the digestion to determine blank bag correction (C_1). Three bags were packed per tray and then stacked on a centre post with each level rotated 120 degrees. A weight was placed on top of the empty 9th tray to keep the bag suspender submerged. Sufficient acetone was poured into bottle to cover bags and secure top. The container was shaken 10 times and the bags were allowed to soak for 10 min. The same steps were repeated with fresh acetone, the acetone was discarded, and the bags were placed on a wire screen to air-dry for approximately 5 min. The bag suspender with samples was placed in the solution in the vessel. The agitator was switched on and the samples were left to heat for 60 min in a closed and sealed vessel.

The drain valve was opened and after the solution has been exhausted the valve was closed and the lid was opened. Then the water was removed, and rinsing was performed three times. After the final rinse, the filter bags were removed from the bag suspender and gently pressed to expel excess water. The bag was soaked for 3 minutes, and excess acetone was removed. The samples were left to dry in an oven at 105ºC for at least 2 hr. After to cool to ambient temperature, the bags were weighed in triplicate. The crude fibre was calculated using the following equation:

$$Crude\ fibre\ content\ (\%)\ = \frac{W3 - (W1\ X\ C1)}{W2}\ X\ 100$$

Where: W_1= Bag tare weight; W_2 = Sample weight; W_3 = Weight after extraction; C_1= blank bag weight (final oven dried weight/original blank weight)

3.2.3.6 Total available (glycaemic) carbohydrates

The carbohydrate content of pre-treated chickpea flour was calculated by subtracting for a sum of mean values for other major food constituents from one hundred as shown in the equation below (Xie *et al.,* 2019). The contents were reported on dry weight basis (% dw).

$$\text{Total carbohydrates (\%)} = 100 - (\text{crude protein} + \text{crude fat} + \text{ash} + \text{moisture} + \text{crude fibre})$$

3.2.4 Enzymatic hydrolysis of chickpea protein isolates

pre-treated chickpea flour was hydrolysed as described by Segura-Campos *et al*. (2012). A random block design was used where the enzyme alcalase was used as block. Reaction times of 5, 15, 30 and 60 min, respectively, were used as the factor to evaluate while the degree of hydrolysis was the response variable. Exactly 5 g of pre-treated chickpea flour was dispersed in 250 ml of deionized water, pH adjusted to 8 with 1N NaOH (5 ml using plastic pipette/dropper), before 25 mg of enzyme was added. The reaction was run at 50°C with constant stirring (IKA KS 130 basic) at 320 rpm for 1 h. Then, 25 ml samples were taken at 0, 5, 15, 30, and 60 min to measure the degree of hydrolysis. The hydrolysis reactions were run in a beaker equipped with a stirrer, thermometer and pH electrode, and the reaction was stopped by heating to 85°C for 15 min. The dissolved protein was centrifuged at 10,000 rpm for 20 min to extract the soluble fraction.

3.2.4.1 Degree of Hydrolysis

The degree of hydrolysis (DH) was determined as described by Segura-Campos *et al*. (2012). Here the value of DH is estimated by measuring soluble nitrogen content in 10% trichloroacetic acid (TCA). A 10 ml sample of pre-treated chickpea flour was mixed with 10 ml of 20% TCA and centrifuged at 12,100 g for 15 min. Soluble nitrogen in the supernatant was assayed using the Kjeldahl method. Firstly, pre-treated chickpea flour was placed on the digestion chambers and 20 ml of sulphuric acid was added in each chamber (6 chambers) and digestion took place at 420 degrees for 60 minutes. The solution was then cooled for 40 minutes. After digestion, the samples were placed in a distillation flask (Buchi distillation unit model K-314, Biostad Analytical). Then 60 ml of distilled water plus 4 drops of an indicator was added to each sample. After distilling, the samples were titrated, and nitrogen content was recorded and later converted to protein by the factor 6.25 and percent DH calculated as:

$$DH\ (\%) = \frac{10\%\ TCA\ solude\ N}{Total\ N} \times 100$$

3.2.5 *In vitro* protein digestibility of chickpea protein hydrolysate

In vitro digestibility of chickpea protein hydrolysates was determined as described by Patil *et al*. (2016). A sample weight of 2% (w/v) was measured and diluted in reverse osmosis (RO) water. Then pepsin (4 units/mg protein basis) was added after adjusting the pH to 2.0 with 1 M HCl (3 to 5 drops). The solution was incubated at 37°C for 60 min. After incubation, the pH was adjusted to 7.0 using 1 M NaOH (3 to 5 drops). Pancreatin (4 units/mg protein basis) was added, and the digestion volume made up to 50 ml. Samples were incubated at 37°C for 120 min. Aliquots of chickpea protein hydrolysates were taken at intervals of 0, 60, 120 and 180 min before being placed in ice to stop enzyme activity, and subsequently centrifuged at 3000 rpm for 5 min. The supernatant was collected for analysis. After digestion, the remaining (supernatant) protein was determined by using the Bradford method.

Firstly, the bovine serum albumin (BSA) protein standards were prepared, and Bradford reagents were stored at 4°C. Five BSA standards were prepared (1 mg/ml, 0.75 mg/ml, 0.5 mg/ml, 0.25 mg/ml, and 0.125 mg/ml) in 0.01 M of Tris-HCl at pH 8. Then, three sets of unknown protein dilutions were prepared to a total volume of 20 µl. The Eppendorf tubes were labelled was standards or the unknown protein dilutions. Exactly 20 µl of each sample (supernatant) was added to 1 ml Bradford reagent and stored at room temperature. Each sample

was then vortexed after addition of Bradford reagents. The samples were then incubated at room temperature for 5 min. Exactly 250 µl of each sample were vortexed again and transferred in triplicate to 96-well microtitre plate. The absorbance was measured at 595 nm using an XPS/EM Microplate Readers (Molecular Devices, San Jose, CA, USA, Spectramax M3). Measurements were taken in triplicate and results were reported as mean ± SD. The percent digestibility was calculated as protein content before digestion and protein content after digestion using the following equation:

$$Digestibility\ (\%) = \frac{soluble\ protein}{total\ protein} \text{X } 100$$

3.2.6 Simulated gastric and intestinal (pepsin-trypsin-α-chymotrypsin) digestion

Chickpea protein hydrolysate samples were digested under simulated gastric and intestinal conditions according to a method described by Alberta (2016) with slight modification. Gastric digestion was initiated by adding pepsin to each dispersed hydrolysate [5% protein (w/w), pH 2.0] at an enzyme: substrate ratio of 1:250 (w/w) and allowed to proceed for 2 hr at 37°C with continuous shaking in a water bath (Memmert, Southern Germany, Model WNB:45). At the end of the gastric phase. the pH was adjusted to 6.5 and trypsin and α-chymotrypsin (4 mg) were added at an enzyme: substrate ratio of 1:250 (w/w) and the reaction continued for an additional 2.5 h. The reaction was terminated by acidification. The clear supernatant obtained after centrifuging (3000 x g, 30 min at 4°C) was freeze-dried (Harvest Right home freeze drier, Salt Lake City, Utah, USA) and stored for further analysis. Protein content was determined following the Bradford method as described in section 3.2.5.

3.2.7 Selected functional properties of chickpea protein hydrolysates

3.2.7.1 Protein solubility

The solubility of the protein hydrolysate was determined using the method of Jain and Anal (2016) (with slight modification). Each protein hydrolysate sample (200 mg) was dissolved in 20 ml of distilled water and pH adjusted using 1 M HCl (5ml) or 1 M NaOH (5 ml). Mixtures were incubated at 30◦C with stirring (320 rpm) for 30 min and then centrifuged at 3000 g for 20 min at room temperature. Protein content in the supernatant was determined by the Bradford

method (1976) using BSA as standard. The protein solubility was calculated using the following equation:

$$Solubility\ (\%) = \frac{Protein\ content\ in\ the\ supernatant}{Total\ protein\ content\ in\ the\ sample} \times 100$$

3.2.7.2 Water absorption capacity

The absorption capacity of chickpea protein was determined according to Xu *et al*. (2017) with slight modification. The protein hydrolysate of ml was placed into each pre-weighed centrifuge tube before 10 ml of water was added to each. The suspensions were stirred for 1 hr at 320 rpm and then centrifuged at 3000 g for 50 min at room temperature. The supernatant was decanted, and the sample were re-weighed. The water absorption capacity was expressed as grams of water per 100 g of dry sample. The estimations were done in triplicate using the following equation:

$$WAC\ (\%) = \frac{weight\ of\ the\ residue\ obtained\ after\ removal\ of\ supernatant\ (g)}{Weight\ of\ the\ sample\ (g)} \times 100$$

3.2.7.3 Oil absorption capacity

The method of analysing oil absorption capacity was determined according to Bai *et al.* (2018). An accurate amount of 4 ml of chickpea protein was mixed with 10 ml of refined canola oil (Woolworth brand) and placed in a 50 ml centrifuge tube and then mixed for 10 sec using a Vortex mixer (VWR, Chemlab Supplies) every 5 min for 30 min. The samples were then centrifuged for 15 min at 1000 g at room temperature before the supernatants were removed. The pellet was weight and recorded and the oil absorption capacity was expressed as g oil/g on a dry basis using the following equation:

$$OAC\ (\%) = \frac{weight\ of\ the\ residue\ obtained\ after\ removal\ of\ supernatant\ (g)}{Weight\ of\ the\ sample\ (g)} \times 100$$

3.2.7.4 Percentage syneresis

The syneresis of chickpea protein hydrolysate was determined according to the method described by Singh *et al.* (2006). Approximately 4 ml of protein hydrolysate was suspended to 10 ml of distilled water and then boiled in a water bath (Memmert GmbH & Co. KG) at 85℃

for 30 min and then cooled with ice water in a 500 ml beaker to room temperature (±25℃). The protein was stored for 120 hrs at 5℃. Syneresis of chickpea protein hydrolysate was measured as the percentage eliminated water divided by total weight of a boiled sample after centrifugation at 3000 rpm for 15 min using the following equation:

$$syneresis\ (\%) = \frac{eliminated\ water\ (g)}{Total\ weight\ of\ gelatinised\ sample\ (g)} \times 100$$

3.2.8 Statistical analysis

Data were analysed using the statistical package for the social sciences (SPSS) version 26.0 for windows (SPSS IBM, New York, NY, USA). Data were assessed by one-way analysis of variance (ANOVA) and significant differences among mean values were determined by the Duncan multiple ranges test (Duncan, 1995). The results were expressed as means values ± standard deviation.

3.2.9 Ethical considerations

The research work was carried out at the Eureka Laboratories, UNISA Science Campus under the supervision of Dr D Beswa. Ethics approval was obtained from the UNISA Ethics Committee (Ethic number: 2019/CAES-HRE C/116) (Appendix 1). The laboratory code of conduct and safety regulations were strictly adhered to, to prevent any harm or injuries to personnel. Plagiarism was avoided and all citations were done in the form of interpretation and reconstruction of public works. All research work was kept strictly confidential until published as completed MCS Thesis.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Proximate composition

4.1.1 Ash concentration

The ash concentration of the pre-treated chickpea flour is presented in Table 2. The ash concentration of isoelectric precipitate from *Kabuli* was significantly higher (4.2%) compared with the control (2.3%) and the micellized precipitate (1.9%). A similar trend was observed for isoelectric precipitate from *Desi* where the ash concentration was almost twice (3.9%) that of the control and micellized precipitate (1.8%). In comparison with the control samples from both cultivars (*Kabuli* 2.3% and *Desi* 1.8%), there was an increase in ash concentration of pre-treated chickpea flour extracted using isoelectric precipitation method and a decrease in ash concentration of pre-treated chickpea flour extracted from micellized precipitates. A study by Karaca *et al.* (2011) involved the use of isoelectric and salt extraction methods for chickpeas, pea protein, faba beans and lentils and their results shows that the ash content of chickpea isoelectric precipitates was higher (3.05%) than that of the control sample (2.72%). Paredes-lopez *et al.* (1991) reported 2.7% ash concentration of chickpea isoelectric precipitates and this was slightly lower than the ash concentration of the control (2.8) while the micellized precipitates recorded significantly lower ash concentration (2.37%) the isoelectric precipitate (2.7%) and the control (2.8%). The findings of the current study agree with the findings of Karaca *et al.*, (2011) and Paredes-lopez *et al.* (1991).

The high ash concentration observed on isoelectric precipitated *Kabuli* may be due to the strong alkali or acid used in isoelectric method that results in salt formation and the salt remaining after dialysis can contribute to higher ash content in the isolates (Sosulski *et al*., 1987). A study by Boye *et al.* (2010) investigated the use of isoelectric and ultrafiltration precipitation techniques and report showed that the ash content of *Kabuli* was 2.76% and *Desi* 3.04%. The isoelectric *Desi* results of this study were 3.9% which is slightly similar (3.04%) to that of Boye *et al.* (2010). Yadahally *et al.* (2012) reported that raw chickpea flour (control) has the ash content of 2.2% which agrees with our results (Table 2). The findings on the current study are also supported by the results reported by Karaca *et al*. (2011) (3.05%); Boye *et al.* (2010) (3.04%); Paredes-lopez *et al.* (1991) (2.7%). The protein content of *Kabuli* is reportedly higher than that of the *Desi* variety (Summo *et al.*, 2019)

Table 2. Proximate composition of pre-treated chickpea flour (dry basis)

Protein isolates	***Kabuli* chickpea**					
	Ash (%)	**Moisture (%)**	**Crude fibre (%)**	**Crude fat (%)**	**Crude protein (%)**	**CHO (%)**
Control	$2.3^{a} \pm 0.8$	$\mathbf{9.2^{c} \pm 0.1}$	$20.7^{a} \pm 1.5$	$\mathbf{10.2^{ab} \pm 2.6}$	$20.5^{c} \pm 0.1$	$37.2^{a} \pm 3.11$
Isoelectric precipitate	$\mathbf{4.2^{b} \pm 0.2}$	$7.2^{a} \pm 0.1$	$20.7^{a} \pm 2.3$	$8.1^{ab} \pm 2.3$	$13.6^{b} \pm 1.1$	$46.1^{ab} \pm 3.1$
Micellized precipitate	$1.9^{a} \pm 0.2$	$6.9^{a} \pm 0.3$	$19.7^{a} \pm 2.7$	$7.9^{a} \pm 0.5$	$\mathbf{14.3^{b} \pm 1.6}$	$\mathbf{49.4^{b} \pm 4.6}$
	***Desi* chickpea**					
Control	$1.8^{a} \pm 0.2$	$\mathbf{8.2^{b} \pm 0.2}$	$19.0^{a} \pm 9.4$	$\mathbf{11.9^{b} \pm 1.7}$	$\mathbf{20.4^{c} \pm 0.5}$	$38.8^{a} \pm 10.9$
Isoelectric precipitate	$\mathbf{3.9^{b} \pm 0.1}$	$6.7^{a} \pm 0.2$	$\mathbf{27.8^{c} \pm 1.5}$	$9.78^{ab} \pm 2.3$	$8.8^{a} \pm 1.2$	$43.9^{ab} \pm 1.9$
Micellized precipitate	$1.8^{a} \pm 0.6$	$7.0^{a} \pm 0.8$	$23.2^{b} \pm 4.8$	$7.5^{a} \pm 2.1$	$10.0^{a} \pm 0.6$	$\mathbf{50.7^{b} \pm 4.5}$

Means ± Standard deviation. Values are mean of three replications and means with different superscript in a column are significantly different at $p< 0.05$ for the above parameters. Where CHO = Available carbohydrates, the control samples are the raw *Desi* chickpea flour and raw *Kabuli* chickpea flour.

4.1.2 Protein concentration

In the current study, it was found that the crude protein concentration of isoelectric precipitated *Kabuli* was significantly lower (13.6%) compared to control sample (20.5%) and micellized *Kabuli* also reported lower crude protein concentration (14.3%) compared to the control sample. A similar trend was observed for *Desi* pre-treated flour the crude protein concentration of isoelectric precipitate was significantly lower (8.8%) than the control sample (20.4%) while the micellized precipitate exhibited a crude protein concentration (10.0%) which was 50% lower than of the control sample (20.5%). These results are like those obtained by Paredes-Lopez *et al*. (1991) that involved the use of isoelectric and micellization precipitation of chickpeas to show that micellization of pre-treated chickpea flour had a higher protein content compared to that in the isoelectric precipitate. A high protein concentration on micellized *Kabuli* and *Desi* may be that the protein-protein interaction was favoured when the ionic strength of the extracted sample was reduced. Some salts have been reported to bind to proteins when present at relatively high concentration (Murray *et al.,* 1981).

The differences in raw chickpea flour and the pre-treated chickpea flour were probably high since the protein structure of the isoelectric and micellized precipitates were altered during precipitation process which affected their nutritional quality (Xu *et al.,* 2017). This is also supported by a study conducted by Agrahar-Murugkar and Jha (2010) who reported that all processing methods resulted in a significant decrease in protein concentration of the chickpea

protein and this decrease may be due to the loss of some soluble protein as well as protein degradation during heating.

4.1.3 Moisture concentration

Controlling the moisture concentration of food is crucial as certain levels of moisture promote the growth of microorganisms. The moisture concentration of the isoelectric-generated *Kabuli* precipitate (7.2%) as well as the micellize-generated *Kabuli* precipitate (6.9%) was significantly lower than the moisture concentration of the control sample (9.2%). The isoelectric precipitate of *Desi* exhibited a moisture concentration of 6.7% which is approximately 2% lower than the control sample (8.2%) while a mean value of 7.0% was reported for the micellized precipitate. Jagannadham *et al.* (2014) reported similar results about the moisture concentration of raw chickpea flour (9.35%) and lower mean moisture values for both *Desi* and *Kabuli* were probably due to differences between the moisture concentration of these cultivars or varieties. In a study by Zhao *et al.* (2019) to investigate the nutritional components, volatile constituents, and antioxidant activities of 6 chickpea species, five *Kabuli* cultivars (T2-3, A1, Benying-1, Y2-32, Y2-514) and the *Desi* cultivar y2-364, the moisture content for the studied chickpea cultivar varied between 6.31% and 8.18%. In the current study, the moisture content for *Desi* and *Kabuli* falls within the range reported by Zhao *et al*. (2019). Generally, chickpea flour contains a relatively low-fat content and that is why it is regarded as a healthy legume.

4.1.4 Fat concentration

In this study, like that obtained from the micellized *Kabuli* (7.9%), the isoelectric precipitation of *Kabuli* exhibited a significantly lower crude fat concentration n (8.1%) when compared to the control sample (10.2%). In contrast, fat concentration analysis of precipitated *Desi* samples showed that isoelectric treatment showed a crude fat a mean value of 9.78% which is approximately 2% lower than its control sample (11.9%). However, the micellization of *Desi* showed a concentration of 7.5%, that is significantly lower than the mean value of the control sample but also around 2% lower that the figure obtained for isoelectric-treated *Desi* material. Statistically, there was no significant difference ($p<0.05$) between the isoelectric *Kabuli* and the micellized *Kabuli* material and the control sample. There was a significant difference between the fat concentration of *Desi* micellized precipitation and the control sample. Espinosa-ramirez *et al*. (2019) studied wet-milled chickpea co-product as an alternative to obtain protein isolates of chickpea and their report include crude fat content of chickpea protein

hydrolysate which as low as 1%. This was probably due to the extraction method used (wet-milling isoelectric precipitation) that more effectively removed fat during the defatting process. Chickpeas contain carbohydrates, fat, minerals, bioactive substances, and anti-nutrients in addition to protein, all of which affect the efficiency of recovery and important quality factors of chickpea protein (Shevkani *et al.,* 2019). Chickpeas have a higher fat concentration than other pulses and some cereals, but a lower fat concentration than other oilseed legumes like soybean and groundnut, with fat content varying from 3.10 % to 5.67 % depending on the chickpea type. Chickpea fat is made up of roughly 66 percent polyunsaturated fatty acids, 19 % monounsaturated fatty acids and 15% saturated fatty acids. Linoleic and oleic acids are the most prevalent fatty acids, with palmitic acid coming in second (51.2% and 61.6%, 32.6% and 22.3% and 9.4% and 9.1 % of total fats, respectively (Grasso *et al.*, 2021) However, these fats are frequently removed from chickpea proteins during manufacturing to improve the protein purity and yielding (Grasso *et al*., 2021). Chickpea and its flour are widely employed in food processing in many countries, and this is because of its perfect cell wall polysaccharide composition, varied functionality, and relatively high content of oil (Sreerama *et al.,* 2019). It is a staple food in Asian cuisines from the south to the southeast and its flour is used in variety of Indian sweets, pastries, and beverages, it is also used to make pasta in Italian and French cuisines to make deserts, noodles, snacks, and main dishes with savoury flavour (Aljaji, 2006).

Jukanti *et al*. (2012) investigated the nutritional quality and health benefits of chickpea and found that the fat content in chickpea is higher than that of pulses such as lentils (1.06 g/100 g), red beans (1.06g/100 g, mung bean (1.15 g/100 g, pigeon pea (1.6 g/100 g) and in cereal such as wheat (1.70 g/100 g) and rice (0.60 g/100 g). The fat content in chickpea protein is one of the most important food constituents in the prevention and management of non-communicable diseases.

4.1.5 Fibre concentration

The chickpea protein from both cultivars showed a high fibre concentration and these results are supported by reports from the literature as to chickpeas being high in dietary fibre (Ghribi *et al.,* 2015; Jukanti *et al*., 2012)., The crude fibre concentration of the isoelectric precipitated *Kabuli* (20.7%) was found to be the same as that of the control sample (20.7%) while the crude fibre concentration of the micellized *Kabuli* precipitate was slightly lower at 19.7%. The fibre concentration mean value (27.8%) of isoelectric precipitated *Desi* was significantly higher compared to the control sample (19%) and that of the micellized *Desi* precipitate (23.2%). Thus, no significant differences were noted when comparing the fibre concentration of the control samples for both the *Kabuli* and *Desi* cultivars. In explaining these results, Jukanti *et*

al. (2012) reported that the *Desi* type had a high total fibre concentration compared to *Kabuli* and this could be due to the thicker hulls and seed coat in the *Desi* type (11.5% of total seed weight) compared with the *Kabuli* type (4.3 - 4.4% of total seed weight). A study by Summo *et al.* (2019) regarding the nutritional, physico-chemical and functional characterization of a global chickpea collection also reported that the beige (*Kabuli*) chickpea contained a lower relative amount of dietary fibre (11%) compared with the brown (*Desi*) cultivar reported at 22%. In contrast, another study by Wood *et al.* (2011) evaluated the morphology of *Desi* and *Kabuli* chickpea seeds to show that *Desi* chickpea had a fibre content of 11.5% while *Kabuli* showed 44% fibre content.

The findings in this current study agree with that of Jukanti *et al.* (2012) and Summo *et al.* (2019) but contrast with the results reported by Woods *et al.* (2011). Nonetheless, the results of the current study support the suggestion that *Desi* precipitates could be highly valuable in terms of their dietary fibre content (Anderson *et al.,* 2009).

4.1.6 Carbohydrate concentration

Results from the current study showed fairly similar results when comparing the results of the isoelectric- and the micellized-precipitated *Kabuli* and *Desi* chickpeas. Thus, the isoelectric precipitate from *Kabuli* recorded a mean carbohydrate concentration value of 46.1%, a figure that was substantially higher than that of the control sample (37.2%) but closer to the micellized *Kabuli* precipitate figure for carbohydrate concentration of 49.4%. The isoelectric precipitated *Desi* had 43.9% which was 4.1% higher than that of the control sample (38.8%) while the micellized *Desi* precipitate results showed a 11.9% higher carbohydrates concentration (50.7%) than the control (38.8). There is limited information on the effect of isoelectric and micellization precipitation of the carbohydrates content of *Kabuli* and *Desi* chickpea. Hence, it is difficult to explain or to give information on results obtained above where carbohydrates of chickpea cultivars show high carbohydrates concentration than the control sample. A study by Sofi *et al.* (2019) evaluated the effect of incorporation of germinated flour and pre-treated chickpea flour from chickpea on different quality characteristics of rice-based noodles; this study reported that the carbohydrate content (isoelectric precipitation) of the noodles supplemented by chickpea ingredient ranged between 79 to 70.44%. Noodles were prepared by substituted rice flour with germinated chickpea flour at ratio of 7%, 15%, 20%, and 30% and chickpea protein at ratio of 3%, 5%, 8%, and 10% with rice noodle as control sample (Sofi *et al.,* 2019). Therefore, this suggest that the addition of chickpea in the preparation of the noodles may have increased available carbohydrate content and hence energy content.

4.2 Degree of hydrolysis (DH)

Table 3 presents the results of the hydrolysis of pre-treated chickpea flour following the action of the enzyme alcalase. The reaction time was used to evaluate this process and the degree of hydrolysis was the response variable. Overall, the study results indicated that the degree of hydrolysis of pre-treated chickpea flour was over 80% by the start of the experiment and that this figure decreased over time. This decrease in the degree of hydrolysis was greater in the *Kabuli* pre-treated flour extracted using isoelectric precipitation. In detail, both *Kabuli and Desi* isoelectric precipitated pre-treated flour exhibited a high degree of hydrolysis (81.6%) for *Kabuli*, 82.8% for *Desi*) at the start of the experiment (T_0) and decreased to 69.6% for *Kabuli* and to 74.2% for *Desi* as hydrolysis time increased to 60 min. There was no significant ($p<0.05$) difference between the hydrolysis for *Kabuli* and *Desi* at times 0 and 5 min when the hydrolysate from the two precipitation reactions were compared. Thus, at these times a decrease in hydrolysis was noted in the proteins that were precipitated using the isoelectric method. After 15 min of hydrolysis, the *Kabuli* proteins precipitated by the isoelectric method showed a further reduction of degree of hydrolysis, from 79,7% to 67.1% while a minor reduction in the degree of hydrolysis was noted in the remaining hydrolysates. By 30 min, the hydrolysis in the isoelectric *Kabuli* proteins had corrected back to 78.5% and a reduction in hydrolysis, from 81.5% to 76.5%, was noted in the isoelectric *Desi* proteins. The hydrolysis of the micellized *Kabuli* and *Desi* protein showed a slight, approximately 2%, increase. After a further 30 min of hydrolysis, the *Kabuli* proteins from both precipitates showed almost a 10% decrease in hydrolysis while the *Desi* proteins from both precipitates showed only around a 2.5% reduction in hydrolysis.

Hydrolysis took place at an initial stage (0 min) even before the reaction analysis began, might be due to the extraction method used during pre-treatment. Isoelectric precipitation has been reported to influence the functional and nutritional properties of legumes as well as to improve their protein digestibility and their physicochemical properties (Boye *et al.,* 2010; potier *et al.,* 2008; Tinus *et al*., 2012). The enzyme alcalase might have played an important role of increasing the degree of hydrolysis at the initial stage as it is an endoenzyme with broad specificity and so it is a very effective proteolytic enzyme (Fathollahy *et al*., 2021)

Pedroche *et al.* (2002) reported a DH of 65% with the use of both alcalase and flavourzyme after a reaction time of 150 min. Hong *et al*. (2005) hydrolysed mung bean protein with alcalase and recorded 22% DH after 10 h. Maria del Mar Yust *et al* 2012 determined the degree of hydrolysis of pre-treated chickpea flour using alcalase and reported an increase in DH over time. The addition of flavourzyme led to a new increase of DH up to 70% after 120 min of hydrolysis.

Table 3. Kinetics of hydrolysis of pre-treated chickpea flour

Hydrolysis time (min)	**Isoelectric precipitation (%)**		**Micellization precipitation (%)**	
	Kabuli	*Desi*	*Kabuli*	*Desi*
0	$81.6^{a} \pm 4.9$	$82.8^{a} \pm 5.2$	$80.2^{a} \pm 5.7$	$84.9^{a} \pm 3.1$
5	$79.7^{b} \pm 6.2$	$84.3^{b} \pm 2.7$	$77.4^{b} \pm 6.4$	$84.3^{b} \pm 2.7$
15	$67.1^{c} \pm 1.9$	$81.5^{d} \pm 7.5$	$77.0^{b} \pm 4.2$	$82.5^{d} \pm 2.0$
30	$78.5^{e} \pm 4.9$	$76.5^{e} \pm 6.3$	$79.7^{c} \pm 6.2$	$84.3^{b} \pm 2.7$
60	$69.6^{f} \pm 4.7$	$74.2^{fg} \pm 2.3$	$69.1^{f} \pm 2.7$	$82.2^{d} \pm 4.6$

Means ± Standard deviation. Mean values followed by different superscript letters in the same column are significantly different at $p<0.05$ (LSD). CK = Chickpea *Kabuli*; CD = Chickpea *Desi*

4.3 *In vitro* digestibility of chickpea protein hydrolysate

The effect of enzymatic modification on *in vitro* digestibility of chickpea protein hydrolysate is presented in Figure 4. Overall, as digestion time increased, the digestibility of chickpea protein hydrolysates decreased. The exception to this involved *Desi* protein isolated using the micellar method that showed an increase in % digestibility at T_{180}. In detail, the results showed that the isoelectric precipitated protein hydrolysate from *Kabuli* exhibited the highest *in vitro* digestibility (77.79%) at T_0 followed by the *Desi* hydrolysate from micellized precipitation (76.2%). It was observed that *Kabuli* hydrolysates extracted by isoelectric precipitation and *Desi* hydrolysates from micellization precipitation had the highest mean values of digestibility (71.15% and 71.23%, respectively). Thus, the lowest digestibility mean values were recorded for isoelectric-precipitated *Desi* protein (D1-M1) (67.64%) and the micellar-isolated *Kabuli* protein (K2-M2) (66.38%). From 0 to 120 min, the digestibility of isoelectric-precipitated *Desi* protein was slightly higher than micellized *Kabuli* hydrolysate.

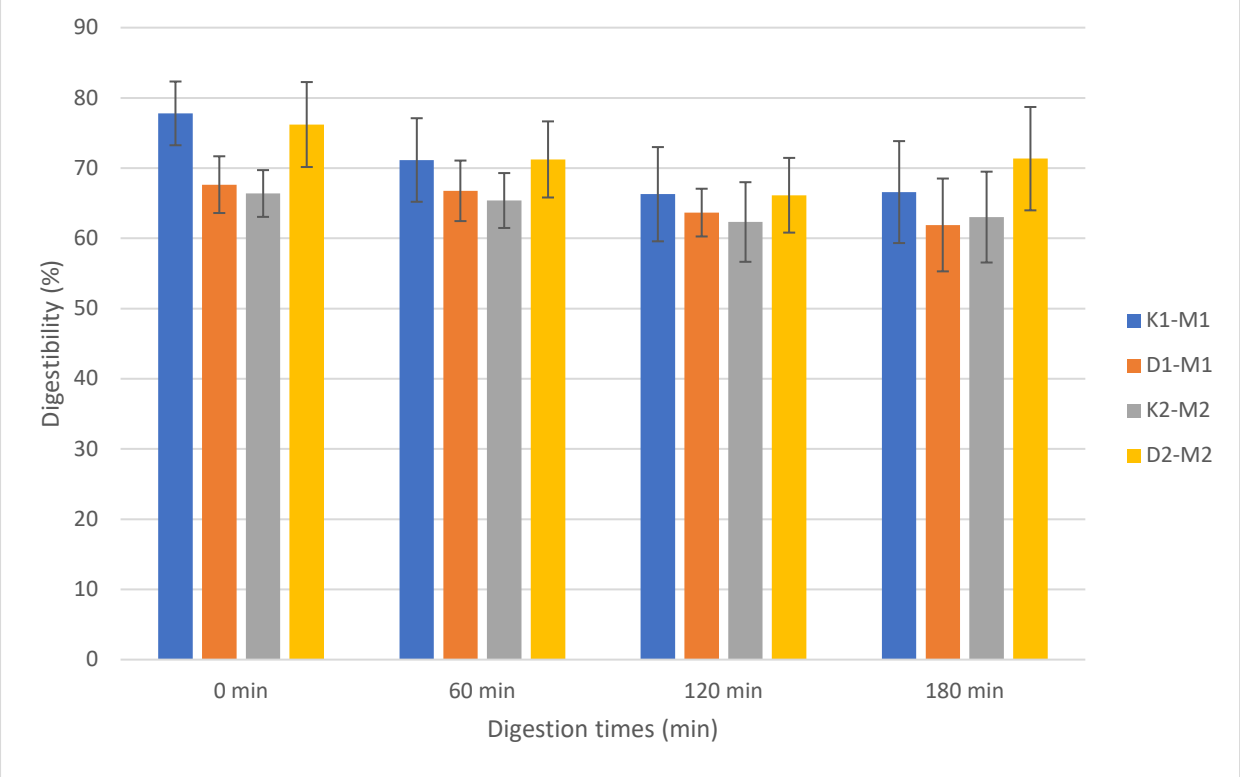

Figure: 4 Effect of enzymatic modification on in-vitro protein digestibility of chickpea protein hydrolysate. K1-M1 (Blue) represents the *Kabuli* hydrolysate from method 1 (isoelectric precipitation) and D1-M1 (orange) represents *Desi* hydrolysate from method 1 (isoelectric precipitation), K2-M2 (grey) represents *Kabuli* hydrolysate from method 2 (micellization precipitation) and D2-M2 (yellow) represents *Desi* hydrolysate from method 2 (micellization precipitation).

The lowest digestibility was displayed by isoelectric-precipitated *Desi* hydrolysates (66.76%) and micellized *Kabuli* precipitate (65.38). Overall, it appears that the hydrolysates were highly digestible at 0 min compared to the other digestion intervals. As pepsin and pancreatin enzymes were added to the sample leading to the pepsin-mediated breakdown of dietary protein into amino acids, it is to be expected for the protein concentration to be high at an initial stage (0 min) and to decrease as digestion time increases (Ofori-Anti *et al*., 2008).

Therefore, at T_0, the protein concentration was high as the proteolysis had just started. As the digestion time increased, the protein degraded and as a result protein digestibility decreased, pepsin will continue digesting peptide bonds, the predominant chemical bonds found in protein until enzyme substrate is reduced to building blocks of amino acids (Allen *et al.,* 2005; Samloff, 1989). As the digestion time increased to 60 min, a significant decrease in digestibility of hydrolysates was observed. At 120 min, isoelectric-precipitated *Kabuli* and *Desi* hydrolysates

continued to show a decrease in digestibility of *Desi* protein having a lower mean value (63.66%) compared to *Kabuli* protein (66.28 The micellized *Kabuli* precipitate showed less digestibility (62.32%) than that of micellized *Desi* precipitate (66.13%).

The lowest % *in vitro* digestibility (62.32%) was shown by *Kabuli* protein extracted using the micellar method (K2-M2) at 180 min and the second lowest figure was for *Desi* protein using the isoelectric method (D1-M1) (63.66%) after 120 minutes of digestion. The *in vitro* protein digestibility of D2-M2 at 180 minutes was 71.34% which was the highest percentage digestibility at 180 min compared to the other results.

According to these results, Figure 4 shows that between 120 and180 min of digestion the *in vitro* protein digestibility of the hydrolysates increased. Here, K1-M1 increased from 66.28% to 66.58%, K2-M2 increased from 62.32% to 63.02%, D2-M2 increased from 66.13 % to 71.34% and lastly D1-M1 was the only hydrolysate whose digestibility continued to decrease until 180 minutes of digestion. In summary, Figure 4 shows that at 120 minutes the *in vitro* protein digestibility of chickpea (both cultivars) hydrolysates is lower compared to other digestion times (0 min, 60 min, 180 min).

The chickpea protein hydrolysates showed high protein digestibility that may indicate increased peptide bond cleavage resulting from modification in protein structure during extraction or processing that might have exposed more peptides bonds and improved enzyme accessibility to cleavage sites. It can also be reported that the extraction methods (isoelectric and micellization precipitation) had a positive effect on the *in vitro* protein digestibility of both cultivars as they all produced high % digestibility and at a similar range. Bhagyawant *et al*. (2018 reported the *in vitro* protein digestibility of chickpea protein to be between 59% and 76%, findings that are close to the mean ranges recorded in the current study.

In comparison with other studies, Alberta *et al.* (2016) reported high protein digestibility values (72.1%-82.4%) of cooked lentil and lentil isolates. Thus, in addition, an increase in protein digestibility might have resulted from heating the digested samples at 50◦C to inactivate protease inhibitors which known to decrease digestibility (Alberta *et al.*, 2016). Wang *et al.,* (2010) reported that the digestibility of *Kabuli* protein was increased compared to that shown by *Desi* protein isolates, whereas the current study revealed that both *Kabuli* and *Desi* hydrolysates digestibility were similar in terms of digestibility. Sara Aviles-Gaxiola *et al.* (2018) reported the *in vitro* protein digestibility of chickpea protein of 62.28% and this

percentage digestibility is like that observed in the current study. In summary, enzymatic modification of extracted chickpea protein using pepsin-pancreatin can indeed improve the *in vitro* digestibility of chickpea protein hydrolysates, a result which addresses study objective 2 of the current study.

4.4 Simulated gastric and intestinal (pepsin-trypsin-α-chymotrypsin) digestion

The results for simulated gastric and intestinal digestion are presented in Table 3 below. The digestibility of the isoelectric-precipitated *Kabuli* and *Desi* hydrolysates were similar (0.56%). A significant increase in protein digestibility (0.56% to 0.72%) of micellized *Kabuli* hydrolysates was observed while the micellized *Desi* hydrolysate exhibited a slight decrease in protein digestibility (0.56% to 0.52%).

Table 4. Protein digestibility of chickpea hydrolysate by simulated gastric and intestinal digestion.

Sample identification	Isoelectric precipitation method 1 (%)	Micellization precipitation method 2 (%)
Kabuli	$0.56^{a} \pm 0.05$	$0.72^{b} \pm 0.10$
Desi	$0.56^{a} \pm 0.04$	$0.52^{a} \pm 0.06$

Means ± Standard deviation. Mean values followed by different superscript letters in the same row are significantly different at $p<0.05$ (LSD). CK = Chickpea *Kabuli*; CD = Chickpea *Desi.*

Theoretically, chickpea *Kabuli* seeds were reported to have high protein digestibility compared to *Desi* seeds (Kaur *et al.,* 2021). This may have been caused by the absence of pancreatin during the digestion as pancreatin helps to stimulate digestion in the small intestine while pepsin stimulates digestion in the stomach - in this study only pepsin, trypsin and α-chymotrypsin were used during digestion (Ribeiro *et al.,* 2017; Kaur *et al.,* 2010).

4.5 Effect of enzymatic modification on selected functional properties of chickpea protein hydrolysates

The chickpea functional properties that were analysed in the current study included syneresis, oil absorption capacity, water absorption capacity (Table 5) and protein solubility (Figure 5.1 and 5.2).

4.5.1 Syneresis

Syneresis is the term used to describe a phenomenon where a liquid or water oozing out of gel or is expelled from gel (Mizrahi, 2010). This functional property is common in a wide variety of foods including jams, jellies, sauces, dairy products, surimi, and tomato juice, as well as meat and soybean products (Boye *et al.,* 2010). Syneresis is undesirable to some food products as it affects the consumer appeal of food products as it results in shrinkage of the gel (Raza *et al.,* 2021). For example, if more serum is released from the gel matrix of the yoghurt, consumers perceive the yoghurt as defective.

Table 5. Selected functional properties of *Kabuli* and *Desi* chickpea hydrolysates

Chickpea variety	Extraction method	Syneresis (%)		OAC (%)	WAC (%)
		0 min	120 min		
	Control	**174.25^{c}** ± 6.16	33.29^{a} ± 2.44	**225.92^{c}** ± 11.00	**191.46^{b}** ± 5.47
Kabuli chickpea	Isoel. precipitate	139.73^{b} ± 13.55	31.70^{a} ± 3.77	69.89^{a} ± 11.68	117.26^{a} ± 22.46
	Micel. precipitate	147.98^{b} ± 8.66	28.06^{a} ± 5.77	90.88^{b} ± 12.70	135.90^{a} ± 3.40
Desi chickpea	Control	117.62^{a} ± 1.76	41.00^{b} ± 1.35	228.39^{b} ± 3.02	191.52^{b} ± 0.82
	Isoel. precipitate	**168. 42^{c}** ± 6.16	33.29^{ab} ± 2.44	225.92^{b} ± 10.80	191.46^{b} ± 5.47
	Micel. precipitate	117.6^{a}± 1.76	41.00^{b} ± 1.35	228.39^{b} ± 3.02	191.52^{b} ± 0.82

Mean ± standard deviation; values in the same column with the same superscript letter are not statistically significantly different from one another at 95% confidence interval. WAC = Water absorption capacity, OAC = Oil absorption capacity, Isoel. precipitate = isoelectric precipitate, Micel. precipitate = micellised precipitate, control= raw desi and raw Kabuli flour.

The Study results show that at 0 min the syneresis of *Kabuli* protein precipitates - both isoelectric and micellized - was significantly lower at 139.7% and 147.98%, respectively, compared to the control (174.25%). The syneresis for isoelectric precipitated *Desi* protein (168.42.%) was significantly higher) than the control (117.62%) and the micellized precipitate (117.6%). Thus, the micellised *Desi* precipitate shows the same syneretic properties as the control sample.

The relatively reduced synergetic performance of the micellised and isoelectric precipitates for *Kabuli* implies that these precipitates would release or expel less liquid or water after gel formation than the control sample. On the other hand, the higher figure for the isoelectric

precipitate for *Desi* indicates that this protein would have more water or liquid released or expelled immediately after gel formation compared to its control sample.

At 120 min, the syneresis of *Kabuli* precipitates (isoelectric and micellization) was slightly lower (31.70% and 28.0%) compared to the control results (33.29%). It appears that storing the isoelectric precipitated *Kabuli* for 120 min did not affect its syneresis properties as it exhibited less liquid release compared to its control. The isoelectric precipitated *Desi* was low at 33.29% which is lower than that of the control sample (41.00%) while micellized precipitated *Desi* showed similar results (41.00%) to that of the control sample (41%).

When comparing syneresis between chickpea cultivar proteins at 0 min, the isoelectric precipitated *Kabuli* was lower (139.73%) than that of micellized precipitated *Kabuli* (147.98%). The percentage syneresis of isoelectric *Desi* at 0 minutes was recorded to be (174.25%) which is higher than the micellized *Desi* (117.6%). At 120 minutes the isoelectric precipitated *Kabuli* was higher (31.70%) than the micellized precipitated *Kabuli* (28.06%). Isoelectric-precipitated *Desi* reported low (33.29%) syneresis compared to micellized *Desi* (41.00%). When checking results recorded in Table 5. it shows that *Desi* protein hydrolysates did not differ significantly from the control. Therefore, it can be assumed that enzymatic hydrolysis did not influence the *Desi* protein. Furthermore, it appears that there is no publication on syneretic properties of Kabuli and Desi protein hydrolysates samples

4.5.2 Oil absorption capacity

Oil absorption capacity (OAC) is one of most important functional properties of pulse flours and proteins as this property refers to the index of the food material's ability to absorb and retain oil **(**Wang *et al.,* 2020) and is known to influence flavour, texture, and mouthfeel of food products such as comminated meats, extenders or analogues and baked dough (Adebowale *et al*., 2005)**.**

In this study, the OAC of isoelectric-precipitated and micellized-precipitated *Kabuli* protein hydrolysates showed significantly low mean values of oil absorption capacity (69.89% and 90.88%, respectively) when compared to the control sample (225.92%). These values suggest that *Kabuli* precipitates (isoelectric and micellised) would exhibit undesirably low capacity to absorb and retain oil when used as ingredients in products such as doughnuts, pancakes, baked goods, desserts, confectioneries, beverages, salad dressings, meats extenders and meat analogues (Wang *et al.,* 2020). It appears that enzymatic hydrolysis did not disrupt the primary

structure of protein in desi chickpeas as their precipitates - isoelectric and micellised - did not significantly differ from the control sample (228.39%).

When comparing the oil absorption capacity between *Kabuli* and *Desi* precipitates, *Desi* exhibited a substantially higher capacity to absorb and retain oil as shown by higher mean values compared to those shown by *Kabuli*. Similar results were reported by Ghribi *et al.* (2015), where a *Desi* protein showed mean oil absorption capacity values between 105 g/100 g and 124 g/100 g. Thus, *Desi* chickpea protein appears to be more suitable to be utilized in food for which fat retention is desirable. Yust *et al*. (2010) reported that oil absorption capacity of chickpea protein hydrolysates that ranged from 443-628 g/100 g depending on the degree of hydrolysis, but the degree of hydrolysis was less than 10%. Results reported by Yust *et al.* (2010) were high compared to the one from the current study. This may be caused by the degree of hydrolysis as their %DH was less than 10% and the %DH of the current study was high, ranging from 60-80%. When the degree of hydrolysis increases further the oil absorption capacity decreases, and this may be attributed to an extensive exposure of ionic groups after hydrolysis.

4.5.3 Water absorption capacity

The water absorption capacity (WAC) refers to the amount of water absorbed per gramme of protein materials (Lam *et al.,* 2018). The WAC can also be defined as the capacity of proteins required to retain water against gravity (Shevkani *et al.,* 2015). Since food products can contain more than 50% water, poor water absorption capacity can later lead to the loss of liquid during processing and negatively change the texture of the products (Lam *et al.,* 2018)

In this study the isoelectric-precipitated *Kabuli* protein showed a WAC of 117.26% and this was lower than the control sample (191.46%). The micellized-precipitated *Kabuli* protein showed a lower %WAC (135.9%) compared to the control sample (191.46%). The isoelectric and micellized precipitated *Desi* had similar (191.46% and 191.52%) water absorption capacity compared to the control sample (191.52%) which implies that there were no changes in the water absorption capacity of the control sample and that of the precipitated samples. This study revealed that cultivars isolated from micellization have high water absorption capacity (*Kabuli;* 135.90%, *Desi*; 191.52%) compared to cultivars from isoelectric precipitation (*Kabuli*; 117.26%, *Desi*; 191.46%). A study by Summo *et al.,* 2019 involving the nutritional, physico-chemical and functional characterisation of a global chickpea collection showed that brown

chickpea (*Desi*) has the highest water absorption capacity compared to beige (*Kabuli*). This may be due to that *Desi* have more hydrophilic constituent such as polysaccharides.

Another study on nutritional composition of and compositional study of *Desi* and *Kabuli* chickpea (Cicer *Arientinum L.*) flours from Tunisian cultivar reported by Ghribi *et al.* (2015) reported that *Kabuli* had 73.89 g/100 g and *Desi* 107.96 g/100 g. The findings of the current study agree with the findings of Summo *et al.* (2019) and Ghribi *et al*. (2015).

Desi protein hydrolysates showed a high WAC, and this might be because isolates have an ability to swell, dissociate and unfold exposing additional binding sites, whereas the carbohydrate and other component present may impair it (Kaur *& Singh*, 2007). *Kabuli* protein showed a low water absorption capacity, and this can be attributed to the presence of carbohydrates and other components that cannot allow the protein to swell, dislocate and unfolds (Ghribi *et al.,* 2015).

4.5.4 Protein solubility

Protein solubility is defined as the amount of protein in a sample that can dissolve into solubilised (Grasso *et al*., 2021). It is a crucial functional property as it influences other functional properties such as emulsification, foaming and gelation (Fathollahy *et al*., 2021). Improving protein solubility, circumventing colloidal instability of protein solutions, and avoiding the formation of intrinsic protein particles is of the upmost importance (Garidel *et al.,* 2010). In this study, the protein solubility of chickpea protein hydrolysate was determined, and the pH of the samples were adjusted to 2, 4, 6, 7 and 10, respectively. The effect of enzymatic modification on protein solubility of chickpea hydrolysates have been determined and the results are presented in Figure 5.

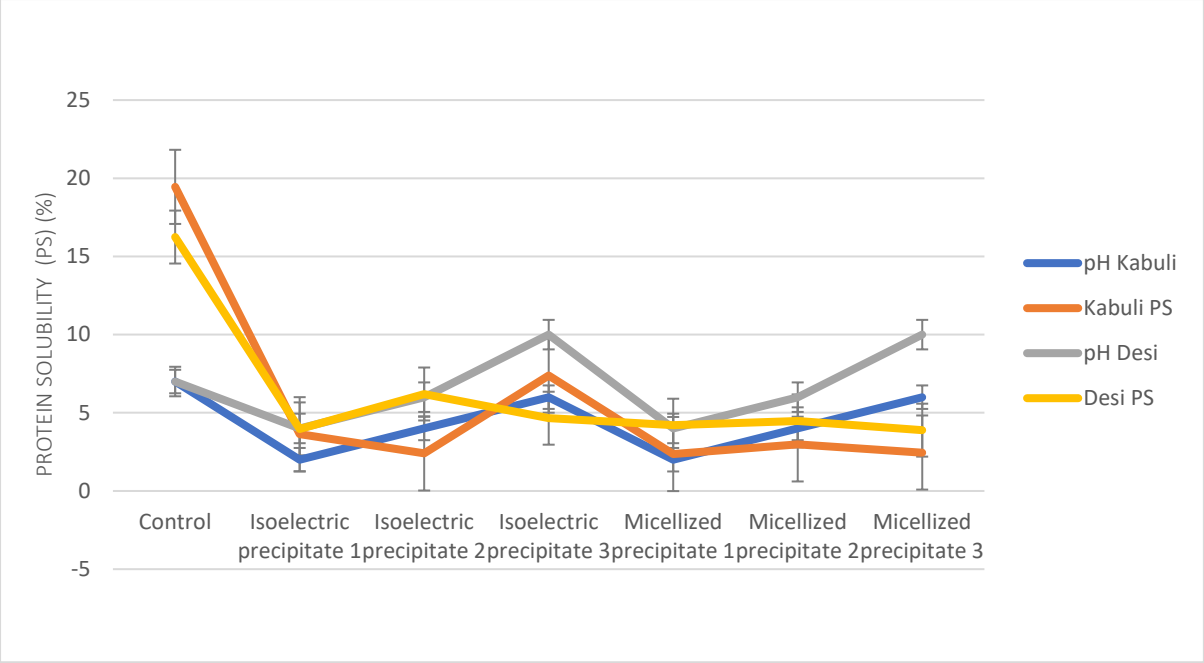

Figure:5. Effect of enzymatic modification on protein solubility of *Kabuli* hydrolysates and *Desi* hydrolysates. Control: represent *Kabuli* control sample and *Desi* control sample, *Kabuli* PS: represents *Kabuli* hydrolysates from method 1 (isoelectric precipitation) and *Kabuli* hydrolysate from method 2 (Micellized precipitation). *Desi* PS: represent *Desi* hydrolysates from method 1 (isoelectric precipitate) and *Desi* hydrolysates from method 2 (micellization precipitation). pH *Desi* are the pH values plotted with *Desi* protein solubility (PS%) values and pH *Kabuli* are the pH values plotted with the *Kabuli* protein solubility (PS%).

The percentage solubility of the isoelectric-precipitated *Kabuli* hydrolysate was lower (4.46%) at pH 2 than the control sample (19.45%) at pH 7, micellized-precipitated *Kabuli* hydrolysate had low (2.60%) protein solubility at pH 6 which is lower than the control sample (19.45%) at pH 7. The isoelectric-precipitated *Desi* hydrolysate recorded low (4.94%) protein solubility at pH 10 compared to the control sample (16.24%) and micellized-precipitated *Desi* hydrolysate (4.19%) also had a low protein solubility at pH 4 compared to control. The results show that at pH 2 and 4 the percentage protein solubility was high and at pH 7 and 10 there was also high percentage protein solubility compared to pH 6 of micellized *Kabuli* hydrolysate (2.60%). A study by Karaca *et al*. (2011) involved the use of micellization (salt extraction) and isoelectric precipitation from chickpea, faba bean and pea protein and their figures for protein solubility were significantly higher for the isolates produced by isoelectric (85.9%) precipitation relative to those produced by salt extraction (61.5%). Pre-treated chickpea flour prepared from isoelectric precipitation showed a higher solubility (91.20%) compared to faba bean (89.65%), pea protein (61.42%), lentil (90.73%). However, isolates prepared by salt extraction reported chickpea isolates with lower protein isolates compared to faba bean (52.54%), lentil (89.88%), pea protein (38.12%). Karaca *et al*. (2011)

The findings of the current study contrast with published results (Karaca *et al.,* 2011; Gamage *et al.,* 2011; Boye *et al.,* 2010;). Boye *et al*. (2010) reported the solubility of chickpea protein to be high between pH 1 to 3 and pH 7 to 10, figures like those shown by other legume proteins (lentils, faba beans and soybeans). This is due to the net zero charge of the protein that reduces the intermolecular electrostatic repulsion and ionic hydration leading to precipitation of protein Kaur & Singh (2007). Based on the above observation, the protein solubility of chickpea protein hydrolysate is mostly around 4% to 5%. Protein solubility at different pH might serve as an important indicator of the performance of the protein isolates in food system. Kaur & Singh (2007) reported that the solubility of *Desi* and *Kabuli* chickpea did not differ significantly at $p< 0.05$. The report from Kaur & Singh (2007) also support part of the current results as there was no significant difference between the solubility of both cultivars. Generally, pea protein isolates display the lowest solubility at pH 4 to 6 irrespective of their extraction method or pea cultivar (Lam *et al.,* 2018). The above statement supports the results from the current study because at pH 4 the solubility of micellized-precipitated *Desi* hydrolysate protein was 4.19% and at pH 6 the solubility of micellized-precipitated *Kabuli* hydrolysate protein was 2.60%.

CHAPTER 5: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Chickpea is considered as healthy because of its high protein concentration and high protein availability. The first objective of this study was to determine the proximate composition of pre-treated chickpea flour. The results show that the protein concentration was affected by enzymatic modification as shown by low protein concentration of both isoelectric precipitated *Kabuli* (13.6%) and micellized *Kabuli* (14.3%) compared to the control sample (20.5%). The protein concentration of the isoelectric precipitated *Desi* and micellized *Desi* protein concentration was also lower than that of the control sample. Nevertheless, *Kabuli* precipitates (isoelectric and micellization precipitation) had a higher protein concentration than *Desi* precipitates (isoelectric and micellization). It appears that micellization precipitation yielded more protein than isoelectric precipitation.

The isoelectric and micellized precipitation methods efficiently extracted carbohydrate in the isolated *Desi* and *Kabuli* pre-treated flour as shown by high carbohydrate concentration of *Kabuli* isoelectric precipitate (46.1%) and micellized precipitates (49.4%) than the control sample (37.2%); *Desi* micellized precipitate carbohydrate concentration (50.7%) compared to the control sample (38.8%). The study demonstrates that both extraction methods can be used to produce pre-treated chickpea flour with a high carbohydrate concentration (40-50%). It can also be concluded that the extraction of chickpea protein by micellization precipitation can improve the carbohydrate concentration from both *Kabuli* and *Desi* cultivars as they showed a slightly higher carbohydrate concentration compared to the isoelectric pre-treated chickpea flour

Isoelectric precipitation method was found to be an excellent method for the extraction of pre-treated chickpea flour with a high ash concentration (4.2% for *Kabuli* and 3.9% for *Desi*) compared to micellized *Desi* (1.8%) and *Kabuli* (1.9%) protein and their control samples (*Kabuli* control: 2.3%; *Desi* control sample: 1.8%). The isoelectric precipitated *Kabuli* and micellized precipitates exhibited low crude fat concentration (8.1% and 7.9%, respectively), compared to control sample (10.2%). Likewise, this study also revealed that the isoelectric precipitated *Desi* (9.78%) and micellized *Desi* (7.5) obtained lower fat concentration compared to the control sample (11.9%). Partial substitution of high fat proteinous ingredients in cereal-based formulations with these protein precipitates could significantly lower the fat

concentration of resultant food products. This would also have a significant contribution towards reducing the problem of obesity.

It appears that both isoelectric and micellization precipitations did not influence on fibre content of the chickpea *Kabuli* while the isoelectric precipitated *Desi* (27.8%) and micellized precipitate of *Desi* (23.2%) showed a higher fibre concentration compared to the control sample (19.0%). Furthermore, both *Desi* precipitates (isoelectric and micellized) showed higher fibre concentration (27.8% and 23.2%, respectively) compared to isoelectric *Kabuli* (20.7%) and micellized *Kabuli* (19.7%). Therefore, this could be useful when formulating fibre-enriched food products.

Kabuli from both isoelectric and micellization precipitation had a higher protein concentration than *Desi* extracted using both these methods (isoelectric and micellization). Therefore, Micellization precipitation is the best method to use to improve the protein concentration of *Desi* and *Kabuli.* Isoelectric and micellization precipitation produces pre-treated chickpea flour with high carbohydrates concentration.

The second objective that this study focused on was to determine the effect of enzymatic modification on *in vitro* digestibility of pre-treated chickpea flour. Enzymatic hydrolysis analysis was conducted, and the degree of hydrolysis was determined. Results from this study revealed that the enzymatic hydrolysis of pre-treated chickpea flour responded well to the extraction methods and to the enzymes used for hydrolysis. This was because the degree of hydrolysis of both *Kabuli* and *Desi* from both micellization and isoelectric precipitation recorded over 80% degree of hydrolysis at the initial stage (0 min) of hydrolysis. After 5 min the degree of hydrolysis from both cultivars and both methods decreased but by 30 min the hydrolysis started to increase (isoelectric *Kabuli*: 78.5%, Micellized *Kabuli*; 79.7%, Micellized *Desi*: 84.3%). However, the isoelectric *Desi* kept on decreasing its degree of hydrolysis. Overall, the enzymatic modification by alcalase had a positive influence on pre-treated chickpea flour since the degree of hydrolysis ranged from 67.1% to 84%. It can also be concluded that both isoelectric precipitation and micellization precipitation produced high degree of hydrolysis of pre-treated chickpea flour (67.1% to 84%)

Overall, the results revealed that pre-treated chickpea flour have high protein digestibility which indicate that there was an increase in peptide bond cleavage resulting from modification of protein structure during these two extraction processes that might have exposed peptide cleavage sites to facilitate enzyme accessibility and proteolysis.

This implies that both chickpea cultivars can be used in the production of legume food products as there are highly digestible it can also be incorporated in cereal products with low digestibility to enhance the product. The food industries can also use the *in vitro* method as it is less expensive and not time consuming and it is not a complicated digestion method.

Chickpea protein hydrolysates were also subjected to simulated gastric and intestinal digestion using a mix of enzymes (pepsin, trypsin, and α-chymotrypsin) and results showed that the isoelectric precipitated *Kabuli* and *Desi* hydrolysates showed a similar percentage digestibility (0.56%). Micellization precipitated *Kabuli* protein recorded a higher percentage digestibility (0.72%) when compared to micellization *Desi* (0.52%). According to the relatively low levels of digestibility obtained in this study, simulated gastric and intestinal digestion using the three enzymes may not really have had much effect on the digestibility of chickpea protein hydrolysates when compared to the *in vitro* protein digestibility method, perhaps because of the absence of pancreatin in the simulation digestion where pancreatin helps to stimulate digestion in the small intestine while pepsin stimulates digestion in the stomach.

Based on the results obtained in this study, the *in vitro* protein digestibility method is the best method to improve the protein digestibility of *Desi* and *Kabuli* when compared to digestion using the simulation and intestinal digestion method.

The last objective of the study was to determine the effect of enzymatic modification on selected functional properties. The selected functional properties include water absorption capacity (WAC), oil absorption capacity (OAC), protein solubility and percentage syneresis. The study revealed that the *Kabuli* and *Desi* protein hydrolysates showed a high percentage of syneresis at 0 minutes and that this syneresis level decreased over time. Results show that *Kabuli* protein hydrolysates from both isolation methods (isoelectric *Kabuli* (139.2%) and micellized *Kabuli* (147.98%) had a significantly lower percentage syneresis when compared to the control sample value (174.25%) at 0 minutes and after 120 mins there was a decrease in the percentage syneresis of isoelectric *Kabuli* and micellized *Kabuli* when compared to results obtained at 0 min. Results obtained at 120 min showed that the isoelectric *Kabuli* and micellized *Kabuli* had a low percentage syneresis when compared to the control sample. Overall, the syneresis showed by isoelectric precipitated *Desi* protein (168.42%) was significantly higher than the control (117.62%) and the micellized precipitate (117.6%). Thus, the micellised *Desi* precipitate shows the same syneresis properties as the control sample.

The OAC observed in this study showed that the isoelectric precipitated and micellized precipitated *Kabuli* protein showed significantly lower mean values of oil absorption capacity (69.89% and 90.88%, respectively) when compared to the control sample (225.92%). When comparing the oil absorption capacity between *Kabuli* and *Desi* hydrolysates, *Desi* exhibited a substantially higher capacity to absorb and retain oil as shown by higher mean values compared to those shown by *Kabuli*.

Overall, *Desi* hydrolysates reported high oil absorption capacity when compared to the Kabuli hydrolysates. Therefore*, Desi* hydrolysates can be utilized in developing products with high oil absorption capacity.

The findings from this study showed that both isoelectric precipitated *Kabuli* (117.26%) and micellised *Kabuli* (135.9%) reported lower water absorption capacity compared to the control sample (191.46%), while the isoelectric and micellized *Desi* showed similar WAC levels (191.46% and 191.5%, respectively) compared to the raw sample (191.52%). The overall results from this study showed that the *Desi* and *Kabuli* hydrolysates had a relatively high percentage WAC. The isoelectric precipitation and micellization including the enzymatic modification did not really address the objective of this study for improving functional properties because the WAC of the hydrolysates was low when compared to the raw chickpea flour. However, *Desi,* and *Kabuli* hydrolysates can still be used as they reported high WAC alone not in comparison with the raw data.

Protein solubility of the *Desi* and *Kabuli* hydrolysates were determined, and results showed that isoelectric *Desi* and *Kabuli* recorded a high percentage protein solubility compared to micellized *Kabuli* and *Desi*. In comparison to *Kabuli* and *Desi* hydrolysates, the control samples recorded a higher protein solubility than the chickpea hydrolysates.

It can be concluded that isoelectric precipitation method produces *Desi* and *Kabuli* hydrolysates with high protein solubility.

Food processors can make use of *Kabuli* and *Desi* isolates and isoelectric and micellization method when producing products with high fibre content. Food processors can also use isoelectric precipitation method to extract chickpea cultivars with high ash content. Isoelectric precipitation is not a time-consuming method which will be very advantageous to the company and food processors, and it is less expensive. Both *Kabuli* and *Desi* isolates cannot be used to enhance fat on food product as the results showed that they have low fat content compared to the raw chickpea cultivar flour, same applies to the moisture content of the isolates. *Kabuli*

hydrolysates are the preferred type as it has high protein content and both isoelectric and micellization can be used to extract *Kabuli* with high protein content. In-vitro method is the most preferred method to improve chickpea protein digestibility. *Desi* is the recommended hydrolysates when developing products with high oil absorption capacity. Isoelectric precipitation can be used to produce chickpea cultivars with high protein solubility. Both *Kabuli* and reported high WAC.

RECOMMENDATIONS

Results from the current study suggest the following recommendations:

- Further studies are required to compare the efficiency of the isoelectric and micellization methods to other extraction methods such as wet extraction and ultrafiltration for extracting pre-treated chickpea flour.
- Studies are required to elucidate the effect of enzymatic modification for improving the functional properties of chickpea protein such as emulsifying and emulsion properties, surface hydrophobicity and foaming properties
- The addition of pancreatin during simulation gastric and intestinal digestion.
- Studies are needed to develop a product incorporating chickpea protein hydrolysates together with other cereal grains such as maize and to determine their sensory/organoleptic characteristics.
- Further investigation is required as to good fats found in the pre-treated chickpea flour and how those fats can help during new products developed by incorporating chickpea protein isolates with other cereal grains
- Determine the effect of isoelectric and micellization precipitation on the carbohydrate content of *Kabuli* and *Desi* chickpea since there is limited information on this aspect.
- Further nutritional profiling of chickpea protein hydrolysates is needed to determine vitamin and amino acid content of such hydrolysates.
- Based on the results of water absorption capacity, *Desi* chickpea protein appears to be more suitable to be utilized in food for which fat retention is desirable.
- The use of both alcalase and flavourzyme during enzymatic hydrolysis of pre-treated chickpea flour as the current study only used alcalase as a block.

- Based on the results obtained in this study it is recommended that *Desi* hydrolysates could be highly valuable in terms of their dietary fibre content when producing new food products for those who need fibre in their daily diet.
- Different enzymes should be used for *Desi* protein hydrolysis

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APPENDICES

Appendix 1: Ethical clearance

UNISA university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 11/06/2019

Dear Ms Matheba

NHREC Registration # : REC-170616-051
REC Reference # : 2019/CAES_HREC/116
Name : Ms N Matheba
Student #: 63286939

Decision: Ethics Approval from 06/06/2019 to 31/05/2020

Researcher(s): Ms N Matheba
63286939@mylife.unisa.ac.za

Supervisor (s): Dr D Beswa
beswad@unisa.ac.za; 011-471-2274

Working title of research:

Effect of enzymatic modification on in vitro digestibility of chickpea protein and its suitability as a functional food ingredient

Qualification: M Consumer Science

Thank you for the application for research ethics clearance by the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for a one-year period, **subject to the clarification required below**. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

Due date for progress report: 31 May 2020

Please note the points below for further action:

1. The researcher indicates that judgemental sampling will be used for the selection of samples. What is the motivation for using this approach?
2. How many samples will be collected?
3. What is the motivation for the use of one-way ANOVA? The treatment and its levels that usually constitute a one-way ANOVA is not discussed. What is the response variable? Is it continuous/count/binary?

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4. What is the motivation for the selection of Duncan multiple range test? Discuss how the model will be fitted, such as method of estimation, how the goodness of fit will be assessed, how the test on regression coefficients will be done and how the model assumptions will be done. Also, what remedial action will be taken if the model assumptions are not met by the data?
5. The researcher should consider amending the title, as the concept of 'functional food ingredient' is not correctly used in this context.

*The low **risk application** was **reviewed** by the UNISA-CAES Health Research Ethics Committee on 06 June 2019 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
2. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
3. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
4. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
5. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
6. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
7. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

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Note:
*The reference number **2019/CAES_HREC/116** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

Prof EL Kempen
Chair of UNISA-CAES Health REC
E-mail: kempeel@unisa.ac.za
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Executive Dean : CAES
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Appendix 2: Turn-It-in Report

turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	M Ndivho
Assignment title:	LCS 2022
Submission title:	M Consumer Science dissertation
File name:	FINAL_THESIS_OF_MATHEBA_NDIVHO-2022.docx
File size:	7.41M
Page count:	93
Word count:	27,881
Character count:	157,298
Submission date:	28-Feb-2022 07:33AM (UTC+0200)
Submission ID:	1772651543

A dissertation

entitled

Effect of enzymatic modification on in vitro digestibility and selected functional properties of chickpea protein

submitted

by

Matheba Ndivho

(u22868439)

in accordance with

the requirements for

the degree of

MASTER OF CONSUMER SCIENCE

in the

DEPARTMENT OF LIFE AND CONSUMER SCIENCES

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

of the

UNIVERSITY OF SOUTH AFRICA

SUPERVISOR: DR D BESTA

February 2022

Appendix 3: Language Editing Letter

John Dewar Tel: +27833210844
PhD, DAHM Email: johndewar65@gmail.com

Dear Dr Beswa,

This letter is to confirm that I completed a language and content edit of a dissertation entitled: **Effect of enzymatic modification on *in vitro* digestibility and selected functional properties of chickpea protein.** This dissertation was prepared by Ms Ndivho Matheba and describes a research study under your supervision. The dissertation will be presented to the Department of Life and Consumer Sciences, College of Agricultural and Environmental Sciences, University of South Africa in fulfilment for the requirements for the degree Masters in Consumer Science.

My edit included the following:

- Spelling and grammar
- Vocabulary and punctuation
- Sentence structure and word usage
- Correct outlay of dissertation

Text formatting included:

- Adjusting legend and size of some figures and tables
- Suggested inclusion of methodology theory
- Suggested alignment of conclusions with study objectives
- Suggested required detail for references in reference list
- Suggested inclusion of appendices

Yours sincerely,

John Dewar

24th February 2022