School of Molecular and Life Sciences

Identifying the Nutritional Properties of Australian Kabuli Chickpeas to Inform Future Novel Food Product Development

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DECLARATION

To the best of my knowledge and belief, this thesis titled "Identifying the nutritional properties of Australian kabuli chickpeas to inform future novel food product development" contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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AWARDS

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ABSTRACT

Abstract

Chickpea (*Cicer arietinum* L.) has been a staple food in many countries for generations. With the steadily growing world population, changing climate, increase in vegetarianism, and veganism, there is a new-found interest in chickpea as a sustainable source of plant-based proteins and other beneficial nutrients, especially in developed countries. Chickpeas are of high nutritional value due to their high concentration of proteins, essential amino acids, complex carbohydrates, and dietary fibre. Chickpeas also contain polyphenols which are known for their health-protective antioxidant effects. Australia is one of the major producers of chickpeas, with over 90% of the production exported. Of the two chickpea types, desi and kabuli, Australian kabuli chickpeas command a premium price due to their high quality including germination, vigour, and seed size. There is, however, a lack of information on the physical, nutritional and antioxidant properties of Australian kabuli chickpea varieties. Thus in this thesis, the physical (i.e. seed weight, volume, density, hydration, and swelling properties), nutritional (i.e. proximate composition, amino acids, individual mineral content), and antioxidant properties (total polyphenol content and antioxidant capacity) of Australian kabuli chickpea varieties were investigated. Thus, a genotype by environment study was conducted to determine the effects of genotype, environment, and their interactions on Australian kabuli chickpea seed properties. And to further understand the effect of processing, non-thermal, highpressure processing was utilised and the textural, nutritional, and antioxidant properties of high pressure processed kabuli chickpeas were determined.

Firstly, five commercially grown kabuli chickpea varieties were sampled to investigate differences between them in terms of seed physical, nutritional and antioxidant properties. The results found large variation in seed physical properties. In particular, the variety Kimberley Large, which is exclusively grown in Western Australia surpassed all other varieties in seed size. Kimberley Large also exhibited the highest values for ash (2.86 g/ 100g, db), starch (48.88 g/ 100g, db) and carbohydrate (by difference) content (52.61 g/ 100g, db) content. In terms of protein content, Genesis Kalkee (24.56 g/ 100 g, db) was the highest. Despite the differences

in physical properties, Kimberley large and Genesis 090 exhibited the highest and very similar total polyphenol contents however, Genesis 090 showed significantly ($p \leq 0.05$) higher DPPH and ABTS antioxidant capacity values. In contrast, oxygen radical antioxidant capacity (ORAC) for Kimberley Large was the highest among the five varieties. These results showcase the important inter-varietal differences between Australian kabuli chickpea varieties, which can be exploited to direct the development of new varieties and nutritious food products in the near future.

Next, four common kabuli chickpea varieties grown in five locations across Australia were investigated to determine the effect of genotype, growing environment, and their interactions on their physical, nutritional and bioactive properties. The results indicated that genotype, environment, and their interactions had significant effects on swelling capacity, proximate composition (i.e. protein, fat, ash, and starch content), total polyphenol content and antioxidant capacities. A strong positive correlation between polyphenol content and antioxidant capacities was identified, whereas a negative correlation between protein and starch content, and protein and antioxidant capacities was observed. Following a principal components analysis (PCA), the first two components (PC1 and PC2) explained over 87 % of the total variation and clearly depicted the separation of varieties based on the growing environment. The results obtained in this study can be used by plant breeders to develop more adaptable varieties with high nutritional and antioxidant properties. Farmers can also utilise this information to enhance desirable properties in kabuli chickpeas based on the available growing environment in their reach.

Finally, for the first time, the potential of highpressure processing for the development of a ready-to-eat chickpea product using Australian kabuli chickpeas was investigated. Three pressure levels (200, 400, 600 MPA) and two treatment times (1 and 5 min) were selected. Previous sensory studies have reported that a soft texture in legumes is more acceptable to consumers. When compared to the conventionally cooked chickpeas, high pressure processed chickpeas were softer, thus had a more desirable texture due to decreased firmness, chewiness, and gumminess. High pressure processing did not affect the proximate composition, individual mineral content and *in vitro* protein digestibility, however, a significant increase in the slowly digestible starch content and an accompanying decrease in rapidly digestible and resistant starch content was recorded. Slowly digestible starch is the most desirable starch fraction as

it offers a slow but steady increase of postprandial blood glucose levels sustaining over a period of time when compared to rapidly digestible starch. It also increases insulin sensitivity, leading to the prevention and management of diabetes. These findings suggest that high pressure processing could be a practical technology to produce a nutritious, ready-to-eat Kabuli chickpea product with increased levels of beneficial slowly digestible starch in less time. Biodegradable packaging can also be used to package kabuli chickpeas, reducing post-consumption waste generation when compared to canned chickpeas.

This thesis has generated original knowledge on the physical, nutritional, and antioxidant properties of Australian kabuli chickpeas and the effect of the growing environment and innovative processing technique on these properties. The information collectively reported in this thesis can be utilised to maximise production of current kabuli varieties, develop and improve future varieties, and maximise the beneficial effects of kabuli chickpea consumption in humans while adding value to the humble chickpea crop

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LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemists
AOAC	Association of Analytical Communities
ABTS	2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ATR- FTIR	Attenuated Total Reflectance- Fourier Transform Infrared spectroscopy
α-GOS	α- Galacto-oligosaccharides
BOM	Bureau of Meteorology
CE	Catechin equivalent
db	Dry basis
DPPH	2-2-diphenyl-1-picrylhydrazy
DRI	Dietary reference intake
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
GAE	Gallic acid equivalent
G × E	Genotype × Environment
GGE	Genotype × Genotype-environment
GRDC	Grains Research and Development Corporation
IDF	Insoluble dietary fibre
LDL	Low density lipoprotein
µmol	Micro moles
MS	Mass spectrometry
nm	Nanometer
NHMRC	National Health and Medical Research Council
NSW	New South Wales
NVT	National Variety Trials

ORAC	Oxygen radical antioxidant capacity
PDA	Photo diode array
RDS	Rapidly digestible starch
RFO	Raffinose family oligosaccharides
SA	South Australia
SCFA	Short chain fatty acids
SD	Standard deviation
SDF	Soluble dietary fibre
SDS	Slowly digestible starch
TC	Total carbohydrate
TE	Trolox equivalent
TFC	Total flavonoid content
TI	Trypsin inhibitor
TPC	Total polyphenol content
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet
Vic	Victoria
WA	Western Australia

CHAPTER 1 General introduction

"Eat food. Not too much. Mostly plants."

Michael Pollan

1.1 Background

The world population is increasing exponentially and according to the United Nations Department of Economic and Social Affairs, is expected to reach 10 billion people by 2050 (United Nations2021). This exponential population growth has been supported by increased food production, with a decrease in worldwide incidences of malnutrition in children from 199.5 million in the year 2000 to 144 million in 2019 (Rosa et al. 2018, UNICEF 2020). Similarly, a reduction in the prevalence of undernourishment in developing countries from 34.75% in 1970 to 12.9% in 2015 has been observed (Roser and Ritchie 2019). Food and Agriculture Organization (FAO) of the United Nations predicts that by 2050, there will be a 76% increase in meat consumption around the world (Alexandratos and Bruinsma 2012), however, these projections are affected by factors such as climate drivers, socio-economic changes, rising income and demand for meat in different geographical regions (Godfray et al. 2018). It is universally accepted that the current trends in meat consumption are unsustainable (Tilman and Clark 2014). Meat production not only results in higher greenhouse gas emissions (carbon dioxide, nitrous oxide and methane in particular) (Herrero et al. 2011) but there is strong evidence linking high meat consumption to colorectal cancer (Norat et al. 2005). Increased awareness towards climate change and health amongst consumers has led to an increase in plant protein consumption in the last 5-10 years in developed and developing countries (Hood-Niefer 2017). The global plant protein market is estimated to expand from USD$ 10.3 billion (2020) - USD$ 15.6 billion by 2026 (Markets and Markets 2020) with chickpeas at the forefront due to their widespread acceptability, availability and high protein content (Sofi, Muzaffar, et al. 2020).

Chickpea (*Cicer arietinum,* L.) is a widely grown legume belonging to the Fabaceae family and is believed to have originated in south-eastern Turkey (Singh 1997). They

are mainly grown in semi-arid tropics and are a main dietary component due to their low cost and high protein content (19.5- 24.4 g/ 100g, dw) (Wood and Grusak 2007). Chickpeas can be classified into two distinct types - desi and kabuli (Jukanti et al. 2012), with over 100 varieties of chickpeas grown in over 50 countries worldwide (Sofi, Muzaffar, et al. 2020). India is the largest chickpea-producing country with a production of 9.93 million tonnes in 2019, accounting for 70% of global production (FAOSTAT 2019). Australia is also a major chickpea producer, the majority being the desi chickpea, due to higher export potential (GRDC 2018a). However, Australian kabuli chickpeas command a premium price due to their high quality and greater seed size. In some parts of the world, chickpeas are mainly grown on stored soil moisture, whereas in other parts the crop is irrigated (Kashiwagi et al. 2015). Chickpeas contribute to soil improvement by fixing atmospheric nitrogen which is then available for subsequent crops (Knights and Hobson 2004).

Genotype and growing environment play a vital role in chickpea seed quality as they affect the physical, nutritional and bioactive properties (Vandemark et al. 2020). Along with the difference in variety and fertilisation regime, factors such as soil type, water availability, and temperature during plant growth and seed development are the key factors influencing chickpea seed physical and nutritional properties (Nisa et al. 2020, Özer et al. 2010, Thangwana and Ogola 2012). Australia being a large country has a wide spectrum of climatic and environmental conditions, resulting in a range of different growing conditions (Grundy et al. 2015, Bell, Moore, and Kirkegaard 2014). Most chickpea research work to date has focussed on desi varieties due to their higher global demand. In contrast, there is limited knowledge on seed quality of kabuli varieties, especially in Australia. To date, no study has characterised the physical, nutritional and antioxidant properties of Australian kabuli chickpea varieties or reported the effect of growing conditions on these properties.

Chickpeas are generally processed using a range of domestic (soaking, germinating, and cooking) and industrial techniques (milling, canning, high pressure cooking) before consumption, which helps to increase their palatability, and reduces antinutrients such as phytic acid, tannins, and trypsin inhibitors (Mittal et al. 2012, Olika, Abera, and Fikre 2019). However, these traditional processing methods also lead to a reduction of the nutritional profile of chickpeas due to high heat exposure and the elongated cooking times make the chickpeas highly palatable, leading to the

leaching of nutrients in the cooking water (Margier et al. 2018, Kaur, Singh, and Sodhi 2005). High pressure processing (HPP) is a novel, non-thermal processing technique, used for the preservation of foods with minimal effects on taste, flavour as well as the nutritional value of food products (Grundy, Lapsley, and Ellis 2016). However, studies have shown that high pressure processing leads to starch gelatinisation (Le Bail et al. 2013), formation of starch – lipid complexes (Chen et al. 2017), and modification of protein digestibility (Kaur et al. 2016). However, there is a lack of knowledge on the effects of HPP on the nutritional and antioxidant properties of kabuli chickpeas, thus this research area needs further exploration.

The aims of this thesis thus are;

1) To determine Australian kabuli chickpea seed quality, which may assist in defining recommended end product use of particular genotypes, based on their physical, nutritional and antioxidant properties.
2) To understand how production factors (such as growing environment and processing), and varietal differences affect the quality of Australian kabuli chickpea.

1.2 Objectives

1) To determine the physical, nutritional and antioxidant properties of commercial Australian kabuli chickpea varieties.
2) To determine the effects of genotype, environment and their interaction on the physical, nutritional and bioactive properties of Australian kabuli chickpeas.
3) To determine the effects of non-thermal high pressure processing on textural, nutritional and antioxidant properties of cooked kabuli chickpeas to explore the potential of this processing technique in the production of a ready-to-eat product.

1.3 Overview of thesis structure and chapters

The thesis comprises ofa series of investigations around the physical, nutritional, and antioxidant properties of Australian kabuli chickpea varieties and is organised as described below.

Chapter 2 provides an outline of the past and current research literature on chickpea seed nutritional properties. This is then extended to include the effects of genotype, growing environment as well as different processing techniques on the nutritional properties of chickpeas.

Chapter 3 describes a detailed preliminary investigation of the physical, nutritional and antioxidant properties of five kabuli chickpea varieties widely grown across Australia, which were collected from three major chickpea growing states (South Australia, Victoria and Western Australia). This is the first comprehensive study on the seed properties of commercially grown Australian kabuli chickpea varieties.

Chapter 4 reports the effects of genotype, environment and their interaction on the physical, nutritional and antioxidant properties of four common Australian kabuli chickpea varieties (viz. Almaz, Genesis 090, Kalkee, and PBA Monarch), collected from five National Variety Trials (www.nvtonline.com.au) in different environments across Australia. The effect of water availability and growing season temperature on the seed properties was identified using multivariate analysis.

Chapter 5 investigated the effects of high pressure processing on the textural, nutritional and antioxidant capacity of cooked kabuli chickpeas. This included the impact of different levels of pressure and time of processing on the textural, nutritional, and antioxidant properties of Australian kabuli chickpeas.

Chapter 6 brings all the research together, summarising and discussing the results in a broader context, and reports the overall conclusion of the research, including the limitations and future perspectives.

The **Appendices** provide supplementary information in relation to particular chapters.

CHAPTER 2 Review of literature

"Impermanence is not something to be afraid of. It's the evolution, a never-ending horizon."

Deepak Chopra

Abstract

Chickpea is one of the most consumed legumes globally. Other than their nutritional benefits such as high protein, starch, and mineral concentration, chickpeas also contain bioactive components with antioxidant properties conferring health benefits as well as prevention of chronic degenerative diseases. However, these nutritional and bioactive properties in chickpea are affected not only by the growing environment but also by the processing technique used to turn them into a product for human consumption. In this review, the nutritional composition of chickpeas and effects of genotype, environment and processing techniques on this composition is critically analysed. The negative and positive effects of these factors and their implications on chickpea product composition are revealed.

2.1 Origin

Chickpea (*Cicer arietinum* L.) is a leguminous pulse crop in the Fabaceae (Leguminosae) family. It was domesticated in south-eastern Turkey around 10,000 years ago, spreading to the south of Europe and North Africa, as well as Ethiopia. The eastward movement of chickpeas to Indo-Asia, and in particular India, began about 4000 years ago (Redden and Berger, 2007). Chickpeas were introduced into Australia in the 1890s, and the first commercial crop of chickpeas was grown in 1979 (Johnston et al., 1992).

The seed coat colour and seed structure of cultivated chickpea varieties can be highly variable, however, chickpeas are primarily classified into two types; desi and kabuli (Fig.1). Desi types have pink flowers, small seeds with a dark irregular shaped seed coat and are grown as a short season crop in the Mediterranean and semi-arid climate regions. kabuli types have white flowers, and seeds that are larger than those of desi

chickpeas with a thin, light coloured seed coat and are typically grown in warm temperate regions of the world (Agriculture and Agri-Food 2008).

Figure 2.1 Chickpea types (a) desi, (b) kabuli

2.2 Agricultural production:

Chickpeas are the second most commonly grown legume crop in the world with a total production of 17.2 million tonnes in 2018 preceded only by dry beans (*Phaseolus vulgaris* L.) with a total world production of 30.4 million tonnes (FAO, 2019). The overall world production of chickpeas has been growing steadily to meet the consumer demand due to steady population growth in India and the Middle East (Merga and Haji 2019) as well as an increased interest in plant-based protein sources in developed countries (Jukanti et al. 2012). Asia is the largest producer of chickpeas followed by Oceania, Northern America, Europe, Africa, Central America and South America (Fig. 2) (FAO, 2018). Traditionally chickpeas are a rain-fed crop, however, in areas with highly variable and insufficient rainfall, irrigation is utilised to ensure a reliable yield (Oweis, Hachum, and Pala 2004). Irrigation has also been shown to significantly increase chickpea yield per unit area when compared to crops grown under rain-fed conditions only (Zhang et al., 2000, Oweis, Hachum, and Pala 2004).

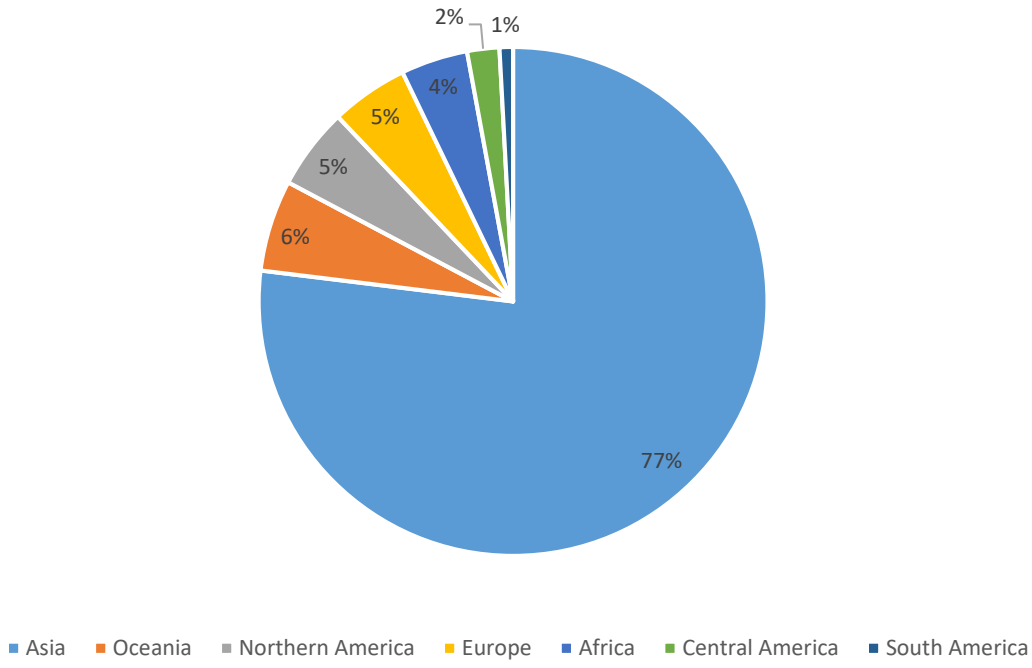

Figure 2.2 Chickpea production (%) around the world (FAO, 2019)

2.3 Chickpea seed composition:

Chickpea seed composition is variable and depends on chickpea type (desi or kabuli), variety, growing condition and post-harvest handling. Desi chickpea seed is typically composed of embryo, seed coat and cotyledons (1.5%, 15.5% and 83%, respectively), resulting in higher dietary fibre content, whereas in kabuli chickpeas, the seed coat fraction is lower (6.5%), resulting in a much higher cotyledon percentage (92%) and in turn higher starch content (Knights and Hobson 2004). Chickpeas are an excellent source of available carbohydrates, proteins, and dietary fibre, however, their levels can be highly irregular based on a range of factors including genotype, environment, agronomic practices and biotic or abiotic stress factors (Wood and Grusak 2007) detailed in Table 1.1.

Table 2. 1 Nutritional composition of kabuli chickpeas (dry basis)

Component (g/100 g, db)	Kabuli chickpeas					
	(Singh et al., 1990)	**(Khan et al., 1995)**	**(Wang and Daun 2004)**	**(Wang et al., 2010)**	**(Bampidis and Christodoulou 2011a)**	**(USDA 2017)**
Protein	19.5-23.4	24.4	24.4	21.2	22.5	20.47
Fat	5.1-6.8	5.1	5.9	5.6	6.2	6.04
Dietary fibre	-	-	-	15.3	-	12.2
Crude fibre	3.0-4.2	3.9	-	-	4.7	-
TC*	-	55.8	-	-	63.7	62.95
Starch	60.1-51.1	-	41.1	45.1	39.4	-
Ash	3.2-3.9	2.8	3.2	3.2	3.4	-
Country of origin	Syria	Pakistan	Canada	Canada	-	USA

TC*= Total carbohydrates

As these factors significantly affect the physical properties and the nutritional composition of chickpeas, it is important to determine their effects on different genotypes of chickpeas grown in different parts of the world. As India is the largest producer of chickpeas in the world and chickpeas are a very important crop to India, multiple studies on the effect of genotype and environment on commercial chickpea varieties from India can be found (Berger et al. 2006, Yadav et al. 2010, Misra et al. 2020). However, there is a lack of information on the composition of commercial chickpea varieties in relation to genotype and environment from the second-largest producer in the world - Australia.

Chickpeas have been known to contain one of the highest amounts of plant proteins (19.5- 24.4 g/ 100 g, db) (Table 1.1) after soy bean (*Glycine max* (L.) Merr.) and lupins (*Lupinus albus* (L.)) and play a crucial role in the diets of vegetarians around the world (Muehlbauer and Rajesh 2008, Bar-El Dadon, Abbo, and Reifen 2017)**.** Chickpea protein is rich in the essential amino acids lysine and arginine but is deficient in methionine and cysteine (Wang, Gao, et al. 2010). The fat content of raw chickpeas (5.1- 6.8 g/ 100g, db) (Table 1.1) is higher than other pulses such as pigeon pea (*Cajanus cajan* (L.) Millsp) (1.64 g/100 g, db), mung bean (*Vigna radiate* (L.) R. Wilczek) (1.15 g/100 g, db), red kidney bean (*Phaseolus vulgaris* spp.) (1.06 g/100 g)

and lentils (*Lens culinaris* spp.) (1.06 g/100 g, db) (Jukanti et al. 2012). Chickpeas are also a rich source of carbohydrates and the carbohydrate content of raw seeds (55.8-63.7 g/ 100g, db) (Table 1.1) is higher than other pulses (viz. pigeon pea, peas, and faba bean (*Vicia faba* (L.)). The dietary fibre content of raw chickpeas can range from 12.2 - 17.4 g/ 100 g (db) (USDA 2017, Jukanti et al. 2012), which is approximately 50% of the recommended daily intake of dietary fibre for an adult (Institute of Medicine2005). Absorbable calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe), zinc (Zn) and potassium (K) can be obtained by consuming 100 g chickpeas (Christodoulou et al. 2005) which fulfils approximately 50% of the recommended daily intake (RDI) for phosphorus, magnesium, zinc and potassium and around 20% RDI for iron and calcium (Bar-El Dadon, Abbo, and Reifen 2017).

2.3.1 Carbohydrate

Carbohydrates are a major component of chickpea seeds that contain free monosaccharides (glucose, ribose, fructose, and galactose), disaccharides (maltose and sucrose) and oligosaccharides (raffinose, verbascose, stachyose and ciceritol) (Jukanti et al. 2012). Pulses are known to contain one of the highest concentrations of oligosaccharides among all crops (Singh et al. 2017). Oligosaccharides are not digested or absorbed by the human digestive system, fermented by the colonic bacteria and are classified as dietary fibre. Polysaccharides act as storage carbohydrates or provide structural support and are also abundant in chickpeas (Singh et al. 2017). Starch is the major polysaccharide found in chickpea seeds and on average is made up of 25% amylose and 75% amylopectin, however, the proportion of amylose and amylopectin varies with genotype (Frimpong et al. 2009). Wang and Daun (2004) reported that the percentage of starch in raw kabuli chickpea ranges from 38.2 to 43.9 g/100 g (db) of which 35% is considered resistant starch (Aguilera et al., 2009a, Aguilera et al., 2009b, Wang and Daun 2004) and does not get digested in the upper gastrointestinal tract but can be fermented by colonic bacteria to give beneficial health effects (Robinson, Balk, and Domoney 2019). A considerable variation has been reported in the percentage of amylose in raw chickpeas ranging from 3.1 - 4.5 g/ 100 g (db) (de Almeida Costa et al. 2006, Chibbar et al. 2004). The starch in kabuli chickpeas has been reported to differ from that in desi chickpeas in relation to higher yield, higher granular size, lower amylose content, higher gelatinisation parameters

and thus will have a different functionality than desi chickpea starch (Miao, Zhang, and Jiang 2009).

Foods containing high amounts of starch can be classified according to their starch digestibility. Analytical methods which mimic the stomach and intestinal digestion process are used to determine the starch digestibility of foods by measuring the glucose released at different times following the commencement of digestion and converting it to percentage dry starch digested (Singh, Dartois, and Kaur 2010). Most starches contain a rapidly digestible starch (RDS) fraction (percentage starch digested in 20 min), a slowly digestible starch (SDS) fraction (percentage starch digested between 20 and 120 min) and a fraction which is considered as digestion resistant starch (RS) (total starch minus amount of starch digest at 120 min) (Englyst et al. 1999). Compared to other pulses such as pea (*Pisum sativum* (L.)) and lentils, chickpeas contain a higher amount of slowly digestible starch, thus making them a healthier choice (Tharanathan and Mahadevamma 2003). Consumption of foods containing slowly digestible starch (SDS) (glucose released between 20-120 min) results in a slow and sustained increase in the postprandial blood glucose levels over time compared to rapidly digestible starch (RDS) (glucose released after 20 min) with its rapid and high peak and rapid decline (Lehmann and Robin 2007). Resistant starch in legumes corresponds to physically inaccessible starch that is trapped in the cellular matrix (Englyst, Kingman, and Cummings 1992), but can be broken down with the help of processing (González-Soto et al. 2006). It works as a prebiotic by escaping digestion in the small intestine and functioning as a substrate for the gut microflora, where it is fermented, resulting in the production of beneficial short-chain fatty acids (SCFA), which protect humans from colon carcinogenesis and can aid in glucose and cholesterol metabolism (Güzel et al. 2011, Cummings 1981). A study by Miao, Zhang, and Jiang (2009) found that isolated kabuli chickpea starch was composed of higher amounts of SDS (46%) compared to desi chickpea starch (around 42%), with a correspondingly lower amount of RDS compared to desi chickpeas and thus contributing to slow increase in postprandial blood glucose levels and satiety (Lehmann and Robin 2007).

Chickpeas contain 10.4-17.0 % (of carbohydrate) of α- galactooligosaccharides (α-GOS) depending on the variety (Dai et al. 2017). The α-GOS are low molecular weight, non-reducing sugars soluble in water and water-alcohol solutions. These sugars are essential for the growth of beneficial bifidobacteria as they reach the colon

intact before fermentation, due to a lack of α- galactosidase in the upper intestinal tract in humans (Bouhnik et al. 2004). But intake of high levels of α-GOS (>3 g/day) (Martínez-Villaluenga et al.2008)) can have negative effects as well. The α-GOS are metabolized in the colon and large amounts of carbon dioxide, hydrogen, methane resulting in flatulence in humans (Naczk, Amarowicz, and Shahidi 1997). The α-GOS responsible for flatus production are raffinose, stachiose, and verbascose. High levels of these α-GOS can also result in disorders such as diarrhoea and abdominal cramps and are considered to be one of the critical factors that discourage people from consuming chickpeas and other legumes (Fares and Menga 2014).

2.3.2 Protein

Chickpeas are inexpensive and due to their suitable amino acid balance, high protein bioavailability and low antinutritional content, are considered a good source of dietary proteins (Singh et al. 2017). A single cup (approx. 150 g) serving of cooked chickpeas provides 13% (FSANZ 2020) of the daily recommended amount of protein-based on canned drained desi chickpeas and a 52 g/ day recommended intake of protein for men (NHMRC 2014). Chickpeas also have a higher bioavailability of proteins when compared to other pulses (Sánchez-Vioque et al.1999, Yust et al. 2003), with protein digestibility ranging from 65.3 - 79.4% (Khattak, Zeb, and Bibi 2008, Khalil et al. 2007, Clemente et al. 1998), higher than mung bean (67.2 - 72.2%), pigeon pea (60.4 - 74.4%), urd bean (*Vigna mungo* (L.) Hepper) (55.7- 63.3 %) and soy bean (62.7 - 71.6%) (Chitra et al. 1995).

No significant differences between amino acids of desi and kabuli chickpeas have been reported previously (Wang, N. et al. 2010, Wang, X. et al. 2010). Due to the limited amounts of sulphur-rich amino acids (cysteine and methionine) in chickpeas and other pulses, they are recommended to be consumed along with cereals rich in sulphur-containing amino acids (Zia-Ul-Haq et al. 2007). Researchers have also reported free radical scavenging and antioxidant properties of chickpea proteins, with chickpea protein hydrolysates performing significantly better than wheat gluten hydrolysates (Li et al. 2008). Chickpea protein hydrolysates successfully inhibited the oxidation of linoleic acid as well as showed hypoglycaemic effects in animal studies (Yust et al. 2012, Amaral et al. 2014). Obesity and oxidative stress are the main factors in the development of neurological and cardiovascular diseases. Chickpea protein

hydrolysates could be utilised as natural preservatives to prevent rancidity and to produce functional foods to confer desirable health benefits in humans (Li et al. 2008, Kim, Je, and Kim 2007, Yust et al. 2012).

2.3.3 Fat

Chickpea oil content ranges from 3 - 10 g/100 g (db) (Ghavidel and Prakash 2006). Unsaturated fatty acids such as omega-6 (linoleic acid) (54 - 56% in oil), omega-9 (oleic acid) (21 - 22% in oil) and omega-3 (linolenic acid) (0.5 - 0.9% in oil) are the main components of chickpea oil (Wood and Grusak 2007). Both omega 3 and omega 6 fatty acids are essential for development, physiological functions and preservation (Pugalenthi et al. 2004). The omega-6 fatty acid is an essential fatty acid for human metabolism and produces prostaglandins which in turn reduce cholesterol levels and regulate smooth muscle contractions (Zia-Ul-Haq et al. 2007), in turn affecting the digestive, circulatory and reproductive system in humans (Hafen and B. 2020).

Dietary intervention studies have reported that chickpea consumption is connected with lower cholesterol levels due to its fatty acid composition (Nobile et al. 2013, Pittaway, Robertson, and Ball 2008). Pittaway, Robertson, and Ball (2008) evaluated the effect of a chickpea supplemented diet for 12 weeks in 45 adults and observed a reduction in cholesterol levels due to increased consumption of polyunsaturated fatty acids from chickpeas. Chickpeas contain phytosterols, squalene and tocopherols which are components of unsaponifiable lipid fractions. Phytosterols, from plants, have a similar structure to cholesterol and are proposed to have a wide range of biological effects such as anti-oxidative, anti-inflammatory, and anti-carcinogenic (Berger, Jones, and Abumweis 2004, de Jong, Plat, and Mensink 2003) and cholesterol-lowering properties by reducing the intestinal absorption (Ryan et al. 2007). Similarly, squalene which is a 30-carbon isoprenoid exists with phytosterols and tocopherols and is a potential cancer chemopreventive agent (Smith 2000).

2.3.4 Dietary fibres

Kabuli chickpeas are a good source of dietary fibre, containing 12.2 - 17.4 g/100 g (db) (Jukanti et al. 2012); they, therefore, provide the benefits of dietary fibre when incorporated into a healthy diet. One cup (approx. 150 g) serving of chickpeas provides 29% of the daily recommended amount of fibre based on canned drained desi chickpea (FSANZ 2020) and 30 g/day recommended intake of DF for men (NHMRC

2019). Kabuli chickpeas contain both soluble fibres (pectin, β-glucans) and insoluble fibres (lignin, cellulose and hemicellulose). In chickpeas, the insoluble fraction is higher than the soluble one at an average of 18.11±0.95 g/100 g and 2.47±0.82 g/ 100 g, respectively (Bar-El Dadon et al.2017).

High dietary fibre consumption leads to a phenomenon known as luminal viscosity which delays the absorption of nutrients in the small intestine and has been associated with managing type 2 diabetes, prevention of cardiovascular diseases and lowering low-density lipoprotein (LDL) cholesterol levels (Silva-Cristobal et al.2010). Insoluble dietary fibres present in chickpeas have also been associated with reducing the incidents of colon cancer (Murphy et al. 2012), weight loss and weight management by promoting satiety (Isken et al. 2010). In a study including 265 cases (105 colon cancer; 144 rectal cancer; and 16 colon and rectal cancer cases) and 252 controls from China, it was reported that the control group consumed higher amounts of total fibre than colorectal cancer patients. An inverse association between fibre intake and colorectal, colon and rectal cancer was also reported indicating beneficial effects of high fibre diet in reducing incidents of colon and rectal cancer (Song et al. 2015). Isken et al. (2010) analysed the effect of insoluble fibre addition (10% weight/weight) to a high-fat diet (45 days) administered to mice prone to obesity that prevented a high fat-induced phenotype and increased insulin sensitivity in these animals.

2.3.5 Minerals

Chickpeas are a rich source of potassium (718-1155 mg/ 100g, db) which helps in maintaining blood pressure levels in humans (Haddy, Vanhoutte, and Feletou 2006). Phosphorus is another mineral found in chickpeas that helps in metabolism (Arnaud and Sanchez 1996). The third major mineral found in chickpeas is calcium (around 49 mg/ 100 g, db), and consumption of 100 g chickpeas contributes to 3.7 and 7% of dietary reference intake (DRI) of calcium in adults and children (1 - 3 years) respectively (Bar-El Dadon, Abbo, and Reifen 2017). Chickpeas also contain copper (0.656 mg/ 100g db), manganese (21.31 mg/ 100 g, db) and zinc (2.76 mg/ 100g, db) (Wallace, Murray, and Zelman 2016). Consumption of 100g cooked chickpeas can provide 40% of the adult DRI for copper and manganese and about 14% RDI for zinc (Bar-El Dadon, Abbo, and Reifen 2017, Wallace, Murray, and Zelman 2016).

2.3.6 Polyphenols

Polyphenols are secondary metabolites of plants and thus are found in plant-based foods. Plant polyphenols have been broadly studied and have been shown to possess biological activities and health benefits for the prevention of cancer, age-related diseases, and cardiovascular diseases (Gorzynik-Debicka et al. 2018, Ganesan and Xu 2017). These phenolic compounds are responsible for biological activities such as anti-ageing, anti-cancer, anti-inflammation, and cardiovascular protection (Li et al. 2014). One of the notable bioactivities of phenolic compounds is the antioxidant activity, and thus they are the main antioxidant constituents in many plant foods (Zhang and Tsao 2016) and are responsible for breaking of oxidation reactions, and as chelators of metal ions that induce oxidation in foods (Bravo 1998, Lopes, Schulman, and Hermes-Lima 1999, Rubilar et al. 2012) as well as radical scavenging activity for health prevention (Giacometti et al. 2016, Sabu, Smitha, and Ramadasan 2002, Gladine et al. 2007). These polyphenols can be found in a free or soluble conjugate form or a bound form as esters, glycosides and bound complexes (Acosta-Estrada, Gutiérrez-Uribe, and Serna-Saldívar 2014).

The main phenolic compounds in chickpea are phenolic acids, isoflavones, anthocyanins, and condensed tannins (Xu, Yuan, and Chang 2007, Fares and Menga 2012). Source of polyphenols such as cinnamic, salicylic, *p*-coumaric, gallic, caffeic, ferulic, and chlorogenic acid (Rachwa-Rosiak, Nebesny, and Budryn 2015), the polyphenol content in raw chickpeas ranges from 7.2 - 18.1 mg/ 100 g (db), which depends strongly on the extraction solvent, and analytical method used (Segev et al. 2010, Xu, Yuan, and Chang 2007, Ahmed et al. 2020). Lapornik, Prošek, and Golc Wondra (2005) reported that aqueous methanol and ethanol extracts of black and red currant contained a higher amount of polyphenols than water extracts and their content increased with an increase in extraction times from 1- 24 hours. Seabuckthorn seeds extracted for 8 hours using methanol exhibited the highest polyphenol extraction rate in comparison with ethyl acetate, chloroform and acetone extracts (Negi et al. 2005). In a recent study, Ahmed et al. (2020) reported that methanol extraction at 60° C for 180 min resulted in the highest polyphenol levels when compared to acetone, ethanol and water extracts of *D. aegyptium* seeds. In light of this, it is important when comparing antioxidant data that these variables are taken into account. Chickpeas are

characterised by a lower level of polyphenols and anthocyanins compared to black beans (*Phaseolus vulgaris* (L.)) and lentils.

2.3.7 Antinutritional factors

Legumes are a popular source of protein and other nutritional components, however, their consumption can sometimes cause deleterious effects on human health due to the presence of antinutritional factors such as phytic acid, tannins, and trypsin inhibitor (Singh et al. 2017). These antinutritional factors are naturally occurring plant secondary metabolites, which are responsible for protecting plants from biological stresses such as pest attacks and extreme temperature fluctuations. However, consumption of foods containing higher amounts of these antinutritional factors, especially trypsin inhibitors (TI) (0.001- 0.002 TI units mg/ 100 g) is of the greatest concern (Avilés-Gaxiola, Chuck-Hernández, and Serna Saldívar 2018). Phytic acid (0.03- 0.09 mg/ 100g, db) found in chickpeas can bind to nutritionally important minerals (e.g. Fe, Zn, Ca, Mg), resulting in the formation of insoluble complexes and making them unavailable for absorption by the human body (Sandberg 2002, Bueckert et al. 2011). In contrast, tannins (0.01-0.1 mg/ 100 g, db) reduce the digestibility of chickpeas by inhibiting enzymes such as trypsin, α- amylase and lipase (Griffiths 1986, Barrett, Farhadi, and Smith 2018), and also make chickpeas astringent (Jukanti et al. 2012, Singh et al. 2015). TI's actively inhibit the activity of trypsin and chymotrypsin (key pancreatic enzymes) by the formation of indigestible complexes, resulting in reduced digestion and absorption of dietary proteins (Gemede and Ratta 2004). However different processing techniques get rid of these antinutritional factors making chickpeas suitable for human consumption (Table 1.2).

2.4 Effect of variety and environment on chickpea seed characteristics and composition

2.4.1 Physical properties

The physical properties of legume seeds can be expressed as seed weight, volume,density, hydration capacity, swelling capacity and cooking time. These physical properties are important for designing processing equipment. Designing equipment without taking into consideration these properties can result in sub-optimal performances, lower work efficiency and increased product losses (Baümler et al.

2006). Thus it is important to determine these properties for legumes and cereal grains. Seed size is important for packing or packed dimension, and heat and mass transfer calculations, whereas volume and density play an important role in seed quality assessment, and transfer of seeds using pneumatic or hydraulic equipment (Kabas et al. 2007). Water absorption during soaking of legume seeds is related to physicochemical properties of seeds and varies due to seed coat thickness, density, and starch and protein content (Mwangwela, Waniska, and Minnaar 2006). The weight and volume increase of chickpeas as a function of soaking time determines the hydration behaviour of the seed; with the weight being related to hydration capacity and volume to swelling capacity. Previous research has shown that hydration and swelling capacities significantly affect the cooking time of legume seeds, with high hydratable and swellable seeds cooking faster (Wani, Sogi, and Gill 2013). Both, Kaur, Singh, and Sodhi (2005) and Sfayhi and Kharrat (2011) have reported that seeds with higher weight and volume took longer to cook and swelling capacity and hydration capacity correlated positively with the cooking time for Tunisian chickpeas.

Genotype by Environment (G x E) interactions have been reported for several physical characteristics of chickpeas and have been related to water availability, soil type and growing conditions. For example, Patanè, Iacoponi, and Raccuia (2004) reported a significant difference in seed weight, density, and large variability in swelling capacity and cooking time between genotypes from Italy. Leport et al. (1999), found that reduced water availability during the growing season reduced the seed size in both desi and kabuli chickpea varieties grown in Australia. However, Cobos et al. (2017) only reported significant genotype effects on seed size and hydration in chickpeas from Spain. Sood, Bhambota, and Gartan (2001) reported a significant G x E interaction for seed weight of 32 chickpea genotypes from India in their study. The type of soil also affects chickpea plant growth, with sandy-loam giving better results when compared to clay soil, thus affecting the overall seed weight (Moloto et al. 2018). Wang, Gangola, et al. (2017) reported that chickpeas grown in a greenhouse had a higher thousand seed weight when compared to field-grown chickpeas due to an ideal growing environment and the genotype, environment and their interaction played a crucial role in seed weight. Similar results were reported by Frimpong et al. (2009). However, Singh, Yadava, and Agrawal (2003) and (Lokare, Patil, and Chavan 2007) observed insignificant influence of environment on chickpea physio-chemical traits.

2.4.2 Nutritional properties

Genotype and environment affect the nutritional profile of chickpeas in different ways. The protein content of chickpeas has been shown to vary widely depending on the genotype, sowing time (spring or winter), environment during seed development, and agronomic and cultural practices (El-Adawy 2002, Tayyar, Egesel, Gül, et al. 2008, Khan et al. 1995). Singh et al. (1993) suggested that the protein content of kabuli chickpeas was more likely to be influenced by the environment than genotype. Singh et al. (1990) reported the effects of spring and winter planting in four chickpea genotypes planted in Syria and Lebanon. The protein content of winter planted kabuli chickpeas was slightly higher and less variable than that of spring-planted kabuli chickpeas. Significant effects of autumn and spring plantings on the protein content of chickpeas genotypes grown in Turkey over two years were found by Tayyar et al. (2008). Chickpeas sown in spring had significantly higher protein content (23.2 g/100 g, db) than those sown in the autumn (20.5 g/100 g, db) and the seed yield was negatively correlated to the protein content, suggesting that increased yield results in lower protein content (Tayyar, Egesel, Gül, et al. 2008). The findings of Cobos et al. (2017) were in agreement with those of Tayyar et al. (2008), who also concluded that sowing times had a significant effect on the protein content of chickpeas, with spring-sown chickpeas having a higher protein content.

The fat content of chickpeas negatively correlates to the starch concentration (Ereifej, Al-Karaki, and Hammouri 2001). A study by Gül, Egesel, and Turhan (2008) reported that autumn-sown chickpeas in Turkey contained 35% and 63% oleic and linoleic acid content respectively, whereas chickpeas sown in spring only had an 18% and 47% oleic and linoleic acid content. Thus, it was concluded that oleic and linoleic acid content in chickpeas varies with planting time (Gül, Egesel, and Turhan 2008). Phytosterols, tocopherols and squalene are components present in the unsaponifiable lipid fraction and their content in plant foods is affected by genotype, location and growing environment (Määttä et al. 1999, Marcone, Kakuda, and Yada 2003, Zhang, Vasanthan, and Wettasinghe 2007). Chickpeas are one of the highest sources of phytosterols after peas and sesame seeds (*Sesamum indicum* (L.) (Ryan et al. 2007, Kalogeropoulos et al. 2010). However, there is no data on the effect of genotype and environment, and their interaction on these compounds (Zia-ul-Haq et al. 2009).

Genotype, growing environment and their interaction also affect the concentrations of glucose, fructose, sucrose and raffinose family oligosaccharides (RFO) such as stachyose, verbascose and raffinose, in both kabuli and desi genotypes (Gangola et al. 2013). Lower concentrations of RFO in chickpeas from a controlled growing environment in a greenhouse (higher photosynthetically active radiation, longer photoperiod, and less temperature variation), when compared to field trials, indicates the physiological function of these oligosaccharides in providing tolerance against abiotic stresses such as drought, salinity and temperature (Krasensky and Jonak 2012).

A G x E study on seven desi and nine kabuli chickpea varieties at multiple sites across the Canadian prairies reported significant G x E interactions on the total starch and amylose concentrations in desi, but not in kabuli chickpeas, concluding that different chickpea types did not perform consistently relative to each other in different environments (Frimpong et al. 2009). A negative correlation between some nutritional components indicates that breeding selection strategy for high seed quality will require consideration of the genotype and G x E interaction for these constituents (Frimpong et al. 2009). Results of Wang et al. (2017) were in agreement with that of Frimpong et.al (2009), showing that genotype, environment and G x E interactions had a significant effect on the starch and amylose content of 180 desi and 49 kabuli chickpea genotypes. Genotype, environment and G x E also showed a significant ($p \leq 0.001$) effect on the concentration of soluble sugars in chickpeas. Starch and protein content in legumes including chickpeas correlate negatively (Wang, Gangola, et al. 2017, Gaur et al. 2016) as both starch and protein compete for photosynthetic substrate (Rolletschek et al. 2002, Tayade et al. 2019). Also, due to a higher activity of α-amylase during a rainy environment, available starch gets catabolized resulting in soluble sugar accumulation and water influx, enhancing uptake of soluble nitrogen (mainly as free amino acids) resulting in higher protein synthesis and a decrease in starch content (Rolletschek et al. 2002). These changes in seed quality traits are supported by other studies on lentil (Tahir et al. 2011) and chickpeas (Frimpong et al. 2009).

Minerals are important for the proper functioning of the human body, from maintaining bone health, nerve impulse transmittance, hormone production to the regulation of the heartbeat (Gharibzahedi and Jafari 2017). In terms of both desi and kabuli chickpeas, no significant main effect of genotype has been reported for mineral

composition. In a study by Ibáñez et al. (1998), 37 cultivars of desi and kabuli chickpeas were analysed for their mineral composition (Cu, Fe, Zn, Mn, Ca, Mg, Na and K). No significant differences ($p \leq 0.05$) were observed between desi and kabuli biotypes (Ibáñez et al. 1998). It has been reported that calcium (Ca) is the main mineral in the seed coat, thus the difference in Ca content between both biotypes can be a consequence of the difference in the percentage of seed coat with desi chickpeas having a higher percentage when compared to the kabuli types. Even within kabuli cultivars, higher seed coat percentage resulted in higher Ca content. A similar trend was observed for magnesium (Mg), where seeds with higher seed coat percentage displayed a higher Mg content. However, for potassium (K) concentration, this was not the case as kabuli types showed a higher concentration (926 mg/ 100 g, db) when compared to desi types (878 mg/ 100 g, db). It was observed that the percentage of K was higher in kabuli chickpea seed coats when compared to desi chickpeas, thus overall, the K content of kabuli chickpeas was higher. However, the K content is significantly influenced by the location where the chickpeas were grown due to differences in soil type and farming practices (Jambunathan and Singh 1981).

Ereifej et al. (2001) analysed the mineral content of four Jordanian chickpea genotypes grown over two years to determine the effect of year and cultivar on the mineral content of these chickpeas. A significant difference in the mineral content between chickpea cultivars was recorded. However, no influence of the growing season on the mineral composition (Mg, Na, Mn, Fe, Cu and Zn) was found. Patanè (2006) analysed ten Sicilian genotypes of chickpeas and compared them to an accession from the International Center for Agricultural Research in the Dry Areas (ICARDA) gene bank and a traditional cultivar from Italy. All genotypes were grown at the same location in the year 1997-98. Significant differences were recorded between cultivars in their Fe, Ca, Mg and K content. Very high significant differences in the Ca content (CV- 39.3%) of cultivars was related to differences in seed coat percentage and are in agreement with Jambunathan and Singh (1981) and Ibáñez et al., (1998). High variability in the Mg content between genotypes was also observed (CV- 9.8%). Significantly higher values for both Mg and K were reported when compared to the available literature, suggesting Sicilian genotypes as a good source of these minerals (Ibáñez et al. 1998, Jambunathan and Singh 1981, El-Adawy 2002).

2.5 Effect of processing methods on the nutritional components in chickpeas

2.5.1 Dehusking and Milling

Decortication is used to remove the seed coat of chickpeas before the cotyledons are split to make dahl and chickpea flour. During this process, the seeds are passed through a milling machine to remove the outer husk and this process has been known to affect the protein and dietary fibre content of the seeds. Dehusking and milling also affect colour; and when compared to the raw seeds, dehusked as well as dehusked and milled chickpeas are significantly lighter in colour (Ravi and Harte 2009, Vasishtha, Srivastava, and Verma 2014). No significant differences in the starch content of dehulled and milled desi and kabuli chickpeas were recorded and can be attributed to the high fibre containing husk in desi chickpeas which reduces the total starch content of the whole seed sample (Jambunathan and Singh 1980). However, an increase of around 2.5 times in the *in vitro* starch digestibility of germinated but dehulled chickpeas and a significant increase in the *in vitro* protein digestibility when compared to raw seeds was observed, which suggests that chickpea husk not only contributes a significant amount in the total weight of the seed but is also less digestible (Ghavidel and Prakash 2007). On dehusking, an increase in the soluble dietary fibre (SDF) content of chickpea was observed by Dalgetty and Baik (2003), confirmed by an increase in the pectin content of the dehusked seeds as SDF mainly consists of pectin. Vasishtha et al. (2014) reported a significant increase in the soluble protein content after decortication of both desi and kabuli chickpeas, confirming that the chickpea husk contains higher amounts of insoluble dietary fibre (IDF) which interferes with protein digestibility. Ghavidel and Prakash (2007) reported a significant decrease in soluble, insoluble and total dietary fibre content in chickpeas after dehulling, which significantly ($p<0.5$) increased the *in vitro* protein and starch digestibility of the dehulled samples.

Dehulling resulted in a four times increase in the bioavailable iron and a 2.5 times increase in the bioavailable calcium (Han and Baik, 2008). Dehusking resulted in a 37% reduction of total polyphenol content of chickpeas, however, this was not translated into the antioxidant activity of the phytochemical extracts from the dehusked seeds. Dehusking does not affect the antioxidant activity of free phytochemicals in chickpeas, thus, it can be concluded that the chickpea hull is an insignificant source of free phytochemicals (Han and Baik 2008). Furthermore,

dehusking results in a reduction of anti-nutrients such as phytic acid (36.42%) and tannins (68.75%) in chickpeas, suggesting a high concentration of phytic acid and tannins in the husk.

2.5.2 Soaking

Soaking is an integral part of treatments such as germination, boiling, cooking, and canning. It comprises of hydrating seeds in water until maximum weight is reached, with or without discarding the soaking medium (Prodanov, Sierra, and Vidal-Valverde 2004). It decreases the total starch and resistant starch content in chickpeas by 8 - 11% and 28% respectively, due to the loss of amylopectin solubilized by α-amylase action (Aguilera et al., 2009). However, soaking chickpeas in an alkaline solution of sodium bicarbonate alone significantly increased the insoluble dietary fibre components thus affecting the total dietary fibre content (Rehman, Rashid, and Shah 2004). The process of soaking in an alkaline solution is governed by a series of kinetic stages of diffusion with a loss of material. Specific changes occur in the pericarp, aleurone layer, and the internal parts of the seed. The alkaline solution reacts with the pericarp structure and results in the opening of new pathways for diffusion of the alkaline solution, and loss of protein of the aleurone layer. Furthermore, extended soaking in an alkaline solution results in a reaction between the protein matrix of the starch granules in the endosperm resulting in the loss of material, leading to an increased dietary fibre content (Laria, Meza, and Peña 2007, Pineda-Gómez, Rosales-Rivera, and Rodríguez-García 2012). Soaking also reduces the available carbohydrates in chickpeas by up to 20%, with no effect of the type of solution used (acidic, alkaline or neutral) (Frias et al. 2000). A significant reduction in zinc, iron and calcium content due to leaching after 24 hours of soaking chickpeas at room temperature has been observed (Olika, Abera, and Fikre 2019).

One of the most influential factors that deter consumers from legume consumption is flatulence caused by a group of α-galactosides from the raffinose family of oligosaccharides (stachyose, verbascose and raffinose) (Gulewicz et al. 2000, Peterbauer and Richter 2007). Soaking chickpeas in water reduces the amount of oligosaccharides, however soaking with ultrasound for three hours reduced the oligosaccharide content by more than 64% (Han and Baik 2006). Similarly, soaking chickpeas with high hydrostatic pressure for just one hour reduced the oligosaccharide

content by 67%, compared to conventional soaking reducing the oligosaccharide content by around 75% after 12 hours (Han and Baik 2006). Some oligosaccharides are bound either to proteins, to macromolecules, or are present as constituents of high molecular polysaccharides and go through non-enzymatic hydrolysis and get released, resulting in an increased oligosaccharide content during cooking (Jambunathan et al. 1994, Han and Baik 2006).

Polyphenols in chickpeas are sensitive to food processing conditions. Jood et al. (1987) reported that soaking for 12 hours significantly reduced (4-8%) the polyphenol content of chickpeas and this trend was observed for all eight varieties included in this study. In contrast, Han and Baik (2008) reported that soaking showed no changes in the total polyphenol content of chickpeas, the antioxidant activity of free phytochemicals increased and no changes were observed in the antioxidant activity of bound phytochemicals. However, a positive result of a reduction in the antinutritional factors - tannins (50%) and phytic acid (15.20%) was observed (Han and Baik 2008).

2.5.3 Germination

Germination is one of the most common practices known to improve the nutritional quality of legumes (Ridge 1991) and is carried out by soaking seeds in excess water, draining the water and letting the seeds germinate for a desired time. During germination, the seed reserve materials get degraded, causing drastic changes in the biochemical, nutritional as well as sensory characteristics of the seeds. Germination of pulses before consumption has been found to decrease the raffinose, stachyose and verbascose content in chickpeas (Wang et al.2010). It not only reduces the amount of anti-nutritive compounds but also boosts the levels of amino acids, carbohydrates, dietary fibre (Vidal-Valverde et al. 2002, Urbano et al. 1995) and as a consequence, increases the amount of bioactive compounds (Frias et al. 2005, Vidal-Valverde et al. 2002). Neves and Lourenço (2001) reported a decrease of 19% and 20.6% in the globulins and albumin fractions of chickpeas respectively after 6 days of germination, whereas, Portari et al. (2005) found a significant increase in the protein content and protein digestibility values after germination for the same period. A significant fat reduction (Mittal et al. 2012, Alajaji and El-Adawy 2006, Ghavidel and Prakash 2007), soluble, insoluble, and total dietary fibre (Ghavidel and Prakash 2007) and crude fibre

content (60.3% reduction) (Mittal et al. 2012) have also been reported in chickpeas following germination.

Phytic acid in chickpeas binds with minerals, rendering them unavailable for absorption and a very significant reduction in both tannins (75% and 21%) and phytic acid (31% and 17%) has been reported after germination (Olika, Abera, and Fikre 2019, Ghavidel and Prakash 2007). The decrease in phytic acid results in significantly higher bioavailability of iron and calcium when compared to raw chickpeas. Germination also modifies the phenolic profile of chickpeas along with other legumes such as peas, lentils and beans (López-Amorós, Hernández, and Estrella 2006, Tarzi et al. 2012) and a significant increase in both the total phenolic content and the antioxidant activity has been observed following germination (Tarzi et al. 2012). Similar results were reported by Wu et al. (2012), who observed that germinated chickpeas had the highest polyphenol content out of nine legumes including chickpeas which when germinated for 3 days had a 3-fold increase in their antioxidant capacity when compared to their raw counterparts.

2.5.4 Thermal processing: boiling, cooking, or dry heating

Food legumes are typically cooked or boiled before consumption, bringing out many changes to their physical characteristics and chemical composition. The ability of fibre to retain water provides volume and consumption of a high fibre diet may cause a feeling of satiety without supplying excessive nutrients, which supports the role of high-fibre diets in treating obesity (Elhardallou and Walker 1993). The water-holding capacity, the amount of water a food material can take up and retain, varies according to the type of fibres present in the food. The water holding capacity of chickpea flour has been observed to significantly increase after cooking and dehydration due to denaturation and unfolding of proteins, which exposes earlier hidden peptide bonds as well as polar side chains to hydrogen bonding, providing higher binding of water molecules (Singh 2001). Besides, the water-holding capacity is also influenced by starch and dietary fibres (Granito, Brito, and Torres 2007). An increase in insoluble dietary fibres and available starch can influence this property in foods (Wagner and Gueguen 1999) including cooked chickpeas (Aguilera et al. 2009b)

In terms of nutritional composition, cooking significantly decreases available carbohydrates and increases the starch content in chickpeas by around 21% due to

modification of resistant starch into digestible starch (Aguilera et al. 2009). A significant decrease in reducing sugars, sucrose, raffinose, stachyose and verbascose after soaking and boiling (Alajaji and El-Adawy 2006) was observed due to the leaching of sugars (sucrose and fructose) in the soaking and cooking liquid in both desi and kabuli chickpeas. The effect of cooking on starch fractions has been controversial. Cooking has been shown to increase the resistant starch content of chickpeas (Wang, Hatcher, and Gawalko 2008, Wang, Hatcher, et al. 2010, Brummer, Kaviani, and Tosh 2015), whereas cooking of chickpeas followed by dry heating reduces the amount of resistant starch when compared to raw seeds, by modifying resistant starch into more digestible starch (Aguilera et al. 2009). An increase in the resistant starch content after cooking and cooling has been attributed to gelatinisation and retrogradation of the starch, while a decrease in resistant starch on cooking can be due to the destruction of amylase inhibitors by heat (Wang, Hatcher, et al. 2010, de Almeida Costa et al. 2006). Although the process of cooking increases starch digestibility, in some instances cooked legumes still retain a considerable amount of slowly digestible starch and resistant starch and can be considered nutritionally beneficial for human health. Further research is required to understand the effect of different processing methods on the resistant starch content of chickpeas.

Cooking also increases the amount of proteins in chickpeas and the reason for an increased protein content can be the greater relative loss of soluble solids such as carbohydrates in the soaking and cooking liquid (de Almeida Costa et al. 2006, Wang, Hatcher, et al. 2010, Candela, Astiasaran, and Bello 1997). Vasishtha et al. (2014) reported around a 20 - 40% increase in soluble protein content of decorticated chickpeas after boiling, whereas Alajaji and El-Adawy (2006) found no significant differences between protein content after cooking of whole chickpeas signifying the role of decortication. Furthermore, Alajaji and El-Adawy (2006) reported a significant increase in the protein digestibility of cooked chickpeas when compared to the raw seeds, due to denaturation of proteins and destruction of antinutrients such as trypsin inhibitors. Contradicting results have also been reported where a reduction in protein content after cooking was observed (Clemente et al. 1998, Attia et al. 1994). Bulbula and Urga (2018) reported a significant decrease in protein, fat and fibre content of chickpeas after boiling followed by dehydration at 55ºC for 24 hr which can be

attributed to leaching, partial breakage of chickpea seeds and loss of material during cooking (Clemente et al. 1998).

Soaking along with cooking increases the amount of dietary fibres (both soluble and insoluble) (Candela, Astiasaran, and Bello 1997) and causes structural changes in the dietary fibre components of chickpeas (Herranz, Vidal-Valverda, & Rojes-Hodelgo, 1981; Roehrig, 1990). A decrease in hemicellulose content and an increase in cellulose, in dehusked and cooked desi and kabuli chickpeas was observed by Vasishtha et al.(2014) and these results were in agreement with Vidal-Valverde and Frias (1991). The softening of chickpeas during cooking due to the disintegration of the cotyledonous tissue is caused by the conversion of native protopectin into pectin, which disintegrates quickly. An increase in the insoluble dietary fibre (e.g. cellulose) can also be attributed to Maillard's reaction, possibly due to the production of Maillard reaction products during thermal processing, resulting in an overall increased cellulose content (Vasishtha, Srivastava, and Verma 2014). Furthermore, the percentage of lignin decreases and the pectin percentage increases on cooking of dehusked chickpeas. These components constitute chickpea dietary fibre, which is thus also altered in the process.

Even though chickpeas are a good source of polyphenols, they rank lower than cowpea (*Vigna unguiculata* L.) and pigeon pea in their antioxidant activity (Ferreira et al. 2004, Gutiérrez-Uribe, Romo-Lopez, and Serna-Saldívar 2011). Soaking followed by domestic cooking significantly decreases the polyphenol content in chickpeas by up to 9 - 12% (Jood, Chauhan, and Kapoor 1987). Han and Baik (2008) reported a decrease in the antioxidant capacity of free polyphenols from cooked chickpeas by up to 30% which could be due to the loss of soluble antioxidant compounds during cooking. Similar results of a decrease in polyphenol content after cooking were reported by Kalogeropoulos et al.,(2010) and Xu and Chang (2008). The possible explanation for this phenomena can be partial leaching of polyphenols in the water during soaking and cooking and thermal/ oxidative deterioration of phenolics. However, no significant changes in the antioxidant activity of bound polyphenols were observed (Kalogeropoulos et al. 2010). Unlike soaking and subsequent cooking, dry roasting alone does not lead to a significant loss in total polyphenol content (Nithiyanantham, Selvakumar, and Siddhuraju 2012).

A reduction in water-soluble anti-nutrients (tannins and phytic acid) and an increase in the protein and starch digestibility of chickpeas after cooking has been reported in multiple studies (Olika, Abera, and Fikre 2019, Ramakrishna, Rani, and Rao 2006), however, even after boiling, chickpeas maintain several other heat-stable immunoreactive proteins (Lobato et al. 2011). The partial removal of tannins and phytic acid due to the thermal treatment creates a large space within the matrix, increasing the susceptibility of chickpeas to enzymatic attack, in turn increasing the digestibility of protein and starch (Rehman and Shah 2005). Overall, cooking the pre-soaked chickpea seeds in water, acidic or basic solution, followed by drying seems to be sufficient to obtain a large reduction in the antinutritional factors such as trypsin inhibitors and α- galactosides in chickpea flour (Frias et al. 2000).

2.5.5 High pressure cooking: including autoclaving and high hydrostatic pressure

Soaking followed by high pressure cooking is a widely used domestic cooking method for the preparation of legumes such as chickpeas due to convenience and effectiveness. It has been shown to slightly increase nutrient availability as well as the insoluble dietary fibre content when compared to raw samples (Tovar and Melito 1996). An increase in the crude fibre content of chickpeas was observed after pressure cooking which could be due to the formation of protein-fibre complexes (Chitra et al. 1995, Mittal et al. 2012). High pressure cooking significantly increased the rapidly digestible starch content in chickpeas, which can be attributed to the digestion of starch by α-amylase. Also, pressure cooked chickpeas tended to have higher levels of resistant starch but lower levels of slowly digestible starch than conventionally cooked chickpeas (Jood, Chauhan, and Kapoor 1987). Compared to conventional cooking, pressure cooking also brings out organoleptic changes in the final product. High pressure cooked chickpeas were found to be darker in colour in comparison to conventionally cooked chickpeas (Güzel and Sayar 2012).

Autoclaving of raw and soaked chickpeas has shown to significantly reduce (10-87%) the total polyphenol content and these significant losses could be credited to the leaching of water-soluble phenolics in cooking water as well as degradation of these compounds under high pressure and temperature (Nithiyanantham, Selvakumar, and Siddhuraju 2012). Whereas, A significant increase in the ferric reducing antioxidant

activity for autoclaved raw and soaked chickpeas was observed and could be attributed to the changes in phenolic compounds (Tsai and She 2006) or the release of bound polyphenols during processing (Dewanto, Wu, and Liu 2002, Nicoli et al. 1997). Mittal et al. (2012) reported a reduction of 13.7% phytic acid and 93.97% tannins after chickpeas were high pressure cooked in 1:2 w/v water for 15 min at 15 psi pressure, and they found that compared to all the processing techniques (germination, boiling and roasting), pressure cooking was the most effective in reducing phytic acid and tannins in chickpeas. Ertaş and Türker (2014) soaked chickpea seeds in different pH solutions (pH 4, 6 and 8) followed by autoclaving at 121 ºC and reported that not only the duration of soaking followed by autoclaving, but the pH of the soaking solution also affects the levels of phytic acid, minerals, and starch.

High pressure processing (HPP) is a novel non-thermal method of food processing, which inactivates pathogenic microorganisms, increases shelf-life, and maintains the colour and freshness of food products. It is usually carried out using intense pressure between 100-1000 MPa, with or without heating (Yordanov and Angelova 2010). HPP is lethal to microorganisms but at lower temperatures (0 – 40 ºC) covalent bonds are almost unaffected, causing minimal effects on the food chemistry (Balny and Masson 1993). Only a handful of studies have determined the effect of high pressure processing on chickpeas. Both Ibarz, González, and Barbosa-Cánovas (2004) and Alsalman and Ramaswamy (2020) reported a reduction in the cooking time of chickpea seeds with an increase in the pressure levels during high pressure processing. High pressure treatment assisted with faster absorption of water by chickpeas, resulting in swelling of starch granules and/ or absorption of water by proteins. Higher protein content in chickpeas results in more water retention at medium pressure (200- 500 MPa) compared to brown rice (Yu et al. 2017b), since protein aggregation allows water retention by forming cavities (Alsalman and Ramaswamy 2020). The highest decrease in percentage phytic acid (86%) and tannin (77%) was obtained in samples pre-soaked for 2 hours, at 40 ºC and high pressure treated at 500 MPa. Kadlec et al. (2006) in their study reported a 93% decrease in flatulence causing α-galactoside content by applying a combination of germination (2 days), pressurization (500 MPa for 10 min) and storage for 21 days in refrigerated conditions. However, effects of high pressure processing on the nutritional

composition, digestibility and antioxidant properties of kabuli chickpeas have not been explored in detail and need further research.

2.5.6 Microwave cooking

The use of microwave ovens has been on the rise due to its speed, convenience and energy-saving nature when compared to conventional cooking methods (Khatoon and Prakash 2004). However, the use of microwaves in the cooking of legumes has been limited. Previous research has shown that microwave cooking of chickpeas significantly reduced the fat, ash and reducing sugars content, whereas an increase of around 30% in the crude fibre content was observed. The *in-vitro* protein digestibility of chickpeas also increased after microwave cooking, and it has been shown to retain more minerals, both macro (Na, Ca, Mg, Mn, P, K) and micro (Mn, Zn, Cu, Fe) compared to other cooking techniques, such as boiling and autoclaving. Furthermore, microwave cooking effectively reduced the amount of anti-nutritional factors such as trypsin inhibitors and phytic acid (Alajaji and El-Adawy 2006). Similarly, Xu et al. (2016), reported a significant decrease in protein, ash, mineral, phytate and tannin content of kabuli chickpeas after microwave cooking in a domestic microwave cooker for 15 min. However, a significant increase in protein digestibility was observed after microwave cooking, possibly due to the destruction of tannins and phytic acid (Mubarak 2005).

Microwave cooking is known to cause a significant decrease in the levels of resistant and poorly digestible starch and can increase the level of rapidly digestible starch when compared to levels of these components in raw chickpeas. It leads to depolymerisation of the cell wall polysaccharides and the temperature and pressure during microwave cooking contributes towards the breaking of cell wall polysaccharide linkages, therefore increasing the soluble dietary fibre levels. However, conventional cooking had similar effects on these attributes, with the only advantage being reduced processing time of microwave cooking (Marconi et al. 2000).

2.5.7 Extrusion

Extrusion technology widely used in the plastic industries is increasing in popularity in the agri-food processing sector due to its versatility, high efficiency and cost-effectiveness (Moscicki 2011). The raw material is cooked at a very high temperature under high pressure (HTHP) and shear stress developed in the barrel of

the extruder, followed by expulsion from a die to shape it. The thermo-mechanical action during extrusion results in protein denaturation, starch gelatinisation, and inactivation of microbes, enzymes, and many anti-nutritional factors (Lobato et al. 2011). Extrusion is considered to be a highly versatile process that can cook, texturize, form, mix and shape food products using conditions that can lead to quality retention, high productivity and low cost (Riaz 2000).

However, twin-screw extrusion of chickpea flour at 150 ºC has been shown to result in the disruption of chickpea flour microstructure (Raza et al. 2019). When compared to the round, oval, or spherical shape of non-extruded chickpea flour, using scanning electron microscopy, extruded flour had a non-uniform and irregular shape which signifies disruption of starch granules and crosslinking of proteins and starches (Raza et al. 2019). Extrusion cooking has little to no effect on the protein, ash or lipid levels in extruded legumes (Abd El-Hady and Habiba 2003, Adamidou et al. 2011, Alonso et al. 2000). However, it does increase the level of digestible starch in the product as a result of a change in resistant starch composition during processing and easier hydrolysis of extruded starch granules during the starch content analysis of starch. Poltronieri, Arêas, and Colli (2000) reported a significant increase in soluble dietary fibre content and a reduction in insoluble dietary fibre and resistant starch content after chickpeas were extruded at 200 rpm and 130 ºC using a single screw extruder; with no effect on the level of polyphenols.

Extrusion being an HTHP process leads to macromolecular degradation of starch and other polysaccharides into simpler, low molecular weight components. Extruded chickpea flour contained insignificantly lower amounts of insoluble dietary fibre (Berrios et al. 2010). However, a significant increase in the *in vitro* protein digestibility when compared to conventional flour has been observed (Milán-Carrillo et al. 2000). This increase could be related to the elimination of antinutritional components such as tannins, phytic acids and trypsin inhibitors due to the high temperature and pressure of the extrusion process. These antinutritional factors have been known to interact and form complexes with proteins, in turn decreasing its digestibility (Mercier 1993, Rathod and Annapure 2016).

Table 2. 2 Summary of the effect of different processing techniques on the nutritional and phytochemical properties of chickpeas

Source	Activity	Temperature	Effect
	Dehulling/ Dehusking and Milling		
Han and Baik (2008)	Dehusking of chickpeas	-	• Increase in antioxidant activity of bound phytochemicals • Decrease in total polyphenol content and antioxidant activity of free phytochemicals
Vasishtha, Srivastava, and Verma (2014)	Drying of chickpea seeds at 70ºC, followed by dehulling	-	• Increase in soluble protein and pectin content • Decrease in cellulose, hemicellulose, and lignin content
Ghavidel and Prakash (2007)	Dehulling of chickpeas from the local market	-	• Increase in protein, fat, bioavailable iron and calcium, *in vitro* starch and protein digestibility • Decrease in soluble, insoluble and total dietary fibre, phytic acid and tannin content
Dalgetty and Baik (2003)	Dehulling and milling	-	• Increase in protein, fat, and soluble dietary fibre content and decrease in insoluble dietary fibre content
Ravi and Harte (2009)	Dehusking and milling	-	• Slight lightening of colour after dehusking, further lightening of colour on milling.

Source	Activity	Temperature	Effect
Dehulling/ Dehusking and Milling (contd.)			
Olika, Abera, and Fikre (2019)	Soaking in distilled water for 6 hr followed by dehulling, drying and milling	Room temperature	• Highest reduction in zinc, iron and calcium content when compared to soaking, germinating, cooking and dry roasting • Significantly high reduction in tannin (68.75%) and phytic acid content (36.42)
Soaking methods			
Aguilera et al (2009)	Soaking in tap water 1:10 w/v (16 h)	20ºC	• Decrease in total starch (8 - 11%) and resistant starch content (up to 28%)
Rehman, Rashid, and Shah (2004)	Soaking in alkaline solution of sodium bicarbonate	-	• Increase in insoluble dietary fibre components- acid detergent fibre, neutral detergent fibre, cellulose and hemicellulose
Han and Baik (2006)	Soaking in water for 12 hr	Room temperature	• 75% decrease in oligosaccharide content
	Soaking in water for 1 hr with ultrasound		• 64% decrease in oligosaccharide content
	Soaking in water for 1 hr with high hydrostatic pressure		• 67% decrease in oligosaccharide content

Source	Activity	Temperature	Effect
	Soaking methods (contd.)		
Frias et al. (2000)	Soaking in water Soaking in citric acid solution (0.1%, pH=2.6) Soaking in sodium bicarbonate solution (0.075, pH=8.4)(for 9 h, in seed to solution ratio 1:3 w/v)	Room temperature	• Decrease in starch content (20 - 21%), no effect of solution employed. • Highest decrease (27%) of α- galactosidase in water-soaked seeds • 12% decrease in trypsin inhibitor activity on water soaking only. No effect of other solutions used.
Olika, Abera, and Fikre (2019)	Soaking for 12hr in distilled water (1:3 w/v ratio)	Room temperature	• Decrease in zinc, iron and calcium content when compared to raw seeds • 50% reduction in tannins and 15.2% reduction in phytic acid
	Germination methods		
Mittal et al. (2012)	Soaked overnight and germinated for 2 days	22 ºC	• Increase in protein, fatty acid (palmitic, linolenic) content • Significant decrease in crude fibre (-60.3%), polyphenol (-81.9%) tannins (-93.25)

Source	Activity	Temperature	Effect
	Germination methods (contd.)		
Tarzi et al. (2012)	Seeds soaked in 0.07% sodium hypochlorite for 30 min, washed and soaked again in distilled water for 5 hr. Transferred to germination trays and germinated for 5 days at 99% humidity.	20 ºC	• Significant increase in the total phenolic content and antioxidant activity
Egli et al. (2002)	Seeds soaked in a covered beaker with water for 16 hr in the dark. Seeds germinated in the dark for 24, 48 and 72 hr	25 ºC	• Significant reduction in phytic acid content (16%) after soaking and 72 hr germination
Ghavidel and Prakash (2007)	Soaking for 12 hr (22 - 25 ºC), followed by germination under a wet muslin cloth for 24 hr	22 - 25 ºC	• Increase in protein content, soluble, insoluble and total dietary fibre content, bioavailable iron and calcium content • Decrease in fat, phytic acid and tannin content
Portari et al. (2005)	Washed in 0.001% benlate solution, soaked in distilled water and germinated for 2, 4 & 6 days in a germination chamber in dark	16 - 18 ºC	• Significant increase in major globulins after 6 days of germination was observed • With increase in germination time, a significant reduction in protein content was observed. • *In vitro* protein digestibility reduced as well after 6 days of germination compared to raw seeds.

Source	Activity	Temperature	Effect
	Thermal processing (Boiling, cooking or dry heating)		
Frias et al. (2000)	Soaking in water + cooking	100 ºC	• Soaking in citric acid + cooking gives highest reduction in fructose content (83%) • Sodium bicarbonate soaking+ cooking results in highest (58%) α- galactosidase reduction • 100% decrease in trypsin inhibitor activity from all soaked and cooked samples.
	Soaking in citric acid solution (0.1%, pH=2.6) + cooking		
	Soaking in sodium bicarbonate solution (0.075, pH=8.4) + cooking (soaking for 9 hr, seed to solution ratio 1:3 w/v, followed by cooking (35 min) in distilled water (1:6.67 w/v ratio in water))		
	Dry heating under pressure (1 atm for 15 min)	120 ºC	• Dry heating resulted in highest reduction in starch content (26%) and only a 27% decrease in trypsin inhibitor activity.
Aguilera et al. (2009)	Soaking in tap water 1:10 w/v (16 hr, 20 ºC) + cooking in boiling water for 70 min	100 ºC	• Increase in the amount of available starch and a decrease in resistant starch was observed
Mittal et al. (2012)	Boiled in ratio 1:7 w/v for 10 min, followed by drying	100 ºC	• Increase in fatty acid (palmitic, oleic), potassium, iron content • Decrease in phytic acid (-12.3%), polyphenols (-86.4%) and tannins (-93.07%)
	Sand roasted in an open pan for 15 min	120 ºC	• Increase in potassium and phosphorus content • > 80% decrease in polyphenols and tannins

Source	Activity	Temperature	Effect
Thermal processing (Boiling, cooking or dry heating) (contd.)			
Olika, Abera, and Fikre (2019)	Boiled in distilled water for 60 min	96 ºC	• Decrease in zinc, iron and calcium content when compared to raw seeds • Highest reduction in tannin (82.5%) and phytic acid (57.35%) content when compared to other processing techniques such as soaking, germinating, dehulling and dry roasting.
	Dry roasting for 30 min	150 ºC	• Dry roasting resulted in lowest reduction in tannin (25%) and phytic acid content (5.89%) when compared to soaking, germinating, dehulling and boiling.
Brummer et al. (2015)	Soaked for 16 hr in distilled water (1:4 w/v ratio) followed by boiling for 22 min	100 ºC	• Significant increase in total starch content after boiling
High pressure cooking			
Alajaji and El-Adawy (2006)	Soaking in distilled water (1:10 w/ v ratio seed:water) for 12 h at room temperature, followed by autoclaving (15 psi) in tap water (35 min)	121 ºC	• Significant increase in crude fibre content • Decrease in sugars (sucrose, raffinose, stachyose and verbascose) • Compared to boiling and microwave cooking, highest reduction in tannins (50.10%) and phytic acid (41.32%)

Source	Activity	Temperature	Effect
	High pressure cooking (contd.)		
Mittal et al. (2012)	High pressure cooking at 15 psi in water (1:2 w/v seed: water) for 15 min	-	• Decrease in fatty acid (oleic, linoleic), potassium, magnesium, and sodium content • Highest decrease in phytic acid (-13.7%), polyphenols (-87.71%), and tannins (-93.97%) out of all processing techniques
Ertaş and Türker (2014)	Soaking (2, 8 &12 hr) in different soaking mediums (pH 4, 6, 8), followed by autoclaving	121 ºC	• With increase in soaking time, the reduction in starch, total zinc and phytic acid content increased • Soaking in pH 8 water increased in vitro protein digestibility, total zinc, and total potassium content.
de Almeida Costa et al. (2006)	Soaking for 16 hr in water (1:2 w/v seed to water ratio), followed by pressure cooking (14.7psi) for 20 and 40 min	-	• Significant increase in insoluble dietary fibre content
Nithiyanantham, Selvakumar, and Siddhuraju (2012)	Soaking for 12 hr in distilled water (1:10 w/v seed to water ratio), followed by autoclaving (1:5 w/v seed to water ratio) for 20 min	121 ºC	• Significant reduction in total phenolics and tannins, after autoclaving

Source	Activity	Temperature	Effect
	Microwave cooking		
(Máñez et al. 2002)	Soaking in water for 24 hr, followed by 5 min of microwave cooking (1400 W)	-	• No significant difference in phytic acid content when compared to raw seeds.
Alajaji and El-Adawy (2006)	Soaking in distilled water for 12 hr at room temperature (1:10 w/v ratio seed: water), followed by microwave cooking on high (tap water)	-	• Slight increase in crude fibre • Reduction in sugars (raffinose, sucrose, stachyose and verbascose). • A further reduction in tannin (48.5%) and phytic acid (38.02%) content compared to raw seeds
Marconi et al. (2000)	Soaking in distilled water for 18hr (1:10 w/v seed: water ratio) at room temperature, followed by microwave cooking (585 W, pressure 12 - 15 psi)	105 ºC	• Significant increase in soluble dietary fibre percentage • Significant decrease in protein, insoluble dietary fibre and resistant starch

Source	Activity	Temperature	Effect
	Extrusion		
Berrios et al. (2010)	Twin screw extrusion of chickpea flour (500 rpm screw speed)	160 ºC	• Reduction in total available carbohydrate content • Insignificant reduction in insoluble dietary fibre content
Raza et al. (2019)	Twin screw extrusion of chickpea flour	150 ºC	• Disruption of microstructure and crosslinking of proteins and starches as confirmed by scanning electron microscopy.
Poltronieri, Arêas, and Colli (2000)	Single screw extrusion of chickpea flour (200 rpm screw speed)	130 ºC	• Increase in iron, soluble fibre • Decrease in insoluble and resistant starch
Milán-Carrillo et al. (2000)	Dehulling, softening and single screw extrusion (189.5 rpm screw speed)	151ºC	• Significant increase in vitro protein digestibility of extruded flour when compared to conventional flour

2.6 Conclusion:

Consumption of chickpeas, being an excellent source of nutrients, provide beneficial health effects to humans and its global demand has been steadily growing. This is not only in developing countries where chickpeas are a staple food, but also in developed countries due to an increasing vegan and vegetarian population, plus a general health awareness resulting in a decreasing consumption of meat. Several factors including the environmental conditions during production, and different processing techniques post-harvest affect the physical, nutritional and bioactive properties of chickpea. Particular growing environment and processing techniques can be selected to obtain the desired nutritional, bioactive and organoleptic properties (aroma, taste, flavour and texture) to meet consumer demand for an alternative source of proteins and bioactive components.

However, very limited information on these conditions on the commercial Australian varieties is currently available and needs further exploring. New, innovative and versatile processing techniques such as high pressure processing are now accessible and can provide better products than traditional processing methods, however, their full effects are unknown. Careful consideration of all these factors is required to obtain the highest nutritional and phytochemical benefits from chickpeas in their journey from farm to table which will establish chickpeas as the next health food for human consumption.

CHAPTER 3. The physical, nutritional and antioxidant properties of Australian kabuli chickpeas

"Variety is a positive requisite even in the character of our food"

John Ruskin

Abstract

Research suggests that different kabuli chickpea varieties have different seed properties, however, the evidence for this phenomenon in Australian kabuli chickpeas is lacking. Thus, to evaluate the intervarietal differences between Australian kabuli chickpeas, five commercially grown varieties were evaluated for their physical, nutritional and antioxidant properties using established methods. The results obtained showed large variation between varieties in terms of seed weight, volume, hydration and swelling characteristics, with Kimberley Large variety outperforming all other varieties due to its large size (9-11mm). The proximate composition was highly variable as well, with Kimberley Large exhibiting the highest values for ash (2.856 g/ 100g, db), starch (48.881 g/ 100g, db) and carbohydrates (52.612 g/ 100g, db), while Genesis Kalkee contained highest protein levels (24. 564 g/ 100 g, db), resulting in higher proportions of the majority of amino acids when compared to other varieties. Both, Kimberley Large and Genesis 090 exhibited almost identical values for total polyphenol content (60.395 and 60.451 mg Gallic acid equivalent/ 100g sample, db), however, Genesis 090 presented significantly ($p \leq 0.05$) high DPPH (60.955 mg trolox equivalent (TE) /100 g, db) and ABTS (84.992 mg TE /100 g, db) antioxidant values when compared to all other varieties. Interestingly, the oxygen radical antioxidant capacity (ORAC) for Kimberley Large was the highest (2808 µmol TE/ 100 g sample, db), also making it the variety with the highest biologically relevant antioxidant capacity. These results showcase important seed quality differences between Australian chickpea varieties and can be utilised by farmers, researchers and food manufacturers.

3.1 Introduction:

Chickpea (*Cicer arietinum* L.) is the third most frequently grown cereal legume crop in the world with a total production of 14.8 million tonnes in 2017 (FAOSTAT 2019). India is the leading producer of chickpeas with 66% of the world production, followed by Turkey, Pakistan, Iran, Canada and the United States of America (Rachwa-Rosiak, Nebesny, and Budryn 2015). Chickpeas can be grouped into two different types- desi and kabuli. Desi chickpeas are small, dark with an irregular- shaped seed coat and is grown in semi-arid tropical regions whereas, kabuli chickpeas are larger than desi chickpeas, have a thin beige or light-coloured seed coat and are generally grown in the temperate regions of the world (Gangola et al. 2013). In Australia, chickpeas are produced in a variety of climates from warm temperate with high humidity to hot arid, cold arid and equatorial with dry winters (Kottek et al. 2006) Total chickpea production in Australia in 2015 was 555,400 tonnes, out of which 43,700 tonnes was of the kabuli type (PULSE Australia, 2017).

Chickpeas are an excellent source of carbohydrates, proteins, and dietary fibre, especially for people in countries where animal protein is very expensive compared to plant protein sources. It is also a good source of bio-actives with good antioxidant activity and bioavailable calcium, phosphorus, magnesium, iron and potassium (Christodoulou et al. 2005, Xue et al. 2015). Not only are desi and kabuli chickpeas morphologically different, but the nutritional composition also differs significantly, especially in terms of the protein and the dietary fibre content. Kimberley Large is a very large-seeded variety of kabuli chickpeas, which is being cultivated in the equatorial climate of northern Western Australia, compared to other commercial vari -eties such as Genesis 090, Genesis Kalkee, PBA Monarch and PBA Royal which are more suitable to the warm temperate climate of the northern and south-eastern parts of Australia (PULSE Australia, 2019).

Although kabuli chickpeas are a good source of nutrients in the human diet, studies have mostly focussed on kabuli chickpea varieties from Asia, Europe and North America (Frimpong et al. 2009, Cobos et al. 2017, Wang et al., 2017, Gil et al., 1996). It is also known that the nutritional composition of chickpeas is affected by factors such as plant variety, time of sowing, cultivation area, weather conditions, and their interactions (Frimpong et al. 2009, Cobos et al. 2017, Nikolopoulou et al. 2006b), consumers should be made aware of the exact nutritional profile of kabuli chickpeas which they are purchasing for consumption. As the production of kabuli chickpeas is increasing in Australia, with new areas of production opening up, it is important to characterise and compare Kabuli chickpea varieties grown under different climates and soil types found in different growing regions of Australia, which has not been previously studied.

The Australian pulse production has been growing steadily from 2.14 million tonnes in 2009-10 to around 4.12 million tonnes in 2016-17, as it is one of the most exported agricultural commodities of Australia and accounts for around $2 billion in revenues (FAOSTAT 2014). Even though wheat and barley are produced in greater quantities, Australian chickpeas are a high-value crop and are sold at more than double the price per tonne of both wheat and barley (Profarmer 2019). Even after being a high-value export crop of Australia, there is limited information regarding the nutritional and polyphenolic composition as well as physical and antioxidant properties of Australian Kabuli chickpea varieties. Thus, in this study, we, therefore, determined the physical, nutritional, and polyphenol content, as well as the antioxidant capacity of Australian chickpea varieties grown in different agricultural regions of Australia to determine the inter-varietal differences.

3.2 Materials and methods

Representative samples of five kabuli chickpea varieties grown in different regions of Australia viz. Kimberley Large (Kununurra, Western Australia), Genesis 090 (Sea Lake, Victoria), Genesis Kalkee (Warracknabeal, Victoria), PBA Monarch and PBA Royal (formerly known as CICA1156) both from Kadina, South Australia, from 2017 harvest were collected (Table 3.1) (Fig 3.1). Approximately 1-2kg sample was collected, sieved, vacuum packaged and stored at 4°C until analysis.

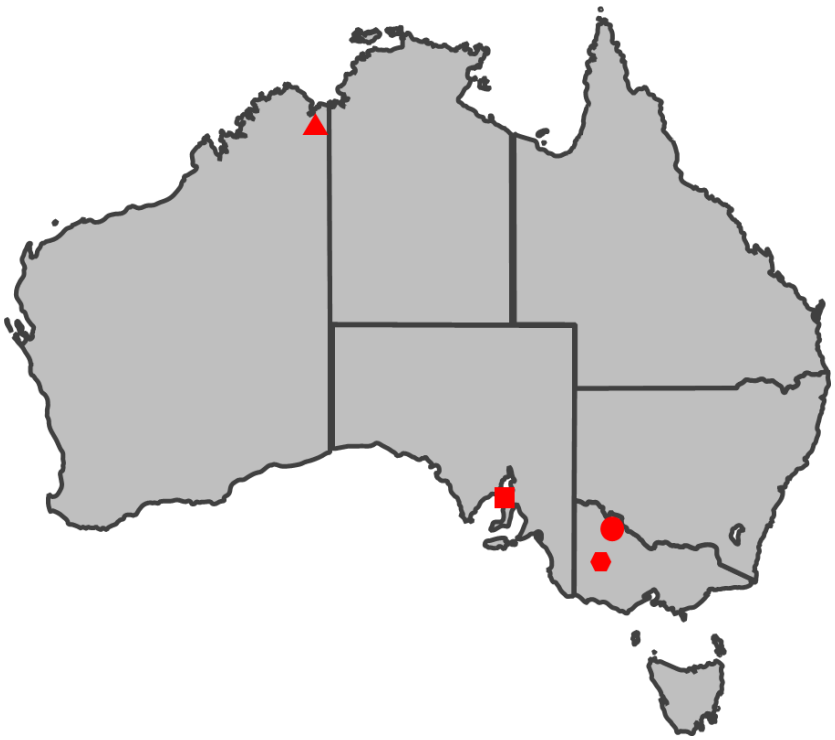

Figure 3.1 Locations where Australian kabuli chickpea crops were grown in 2017:

(1) ▲ Kununurra, WA (2) ■ Kadina, SA

(3) ● Sea Lake, VIC, (4) ⬣ Warracknabeal, VIC.

Table 3. 1 Climate and soil type at Kabuli chickpea growing locations

Kabuli chickpea variety	Growing location	Latitude	Longitude	Climate (Beck et al. 2018)	Soil type (CSIRO 2012)
Kimberley Large	Kununurra, WA	15.604949 °S	128.765468 °E	Tropical, dry winter	Clay
Genesis 090	Sea Lake, VIC	35.505541 °S	142.85269 °E	Arid, steppe, cold	Sandy clay, clay loam
Genesis Kalkee	Warracknabeal, VIC	36.252755 °S	142.391955 °E	Arid, steppe, cold	Clay loam, sandy clay, clay
PBA Monarch	Kadina, SA	33.963265 °S	137.714859 °E	Arid, steppe, cold	Clay, clay loam
PBA Royal	Kadina, SA	33.963265 °S	137.714859 °E	Arid, steppe, cold	Clay, clay loam

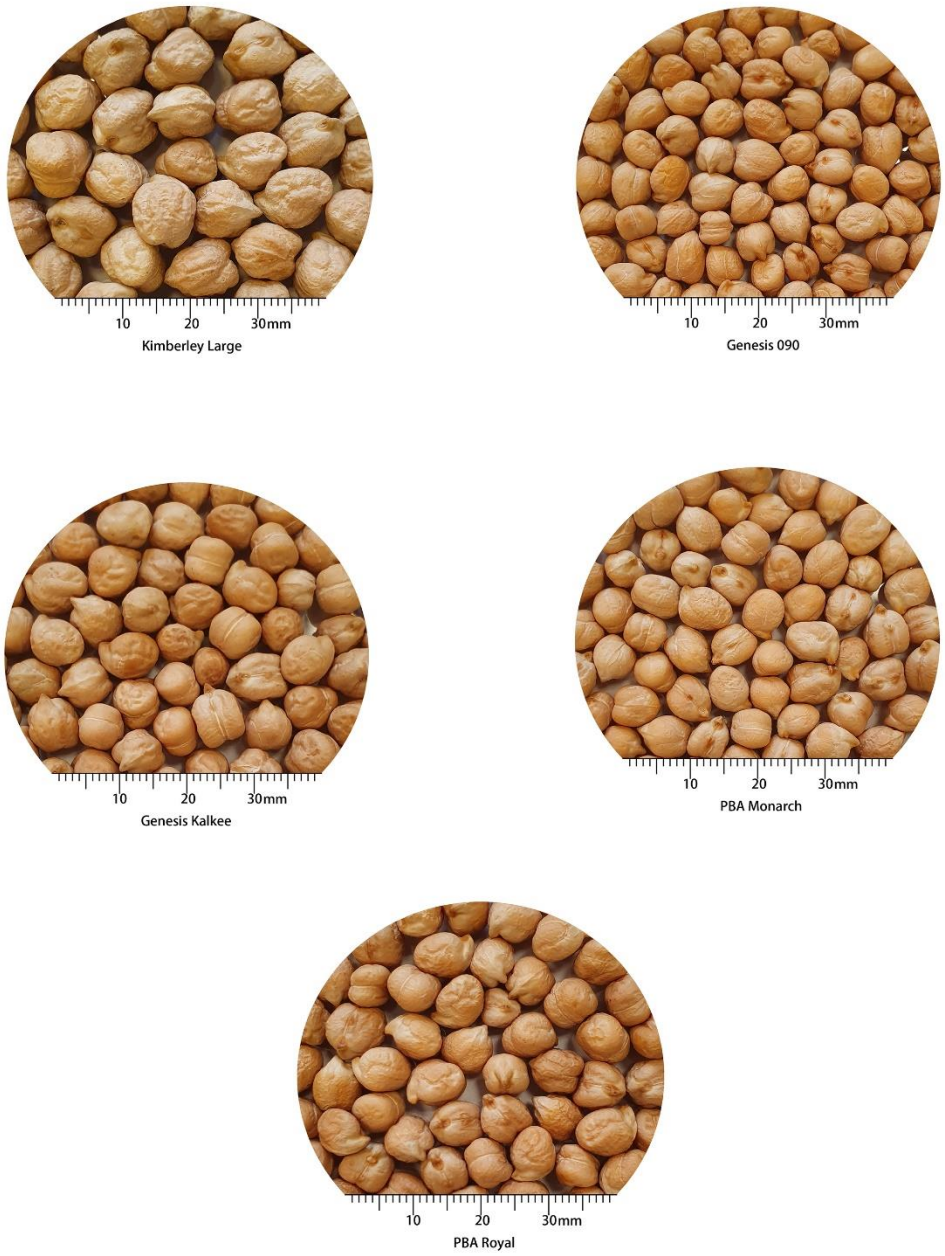

Figure 3.2 Representative samples of the five modern genotypes of Australian kabuli chickpeas grown in 2017

3.3 Physical analysis

Seed size (g), volume (mL), density(g/mL), hydration capacity(g), hydration index, swelling capacity(mL), and swelling index were evaluated following the methods of Williams *et al*.(1983) and Khattak *et al.* (2006). Three random samples of 50 seeds from each cultivar were weighed and the values were converted into grams per seed. The seed volume was determined by transferring 50 seeds into a 100mL measuring cylinder, with the addition of 25mL distilled water. The increase in volume was taken as the volume occupied by seeds. Seed density was calculated by dividing seed weight by seed volume. Seeds were soaked in distilled water overnight (12h), and hydration capacity was calculated using gain in weight after overnight soaking. The hydration index was determined by diving hydration capacity by original seed weight. Similarly, swelling capacity was determined using gain in volume after overnight soaking, and the swelling index was calculated as swelling capacity divided by the original seed volume.

3.4 Colour measurement

The surface colour of chickpea grits was measured using a BYK Gardener handheld spectrophotometer (Model 6801, BYK, Germany). Chickpea grits of uniform size contained in a petri dish were placed on a white surface and L*(lightness), a* (redness and greenness) and b* (yellowness and blueness) colour values were recorded (Kaur, Singh, and Sodhi 2005).

3.5 Chemical analyses

3.5.1 Sample preparation

Kabuli chickpea seeds were milled using a CEMOTEC 1090 grinder (Foss Tecator, Hoganäs, Sweden) to enable them to pass 100% through a 500-micron sieve, then vacuum packaged and stored at~-20 ± 2 °C for two weeks to kill any insects present in the samples, and then stored at 15°C for 18 months.

3.5.2 General nutrient analyses

General nutrient analyses of chickpea samples were performed in triplicate using the following standard methods: Moisture by the Modified Solids (total) and moisture in flour- oven drying method (Method 925.10, AOAC International 2008); Protein by Kjeldahl assay: digestion, distillation and titration using a nitrogen conversion factor

of 6.25 (Method 920.87, AOAC International 2005b); Fat by the Soxhlet solvent extraction technique, (Method 963.15, AOAC International 2006); Ash by the Ash of flour method (Method 923.03, AOAC International 2005a) and Dietary fibre was determined using Enzymatic – Gravimetric method (AACC 2009) (AOAC 2005) and available carbohydrates were calculated by difference (FSANZ 2017).

3.5.3 Amino acid profile

Eighteen essential and non-essential amino acids were analysed using ultra-performance liquid chromatography with a combination of Photo Diode array and tandem mass spectrometric detectors (UPLC-PDA-MSMS) using an in-house developed, validated and NATA accredited method at the National Measurement Institute, Port Melbourne, Victoria, Australia. The instrumentation included an H class Waters™ Acquity UPLC/ MS system equipped with a photodiode array and Waters™ QuattroMicro mass spectrometer (Milford, MA, USA) were used. Samples were prepared by thoroughly homogenising and weighing approximately and accurately 100mg of sample. A test portion of 2g of sample was further mixed with 0.1M HCl to extract free amino acids, whereas another test portion of 100mg was base hydrolysed for 24 h using 6 M NaOH at 110 °C, followed by neutralisation and filtration. An aliquot of the free amino acid solution and the neutralised hydrolysate solution was analysed by UPLC-PDA-MSMS. The derivative amino acids from sample extracts were separated, detected and quantified using UPLC-PDA-MSMS. The confirmation of the identity of each amino acid was performed using PDA retention times of the reference standards against the retention times of amino acid obtained from the sample. Furthermore, the unique MRM transitions of the MSMS were used as a secondary form of confirmation. All results were expressed as mg/kg sample.

3.5.4 Total starch content:

Starch content was determined in duplicate using the standard colourimetric technique (AOAC International 2005c) using a total starch assay kit (AA/AMG) from Megazyme International Ltd (Bray, County Wicklow, Ireland).

3.5.5 Mineral content:

Content of individual minerals (mg) was determined in duplicate using an inductively coupled plasma optical emission spectrometer (ICP- OES) (Varian, Palo Alto, USA)

at the National Measurement Institute, Perth, Western Australia. Concentrated nitric acid (HNO_3) (3 mL) and concentrated HCl (3 mL) were added to 1g of chickpea flour, and this mixture was digested at 95 ℃ for 2 hours in a DEENA automated digestion block (Thomas Cain, Omaha, Nebraska, USA). After digestion, the sample tube volume was made up to 40 mL using distilled water, and the solutions were left to settle. Afterwards, all samples were diluted 5-folds with distilled water before being analysed using ICP-OES. Appropriate emission wavelengths with higher sensitivity and lower interferences of 238.204 nm, 317.933 nm, 213.618 nm, 213.857 nm, 279.078 nm, 766.491 nm, and 589.592 nm were chosen to analyze Fe, Ca, P, Zn, Mg, K and Na respectively (Wu, Johnson, Bornman, Bennett, Singh, et al. 2016). All results were expressed as mg/ kg sample.

3.5.6 Polyphenol extraction

Total polyphenols were extracted as per Segev et al.(2010) with a few modifications. In brief, whole chickpea seeds were milled in a CEMOTEC 1090 grinder (Foss Tecator, Hoganäs, Sweden) until 100% passed through a 500 µm sieve. Whole chickpea flour (1g) was mixed with 10 mL 50% acetone solution. The mixture was shaken at ambient temperature at 60 rpm for 3 hours (h) using a suspension mixer (RSM7DC, Ratek, Boronia, Victoria, Australia). The mixture was extracted for an additional 12 to 16 h in the dark overnight. The extract was centrifuged using an Eppendorf centrifuge (Model- 5810r, Hamburg, Germany) at 12000 g, for 10 min at room temperature, and the extracts were kept in the dark at -20 °C until used for determination of total phenolic content (TPC), total flavonoid content (TFC), 2-2-diphenyl-1-picrylhydrazyl (DPPH), 3-ethylbenzothiazoline-6-sulfonic acid diammoni -umsalt (ABTS) and oxygen radical absorbance capacity (ORAC).

3.5.7 Total polyphenol and flavonoid content

Total polyphenol content was determined using Folin-Ciocalteu method (Wu et al. 2017). In brief, 100 µL of polyphenol extracts were mixed with 2.5 mL of 0.2 N Folin-Ciocalteu reagent, followed by 2 mL of saturated sodium carbonate solution (75g/L) addition. After a reaction time of 2 h at room temperature in the dark, the absorbance of obtained mixtures was determined using a UV-1800 Spectrophotometer (Shimadzu, Canby, USA) at 765 nm. A standard curve was prepared using Gallic acid

(0-360mg/L) and results were expressed as mg Gallic acid equivalents (GAE)/ 100g dry basis (db). All extract were analysed in duplicate.

The total flavonoid content was determined as per Wu et al.(2017). In brief, 250µL extract was mixed with 5% $NaNO_2$ (75 µL) and 1 mL water in a 2.5mL microfuge tube. After 5 min of reaction time, 10% $AlCl_3$ (75 µl) was added. After 6 min, 500 µl of 1 mol/L NaOH and 600 µL of ultrapure water was added. The absorbance of the mixture obtained was immediately determined at 510 nm and the results were expressed in terms of mg catechin equivalents (CE)/ 100g db. Catechin (20–100 mg/L) was used to prepare a standard curve and all extracts were analyzed in duplicate.

3.5.8 Antioxidant capacity

The method of Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Byrne (2006) was used to determine the antioxidant capacities of the chickpea varieties. The 2-2-diphenyl-1-picrylhydrazyl (DPPH), 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS), and oxygen radical absorbance capacity assay were used to estimate the antioxidant capacity of the polyphenolic extracts obtained from Kabuli chickpeas as described below.

For the DPPH assay, firstly, DPPH (24 mg) was added into 100 mL methanol to prepare the stock solution, which was stored at -20 °C in the dark until use. To prepare the working solution, a 10mL stock solution was diluted with 50 mL methanol, giving an absorbance of 1.1 ± 0.02 units at 515 nm. Duplicate kabuli chickpea phenolic extracts (150µL) were mixed with the DPPH working solution (2850µL) and reacted for two hours in the dark, after which the absorbance was determined at 515 nm and results were expressed in terms of mg Trolox equivalents (TE)/g db. Different concentrations of trolox (20–250 mg/L) were used to prepare a standard curve for DPPH antioxidant activity determination.

For the ABTS assay, a fresh stock solution was prepared by mixing 7.4 mM ABTS and 2.6 mM potassium persulphate in equal amounts, which were kept for the duration of 12 hours in the dark. Using the stock solution, a fresh ABTS working solution was prepared by diluting 1 mL of the stock solution with 60 mL of methanol to get a 1.1 ± 0.02 units absorbance at 734 nm. The kabuli chickpea phenolic extract (150µL) was mixed with the ABTS working solution (2850 µL) and incubated in the dark for two hours, followed by absorbance determination at 734 nm. The standard used for the ABTS assay was Trolox (20–250 mg/L) and the results were expressed in terms of mg Trolox equivalents (TE)/g db, with all extracts being analysed in duplicate. The ORAC assay was performed using the procedure of Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002) in a 96-well black-walled, clear-bottom Polypropylene microplate. On all sides of the plate, the exterior wells were left unused as there was high variation from the results when compared to the interior wells. Polyphenol extract (25 µL) from each chickpea variety was mixed with 0.084 µmol/ L fluorescein (150 µL) and incubated for 30 min at 37 °C. Then, 25 µL of 153 mmol/ L AAPH (2, 2'-Azobis (2-methylpropionamidine) Dihydrochloride) was quickly added to start the reaction, followed by shaking for 10s in a microplate shaker at maximum intensity. Fluorescence intensity was kinetically monitored at 485 nm excitation and 528 nm emission every minute over 120 mins (at 37 °C) using a multi-detection micro-plate reader (Synergy HT, Biotek Instruments, Winooski, USA). The area under the average fluorescence-reaction time kinetic curve (AUC) for both blank and samples were calculated. All extracts were analysed in duplicate and the final antioxidant activity results were calculated using Trolox as a standard (0-50 µmol/ L), and expressed as µmol TE/100 g sample, db.

3.5.9 Statistical analysis

The statistical analysis was performed using SPSS v.26.0 (SPSS Inc.; Chicago, IL). Data were evaluated using analysis of variance with Tukey's post hoc test to determine the significant differences ($p \leq 0.05$) between varieties. Pearson correlation coefficients were calculated to compare analytical determinations to reveal associations ($p \leq 0.05$).

3.6 Results and Discussion

3.6.1 Physical analysis

Significant differences in the physical characteristics of kabuli chickpeas analysed in this study were observed and are reported in Table 3.2. The seed weight of the different cultivars varied significantly ($p<0.05$). Greatest seed weight and volume of 0.61g and 0.51mL per seed was observed in Kimberley Large compared to the lowest seed weight (0.30 g/seed) and volume (0.25 mL/ seed) in Genesis090, with the average seed weight of varieties decreasing from Kimberley Large > Genesis Kalkee > PBA Royal > PBA Monarch > Genesis 090. In legumes, seed size controls the distance to which water must travel to reach the seeds innermost portion, in turn affecting the cooking time (Kaur, Singh, and Sodhi 2005, Khan et al. 1995). Thus it can be concluded that Kimberley Large with the largest seed size will take the longest to cook. Similar results have been reported by Kaur *et al.*(2005), who reported that the cooking time for Kabuli chickpea was longer than desi chickpeas due to their larger seed size. Seed density ranged from 1.177 and 1.337g/ mL, being the lowest for Genesis Kalkee and highest for PBA Royal. Significant differences in the hydration capacity and index were observed and hydration capacity and hydration index for chickpea varieties ranged between 0.31-0.64 g/seed and 95.05-110.16, respectively. Hydration capacity was positively correlated to seed weight ($r=0.974$, $p<0.01$) and volume ($r=0.980$, $p<0.01$) (Table 3), the lowest hydration capacity recorded in Genesis 090, the variety with the lowest seed weight. Both, seed size and seed coat thickness play an important role in the water absorption qualities of legume seeds (Kaur, Singh, and Sodhi 2005).

Water uptake is also a characteristic of seed pore structure which increases with an increase in permeability, and a decrease in tortuosity (Joshi et al. 2010). Kimberley Large variety had the largest seed size, resulting in the highest values for hydration capacity and hydration index when compared to other samples. Similarly, significant differences ($p<0.05$) were observed between swelling capacity and swelling index of chickpea samples. The swelling capacity of the different chickpea varieties ranged between 0.3-0.69 mL/ seed, whereas the swelling index per seed ranged from 112.9-137.2. Kaur et al. (2005) reported similar results for swelling capacity (0.23) and swelling index (136.5) for kabuli chickpea (variety L-550) in their study. A positive correlation was recorded between seed weight and swelling capacity ($r=0.949$, $p<0.01$) (Table 3.3). There is an association between the swelling index and the gelatinization

of starch which represents the uptake of water by hydrogen bonding as well as by non-starch polysaccharides and proteins. The water absorption capacity in seeds is affected by the seed composition, cell structure, and cell compactness, in turn affecting both the hydration and swelling of seeds (Mokni et al. 2015).

Table 3. 2 Physical analyses of Kabuli chickpea varieties grown in Australia compared to Indian cultivars (Kaur, Singh, and Sodhi 2005)

Property	Kimberley Large	Genesis90	Genesis Kalkee	PBA Royal	PBA Monarch	Kaur et al.(2005)
Seed weight (g)	0.61 ± 0.02^{a}	0.3 ± 0.014^{b}	0.43 ± 0.01^{c}	0.43 ± 0.02^{c}	0.38 ± 0.02^{d}	0.21
Seed volume (mL)	0.51 ± 0.02^{a}	0.25 ± 0.01^{b}	0.37 ± 0.02^{d}	0.32 ± 0.02^{c}	0.29 ± 0.01^{bc}	0.17
Density (g/ mL)	1.21 ± 0.02^{ab}	1.19 ± 0.05^{ab}	1.18 ± 0.07^{b}	1.34 ± 0.12^{a}	1.30 ± 0.03^{ab}	1.29
Hydration capacity (g/ seed)	0.64 ± 0.01^{a}	0.31 ± 0.01^{d}	0.45 ± 0.02^{b}	0.41 ± 0.01^{c}	0.42 ± 0.02^{bc}	0.20
Hydration Index	104.6 ± 2.9^{a}	103.49 ± 1.3^{ab}	105.05 ± 5.9^{ab}	95.06 ± 5.07^{b}	$110.16\ \pm3.31^{a}$	90.4
Swelling capacity (mL/ seed)	0.69 ± 0.02^{a}	0.3 ± 0.02^{c}	0.42 ± 0.02^{b}	0.36 ± 0.02^{bc}	0.37 ± 0.04^{bc}	0.23
Swelling index	137.18 ± 11.1^{a}	118.59 ± 9.5^{a}	115.07 ± 12.7^{a}	112.93 ± 11.9^{a}	$126.35\ \pm14.2^{a}$	136.5

All values are presented as mean ± standard deviation. Following Tukey's post hoc test, values followed by the same superscript within a row do not differ significantly ($p<0.05$)

Table 3. 3 Pearson's correlation coefficients (r) among physical properties of Kabuli chickpea seeds

Trait		**Seed volume**	**Hydration capacity**	**Swelling capacity**
Seed weight (g)	-	0.979**	0.974**	0.949**
Seed volume (mL)	-	-	0.980**	0.959**
Hydration capacity	-	-	-	0.977**

Note: ** Significant at $p<0.01$

3.6.2 Colour

Colour measurement values (L*, a*, b*) are shown in Table 3.4. Among different chickpea cultivars, significant differences ($p \leq 0.05$) in colour values were observed. The L* and a* (redness and greenness) values ranged between 86.15 and 90.54 and 2.55 and 3.83 respectively. The b* value (yellowness and blueness) ranged from 16.39 and 19.34, being the lowest for PBA Royal and highest for Genesis090. L* value for PBA monarch indicated that these seeds were lightest in colour compared to Genesis 090 which had the lowest L* value and highest a* value, indicating that these seeds were the darkest out of all cultivars and had a faint reddish tinge to them. Kaur et al. (2005) also reported colour values for kabuli chickpea samples, however as can be seen from the data in Table 4, their samples were darker (lower L*) with a higher b*(yellowness and blueness) compared to samples in this study. Harvest times have shown to affect chickpea seed colour, with an early harvest giving a more bright and intense colour to the seeds (Cassells and Caddick 2000). Past studies have also linked chickpea seed colour to establishment on sowing, seed yield, and ease of decortication, seed with the darker seeds being more favourable (Knights and Mailer 1989).

3.6.3 General nutrient analyses

The concentration of major components in chickpea varieties is reported in Table 3.5. The statistically significant differences ($p<0.05$) in terms of moisture, protein, fat, ash, dietary fibre and starch content between varieties were observed. The moisture content of whole chickpea flours ranged from 6.649 to 9.041 g/100g with the moisture content of Kimberley Large variety being significantly lower than all other varieties. The observed difference in moisture content could be due to a difference in the postharvest handling of chickpeas (Abu, Arogba, and Ugwu 1999). A study by the Grains Research and Development Corporation (GRDC) concluded that over a period of 9 months of post-harvest storage, chickpea seeds lost about 2% of moisture due to conditions such as moisture content upon harvesting, storage temperature and aeration (GRDC 2018a).

A significant difference in protein content was observed as well, ranging from 18.163 to 24.564 g/100g sample (db). Genesis Kalkee had the highest protein content and Kimberley Large had the lowest amongst all samples. The protein content in some Australian chickpea varieties was comparatively higher than that found in varieties from Syria, Canada and India which varied from 17.12 to 19.81g/ 100 g (db) sample (Frimpong et al. 2009) and could be due to the difference in the cultivar and growing conditions (Mokni et al. 2015). The fat content in chickpeas ranged from 5.871-6.707 g/ 100 g sample (db) and was similar to results obtained by El Adawy (2002) and Nikolopoulou et al.(2006b) for varieties from Egypt and Greece, respectively. Compared to other legumes such as black beans and lentils, chickpeas generally have a higher fat content. The higher fat content in chickpeas is of importance as during starch gelatinisation, amylo-lipid complexes are formed resulting in a lower starch bioavailability (Silva-Cristobal et al. 2010).

The ash content is an indicator of the mineral content of a material and its value in plant sources is quite variable when compared to animal sources, making it an important part of the proximate analysis (Marshall 2010). The ash content in chickpea samples ranged from 2.433 to 2.856 g/100g (db) sample, with significant differences observed between chickpea varieties. These observations are in agreement with the findings of Özer et al.(2010) and Xu et al. (2013), who also reported significant differences in ash content between different Kabuli chickpea varieties.

Legumes have been known to contain one of the highest percentages of dietary fibre in seed groups (Rosin, Lajolo, and Menezes 2002), with high levels reported in common beans, chickpeas and lentils. The dietary fibre (DF) content of the chickpea samples in this study ranged from 14.3 to 18.234 g/100g (db). Wang et al. (2010) reported an average DF content in kabuli chickpeas of 15.3 g/100g dry sample whereas, Jukanti et al.(2012) reported an average DF content of 17.4 g/100g (db). The values reported in both of these studies are within the range of this study. In contrast, Tosh and Yada (2010) reported a higher DF content of 18-22g in kabuli chickpeas. Similar to protein content, DF content has been known to vary due to differences in seed size, and coat thickness (as DF is mainly found in the seed coat), chickpea type (desi or kabuli) as well as the analytical method used for the estimation

of DF (Rincón, Martínez, and Ibáñez 1998, Silva-Cristobal et al. 2010). Higher DF contents have been reported in desi chickpea varieties (Gil, Nadal, Luna, Moreno, and Haro 1996) and are linked to the thicker seed coat of desi chickpeas which accounts for 14.5-16.5% of the seed weight compared to kabuli chickpeas, where it accounts for only 4.3-4.4% (Sosulski and Gadan 1988). This is despite the larger seed size of kabuli chickpeas compared to desi chickpeas. A high intake of DF has been shown to have beneficial health effects (Saura-Calixto et al. 2000).

In general, the nutrient composition of Australian kabuli chickpeas was in agreement with the available literature. However the variation in the characteristics presented in this study compared to the previously published data can be explained by the differences in genotype, growing conditions, sowing time (autumn or spring), post-harvest handling as well as different techniques of analysis (Gül, Egesel, and Turhan 2008, El-Adawy 2002, Tayyar, Egesel, Gul, et al. 2008, Silva-Cristobal et al. 2010).

Table 3. 4 Colour (L*, a*, b*) of Kabuli chickpea varieties grown in Australia compared to published literature

Colour components	Kimberley Large	Genesis90	Genesis Kalkee	PBA Royal	PBA Monarch	Literature
L*	88.29±0.12^{a}	86.15±0.79^{b}	88.10±0.36^{a}	90.41±0.06^{c}	90.54±0.54^{c}	76.78
a*	2.94±0.01^{ac}	3.83±0.18^{b}	3.34±0.25^{ab}	2.55±0.09^{c}	2.75±0.13^{ac}	1.54
b*	16.47±0.16^{a}	19.34±0.39^{b}	18.87±0.08^{b}	16.39±0.07^{a}	16.72±0.34^{a}	22.34

All values are presented as Mean± standard deviation. Values followed by the same superscript within a row do not differ significantly ($p<0.05$).

Literature: Kaur et al.(2005)

Table 3. 5 General nutrient composition of Kabuli chickpea varieties grown in Australia compared to published literature

Component (g/ 100 g, db)	Kimberley Large	Genesis 090	Genesis Kalkee	PBA Royal	PBA Monarch	Literature
Moisture	6.649±0.13[a]	9.041±0.19[c]	8.158±0.22[b]	8.795±0.11[c]	8.075±0.32[b]	7.68
Protein	18.163±0.21[a]	20.297±.07[b]	24.564±0.05[c]	24.026±0.12[d]	22.925±0.12[e]	20.47
Fat	5.921±0.45[a]	6.707±0.19[a]	5.837±0.36[a]	5.923±1.25[a]	5.871±0.35[a]	4.4-6.04[×]
Ash content	2.856±0.00[a]	2.588±0.05[b]	2.688±0.01[b]	2.564±0.03[c]	2.433±0.01[bc]	2.85
Dietary fibre	16.485 ±0.02[a]	16.536±0.65[a]	18.234±2.33[a]	14.300±0.39[a]	16.181±0.12[a]	12.2-22[+]
Carbohydrate by difference	52.612	44.831	40.519	44.392	44.515	62.95
Starch content	48.881±1.60[a]	32.684±3.67[b]	38.778±1.88[b]	36.596±2.74[b]	38.881±1.10[b]	38.2-43.9*

All values are presented as mean ± standard deviation. Following Tukey's post hoc test, values followed by the same superscript within a row do not differ significantly ($p<0.05$). Literature: USDA(2019), [+] Tosh and Yada(2010), * Wang and Daun (2004), [×] Iqbal, Khalil, Ateeq, & Sayyar Khan,(2006)

3.6.4 Total starch content

The total starch content (Table 3.5) of Kabuli chickpeas ranged from 32.684 to 48.881 g/ 100 g (db) with the highest value for Kimberley Large and the lowest for Gensis090. These values are in agreement with the starch content reported by Özer et al.(2010), but the starch content for Kimberley Large chickpeas is significantly higher than previously reported by Wang and Daun (2004) for Canadian kabuli chickpeas (41.1 g/ 100 g, db). Compared to cereals, legume starches have lower digestibility due to higher resistant starch content (Hoover and Zhou 2003), which makes them highly desirable as resistant starch has been known to have the potential to prevent diabetes, colon cancer and obesity-related complications (Sharma, Yadav, and Ritika 2008). Xu et al. (2013) reported a starch content range of 38.6 to 49.7 g per 100 g (db) for Kabuli chickpeas grown in Virginia, USA, which is also similar to the range obtained in this study. Compared to other legumes such as green gram, black gram, haricot bean and moth bean, Kimberley Large kabuli chickpea from this study have a greater starch percentage (Bravo, Siddhuraju, and Saura-Calixto 1999), which is also similar to the starch content found in cowpeas (49.6 g per 100 g, db) (Huang et al. 2007). The difference in the starch content of Australian Kabuli chickpeas when compared to other varieties of chickpeas can be due to different sowing times, genotypic as well as climatic differences at the growing location.

3.6.5 Amino acid profile

Amino acids (AA) are responsible for protein synthesis, regulated hormone secretion, immune responses, and cell signalling and other essential metabolic processes in the human body. They can also act as antioxidants (Cysteine) or are involved in the synthesis of other amino acids (Wu 2009). Amino acids can be classified as “essential” and “nonessential” based on whether they can be produced in the human body (nonessential) or need to be supplemented through diet (essential) (Wu 2016). The most abundant AA in all chickpea varieties was arginine, aspartic acid, glutamic acid, leucine, lysine and phenylalanine (Table 3.6). Significant ($p \leq 0.05$) differences were observed in the AA profile of chickpea varieties, with Genesis Kalkee containing the highest and Kimberley Large the lowest concentration of most AA. Methionine was a limiting amino acid across all varieties and this finding is in agreement with Wang and Daun (2004) and Jukanti et al.(2012). Higher concentrations of aspartic acid,

glutamic acid, arginine and proline were observed in some chickpea varieties in this study when compared to the previous studies (Bampidis and Christodoulou 2011b, Brenes et al. 2008).

Table 3. 6 Amino acid content of Kabuli chickpea varieties grown in Australia compared to published literature

Amino acids (mg/ kg, db)	Kimberley Large	Genesis 090	Genesis Kalkee	PBA Royal	PBA Monarch	Literature*
Aspartic	21500 ± 707^{a}	22000 ± 0.00^{a}	26000 ± 0.00^{b}	25000 ± 0.00^{b}	26000 ± 0.00^{b}	20400-24000
Serine	9750 ± 212^{a}	10500 ± 707^{ab}	12000 ± 0.00^{b}	11500 ± 707^{b}	12000 ± 0.00^{ab}	12800-13600
Glutamic	31500 ± 707^{a}	32500 ± 707^{a}	38500 ± 707^{b}	36000 ± 0.00^{b}	38500 ± 707^{b}	19400-34300
Glycine	7800 ± 141^{a}	7850 ± 71^{a}	9100 ± 0.00^{c}	8550 ± 71b^{c}	8800 ± 141^{b}	10400-11200
Histidine	4000 ± 141^{a}	4350 ± 71^{b}	5000 ± 0.00^{c}	4700 ± 0.00^{c}	4950 ± 71^{c}	2000-5500
Argenine	14000 ± 0.00^{a}	16000 ± 0.00^{b}	22500 ± 707^{c}	18000 ± 0.00^{d}	19000 ± 0.00^{d}	12700-17800
Threonine	7100 ± 141^{a}	7100 ± 0.00^{a}	7950 ± 71^{b}	7650 ± 71^{b}	7650 ± 71^{b}	8600-9300
Alanine	7800 ± 141^{a}	7950 ± 71^{a}	9100 ± 141^{b}	8550 ± 71^{bc}	8950 ± 71^{c}	10200
Proline	7500 ± 141^{a}	8000 ± 0.00^{b}	9250 ± 71^{c}	8600 ± 141^{c}	9100 ± 0.00^{d}	7600
Tyrosine	4450 ± 71^{a}	4400 ± 0.00^{b}	5250 ± 71^{c}	4850 ± 71^{cd}	5050 ± 71^{d}	6800-8000
Valine	7700 ± 141^{a}	8100 ± 283^{ab}	9150 ± 71^{c}	8800 ± 0.00^{c}	9400 ± 283^{bc}	9800-11500
Lysine	12500 ± 707^{a}	13000 ± 0.00^{ab}	14000 ± 0.00^{b}	14000 ± 0.00^{b}	14000 ± 0.00^{b}	14900-15300
Isoleucine	7350 ± 71^{a}	7650 ± 212^{a}	8750 ± 71^{b}	8450 ± 71^{b}	8900 ± 283^{b}	9100-9600

Table 3.6 Amino acid content of Kabuli chickpea varieties grown in Australia compared to published literature (contd.)

Amino acids (mg/ kg, db)	Kimberley Large	Genesis 090	Genesis Kalkee	PBA Royal	PBA Monarch	Literature*
Leucine	13000 ± 0.00^{a}	14000 ± 0.00^{ab}	15500 ± 707^{c}	15000 ± 0.00^{c}	16000 ± 0.00^{bc}	17000-19000
Phenylalanine	10000 ± 0.00^{a}	11000 ± 0.00^{a}	13000 ± 0.00^{a}	12000 ± 0.00^{a}	13000 ± 0.00^{a}	13100
Methionine	2050 ± 71^{a}	1900 ± 0.00^{a}	2550 ± 212^{a}	2200 ± 283^{a}	2350 ± 71^{a}	2800

All values are presented as mean ± standard deviation. Following Tukey's post hoc test, values followed by the same superscript within a row do not differ significantly ($p<0.05$). Literature*: Brenes et al. (2008), Bampidis and Christodoulou (2011b)

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3.6.6 Individual mineral content

Legumes are characterised by high mineral levels, however, mineral levels depend on the species, agronomic cultivar as well as the soil where the plant was grown (Silva-Cristobal et al. 2010). The individual mineral content of chickpea varieties shown in Table 3.7. Significant ($p<0.05$) differences between varieties were observed. Kimberley Large being the only very large-seeded variety had the highest iron content followed by Genesis Kalkee, Genesis 090, PBA monarch and PBA Royal with the lowest iron content. Kimberley Large also had the highest level of potassium, phosphorus and zinc content however, the sodium content was the lowest out of all varieties. Genesis 090 had the highest amount of magnesium at 1429 mg/ kg (db), whereas PBA monarch had the highest concentration of sodium at 239.33 mg/ kg (db). Compared to other legumes such as cowpea, lentils and green pea, chickpeas are a good source of iron, zinc, and calcium and have similar sodium and magnesium levels (Iqbal et al. 2006). All the chickpea samples analysed in this study have higher iron, magnesium and phosphorus values when compared to the chickpeas analysed by Iqbal et al. (2006).

Several studies have linked the Ca:P ratio in foods to bone health and obesity. A Ca:P ratio of 1.3:1.0 has been suggested to maintain bone health (Kemi et al. 2010, Whybro et al. 1998), whereas a Ca:P ratio above the median of 0.57 has shown to reduce the risk of central obesity based on waist to height ratio (Pereira et al. 2013). None of the varieties in this study meets the Ca:P ratio requirements. The excess of potassium in chickpeas could also be detrimental to the absorption and utilization of other minerals by humans. Both the above-mentioned issues can be corrected by supplementation of other minerals along with chickpea consumption (Iqbal et al. 2006). However, according to the Australian Government Ministry of Health guidelines, the chickpeas analysed in this study can help in meeting the recommended daily intake of iron, zinc and phosphorus in adults (NRV 2017).

Table 3. 7 Individual mineral content of Kabuli chickpea varieties grown in Australia compared to published literature

Minerals (mg/ kg, db)	Kimberley Large	Genesis 090	Genesis Kalkee	PBA Royal	PBA Monarch	Literature
Iron	55.667±0.58a	43.33±0.58bc	45.33±0.58c	36.67±0.58d	43.00±2.00b	30-43.1
Calcium	689.33±6.66a	971.33±29.57b	1012.67±9.71b	1031.00±11.53b	968.33±7.51b	570-1970
Magnesium	1357±62.39a	1429±2.89c	1307±3.51ab	1279±61.78ab	1197±4.00b	46-790
Potassium	10712±15.50a	9821±184.91c	10525±128.51ab	9247±63.52d	10117±73.51bc	7180-11550
Sodium	33.67±0.57a	175.67±0.58b	119.67 ±0.58c	142.67 ±0.58d	239.33±0.58e	240-1010
Zinc	35.33±0.57a	31.00±0.00b	34.00±0.00ab	24.33±0.58d	28.33±1.15bc	27.6-68
Phosphorus	4071±104.62a	3811±59.30ab	3992±55.08a	3253±59.77c	3336±53.11bc	2510-2520
Ca:P ratio	0.169	0.254	0.253	0.316	0.290	0.78

All values are presented as mean ± standard deviation. Following Tukey's post hoc test, values followed by the same superscript within a row do not differ significantly ($p<0.05$). Literature: USDA (2019), Iqbal et al.(2006).

3.6.7 Total polyphenol content and flavonoid content

The total polyphenol (TPC) and flavonoid content of the kabuli chickpea varieties in this study is shown in Table 3.8. The varieties had significantly ($p<0.05$) different amounts of total polyphenol content. Total polyphenol content ranged from 40.757 to 60.451 mg GAE/ 100 g dry sample, with Genesis090 recording the highest and PBA Monarch the lowest polyphenol levels. Segev et al. (2010) reported the effect of different solvents on the extractability of polyphenols and reported significantly different results in the total polyphenol content of the same sample due to difference in the extraction solvent. Acetone (50% v/v) was found to be the best solvent for the extraction of whole seed chickpea polyphenols when compared to 5 other solvents. Thus in this study, 50% acetone was utilised for polyphenol extraction as well. Similar results (57mg TE/100g sample, db) have been reported by Heiras-Palazuelos et al.(2013) in a Mexican kabuli chickpea variety that was extracted using 80% ethanol. In contrast, slightly higher results were reported by Giusti et al.(2017) at 88mg GAE/100g sample (db) for Kabuli chickpeas from the USA which were extracted using 70% ethanol.

As presented in Table 3.8, total flavonoid contents (TFC) were significantly different ($p<0.05$) when comparing varieties. The highest flavonoid content (8.248 mg CE/100g, db) was found in Genesis Kalkee whereas, PBA Royal exhibited the lowest flavonoid content (6.079mg CE/ 100g, db). Slightly higher values were reported by Segev et al. (2010) at 11mg CE/100g sample (db), whereas, Heiras-Palazuelos et al.(2013) reported a flavonoid content of 15mg CAE/ 100 g sample (db) from undigested free polyphenol extracts of kabuli chickpeas. The values obtained are similar to the total flavonoid content of some varieties of legumes such as yellow pea (8.6 mg CE/100g), green peas (6.5 mg CE/ 100g), common beans (9.2 mg CE/ 100 g) and soybean (10.6 mg CE/ 100 g) (Xu, Yuan, & Chang 2007).

Plants have been known to be equipped with antioxidant machinery for combating oxidative stress which is either present in their natural environment or is induced by beneficial microbes or stress conditions (Mittler et al. 2004). It has also been reported that the presence of beneficial microbes (one or a combination of a few) can enhance the total polyphenols and flavonoid levels in chickpeas, in turn increasing the antioxidant capacity (Singh et al. 2014). Thus, chickpeas grown using different

agriculture practices, in different soils around the world will inherently have different levels of bioactive components.

3.6.8 Antioxidant capacity

Three assays (DPPH, ABTS and ORAC) were chosen to determine the antioxidant capacity and the data is reported in Table 3.8. The DPPH and ABTS radical scavenging activity of kabuli chickpea phenolic compounds showed a significant variation ($p \leq 0.05$) between kabuli chickpea varieties. The results demonstrated that Genesis090, the variety with the smallest seed size had the highest DPPH and ABTS antioxidant capacity, while Kimberley large had the lowest values. The descending order of antioxidant capacity of kabuli chickpeas for both antioxidant methods DPPH and ABTS is:

Genesis 090> Genesis Kalkee> PBA Royal > PBA Monarch> Kimberley Large

Both DPPH and ABTS antioxidant capacity from our study corresponded to values reported by Quintero-Soto et al. (2018), who analysed 9 Kabuli chickpea varieties from Mexico, whereas, Marathe et al. (2011) reported slightly higher values for ABTS scavenging activity for Indian kabuli chickpeas. The differences found in our results when compared to previously reported could be due to the difference in variety as well as the extraction method.

It is very important to emphasize that both DPPH and ABTS assay are technically very simple and are based on electron transfer mechanisms and have some limitations compared to the ORAC assay, which is based on the hydrogen atom transfer mechanism. Peroxyl radicals have been shown to react very slowly or being inert to 2-2-diphenyl-1-picrylhydrazyl (DPPH), whereas, the ABTS values for pure antioxidant compounds such as α-tocopherol, ascorbic acid and uric acid are almost the same and show no correlation with the number of electrons they can transfer. Compounds with similar chemical structures have also shown to have significantly different ABTS values (quercetin and kaempferol) (Institute of Medicine Panel2000).

Table 3. 8 Total polyphenol content, total flavonoid content and contents of total antioxidant capacity in Kabuli chickpea whole seed measured using the DPPH (mg TE/g, sample db) and ABTS (mg TE/g sample, db) assay

Property	Kimberley Large	Genesis 090	Genesis Kalkee	PBA Royal	PBA Monarch	Literature
TPC(mg GAE/100g, db)	60.395±0.98a	60.451±0.57a	51.758±0.31b	46.419±0.65c	40.757±0.94d	46-88 (Heiras-Palazuelos et al. 2013, Giusti et al. 2017, Marathe et al. 2011)
TFC(mg CE/100g, db)	6.214±0.24a	6.719±0.19ac	8.428±0.241b	6.079±0.00a	7.103±0.09c	11-15 (Segev et al. 2010, Heiras-Palazuelos et al. 2013)
DPPH (mg TE/100g, db)	42.939±1.31a	60.955±0.89b	53.712±0.07c	51.008±0.15cd	50.044±1.16d	9.76-51.56 (Quintero-Soto et al. 2018)
ABTS(mg TE/100g, db)	78.947±0.50a	84.992±0.96b	84.541±2.55b	84.386±1.52b	81.639±0.10ab	69.58-103.37 (Quintero-Soto et al. 2018)

Abbreviations: TPC- total polyphenol content; TFC- total flavonoid content; DPPH- 2-diphenyl-1- picryhydrazyl radical scavenging activity; ABTS- 3 ethylbenzothiazoline-6-sulfonic acid diammonium salt assay. All values are presented as mean ± standard deviation. Following Tukey's post hoc test, values followed by the same superscript within a row do not differ significantly ($p<0.05$).

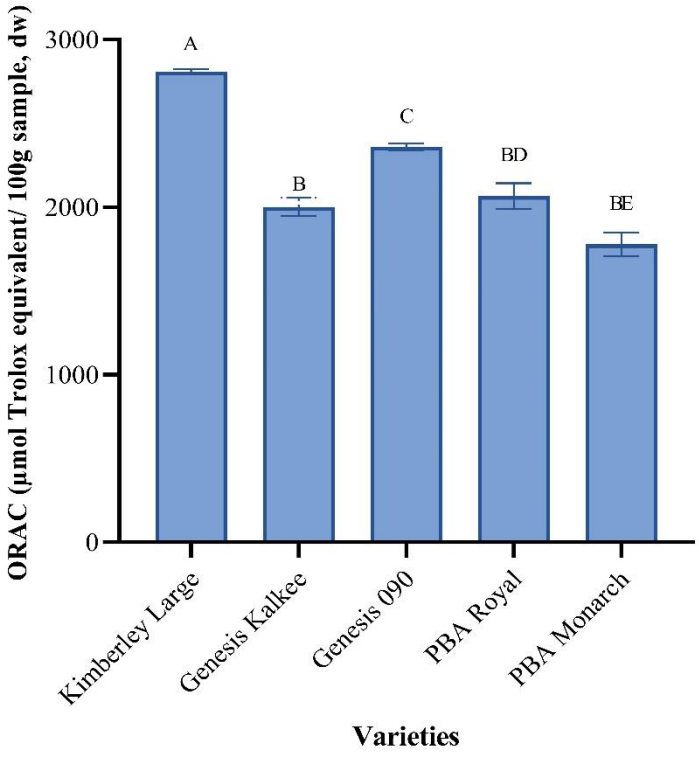

Figure 3.3 Oxygen radical absorbance capacity (ORAC) of Kabuli chickpea varieties. Different letters above bars indicate significant differences ($p \leq 0.05$) following Tukey's post hoc test.

The ORAC values for kabuli chickpea extracts have been reported in Fig. 3.3 and significant differences ($p \leq 0.05$) between ORAC values were observed. The ORAC assay is based on hydrogen atom transfer and it is also the only method that considers complete free radical action, uses area under the curve (AUC) technique for determination of the antioxidant capacity value, thus combining both percentage of inhibition and the inhibition time of the free radical actions by antioxidants in one value (Prior and Cao 1999). It is also considered more accurate due to the use of a biologically relevant free radical source (Prior et al. 2003). Based on the ORAC assay, the descending order of antioxidant capacity of kabuli chickpea varieties is:

Kimberley Large> Genesis 090> PBA Royal >Genesis Kalkee> PBA Monarch

A significant ($p \leq 0.01$) positive correlation was found between TPC and ORAC values ($r=0.834$). In a study by Xu et al. (2007) on cool-season legumes, it was reported that both the DPPH and ABTS assay results were highly correlated in contrast to ORAC assay results. The food and nutraceutical industries have accepted the method to a

point that they have started including ORAC values in their product labels (Bank and Schauss 2004), making it the most relevant method out of all three described in this study. Thus from the results presented in Fig 3.3, it can be concluded that Kimberley Large has the highest and PBA monarch has the lowest biologically relevant antioxidant capacity amongst all the varieties.

3.7 Conclusion

The present work is the first comparative and detailed study on the physical, nutritional and bioactive properties of kabuli chickpea varieties grown exclusively in Australia. This study establishes that Australian kabuli chickpeas are on par or even superior in some of these traits when compared to Indian, Canadian, Sicilian or Mexican kabuli chickpea varieties. The differences found between varieties could be due to the genotype effect or possibly due to the different recommended locations where these chickpeas were grown. The data has also been shown to be comparable to the USDA Nutrient Database as well as widely available literature. Furthermore, the values found for bioactive compounds fit the ranges reported in the literature. Some varieties in this study showed higher antioxidants capacity values and it is suggested that these varieties can be utilized in breeding programs for the selection of chickpea varieties with improved nutraceutical traits.

3.8 Acknowledgement

Our sincere thanks to the National Measurement Institute for their expertise with amino acid analysis.

CHAPTER 4 Effect of genotype by environment, and their interactions, on the physical, nutritional and bioactive properties of Australian kabuli chickpeas

"A fertile soil alone does not carry agriculture to perfection."

E. H. Derby

Abstract

Chickpeas are one of the most widely consumed legumes in the world and for the development of higher quality seed and better-adapted chickpea varieties, it is important to understand the effects of genotype, growing environmental conditions and their interactions on seed properties. Thus, in this chapter, the physical properties, proximate composition, polyphenol content and antioxidant capacities of four common Australian kabuli chickpea varieties grown at five locations around Australia in the 2018 National Variety Trials were evaluated. The results indicate that genotype, environmental conditions and their interactions had significant effects on the physical property of swelling capacity, proximate composition, polyphenol content and antioxidant capacities. Significant positive correlations between polyphenol content and antioxidant capacities were identified, as well as a negative correlation between protein and starch content, and protein and antioxidant capacities. A comparison of seed properties using the principal components analysis (PCA), where the first four principal components explained over 87.86% of the variation, showed a clear separation of the chickpea varieties based on the growing environment. Given the significant effects of genotype, environment and their interaction on chickpea seed properties, the results obtained in this study will provide plant breeders valuable information to develop varieties that are better able to respond to growing season environment with high nutritional and antioxidant properties. Farmers can utilise this information to enhance desirable properties in kabuli chickpeas based on the growing environments on the farm and may have the potential to receive higher returns by targeting specific food markets.

4.1 Introduction

Chickpeas are an important pulse crop with a total world production of 14 Mt in 2019, with Asia being the largest producing region followed by Oceania (FAOSTAT 2019). It is a staple food in India, the Americas, North and East Africa, and southern Europe (Frimpong et al. 2009). Chickpeas are an important winter/spring grown pulse crop in Australia, with over 95% of the production exported. Kabuli chickpeas are grown in Australia command a price premium of around AUD$100/ tonne greater than desi types due to demand and their high quality (high germination rate, vigour and seed weight) (GRDC 2018b).

Chickpeas are a popular alternative to animal protein and have been incorporated into a number of products such as biscuits (Yadav, Yadav, and Dhull 2012) and bread (Mohammed, Ahmed, and Senge 2014, Man et al. 2015), as well as in chickpea blended meat products such as chicken nuggets, sausages and meatballs (Verma, Banerjee, and Sharma 2012, Thushan Sanjeewa et al. 2010, Asgar et al. 2010). More recently, chickpeas have been incorporated into rice noodles to increase the product protein content (Sofi, Singh, et al. 2020) and to replace meat in healthy burgers (Argel et al. 2020), highlighting the varied functionality of chickpeas. A steadily growing demand and an increased interest in plant-based protein sources make chickpeas an economically important crop for the future (Jukanti et al. 2012, Merga and Haji 2019). However, like many other crops, chickpea seed properties and chemical composition are altered when grown under different environmental conditions through changes in metabolic and enzymatic activities (Varol et al. 2020, Carvalho, Ricardo, and Chaves 2004, Frimpong et al. 2009). Awasthi et al. (2014) observed a significant decrease in sucrose and starch content in chickpeas of Indian origin due to heat stress and water deficit, with higher reductions under combined stress conditions. Carvalho, Ricardo, and Chaves (2004) found that water deficit during grain filling in lupin plants, an example of another legume crop, resulted in a decrease in fat and total soluble sugar content in seeds. No studies however have yet characterised the quality variables of Australian kabuli chickpeas in detail since they have focused on desi types or varieties of a different origin such as India, Syria (Yadav et al. 2010), Canada (Frimpong et al. 2009), Spain (Cobos et al. 2017), Turkey (Tayyar, Egesel, Gül, et al. 2008) and the United States (Vandemark et al. 2020).

In terms of studies on the effects of genotype and environment and their interaction, Wang, Gangola, et al. (2017) reported on over 49 chickpea genotypes of international origin whereas Kaloki, Trethowan, and Tan (2019) identified effects of genotype and environment on Australian desi chickpea yield and performance consistency. However, no information on the effects of genotype, environment and their interaction on Australian kabuli chickpea physical, nutritional and bioactive composition has been reported. In this context, this study investigated the physical and compositional properties, along with antioxidant capacities of four Australian kabuli chickpea varieties grown in different environments at five locations across Australia in 2018. The results of this study will help inform selection of cultivar and production location by farmers to target specific consumer markets.

4.2 Materials and methods

Four representative common chickpea varieties (viz. Almaz, Genesis 090, Kalkee and PBA Monarch) (Fig. 1) were collected from five 2018 National Variety Trials (GRDC NVT Online https://www.nvtonline.com.au/) (Birchip, Vic; Kununurra, WA; Rainbow, Vic; Tarranyurk, Vic; and Walgett, NSW) selected with different environmental growing season environments (Table 1). After receival, samples were sieved to remove unwanted material (broken seeds, dirt and stones), vacuum packaged and stored at 4°C until analysis. Figure 2 details the major seasonal rainfall zones of Australia (Bureau of Meteorology, 2021) with Birchip, Rainbow and Tarranyurk described as having a winter rainfall, Kununurra a summer dominant rainfall and Walgett with wet summers and low winter rainfall. Based on temperature and humidity, Kununurra can be classified as a hot humid summer zone, whereas Walgett has a wet summer and the other locations have hot dry summers and cold winters (Bureau of Meteorology, 2021).

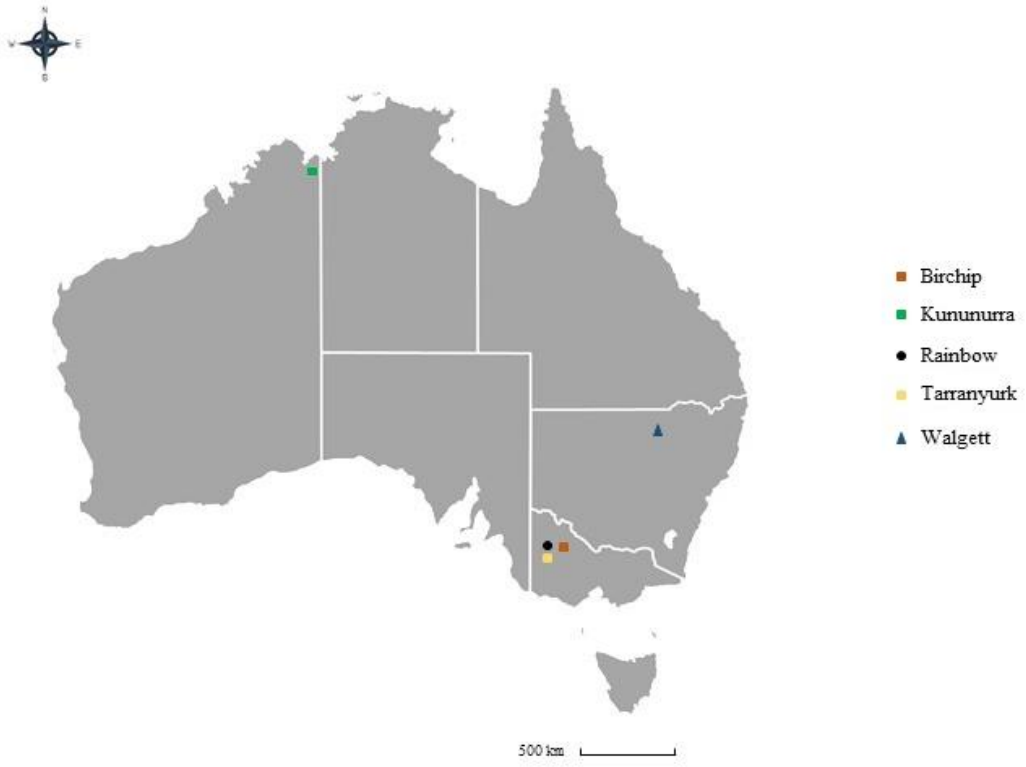

Figure 4.1 Map of Australia showing the location of the five National Variety Trials in 2018 (GRDC NVT Online https://www.nvtonline.com.au/) where kabuli chickpea varieties selected for the study were grown.

Table 4.1 Geographic variables of chickpea trial production environments

Environment	Latitude	Longitude	Sowing date	Harvest date	Soil type*
Birchip, Vic.	35.9833° S	142.9146° E	14/05/2018	25/11/2018	clay
Kununurra, WA	15.6049° S	128.7655° E	18/05/2018	8/10/2018	sandy-loam / loam
Rainbow, Vic.	35.9008° S	141.9972° E	11/05/2018	26/11/2018	sandy-loam
Tarranyurk, Vic.	36.2128° S	142.0340° E	4/06/2018	20/12/2018	clay
Walgett, NSW	30.0268° S	148.1231° E	30/05/2018	14/11/2018	clay

*(CSIRO 2020)

Figure 4.2 Major seasonal rainfall zones across Australia (Bureau of Meteorology 2018)

Figure 4.3 Representative samples of four kabuli chickpea genotypes (Almaz, Genesis 090, PBA Kalkee and PBA Monarch) grown at five Australian locations (Birchip, Vic; Kununurra, WA; Rainbow, Vic; Tarranyurk, Vic and Walgett, NSW) in 2018.

4.3 Physical analysis

Two random samples of 50 seeds from each variety were withdrawn before the analysis. Physical properties of the whole seeds were analysed in duplicate by determining the following using standards methods (Kaur, Singh, and Sodhi 2005, Williams 1983) as previously described in Chapter 3 (Section 3.3):

- Seed weight (S.W.) (g): Obtained weight of 50 seeds, converted to weight per seed.
- Volume (mL): obtained volume of 50 seeds, converted to volume per seed.
- Density (g/ mL): The ratio between weight and volume.
- Hydration capacity (H.C.): calculated using the formula: $HC = (W_s - W_0)/ 100$, where W_s is weight of 50 seeds after soaking for 12 hours, W_0 is weight of seeds before soaking.
- Hydration index (H.I.): Hydration capacity/ seed weight.
- Swelling capacity (S.C.): calculated using the formula: $SC = (V_s - V_0)/ 100$, where V_s is volume of 50 seeds after soaking for 12 hours and V_0 is volume of 50 seeds before soaking.
- Swelling index (S.I.) (%): Swelling capacity/ seed volume.

4.4 Chemical analyses

4.4.1 Sample preparation

A random sample of 200g seeds of each variety was milled using a CEMOTEC 1090 grinder (Foss Tecator, Hoganäs, Sweden) to allow them to pass 100% through a 500-micron sieve. The milled samples were then vacuum packaged and stored at -20 ± 2°C for two weeks to inactivate any living insects which might be present in the samples, followed by storage at 4°C until analysis.

4.4.2 General nutrient analyses

General nutrient analyses were performed in triplicate using the following standard methods:

- Moisture content (g/ 100 g) using the "Modified Solids (total) and Moisture in Flour - oven drying method" (Method 925.10, AOAC International 2008).

- Protein (g/ 100 g) by Kjeldahl digestion, distillation and titration with a nitrogen conversion factor of 6.25 (Method 920.87, AOAC International 2005b).
- Fat (g/ 100 g) by the Soxhlet solvent extraction technique (Method 963.15, AOAC International 2006).
- Ash (g/ 100 g) by the "*Ash of Flour*" method (Method 923.03, AOAC International 2005a).

4.4.3 Total starch content

Starch content (g/ 100g, db) was determined in duplicate using the standard colorimetric
technique (AOAC International 2005c) using the total starch assay kit (AA/AMG) from Megazyme International Ltd (Bray, County Wicklow, Ireland).

4.4.4 Polyphenol extraction and total polyphenol content

Total polyphenols were extracted as per Segev et al. (2010) with a few modifications as previously described in Chapter 3 (Section 3.5.6) of this thesis. They were then quantified in duplicate using the Folin-Ciocalteu method (Wu et al. 2017) as previously described in section 3.5.7 of this thesis.

4.4.5 Antioxidant capacity

The antioxidant capacities of the total polyphenols extracts were determined in duplicate using the 2-2-diphenyl-1-picrylhydrazyl (DPPH), 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS), and oxygen radical absorbance capacity colorimetric methods (Thaipong et al. 2006). These methods were previously describe in more detail in Chapter 3, Section 3.5.8 of this thesis.

4.5 Statistical analysis

Data is presented as combined means of variety and environment. All statistical analyses were performed using Genstat Version- 20.1.2.24528 (VSN International Ltd, UK). Two-way ANOVA was performed to investigate the main effects of genotype, environment and their interaction. Where significant results ($p<0.05$) were returned, Tukey's post hoc test was used to separate the mean values within each genotype or environments. Pearson's correlation was used to reveal associations between seed quality variables prior to running a genotype main effect by- genotype

x environment (GGE) biplot analysis. The GGE biplot analysis, which uses a bi-plot to present factors (genotype and genotype x environment (G × E) interaction), was utilised to visualise the multi-environment data. It was used to graphically display the G x E interaction of a two-way table (Yan 2011) and to determine the "which-won-where" pattern for recommending specific genotypes to environments (Mattos et al. 2013, Tena et al. 2019). An unequivocal display of "which-won-where" pattern was obtained by the polygon view of a GGE biplot with symmetric scaling. The polygon was formed by connecting genotypes located in different sectors, which represented the extent of response of that variety to the tested environments. Other genotypes can have smaller vectors, and thus be contained within the polygon i.e. in relation to the interaction of environments in that sector. These genotypes are therefore less responsive to the growing environment for that particular trait. A set of perpendicular lines intersect the sides of the polygon, dividing the biplot into several sectors. Genotypes furthest away from the origin in a sector were the best performing compared to the other genotypes in that sector (Yan and Rajcan 2002, Yan et al. 2001, Mohammadi et al. 2009). One or more environment within the same sector can be enclosed within an oval, forming a mega-environment. The genotype on the vertex of the polygon, contained in a sector along with an environment or a mega-environment, performs best in the environment furthest away from the origin of the biplot and is one of the best performing genotypes in the other environments located in the same sector (Yan and Rajcan 2002). Environments near the origin have little to no influence on the quality variable being investigated and does not affect the genotypes performance in relation to that quality variable.

As the GGE biplot can visually represent the G × E interaction of only one variable at a time, a principal components analysis (PCA), was used in order to visually represent the overall effect of all quality variables. Within the principal components analysis, the correlation matrix in principal components analysis was used to convert all the variables to a common scale (Bennett and Bullitta 2003) and the results of Pearson's correlation were used to exclude certain highly correlating variables to prevent potential bias.

4.6 Growing season environment

The growing period, number of extreme high (temperature above 30°C) and low-temperature instances (temperatures below 0°C) within the growing season and the

number of rainfall instances have been reported for all growing environments in Table 4.2. Out of all the environments, Kununurra had the shortest growing period of 142 days, whereas Tarranyurk reported the longest growing period (199 days). Kununurra also exhibited the highest number of extreme high temperature instances, followed by Walgett and Tarranyurk. The number of extreme low temperature instances differed in each environment, the highest for Walgett (23 days), followed by Birchip and Tarranyurk (14 days), and Rainbow (9 days). Kununurra was the only location to record no extreme low temperature instances. Tarranyurk received the highest number of rainfall instances during the growing period (75 days), followed by Rainbow and Birchip. As Kununurra has a summer dominant rainfall season, chickpeas in this environment were grown on stored soil moisture with irrigation. All the environments from the state of Victoria (Birchip, Rainbow and Tarranyurk) exhibited very high mean rainfall in the month of December 2018, as shown in Fig. 4.4.

Table 4.2 Summary of growing period, number of days recording extreme high and low temperatures, and number of rainfall days at five NVT trials

Location	**Growing period (days)**	**Extreme high temperature instances (above 30° C)**	**Extreme low temperature instances (below 0° C)**	**Rainfall instances**
Birchip, Vic	194	10	14	53
Kununurra, WA*	142	119	0	1
Rainbow, Vic	198	8	9	62
Tarranyurk, Vic	199	19	14	75
Walgett, NSW	169	35	23	24

***irrigated

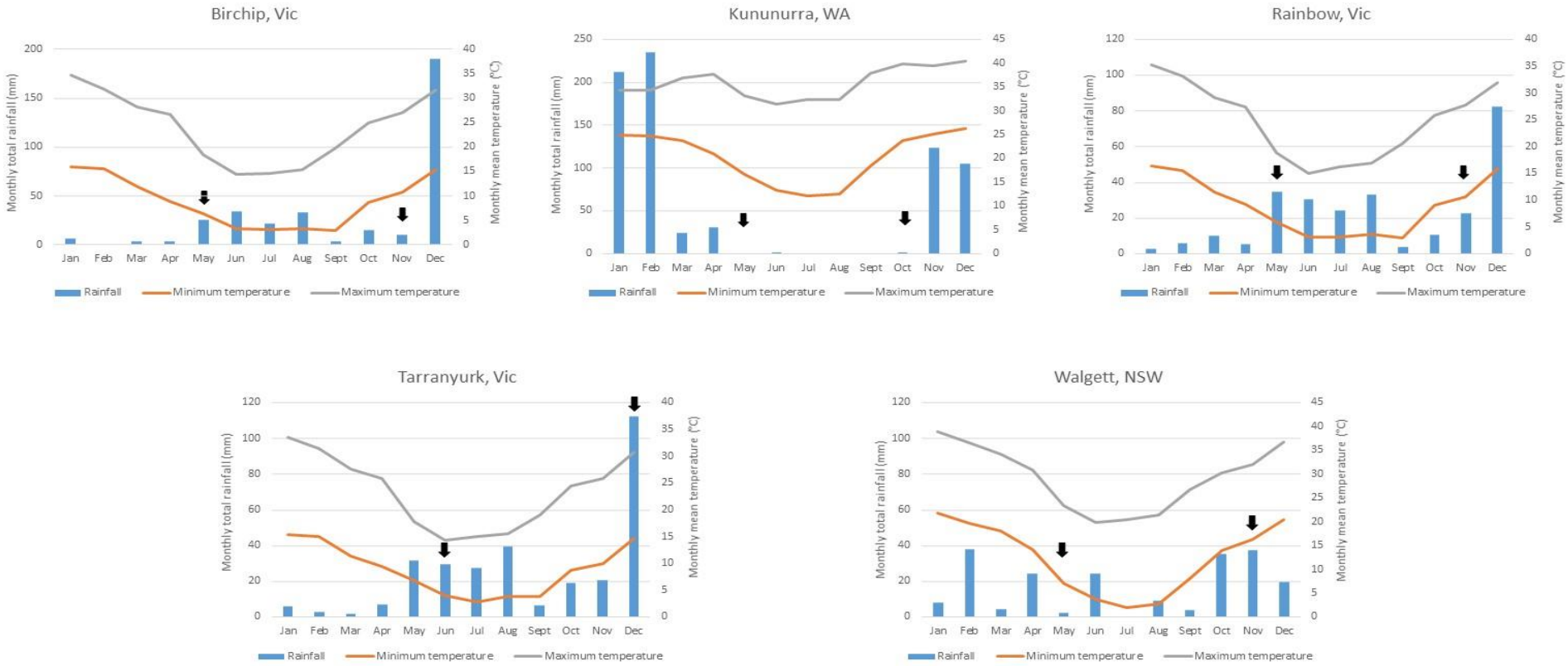

Figure 4.4 Total monthly rainfall and mean minimum and maximum monthly temperatures at the five growing environments (Birchip, Vic; Kununurra, WA; Rainbow, Vic; Tarranyurk, Vic; Walgett, NSW) in the year 2018 (BOM, 2018). First black arrow represents sowing month, with the second black arrow representing harvest month.

4.7 Results and discussion

A significant ($p \leq 0.05$) effect of genotype (chickpea variety), environment, and their interactions were observed for kabuli chickpea varieties grown in five NVT trials across Australia for their physical properties, nutritional composition, and polyphenol and anti-nutritional factors.

4.7.1 The effect of genotype, environment and their interaction on the physical properties of Australian kabuli chickpea varieties

The main effects of variety, environment and their interaction on physical properties of chickpea varieties are reported in Table 4.3. Significant ($p<0.05$) main effects of genotype and environment were observed for all seed physical properties (except density with no main effects of variety). Significant ($p<0.05$) G × E interactions were also observed for hydration index, swelling capacity and swelling index (Table 4.3). The results indicate variability in seed properties among varieties and their response to different growing environments (genotypic plasticity), however, a higher main effect of growing environment was observed on chickpea seed properties.

Out of all the environments, chickpeas grown at Tarranyurk had the highest mean seed weight (S.W.) (0.364 g) as Tarranyurk received higher rainfall during the growing season (Table 4.2) and an unusually high mean rainfall (around 112 mm) in the harvest month of December (Fig. 4.4). Higher water availability and cooler temperatures led to an increased seed weight in chickpeas grown at Tarranyurk when compared to other environments. Our results are in agreement with those of Zhong et al. (2020), who also reported higher seed weight in Australian lupin varieties grown at Wongan Hills (WA) which received higher mean rainfall and had cooler temperatures compared to Eradu (WA). The highest volume was recorded for chickpeas from Tarranyurk (0.383 mL) and lowest for chickpeas from Rainbow (0.218 mL). Seed weight across varieties was significantly ($p<0.05$) different, with Kalkee showing the highest seed weight (0.387g) and Genesis 090 the lowest (0.293g) averaged across all environments. Similarly, Kalkee and PBA monarch showed the highest seed volume at 0.296 mL and 0.277 mL respectively and Genesis 090 the lowest (0.224 mL).

Table 4. 3 Mean squares of ANOVA for each variable, along with the percentage coefficient of variation (CV) for four kabuli chickpea varieties grown in five different environments in Australia (** Significant at p< 0.001, * significant at p<0.05)

Trait	Environment (E)	Genotype (G)	G x E	CV (%)
Seed weight	0.0033**	0.016**	0.0006	10.3
Volume	0.012**	0.010**	0.0004	10.6
Density	0.180**	0.001	0.004	0.8
Hydration capacity	0.001*	0.019**	0.0009	11
Hydration index	97.61**	121.89**	12.761*	2.9
Swelling capacity	0.003**	0.016**	0.001*	9.2
Swelling index	6755.8**	167.67*	158.84*	2.4
Moisture	28.377**	0.189	0.961**	1.7
Protein	41.125**	1.334*	0.891*	1.5
Fat	0.648**	0.258**	0.634**	2.8
Ash	0.805**	0.032**	0.009**	1.8
Starch	240.36**	16.250**	18.707**	4.9
TPC	732.280**	123.296**	55.711**	5.9
DPPH	39.140**	3.475*	6.479*	3.3
ABTS	510.77**	136.957**	80.242**	4.7
ORAC	112272.8**	26762.6**	1997.2**	11.1

Where TPC = total polyphenol content; DPPH = 2-2 diphenyl-1-picrylhydrazyl antioxidant assay; ABTS = 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt antioxidant assay; ORAC= oxygen radical antioxidant capacity

In contrast to seed weight, chickpeas grown at Tarranyurk recorded the lowest density values (1.154 g/ mL) and those from Rainbow the highest (1.477 g/ mL). Kaur, Singh, and Sandhu (2004) reported a positive correlation between seed density and setback viscosity (an indication of the tendency of starch pastes to retrograde) in black gram seeds. A high setback viscosity is indicative of low cooking quality as the seed starch has a greater tendency to retrograde and thus become too firm upon cooking (Fazeli Burestan, Afkari Sayyah, and Safi 2021). Thus, chickpeas from Rainbow may exhibit low cooking quality due to their high seed density, when compared to chickpeas from Tarranyurk, and the cooking quality of Almaz chickpeas will be superior to PBA Monarch chickpeas due to their low seed density. Density also affects structural load and thus is an important factor in the design of drying and storage equipment (Baryeh 2001).

Previous studies have reported a positive relationship between moisture content, and seed weight and volume, and a negative relationship with seed density in lentils (Amin, Hossain, and Roy 2004), kidney bean, pea and black eyed peas (Altuntas and Demirtola 2007), chickpeas (Nikoobin et al. 2009), and faba bean (Altuntaş and Yıldız 2007). However, as the moisture content of all varieties in our study was very similar, the effect of moisture content on seed weight, volume and density was not apparent. Instead, all four varieties grown at Birchip had high seed weight, whereas seeds of varieties grown at Rainbow were denser.

Similar to volume, the hydration capacity of chickpeas from Tarranyurk was the highest (0.362 g/ seed) and the lowest values were obtained for chickpeas from Rainbow (0.332 g/ seed). Chickpeas from Kununurra had the highest hydration index (105.8) and those from Birchip the lowest (98.2). The highest swelling capacity was found in seeds from Birchip (0.403 mL/ seed) and the highest swelling index was found in seeds from Rainbow (179.8), whereas seeds from Tarranyurk had the lowest values for both of these variables (0.354 mL/ seed, 112.4). Between varieties, hydration and swelling capacity followed the same pattern as seed weight, with the highest values for Kalkee (0.386 g/ seed and 0.411 mL/ seed respectively) and lowest for Genesis 090 (0.294 g/ seed and 0.327 mL/ seed respectively) were found. Saha et al. (2009) reported very similar values to those in this thesis for hydration capacity (0.43 g/ seed) and swelling capacity (0.40 mL/ seed) in common beans from India, whereas the swelling capacity for 91 Turkish chickpea varieties ranged from 0.14 to

0.32 mL/ seed (Özer et al. 2010), which is lower than the swelling capacity of chickpea varieties from our study (Table 4). In a study by Kaur, Singh, and Sodhi (2005), the swelling capacity and swelling index for different Indian chickpea varieties ranged from 0.11-0.23 mL/ seed and 103.1-136.5 respectively, and these values are lower than those found in our study due to the lower seed weight of chickpeas from India.

Knowledge of seed physical properties is vital to assist and improve the design of processing and storage equipment as it impacts greatly on the functioning of these equipment (Yalçın and Özarslan 2004). Hydration capacity and hydration index is directly related to the conclusion temperature (T_c) of starch gelatinisation in legumes (Kaur, Singh, and Sandhu 2004) and is influenced by differences in variety and growing conditions (Ma et al. 2017). Low T_c indicates lower starch gelatinisation temperature, attributed to the content of short amylopectin chains in legume starches (Hoover and Ratnayake 2002) and translates into increased enzymatic digestion of starch (Srichuwong et al. 2005). Seed size and seed coat thickness play a major role in water absorption properties, and chickpeas with a highly permeable seed coat may exhibit higher swelling capacity (Özer et al. 2010). Swelling capacity is an important parameter in food products (such as canned chickpeas) where a change in volume post-processing enhances the acceptability of the final product (Ayodele and Beatrice I. O. 2015).

4.7.2 Nutritional composition

The content of all the nutritional components varied significantly ($p<0.05$) between varieties, environments and their interaction (Table 4.3), except moisture content which had no significant interaction with the environment. The average protein content across environments was 21.36 g/ 100 g (db) with the highest protein content found in chickpeas grown at Rainbow (23.69 g/ 100 g, db) and Birchip (23.59 g/ 100 g, db) and the lowest protein content in chickpeas grown at Walgett (18.77 g/ 100 g, db). Between varieties, the protein content ranged from 20.83- 21.68 g/ 100 g (db), with Genesis 090 containing the highest and PBA Monarch the lowest concentration. Overall, the variety Kalkee from Rainbow contained the highest protein levels (24.34 g/ 100g, db) and PBA Monarch from Walgett contained the lowest (18.23 g/ 100 g, db) (Appendix 1, Table S 1). The protein content for varieties in this study was similar to the level previously reported in the literature (Nikolopoulou et al. 2006a, Frimpong

et al. 2009). Sowing times significantly affect the total dry matter of chickpeas (McKenzie and Hill 1995), in turn influencing the protein content in the seeds (López-Bellido et al. 2008, Tayyar, Egesel, Gül, et al. 2008, Varol et al. 2020). Tao et al. (2017) reported that a wetter climate during the vegetative growth period of field pea plants resulted in higher protein content in dry pea seeds The earlier sowing dates at Birchip and Rainbow (Table 4.1) in combination with a lower number of extreme temperature days (days with temperatures above 30° C) and availability of water had a positive effect on the protein content of all chickpea varieties from these growing environments (Khan et al. 2011, Mafakheri et al. 2011, Tao et al. 2017, Croser et al. 2003).

The average fat content across environments was 4.96 g/ 100g (db) (Table 4.4) and the highest fat content recorded in chickpeas grown at Tarranyurk (5.317 g/ 100g). The fat content between varieties ranged from 4.765 to 5.144 g/ 100 g (db), with PBA Monarch recording the highest levels and Almaz the lowest. The overall highest fat content was observed in variety Kalkee from Tarranyurk (5.59 g/ 100 g, db) and Almaz grown in Walgett contained the lowest fat concentrations (4.09 g/ 100 g, db) (Appendix 1, Table S 2). The fat content of chickpea varieties from this study was in agreement with the levels reported by Özer et al. (2010) (4.45- 6.11 g/ 100g sample) and Ravi and Harte (2009) (5.31 g/ 100 g sample) for Turkish and Indian genotypes respectively, but slightly higher than those reported by Summo, De Angelis, Ricciardi, et al. (2019) (3.3- 4.0 g/ 100 g sample) in four different genetic clusters in their research. Water availability during the growing season significantly affects the fat content in legumes, with lower water availability leading to a lower fat content in harvested seeds (Chibarabada, Modi, and Mabhaudhi 2017). In this study, the highest fat content was observed in chickpeas grown at Tarranyurk, which also received the highest rainfall (265 mm) during the growing season out of the five environments. Our results are in agreement with those of Chibarabada, Modi, and Mabhaudhi (2017), which reported similar patterns of reduction in the fat content of groundnut (*Arachis hypogaea*), bambara groundnut (*Vigna subterranean*) and cowpea (*Vigna unguiculata)* seeds as a result of lower water availability when compared to the seeds grown with optimum irrigation. Similarly, in agreement with our present findings, Ali, Ashraf, and Anwar (2010) also reported a significant decrease in the oil content of corn grown under drought stress in Pakistan.

The mean starch and ash content across all growing environments was found to be 22.74 g/ 100 g (db) and 2.672 g/ 100 g (db). Both ash and starch content was found to be the highest in seeds from Kununurra (3.069 g/ 100 g and 28.72 g/ 100 g respectively) which also received the lowest rainfall out of the five growing environments. Whereas, chickpeas from Rainbow contained the lowest amount of starch (14.08 g/ 100 g) and those from Birchip (2.269 g/ 100 g) had the lowest ash content. The highest levels of ash were obtained in variety Almaz (Kununurra) at 3.13 g/ 100 g (db) and the lowest levels were obtained in Genesis 090 (Birchip) at 2.175 g/ 100 g (db) (Appendix 1, Table S 3). The highest total starch content was found in Genesis 090 from Kununurra (31.69 g/ 100 g, db), whereas variety Kalkee grown at Rainbow exhibited the lowest levels (12.43 g/ 100 g, db) (Appendix 1, S 4). A rainy environment has previously been linked to reduced starch accumulation in wheat from the Czech Republic (Faměra et al. 2015) and chickpeas from 26 countries (Wang, Gangola, et al. 2017), due to α- amylase activity, and our results are in agreement with these studies. The starch content across varieties was similar, however, PBA Monarch exhibited the highest ($p \leq 0.05$) ash content (2.728 g/ 100 g, db). Özer et al. (2010) reported an ash content of 2.54- 3.48 g/ 100g for 91 Turkish chickpea genotypes and our results were slightly lower than these ash levels.

4.7.3 Total polyphenol content and antioxidant activity

Plant polyphenolics execute a wide spectrum of biological functions from being structural components in cell walls to participating in growth and developmental processes (Brown et al. 2001). However, one of their main functions in plants is protection from severe environmental stress due to unfavourable growing conditions (Bautista et al. 2015, Di Ferdinando et al. 2014). Plant polyphenols have been shown to reduce the effects of harmful ultraviolet rays, oxidation inhibition and improving water retention in plants facing thermal stress (Agati et al. 2012). The total polyphenol content and antioxidant capacity values were significantly ($p<0.05$) affected by genotype (variety), environment and G × E interaction (Table 4.3).

Varieties grown at Kununurra and Walgett had significantly higher total polyphenol content when compared to genotypes from other locations Chickpeas from Walgett and Kununurra contained the highest TPC levels (67 and 66.66 mg GAE/ 100 g sample (db), respectively), whereas samples from Birchip contained the lowest levels (43.62

mg GAE/ 100 g sample, db) (Table 4.5). Out of the 20 samples analysed, Genesis 090 chickpeas from Walgett contained the highest polyphenol levels, whereas Kalkee from Birchip contained the lowest (Appendix 1, Table S 5). Kununurra faced the most high temperature instances out of all locations, whereas Walgett had the 2nd highest number and the highest number of low-temperature instances (Table 4.2). These temperature extremes may have resulted in the high polyphenol content in samples from these locations as has been suggested in previous research on other grains (Wu, Johnson, Bornman, Bennett, Clarke, et al. 2016, Sallas et al. 2003). Across varieties, the highest TPC was exhibited by Genesis 090 (62.88 mg GAE/ 100g sample, db) and the lowest TPC was obtained for PBA Monarch (54.59 mg GAE/ 100g sample, db), significant at $p<0.05$. The mean TPC (59.48 mg TE/ 100g sample, db) for varieties from this study is very similar to TPC levels reported by Heiras-Palazuelos et al. (2013) for Mexican kabuli chickpeas (57 mg TE/ 100g sample, db), but the study highlights the variability around this value when grown in different environments and between varieties.

The values for DPPH, ABTS and ORAC antioxidant capacity of chickpeas followed the same pattern as TPC (Table 4.5), with chickpeas from Walgett containing significantly ($p\leq0.05$) higher values for DPPH (19.65 mg TE/ 100 g sample, db), ABTS (85.19 mg TE/ 100 g sample, db) and ORAC (583.0 µmol TE/ 100 g sample, db) antioxidant capacity respectively when compared to other environments. The only exception observed was the ABTS antioxidant capacity of chickpeas from Kununurra (86.53 mg TE/ 100 g sample, db) being similar to that of chickpeas from Walgett. In contrast, chickpeas from Birchip had the lowest antioxidant capacities (DPPH - 14.44 mg TE/ 100 g sample, ABTS - 66.64 mg TE/ 100 g sample and ORAC - 290.2 µmol TE/ 100 g) (Table 4.5). Our results for DPPH and ABTS antioxidant capacity were similar to those reported by Quintero-Soto et al. (2018) for nine Mexican kabuli chickpea genotypes grown under irrigation. The mean value for total polyphenol content, DPPH and ABTS antioxidant capacity values for Genesis 090 chickpeas from all growing environments was the highest ($p<0.05$), in contrast to PBA monarch with the lowest values. PBA monarch recorded significantly ($P<0.05$) lower ORAC value (403.8 µmol TE/ 100 g sample) in comparison to both Genesis 090 (498.7 µmol TE/ 100 g sample, db) and Kalkee (516.8 µmol TE/ 100 g sample, db), which had the highest levels. Out of all the varieties, Kalkee when grown at Walgett exhibited the

highest ORAC values and PBA Monarch when grown at Birchip exhibited the lowest (Appendix 1, Table S 6).

Similar to the TPC values, all antioxidant capacity assay values viz. DPPH, ABTS and ORAC were found to be significantly ($p< 0.05$) higher in varieties grown at Kununurra and Walgett. It can be suggested that the extreme temperature instances recorded at Kununurra and Walgett during the seed development and maturation periods (September and November 2018) resulted in increased production of polyphenols in the plants, which were then distributed to the different plant organs, including the seeds, reflecting as higher values for TPC, DPPH, ABTS and ORAC (see next section for correlations).

To date, no information relating to the effects of genotype and growing environment on kabuli chickpea polyphenols is available and thus, our results are discussed in relation to similar studies on other crops. Both Rivero et al. (2001) and Wu, Johnson, Bornman, Bennett, Clarke, et al. (2016) found similar trends of increased polyphenol content in tomatoes and sorghum genotypes subjected to high-temperature stress during growth. A significant increase in polyphenol content resulting in enhanced antioxidant capacity was also reported in strawberries (Wang and Zheng 2001)and linked to increased growing temperatures (30/ 22°C compared to 18/12°C). A recent study also reported that cold growing climates can result in increased polyphenol levels in legume roots, stems and seeds (Kabtni et al. 2020) due to increased gene expression of enzymes involved in flavonoid and procyanidin synthesis pathways, thus resulting in increased antioxidant capacity (MacGregor et al. 2015). Chickpeas grown at Walgett experienced the highest number of low temperature instances, also resulting in increased polyphenol content and antioxidant capacity in these samples.

Table 4.4 Average physical and nutritional variables of four kabuli chickpea varieties evaluated across five National Variety Trials

	Seed weight (g)	Volume (mL)	Density (g/ mL)	Hydration capacity (g/ seed)	Hydration index	Swelling capacity (mL/ seed)	Swelling index	Moisture (g/ 100 g)	Protein (g/ 100 g, db)	Fat (g/ 100 g, db)	Ash (g/ 100 g, db)	Starch (g/ 100 g, db)
Variety												
Almaz	0.325^{b}	0.247^{a}	1.340^{a}	0.331^{b}	101.8^{a}	0.367^{b}	153.2^{a}	7.115^{a}	21.48^{b}	4.765^{a}	2.709^{c}	22.47^{ab}
Genesis 090	0.293^{a}	0.224^{a}	1.326^{a}	0.294^{a}	100.6^{a}	0.327^{a}	149.5^{a}	7.039^{a}	21.68^{b}	5.033^{bc}	2.607^{a}	23.73^{b}
Kalkee	0.387^{d}	0.296^{b}	1.323^{a}	0.386^{c}	99.8^{a}	0.411^{c}	144.8^{a}	7.314^{a}	21.46^{b}	4.935^{ab}	2.644^{b}	21.04^{a}
PBA Monarch	0.357^{c}	0.277^{b}	1.311^{a}	0.383^{c}	107.5^{b}	0.410^{c}	153.7^{a}	7.008^{a}	20.83^{a}	5.144^{c}	2.728^{c}	23.71^{b}
Mean	0.340	0.261	1.325	0.348	102.4	0.378	150.3	7.119	21.36	4.96	2.672	22.74
CV	10.3	10.6	0.8	11.0	2.9	9.2	2.4	1.7	1.5	2.8	1.8	4.9
Pooled SE	0.008	0.009	0.023	0.007	0.8	0.008	3.3	0.113	0.207	0.074	0.011	0.519
Location												
Birchip	0.361^{b}	0.256^{bc}	1.420^{b}	0.355^{ab}	98.2^{a}	0.403^{b}	165.5^{c}	5.816^{a}	23.59^{d}	4.950^{b}	2.269^{a}	22.82^{b}
Kununurra	0.323^{a}	0.231^{ab}	1.402^{b}	0.342^{ab}	105.8^{b}	0.381^{ab}	167.1^{c}	5.829^{a}	19.50^{b}	4.898^{b}	3.069^{e}	28.72^{d}
Rainbow	0.322^{a}	0.218^{a}	1.477^{b}	0.332^{a}	103.2^{b}	0.396^{b}	179.8^{d}	8.918^{b}	23.69^{d}	4.553^{a}	2.907^{d}	14.08^{a}
Tarranyurk	0.364^{b}	0.315^{d}	1.154^{a}	0.362^{b}	99.5^{a}	0.354^{a}	112.4^{a}	9.424^{c}	21.26^{c}	5.317^{c}	2.535^{b}	22.31^{b}
Walgett	0.332^{a}	0.283^{c}	1.173^{a}	0.352^{ab}	105.6^{b}	0.360^{a}	126.6^{b}	5.609^{a}	18.77^{a}	5.128^{bc}	2.580^{c}	25.77^{c}
Mean	0.340	0.261	1.325	0.348	102.4	0.378	150.3	7.119	21.36	4.96	2.672	22.74
CV	6.0	15.1	11.3	3.4	3.4	5.7	19.3	26.5	10.6	5.7	11.9	24.1
Pooled SE	0.008	0.010	0.026	0.007	0.9	0.009	3.7	0.127	0.232	0.083	0.012	0.580

Values presented are averaged by variety and averaged by location. Following Tukey's post hoc test, values followed by the same superscript within a column (between varieties, or between locations) do not differ significantly ($p<0.05$). Nitrogen conversion factor = 6.25.

Table 4.5 Total polyphenol content, DPPH, ABTS and ORAC antioxidant capacities of four kabuli chickpea varieties evaluated across five National Variety Trials

	TPC (mg GAE/ 100g sample, db)	**DPPH** (mg TE/ 100 g sample, db)	**ABTS** (mg TE/ 100 g sample)	**ORAC** (µmol TE/ 100 g sample)
Variety				
Almaz	59.80[b]	18.12[b]	79.84[b]	443.2[b]
Genesis 090	62.88[bc]	18.26[b]	83.18[c]	498.7[c]
Kalkee	60.66[c]	17.82[ab]	80.62[bc]	516.8[c]
PBA Monarch	54.59[a]	16.95[a]	74.38[a]	403.8[a]
Mean	59.48	17.79	79.51	465.63
CV	5.9	3.3	4.7	11.1
Pooled SE	0.887	0.387	0.931	7.173
Location				
Birchip	43.62[a]	14.44[a]	66.64[a]	290.2[a]
Kununurra	66.66[d]	19.61[c]	86.53[d]	570.2[c]
Rainbow	58.33[b]	16.77[b]	77.55[b]	434.5[b]
Tarranyurk	61.78[c]	18.48[c]	81.62[c]	450.3[b]
Walgett	67.00[d]	19.65[c]	85.19[d]	583.0[c]
Mean	59.48	17.79	79.51	465.64
CV	16.1	12.4	10.1	25.6
Pooled SE	0.992	0.432	1.041	8.020

Values presented are averaged by variety and averaged by location. Following Tukey's post hoc test, values followed by the same superscript within a column (between varieties, or between locations) do not differ significantly ($p<0.05$). (Where TPC= total polyphenol content; DPPH = 2-2 diphenyl-1-picrylhydrazyl antioxidant assay; ABTS = 3 ethylbenzothiazoline-6-sulfonic acid diammonium salt antioxidant assay; ORAC= oxygen radical antioxidant capacity).

4.7.4 Pearson's correlation

Pearson's correlation coefficients among different variables are reported in Table 4.6. A high positive correlation was detected between seed weight and volume ($r = 0.818$, $p \leq 0.05$, seed weight and hydration capacity ($r = 0.925$, $p \leq 0.01$) and swelling capacity ($r = 0.684$, $p \leq 0.01$). A positive correlation between seed weight and cooking time has been previously reported, indicating seeds of higher weight will take longer to cook (Kaur, Singh, and Sodhi 2005, Sefadedeh and Stanley 1979). Kaur and Singh (2006) reported high positive correlations between seed weight and hydration capacity and swelling capacity for Indian chickpea varieties in their study. High values for volume and swelling capacity in seeds also indicate longer cooking times (Kaur and Singh 2006). A significant ($r = 0.575$, $p \leq 0.05$) positive correlation between density and protein content was also observed, implying seed density may be useful to identify high or low protein concentrations in chickpea varieties (Li and Burton 2002, Carbonaro et al. 2015). In soybeans, the correlation between seed density and protein content ranges from 0.06 to 0.71, with low-density soybeans exhibiting lower protein content when compared to high-density soybeans, indicating the indirect role of seed density in selection for high protein varieties (Li and Burton 2002).

A significant ($r = -0.667$, $p \leq 0.05$) negative correlation between protein and starch content was obtained (Table 4.6) and was substantiated by the highest mean protein content of chickpeas from Rainbow which showed the lowest levels of starch. These results suggest that selecting a variety for high protein content will give low starch content, and vice versa. This negative correlation between protein and starch content has been previously reported by Frimpong et al. (2009) in Canadian kabuli chickpeas, by Wang, Gangola, et al. (2017) in chickpeas of Syrian and Turkish origin and in soybeans from Korea (Koo et al. 2014). Both protein and starch compete for the same substrate for photosynthesis in plants and thus are negatively correlated to each other (Rolletschek et al. 2002, Wang, Gangola, et al. 2017). Our results are also in agreement with the findings of Wang and Daun (2006), who reported a strong varietal and environmental effect on the starch content of lentils, where, with an increase in protein content, the starch content decreased. Although it is recommended to independently select varieties for starch and protein content, their correlation and its effect on seed quality cannot be neglected (Gaur et al. 2016).

Protein and TPC ($r = -0.669$, $p \leq 0.05$) negatively correlated to each other (Table 4.6). Protein was also negatively correlated with DPPH ($r = -0.709$, $p \leq 0.05$), ABTS ($r = -0.613$, $p \leq 0.05$) and ORAC ($r = -0.731$, $p \leq 0.05$) values (Table 4.6). Polyphenols are known to interact with proteins by forming soluble or insoluble complexes (Baxter et al. 1997) and such protein binding may also influence the extractability and physiological effects of polyphenols, including their bioavailability (Wollgast and Anklam 2000). Some studies have also uncovered that the antioxidant capacity of polyphenols can be modified by the presence of proteins (Arts et al. 2002, Riedl and Hagerman 2001), negatively affecting the availability of both components and resulting in a loss of beneficial health properties (Bandyopadhyay, Ghosh, and Ghosh 2012, Liang, Tajmir-Riahi, and Subirade 2008, Naczk et al. 2006).

A very high positive correlation between TPC and DPPH ($r = 0.943$, $p \leq 0.05$), TPC and ABTS ($r = 0.971$, $p \leq 0.05$) and TPC and ORAC ($r = 0.889$, $p \leq 0.05$) values in our study, indicating the major role of polyphenols in the antioxidant capacity of the kabuli chickpeas (Xu, Yuan, and Chang 2007, Segev et al. 2010). A number of studies have reported the role of oxidative stress in the pathogenesis of degenerative diseases in humans including aging, cancer, cataract, Alzheimer's or Parkinson's diseases, and the role of polyphenols in reducing this oxidative stress (Golden, Hinerfeld, and Melov 2002, Finaud, Lac, and Filaire 2006, Scalbert, Johnson, and Saltmarsh 2005, Hollman 2014, Yan et al. 2020). Polyphenols regulate the oxidoreductase system and improve the antioxidation ability in the human body (Ahmed et al. 2017), revealing the direct link between polyphenols and antioxidant capacity.

Table 4. 6 Pearson's correlation coefficients (r) between different physical and nutritional properties in four kabuli chickpea varieties evaluated across five NVT trials in Australia

Trait	S.W.	Volume	Density	H.C.	H.I.	S.C.	S.I.	Moisture	Protein	Fat	Ash	Starch	TPC	DPPH	ABTS	ORAC
S.W.	-	**0.818****	-0.215	**0.925****	-0.193	**0.684****	-0.268	0.115	0.052	0.043	-0.274	-0.083	-0.333*	-0.267	-0.337*	-0.274
Volume		-	**-0.732****	0.77**	-0.132	0.242	**-0.739****	0.145	-0.294	0.275	-0.356*	0.117	-0.011	0.051	-0.059	-0.002
Density			-	-0.218	0.005	0.390*	**0.946****	-0.061	**0.575****	-0.396*	0.298	-0.306	-0.374*	**-0.407****	-0.305	-0.334*
H.C.				-	0.192	**0.763****	-0.194	0.012	-0.135	0.053	-0.068	0.028	-0.244	-0.156	-0.279	-0.173
H.I.					-	0.201	0.199	-0.256	**-0.495****	0.001	**0.552****	0.267	0.254	0.309	0.184	0.281
S.C.						-	0.429*	-0.176	0.227	-0.212	0.118	-0.210	**-0.437****	-0.365*	**-0.439****	-0.333*
S.I.							-	-0.176	**0.469****	-0.395*	0.388*	-0.258	-0.327*	-0.335*	-0.284	-0.276
Moisture								-	**0.418****	-0.113	0.073	**-0.668****	-0.033	-0.166	-0.062	-0.177
Protein									-	-0.349*	-0.287	**-0.667****	**-0.669****	**-0.709****	**-0.613****	**-0.731****
Fat										-	-0.307	**0.456****	0.077	0.157	0.01	0.154
Ash											-	0.016	**0.531****	**0.444****	0.479**	**0.488****
Starch												-	0.249	0.368*	0.242	0.340
TPC													-	**0.943****	**0.971****	**0.889****
DPPH														-	**0.939****	**0.796****
ABTS															-	**0.826****

Note: ** significant at $p < 0.001$, * significant at $p < 0.05$

4.7.5 Mean performance of four kabuli chickpea varieties using GGE biplot

The GGE biplot for six variables (seed weight, moisture, protein, fat, starch and total polyphenol content) can be seen in Fig. 4.5(a-f). These variables were chosen due to their key role in chickpea seed quality. In general, seed yield is one of the most important commercial factors, however, to maintain a high-quality product, nutritional properties cannot be ignored. For example, Tena et al. (2019) utilised GGE biplot to determine the best performing genotype for sugar yield from sugar cane grown in eight different growing environments in Ethiopia, and Vaezi et al. (2017) used GGE biplot to determine the highest yielding barley genotypes in four different locations across Iran. However, recent GGE biplot analysis studies have also focussed on seed quality characteristics in different grains and pulses (Netyam et al. 2017, Yildirim et al. 2018, Silva et al. 2017, Kargiotidou et al. 2019, Subedi et al. 2021), and thus GGE biplot can be considered a key tool to visually determine the performance of genotypes in different growing environments pertaining to a particular seed quality trait.

In the case of seed weight (Fig 4.5a), the values for PC1 and PC2 were 90% and 9% respectively, following the GGE biplot which jointly accounted for 99% of G × E. In the GGE biplot for seed weight, all environments (Birchip, Kununurra, Rainbow, Tarranyurk and Walgett) fall into the same sector as variety Kalkee and are also outside the polygon formed by all varieties, making Kalkee the best performing variety in terms of seed weight across all environments. In terms of moisture content (Fig. 4.5b), the biplot accounts for 99% of the variation across both principal components. Kalkee had the highest moisture content out of all genotypes when grown at Birchip, whereas the other environments did not affect the moisture content of varieties due to their closeness to the biplot origin. Being a main source of proteins for a large number of people, protein content in chickpeas is an important quality parameter (Subedi et al. 2021). For the protein content biplot, the PC1 and PC2 values were 58% and 33% respectively. Kununurra and Tarranyurk, formed a mega-environment in which Genesis 090 was the best performing variety, whereas Kalkee performed best in Birchip, Walgett, and Rainbow out of all the other varieties grown in these environments (Fig 4.5c).

The biplot for fat content accounted for 90% of the total variation across both principal components. The fat content of PBA Monarch and Kalkee grown at Kununurra and

Walgett was the highest out of all varieties from these environments, whereas Almaz had the highest starch content when grown at Birchip and performed better at Rainbow than other varieties (Fig 4.5d). The biplot for polyphenol content accounted for around 94% variation. Kalkee had the highest total polyphenol content out of all varieties when grown at Tarranyurk followed by Rainbow. Both Walgett and Kununurra experienced extreme temperature instances (Table 4.2) and the importance of this is highlighted, by these two locations forming a mega environment following GGE biplot analysis (Fig. 4.5f). Genesis 090 when grown at Walgett exhibited the highest total polyphenol content possibly due to the environmental stress, while also performing well at Kununurra.

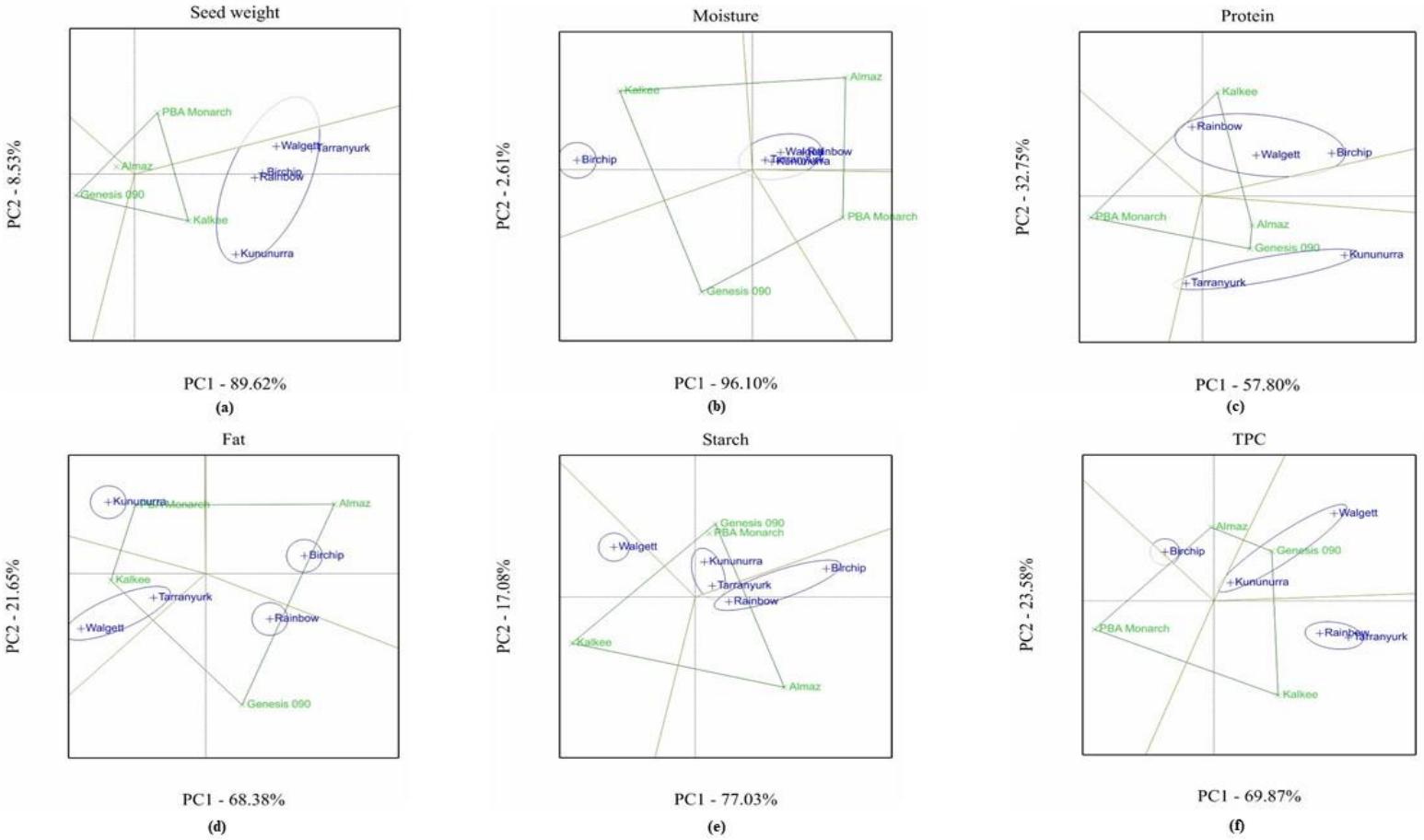

Figure 4.5 Genotype main effects + genotype x environment interactions shown as a (GGE) biplot, representing (a) seed weight, (b) moisture, (c) protein (d) fat (e) starch, (f) TPC (total polyphenol content), of four chickpea genotypes grown in five environments in 2018.

4.7.6 Principal component analysis:

Principal component analysis of four kabuli chickpea varieties grown at five locations was carried out using 11 variables (seed weight, density, H.C., S.W., moisture, protein, fat, ash, starch, TPC and ORAC). These were selected from 16 possible variables, as they were the variables with the lowest correlations between each other, thus reducing potential bias that comes from highly correlated variables (Jiang and Eskridge 2000). The first four principal components expressed 87.86% of the variation (Table 7). Principal component 1 (PC1) and principal component 2 (PC2) accounted for 36.35% and 19.72% of the variation respectively.

All variables with the exception of protein content had positive loadings on PC1 (Table 4.7), with protein content exhibiting a negative relation with total polyphenol content in PC1. This result can be explained as protein-phenolic interactions lead to the formation of complexes, reducing polyphenol extractability, bioavailability, and antioxidant capacity (Wollgast and Anklam 2000, Ozdal et al. 2019). Similar to the negative correlation ($r = -0.667$, $p \leq 0.01$) between protein and starch content reported earlier (Table 4.6), PC1 also showed a negative relationship between these components, highlighting the competition for the same substrate involved in the biosynthesis of both components (Rolletschek et al. 2002).

The second component (PC2) was strongly represented by fat and ash content (Table 4.7), ash having the highest loading (0.575) on this component. On principal component 2 (PC2), density and ash content had positive loadings, whereas fat content had a negative loading. Density was represented to a lesser extent (loading= 0.411), indicating that seeds with a higher density will contain higher ash and lower fat content.

PC3 exhibited positive loadings for hydration index (0.372) and swelling capacity (0.556) whereas PC4 had a large positive loading for seed weight and moisture content. A positive relationship between moisture content and seed weight has previously been reported in legume seeds (Amin, Hossain, and Roy 2004, Altuntas and Demirtola 2007, Altuntaş and Yıldız 2007). High moisture content in seeds leads to higher seed weight, in turn affecting other moisture dependent seed properties such as volume and hydration properties (Zewdu and Solomon 2007).

The distribution of varieties on the biplot of the principal component loadings for PC1 and PC2 overlaid over the principal component scores is shown in Fig. 4.6. The plot of PC1 by PC2 shows that varieties grown at a particular location form separate clusters highlighting that the variation between locations is greater than the variation between varieties (Fig.4.6A). Variety Kalkee grown at Birchip is separated from the rest of the varieties from this location due to its higher protein content. Similarly, variety Almaz from Walgett is separated from the other varieties grown at this location due to its higher TPC and ORAC content. With the exception of these, all other varieties showed high dispersion in the principal component plot in relation to location, depicting the variability of varieties in response to growing environment. It also highlights that seed quality traits related to PC1 (TPC, ORAC, starch) were highest in chickpeas grown at Kununurra and Walgett, but that these contained low protein levels due to a negative loading for protein in PC1 (-0.460). Similarly, chickpeas grown at Rainbow had the highest values for components related to PC2 (ash, density) and possessed a low-fat content due to a negative loading on PC2.

Table 4. 7 Results of principal components analysis (PCA) of five growing locations analysed together

	PC1	**PC2**	**PC3**	**PC4**
Component				
Eigen value	3.99	2.17	1.83	1.33
Cumulative % variance explained	36.35	19.72	16.7	12.09
Variable loadings				
Seed weight	-0.187	-0.230	0.363	**0.572**
Density	-0.253	**0.411**	0.136	-0.397
Hydration index	0.237	0.301	**0.372**	0.054
Swelling capacity	-0.240	0.156	**0.556**	0.289
Moisture	-0.190	0.092	-0.443	**0.494**
Protein	**-0.460**	0.076	-0.178	-0.156
Fat	0.179	**-0.457**	0.045	-0.0004
Ash	0.193	**0.575**	0.064	0.131
Starch	**0.333**	-0.243	0.317	-0.278
TPC	**0.416**	0.174	-0.233	0.193
ORAC	**0.43**	0.138	-0.109	0.167

Where, PC1= principal component 1; PC2= principal component 2; PC3= principal component 3; PC4= principal component 4; TPC= total polyphenol content; ORAC= oxygen radical antioxidant capacity.

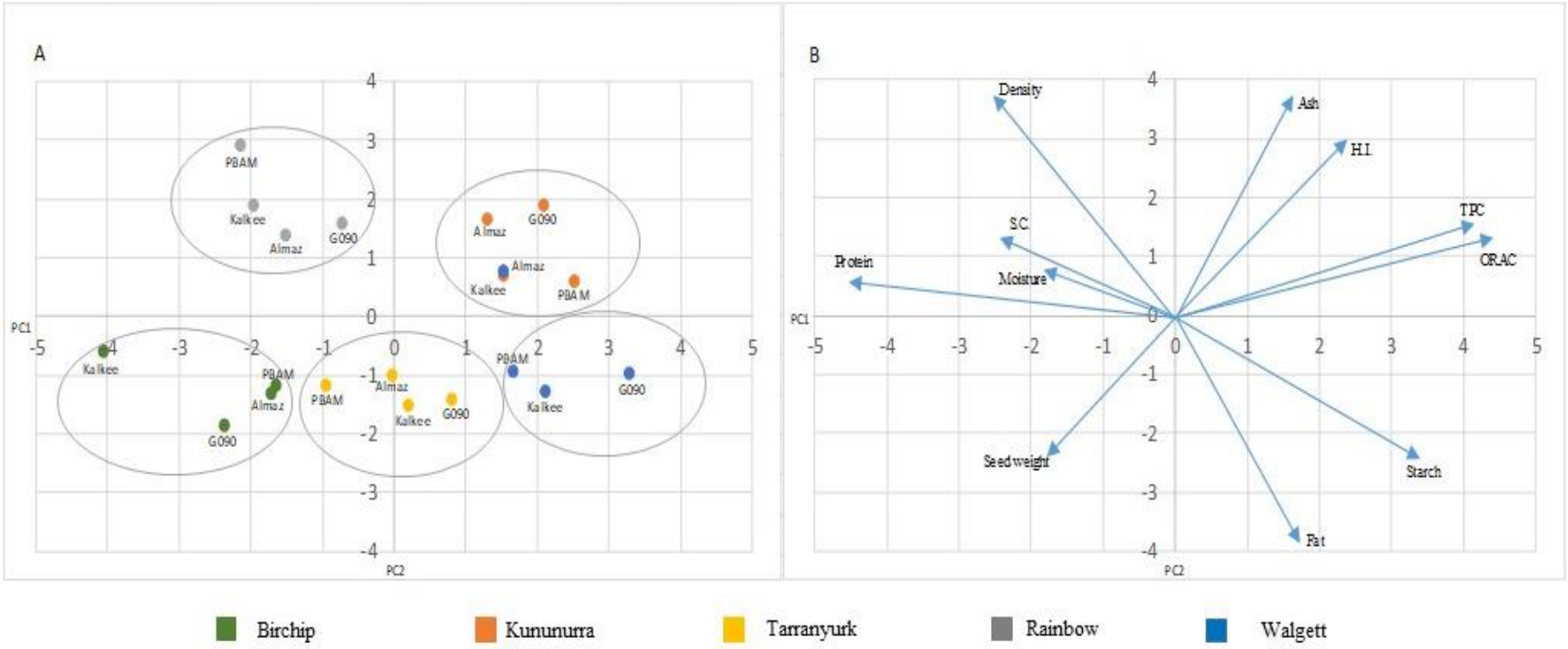

Figure 4.6 Principal components analysis of four kabuli chickpea varieties grown at five NVT locations; (A) scores of variety by location, (B) biplot of the component loadings. Variety abbreviations: G090= Genesis 090; PBAM= PBA Monarch, and variable abbreviations: H.I. = hydration index; S.C. = swelling capacity; TPC= total polyphenol content; and ORAC= oxygen radical antioxidant capacity)

4.8 Conclusion

This chapter examined the physical, nutritional and antioxidant capacities of four Australian kabuli chickpeas varieties grown in five locations across Australia. Significant genotype, environment and G x E effects were found in the properties of the chickpea seeds, findings that have not previously been reported for Australian kabuli chickpeas but have been explored for chickpeas of other origins. Contents of nutritional components and antioxidant properties were significantly affected by the growing environment, with decreased protein content and increased polyphenol levels in extreme temperature environments and thus it appears reasonable to suggest that specific varieties and growing environments can be selected to improve the nutritional and antioxidant properties of Australian kabuli chickpeas. This information can be used to make further progress in enhancing various quality characteristics of kabuli chickpeas by strict selection of growing environments with particular stress characteristics to increase the amount of desirable seed constituents.

The results included in this chapter are from analyses conducted on raw chickpea seeds. Chickpeas are consumed by humans only after processing by methods such as soaking (Vasishtha and Srivastava 2012), cooking (Kaur, Singh, and Sodhi 2005, Alajaji and El-Adawy 2006), germination (Khalil et al. 2007), and roasting (Ouazib et al. 2015), which can result in changes to the nutritional and bioactive properties of the seed (Summo, De Angelis, Rochette, et al. 2019). High pressure processing is a novel processing method, mainly used for the preservation of foods while retaining colour, flavour, and nutritional components (Oey et al. 2008). However, its effect on Australian kabuli chickpea properties has not been explored. Therefore, the following chapter will determine the effects of high pressure processing on Australian kabuli chickpea textural, nutritional and bioactive properties.

4.9 Acknowledgement

We would like to acknowledge the significant cooperation and assistance of the Grains Research Development Corporation (GRDC) with chickpea sample collection obtained from the 2018 GRDC National Variety Trials.

CHAPTER 5. The effect of high pressure processing on the textural, bioactive and digestibility properties of cooked Kimberley Large kabuli chickpeas

"To eat is a necessity, but to eat intelligently is an art."

Francois de la Rochefoucauld

Abstract

High pressure processing is a non-thermal method used for the preservation of various foods while retaining nutritional value and can be utilised for the development of ready-to-eat products. This chapter investigated the capacity of high pressure processing for the development of a ready-to-eat chickpea product using Australian kabuli chickpeas. Three pressure levels (200, 400, 600 MPA) and two treatment times (1 and 5 min) were selected to provide six distinct samples. When compared to the conventionally cooked chickpeas, high pressure processed chickpeas had a more desirable texture due to a decrease in firmness, chewiness and gumminess. The general nutrient composition and individual mineral content were not affected by high pressure processing, however, a significant increase in the slowly digestible starch from 50.53- 60.92 g/ 100 g starch and a concomitant decrease in rapidly digestible starch (11.10- 8.73 g/ 100g starch) as well as resistant starch (50.53- 30.35 g/ 100g starch) content was observed. Increased starch digestibility due to high pressure processing was noticed, whereas *in vitro* protein digestibility was unaffected. These findings suggest that high pressure processing could be utilised to produce a functional, ready to eat kabuli chickpea product with increased levels of beneficial slowly digestible starch.

5.1 Introduction

High pressure processing (HPP) is an innovative method of preserving foods, which is an alternative to standard heat treatment methods. It produces high quality and safe food which have a longer shelf life than conventionally stored food due to a reduction of food spoiling microorganisms (Huang et al. 2017, Krebbers et al. 2002).

High pressure processing has been shown to retain quality attributes such as colour, flavour and the nutritional value of foods (Oey et al. 2008, Huang et al. 2017). In brief, during high pressure processing, at any given time, the pressure is transmitted uniformly by the pressure transmitting medium and is independent of the shape and size of the product being treated. The effectiveness of high pressure treatment depends on treatment time, pressure and temperature level, rate of pressurisation/ decompression, and the composition of the food (Knorr 2002).

The demand for plant-based protein is steadily growing around the world and is estimated to be a USD 14.5 billion market by 2025 (Markets and Markets 2020). In the last 5 years, an increasing number of people shifted animal-based proteins to plant-based proteins sources in their meals (Maphosa and Jideani 2017, Tziva et al. 2020). Chickpeas (*Cicer arietinum* L.) are widely grown worldwide, are already a popular food source for humans and are an excellent source of proteins (Bar-El Dadon, Abbo, and Reifen 2017). However, the time required to cook chickpeas makes them a less popular choice amongst the younger generation, which prefers healthy and convenient food options requiring less preparation time (Linsberger-Martin et al. 2013).

As HPP is known to increase the shelf life of foods without affecting the sensory and nutritional properties (Huang et al. 2017), cooked and high pressure processed chickpeas could be introduced as a ready-to-eat option, with desirable textural, organoleptic and nutritional properties. However, there is a scarcity of knowledge on the effects of HPP on the textural, nutritional and bioactive properties of legumes in general. HPP denatures proteins reversibly and irreversibly. A moderate pressure (<300 MPa) affects the speed of enzyme action whereas, a pressure above 300 MPa induces protein denaturation and inactivation of enzymes (Thakur and Nelson 1998, Oey et al. 2008). Previous studies have reported the effect of high pressure on lentil starch (Ahmed et al. 2016), mung bean starch (Li et al. 2011) and pea starch (Leite et al. 2017), however, no information is available on both protein and starch digestibility of HPP chickpeas.

A small decrease in the total polyphenols (Linsberger-Martin et al. 2013) and antioxidant properties of HPP vegetables have been reported by Butz et al. (2002) whereas Doblado et al. (2007) reported slight changes in the antioxidant capacity and vitamin C content of HPP germinated cowpeas (*Vigna unguiculata* (L.), a grain

legume common in sub-Saharan Africa, following high pressure treatment. A recent study by Alsalman and Ramaswamy (2020) reported changes in the texture, colour and antinutrient (tannins, phytic acid) content of raw Canadian kabuli chickpeas, but did not explore the changes in starch or protein digestibility, polyphenol content and antioxidant capacities of cooked chickpeas. Therefore, there is a need to investigate the effect of high pressure processing on the texture profile, general nutrient composition, starch and protein digestibility, polyphenol content and antioxidant capacity of cooked and HPP kabuli chickpeas, compared to cooked only samples to inform ready to eat product development in the near future. To fill this knowledge gap, the objective of this chapter was to examine the effects of HPP (200, 400 and 600 MPa for 1 and 5min) on the textural, nutritional, and bioactive properties of cooked Australian kabuli chickpeas.

5.2 Materials and methods

Representative samples of the cultivar Kimberley Large kabuli chickpeas (Siddique and Regan 2005) harvested in 2017 from the Ord River region of Western Australia were collected, sieved, vacuum packaged and stored at 4°C until analysis. The kabuli chickpeas were soaked in excess water overnight (12 h) at room temperature (22°C). After 12 h, the remaining water was drained off and chickpeas were cooked in fresh boiling water for 30 min. Following cooking, the chickpeas were cooled by washing them under running tap water and excess moisture was removed using a paper towel. The cooked and cooled chickpeas were vacuum packaged in a 100-micron vacuum packaging bag using a Multivac double chamber vacuum packaging machine (Model-C450, Multivac, Keilor Park, Victoria, Australia) and stored at 4° C until high pressure processing.

5.2.1 High pressure processing

Cooked and vacuum packaged Kimberley Large kabuli chickpeas were commercially high pressure processed at Preshafoods Pty. Ltd. (Derrimut, Victoria, Australia) using hyperbaric high pressure processing equipment (Model- Hyperbaric 300, Burgos, Spain, EU) at the following settings detailed in Table 5.1.

Table 5.1 Pressure and time combinations for high pressure processing of cultivar Kimberley Large chickpea samples

Pressure / **Time**	**200 MPa**	**400 MPa**	**600 MPa**
1 min	2000 MPa, 1 min (2K1)	4000 MPa , 1min (4K1)	6000 MPa , 1min (6K1)
5 min	2000 MPa , 5min (2K5)	4000 MPa , 5min (4K5)	6000 MPa , 5min (6K5)

The time required to reach 200 MPa, 400 MPa and 600 MPa was 60, 130 and 240 seconds respectively, and the decompression was instant. Purified water was used as the pressurization media and all the samples were pressurised at 4°C. After processing, the samples were stored at 4°C and transported to Curtin University (Bentley, Western Australia) under refrigerated conditions. Cooked and vacuum packaged Kimberley Large kabuli chickpeas not subjected to high pressure processing were used as a control.

5.2.2 Texture profile analysis

Texture profile analysis of the control and high pressure processed chickpeas was conducted using a Perten Texture analyser (TVT6700, Hägersten, Sweden) (Wang, Daun, and Malcolmson 2003). The samples were subjected to 50% compression with a perspex cylindrical probe (25 mm diameter) at a crosshead speed of 1 mm/s twice in two cycles using a 5 kg load cell. The texture profile was expressed as firmness, cohesiveness, springiness, gumminess and chewiness. Forty replicates were analysed for control and each HPP sample.

5.2.3 Moisture content

Moisture content was determined by the modified solids- (total) and moisture in flour-oven drying method (Method 925.10, AOAC International 2008).

5.2.4 Protein content

Protein content was determined in duplicate by Kjeldahl assay: digestion, distillation and titration using a nitrogen conversion factor of 6.25 (Method 920.87, AOAC International 2005b).

5.2.5 Total starch content

Starch content was determined in duplicate using the standard colourimetric technique (Method 996.11, AOAC International 2005c) using the total starch assay kit (AA/AMG) from Megazyme International Ltd (Bray, County Wicklow, Ireland).

5.2.6 Freeze drying

The chickpea samples were placed in a freezer over night at -20°C. The samples were transferred to a freeze dryer (Christ Alpha 1-4LD Plus, Germany) and freeze dried at 1 mbar pressure and -50°C for four days. The freeze dried samples were milled (Grindomix, GM200, Retsch, Haan, Germany) to get a fine powder, passing 100% through 500 microns.

5.2.7 Mineral content:

Content of individual minerals (mg) in freeze dried control and highest HPP treated sample (6K5) was determined in duplicate at the National Measurement Institute, Perth, Western Australia using an inductively coupled plasma optical emission spectrometer (ICP- OES) (Varian, Palo Alto, USA) (Wu, Johnson, Bornman, Bennett, Singh, et al. 2016) as discussed in detail in Chapter 3 (3.5.5).

5.2.8 Polyphenol extraction and total polyphenol content

Total polyphenols were extracted from the freeze dried control and HPP samples as per Segev et al. (2010) with a few modifications as described in detail in section 3.5.6 of Chapter 3. Total polyphenol content was determined in duplicate samples using the Folin-Ciocalteu method (section 3.5.7) (Wu et al. 2017)

5.2.9 Antioxidant capacity

The method of Thaipong et al. (2006) was used to determine the antioxidant capacities of polyphenol extracts of control and high pressure processed samples. The 2-2-diphenyl-1-picrylhydrazyl (DPPH), 3-ethylbenzothiazoline-6-sulfonic acid

diammonium salt (ABTS), and oxygen radical absorbance capacity (ORAC) assay were used to estimate the antioxidant capacity of phenolic compounds in polyphenolic extracts of duplicate samples as described in Chapter 3 (3.5.8).

5.2.10 Starch Isolation

Starch from the freeze dried control and high pressure processed chickpea samples was isolated using the method of Sun et al. (2014). Freeze dried high pressure processed chickpea sample (250 g) was steeped in water containing 0.1% sodium sulphite for 12 h at 25°C. The slurry obtained was then diluted with 100 mL distilled water, and the pH was adjusted to pH 10 using 0.5 M sodium hydroxide (NaOH). The slurry was continuously mixed using a magnetic stirrer (500 rpm) for 1 h and then filtered through a 100 mesh sieve (0.149 mm nominal sieve opening) to separate the fibre from the starch. The filtered slurry was centrifuged at 3000 g for 10 min at 10°C (Eppendorf Centrifuge 5810R made in Germany) and the supernatant was discarded. The centrifugation step was repeated six times until a clear supernatant was obtained whereas, pure starch was the white sediment remaining at the bottom. The isolated starch was dried using a digital hot air oven (Thermotec 200, Contherm, Lower Hutt, New Zealand) at 40°C for 48 h and ground into a fine powder using a pestle and mortar.

5.2.11 Light microscopy

Isolated starch samples (~0.01 g) were mounted on a glass slide with a few drops of water and covered with a cover slip. The samples were observed under a microscope (Alphaphot-2 YS2, Nikon, Melville, USA) equipped with a 10x lens (100x magnification) and a 3.2 M pixel camera (Pro-MicroScan 5888, Prague, Czech Republic). Digital images were captured using ScopePhoto software (version x86,3.1.615, Scopetek).

5.2.12 Scanning electron microscopy

Isolated starch sample morphology was investigated using Tescan Mira3 FESEM with Oxford Instruments X-MaxN 150 silicon drift x-ray detector and Aztec software. Starch samples were fixed on a circular metallic microscope stub with carbon aluminium tape and then coated with a 5 nm platinum coating using a sputter coater (208HR, Cressington). The scanning electron microscopy was performed at an accelerating voltage of 2 kV.

5.2.13 ATR-FTIR analysis

Attenuated total reflectance Fourier transform infrared spectra (ATR-FTIR) were obtained using a Nicolet IFS50 FTIR spectrometer, and a single bounce diamond ATR accessory. Spectra were collected by placing the milled freeze dried chickpea sample in contact with the ATR accessory. Spectra were recorded across 4000 – 400 cm^{-1} spectral range at 4 cm^{-1} spectral resolution, and with 128 co-added scans. The background spectrum was collected from blank diamond ATR crystals. Spectra were post normalised using vector normalisation across the spectra range 875 – 1190 cm^{-1}, using OPUS software (v7.0). Second-derivatives of the FTIR spectra were then calculated using a 17 smoothing point Savitzky-Golay function.

5.2.14 In vitro slowly digested starch

The amount of *In-vitro* slowly digested starch was determined in duplicate on fresh chickpea samples using a rapid glucometer method based on that of Sopade and Gidley (2009). A weighed sample of 250 mg was placed in a glass jar (150 mL, Ergo Flint Glass 70 mm, Plasdene Glass-Pak, Canning Vale, Australia) and 1 mL of porcine α-amylase (250 U/mL in 0.2 M pH 7 carbonate buffer) was added to it. After, 20 seconds, 5 mL of pepsin suspension [9 mg of pepsin, (2500 units/mg) and 5 mL of 0.02 molar hydrochloric acid was added to lower the pH to 2.0 (approximate pH of *in vivo* gastric conditions). The mixture was then incubated in a reticulating water bath (37°C, 85 rpm) for a duration of 30 min before neutralisation using 5 mL 0.02 M NaOH and 50 mL 0.2 M pH 6 sodium acetate buffer. This was then followed by the addition of 5 mL pancreatin mixture [0.095 g pancreatin + 0.2 M acetate buffer (44.175 mL, pH 6.0)]. Incubation was continued in a reticulating water bath (37°C, 85 rpm). Glucose readings in duplicate were made at 0, 5, 10, 20, 40, 60, 90 and 120 min from the time of addition of the pancreatin mixture by an Accu Check Performa glucometer (Roche Diagnostics Aust. Pty. Ltd, Castle Hill, Australia). Slowly digested starch (SDS) was calculated using equation (1) and expressed in terms of g/ 100 g starch (db).

$$\mathrm{SDS} = \frac{0.9 \times (\mathrm{G120} - \mathrm{G20}) \times 180 \times \mathrm{V}}{\mathrm{W} \times \mathrm{S}(100 - \mathrm{M})} \qquad (1)$$

Where; G120 = glucometer reading (mM) at 120 min, G20=glucometer reading (mM) at 20 min, V= digesta volume (mL), 180 = molecular weight of glucose, W = sample weight (g), S= sample starch content (g/ 100 g db), M = sample moisture content (g

/100g sample), and 0.9 = stoichiometric constant for starch from glucose contents. Using known quantity of glucose in the digesta, the glucometer was corrected (37°C).

5.2.15 Starch digestion kinetics

First order kinetics was used to investigate the starch digestion kinetics of the control and HPP samples under simulated digestion conditions. This is given by equation (2):

$$C = 1 - e^{-kt} \qquad (2)$$

Where; C is the starch fraction digested at time t (and 1- C is the undigested fraction); k is the fractional-digestion rate coefficient (h^{-1}), t is the incubation or digestion time. For data fitting, the value of k was obtained by linear-least-squares fit of the solution of eq. (2), viz., as the slope of a plot of ln(1-C) against t. The linearity of this plot will provide a measure of the applicability of first-order kinetics (Al-Rabadi, Gilbert, and Gidley 2009).

5.2.16 Protein digestibility

The *in-vitro* protein digestibility of samples (control and HPP (6K5)) was determined following the method of Villarino et al. (2015) with a few modifications. Approximately, 100 mg freeze-dried chickpea sample was incubated with a mixture of pepsin (0.75 mg, 2500 units/mg activity; Chem-Supply, Gillman, SA, Australia) and 0.1 N hydrochloric acid (7.5 mL) at 37°C for 5 h. Using 3.75 ml of 0.2 N NaOH, the solution was neutralized. By the addition of 25 ml of 10% tricholoroacetic acid and centrifuging for 30 min (2000×g at 22°C), the undigested proteins present in 15 ml of digesta were precipitated. The total nitrogen content of the supernatant was then determined using the Kjeldahl method and the *in-vitro* protein digestibility (IVPD) was calculated according to Eq. (3).

$$\text{IVPD (\%)} = \frac{(\text{Total nitrogen(g/100mL)} - \text{nitrogen in supernatant(g/100mL)})}{\text{Total nitrogen (g/100mL)}} \times 100 \qquad (3)$$

5.2.17 Statistical analysis

Two-way analysis of variance (ANOVA) was used to compare means of replicate analyses per treatment followed by Duncan's multiple range test to separate means when F was significant (<0.05). Dunnett's post-hoc test was used to compare the mean values of the control against those of high pressure treated samples. All tests were performed using Genstat statistical tool (Version- V.20.1.2.24528, VSN International Ltd, UK).

5.3 Results and discussion

5.3.1 Texture Profile analysis

A typical texture profile analysis (TPA) curve for cooked kabuli chickpea samples is shown in Fig. 5.1. Texture measurements of control and HPP samples are presented in Fig. 5.2 and Fig. 5.3, where high pressure processing resulted in significant ($p<0.05$) changes in TPA parameters when compared to the control following ANOVA. In addition, the 5 min application at all pressures lead to a significant ($p<0.05$) reduction in TPA values than samples treated for 1 min. The firmness of HPP samples ranged from 15.27 – 25.28 N and was highest for the control and lowest for 6K5. Two way analysis of variance (ANOVA) showed a significant effect ($P<0.05$) of pressure (P) on the firmness of HPP samples, however, no effect of time (T) or pressure and time (P x T) interaction was observed. Samples treated at 200 and 400 MPa had similar firmness values, however, with the increase in treatment pressure to 600 MPa, a significant reduction in the firmness of HPP samples was observed. When compared to the control, even the lowest processing treatment (2K1) significantly ($p<0.05$) reduced the firmness of cooked chickpeas and all high pressure treated samples were significantly ($p<0.05$) less firm than the control, with 6K5 being the softest. Alsalman and Ramaswamy (2020) also reported a decrease in the firmness of chickpeas with an increase in pressure (from 100 to 400 MPa). However, an increase in pressure to 600 MPa led to an increase in firmness, possibly due to the aggregation of protein in the uncooked samples used in this study (Oliveira et al. 2017). Internal redistribution of moisture, weakened hydrophobic interactions of the protein matrix and tissue collapse can be attributed to texture degradation (Koca, Balasubramaniam, and Harper 2011), resulting in loss of firmness.

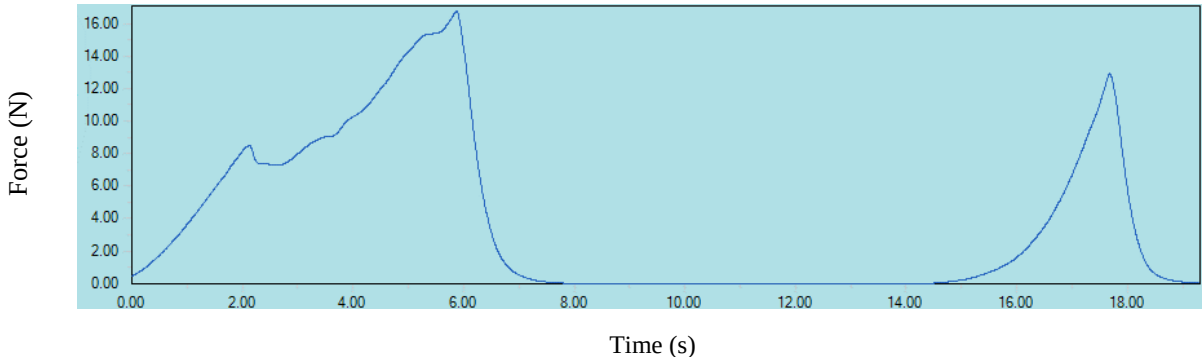

Figure 5.1 A typical texture profile curve for cooked kabuli chickpeas

Cohesiveness in the texture profile analysis indicates how well a product withstands a second deformation following the first. Cohesiveness for cooked and HPP chickpeas ranged between 0.16 – 0.23 and was lowest for 6K5 and highest for the control. Cohesiveness can be influenced by the depressurization process and hence a more pronounced effect can be observed for samples treated at 600 MPa. Springiness, gumminess, and chewiness for the cooked and HPP samples ranged between 0.80-0.96 mm, 2.53- 5.77 N, and 2.32- 4.68 N mm, respectively. Similar to the firmness, cohesiveness, gumminess and chewiness of HPP treated samples were significantly ($p<0.05$) affected by the pressure treatment (P), however, there was no effect of time (T) or P x T interaction on these texture properties. Cohesiveness values for 2K1, 4K5, 6K1 and 6K5 were significantly ($P < 0.05$) lower than the control. Also, all HPP samples were significantly ($P < 0.05$) less gummy (except 4K1) and less chewy than the control. Springiness is the degree to which cooked grains can return to their original shape after partial compression. Springiness negatively correlates with hardness and chewiness, suggesting that springier samples are less hard and less chewy (Meullenet et al. 1998). No effect of high pressure processing on the springiness of cooked kabuli chickpeas was observed in this study.

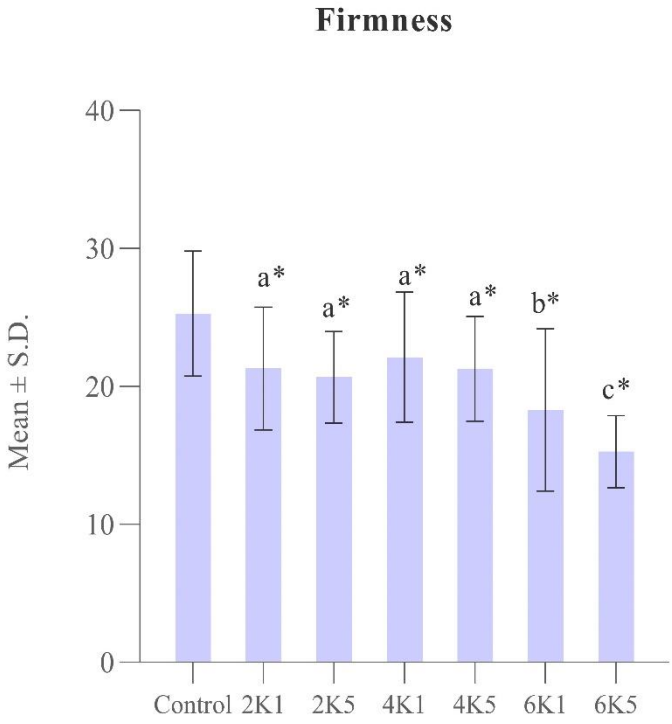

Figure 5.2 Firmness (N) of control and HPP chickpea samples (2K1= 200 MPa, 1min; 2K5= 200 MPa, 5 min; 4K1= 400 MPa, 1 min; 4K5= 400 MPa, 5 min; 6k1= 600 MPa, 1 min; and, 6K5 = 600 MPa, 5min). Bars bearing different letter are significantly ($p<0.05$) different to each other. Bars bearing * are significantly ($p<0.05$) different to the control.

The effect of high pressure on firmness, cohesiveness, springiness, gumminess, and chewiness has been investigated in previous studies on the texture of beetroot (Paciulli et al. 2016), meat (Sun and Holley 2010, Oliveira et al. 2017) and chickpeas (Alsalman and Ramaswamy 2020),

However, due to the conflicting results of these studies, a conclusion based on the available data to support the clear effects of high pressure processing on these textural properties cannot be reached. Our result for firmness was in agreement with that of Koca, Balasubramaniam, and Harper (2011) for cheese, Yu et al. (2017a) for brown rice and Alsalman and Ramaswamy (2020) for chickpeas, which also reported that increasing pressure during HPP results in a reduction in sample firmness.

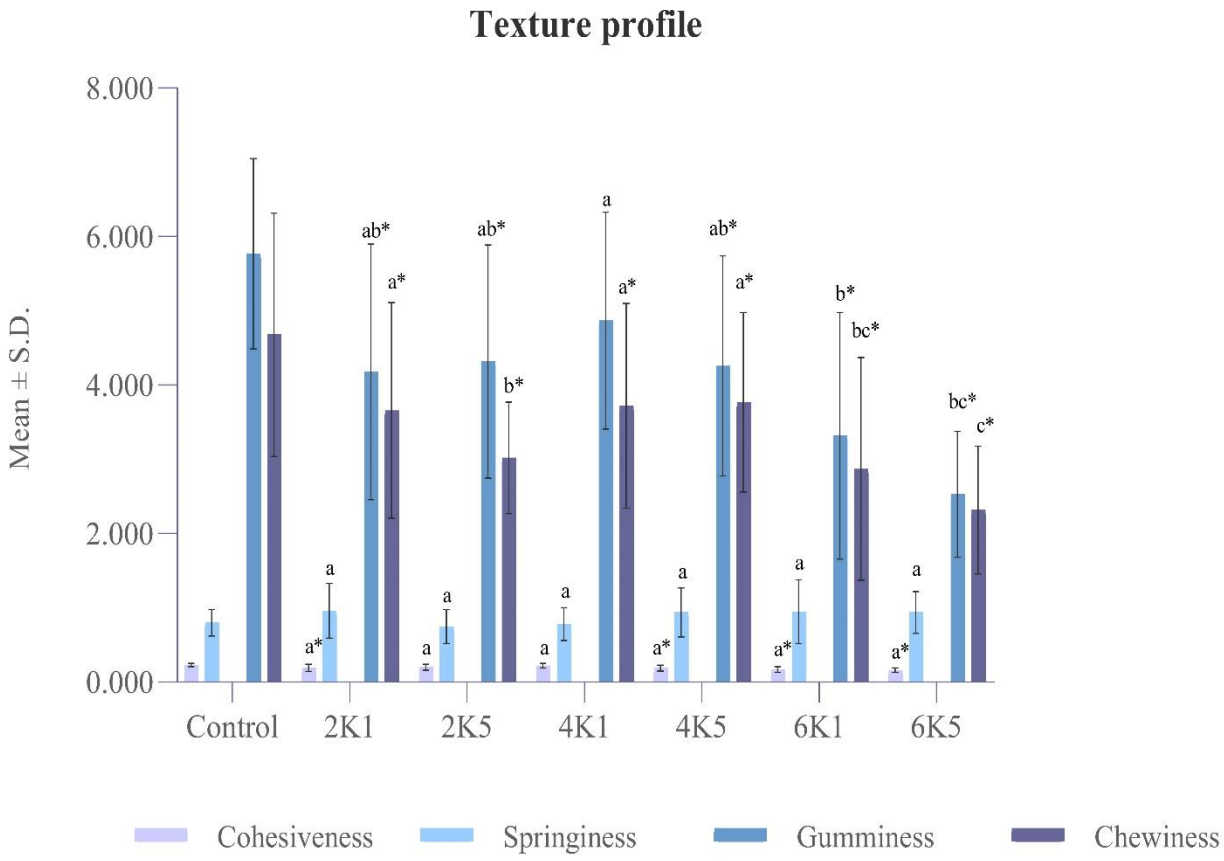

Figure 5.3 Texture profile of control and HPP chickpea samples (where, 2K1= 200 MPa, 1min; 2K5= 200 MPa, 5 min; 4K1= 400 MPa, 1 min; 4K5= 400 MPa, 5 min; 6k1= 600 MPa, 1 min; and, 6K5 = 600 MPa, 5min). Bars bearing different letter for the same parameter are significantly ($p<0.05$) different. Bars bearing * for the same parameter are significantly ($p<0.05$) different to the control.

In our study, the softest texture was chosen as the most desirable based on previous results of sensory studies on legumes such as green gram (*Phaseolus aureus* Roxb), bengal gram (*Cicer arietinum*), lentils (*Lens esculenta*) (Khatoon and Prakash 2006) and beans (*Phaseolus vulgaris*) (Ghasemlou, Gharibzahedi, and Emam-Djomeh 2013). Among all the samples, 6K5 showed the lowest firmness, cohesiveness, gumminess and chewiness values, whereas the control had the highest values for these textural properties. Thus, based on the instrumental analysis we can concur that 6K5 had the most desirable textural properties out of all the samples in this study.

5.3.2 General nutrient composition

The proximate and dietary fibre content of the control and HPP chickpea samples is given in Table 5.2. The moisture content of the HPP samples ranged from 61.01- 64.13 g/100 g, and the protein content ranged from 17.81- 19.02 g/100 g (db). None of these measurements were significantly different to the control. On the other hand, the starch content of the HPP samples ranged from 46.13- 54.97 g/ 100 g, and was significantly ($P<0.05$) affected by treatment pressure levels, with an increase in pressure, leading to a decrease in starch content. When compared to the control, 4K1, 4K5, 6K1 and 6K5 had significantly ($P<0.05$) less starch content. The reason for the reduction in starch content is the leaching of starch due to rupture of cell walls by the high pressure applied as observed in common beans as well (Belmiro, Tribst, and Cristianini 2020). Based on these results, HPP can be potentially used to reduce the starch content of other high starch grains and pulses for designing lower calorie alternatives.

The soluble dietary fibre (SDF), insoluble dietary fibre and total dietary fibre content ranged from 2.244 - 3.189 g/100 g, (db) 18.571 - 21.417 g/ 100 g, (db) and 21.388 - 23.662 g/ 100 g (db) respectively. Treatment time (T) had a significant ($P<0.05$) effect on the SDF content of the HPP samples, with the increase in treatment time from 1 min to 5 min, resulting in a marked increase in SDF levels. Whereas, pressure x time (P x T) interaction had a significant ($p<0.05$) negative effect on the IDF and total dietary fibre (TDF) levels. With an increase in treatment pressure (from 200-600 MPa) and time (1-5 min), the IDF and TDF levels decreased markedly. However, when compared to the control, all the samples had similar SDF, IDF and TDF levels, with only 6K5 having a significantly ($P<0.05$) higher SDF content. The significant increase in SDF content of sample 6K5 and the marked reduction in IDF and TDF levels of all samples can be a result of cleavage of glycosidic linkages and/ or breakage of weak bonds between polysaccharides (Wennberg and Nyman 2004). Consumption of SDF has shown to reduce postprandial glucose absorption in humans, acting as a glycaemic lowering ingredient. Thus HPP can be used in further research and development of legume-based functional foods.

5.3.3 Mineral content

The mineral content of the control and HPP sample (6K5) is reported in Table 5.3. Potassium was the most abundant mineral (5371 - 6131 mg/kg), followed by

phosphorus (3749 - 4173 mg/ kg), magnesium (1267 - 1340 mg/ kg) and calcium (917 - 994 mg/ kg). Other studies have reported that compared to their raw counterparts, soaked and/ or cooked legumes have a reduced mineral content (Bains, Uppal, and Kaur 2014, Barampama and Simard 1995, Wang, Hatcher, et al. 2010). The cooked samples from this study demonstrated higher iron, phosphorus, and magnesium levels when compared to uncooked chickpea samples from Pakistan (Iqbal et al. 2006), however, the effect of high pressure processing on the mineral content of cooked kabuli chickpeas has previously not been reported.

High hydrostatic pressure processing is known to not influence smaller molecules including pigments, vitamins, volatiles and compounds related to organoleptic, nutritional and health-promoting effects (Oey et al. 2008, Briones-Labarca et al. 2011). In our study, no significant ($P<0.05$) differences were observed in the mineral content of the control and the highest treatment level sample, meaning when consumers ingest HPP chickpeas, they receive the same total intake of minerals as with traditionally cooked chickpeas. Our results are also in agreement with studies on other foods treated with HPP such as Andrés, Villanueva, and Tenorio (2016) who reported no effect of HPP on the mineral content of milk and soy smoothies containing fruits (orange, papaya, melon and carrot). As no difference between the control and highest treatment level sample was found, samples with lower HPP treatments were not analysed for their mineral content.

5.3.4 Polyphenol content

The polyphenol content of the control and HPP samples is reported in Table 5.4. It ranged from 31.704 - 52.321 mg Gallic acid equivalent/ 100g sample (db), with the highest recorded for 2K1 and the lowest for 6K5. Main and significant ($P<0.05$) effects of pressure and treatment time were observed, suggesting that with an increase in pressure and treatment time, total polyphenol content (TPC) in samples decreased. However, when compared to the control, only samples treated at 600 MPa were significantly ($P<0.05$) lower in their TPC.

Similar results have been reported by Rodríguez-Roque et al. (2015) and Barba et al. (2012), who also found a slight decrease in the polyphenol content of orange juice following high pressure treatment above 400 MPa. Linsberger-Martin et al. (2013) also reported a reduction in the total phenolic content in split peas and whole white

beans after HPP. In contrast, Patras, Brunton, Da Pieve, and Butler (2009), Wang, He, and Chen (2014), Patras, Brunton, Da Pieve (2009), and Butler (2009) Sánchez-Moreno et al. (2006), reported an increase in the polyphenol content of different plant foods (cereals; tomato puree; tomato, carrot; and strawberry, blueberry respectively) after high pressure processing.

The type of food, location of phenolic compounds in food, pre-processing treatment, as well as the duration and intensity of the HPP treatment affects the concentration of phenolic compounds in the extract (Chandrasekara, Naczk, and Shahidi 2012). An increase in the total phenolic content of certain foods (in case certain fruits and vegetables) can be due to the disruption of cell wall structures, inactivation of enzymes related to loss of phenolic substances or improved extractability of the antioxidant components following high pressure treatment (Barba et al. 2012). However, changes in the physicochemical characteristics (Rodríguez-Roque et al. 2015) and enhanced chemical and enzymatic oxidation of polyphenols (Clariana et al. 2011) can occur due to processing, resulting in a lower availability.

5.3.5 Antioxidant capacity

An effect of high pressure processing on the antioxidant capacity of cooked kabuli chickpeas was observed and the values are reported in Table 5.4. The DPPH, ABTS and ORAC values for HPP samples ranged from 29.722 - 58.480 mg trolox equivalent/ 100g, 85.506 - 91.022 mg TE/ 100g, and 1253 - 1993 µmol TE/ 100g, respectively. For DPPH and ORAC antioxidant capacity, significant ($P < 0.05$) negative effects of pressure and time were observed. Thus, with an increase in pressure (200 – 600 MPa) and treatment time (1-5min), the DPPH and ORAC values significantly decreased. However, the reduction in ORAC values was only observed at the highest pressure level (600 MPa). Significant ($P<0.05$) main effects of pressure (P), time (T) and PxT interaction were observed for ABTS antioxidant capacity. However, following Duncan's post hoc test, only sample 6K5 showed a significantly ($P<0.05$) lower ABTS value when compared to all other samples including control. When compared to the control, 4K1, 4K5 6K1 and 6K5 showed significantly ($P<0.05$) lower DPPH antioxidant values, whereas only 6K5 expressed significantly ($p<0.05$) lower ABTS and ORAC value than the control. Sample 6K5 had the overall highest decrease in the antioxidant capacities out of all samples analysed.

The DPPH and ABTS assays are considered more accurate and reliable when compared to other methods such as FRAP (Ferric reducing antioxidant power) because of their rapidity, robustness and reliability in assessing the antioxidant capacity of plant materials (Piluzza and Bullitta 2011). On the other hand, ORAC assay uses biologically relevant free radicals, is standardised and integrates both degree and time of antioxidant reaction unlike other chemical assays (Zulueta, Esteve, and Frígola 2009). Previous studies have recommended using at least 2 assays for the antioxidant capacity analysis of plant materials (Schlesier et al. 2002, Piluzza and Bullitta 2011), and thus we have chosen these 3 assays for this study to validate our results.

Negative effects of thermal treatments on the antioxidant capacity of legumes such as chickpeas, soybeans, kidney beans (Gujral et al. 2013), common beans (Rocha-Guzmán et al. 2007), and cowpeas (Barros et al. 2017) have been reported in previous studies. However, very limited information on the effects of high pressure processing on the antioxidant capacity of legumes or foods, in general, is available. Doblado, Frías, and Vidal-Valverde (2007) observed a significant decrease in the ABTS antioxidant capacity of germinated cowpeas after HPP of up to 500 MPa (room temperature/ 15 min), and Butz et al. (2002) reported an 11% decrease in the ABTS antioxidant capacity of carrots after HPP at 500 MPa for 5 min (4 °C). In another study, a decrease in ABTS antioxidant capacity of orange juice was observed when the HPP pressure level was increased from 100- 800 MPa (at 30–65 °C) (Indrawati, Van Loey, and Hendrickx 2004) due to ascorbic acid degradation. In contrast, the DPPH antioxidant capacity of tomato puree was unchanged by HPP at 400 MPa/ 25 °C/ 15 min (Sánchez-Moreno et al. 2006) and Briones-Labarca et al. (2011) reported an increase in the DPPH antioxidant activity of uncooked Algarrobo seeds, an underutilised legume after high pressure processing at 500 MPA for 10 min compared to untreated seeds and seeds treated at 500 MPa for 2, 4 and 8 min (20 °C). The reduced antioxidant activity recorded in the present study may have occurred because of the combination of cooking and high pressure processing destroying the bioactive components or due to the formation of new compounds with pro-oxidant action.

The correlation between total polyphenol content (TPC) and the antioxidant activity revealed by three assays (DPPH, ABTS and ORAC) is also represented in Table 5.5. A very high and significant ($P<0.001$) positive correlation was observed between TPC and DPPH values ($r=0.922$), and slightly lower but significant ($P<0.001$) positive

correlations with ABTS (r =0.669), and ORAC (r = 0.727) assays were also observed. These results indicate that changes in the antioxidant capacity due to high pressure processing were closely related to the polyphenol content of the samples, supporting previous reports (Marathe et al. 2011, Wu et al. 2017).

Table 5. 2 Moisture, protein, starch and dietary fibre (soluble, insoluble and total dietary fibre) content of control and high pressure processed cooked chickpeas

Component (g/100g, db)	**Control**	**200 MPa, 1min**	**200 MPa, 5min**	**400 MPa, 1min**	**400mPa, 5min**	**600 MPa, 1min**	**600 MPa, 5min**
Moisture	62.65 ± 1.30	61.01 ± 1.46^{a}	62.53 ± 3.40^{a}	62.60 ± 1.49^{a}	61.44 ± 1.51^{a}	64.13 ± 1.41^{a}	62.62 ± 1.23^{a}
Protein	17.09 ± 1.21	17.81 ± 1.43^{a}	17.94 ± 1.24^{a}	19.02 ± 1.57^{a}	17.44 ± 0.17^{a}	18.65 ± 0.78^{a}	17.96 ± 2.12^{a}
Starch	54.48 ±2.18	54.97 ± 2.26^{a}	53.86 ± 1.30^{ab}	49.46 ± 4.57^{bc*}	47.41 ± 1.99^{c*}	47.29 ± 1.26^{c*}	46.13 ±0.59^{c*}
Dietary fibre							
SDF	2.408 ± 0.03	2.244 ± 0.39^{a}	2.817 ± 0.18^{a}	2.267 ± 0.10^{a}	3.094 ± 0.28^{a}	2.412 ± 0.49^{a}	3.189 ± 0.06^{a*}
IDF	20.951 ± 0.30	21.417 ± 0.56^{a}	18.571 ± 0.68^{b}	20.373 ± 0.07^{ab}	20.026 ± 0.61^{ab}	20.414 ± 0.16^{ab}	19.409 ± 0.39^{ab}
Total	23.359 ± 0.33	23.662 ± 0.96^{a}	21.388 ± 0.50^{a}	22.64 ± 0.02^{a}	23.12 ± 0.88^{a}	22.826 ± 0.65^{a}	22.598 ± 0.33^{a}

Where, SDF = soluble dietary fibre; and IDF= insoluble dietary fibre. All values are presented as mean ± standard deviation. Following Duncan's post hoc test, values followed by the same superscript within a row do not differ significantly ($p<0.05$). * Denotes significant difference ($p<0.05$) between control and HPP sample following Dunnett's test.

Table 5.3 In vitro Protein digestibility and mineral content of the control and high pressure processed cooked chickpeas

Parameters	Control	600 MPa, 5 min
IVPD (%)	78.078 ± 1.25[a]	79.046 ± 3.29[a]
Minerals (mg/kg, db)		
Calcium	994 ± 59[a]	917 ± 10[a]
Iron	62 ± 4.9[a]	59 ± 1.4[a]
Potassium	6131 ± 416[a]	5371 ± 18[a]
Magnesium	1340 ± 157[a]	1267 ± 76[a]
Sodium	75 ± 4.9[a]	74 ± 1.4[a]
Phosphorus	4173 ± 108[a]	3749 ± 12[a]
Zinc	42 ± 4.2[a]	40 ± 0.7[a]

Where, IVPD = *In vitro* protein digestibility. All values are presented as mean ± standard deviation. Following Duncan's post hoc test, values followed by the same superscript within a row do not differ significantly ($p<0.05$).

Table 5. 4 Polyphenol content and antioxidant capacity of control and high pressure processed cooked chickpeas

Parameters	Control	200 MPa, 1 min	200 MPa, 5 min	400 MPa, 1 min	400 MPa, 5 min	600 MPa, 1 min	600 MPa, 5 min
TPC (mg GAE/ 100 g, db)	49.390 ±1.05	52.321 ± 3.16^{a}	51.478 ± 1.56^{a}	51.341 ± 1.81^{a}	49.712 ± 1.53^{a}	34.159 ± 1.61^{b*}	31.704 ± 1.01^{b*}
DPPH (mg TE/ 100 g, db)	55.511 ±0.92	58.480 ± 1.50^{a}	56.542 ±0.61^{a}	48.731 ± 2.81^{b*}	46.351 ± 1.79^{b*}	33.693 ± 2.02^{c*}	29.722 ± 1.97^{c*}
ABTS (mg TE/ 100 g, db)	91.050 ±2.09	91.022 ± 3.38^{a}	90.309 ± 0.89^{a}	90.783 ± 1.07^{a}	90.678 ± 1.34^{a}	89.813 ± 1.46^{a}	85.506 ± 0.82^{b*}
ORAC (µmol TE/ 100 g, db)	1996 ± 72	1993 ± 97^{a}	1875 ± 224^{a}	2054 ± 267^{a}	1937 ± 128^{a}	1723 ± 196^{a}	1253 ± 233^{b*}

Where, TPC = total polyphenol content; DPPH = 2-2-diphenyl-1-picrylhydrazyl antioxidant assay; ABTS = 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt antioxidant assay; ORAC= oxygen radical antioxidant capacity. All values are presented as mean ± standard deviation. Following Duncan's post hoc test, values followed by the same superscript within a row do not differ significantly ($p<0.05$). * Denotes significant difference ($p<0.05$) between control and HPP samples following Dunnett's test.

Table 5.5 Pearson's correlation between TPC, DPPH, ABTS and ORAC values

Parameters	DPPH	ABTS	ORAC
TPC	0.922*	0.669*	0.727*
DPPH		0.618*	0.703*
ABTS			0.655*

*Significant ($p<0.001$). Where, TPC = total polyphenol content; DPPH = 2-2-diphenyl-1-picrylhydrazyl antioxidant assay; ABTS = 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt antioxidant assay; ORAC= oxygen radical antioxidant capacity

5.3.6 Light microscopy

Fig. 5.4 shows typical light microscopy images of the isolated starch powder from raw, cooked and HPP kabuli chickpeas. Swelling and distortion of cooked and HPP starch granules (Fig.5.4b-h) were evident when compared to the raw starch granules (40x) (Fig.5.4a). The smooth-surfaced, round or oval-shaped native chickpea starch granules transformed into irregular and elongated granules with a rough surface after high-temperature cooking and high pressure processing due to gelatinization (Fig.5.4 b-h) (Yniestra Marure et al. 2019). Similar results have been reported by Henry, Hudson, and Piperno (2009) and Crowther (2012), who also observed changes in chickpea starch granule structure on cooking. To understand the effect of cooking and HPP in more detail scanning electron microscopy was used to obtain high-resolution images of the starch granules.

5.3.7 Scanning electron microscopy

Morphological characteristics of starch granules from raw, cooked and HPP kabuli chickpeas was observed using scanning electron microscope (SEM). Raw chickpea starch (Fig. 5.5a), which was used as an internal control exhibited smooth, oval-shaped granules with no evidence of fissures or damage. Similar observations have been reported in previous studies on chickpea starch and starches from other legumes such as adzuki, black and kidney bean (Zhang et al. 2019, Wani, Sogi, and Gill 2015). When compared to starch granules from the raw samples, starch granules from the cooked and cooked+ HPP sample were a lot bigger (Fig 5.5a-h) in response to soaking and subsequent cooking. Scanning electron microscopy confirmed that high pressure

processing altered the starch granule structure (Fig. 5.5 c-h). SEM pictures showed that, like the control (5.5b), the majority of starch granules retain their granular shape. However, differences in their surface morphology were evident. HPP resulted in the formation of fissures and caused surface damage to the starch granules of HPP samples. Similar results have been reported in previous studies on high pressure processing of barley (Stolt, Oinonen, and Autio 2000) and potato (Błaszczak, Valverde, and Fornal 2005) starch.

During high pressure treatment, the available water is forced into the chickpea grains causing rapid hydration driven by the applied external pressure, resulting in rapid swelling even at ambient temperatures (Alsalman and Ramaswamy 2020). Błaszczak, Valverde, and Fornal (2005) reported significant deformations to freeze-dried potato starch granules caused by high pressure processing (Błaszczak, Valverde, and Fornal 2005), which can be observed in samples from this study as well. Liu et al.(2016) reported that high pressure contributed to a strong interaction between amylose and amylopectin chains leading to the formation of fractures and fissures on the surface of starch granules. This may also indicate significant changes in the internal structure of the granule. Starch undergoes a structural collapse that causes an alteration of the granular shape due to the simultaneous diffusion of solvents and gelatinization of starch (Pineda-Gómez, Rosales-Rivera, and Rodríguez-García 2012). Taken at the same magnification as the control, the pictures of starch granules (Fig.5.5g) depicts that very high pressure of 600MPa is responsible for greater destruction of the granule integrity leading to further damage. As differences in the morphology of the isolated starch granules were observed, an *in vitro* starch digestibility assay was performed to understand the effect of HPP on starch digestibility of all samples.

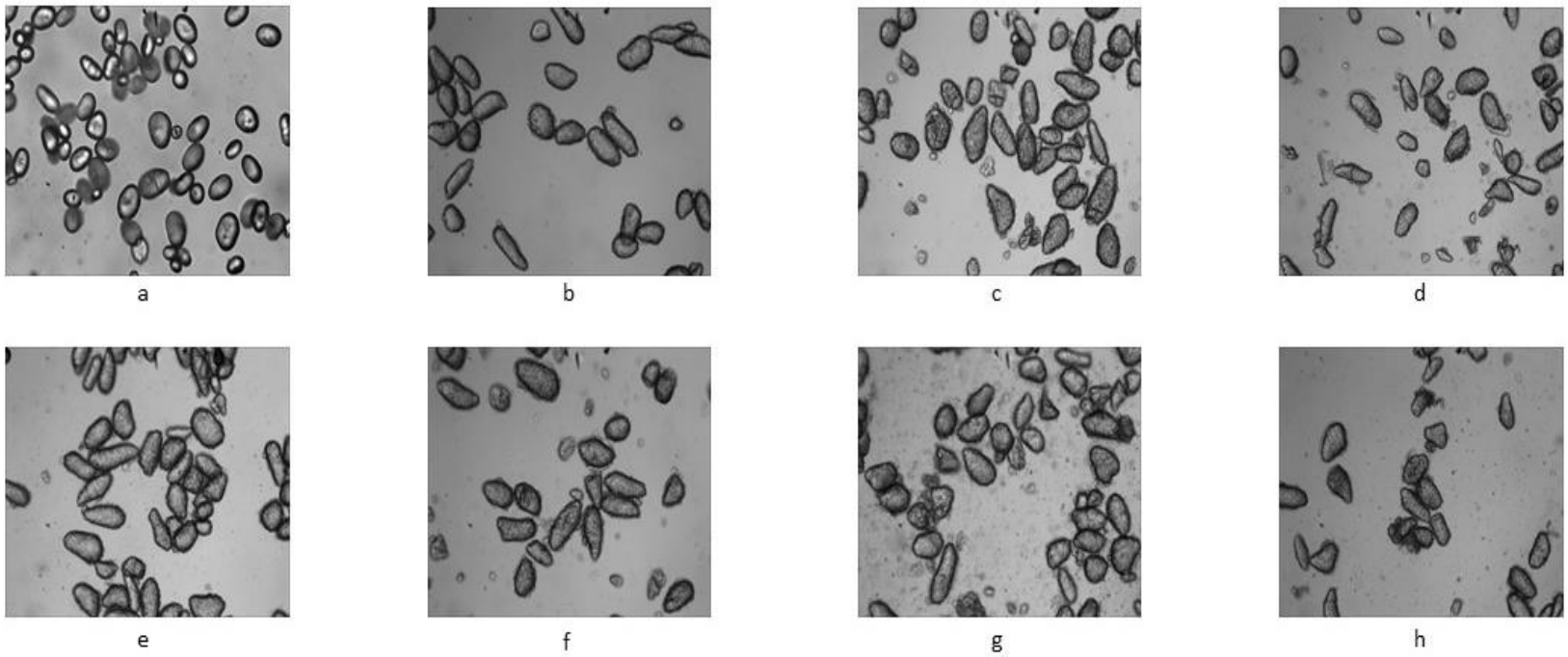

Figure 5.4 Light microscopic images (10 x magnification) of isolated native starch (a), control (b), and high pressure processed cooked samples (where, c= 200 MPa, 1min; d = 200 MPa, 5 min; e = 400 MPa, 1 min; f = 400 MPa, 5 min; g = 600 MPa, 1 min; and, h = 600 MPa, 5min)

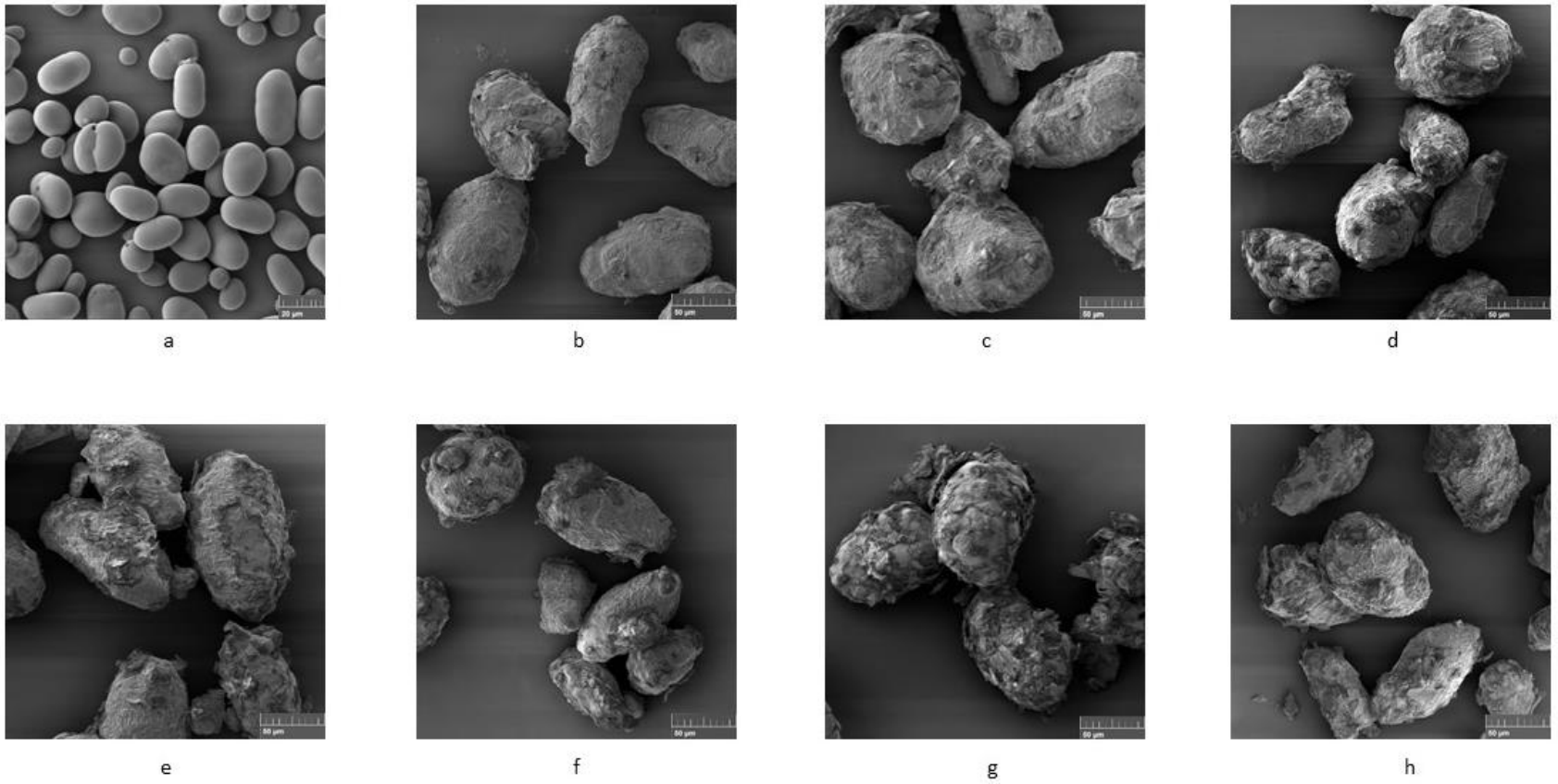

Figure 5.5 Scanning electron microscopy images (scale 20 to 50 µm) of isolated native starch (scale 20 µm) (a), control (b), and high pressure processed cooked chickpeas (where, c= 200 MPa, 1min; d = 200 MPa, 5 min; e = 400 MPa, 1 min; f = 400 MPa, 5 min; g = 600 MPa, 1 min; and, h = 600 MPa, 5min)

5.3.8 ATR-FTIR analysis

ATR-FTIR spectroscopy has previously been used to investigate the crystallinity, structure, and intermolecular interactions of starch granules (Warren, Gidley, and Flanagan 2016). However, in previous studies using purified starch samples specific structural information relating to starch was analysed (Xiong et al. 2017, Chavez-Murillo, Orona-Padilla, and de la Rosa Millan 2019). In this study, control and HPP chickpeas have been analysed with ATR- FTIR, and the spectra, therefore, contain contributions from proteins and lipids, in addition to starch. In this study, it is therefore not possible to draw absolute conclusions concerning starch structure, due to the spectra overlap of starch absorbance bands with the absorbance bands of proteins and lipids. Nonetheless, ATR-FTIR is still a useful method to investigate relative changes in the starch bonding environment and/or structure, as a consequence of high pressure treatments at different times.

The major adsorption bands arising from starch can be observed in the region 1200-1000cm^{-1} and it has been shown that bands at 1047 cm^{-1} and 1022cm^{-1} respectively, describe the crystalline and amorphous indices of starch (Kaptso et al. 2015). The ATR-FTIR results (Fig. 5.6) reveal distinct absorbance bands attributed to starch, which are easily viewed as “negative peaks” in the second-derivative spectra (Fig.5.6 a, b). Three specific bands of interest are those at 1140, 1040, and 1020 cm^{-1} (assigned to ν(C-O) modes) (Warren, Gidley, and Flanagan 2016). Interestingly, an increased spectra shift to lower wavenumbers was observed at the bands for HPP samples treated at 200, 400 and 600 MPa, indicating treatment-induced alteration to the starch structure and intermolecular bonding environment.

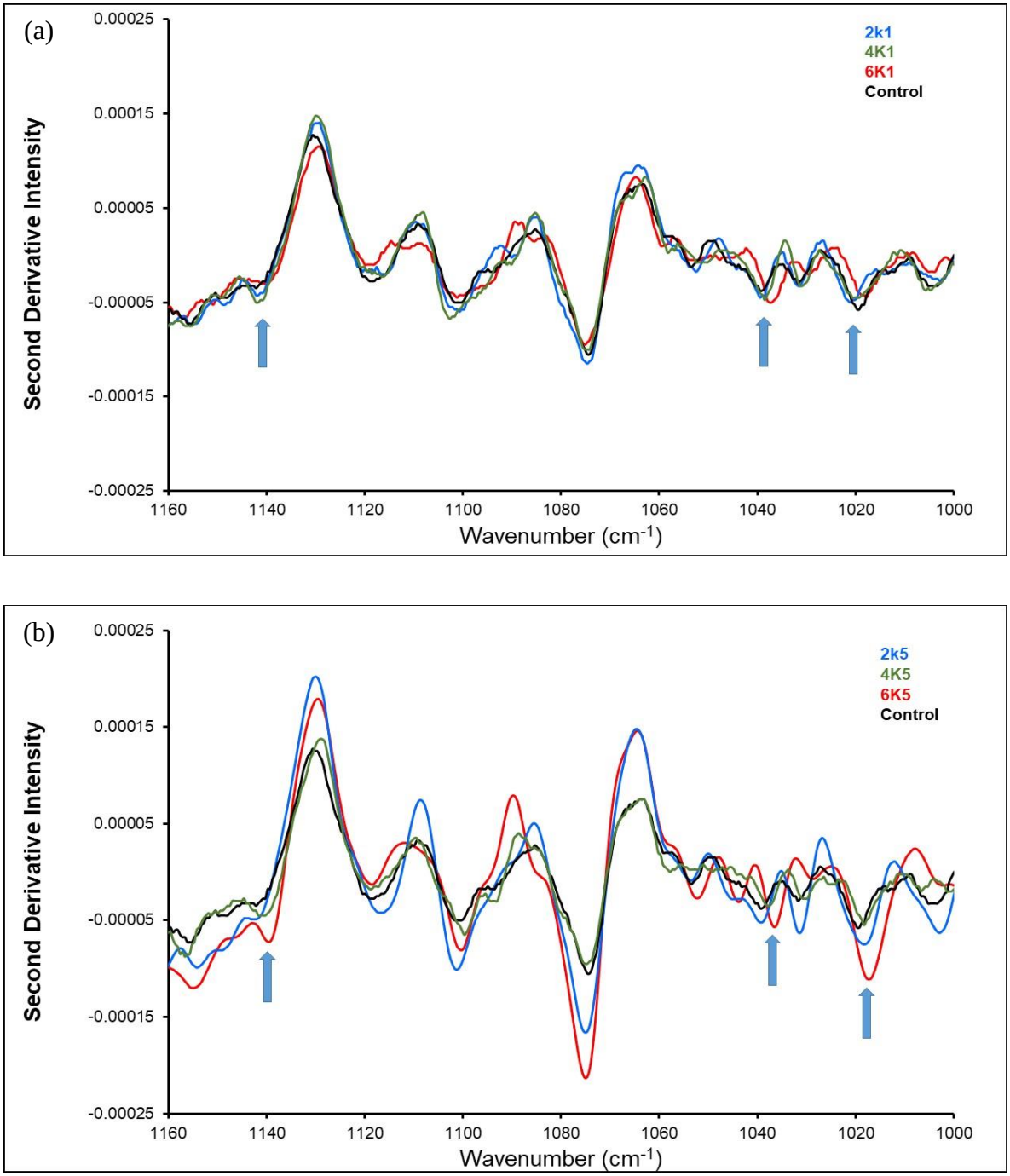

Figure 5.6 ATR- FTIR analysis of control and HPP kabuli chickpeas with upward arrows indicating shift in spectra at 1140 cm^{-1}, 1040 cm^{-1} and 1020 cm^{-1} (where 2K1= 200 MPa, 1min; 2K5= 200 MPa, 5 min; 4K1= 400 MPa, 1 min; 4K5= 400 MPa, 5 min; 6k1= 600 MPa, 1 min; and, 6K5 = 600 MPa, 5min)

Also, the intensity of the bands increased with an increase in treatment pressure and time (Fig. 5.6b), suggesting conversion of starch molecules to their ruptured form and disruption of the chemical bonds (Mir et al. 2016). High pressure processing has been

known to decrease the relative crystallinity of starch granules, which in turn lowers starch digestibility (Katopo, Song, and Jane 2002, Colussi et al. 2018). Pressurisation during high pressure processing partially gelatinises starch granules, whereas depressurisation promotes retrogradation which results in recrystallization of amylopectin and further reduces starch digestion (Fredriksson et al. 2000).

5.3.9 In vitro starch digestibility

Several factors such as the crystalline structure, source of starch, amylose-amylopectin ratio and granule size affect the enzymatic susceptibility of starches, with amylose content and crystallinity being the most important factors (Zavareze and Dias 2011). Starch gelatinisation occurs when starch is heated in the presence of excess water, resulting in a change from crystalline to a more amorphous structure (Zavareze and Dias 2011), thus becoming more digestible. However, starch entrapped within the cells of edible plant material (e.g. seeds) undergoes limited starch gelatinisation (Brummer, Mina, and Susan 2015), retaining its crystalline nature and resulting in a lower digestibility (Baldwin et al. 2015). Intracellular components such as proteins also impose restrictions on starch granule swelling, thus affecting starch digestibility (Shomer 1995).

As shown in Fig. 5.7, with an increase in treatment pressure and time, the contents of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) in the HPP samples also changed. Significant ($P<0.05$) main effects of pressure (P) and time (T) on RDS, SDS and RS levels were observed. It was observed that HPP at higher pressures and longer times (2K5, 4K5, 6K5) can significantly ($P < 0.05$) increase the SDS levels in cooked chickpeas when compared to lower pressures and shorter treatment durations (2K1, 4K1, 6K1), and when the pressure level reached the maximum value (600 MPa, 5min) of the machine, the SDS fraction was at the highest amount (60.92 g/ 100g starch). However, only the SDS content of the 2K5, 6K1 and 6K5 samples and the RS content of the 2K5 and 6K5 samples were significantly ($p<0.05$) different from the control following the starch fractions determination.

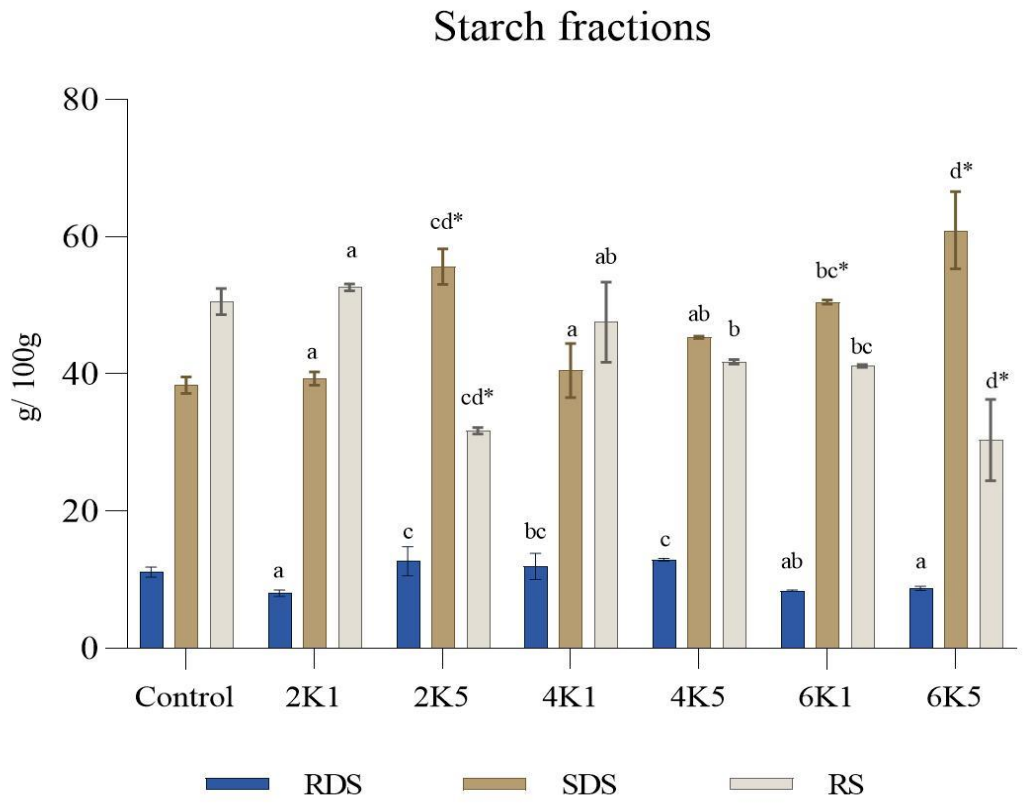

Figure 5.7 Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) in the control and HPP chickpea samples. Bars bearing different letter for the same fraction are significantly ($p<0.05$) different. Bars bearing * for the same fraction are significantly ($p<0.05$) different than the control (where, 2K1= 200 MPa, 1min; 2K5= 200 MPa, 5 min; 4K1= 400 MPa, 1 min; 4K5= 400 MPa, 5 min; 6k1= 600 MPa, 1 min; and, 6K5 = 600 MPa, 5min)

Previous research has shown that high pressure processing can completely damage uncooked rice starch granules as well (Hu et al. 2011), while also promoting a wider distribution of water molecules in the crystalline regions of the starch granules. This in turn disrupts the amylopectin chains (Li et al. 2012) and forms imperfect crystallites, which are directly linked to high SDS levels (Tian et al. 2014). Another reason for the increase in SDS content might be the formation of amylose-lipid complexes in starch granules in smaller volumes as a result of HPP (Lullien-Pellerin and Balny 2002).

Starch digestibility of cooked, waxy wheat starch (Hu et al. 2017), buckwheat (Liu et al. 2016) and brown rice (Xia et al. 2017) have previously been modified by the use of high pressure treatment when compared to their raw counterparts. It was also

observed in these studies that HPP increased the amount of slowly digestible starch (SDS), decreasing the overall starch digestibility of these samples. Slowly digestible starch has been shown to provide nutritional benefits for humans (Sang and Seib 2006), as it leads to a slow and prolonged postprandial release of glucose in the bloodstream, thus providing a lower glycemic response (Zhang, Ao, and Hamaker 2006). It has been suggested that HPP has potential in producing products for glycemic control (Xia et al. 2017), which can help in the prevention of diseases such as diabetes, cardiovascular diseases and colon cancer (Jenkins et al. 2002).

5.3.10 Starch digestion kinetics

Disregarding the type of treatment, all samples in this study displayed a monophasic digestogram (Fig. 5.8). With the increase in enzyme-substrate contact time, an increase in starch digestion was observed. Starch digestion is affected by several factors such as starch granule size, crystallinity, surface channels/ pores, and availability and interaction of non-starch components with the starch (Noda et al. 2008, Mahasukhonthachat, Sopade, and Gidley 2010). As shown in Fig 8, the digestion rate of the 6K5 sample was the highest, followed by 6K1, 2K5, 4K1, 4K5, control and 2K1 in the descending order. The starch digestion progress curves were fitted using the general first order (Al-Rabadi, Gilbert, and Gidley 2009) and the digestion rate coefficient (k) was determined. The extent of starch hydrolysis after 120 min of digestion for the cooked and HPP samples were 53, 48, 71, 65, 66, 75 and 79% for the control, 2K1, 2K5, 4K1, 4K5, 6K1 and 6K5 respectively. Kinetic analysis using Al-Rabadi's first order showed a distinct linear phase with the corresponding calculated first order rates ($k \times 10^{-3}$ value, min^{-1}) of 5.8, 5.1, 9.5, 8.3, 7.8, 10.7 and 11.6 respectively for the control and high pressure treated samples (Appendix 1, S Fig. 1, Table S 7). The corresponding calculations using Al-Rabadi's first order model revealed that the digestion rate coefficient (k) for the highest treatment level (6K5) was markedly higher than all other samples including control, and was closely followed by the second highest treatment level (6K1). Previous studies have reported that starch crystalline structural features do not affect the starch digestion coefficient (Xiong et al. 2018, Wang, Wang, et al. 2017), and only access or binding of digestive enzymes and starch dominates the digestion process in pulses (Dhital et al. 2016).

As HPP treatment involves very high pressure levels, it can encourage the distribution of water molecules in starch, leading to the formation of more imperfect crystallites, thus affecting starch digestibility (Zhou et al. 2015). Cell wall porosity is considered another major factor that can affect starch digestibility in pulses such as chickpeas (Dhital et al. 2016, Xiong et al. 2018, Bhattarai et al. 2017). Instantaneous pulse softening (IPS) is a phenomenon, known to be responsible for softening of cell wall tissue during high pressure processing due to membrane disruption (Trejo Araya et al. 2007, Basak and Ramaswamy 1998), leading to higher accessibility of starch by digestive enzymes, in turn, increasing the coefficient of digestibility, and is supported in this study.

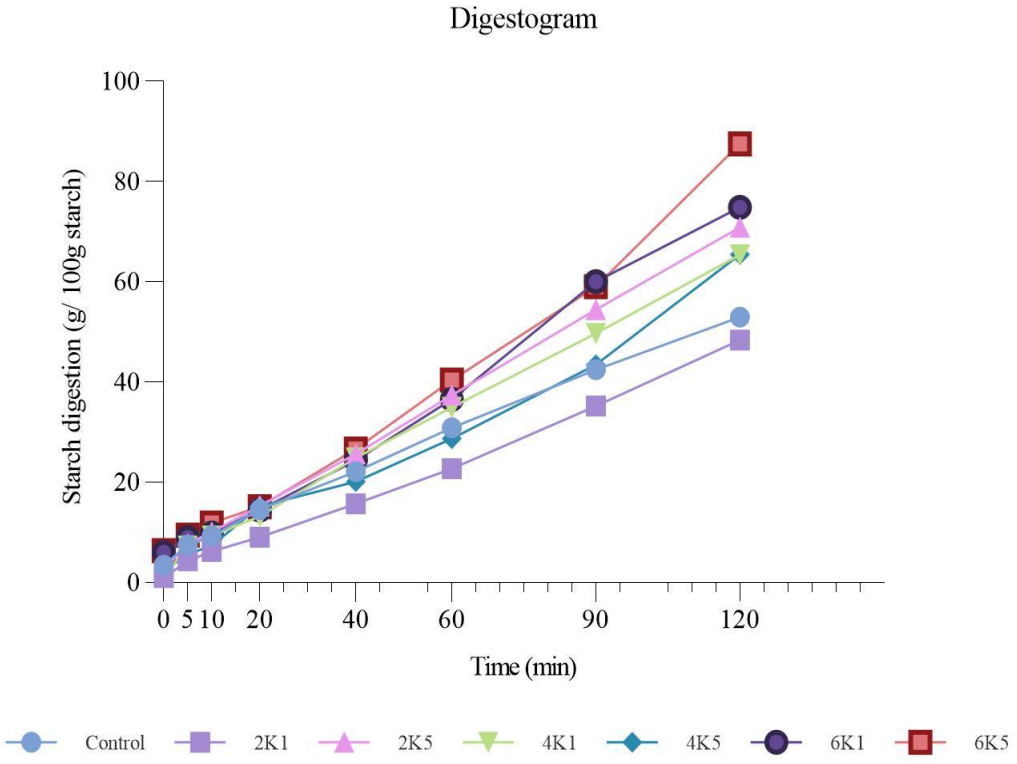

Figure 5.8 A digestogram of control and HPP samples (where, 2K1= 200 MPa, 1min; 2K5= 200 MPa, 5 min; 4K1= 400 MPa, 1 min; 4K5= 400 MPa, 5 min; 6k1= 600 MPa, 1 min; and, 6K5 = 600 MPa, 5min)

5.3.11 In vitro protein digestibility

The percentage in vitro protein digestibility (IVPD) values for the control and the highest HPP treatment (600 MPa, 5min) are reported in Table 5.3. There was no

significant ($P<0.05$) effect of pressure, time or their interaction on the IVPD values for the control and HPP sample. Cooking and thermal heat treatments (autoclaving) have been shown to improve the protein digestibility of raw legumes such as black grams, chickpeas, lentils, and red kidney beans due to increased accessibility of the protein component for enzymatic attack (Rehman and Shah 2005), and/ or inactivation of proteinacious anti-nutritional factors (van der Poel, Gravendeel, and Boer 1991). High pressure cooking of rice has been shown to reduce its protein digestibility (Liu, Zheng, and Chen 2019), whereas Laguna et al. (2017) reported a small reduction (8%) in the protein digestibility of apple puree enriched with pea protein following HPP, with no effect of HPP on carrot puree enriched with pea protein. However, the effect of high pressure processing on the protein digestibility of chickpeas is not widely known.

Deol and Bains (2010) reported an increase in protein digestibility of cowpeas after steam pressure cooking mainly due to the eradication of antinutrients. Linsberger-Martin et al. (2013) reported a 3.5% increase in the protein digestibility of split peas and a 6% increase in the protein digestibility of whole white beans after HPP at 600 MPa for 60 min. However, a small but significant reduction in protein digestibility was also reported in both split peas and whole white beans after HPP at 100 MPA for 60 min and 350 MPA for 45 min (Linsberger-Martin et al. 2013). A possible explanation for this reduction in protein digestibility can be due to the formation of intra/ intermolecular disulphide bridges or a protein network (Angioloni and Collar 2012). While the mechanism of pressure induced protein unfolding is not completely understood, the underlying mechanism of pressure induced protein denaturation involves water penetration into cavities within the molecule resulting in varying populations of molecular conformations (Silva, Foguel, and Royer 2001), resulting in increased protein digestibility. However, as in our study, all the samples were highly saturated with water (12 h soaking + 30min boiling) before the HP treatment, no significant changes in the protein digestibility of control and HPP sample were observed. As there was no significant difference between the control and the highest treatment level (6k5), the remaining samples were not subjected to the this analysis.

5.4 Conclusion

This study demonstrated that the textural and starch digestibility properties of cooked kabuli chickpea is significantly improved by the application of high pressure processing. Significant effects of treatment pressure and time on the starch, polyphenol and antioxidant capacity were also revealed. These combined findings highlight the potential of utilizing non-thermal processing technologies to increase consumer acceptance of plant-based protein sources with desirable textural properties. Our findings suggest that high pressure processing has the potential to enable the design of products with a low glycaemic index, compared to traditional products, while retaining dietary fibre percentages, minerals and digestible proteins and thus providing beneficial health effects to consumers. However, the shelf stability of cooked + high pressure processed ready-to-eat chickpea product is unknown and thus further research is required to understand this property while maintaining high consumer acceptability and maximum potential nutritional and health benefits.

5.5. Acknowledgement

I wish to acknowledge Preshafoods Pty Ltd, Victoria (Australia) for their assistance with high pressure processing, Dr Mark J. Hackett, School of Molecular and Life Sciences, Curtin University for assistance with the FTIR analysis and interpretation, and the John de Laeter Centre, Curtin University, Bentley, WA for the microscopy equipment.

CHAPTER 6 Final conclusion

"I feel it is an obligation to help people understand the relation of food and agriculture and the relation of food to culture."

Alice Waters

6.1 Introduction

This thesis aimed to further our understanding of Australian kabuli chickpea seed properties and the effects of different factors (genotype, environment and processing) on these properties. Chickpeas are an important global pulse crop and could be a key source of essential proteins in the challenging circumstances of an ever-growing world population. The results from this research can be included in recommended guidelines for chickpea growers and breeders, food manufacturers and consumers for improving the nutritional composition of chickpeas, location selection for growing particular varieties, and increasing consumption of Australian kabuli chickpeas by adding value through appropriate processing.

Proteins are an essential macronutrient for human nutrition (Adenekan et al. 2018), and the current unsustainable trend of high global animal protein consumption results in significant greenhouse gas emissions when compared to consumption of plant proteins, contributing directly to a changing climate (Alemayehu, Bendevis, and Jacobsen 2015, Pojić, Mišan, and Tiwari 2018). This research has underlined the seed properties of Australian kabuli chickpeas for its wider use as a source of plant-based proteins and antioxidants for human nutrition and prevention of chronic illnesses. Increasing demand for plant proteins and an increase in vegetarianism or veganism, especially in developed countries such as Australia, New Zealand, Sweden and Israel, with over 10% of the total population representing this demographic, have been observed in the last 5-10 years (Pojić, Mišan, and Tiwari 2018). Chickpeas being a rich source of proteins, widely available at low cost are therefore one of the best alternatives to animal proteins, and the application of sustainable farming practices can lead to low carbon-emitting food system (Lal 2004).

6.2 General discussion

The main purpose of this study was to determine the physical, nutritional and antioxidant properties of Australian kabuli chickpeas and to understand the effects of growing environment and processing on these properties.

The first objective of this thesis was to determine the seed properties of the most widely grown commercial Australian kabuli chickpea varieties. The findings of this chapter revealed high variability between Australian kabuli chickpea physical, nutritional and bioactive properties. Kimberley large kabuli chickpeas, grown exclusively in the Kimberley region of Western Australia exhibited the highest values for physical properties (seed weight, volume, hydration and swelling capacity) due to its large seed size (9-11mm). The seed weight of this particular variety was comparatively higher when compared to kabuli chickpeas from India (Kaur, Singh, and Sodhi 2005, Sundaram et al. 2019), Pakistan (Hameed et al. 2009) and Canada (Frimpong et al. 2009), and thus demands a premium price in the global export market (Dixit, Srivastava, and Singh 2019). Kimberley large also contained the highest starch content (48.9g/ 100g, db) and biologically relevant antioxidant capacity (ORAC) level (2808 µmol TE/ 100 g sample, db), but lacked protein (18.2 g/ 100g, db) when compared even to the smallest seeded variety, Genesis 090 (20.3 g/ 100g, db) from this study. Genesis Kalkee had the highest protein content (24.6 g/ 100 g, db), making it the highest protein containing Australian kabuli chickpea variety from our study. The protein content of Genesis Kalkee when compared to some Mexican (Arevalo et al. 2020), Italian (Summo, De Angelis, Rochette, et al. 2019), Canadian (Frimpong et al. 2009), Jordanian (Ereifej, Al-Karaki, and Hammouri 2001) and Turkish (Özer et al. 2010) kabuli chickpeas was higher, making these Australian kabuli varieties more attractive in the global chickpea market. The Kabuli chickpea varieties analysed in this first objective of the thesis were commercially grown in the environments best suited to them. However, as reported in multiple studies (Frimpong et al. 2009, Cobos et al. 2017, Kaloki, Trethowan, and Tan 2019, Vandemark et al. 2020), chickpea seed properties are significantly affected by the growing environment as well variety.

Thus, the second objective of this thesis was to determine the effects of genotype, environment and their interactions on the physical, nutritional and antioxidant properties of Australian kabuli chickpeas. The outcomes of this study demonstrate that genotype, environment and their interaction play a crucial role in variation in kabuli

chickpea seed properties, with the growing environment being the most important factor. Previous research has reported that consumption of high polyphenol containing foods is beneficial to human health due to their protective effects against type 2 diabetes, neurodegenerative and cardiovascular diseases (Fraga et al. 2019, Cory et al. 2018, Fujiki et al. 2015, Xiao and Hogger 2015). The amount of chickpea polyphenols and their antioxidant properties significantly increased when chickpeas were grown in environments, such as Kununurra and Walgett, with a high number of extreme temperature occurrences (both high and low temperatures) as reported in Chapter 3, Table 4.2, whereas, the mean seed weight, and mean protein content decreased due to these extreme temperature conditions (Table 4.4). Furthermore, chickpeas grown in the environment with the highest rainfall (Tarranyurk) contained the highest mean fat content (5.3 g/ 100g, db), whereas, chickpeas grown in the environment with the lowest rainfall, but sufficient water due to irrigation, exhibited the highest mean starch levels (28.7 g/ 100 g, db) (Table 4.4). Kabuli chickpeas grown in locations with extreme high (above 30° C) or low temperatures (below 0° C) can be an inexpensive source of dietary polyphenols, thus providing the beneficial effects associated with it to a wider consumer base. By appropriate marketing, it is suggested that farmers could get higher return on chickpeas grown in regions experiencing extreme temperatures as these chickpeas may command a higher price due to their high antioxidant capacity.

The third objective of this study was to determine the effects of high pressure processing on textural, nutritional and antioxidant properties of cooked kabuli chickpeas. Being a non-thermal processing method, high pressure processing has been utilised for production of high quality packaged dairy, fruits, vegetables, meat and seafood. However, its application in grain and pulse processing industry is limited. Consumption of whole chickpeas is beneficial due to starch confinement within chickpea cell walls, leading to limited release of digestion products in the human gut post consumption (Martinez 2021). The findings of Chapter 5 demonstrated that high pressure processing (HPP) alters the texture profile of cooked kabuli chickpeas, making them softer, less chewy (Section 5.3.1) and thus more acceptable by consumers (Khatoon and Prakash 2006, Ghasemlou, Gharibzahedi, and Emam-Djomeh 2013). The proximate composition and protein digestibility of cooked and HPP kabuli chickpeas were similar (Table 5.2 and 5.3), concluding that humans will receive the same amount of nutrients after consuming either. The attenuated total reflectance-

Fourier transform infrared (ATR-FTIR) spectroscopy analysis showed changes in starch structure and isolated starch globules were damaged through the formation of fissures due to high pressure processing. A further starch digestibility analysis confirmed that the highest HPP levels modified the starch fractions (rapidly digestible, slowly digestible and resistant starch) significantly ($p<0.05$) by increasing the slowly digestible starch (SDS), from 38.36 to 60.92 g/ 100 g starch (db), and reducing the rapidly digestible starch (RDS), from 50.53- 30.35 g/ 100g starch (db), and resistant starch, 11.10- 8.73 g/ 100 g starch, (db) content in chickpeas. Unlike RDS, digestion of SDS does not begin in the oral cavity by salivary α- amylase, or in the stomach lining by gastric acids. SDS is mainly digested in the duodenum by the enzymes secreted by the pancreas, resulting in the prolonged release of glucose in the bloodstream (Miao et al. 2015). This in turn makes high pressure processed kabuli chickpeas a healthier and desirable food option, especially for people suffering from chronic conditions such as diabetes and cardiovascular diseases (Rizkalla et al. 2004).

In conclusion, this study showed that new knowledge on seed properties of Australian kabuli chickpea varieties as well as the effects of genotype, environment and processing presented in this thesis are important for chickpea breeders, farmers and food manufacturers to enable them to make informed decisions on the chickpea varieties, growing locations, and design of novel food products with high nutritional and antioxidant properties to target a wide spectrum of consumers.

6.3 Significance

There is a lack of studies on the physical and compositional properties of kabuli chickpeas, in particular Australian kabuli chickpeas. In addition to this, limited research has been conducted to determine the effects of genotype, environment, and non-thermal processing methods on the physical, nutritional, and antioxidant properties of kabuli chickpeas. Therefore, this thesis can provide:

1. Vital new knowledge on Australian kabuli chickpea seed quality assessed by determination of chickpea seed properties.
2. Valuable information to chickpea farmers for selecting specific growing environments to get desirable nutritional properties in their produce, followed by appropriate marketing to encourage consumers to utilise Australian kabuli chickpeas to meet their nutritional requirements.

3. New information on the significant role of growing environment on kabuli chickpea physical and nutritional properties not captured by the currently available literature.
4. Food manufacturers an opportunity to utilise Australian kabuli chickpeas grown specifically for; (a) high protein and (b) high antioxidant content to produce novel food products to maximise the beneficial effects of kabuli chickpea consumption.

These outputs lead to the following outcomes:

1. This information can be used for the improvement of future Australian kabuli chickpea cultivars by enhancing their nutritional and bioactive composition.
2. Chickpea growers may benefit by growing kabuli chickpeas in specific growing environments to target particular food markets e.g. high polyphenol containing chickpeas grown in harsh climates to target the functional foods market.
3. Product developers can use this information to target specific consumer markets, benefitting chickpea growers due to increased demand.
4. Food industries can utilise the information on kabuli chickpea nutritional composition and high pressure processing of cooked kabuli chickpeas to design healthier food products for consumers focussed on plant protein sources and health.

Despite the significance of the results, there were certain limitations to this study: (a) a more sensitive method for total polyphenol content estimation (Fast Blue BB) can now be utilised in place of Folin Ciocalteu method due to recent method development (Pico et al. 2020) ;(b) the individual polyphenols in different chickpea varieties (Chapter 3) could be identified using advanced techniques such as nuclear magnetic resonance imaging, high performance liquid chromatography and mass spectrometry, and triple quadrupole mass spectrometry to understand the role of specific polyphenols in chickpea antioxidant capacity as detailed by Forino et al. (2016) and Dzah et al. (2020); (c) the anti-nutrients (such as α- galactosidase, phytic acid, tannins and trypsin inhibitors) responsible for causing discomfort, hindering digestion, reducing the bioavailability of macro and micronutrients in humans content were not determined in

chickpea varieties (Chapter 3); (d) the antioxidant activity of kabuli chickpea polyphenol extracts was evaluated using in vitro chemical assays (DPPH, ABTS, and ORAC), and even though the ORAC assay uses a biologically relevant radical source, these methods cannot predict the actual effects of the chickpea polyphenol extracts in humans (Prior et al. 2003, Wolfe and Liu 2007); (e) The G × E study did not include National Variety Trial samples from 2 growing seasons (different years), which could help in understanding the stability in performance of kabuli chickpea varieties within an environment (Chapter 4); (f) the high pressure processing time settings were constrained by the limitations of the equipment (Chapter 5); (g) instead of animal or human trials, in vitro digestion model was used to evaluate the starch and protein digestibility of high pressure processed kabuli chickpeas, wherein fermentation in-colon was not included. Thus the results may not reflect actual values in humans (Hur et al. 2011, Bohn et al. 2018).

6.4 Future perspectives

The current findings of this study urge us to further explore this research area. A detailed characterisation of the physical, nutritional, and antioxidant properties of other Australian chickpea varieties can be conducted to update nutrition composition databases (e.g. FSANZ) to depict the actual nutritional composition of specific kabuli chickpea varieties. The knowledge generated will also be helpful to growers to help increase the nutritional and antioxidant properties of Australian kabuli chickpeas by careful selection of chickpea growing environment. Researchers and food industries can utilise chickpea composition data to design innovative food products.

Other recommended studies for the future include shelf-life determination of high pressure processed kabuli chickpeas. High pressure processing has been utilised as a preservation technique for fruit and dairy products, however, its preservation effects on legumes are still underexplored. Another area of interest is the reduction in anti-nutrients which deter people from consuming legumes. A reduction or complete eradication of anti-nutrients responsible for digestive discomfort will likely increase chickpea consumption.

In the words of Thomas Jefferson-"*Agriculture is our wisest pursuit because it contributes most to real wealth*", we can conclude that findings reported in this thesis have a broad range of potential beneficial outcomes, one being increased demand for

Australian kabuli chickpeas generating higher financial returns for chickpea farmers. Chickpea breeders will possess new information that may assist in producing more adaptable varieties that perform consistently in unfavourable growing environments. Chickpeas with specific seed properties (e.g. high polyphenol content) can be packaged and marketed to target specific consumer markets and food industries may utilise the information to produce new chickpea-based ingredients and products with specific nutritional functionality for benefitting consumer health.

REFERENCES

Abd El-Hady, E. A., and R. A. Habiba. 2003. "Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds." *LWT - Food Science and Technology* 36 (3):285-293. doi: https://doi.org/10.1016/S0023-6438(02)00217-7.

Abu, JD, SS Arogba, and FM Ugwu. 1999. "The effects of post-harvest handling on physical, chemical and functional properties of cowpea (*Vigna unguiculata*) seed." *Journal of the Science of Food and Agriculture* 79 (11):1325-1330. doi: 10.1002/(sici)1097-0010(199908)79:11<1325::Aid-jsfa333>3.0.Co;2-c.

Acosta-Estrada, Beatriz A., Janet A. Gutiérrez-Uribe, and Sergio O. Serna-Saldívar. 2014. "Bound phenolics in foods, a review." *Food Chemistry* 152:46-55. doi: https://doi.org/10.1016/j.foodchem.2013.11.093.

Adamidou, S., I. Nengas, K. Grigorakis, D. Nikolopoulou, and K. Jauncey. 2011. "Chemical Composition and Antinutritional Factors of Field Peas (Pisum sativum), Chickpeas (Cicer arietinum), and Faba Beans (Vicia faba) as Affected by Extrusion Preconditioning and Drying Temperatures." *Cereal Chemistry* 88 (1):80-86. doi: 10.1094/cchem-05-10-0077.

Adenekan, M. K., G. J. Fadimu, L. A. Odunmbaku, and E. K. Oke. 2018. "Effect of isolation techniques on the characteristics of pigeon pea (*Cajanus cajan*) protein isolates." *Food Science and Nutrition* 6 (1):146-152. doi: 10.1002/fsn3.539.

Agati, G., E. Azzarello, S. Pollastri, and M. Tattini. 2012. "Flavonoids as antioxidants in plants: location and functional significance." *Plant Science* 196:67-76. doi: 10.1016/j.plantsci.2012.07.014.

Agriculture, and Canada Agri-Food. 2008. "Dry peas: Situation and outlook." accessed 10th Feb. http://www.publications.gc.ca/collections/Collection/A27-18-13-16E.pdf.

Aguilera, Y., R. M. Esteban, V. Benitez, E. Molla, and M. A. Martin-Cabrejas. 2009. "Starch, functional properties, and microstructural characteristics in chickpea and lentil as affected by thermal processing." *Journal of Agriculture and Food Chemistry* 57 (22):10682-8. doi: 10.1021/jf902042r.

Aguilera, Yolanda, Rosa M. Esteban, Vanesa Benítez, Esperanza Mollá, and María A. Martín-Cabrejas. 2009b. "Starch, Functional Properties, and Microstructural Characteristics in Chickpea and Lentil As Affected by Thermal Processing." *Journal of Agricultural and Food Chemistry* 57 (22):10682-10688. doi: 10.1021/jf902042r.

Aguilera, Yolanda, María A. Martín-Cabrejas, Vanesa Benítez, Esperanza Mollá, Francisco J. López-Andréu, and Rosa M. Esteban. 2009a. "Changes in carbohydrate fraction during dehydration process of common legumes." *Journal of Food Composition and Analysis* 22 (7–8):678-683. doi: http://dx.doi.org/10.1016/j.jfca.2009.02.012.

Ahmed, J., L. Thomas, A. Taher, and A. Joseph. 2016. "Impact of high pressure treatment on functional, rheological, pasting, and structural properties of lentil starch dispersions." *Carbohydrate Polymers* 152:639-647. doi: 10.1016/j.carbpol.2016.07.008.

Ahmed, Mohamed Ismael, Xueming Xu, Abdellatief A. Sulieman, Amer Ali Mahdi, and Yang Na. 2020. "Effect of extraction conditions on phenolic compounds and antioxidant properties of koreeb (*Dactyloctenium aegyptium*) seeds flour." *Journal of Food Measurement and Characterization* 14 (2):799-808. doi: 10.1007/s11694-019-00328-9.

Ahmed, Nawal A., Nasr M. Radwan, Heba S. Aboul Ezz, and Noha A. Salama. 2017. "The antioxidant effect of Green Tea Mega EGCG against electromagnetic radiation-induced oxidative stress in the hippocampus and striatum of rats." *Electromagnetic Biology and Medicine* 36 (1):63-73. doi: 10.1080/15368378.2016.1194292.

Al-Rabadi, Ghaid J. S., Robert G. Gilbert, and Michael J. Gidley. 2009. "Effect of particle size on kinetics of starch digestion in milled barley and sorghum grains by porcine alpha-amylase." *Journal of Cereal Science* 50 (2):198-204. doi: 10.1016/j.jcs.2009.05.001.

Alajaji, Saleh A., and Tarek A. El-Adawy. 2006. "Nutritional composition of chickpea (Cicer arietinum L.) as affected by microwave cooking and other traditional cooking methods." *Journal of Food Composition and Analysis* 19 (8):806-812. doi: https://doi.org/10.1016/j.jfca.2006.03.015.

Alemayehu, F. R., M. A. Bendevis, and S. E. Jacobsen. 2015. "The Potential for Utilizing the Seed Crop Amaranth (Amaranthus spp.) in East Africa as an Alternative Crop to Support Food Security and Climate Change Mitigation." *Journal of Agronomy and Crop Science* 201 (5):321-329. doi: 10.1111/jac.12108.

Alexandratos, N. and J. Bruinsma. 2012. World agriculture towards 2030/2050: the 2012 revision, ESA Working Papers 288998. Rome: Food and Agriculture Organization of the United Nations, Agricultural Development Economics Division (ESA).

Ali, Qasim, Muhammad Ashraf, and Farooq Anwar. 2010. "Seed Composition and Seed Oil Antioxidant Activity of Maize Under Water Stress." *Journal of the American Oil Chemists' Society* 87 (10):1179-1187. doi: https://doi.org/10.1007/s11746-010-1599-5.

Alonso, R., G. Grant, P. Dewey, and F. Marzo. 2000. "Nutritional assessment in vitro and in vivo of raw and extruded peas (*Pisum sativum* L.)." *J Agric Food Chem* 48 (6):2286-90.

Alsalman, Fatemah B., and Hosahalli Ramaswamy. 2020. "Reduction in soaking time and anti-nutritional factors by high pressure processing of chickpeas." *Journal of Food Science and Technology* 57 (7):2572-2585. doi: 10.1007/s13197-020-04294-9.

Altuntas, Ebubekir, and Hilal Demirtola. 2007. "Effect of moisture content on physical properties of some grain legume seeds." *New Zealand Journal of Crop and Horticultural Science* 35 (4):423-433. doi: 10.1080/01140670709510210.

Altuntaş, Ebubekir, and Mehmet Yıldız. 2007. "Effect of moisture content on some physical and mechanical properties of faba bean (*Vicia faba* L.) grains." *Journal of Food Engineering* 78 (1):174-183. doi: https://doi.org/10.1016/j.jfoodeng.2005.09.013.

Amaral, A. L., E. S. Ferreira, V. A. Neves, and A. Demonte. 2014. "Legumin from chickpea: hypolipidemic effect in the liver of hypercholesterolemic rats." *Nutrition & Food Science* 44 (5):378-388. doi: 10.1108/Nfs-10-2013-0115.

Amin, M. N., M. A. Hossain, and K. C. Roy. 2004. "Effects of moisture content on some physical properties of lentil seeds." *Journal of Food Engineering* 65 (1):83-87. doi: https://doi.org/10.1016/j.jfoodeng.2003.12.006.

Andrés, Víctor, María-José Villanueva, and María-Dolores Tenorio. 2016. "Influence of high pressure processing on microbial shelf life, sensory profile, soluble sugars, organic acids, and mineral content of milk- and soy-smoothies." *LWT - Food Science and Technology* 65:98-105. doi: https://doi.org/10.1016/j.lwt.2015.07.066.

Angioloni, A., and C. Collar. 2012. "Effects of pressure treatment of hydrated oat, finger millet and sorghum flours on the quality and nutritional properties of composite wheat breads." *Journal of Cereal Science* 56 (3):713-719. doi: https://doi.org/10.1016/j.jcs.2012.08.001.

AOAC International. 2005a. AOAC Official Method 923.03. Gaithersburg, Md.: Official Methods of Analysis AOAC International.

AOAC International. 2005b. AOAC Official Method 991.43. Gaithersburg, Md.: Official Methods of Analysis AOAC International.

AOAC International. 2005c. AOAC Official Method 996.11 Gaithersburg, Md.: Official Methods of Analysis AOAC International.

AOAC International. 2006. AOAC Official Method 963.15. Gaithersburd, Md.: Official Methods of Analysis AOAC International.

AOAC International. 2008. Official method 925.10. Gaithersbug, Md.: Official Methods of Analysis AOAC International.

Arevalo, Isabel, Salvador Horacio Guzmán-Maldonado, Sara Meyeth Mata Sanchez, and Jorge Alberto Acosta-Gallegos. 2020. "Steaming and Toasting Reduce the Nutrimental Quality, Total Phenols and Antioxidant Capacity of Fresh Kabuli Chickpea (*Cicer arietinum* L.)." *Plant Foods for Human Nutrition* 75 (4):628-634. doi: 10.1007/s11130-020-00857-5.

Argel, Natalia Soledad, Natalia Ranalli, Alicia Noemí Califano, and Silvina Cecilia Andrés. 2020. "Influence of partial pork meat replacement by pulse flour on physicochemical and sensory characteristics of low-fat burgers." *Journal of the Science of Food and Agriculture* 100 (10):3932-3941. doi: https://doi.org/10.1002/jsfa.10436.

Arnaud, CD, and SD Sanchez. 1996. "Calcium and phosphorus." *Present Knowledge in Nutrition, 7th edition. Ilsi Press, Washington DC*:245-255.

Arts, Mariken J. T. J., Guido R. M. M. Haenen, Lonneke C. Wilms, Sasja A. J. N. Beetstra, Chantal G. M. Heijnen, Hans-Peter Voss, and Aalt Bast. 2002. "Interactions between Flavonoids and Proteins: Effect on the Total Antioxidant Capacity." *Journal of Agricultural and Food Chemistry* 50 (5):1184-1187. doi: 10.1021/jf010855a.

Asgar, M.A., A. Fazilah, Nurul Huda, Rajeev Bhat, and A.A. Karim. 2010. "Nonmeat Protein Alternatives as Meat Extenders and Meat Analogs." *Comprehensive Reviews in Food Science and Food Safety* 9 (5):513-529. doi: https://doi.org/10.1111/j.1541-4337.2010.00124.x.

Attia, R. S., A. M. El-Tabey Shehata, M. E. Aman, and M. A. Hamza. 1994. "Effect of cooking and decortication on the physical properties, the chemical composition and the nutritive value of chickpea (*Cicer arietinum* L.)." *Food Chemistry* 50 (2):125-131. doi: https://doi.org/10.1016/0308-8146(94)90108-2.

Australia, PULSE. 2017. "Australian Kabuli chickpea production 2010/11 to 2015/16." Pulse Australia Limited, accessed 13/07. http://www.pulseaus.com.au/storage/app/media/industry/AU-Kchickpea-production.pdf.

Avilés-Gaxiola, Sara, Cristina Chuck-Hernández, and Sergio O. Serna Saldívar. 2018. "Inactivation Methods of Trypsin Inhibitor in Legumes: A Review." *Journal of Food Science* 83 (1):17-29. doi: 10.1111/1750-3841.13985.

Awasthi, Rashmi, Neeru Kaushal, Vincent Vadez, Neil C. Turner, Jens Berger, Kadambot H. M. Siddique, and Harsh Nayyar. 2014. "Individual and combined effects of transient drought and heat stress on carbon assimilation and seed filling in chickpea." *Functional Plant Biology* 41 (11):1148-1167. doi: https://doi.org/10.1071/FP13340.

Ayodele, O. M., and A. O. Beatrice I. O. 2015. "Some functional and physical properties of selected underutilised hard-to-cook legumes in nigeria." *American Journal of Food Science and Nutrition* 2 (5):73-81.

Bains, Kiran, Veny Uppal, and Harpreet Kaur. 2014. "Optimization of germination time and heat treatments for enhanced availability of minerals from leguminous sprouts." *Journal of Food Science and Technology* 51 (5):1016-1020. doi: 10.1007/s13197-011-0582-y.

Baldwin, Andrew J., Danielle L. Egan, Fredrick J. Warren, Paul D. Barker, Christopher M. Dobson, Peter J. Butterworth, and Peter R. Ellis. 2015. "Investigating the mechanisms of amylolysis of starch granules by solution-state NMR." *Biomacromolecules* 16 (5):1614-1621. doi: 10.1021/acs.biomac.5b00190.

Balny, Claude, and Patrick Masson. 1993. "Effects of high pressure on proteins." *Food Reviews International* 9 (4):611-628. doi: 10.1080/87559129309540980.

Bampidis, V. A., and V. Christodoulou. 2011a. "Chickpeas (*Cicer arietinum* L.) in animal nutrition: A review." *Animal Feed Science and Technology* 168 (1):1-20. doi: https://doi.org/10.1016/j.anifeedsci.2011.04.098.

Bampidis, V. A., and V. Christodoulou. 2011b. "Chickpeas (*Cicer arietinum* L.) in animal nutrition: A review." *Animal Feed Science and Technology* 168 (1-2):1-20. doi: 10.1016/j.anifeedsci.2011.04.098.

Bandyopadhyay, Prasun, Amit K. Ghosh, and Chandrasekhar Ghosh. 2012. "Recent developments on polyphenol–protein interactions: effects on tea and coffee taste, antioxidant properties and the digestive system." *Food & Function* 3 (6):592-605. doi: 10.1039/C2FO00006G.

Bank, G., and A. Schauss. 2004. "Antioxidant Testing: An ORAC update." *Nutraceuticals World*.

Bar-El Dadon, Shimrit, Shahal Abbo, and Ram Reifen. 2017. "Leveraging traditional crops for better nutrition and health - The case of chickpea." *Trends in Food Science & Technology* 64:39-47. doi: http://doi.org/10.1016/j.tifs.2017.04.002.

Barampama, Zacharie, and Ronald E. Simard. 1995. "Effects of soaking, cooking and fermentation on composition, in-vitro starch digestibility and nutritive value of common beans." *Plant Foods for Human Nutrition* 48 (4):349-365. doi: 10.1007/BF01088494.

Barba, Francisco J., Clara Cortés, María J. Esteve, and Ana Frígola. 2012. "Study of Antioxidant Capacity and Quality Parameters in An Orange Juice–Milk Beverage After High-Pressure Processing Treatment." *Food and Bioprocess Technology* 5 (6):2222-2232. doi: 10.1007/s11947-011-0570-2.

Barrett, Ann H., Nicole F. Farhadi, and Tracey J. Smith. 2018. "Slowing starch digestion and inhibiting digestive enzyme activity using plant

flavanols/tannins— A review of efficacy and mechanisms." *LWT* 87:394-399. doi: https://doi.org/10.1016/j.lwt.2017.09.002.

Barros, Nara Vanessa dos Anjos, Maurisrael de Moura Rocha, Maria Beatriz Abreu Glória, Marcos Antônio da Mota Araújo, and Regilda Saraiva dos Reis Moreira-Araújo. 2017. "Effect of cooking on the bioactive compounds and antioxidant activity in grains cowpea cultivars." *Revista Ciência Agronômica* 48:824-831.

Baryeh, Edward A. 2001. "Physical properties of bambara groundnuts." *Journal of Food Engineering* 47 (4):321-326. doi: https://doi.org/10.1016/S0260-8774(00)00136-9.

Basak, S., and H. S. Ramaswamy. 1998. "Effect of high pressure processing on the texture of selected fruits and vegetables." *Journal of Texture Studies* 29 (5):587-601. doi: 10.1111/j.1745-4603.1998.tb00185.x.

Baümler, Erica, Adela Cuniberti, Susana M. Nolasco, and Isabel C. Riccobene. 2006. "Moisture dependent physical and compression properties of safflower seed." *Journal of Food Engineering* 72 (2):134-140. doi: https://doi.org/10.1016/j.jfoodeng.2004.11.029.

Bautista, Inmaculada, Monica Boscaiu, Antonio Lidón, Josep V. Llinares, Cristina Lull, Mª Pilar Donat, Olga Mayoral, and Oscar Vicente. 2015. "Environmentally induced changes in antioxidant phenolic compounds levels in wild plants." *Acta Physiologiae Plantarum* 38 (1):9. doi: 10.1007/s11738-015-2025-2.

Baxter, N. J., T. H. Lilley, E. Haslam, and M. P. Williamson. 1997. "Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation." *Biochemistry* 36 (18):5566-5577. doi: 10.1021/bi9700328.

Beck, Hylke E., Niklaus E. Zimmermann, Tim R. McVicar, Noemi Vergopolan, Alexis Berg, and Eric F. Wood. 2018. "Present and future Köppen-Geiger climate classification maps at 1-km resolution." *Scientific Data* 5 (1):180214. doi: 10.1038/sdata.2018.214.

Bell, Lindsay W., Andrew D. Moore, and John A. Kirkegaard. 2014. "Evolution in crop–livestock integration systems that improve farm productivity and environmental performance in Australia." *European Journal of Agronomy* 57:10-20. doi: https://doi.org/10.1016/j.eja.2013.04.007.

Belmiro, Ricardo Henrique, Alline Artigiani Lima Tribst, and Marcelo Cristianini. 2020. "Effects of High Pressure Processing on Common Beans (Phaseolus Vulgaris L.): Cotyledon Structure, Starch Characteristics, and Phytates and Tannins Contents." *Starch - Stärke* 72 (3-4):1900212. doi: 10.1002/star.201900212.

Bennett, Sarita, and Simonetta Bullitta. 2003. "Ecogeographical analysis of the distribution of six Trifolium species in Sardinia." *Biodiversity and Conservation* 12:1455-1466. doi: 10.1023/A:1023677726849.

Berger, Alvin, Peter J. H. Jones, and Suhad S. Abumweis. 2004. "Plant sterols: factors affecting their efficacy and safety as functional food ingredients." *Lipids in health and disease* 3:5-5. doi: 10.1186/1476-511X-3-5.

Berger, J. D., M. Ali, P. S. Basu, B. D. Chaudhary, S. K. Chaturvedi, P. S. Deshmukh, P. S. Dharmaraj, S. K. Dwivedi, G. C. Gangadhar, P. M. Gaur, J. Kumar, R. K. Pannu, K. H. M. Siddique, D. N. Singh, D. P. Singh, S. J. Singh, N. C. Turner, H. S. Yadava, and S. S. Yadav. 2006. "Genotype by environment studies demonstrate the critical role of phenology in adaptation of chickpea (*Cicer arietinum* L.) to high and low yielding environments of India." *Field Crops Research* 98 (2):230-244. doi: https://doi.org/10.1016/j.fcr.2006.02.007.

Berrios, J. De J., P. Morales, M. Cámara, and M. C. Sánchez-Mata. 2010. "Carbohydrate composition of raw and extruded pulse flours." *Food Research International* 43 (2):531-536. doi: https://doi.org/10.1016/j.foodres.2009.09.035.

Bhattarai, Rewati R., Sushil Dhital, Peng Wu, Xiao Dong Chen, and Michael J. Gidley. 2017. "Digestion of isolated legume cells in a stomach-duodenum model: three mechanisms limit starch and protein hydrolysis." *Food & Function* 8 (7):2573-2582. doi: 10.1039/C7FO00086C.

Błaszczak, W., S. Valverde, and J. Fornal. 2005. "Effect of high pressure on the structure of potato starch." *Carbohydrate Polymers* 59 (3):377-383. doi: https://doi.org/10.1016/j.carbpol.2004.10.008.

Bouhnik, Y., L. Raskine, G. Simoneau, E. Vicaut, C. Neut, B. Flourié, F. Brouns, and F. R. Bornet. 2004. "The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: A double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study." *American Journal of Clinical Nutrition* 80 (6):1658-1664.

Bravo, L. 1998. "Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance." *Nutrition Review* 56 (11):317-33. doi: 10.1111/j.1753-4887.1998.tb01670.x.

Bravo, Laura, Perumal Siddhuraju, and Fulgencio Saura-Calixto. 1999. "Composition of underexploited Indian pulses. Comparison with common legumes." *Food Chemistry* 64 (2):185-192. doi: https://doi.org/10.1016/S0308-8146(98)00140-X.

Brenes, A., A. Viveros, C. Centeno, I. Arija, and F. Marzo. 2008. "Nutritional value of raw and extruded chickpeas (*Cicer arietinum* L.) for growing chickens." *Spanish Journal of Agricultural Research* 6 (4):537-545. doi: DOI 10.5424/sjar/2008064-348.

Briones-Labarca, Vilbett, Gabriela Venegas-Cubillos, Susana Ortiz-Portilla, Marcelo Chacana-Ojeda, and Hector Maureira. 2011. "Effects of high hydrostatic pressure (HHP) on bioaccessibility, as well as antioxidant activity, mineral and starch contents in Granny Smith apple." *Food Chemistry* 128 (2):520-529. doi: https://doi.org/10.1016/j.foodchem.2011.03.074.

Brown, D. E., A. M. Rashotte, A. S. Murphy, J. Normanly, B. W. Tague, W. A. Peer, L. Taiz, and G. K. Muday. 2001. "Flavonoids act as negative regulators of auxin transport in vivo in arabidopsis." *Plant Physiology* 126 (2):524-35. doi: 10.1104/pp.126.2.524.

Brummer, Yolanda, Mina Kaviani, and Susan M. Tosh. 2015. "Structural and functional characteristics of dietary fibre in beans, lentils, peas and chickpeas." *Food Research International* 67:117-125. doi: https://doi.org/10.1016/j.foodres.2014.11.009.

Brummer, Yolanda, Kaviani Mina, and M. Tosh Susan. 2015. "Structural and functional characteristics of dietary fibre in beans, lentils, peas and chickpeas." *Food Research International (Ottawa, Ont.)* 67:117-125. doi: 10.1016/j.foodres.2014.11.009.

Bueckert, Rosalind A., Dil Thavarajah, Pushparajah Thavarajah, and Janet Pritchard. 2011. "Phytic acid and mineral micronutrients in field-grown chickpea (*Cicer arietinum* L.) cultivars from western Canada." *European Food Research and Technology* 233 (2):203-212. doi: 10.1007/s00217-011-1495-8.

Bureau of Meteorology. 2018. Climate Summaries Archive. Beureau of Meteorology, Australian Government.

Butz, Peter, R. Edenharder, Avelina Fernandez, H. Fister, C. Merkel, and Bernhard Tauscher. 2002. *Changes in functional properties of vegetables induced by high pressure treatment*. Vol. 35.

Candela, Montserrat, Iciar Astiasaran, and José Bello. 1997. "Cooking and Warm-Holding: Effect on General Composition and Amino Acids of Kidney Beans (*Phaseolus vulgaris*), Chickpeas (*Cicer arietinum*), and Lentils (*Lens culinaris*)." *Journal of Agricultural and Food Chemistry* 45 (12):4763-4767. doi: 10.1021/jf9702609.

Carbonaro, Marina, Mirella Nardini, Paola Maselli, and Alessandro Nucara. 2015. "Chemico-physical and nutritional properties of traditional legumes (lentil, *Lens culinaris* L., and grass pea, *Lathyrus sativus* L.) from organic agriculture: an explorative study." *Organic Agriculture* 5 (3):179-187. doi: 10.1007/s13165-014-0086-y.

Carvalho, I. S., C. P. Ricardo, and M. Chaves. 2004. "Quality and distribution of assimilates within the whole plant of lupines (*L. albus* and *L. mutabilis*) influenced by water stress." *Journal of Agronomy and Crop Science* 190 (3):205-210. doi: 10.1111/j.1439-037X.2004.00094.x.

Cassells, Julie, and Len Caddick. 2000. Harvesting and storage of desi type chickpeas. Stored Grain Research Laboratory, CSIRO Entomology, Acton, ACT.

Chandrasekara, Anoma, Marian Naczk, and Fereidoon Shahidi. 2012. "Effect of processing on the antioxidant activity of millet grains." *Food Chemistry* 133 (1):1-9. doi: https://doi.org/10.1016/j.foodchem.2011.09.043.

Chavez-Murillo, Carolina E., Jose L. Orona-Padilla, and Julian de la Rosa Millan. 2019. "Physicochemical, functional properties and ATR-FTIR digestion analysis of thermally treated starches isolated from black and bayo beans." *Starch - Stärke* 71 (3-4):1800250. doi: https://doi.org/10.1002/star.201800250.

Chen, B., S. Zeng, H. Zeng, Z. Guo, Y. Zhang, and B. Zheng. 2017. "Properties of lotus seed starch–glycerin monostearin complexes formed by high pressure homogenization." *Food Chemistry* 226:119-127. doi: 10.1016/j.foodchem.2017.01.018.

Chibarabada, Tendai Polite, Albert Thembinkosi Modi, and Tafadzwanashe Mabhaudhi. 2017. "Nutrient content and nutritional water productivity of selected grain legumes in response to production environment." *International Journal of Environmental Research and Public Health* 14 (11):1300.

Chibbar, R. N., S. Ganeshan, M. Båga, and R. L. Khandelwal. 2004. "CARBOHYDRATE METABOLISM." In *Encyclopedia of Grain Science*, edited by Colin Wrigley, 168-179. Oxford: Elsevier.

Chitra, U., V. Vimala, U. Singh, and P. Geervani. 1995. "Variability in phytic acid content and protein digestibility of grain legumes." *Plant Foods Hum Nutr* 47 (2):163-72. doi: 10.1007/bf01089266.

Christodoulou, V., V. A. Bampidis, B. Hučko, K. Ploumi, C. Iliadis, P. H. Robinson, and Z. Mudřik. 2005. "Nutritional value of chickpeas in rations of lactating ewes and growing lambs." *Animal Feed Science and Technology* 118 (3–4):229-241. doi: http://dx.doi.org/10.1016/j.anifeedsci.2004.10.017.

Clariana, Maria, Juan Valverde, Hilde Wijngaard, Anne Maria Mullen, and Begonya Marcos. 2011. "High pressure processing of swede (*Brassica napus*): Impact on quality properties." *Innovative Food Science & Emerging Technologies* 12 (2):85-92. doi: https://doi.org/10.1016/j.ifset.2011.01.011.

Clemente, Alfonso, Raúl Sánchez-Vioque, Javier Vioque, Juan Bautista, and Francisco Millán. 1998. "Effect of cooking on protein quality of chickpea (*Cicer arietinum*) seeds." *Food Chemistry* 62 (1):1-6. doi: https://doi.org/10.1016/S0308-8146(97)00180-5.

Cobos, Maria J., Inmaculada Izquierdo, Miguel A. Sanz, Antonio Tomás, Juan Gil, Fernando Flores, and Josefa Rubio. 2017. "Genotype and environment effects on sensory, nutritional, and physical traits in chickpea (*Cicer arietinum* L.)." *2017* 14 (4). doi: 10.5424/sjar/2016144-8719.

Colussi, Rosana, Lovedeep Kaur, Elessandra da Rosa Zavareze, Alvaro Renato Guerra Dias, R. B. Stewart, and Jaspreet Singh. 2018. "High pressure processing and retrogradation of potato starch: Influence on functional properties and gastro-small intestinal digestion in vitro." *Food Hydrocolloids* 75:131-137. doi: https://doi.org/10.1016/j.foodhyd.2017.09.004.

Cory, Hannah, Simone Passarelli, John Szeto, Martha Tamez, and Josiemer Mattei. 2018. "The Role of Polyphenols in Human Health and Food Systems: A Mini-Review." *Frontiers in Nutrition* 5 (87). doi: 10.3389/fnut.2018.00087.

Croser, J. S., H. J. Clarke, K. H. M. Siddique, and T. N. Khan. 2003. "Low-Temperature Stress: Implications for Chickpea (*Cicer arietinum* L.) Improvement." *Critical Reviews in Plant Sciences* 22 (2):185-219. doi: 10.1080/713610855.

Crowther, Alison. 2012. "The differential survival of native starch during cooking and implications for archaeological analyses: a review." *Archaeological and Anthropological Sciences* 4 (3):221-235. doi: 10.1007/s12520-012-0097-0.

CSIRO. 2012. Soilmapp Database for android. CSIRO.au: CSIRO.

CSIRO. 2020. SoilMapp. Commonwealth Scientific and Industrial Research Organisation.

Cummings, J. H. 1981. "Short chain fatty acids in the human colon." *Gut* 22 (9):763-779.

Dai, Zhuqing, Wanyong Lyu, Minhao Xie, Qingxia Yuan, Hong Ye, Bing Hu, Li Zhou, and Xiaoxiong Zeng. 2017. "Effects of α-Galactooligosaccharides from Chickpeas on High-Fat-Diet-Induced Metabolic Syndrome in Mice." *Journal of Agricultural and Food Chemistry* 65 (15):3160-3166. doi: 10.1021/acs.jafc.7b00489.

Dalgetty, David D., and Byung-Kee Baik. 2003. "Isolation and Characterization of Cotyledon Fibers from Peas, Lentils, and Chickpeas." *Cereal Chemistry* 80 (3):310-315. doi: 10.1094/cchem.2003.80.3.310.

de Almeida Costa, Giovana Ermetice, Keila da Silva Queiroz-Monici, Soely Maria Pissini Machado Reis, and Admar Costa de Oliveira. 2006. "Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes." *Food Chemistry* 94 (3):327-330. doi: https://doi.org/10.1016/j.foodchem.2004.11.020.

de Jong, A., J. Plat, and R. P. Mensink. 2003. "Metabolic effects of plant sterols and stanols (Review)." *J Nutr Biochem* 14 (7):362-9. doi: 10.1016/s0955-2863(03)00002-0.

Deol, Jasraj K., and Kiran Bains. 2010. "Effect of household cooking methods on nutritional and anti nutritional factors in green cowpea (*Vigna unguiculata)*

pods." *Journal of Food Science and Technology* 47 (5):579-581. doi: 10.1007/s13197-010-0112-3.

Dewanto, V., X. Wu, and R. H. Liu. 2002. "Processed sweet corn has higher antioxidant activity." *Journal of Agricultural and Food Chemistry* 50 (17):4959-4964. doi: 10.1021/jf0255937.

Dhital, Sushil, Rewati R. Bhattarai, John Gorham, and Michael J. Gidley. 2016. "Intactness of cell wall structure controls the in vitro digestion of starch in legumes." *Food & Function* 7 (3):1367-1379. doi: 10.1039/C5FO01104C.

Di Ferdinando, Martina, Cecilia Brunetti, Giovanni Agati, and Massimiliano Tattini. 2014. "Multiple functions of polyphenols in plants inhabiting unfavorable Mediterranean areas." *Environmental and Experimental Botany* 103:107-116. doi: https://doi.org/10.1016/j.envexpbot.2013.09.012.

Dida Bulbula, Dejene, and Kelbessa Urga. 2018. "Study on the effect of traditional processing methods on nutritional composition and anti nutritional factors in chickpea (*Cicer arietinum*)." *Cogent Food & Agriculture* 4 (1):1422370. doi: 10.1080/23311932.2017.1422370.

Dixit, G. P., A. K. Srivastava, and N. P. Singh. 2019. "Marching towards self-sufficiency in chickpea." *Current Science* 116 (2):239-242. doi: 10.18520/cs/v116/i2/239-242.

Doblado, Rosa, Juana Frías, and Concepción Vidal-Valverde. 2007. "Changes in vitamin C content and antioxidant capacity of raw and germinated cowpea (*Vigna sinensis* var. carilla) seeds induced by high pressure treatment." *Food Chemistry* 101 (3):918-923. doi: https://doi.org/10.1016/j.foodchem.2006.02.043.

Dzah, Courage Sedem, Yuqing Duan, Haihui Zhang, Nana Adwoa Serwah Boateng, and Haile Ma. 2020. "Latest developments in polyphenol recovery and purification from plant by-products: A review." *Trends in Food Science & Technology* 99:375-388. doi: https://doi.org/10.1016/j.tifs.2020.03.003.

Egli, I., L. Davidsson, M.A. Juillerat, D. Barclay, and R.F. Hurrell. 2002. "The Influence of Soaking and Germination on the Phytase Activity and Phytic Acid Content of Grains and Seeds Potentially Useful for Complementary Feedin." *Journal of Food Science* 67 (9):3484-3488. doi: 10.1111/j.1365-2621.2002.tb09609.x.

El-Adawy, T. A. 2002. "Nutritional composition and antinutritional factors of chickpeas (Cicer arietinum L.) undergoing different cooking methods and germination." *Plant Foods for Human Nutrition* 57 (1):83-97.

Elhardallou, Sirelkhatim B., and Ann F. Walker. 1993. "The water-holding capacity of three starchy legumes in the raw, cooked and fibre-rich fraction forms." *Plant Foods for Human Nutrition* 44 (2):171-179. doi: 10.1007/bf01088382.

Englyst, H. N., S. M. Kingman, and J. H. Cummings. 1992. "Classification and measurement of nutritionally important starch fractions." *European Journal of Clinical Nutrition* 46 Suppl 2:S33-50.

Englyst, K. N., H. N. Englyst, G. J. Hudson, T. J. Cole, and J. H. Cummings. 1999. "Rapidly available glucose in foods: An in vitro measurement that reflects the glycemic response." *American Journal of Clinical Nutrition* 69 (3):448-454. doi: 10.1093/ajcn/69.3.448.

Ereifej, K. I., G. N. Al-Karaki, and M. K. Hammouri. 2001. "Seed chemical composition of improved chickpea cultivars grown under semiarid mediterranean conditions." *International Journal of Food Properties* 4 (2):239-246. doi: 10.1081/JFP-100105190.

Ertaş, Nilgün, and Selman Türker. 2014. "Bulgur processes increase nutrition value: possible role in in-vitro protein digestability, phytic acid, trypsin inhibitor activity and mineral bioavailability." *Journal of food science and technology* 51 (7):1401-1405. doi: 10.1007/s13197-012-0638-7.

Faměra, O., M. Mayerová, I. Burešová, L. Kouřimská, and M. Prášilová. 2015. "Influence of selected factors on the content and properties of starch in the grain of non-food wheat." *Plant, Soil and Environment* 61 (6):241-246. doi: 10.17221/13/2015-PSE.

FAOSTAT. 2014. "Statistical data on Chickpea production in Australia." Food and Agriculture Organisation of United Nations. http://www.fao.org/faostat/en/#data/QC.

FAOSTAT. 2018. Crops. In *Data*: Food and Agriculture Organisation of the United Nations.

FAOSTAT. 2019. "Statistical Data on Total Chickpea production worlwide ", accessed 10 December. http://www.fao.org/faostat/en/#data/QC.

Fares, Clara, and Valeria Menga. 2012. "Effects of toasting on the carbohydrate profile and antioxidant properties of chickpea (*Cicer arietinum* L.) flour added to durum wheat pasta." *Food Chemistry* 131 (4):1140-1148. doi: http://dx.doi.org/10.1016/j.foodchem.2011.09.080.

Fares, Clara, and Valeria Menga. 2014. "Chapter 41 - Chickpea (*Cicer arietinum* L.) Fortification of Cereal-Based Foods to Increase Fiber and Phytochemical Content A2 - Watson, Ronald Ross." In *Wheat and Rice in Disease Prevention and Health*, edited by Victor R. Preedy and Sherma Zibadi, 533-546. San Diego: Academic Press.

Fazeli Burestan, Nasrollah, Amir Hossein Afkari Sayyah, and Mahdi Safi. 2021. "Prediction of amylose content, protein content, breakdown, and setback viscosity of Kadus rice and its flour by near-infrared spectroscopy (NIRS) analysis." *Journal of Food Processing and Preservation* 45 (1):e15069. doi: https://doi.org/10.1111/jfpp.15069.

Ferreira, E. C., A. R. A. Nogueira, G. B. Souza, and L. A. R. Batista. 2004. "Effect of drying method and length of storage on tannin and total phenol concentrations in Pigeon pea seeds." *Food Chemistry* 86 (1):17-23. doi: 10.1016/j.foodchem.2003.08.024.

Finaud, Julien, Gérard Lac, and Edith Filaire. 2006. "Oxidative Stress." *Sports Medicine* 36 (4):327-358. doi: 10.2165/00007256-200636040-00004.

Forino, M., L. Tartaglione, C. Dell'Aversano, and P. Ciminiello. 2016. "NMR-based identification of the phenolic profile of fruits of *Lycium barbarum* (goji berries). Isolation and structural determination of a novel N-feruloyl tyramine dimer as the most abundant antioxidant polyphenol of goji berries." *Food Chemistry* 194:1254-1259. doi: 10.1016/j.foodchem.2015.08.129.

Fraga, César G., Kevin D. Croft, David O. Kennedy, and Francisco A. Tomás-Barberán. 2019. "The effects of polyphenols and other bioactives on human health." *Food & Function* 10 (2):514-528. doi: 10.1039/C8FO01997E.

Fredriksson, H., I. Björck, R. Andersson, H. Liljeberg, J. Silverio, A. C. Eliasson, and P. Åman. 2000. "Studies on α-amylase degradation of retrograded starch gels from waxy maize and high-amylopectin potato." *Carbohydrate Polymers* 43 (1):81-87. doi: https://doi.org/10.1016/S0144-8617(99)00205-2.

Frias, J., C. Vidal-Valverde, C. Sotomayor, C. Diaz-Pollan, and G. Urbano. 2000. "Influence of processing on available carbohydrate content and antinutritional factors of chickpeas." *European Food Research and Technology* 210 (5):340-345. doi: 10.1007/s002170050560.

Frias, Juana, Martha L. Miranda, Rosa Doblado, and Concepción Vidal-Valverde. 2005. "Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of Lupinus albus L. var. Multolupa." *Food Chemistry* 92 (2):211-220. doi: https://doi.org/10.1016/j.foodchem.2004.06.049.

Frimpong, Adams, Anupam Sinha, Bunyamin Tar'an, Tom D. Warkentin, Bruce D. Gossen, and Ravindra N. Chibbar. 2009. "Genotype and growing environment influence chickpea (*Cicer arietinum* L.) seed composition." *Journal of the Science of Food and Agriculture* 89 (12):2052-2063. doi: 10.1002/jsfa.3690.

FSANZ. 2020. Australian Food Composition Database. In *F002880: Chickpea, canned, drained*. www.foodstandards.gov.au: Food Standards Australia New Zealand.

Fujiki, Hirota, Eisaburo Sueoka, Tatsuro Watanabe, and Masami Suganuma. 2015. "Primary cancer prevention by green tea, and tertiary cancer prevention by the combination of green tea catechins and anticancer compounds." *Journal of cancer prevention* 20 (1):1-4. doi: 10.15430/JCP.2015.20.1.1.

Ganesan, Kumar, and Baojun Xu. 2017. "A Critical Review on Polyphenols and Health Benefits of Black Soybeans." *Nutrients* 9 (5):455.

Gangola, M. P., Y. P. Khedikar, P. M. Gaur, M. Baga, and R. N. Chibbar. 2013. "Genotype and growing environment interaction shows a positive correlation between substrates of raffinose family oligosaccharides (RFO) biosynthesis and their accumulation in chickpea (*Cicer arietinum* L.) seeds." *Journal of Agriculture and Food Chemistry* 61 (20):4943-52. doi: 10.1021/jf3054033.

Gaur, Pooran M., Muneendra K. Singh, Srinivasan Samineni, Sobhan B. Sajja, Aravind K. Jukanti, Suresh Kamatam, and Rajeev K. Varshney. 2016. "Inheritance of protein content and its relationships with seed size, grain yield and other traits in chickpea." *Euphytica* 209 (1):253-260. doi: 10.1007/s10681-016-1678-2.

Gemede, H. F., and N. Ratta. 2004. "Antinutritional factors in plant foods: potential health benefits and adverse effects." *Global Advanced Research Journal of Food Science and Technology* 3 (4):103-17.

Gharibzahedi, Seyed Mohammad Taghi, and Seid Mahdi Jafari. 2017. "The importance of minerals in human nutrition: bioavailability, food fortification, processing effects and nanoencapsulation." *Trends in Food Science & Technology* 62:119-132. doi: https://doi.org/10.1016/j.tifs.2017.02.017.

Ghasemlou, Mehran, Seyed Mohammad Taghi Gharibzahedi, and Zahra Emam-Djomeh. 2013. "Relating consumer preferences to textural attributes of cooked beans: Development of an industrial protocol and microstructural observations." *LWT - Food Science and Technology* 50 (1):88-98. doi: https://doi.org/10.1016/j.lwt.2012.06.018.

Ghavidel, Reihaneh Ahmadzadeh, and Jamuna Prakash. 2006. "Effect of germination and dehulling on functional properties of legume flours." *Journal of the Science of Food and Agriculture* 86 (8):1189-1195. doi: 10.1002/jsfa.2460.

Ghavidel, Reihaneh Ahmadzadeh, and Jamuna Prakash. 2007. "The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds." *LWT - Food Science and Technology* 40 (7):1292-1299. doi: https://doi.org/10.1016/j.lwt.2006.08.002.

Giacometti, Jasminka, Damir Muhvić, Adriano Pavletić, and Luka Đudarić. 2016. "Cocoa polyphenols exhibit antioxidant, anti-inflammatory, anticancerogenic, and anti-necrotic activity in carbon tetrachloride-intoxicated mice." *Journal of Functional Foods* 23:177-187. doi: https://doi.org/10.1016/j.jff.2016.02.036.

Gil, J., S. Nadal, D. Luna, M. T. Moreno, and A. DeHaro. 1996. "Variability of some physico-chemical characters in desi and kabuli chickpea types." *Journal of the Science of Food and Agriculture* 71 (2):179-184.

Gil, Juan, Salvador Nadal, Diego Luna, M Teresa Moreno, and Antonio De Haro. 1996. "Variability of Some Physico-chemical Characters in Desi and Kabuli Chickpea Types." *Journal of the Science of Food and Agriculture* 71 (2):179-184. doi: 10.1002/(sici)1097-0010(199606)71:2<179::Aid-jsfa566>3.0.Co;2-7.

Giusti, Federica, Giovanni Caprioli, Massimo Ricciutelli, Sauro Vittori, and Gianni Sagratini. 2017. "Determination of fourteen polyphenols in pulses by high performance liquid chromatography-diode array detection (HPLC-DAD) and correlation study with antioxidant activity and colour." *Food Chemistry* 221:689-697. doi: https://doi.org/10.1016/j.foodchem.2016.11.118.

Gladine, C., C. Morand, E. Rock, D. Bauchart, and D. Durand. 2007. "Plant extracts rich in polyphenols (PERP) are efficient antioxidants to prevent lipoperoxidation in plasma lipids from animals fed n−3 PUFA supplemented diets." *Animal Feed Science and Technology* 136 (3):281-296. doi: https://doi.org/10.1016/j.anifeedsci.2006.09.003.

Godfray, H. S. J., P. Aveyard, T. Garnett, J. W. Hall, T. J. Key, J. Lorimer, R. T. Pierrehumbert, P. Scarborough, M. Springmann, and Susan A. Jebb. 2018. "Meat consumption, health, and the environment." *Science* 361 (6399):eaam5324. doi: 10.1126/science.aam5324.

Golden, Tamara R., Douglas A. Hinerfeld, and Simon Melov. 2002. "Oxidative stress and aging: beyond correlation." *Aging Cell* 1 (2):117-123. doi: https://doi.org/10.1046/j.1474-9728.2002.00015.x.

González-Soto, R. A., L. Sánchez-Hernández, J. Solorza-Feria, C. Núñez-Santiago, E. Flores-Huicochea, and L. A. Bello-Pérez. 2006. "Resistant Starch Production from Non-conventional Starch Sources by Extrusion." *Food Science and Technology International* 12 (1):5-11. doi: 10.1177/1082013206060735.

Gorzynik-Debicka, Monika, Paulina Przychodzen, Francesco Cappello, Alicja Kuban-Jankowska, Antonella Marino Gammazza, Narcyz Knap, Michal Wozniak, and Magdalena Gorska-Ponikowska. 2018. "Potential Health Benefits of Olive Oil and Plant Polyphenols." *International Journal of Molecular Sciences* 19 (3):686.

Granito, Marisela, Yannellis Brito, and Alexia Torres. 2007. "Chemical composition, antioxidant capacity and functionality of raw and processed Phaseolus lunatus." *Journal of the Science of Food and Agriculture* 87 (15):2801-2809. doi: doi:10.1002/jsfa.2926.

GRDC. 2018a. GRDC Grownotes *Chickpea, Section-13*. Accessed 16/06/2020.

GRDC. 2018b. GRDC Grownotes - Chickpea. Grains Research Development Coropration.

Griffiths, David Wynne. 1986. "The Inhibition of Digestive Enzymes by Polyphenolic Compounds." In *Nutritional and Toxicological Significance of Enzyme*

Inhibitors in Foods, edited by Mendel Friedman, 509-516. Boston, MA: Springer US.

Grundy, M. J., R. A. Viscarra Rossel, R. D. Searle, P. L. Wilson, C. Chen, and L. J. Gregory. 2015. "Soil and Landscape Grid of Australia." *Soil Research* 53 (8):835-844. doi: https://doi.org/10.1071/SR15191.

Grundy, Myriam Marie-Louise, Karen Lapsley, and Peter Rory Ellis. 2016. "A review of the impact of processing on nutrient bioaccessibility and digestion of almonds." *International Journal of Food Science & Technology* 51 (9):1937-1946. doi: https://doi.org/10.1111/ijfs.13192.

Gujral, Hardeep Singh, Paras Sharma, Neha Gupta, and Ali Abbas Wani. 2013. "Antioxidant properties of legumes and their morphological fractions as affected by cooking." *Food Science and Biotechnology* 22 (1):187-194. doi: 10.1007/s10068-013-0026-8.

Gül, Muhammet Kemal, Cem Ömer Egesel, and Hakan Turhan. 2008. "The effects of planting time on fatty acids and tocopherols in chickpea." *European Food Research and Technology* 226 (3):517-522. doi: 10.1007/s00217-007-0564-5.

Gulewicz, P., D. Ciesiolka, J. Frias, C. Vidal-Valverde, S. Frejnagel, K. Trojanowska, and K. Gulewicz. 2000. "Simple method of isolation and purification of alpha-galactosides from legumes." *Journal of Agriculture and Food Chemistry* 48 (8):3120-3.

Gutiérrez-Uribe, J. A., I. Romo-Lopez, and S. O. Serna-Saldívar. 2011. "Phenolic composition and mammary cancer cell inhibition of extracts of whole cowpeas (*Vigna unguiculata*) and its anatomical parts." *Journal of Functional Foods* 3 (4):290-297. doi: https://doi.org/10.1016/j.jff.2011.05.004.

Güzel, Demet, and Sedat Sayar. 2012. "Effect of cooking methods on selected physicochemical and nutritional properties of barlotto bean, chickpea, faba bean, and white kidney bean." *Journal of Food Science and Technology* 49 (1):89-95. doi: 10.1007/s13197-011-0260-0.

Haddy, Francis J., Paul M. Vanhoutte, and Michel Feletou. 2006. "Role of potassium in regulating blood flow and blood pressure." *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 290 (3):R546-R552. doi: 10.1152/ajpregu.00491.2005.

Hafen, B. B , and Burns B. 2020. "Physiology, Smooth Muscle." StatPearls Publishing, accessed Feb. https://www.ncbi.nlm.nih.gov/books/NBK526125/.

Hameed, A., T. M. Shah, B. M. Atta, N. Iqbal, M. A. Haq, and H. Ali. 2009. "Comparative Seed Storage Protein Profiling of Kabuli Chickpea Genotypes." *Pakistan Journal of Botany* 41 (2):703-710.

Han, Hwa, and Byung-Kee Baik. 2008. "Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum*

L.) and soybeans (*Glycine max*), and their quantitative changes during processing." *International Journal of Food Science & Technology* 43 (11):1971-1978. doi: 10.1111/j.1365-2621.2008.01800.x.

Han, In Hwa, and Byung-Kee Baik. 2006. "Oligosaccharide Content and Composition of Legumes and Their Reduction by Soaking, Cooking, Ultrasound, and High Hydrostatic Pressure." *Cereal Chemistry* 83 (4):428-433. doi: doi:10.1094/CC-83-0428.

Heiras-Palazuelos, Mar J., Mirna I. Ochoa-Lugo, Roberto Gutiérrez-Dorado, José A. López-Valenzuela, Saraid Mora-Rochín, Jorge Milán-Carrillo, José A. Garzón-Tiznado, and Cuauhtémoc Reyes-Moreno. 2013. "Technological properties, antioxidant activity and total phenolic and flavonoid content of pigmented chickpea (*Cicer arietinum* L.) cultivars." *International Journal of Food Sciences and Nutrition* 64 (1):69-76. doi: 10.3109/09637486.2012.694854.

Henry, Amanda G., Holly F. Hudson, and Dolores R. Piperno. 2009. "Changes in starch grain morphologies from cooking." *Journal of Archaeological Science* 36 (3):915-922. doi: https://doi.org/10.1016/j.jas.2008.11.008.

Herrero, M., P. Gerber, T. Vellinga, T. Garnett, A. Leip, C. Opio, H. J. Westhoek, P. K. Thornton, J. Olesen, N. Hutchings, H. Montgomery, J. F. Soussana, H. Steinfeld, and T. A. McAllister. 2011. "Livestock and greenhouse gas emissions: The importance of getting the numbers right." *Animal Feed Science and Technology* 166-167:779-782. doi: https://doi.org/10.1016/j.anifeedsci.2011.04.083.

Hollman, Peter C. H. 2014. "Unravelling of the health effects of polyphenols is a complex puzzle complicated by metabolism." *Archives of Biochemistry and Biophysics* 559:100-105. doi: https://doi.org/10.1016/j.abb.2014.04.013.

Hood-Niefer, S. 2017. "Insights into Extrusion of Protein." *Cereal Foods World* 62 (4):148-150. doi: 10.1094/Cfw-62-4-0148.

Hoover, R., and W. S. Ratnayake. 2002. "Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada." *Food Chemistry* 78 (4):489-498. doi: http://dx.doi.org/10.1016/S0308-8146(02)00163-2.

Hoover, R., and Y. Zhou. 2003. "In vitro and in vivo hydrolysis of legume starches by α-amylase and resistant starch formation in legumes—a review." *Carbohydrate Polymers* 54 (4):401-417. doi: https://doi.org/10.1016/S0144-8617(03)00180-2.

Hu, Xiao-Pei, Bao Zhang, Zheng-Yu Jin, Xue-Ming Xu, and Han-Qing Chen. 2017. "Effect of high hydrostatic pressure and retrogradation treatments on structural and physicochemical properties of waxy wheat starch." *Food Chemistry* 232:560-565. doi: https://doi.org/10.1016/j.foodchem.2017.04.040.

Hu, Xiuting, Xueming Xu, Zhengyu Jin, Yaoqi Tian, Yuxiang Bai, and Zhengjun Xie. 2011. "Retrogradation properties of rice starch gelatinized by heat and high hydrostatic pressure (HHP)." *Journal of Food Engineering* 106 (3):262-266. doi: https://doi.org/10.1016/j.jfoodeng.2011.05.021.

Huang, Hsiao-Wen, Sz-Jie Wu, Jen-Kai Lu, Yuan-Tay Shyu, and Chung-Yi Wang. 2017. "Current status and future trends of high-pressure processing in food industry." *Food Control* 72:1-8. doi: https://doi.org/10.1016/j.foodcont.2016.07.019.

Huang, Junrong, Henk A. Schols, Jeroen J. G. van Soest, Zhengyu Jin, Ellen Sulmann, and Alphons G. J. Voragen. 2007. "Physicochemical properties and amylopectin chain profiles of cowpea, chickpea and yellow pea starches." *Food Chemistry* 101 (4):1338-1345. doi: https://doi.org/10.1016/j.foodchem.2006.03.039.

Ibáñez, M. Victoria, Francisco Rincón, Manuel Amaro, and Beatriz Martínez. 1998. "Intrinsic variability of mineral composition of chickpea (*Cicer arietinum*, L.)." *Food Chemistry* 63 (1):55-60. doi: https://doi.org/10.1016/S0308-8146(97)00221-5.

Ibarz, A., C. González, and G. V. Barbosa-Cánovas. 2004. "Kinetic models for water adsorption and cooking time in chickpea soaked and treated by high pressure." *Journal of Food Engineering* 63 (4):467-472. doi: https://doi.org/10.1016/j.jfoodeng.2003.09.008.

Indrawati, A. Van Loey, and M. Hendrickx. 2004. "Pressure and temperature stability of water-soluble antioxidants in orange and carrot juice: A kinetic study." *European Food Research and Technology* 219 (2):161-166. doi: 10.1007/s00217-004-0929-y.

Iqbal, Amjad, Iqtidar A. Khalil, Nadia Ateeq, and Muhammad Sayyar Khan. 2006. "Nutritional quality of important food legumes." *Food Chemistry* 97 (2):331-335. doi: https://doi.org/10.1016/j.foodchem.2005.05.011.

Isken, Frank, Susanne Klaus, Martin Osterhoff, Andreas F. H. Pfeiffer, and Martin O. Weickert. 2010. "Effects of long-term soluble vs. insoluble dietary fiber intake on high-fat diet-induced obesity in C57BL/6J mice." *The Journal of Nutritional Biochemistry* 21 (4):278-284. doi: https://doi.org/10.1016/j.jnutbio.2008.12.012.

Jambunathan, R., H. L. Blain, K. S. Dhindsa, L. A. Hussein, K. Kogure, L. Li-Juan, and M. M. Youssef. 1994. "Diversifying use of cool season food legumes through processing." In *Expanding the Production and Use of Cool Season Food Legumes: A global perspective of peristent constraints and of opportunities and strategies for further increasing the productivity and use of pea, lentil, faba bean, chickpea and grasspea in different farming systems*, edited by F. J. Muehlbauer and W. J. Kaiser, 98-112. Dordrecht: Springer Netherlands.

Jambunathan, R., and U. Singh. 1980. "Studies on desi and kabuli chickpea (*Cicer arietinum* L.) cultivars. 1. Chemical composition." Patancheru.

Jambunathan, Ramamurthi, and Umaid Singh. 1981. "Studies on desi and kabuli chickpea (*Cicer arietinum* L.) cultivars. 3. Mineral and trace elements composition." *Journal of Agricultural and Food Chemistry* 29 (5):1091-1093. doi: 10.1021/jf00107a050.

Jenkins, D. J. A., C. W. C. Kendall, L. S. A. Augustin, S. Franceschi, M. Hamidi, A. Marchie, A. L. Jenkins, and M. Axelsen. 2002. "Glycemic index: overview of implications in health and disease." *American Journal of Clinical Nutrition* 76 (1):266s-273s.

Jiang, H., and K. M. Eskridge. 2000. "Bias in principal components analysis due to correlated observations." Conference on Applied Statistics in Agriculture.

Johnston, B., T. Healt, J. Ions, and M. Mcgregor. 1992. Rural research- the pay-off. The returns from research undertaken by CSIRO. Institute of Plant production and Processing

Jood, Sudesh, Bhag Mal Chauhan, and Amin C. Kapoor. 1987. "Polyphenols of chickpea and blackgram as affected by domestic processing and cooking methods." *Journal of the Science of Food and Agriculture* 39 (2):145-149. doi: 10.1002/jsfa.2740390207.

Joshi, Matina, Benu Adhikari, Joe Panozzo, and Peter Aldred. 2010. "Water uptake and its impact on the texture of lentils (*Lens culinaris*)." *Journal of Food Engineering* 100 (1):61-69. doi: https://doi.org/10.1016/j.jfoodeng.2010.03.028.

Jukanti, A. K., P. M. Gaur, C. L. Gowda, and R. N. Chibbar. 2012. "Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review." *British Journal of Nutrition* 108 Suppl 1:S11-26. doi: 10.1017/S0007114512000797.

Kabas, Onder, Erdem Yilmaz, Aziz Ozmerzi, and İbrahim Akinci. 2007. "Some physical and nutritional properties of cowpea seed (*Vigna sinensis* L.)." *Journal of Food Engineering* 79 (4):1405-1409. doi: https://doi.org/10.1016/j.jfoodeng.2006.04.022.

Kabtni, Souhir, Dorra Sdouga, Ines Bettaib Rebey, Mattew Save, Neila Trifi-Farah, Marie-Laure Fauconnier, and Sonia Marghali. 2020. "Influence of climate variation on phenolic composition and antioxidant capacity of Medicago minima populations." *Scientific Reports* 10 (1):8293. doi: 10.1038/s41598-020-65160-4.

Kadlec, Pavel, Jana Dostálová, Milan Houška, Jan Strohalm, Jana Culková, Andrea Hinková, and Helena Štarhová. 2006. "High pressure treatment of germinated chickpea *Cicer arietinum* L. seeds." *Journal of Food Engineering* 77 (3):445-448. doi: https://doi.org/10.1016/j.jfoodeng.2005.07.012.

Kalogeropoulos, Nick, Antonia Chiou, Maria Ioannou, Vaios T. Karathanos, Maria Hassapidou, and Nikolaos K. Andrikopoulos. 2010. "Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries." *Food Chemistry* 121 (3):682-690. doi: https://doi.org/10.1016/j.foodchem.2010.01.005.

Kaloki, Peter, Richard Trethowan, and Daniel K. Y. Tan. 2019. "Effect of genotype × environment × management interactions on chickpea phenotypic stability." *Crop and Pasture Science* 70 (5):453-462. doi: https://doi.org/10.1071/CP18547.

Kaptso, Kuaté Giscard, Yanou Nicolas Njintang, Mbouga Marie Goletti Nguemtchouin, Joël Scher, Joseph Hounhouigan, and Carl Moses Mbofung. 2015. "Physicochemical and micro-structural properties of flours, starch and proteins from two varieties of legumes: bambara groundnut (*Vigna subterranea*)." *Journal of Food Science and Technology* 52 (8):4915-4924. doi: 10.1007/s13197-014-1580-7.

Kargiotidou, A., F. Papathanasiou, D. Baxevanos, D. N. Vlachostergios, S. Stefanou, and I. Papadopoulos. 2019. "Yield and stability for agronomic and seed quality traits of common bean genotypes under Mediterranean conditions." *Legume Research* 42 (3):308-313. doi: 10.18805/Lr-437.

Kashiwagi, J., L. Krishnamurthy, R. Purushothaman, H. D. Upadhyaya, P. M. Gaur, C. L. L. Gowda, O. Ito, and R. K. Varshney. 2015. "Scope for improvement of yield under drought through the root traits in chickpea (*Cicer arietinum* L.)." *Field Crops Research* 170:47-54. doi: https://doi.org/10.1016/j.fcr.2014.10.003.

Katopo, Herman, Y. Song, and Jay-lin Jane. 2002. "Effect and mechanism of ultrahigh hydrostatic pressure on the structure and properties of starches." *Carbohydrate Polymers* 47 (3):233-244. doi: https://doi.org/10.1016/S0144-8617(01)00168-0.

Kaur, Lovedeep, Thierry Astruc, Annie Vénien, Olivier Loison, Jian Cui, Marion Irastorza, and Mike Boland. 2016. "High pressure processing of meat: effects on ultrastructure and protein digestibility." *Food & Function* 7 (5):2389-2397. doi: 10.1039/C5FO01496D.

Kaur, M., N. Singh, and N. S. Sodhi. 2005. "Physicochemical, cooking, textural and roasting characteristics of chickpea (*Cicer arietinum* L.) cultivars." *Journal of Food Engineering* 69 (4):511-517. doi: https://doi.org/10.1016/j.jfoodeng.2004.09.002.

Kaur, Maninder, and Narpinder Singh. 2006. "Relationships Between Selected Properties of Seeds, Flours, and Starches from Different Chickpea Cultivars." *International Journal of Food Properties* 9 (4):597-608. doi: 10.1080/10942910600853774.

Kaur, Maninder, Narpinder Singh, and Kawaljit Singh Sandhu. 2004. "Relationships Between Selected Properties of Black Gram Seeds and Their Composition." *International Journal of Food Properties* 7 (3):541-552. doi: 10.1081/JFP-200032966.

Kemi, V. E., M. U. Kärkkäinen, H. J. Rita, M. M. Laaksonen, T. A. Outila, and C. J. Lamberg-Allardt. 2010. "Low calcium:phosphorus ratio in habitual diets affects serum parathyroid hormone concentration and calcium metabolism in healthy women with adequate calcium intake." *British Journal of Nutrition* 103 (4):561-8. doi: 10.1017/s0007114509992121.

Khalil, A. W., A. Zeb, F. Mahmood, S. Tariq, A. B. Khattak, and H. Shah. 2007. "Comparison of sprout quality characteristics of desi and kabuli type chickpea cultivars (*Cicer arietinum* L.)." *Lebensmittel-Wissenschaft + [i.e. und] Technologie. Food science + technology. Science + technologie alimentaire* 40 (6):937-945. doi: 10.1016/j.lwt.2006.05.009.

Khan, A. Z., P. Shah, H. Khan, S. Nigar, S. Perveen, M. K. Shah, Amanullah, S. K. Khalil, S. Munir, and M. Zubair. 2011. "Seed quality and vigor of soybean cultivars as influenced by canopy temperature." *Pakistan Journal of Botany* 43 (1):643-648.

Khan, M. Akmal, Neghmana Akhtar, Ihsan Ullah, and Saeeda Jaffery. 1995. "Nutritional evaluation of desi and kabuli chickpeas and their products commonly consumed in Pakistan." *International Journal of Food Sciences and Nutrition* 46 (3):215-223. doi: 10.3109/09637489509012551.

Khatoon, Naveeda, and Jamuna Prakash. 2004. "Nutritional quality of microwave-cooked and pressure-cooked legumes." *International Journal of Food Sciences and Nutrition* 55 (6):441-448. doi: 10.1080/09637480400009102.

Khatoon, Naveeda, and Jamuna Prakash. 2006. "Nutritive value and sensory profile of microwave- and pressure-cooked decorticated legumes (dhals)." *Journal of Food Processing and Preservation* 30 (3):299-313. doi: https://doi.org/10.1111/j.1745-4549.2006.00066.x.

Khattak, A. B., A. Zeb, and N. Bibi. 2008. "Impact of germination time and type of illumination on carotenoidcontent, protein solubility and in vitro protein digestibility of chickpea (*Cicer arietinum* L.) sprouts." *Food Chem* 109 (4):797-801. doi: 10.1016/j.foodchem.2008.01.046.

Khattak, Amal Badshah, Gul Sanat Shah Khattak, Zahid Mahmood, Nizakat Bibi, and Ihsanullah Ihsanullah. 2006. "Study of selected quality and agronomic characteristics and their interrelationship in Kabuli-type chickpea genotypes (*Cicer arietinum* L.)." *International Journal of Food Science & Technology* 41 (s2):1-5. doi: 10.1111/j.1365-2621.2006.01193.x.

Kim, Soo-Yong, Jae-Young Je, and Se-Kwon Kim. 2007. "Purification and characterization of antioxidant peptide from hoki (*Johnius belengerii*) frame

protein by gastrointestinal digestion." *The Journal of Nutritional Biochemistry* 18 (1):31-38. doi: https://doi.org/10.1016/j.jnutbio.2006.02.006.

Knights, E. J., and K. B. Hobson. 2004. "Chickpea: Overview." In *Encyclopedia of Food Grains (Second Edition)*, 316-323. Oxford: Academic Press.

Knights, E. J., and R. J. Mailer. 1989. "Association of seed type and colour with establishment, yield and seed quality in chickpea (*Cicer arietinum*)." *The Journal of Agricultural Science* 113 (3):325-330. doi: 10.1017/S0021859600070003.

Knorr, Dietrich. 2002. "High Pressure Processing for Preservation, Modification and Transformation of Foods." *High Pressure Research* 22 (3-4):595-599. doi: 10.1080/08957950212411.

Koca, N., V.M. Balasubramaniam, and W.J. Harper. 2011. "High-Pressure Effects on the Microstructure, Texture, and Color of White-Brined Cheese." *Journal of Food Science* 76 (5):E399-E404. doi: 10.1111/j.1750-3841.2011.02201.x.

Koo, SungCheol, MyeongGi Jeon, YoungHoon Lee, HyunYoung Kim, BeomKyu Kang, JongMin Go, InYoul Baek, HongTai Yun, InYoul Baek, and ManSoo Choi. 2014. "Screening of soybean germplasm with high starch content." *Korean Journal of Breeding Science* 46 (1):52-57.

Kottek, Markus, Jürgen Grieser, Christoph Beck, Bruno Rudolf, and Franz Rubel. 2006. "World Map of the Köppen-Geiger Climate Classification Updated." *Meteorologische Zeitschrift* 15:259-263. doi: 10.1127/0941-2948/2006/0130.

Krasensky, J., and C. Jonak. 2012. "Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks." *J Exp Bot* 63 (4):1593-608. doi: 10.1093/jxb/err460.

Krebbers, B., A. M. Matser, M. Koets, and R. W. Van den Berg. 2002. "Quality and storage-stability of high-pressure preserved green beans." *Journal of Food Engineering* 54 (1):27-33. doi: https://doi.org/10.1016/S0260-8774(01)00182-0.

Laguna, Laura, Pierre Picouet, M. Dolors Guàrdia, Catherine M. G. C. Renard, and Anwesha Sarkar. 2017. "In vitro gastrointestinal digestion of pea protein isolate as a function of pH, food matrices, autoclaving, high-pressure and re-heat treatments." *LWT* 84:511-519. doi: https://doi.org/10.1016/j.lwt.2017.06.021.

Lal, R. 2004. "Carbon emission from farm operations." *Environment International* 30 (7):981-990. doi: https://doi.org/10.1016/j.envint.2004.03.005.

Lapornik, Brigita, Mirko Prošek, and Alenka Golc Wondra. 2005. "Comparison of extracts prepared from plant by-products using different solvents and extraction time." *Journal of Food Engineering* 71 (2):214-222. doi: https://doi.org/10.1016/j.jfoodeng.2004.10.036.

Laria, J., E. Meza, and J. L. Peña. 2007. "Water and calcium uptake by corn kernel during alkaline treatment with different temperature profiles." *Journal of Food Engineering* 78 (1):288-295. doi: https://doi.org/10.1016/j.jfoodeng.2005.09.031.

Le Bail, P., B. Chauvet, H. Simonin, C. Rondeau-Mouro, B. Pontoire, M. De Carvalho, and A. Le-Bail. 2013. "Formation and stability of amylose ligand complexes formed by high pressure treatment." *Innovative Food Science and Emerging Technologies* 18:1-6. doi: 10.1016/j.ifset.2012.10.006.

Lehmann, U., and F. Robin. 2007. "Slowly digestible starch – its structure and health implications: a review." *Trends in Food Science & Technology* 18 (7):346-355. doi: http://dx.doi.org/10.1016/j.tifs.2007.02.009.

Leite, T. S., A. L. T. de Jesus, M. Schmiele, A. A. L. Tribst, and M. Cristianini. 2017. "High pressure processing (HPP) of pea starch: Effect on the gelatinization properties." *LWT - Food Science and Technology* 76:361-369. doi: 10.1016/j.lwt.2016.07.036.

Leport, L., N. C. Turner, R. J. French, M. D. Barr, R. Duda, S. L. Davies, D. Tennant, and K. H. M. Siddique. 1999. "Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment." *European Journal of Agronomy* 11 (3):279-291. doi: https://doi.org/10.1016/S1161-0301(99)00039-8.

Li, A. N., S. Li, Y. J. Zhang, X. R. Xu, Y. M. Chen, and H. B. Li. 2014. "Resources and Biological Activities of Natural Polyphenols." *Nutrients* 6 (12):6020-6047. doi: 10.3390/nu6126020.

Li, Hongxia, and Joseph W. Burton. 2002. "Selecting Increased Seed Density to Increase Indirectly Soybean Seed Protein Concentration." *Crop Science* 42 (2):393-398. doi: https://doi.org/10.2135/cropsci2002.3930.

Li, W., Y. Bai, S. A. S. Mousaa, Q. Zhang, and Q. Shen. 2012. "Effect of High Hydrostatic Pressure on Physicochemical and Structural Properties of Rice Starch." *Food and Bioprocess Technology* 5 (6):2233-2241. doi: 10.1007/s11947-011-0542-6.

Li, W., F. Zhang, P. Liu, Y. Bai, L. Gao, and Q. Shen. 2011. "Effect of high hydrostatic pressure on physicochemical, thermal and morphological properties of mung bean (*Vigna radiata* L.) starch." *Journal of Food Engineering* 103 (4):388-393. doi: 10.1016/j.jfoodeng.2010.11.008.

Li, Yanhong, Bo Jiang, Tao Zhang, Wanmeng Mu, and Jian Liu. 2008. "Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH)." *Food Chemistry* 106 (2):444-450. doi: https://doi.org/10.1016/j.foodchem.2007.04.067.

Liang, L., H. A. Tajmir-Riahi, and M. Subirade. 2008. "Interaction of beta-lactoglobulin with resveratrol and its biological implications." *Biomacromolecules* 9 (1):50-6. doi: 10.1021/bm700728k.

Linsberger-Martin, Gertrud, Karin Weiglhofer, Thao Phan Thi Phuong, and Emmerich Berghofer. 2013. "High hydrostatic pressure influences antinutritional factors and in vitro protein digestibility of split peas and whole white beans." *LWT - Food Science and Technology* 51 (1):331-336. doi: https://doi.org/10.1016/j.lwt.2012.11.008.

Liu, Hang, Lijing Wang, Rong Cao, Huanhuan Fan, and Min Wang. 2016. "In vitro digestibility and changes in physicochemical and structural properties of common buckwheat starch affected by high hydrostatic pressure." *Carbohydrate Polymers* 144:1-8. doi: https://doi.org/10.1016/j.carbpol.2016.02.028.

Liu, Kunlun, Jiabao Zheng, and Fusheng Chen. 2019. "Effect of domestic cooking on rice protein digestibility." *Food Science & Nutrition* 7 (2):608-616. doi: 10.1002/fsn3.884.

Lobato, L. P., D. Anibal, M. M. Lazaretti, and M. V. E. Grossmann. 2011. "Extruded puffed functional ingredient with oat bran and soy flour." *LWT - Food Science and Technology* 44 (4):933-939. doi: http://dx.doi.org/10.1016/j.lwt.2010.11.013.

Lokare, Y. A. , J. V. Patil, and U. D. Chavan. 2007. "Genetic analysis of yiled and qality traits in kabuli chickpeas." *Journa of Food Legumes* 2:147-149.

Lopes, George K. B., Herbert M. Schulman, and Marcelo Hermes-Lima. 1999. "Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions1This study is dedicated to the memory of Botany Professor Luiz F.G. Labouriau (1921–1996).1." *Biochimica et Biophysica Acta (BBA) - General Subjects* 1472 (1):142-152. doi: https://doi.org/10.1016/S0304-4165(99)00117-8.

López-Amorós, M. L., T. Hernández, and I. Estrella. 2006. "Effect of germination on legume phenolic compounds and their antioxidant activity." *Journal of Food Composition and Analysis* 19 (4):277-283. doi: https://doi.org/10.1016/j.jfca.2004.06.012.

López-Bellido, Francisco J., Rafael J. López-Bellido, Shawkat Kasem Khalil, and Luis López-Bellido. 2008. "Effect of Planting Date on Winter Kabuli Chickpea Growth and Yield under Rainfed Mediterranean Conditions." *Agronomy Journal* 100 (4):957-964. doi: https://doi.org/10.2134/agronj2007.0274.

Lullien-Pellerin, Valérie, and Claude Balny. 2002. "High-pressure as a tool to study some proteins' properties: conformational modification, activity and oligomeric dissociation." *Innovative Food Science & Emerging Technologies* 3 (3):209-221. doi: https://doi.org/10.1016/S1466-8564(02)00045-0.

Ma, Mengting, Yijing Wang, Meixia Wang, Jay-lin Jane, and Shuang-kui Du. 2017. "Physicochemical properties and in vitro digestibility of legume starches." *Food Hydrocolloids* 63:249-255. doi: https://doi.org/10.1016/j.foodhyd.2016.09.004.

Määttä, Kaisu, Anna-Maija Lampi, Jan Petterson, Birgitta M Fogelfors, Vieno Piironen, and Afaf Kamal-Eldin. 1999. "Phytosterol content in seven oat cultivars grown at three locations in Sweden." *Journal of the Science of Food and Agriculture* 79 (7):1021-1027. doi: 10.1002/(sici)1097-0010(19990515)79:7<1021::Aid-jsfa316>3.0.Co;2-e.

MacGregor, D. R., S. L. Kendall, H. Florance, F. Fedi, K. Moore, K. Paszkiewicz, N. Smirnoff, and S. Penfield. 2015. "Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylpropanoid metabolism." *New Phytologist* 205 (2):642-52. doi: 10.1111/nph.13090.

Mafakheri, A., A. Siosemardeh, B. Bahramnejad, P. C. Struik, and Y. Sohrabi. 2011. "Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (*Cicer arietinum*) cultivars." *Australian Journal of Crop Science* 5 (10):1255-1260.

Mahasukhonthachat, K., P. A. Sopade, and M. J. Gidley. 2010. "Kinetics of starch digestion in sorghum as affected by particle size." *Journal of Food Engineering* 96 (1):18-28. doi: https://doi.org/10.1016/j.jfoodeng.2009.06.051.

Man, S., A. Paucean, S. Muste, and A. Pop. 2015. "Effect of the Chickpea (*Cicer arietinum* L.) Flour Addition on Physicochemical Properties of Wheat Bread." *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca-Food Science and Technology* 72 (1):41-49. doi: 10.15835/buasvmcn-fst:11023.

Máñez, G., A. Alegría, R. Farré, and A. Frígola. 2002. "Effect of traditional, microwave and industrial cooking on inositol phosphate content in beans, chickpeas and lentils." *International Journal of Food Sciences and Nutrition* 53 (6):503-508. doi: 10.1080/09637480220164343.

Maphosa, Y., and V. A. Jideani. 2017. "The role of legumes in human nutrition." In *Functional Food: Improve Health through adequate food*, edited by María Chávarri Hueda. Crostia: InTech.

Marathe, Sushama A., V. Rajalakshmi, Sahayog N. Jamdar, and Arun Sharma. 2011. "Comparative study on antioxidant activity of different varieties of commonly consumed legumes in India." *Food and Chemical Toxicology* 49 (9):2005-2012. doi: https://doi.org/10.1016/j.fct.2011.04.039.

Marcone, M. F., Y. Kakuda, and R. Y. Yada. 2003. "Amaranth as a rich dietary source of beta-sitosterol and other phytosterols." *Plant Foods for Human Nutrition* 58 (3):207-11. doi: 10.1023/b:qual.0000040334.99070.3e.

Marconi, E., S. Ruggeri, M. Cappelloni, D. Leonardi, and E. Carnovale. 2000. "Physicochemical, nutritional, and microstructural characteristics of chickpeas (*Cicer arietinum* L.) and common beans (*Phaseolus vulgaris* L.) following microwave cooking." *J Agric Food Chem* 48 (12):5986-94.

Margier, Marielle, Stéphane Georgé, Noureddine Hafnaoui, Didier Remond, Marion Nowicki, Laure Du Chaffaut, Marie-Josèphe Amiot, and Emmanuelle Reboul. 2018. "Nutritional composition and bioactive content of legumes: characterization of pulses frequently consumed in france and effect of the cooking method." *Nutrients* 10 (11):1668.

Markets, and Markets. 2020. "Plant-based Protein Market by Source (Soy, Wheat, and Pea), Type (Isolates, Concentrates, and Textured), Form (Dry and Liquid), Application (Food and Feed), and Region (North America, Europe, APAC, South America, RoW) - Global Forecast to 2025." accessed 23/10. https://www.marketsandmarkets.com/Market-Reports/plant-based-protein-market-14715651.html.

Marshall, Maurice R. 2010. "Ash Analysis." In *Food Analysis*, 105-115. Boston, MA: Springer US.

Martinez, M. M. 2021. "Starch nutritional quality: beyond intraluminal digestion in response to current trends" *Current Opinion in Food Science* 38:112-121. https://doi.org/10.1016/j.cofs.2020.10.024

Martínez-Villaluenga, Cristina, Juana Frias, and Concepción Vidal-Valverde. 2008. "Alpha-Galactosides: Antinutritional Factors or Functional Ingredients?" *Critical Reviews in Food Science and Nutrition* 48 (4):301-316. doi: 10.1080/10408390701326243.

Mattos, Pedro Henrique Costa de, Ricardo Augusto de Oliveira, João Carlos Bespalhok Filho, Edelclaiton Daros, and Mario Alvaro Aloiso Veríssimo. 2013. "Evaluation of sugarcane genotypes and production environments in Paraná by GGE biplot and AMMI analysis." *Crop Breeding and Applied Biotechnology* 13:83-90.

McKenzie, B. A., and G. D. Hill. 1995. "Growth and yield of two chickpea (Cicer arietinum L.) varieties in Canterbury, New Zealand." *New Zealand Journal of Crop and Horticultural Science* 23 (4):467-474. doi: 10.1080/01140671.1995.9513925.

Medicine, Institute of. 2005. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Washington, D. C., USA: The National Academic Press.

Mercier, C. 1993. "Nutritional appraisal of extruded foods." *International hournal of Food Science and Nutrition* 44:545-553.

Merga, Bulti, and Jema Haji. 2019. "Economic importance of chickpea: Production, value, and world trade." *Cogent Food & Agriculture* 5 (1). doi: 10.1080/23311932.2019.1615718.

Meteorology, Bureau of. 2021. "Climate Classification maps." Australian Government, accessed 04 March. http://www.bom.gov.au/jsp/ncc/climate_averages/climate-classifications/index.jsp?maptype=seasgrpb#maps.

Meullenet, Jean-Francois, B.G. Lyon, John A. Carpenter, and C.E. LYON. 1998. "Relationship between sensory and instrumental texture profile attributes." *Journal of Sensory Studies* 13 (1):77-93. doi: https://doi.org/10.1111/j.1745-459X.1998.tb00076.x.

Miao, Ming, Bo Jiang, Steve W. Cui, Tao Zhang, and Zhengyu Jin. 2015. "Slowly Digestible Starch—A Review." *Critical Reviews in Food Science and Nutrition* 55 (12):1642-1657. doi: 10.1080/10408398.2012.704434.

Miao, Ming, Tao Zhang, and Bo Jiang. 2009. "Characterisations of kabuli and desi chickpea starches cultivated in China." *Food Chemistry* 113 (4):1025-1032. doi: http://dx.doi.org/10.1016/j.foodchem.2008.08.056.

Milán-Carrillo, J., C. Reyes-Moreno, E. Armienta-Rodelo, A. Carábez-Trejo, and R. Mora-Escobedo. 2000. "Physicochemical and Nutritional Characteristics of Extruded Flours from Fresh and Hardened Chickpeas (*Cicer arietinum* L)." *LWT - Food Science and Technology* 33 (2):117-123. doi: https://doi.org/10.1006/fstl.1999.0620.

Mir, Shabir Ahmad, Sowriappan John Don Bosco, Manzoor Ahmad Shah, and Mohammad Maqbool Mir. 2016. "Effect of puffing on physical and antioxidant properties of brown rice." *Food Chemistry* 191:139-146. doi: https://doi.org/10.1016/j.foodchem.2014.11.025.

Misra, G., A. Joshi-Saha, D. Salaskar, K. S. Reddy, G. P. Dixit, A. K. Srivastava, V. Jayalakshmi, M. S. Pithia, and P. M. Gaur. 2020. "Baseline status and effect of genotype, environment and genotype × environment interactions on iron and zinc content in Indian chickpeas (*Cicer arietinum* L.)." *Euphytica* 216 (9):137. doi: 10.1007/s10681-020-02673-z.

Mittal, Rajni, H. P. S. Nagi, Priyanka Sharma, and Savita Sharma. 2012. "Effect of processing on chemical composition and antinutritional factors in chickpea flour." *Journal of Food Science and Engineering* 2. doi: 10.17265/2159-5828/2012.03.008.

Mittler, Ron, Sandy Vanderauwera, Martin Gollery, and Frank Van Breusegem. 2004. "Reactive oxygen gene network of plants." *Trends in Plant Science* 9 (10):490-498. doi: https://doi.org/10.1016/j.tplants.2004.08.009.

Mohammadi, Reza, Reza Haghparast, Ahmed Amri, and Salvatore Ceccarelli. 2009. "Yield stability of rainfed durum wheat and GGE biplot analysis of multi-

environment trials." *Crop and Pasture Science* 61 (1):92-101. doi: https://doi.org/10.1071/CP09151.

Mohammed, I., Abdelrahman R. Ahmed, and B. Senge. 2014. "Effects of chickpea flour on wheat pasting properties and bread making quality." *Journal of Food Science and Technology* 51 (9):1902-1910. doi: 10.1007/s13197-012-0733-9.

Mokni, Abir, Ines Maklouf, Christophe Blecker, Hamadi Attia, and Souhail Besbes. 2015. "Nutritional and Compositional Study of Desi and Kabuli Chickpea (*Cicer Arietinum* L.) Flours from Tunisian Cultivars." *Advancess in food technology and nutritional sciences* 1:38-47. doi: 10.17140/AFTNSOJ-1-107.

Moloto, R. M., M. D. Lengwati, P. Soundy, F. D. Dakora, and S. T. Maseko. 2018. "Effect of soil type and biostimulants on growth parameters of chickpea." *South African Journal of Botany* 115:300. doi: https://doi.org/10.1016/j.sajb.2018.02.088.

Moscicki, L. 2011. *Extrusion-cooking techniques: applications, theory and sustainability*. Weinheim: WILEY-VCH Verlag GMBH & Co. KGaA.

Mubarak, A. E. 2005. "Nutritional composition and antinutritional factors of mung bean seeds (Phaseolus aureus) as affected by some home traditional processes." *Food Chemistry* 89 (4):489-495. doi: https://doi.org/10.1016/j.foodchem.2004.01.007.

Muehlbauer, Fred J., and P.N. Rajesh. 2008. "Chickpea, a Common Source of Protein and Starch in the Semi-Arid ropics." In *Genomics of Tropical Crop Plants*, edited by Paul H. Moore and Ray Ming, 171-186. New York, NY: Springer New York.

Murphy, Neil, Teresa Norat, Pietro Ferrari, Mazda Jenab, Bas Bueno-de-Mesquita, Guri Skeie, Christina C. Dahm, Kim Overvad, Anja Olsen, Anne Tjønneland, Françoise Clavel-Chapelon, Marie Christine Boutron-Ruault, Antoine Racine, Rudolf Kaaks, Birgit Teucher, Heiner Boeing, Manuela M. Bergmann, Antonia Trichopoulou, Dimitrios Trichopoulos, Pagona Lagiou, Domenico Palli, Valeria Pala, Salvatore Panico, Rosario Tumino, Paolo Vineis, Peter Siersema, Franzel van Duijnhoven, Petra H. M. Peeters, Anette Hjartaker, Dagrun Engeset, Carlos A. González, Maria-José Sánchez, Miren Dorronsoro, Carmen Navarro, Eva Ardanaz, José R. Quirós, Emily Sonestedt, Ulrika Ericson, Lena Nilsson, Richard Palmqvist, Kay-Tee Khaw, Nick Wareham, Timothy J. Key, Francesca L. Crowe, Veronika Fedirko, Petra A. Wark, Shu-Chun Chuang, and Elio Riboli. 2012. "Dietary Fibre Intake and Risks of Cancers of the Colon and Rectum in the European Prospective Investigation into Cancer and Nutrition (EPIC)." *PLOS ONE* 7 (6):e39361. doi: 10.1371/journal.pone.0039361.

Mwangwela, Agnes M, Ralph D Waniska, and Amanda Minnaar. 2006. "Hydrothermal treatments of two cowpea (*Vigna unguiculata* L. Walp) varieties: effect of micronisation on physicochemical and structural characteristics." *Journal of the Science of Food and Agriculture* 86 (1):35-45. doi: 10.1002/jsfa.2275.

Naczk, M., S. Grant, R. Zadernowski, and E. Barre. 2006. "Protein precipitating capacity of phenolics of wild blueberry leaves and fruits." *Food Chemistry* 96 (4):640-647. doi: https://doi.org/10.1016/j.foodchem.2005.03.017.

Naczk, Marian, Ryszard Amarowicz, and Fereidoon Shahidi. 1997. "α-Galactosides of Sucrose in Foods: Composition, Flatulence-Causing Effects, and Removal." In *Antinutrients and Phytochemicals in Food*, 127-151. American Chemical Society.

Nations, United. 2021. World Population Prospects. United Nations Department of Economic and Social Affairs.

Negi, P. S., A. S. Chauhan, G. A. Sadia, Y. S. Rohinishree, and R. S. Ramteke. 2005. "Antioxidant and antibacterial activities of various seabuckthorn (*Hippophae rhamnoides* L.) seed extracts." *Food Chemistry* 92 (1):119-124. doi: https://doi.org/10.1016/j.foodchem.2004.07.009.

Netyam, Kannababu, Sujay Rakshit, Ragimasalawada Madhusudhana, Vilas Tonapi, I. K. Das, and K. Raghunath. 2017. "Identification of superior parental lines for seed quality and storability through GGE biplot analysis of line × tester data in grain sorghum." *Indian Journal of Genetics and Plant Breeding (The)* 77:278. doi: 10.5958/0975-6906.2017.00037.2.

Neves, Valdir, and E. Lourenço. 2001. "Changes in protein fractions, trypsin inhibitor and proteolytic activity in the cotyledons of germinating Chickpea." *Archivos latinoamericanos de nutrición* 51:269-75.

NHMRC. 2014. "Nutrient Reference Values for Australia and New Zealand- Proteins." NHMRC accessed March. https://www.nrv.gov.au/nutrients/protein.

NHMRC. 2019. "Nutrient reference values for Australia and New Zealand- Dietary fibre." NHMRC, accessed March. https://www.nrv.gov.au/nutrients/dietary-fibre.

Nicoli, M. C., M. Anese, M. T. Parpinel, S. Franceschi, and C. R. Lerici. 1997. "Loss and/or formation of antioxidants during food processing and storage." *Cancer Letters* 114 (1-2):71-74. doi: 10.1016/S0304-3835(97)04628-4.

Nikolopoulou, D., K. Grigorakis, M. Stasini, M. Alexis, and K. Iliadis. 2006a. "Effects of cultivation area and year on proximate composition and antinutrients in three different kabuli-type chickpea." *European Food Research Technology* 223:737-741.

Nikolopoulou, Demetra, Kriton Grigorakis, Maria Stasini, Maria Alexis, and Konstantinos Iliadis. 2006b. "Effects of cultivation area and year on proximate composition and antinutrients in three different kabuli-type chickpea (*Cicer arientinum*) varieties." *European Food Research and Technology* 223 (6):737-741. doi: 10.1007/s00217-006-0261-9.

Nikoobin, M., F. Mirdavardoost, M. Kashaninejad, and A. Soltani. 2009. "Moisture-dependent physical properties of chickpea seeds." *Journal of Food Process Engineering* 32 (4):544-564. doi: https://doi.org/10.1111/j.1745-4530.2007.00231.x.

Nisa, Zaib Un, Anjuman Arif, Muhammad Qandeel Waheed, Tariq Mahmood Shah, Ayesha Iqbal, Amna Jabbar Siddiqui, Muhammad Iqbal Choudhary, Hesham R. El-Seedi, and Syed Ghulam Musharraf. 2020. "A comparative metabolomic study on desi and kabuli chickpea (*Cicer arietinum* L.) genotypes under rainfed and irrigated field conditions." *Scientific Reports* 10 (1):13919. doi: 10.1038/s41598-020-70963-6.

Nithiyanantham, Srinivasan, Subramanian Selvakumar, and Perumal Siddhuraju. 2012. "Total phenolic content and antioxidant activity of two different solvent extracts from raw and processed legumes, *Cicer arietinum* L. and *Pisum sativum* L." *Journal of Food Composition and Analysis* 27 (1):52-60. doi: https://doi.org/10.1016/j.jfca.2012.04.003.

Nobile, Carla G Marioli, Julia Carreras, Rubén Grosso, Marcela Inga, Mercedes Silva, Roxana Aguilar, María José Allende, Raúl Badini, and María José Martinez. 2013. "Proximate composition and seed lipid components of "kabuli"-type chickpea (*Cicer arietinum* L.) from Argentina." *Agricultural Sciences* 2013.

Noda, T., S. Takigawa, C. Matsuura-Endo, T. Suzuki, N. Hashimoto, N. S. Kottearachchi, H. Yamauchi, and I. S. M. Zaidul. 2008. "Factors affecting the digestibility of raw and gelatinized potato starches." *Food Chemistry* 110 (2):465-470. doi: https://doi.org/10.1016/j.foodchem.2008.02.027.

Norat, T., S. Bingham, P. Ferrari, N. Slimani, M. Jenab, M. Mazuir, K. Overvad, A. Olsen, A. Tjønneland, F. Clavel, M. C. Boutron-Ruault, E. Kesse, H. Boeing, M. M. Bergmann, A. Nieters, J. Linseisen, A. Trichopoulou, D. Trichopoulos, Y. Tountas, F. Berrino, D. Palli, S. Panico, R. Tumino, P. Vineis, H. B. Bueno-de-Mesquita, P. H. Peeters, D. Engeset, E. Lund, G. Skeie, E. Ardanaz, C. González, C. Navarro, J. R. Quirós, M. J. Sanchez, G. Berglund, I. Mattisson, G. Hallmans, R. Palmqvist, N. E. Day, K. T. Khaw, T. J. Key, M. San Joaquin, B. Hémon, R. Saracci, R. Kaaks, and E. Riboli. 2005. "Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition." *Journal of National Cancer Institute* 97 (12):906-16. doi: 10.1093/jnci/dji164.

NRV. 2017. "Nutrient Reference Values for Australia and New Zealand." National Health and Mecial Research Council

Ministry of Health, accessed 25th May. https://www.nrv.gov.au/nutrients.

Oey, Indrawati, Iesel Van der Plancken, Ann Van Loey, and Marc Hendrickx. 2008. "Does high pressure processing influence nutritional aspects of plant based food systems?" *Trends in Food Science & Technology* 19 (6):300-308. doi: https://doi.org/10.1016/j.tifs.2007.09.002.

Olika, Ebisa, Solomon Abera, and Asnake Fikre. 2019. "Physicochemical properties and effect of processing methods on mineral composition and antinutritional factors of improved chickpea (*Cicer arietinum* L.) varieties grown in Ethiopia." *International Journal of Food Science* 2019:7. doi: 10.1155/2019/9614570.

Oliveira, Fabiano Alves de, Otávio Cabral Neto, Lígia Marcondes Rodrigues dos Santos, Elisa Helena Rocha Ferreira, and Amauri Rosenthal. 2017. "Effect of high pressure on fish meat quality – A review." *Trends in Food Science & Technology* 66:1-19. doi: https://doi.org/10.1016/j.tifs.2017.04.014.

Ouazib, M., N. Moussou, B. D. Oomah, F. Zaidi, and J. P. Wanasundara. 2015. "Effect of processing and germination on nutritional parameters and functional properties of chickpea (*Cicer arietinum* L.) from Algeria." *Food Legumes* 28 (2):133-140.

Oweis, Theib, Ahmed Hachum, and Mustafa Pala. 2004. "Water use efficiency of winter-sown chickpea under supplemental irrigation in a mediterranean environment." *Agricultural Water Management* 66 (2):163-179. doi: https://doi.org/10.1016/j.agwat.2003.10.006.

Ozdal, Tugba, İpek Ekin Yalcinkaya, Gamze Toydemir, and Esra Capanoglu. 2019. "Polyphenol-Protein Interactions and Changes in Functional Properties and Digestibility." In *Encyclopedia of Food Chemistry*, edited by Laurence Melton, Fereidoon Shahidi and Peter Varelis, 566-577. Oxford: Academic Press.

Özer, Sertaç, Tolga Karaköy, Faruk Toklu, Faheem S. Baloch, Benjamin Kilian, and Hakan Özkan. 2010. "Nutritional and physicochemical variation in Turkish kabuli chickpea (*Cicer arietinum* L.) landraces." *Euphytica* 175 (2):237-249. doi: 10.1007/s10681-010-0174-3.

Paciulli, Maria, Ilce Gabriela Medina-Meza, Emma Chiavaro, and Gustavo Victor Barbosa-Cánovas. 2016. "Impact of thermal and high pressure processing on quality parameters of beetroot (Beta vulgaris L.)." *LWT - Food Science and Technology* 68:98-104. doi: https://doi.org/10.1016/j.lwt.2015.12.029.

Panel, Institute of Medicine. 2000. "Institute of Medicine Panel on dietary, antioxidants and related compounds." In *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington (DC): National Academies Press (US) Copyright 2000 by the National Academy of Sciences. All rights reserved.

Patanè, Cristina. 2006. "Variation and relationships among some nutritional traits in sicilian genotypes of chickpea (*Cicer Arietinum* L.)." *Journal of Food Quality* 29:282-293. doi: 10.1111/j.1745-4557.2006.00074.x.

Patanè, Cristina, Elisa Iacoponi, and Salvatore A. Raccuia. 2004. "Physico-chemical characteristics, water absorption, soaking and cooking properties of some Sicilian populations of chickpea (*Cicer arietinum* L.)." *International Journal of Food Sciences and Nutrition* 55 (7):547-554. doi: 10.1080/09637480400015836.

Patras, Ankit, Nigel Brunton, Sara Da Pieve, Francis Butler, and Gerard Downey. 2009. "Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées." *Innovative Food Science & Emerging Technologies* 10 (1):16-22. doi: https://doi.org/10.1016/j.ifset.2008.09.008.

Patras, Ankit, Nigel P. Brunton, Sara Da Pieve, and Francis Butler. 2009. "Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées." *Innovative Food Science & Emerging Technologies* 10 (3):308-313. doi: https://doi.org/10.1016/j.ifset.2008.12.004.

Pereira, Danielle de Carvalho, Raquel Patrícia Ataíde Lima, Roberto Teixeira de Lima, Maria da Conceição Rodrigues Gonçalves, Liana Clébia Soares Lima de Morais, Sylvia do Carmo Castro Franceschini, Rosália Gouveia Filizola, Ronei Marcos de Moraes, Luiza Sonia Rios Asciutti, and Maria José de Carvalho Costa. 2013. "Association between obesity and calcium:phosphorus ratio in the habitual diets of adults in a city of Northeastern Brazil: an epidemiological study." *Nutrition Journal* 12 (1):90. doi: 10.1186/1475-2891-12-90.

Peterbauer, Thomas, and Andreas Richter. 2007. "Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds." *Seed Science Research* 11 (3):185-197. doi: 10.1079/SSR200175.

Pico, J., Pismag, R. Y., Laudouze, M., & Martinez, M. M. (2020)."Systematic evaluation of the Folin–Ciocalteu and Fast Blue BB reactions during the analysis of total phenolics in legumes, nuts and plant seeds". *Food & Function* 11(11): 9868-9880.

Piluzza, Giovanna, and Simonetta Bullitta. 2011. "Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area." *Pharmaceutical Biology* 49 (3):240-247. doi: 10.3109/13880209.2010.501083.

Pineda-Gómez, P., A. Rosales-Rivera, and M. E. Rodríguez-García. 2012. "Modeling calcium and water intake in threshed corn grain during thermo-alkaline treatment." *Journal of Food Engineering* 113 (3):434-441. doi: https://doi.org/10.1016/j.jfoodeng.2012.06.026.

Pittaway, J. K., I. K. Robertson, and M. J. Ball. 2008. "Chickpeas may influence fatty acid and fiber intake in an ad libitum diet, leading to small improvements in serum lipid profile and glycemic control." *Journal of the American Dietetic Association* 108 (6):1009-13. doi: 10.1016/j.jada.2008.03.009.

Pojić, M., A. Mišan, and B. Tiwari. 2018. "Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin." *Trends in Food Science and Technology* 75:93-104. doi: 10.1016/j.tifs.2018.03.010.

Poltronieri, F., J. A. G. Arêas, and C. Colli. 2000. "Extrusion and iron bioavailability in chickpea (*Cicer arietinum* L.)." *Food Chemistry* 70 (2):175-180. doi: https://doi.org/10.1016/S0956-7135(99)00113-9.

Portari, Guilherme Vanucchi, Olga Luisa Tavano, Maraiza A. da Silva, and Valdir Augusto Neves. 2005. "Effect of chickpea (*Cicer arietinum* L.) germination on the major globulin content and in vitro digestibility." *Food Science and Technology* 25:807-812.

Prior, R. L., and G. Cao. 1999. "In vivo total antioxidant capacity: comparison of different analytical methods." *Free Radic Biol Med* 27 (11-12):1173-81. doi: 10.1016/s0891-5849(99)00203-8.

Prior, R. L., H. Hoang, L. Gu, X. Wu, M. Bacchiocca, L. Howard, M. Hampsch-Woodill, D. Huang, B. Ou, and R. Jacob. 2003. "Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples." *Journal of Agricultural and Food Chemistry* 51 (11):3273-9. doi: 10.1021/jf0262256.

Prodanov, Marin, Isabel Sierra, and Concepción Vidal-Valverde. 2004. "Influence of soaking and cooking on the thiamin, riboflavin and niacin contents of legumes." *Food Chemistry* 84 (2):271-277. doi: https://doi.org/10.1016/S0308-8146(03)00211-5.

Profarmer. 2019. https://www.profarmergrain.com.au/

Pugalenthi, M., V. Vadivel, P. Gurumoorthi, and K. Janardhanan. 2004. "Comparative nutritional evaluation of little known legumes, Tamarindus indica, Erythrina indica and Sesbania bispinosa." *Tropical and Subtropical Agroecosystems* 4:107-123.

PULSE, Australia. 2019. "Chickpea- Variety selection." Pulse Australia. http://www.pulseaus.com.au/growing-pulses/bmp/chickpea.

Quintero-Soto, Maria F., Ana G. Saracho-Peña, Jeanett Chavez-Ontiveros, Jose A. Garzon-Tiznado, Karen V. Pineda-Hidalgo, Francisco Delgado-Vargas, and Jose A. Lopez-Valenzuela. 2018. "Phenolic profiles and their contribution to the antioxidant activity of selected chickpea genotypes from Mexico and ICRISAT collections." *Plant Foods for Human Nutrition* 73 (2):122-129. doi: 10.1007/s11130-018-0661-6.

Rachwa-Rosiak, Danuta, Ewa Nebesny, and Grażyna Budryn. 2015. "Chickpeas—Composition, Nutritional Value, Health Benefits, Application to Bread and Snacks: A Review." *Critical Reviews in Food Science and Nutrition* 55 (8):1137-1145. doi: 10.1080/10408398.2012.687418.

Ramakrishna, V., P. J. Rani, and P. R. Rao. 2006. "Anti-nutritional factors during germination in Indian bean (*Dolichos lablab* L.) seeds." *World Journal of Dairy & Food Sciences* 1 (1):6-11.

Rathod, Rahul P., and Uday S. Annapure. 2016. "Effect of extrusion process on antinutritional factors and protein and starch digestibility of lentil splits." *LWT - Food Science and Technology* 66:114-123. doi: https://doi.org/10.1016/j.lwt.2015.10.028.

Ravi, Ramasamy, and Janice B. Harte. 2009. "Milling and physicochemical properties of chickpea (*Cicer arietinum* L.) varieties." *Journal of the Science of Food and Agriculture* 89 (2):258-266. doi: 10.1002/jsfa.3435.

Raza, Husnain, Kashif Ameer, Farah Zaaboul, Hafiz Rizwan Sharif, Barkat Ali, Muhammad Shoaib, Wasim Akhtar, and Lianfu Zhang. 2019. "Effects of ball-milling on physicochemical, thermal and functional properties of extruded chickpea (*Cicer arietinum* L.) powder." *CyTA - Journal of Food* 17 (1):563-573. doi: 10.1080/19476337.2019.1617352.

Rehman, Zia-ur, M. Rashid, and W. H. Shah. 2004. "Insoluble dietary fibre components of food legumes as affected by soaking and cooking processes." *Food Chemistry* 85 (2):245-249. doi: https://doi.org/10.1016/j.foodchem.2003.07.005.

Rehman, Zia-ur, and W. H. Shah. 2005. "Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes." *Food Chemistry* 91 (2):327-331. doi: https://doi.org/10.1016/j.foodchem.2004.06.019.

Riaz, Mian N. 2000. *Extruders in food applications*. Lancaster, Pa.: Technomic Pub. Co.

Ridge, I. 1991. "The regulation of plant growth." In *Plant Physiology*, edited by I. Ridge, 282-333. London, UK: Hodder and Stoughton.

Riedl, Ken M., and Ann E. Hagerman. 2001. "Tannin−Protein Complexes as Radical Scavengers and Radical Sinks." *Journal of Agricultural and Food Chemistry* 49 (10):4917-4923. doi: 10.1021/jf010683h.

Rincón, Francisco, Beatríz Martínez, and M. Victoria Ibáñez. 1998. "Proximate composition and antinutritive substances in chickpea (*Cicer arietinum* L) as affected by the biotype factor." *Journal of the Science of Food and Agriculture* 78 (3):382-388. doi: 10.1002/(sici)1097-0010(199811)78:3<382::Aid-jsfa128>3.0.Co;2-j.

Rivero, Rosa M., Juan M. Ruiz, Pablo C. García, Luis R. López-Lefebre, Esteban Sánchez, and Luis Romero. 2001. "Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants." *Plant Science* 160 (2):315-321. doi: https://doi.org/10.1016/S0168-9452(00)00395-2.

Rizkalla, Salwa W., Laika Taghrid, Muriel Laromiguiere, Dorothée Huet, Josette Boillot, Aude Rigoir, Fabienne Elgrably, and Gerard Slama. 2004. "Improved Plasma Glucose Control, Whole-Body Glucose Utilization, and Lipid Profile

on a Low-Glycemic Index Diet in Type 2 Diabetic Men." *A randomized controlled trial* 27 (8):1866-1872. doi: 10.2337/diacare.27.8.1866.

Robinson, G. H. J., J. Balk, and C. Domoney. 2019. "Improving pulse crops as a source of protein, starch and micronutrients." *Nutrition Bulletin* 44 (3):202-215. doi: https://doi.org/10.1111/nbu.12399.

Rocha-Guzmán, Nuria E., Rubén F. González-Laredo, Francisco J. Ibarra-Pérez, Cynthia A. Nava-Berúmen, and José-Alberto Gallegos-Infante. 2007. "Effect of pressure cooking on the antioxidant activity of extracts from three common bean (*Phaseolus vulgaris* L.) cultivars." *Food Chemistry* 100 (1):31-35. doi: https://doi.org/10.1016/j.foodchem.2005.09.005.

Rodríguez-Roque, María Janeth, Begoña de Ancos, Concepción Sánchez-Moreno, M. Pilar Cano, Pedro Elez-Martínez, and Olga Martín-Belloso. 2015. "Impact of food matrix and processing on the in vitro bioaccessibility of vitamin C, phenolic compounds, and hydrophilic antioxidant activity from fruit juice-based beverages." *Journal of Functional Foods* 14:33-43. doi: https://doi.org/10.1016/j.jff.2015.01.020.

Rolletschek, H., M. R. Hajirezaei, U. Wobus, and H. Weber. 2002. "Antisense-inhibition of ADP-glucose pyrophosphorylase in Vicia narbonensis seeds increases soluble sugars and leads to higher water and nitrogen uptake." *Planta* 214 (6):954-964. doi: 10.1007/s00425-001-0710-4.

Rosa, Lorenzo, Maria Cristina Rulli, Kyle Frankel Davis, Davide Danilo Chiarelli, Corrado Passera, and Paolo D'Odorico. 2018. "Closing the yield gap while ensuring water sustainability." *Environmental Research Letters* 13 (10):104002. doi: 10.1088/1748-9326/aadeef.

Roser, M., and H. Ritchie. 2019. Hunger and Undernourishment. OurWorldInData.org.

Rosin, Patricia M., Franco M. Lajolo, and Elizabete W. Menezes. 2002. "Measurement and Characterization of Dietary Starches." *Journal of Food Composition and Analysis* 15 (4):367-377. doi: https://doi.org/10.1006/jfca.2002.1084.

Rubilar, Mónica, Eduardo Morales, Roberto Sáez, Francisca Acevedo, Barbara Palma, Mario Villarroel, and Carolina Shene. 2012. "Polyphenolic fractions improve the oxidative stability of microencapsulated linseed oil." *European Journal of Lipid Science and Technology* 114 (7):760-771. doi: https://doi.org/10.1002/ejlt.201100230.

Ryan, E., K. Galvin, T. P. O'Connor, A. R. Maguire, and N. M. O'Brien. 2007. "Phytosterol, Squalene, Tocopherol Content and Fatty Acid Profile of Selected Seeds, Grains, and Legumes." *Plant Foods for Human Nutrition* 62 (3):85-91. doi: 10.1007/s11130-007-0046-8.

Sabu, M. C., K. Smitha, and K. Ramadasan. 2002. "Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental

diabetes." *Journal of Ethnopharmacology* 83 (1):109-116. doi: https://doi.org/10.1016/S0378-8741(02)00217-9.

Saha, Supradip, Gyanendra Singh, V. Mahajan, and H. S. Gupta. 2009. "Variability of Nutritional and Cooking Quality in Bean (*Phaseolus vulgaris* L) as a Function of Genotype." *Plant Foods for Human Nutrition* 64 (2):174-180. doi: 10.1007/s11130-009-0121-4.

Sallas, L., E. M. Luomala, J. Ultriainen, P. Kainulainen, and J. K. Holopainen. 2003. "Contrasting effects of elevated carbon dioxide concentration and temperature on Rubisco activity, chlorophyll fluorescence, needle ultrastructure and secondary metabolites in conifer seedlings." *Tree Physiology* 23 (2):97-108. doi: 10.1093/treephys/23.2.97.

Sánchez-Moreno, C., L. Plaza, B. De Ancos, and M. P. Cano. 2006. "Impact of high-pressure and traditional thermal processing of tomato purée on carotenoids, vitamin C and antioxidant activity." *Journal of the Science of Food and Agriculture* 86 (2):171-179. doi: 10.1002/jsfa.2321.

Sánchez-Vioque, R., A. Clemente, J. Vioque, J. Bautista, and F. Millán. 1999. "Protein isolates from chickpea (*Cicer arietinum* L.): chemical composition, functional properties and protein characterization." *Food Chemistry* 64 (2):237-243. doi: http://dx.doi.org/10.1016/S0308-8146(98)00133-2.

Sandberg, A. S. 2002. "Bioavailability of minerals in legumes." *British Journal of Nutrition* 88 Suppl 3:S281-5. doi: 10.1079/bjn/2002718.

Sang, Yijun, and Paul A. Seib. 2006. "Resistant starches from amylose mutants of corn by simultaneous heat-moisture treatment and phosphorylation." *Carbohydrate Polymers* 63 (2):167-175. doi: https://doi.org/10.1016/j.carbpol.2005.07.022.

Saura-Calixto, Fulgencio, Alejandra García-Alonso, Isabel Goñi, and Laura Bravo. 2000. "In Vitro Determination of the Indigestible Fraction in Foods: An Alternative to Dietary Fiber Analysis." *Journal of Agricultural and Food Chemistry* 48 (8):3342-3347. doi: 10.1021/jf0000373.

Scalbert, Augustin, Ian T Johnson, and Mike Saltmarsh. 2005. "Polyphenols: antioxidants and beyond." *The American Journal of Clinical Nutrition* 81 (1):215S-217S. doi: 10.1093/ajcn/81.1.215S.

Schlesier, K., M. Harwat, V. Böhm, and R. Bitsch. 2002. "Assessment of Antioxidant Activity by Using Different In Vitro Methods." *Free Radical Research* 36 (2):177-187. doi: 10.1080/10715760290006411.

Sefadedeh, S., and D. W. Stanley. 1979. "Textural Implications of the Microstructure of Legumes." *Food Technology* 33 (10):77-83.

Segev, Aharon, Hana Badani, Yoram Kapulnik, Ilan Shomer, Michal Oren-Shamir, and Shmuel Galili. 2010. "Determination of Polyphenols, Flavonoids, and

Antioxidant Capacity in Colored Chickpea (*Cicer arietinum* L.)." *Journal of Food Science* 75 (2):S115-S119. doi: 10.1111/j.1750-3841.2009.01477.x.

Sfayhi, D., and M. Kharrat. 2011. "Physicochemical and cookin characteristics of tunisian chickpea varieties " *Food and Chemical Toxicology* (Special Isssue 1):68-72.

Sharma, Alka, Baljeet Singh Yadav, and Ritika. 2008. "Resistant Starch: Physiological Roles and Food Applications." *Food Reviews International* 24 (2):193-234. doi: 10.1080/87559120801926237.

Shomer, Ilan. 1995. "Swelling behaviour of cell wall and starch in potato (*Solanum tuberosum* L.) tuber cells — I. Starch leakage and structure of single cells." *Carbohydrate Polymers* 26 (1):47-54. doi: https://doi.org/10.1016/0144-8617(95)98834-4.

Siddique, K.H.M., and K.L. Regan. 2005. "Registration of 'Kimberley Large' Kabuli Chickpea." *Crop Science* 45 (4):1659-1660. doi: https://doi.org/10.2135/cropsci2004.0405.

Silva-Cristobal, L., P. Osorio-Díaz, J. Tovar, and L. A. Bello-Pérez. 2010. "Chemical composition, carbohydrate digestibility, and antioxidant capacity of cooked black bean, chickpea, and lentil Mexican varieties Composición química, digestibilidad de carbohidratos, y capacidad antioxidante de variedades mexicanas cocidas de frijol negro, garbanzo, y lenteja." *CyTA - Journal of Food* 8 (1):7-14. doi: 10.1080/19476330903119218.

Silva, Jerson L., Debora Foguel, and Catherine A. Royer. 2001. "Pressure provides new insights into protein folding, dynamics and structure." *Trends in Biochemical Sciences* 26 (10):612-618. doi: https://doi.org/10.1016/S0968-0004(01)01949-1.

Silva, K. B., A. T. Bruzi, E. V. Zambiazzi, I. O. Soares, Jlar Pereira, and M. L. M. Carvalho. 2017. "Adaptability and stability of soybean cultivars for grain yield and seed quality." *Genet Mol Res* 16 (2). doi: 10.4238/gmr16029646.

Singh, Akanksha, Akansha Jain, Birinchi K. Sarma, Ram S. Upadhyay, and Harikesh B. Singh. 2014. "Beneficial compatible microbes enhance antioxidants in chickpea edible parts through synergistic interactions." *LWT - Food Science and Technology* 56 (2):390-397. doi: https://doi.org/10.1016/j.lwt.2013.11.030.

Singh, Balwinder, Jatinder Pal Singh, Khetan Shevkani, Narpinder Singh, and Amritpal Kaur. 2017. "Bioactive constituents in pulses and their health benefits." *Journal of Food Science and Technology* 54 (4):858-870. doi: 10.1007/s13197-016-2391-9.

Singh, Jaspreet, Anne Dartois, and Lovedeep Kaur. 2010. "Starch digestibility in food matrix: a review." *Trends in Food Science & Technology* 21 (4):168-180. doi: https://doi.org/10.1016/j.tifs.2009.12.001.

Singh, K B, P C Williams, and H Nakkoul. 1990. "Influence of growing season, location and planting time on some quality parameters of kabuli chickpea." *Journal of the Science of Food and Agriculture* 53 (4):429-441. doi: 10.1002/jsfa.2740530402.

Singh, K. B. 1997. "Chickpea (*Cicer arietinum* L.)." *Field Crops Research* 53 (1):161-170. doi: https://doi.org/10.1016/S0378-4290(97)00029-4.

Singh, O. P., H. S. Yadava, and S. C. Agrawal. 2003. "Divergence analysis for quality traits in chickpea." *Indian Journal of Pulse Research* 1:12-13.

Singh, Pramod, Nidhi Srivastava, Bechan Sharma, and Sameer Bhagyawant. 2015. "Effect of Domestic Processes on Chickpea Seeds for Antinutritional Contents and Their Divergence." 3:111-117. doi: 10.12691/ajfst-3-4-3.

Singh, U. 2001. *Functional properties of grain legume flours*. Vol. 38.

Singh, U., J. Kumar, and C. L. L. Gowda. 1993. "The protein content of chickpea (*Cicer arietinum* L.) grown at different locations." *Plant Foods for Human Nutrition* 32 (2):179-184. doi: 10.1007/bf01091338.

Singh, Umaid, Nukala Subrahmanyam, and Jagdish Kumar. 1991. "Cooking quality and nutritional attributes of some nely developed cultivars of chickpea (*Cicer arietinum*)." *Journal of the Science of Food and Agriculture* 55 (1):37-46. doi: 10.1002/jsfa.2740550106.

Smith, Theresa J. 2000. "Squalene: potential chemopreventive agent." *Expert Opinion on Investigational Drugs* 9 (8):1841-1848. doi: 10.1517/13543784.9.8.1841.

Sofi, Sajad Ahmad, Khalid Muzaffar, Shafia Ashraf, Isha Gupta, and Shabir Ahmad Mir. 2020. "Chickpea." In *Pulses: Processing and Product Development*, edited by A. Manickavasagan and Praveena Thirunathan, 55-76. Cham: Springer International Publishing.

Sofi, Sajad Ahmad, Jagmohan Singh, Navnidhi Chhikara, and Anil Panghal. 2020. "Effect of incorporation of germinated flour and protein isolate from chickpea on different quality characteristics of rice-based noodle." *Cereal Chemistry* 97 (1):85-94. doi: https://doi.org/10.1002/cche.10192.

Song, Y., M. Liu, F. G. Yang, L. H. Cui, X. Y. Lu, and C. Chen. 2015. "Dietary fibre and the risk of colorectal cancer: a case- control study." *Asian Pacific Journal on Cancer Prevention* 16 (9):3747-52. doi: 10.7314/apjcp.2015.16.9.3747.

Sood, B. C., S. K. Bhambota, and S. L. Gartan. 2001. "Studies on genotype x environment interaction and stability in chickpea." *Indian Journal of Pulses Research.* 14 (1):31-33.

Sopade, Peter A., and Michael J. Gidley. 2009. "A Rapid In-vitro Digestibility Assay Based on Glucometry for Investigating Kinetics of Starch Digestion." *Starch - Stärke* 61 (5):245-255. doi: 10.1002/star.200800102.

Sosulski, F. W., and H. M. Gadan. 1988. "Variations in lipid composition among chickpea cultivars." *Journal of the American Oil Chemists' Society* 65 (3):369-372. doi: 10.1007/bf02663079.

Srichuwong, Sathaporn, Titi Candra Sunarti, Takashi Mishima, Naoto Isono, and Makoto Hisamatsu. 2005. "Starches from different botanical sources II: Contribution of starch structure to swelling and pasting properties." *Carbohydrate Polymers* 62 (1):25-34. doi: https://doi.org/10.1016/j.carbpol.2005.07.003.

Stolt, M., S. Oinonen, and K. Autio. 2000. "Effect of high pressure on the physical properties of barley starch." *Innovative Food Science & Emerging Technologies* 1 (3):167-175. doi: https://doi.org/10.1016/S1466-8564(00)00017-5.

Subedi, Maya, Hamid Khazaei, Gene Arganosa, Emediong Etukudo, and Albert Vandenberg. 2021. "Genetic stability and genotype × environment interaction analysis for seed protein content and protein yield of lentil." *Crop Science* 61 (1):342-356. doi: https://doi.org/10.1002/csc2.20282.

Summo, Carmine, Davide De Angelis, Luigi Ricciardi, Francesco Caponio, Concetta Lotti, Stefano Pavan, and Antonella Pasqualone. 2019. "Nutritional, physico-chemical and functional characterization of a global chickpea collection." *Journal of Food Composition and Analysis* 84:103306. doi: https://doi.org/10.1016/j.jfca.2019.103306.

Summo, Carmine, Davide De Angelis, Isabelle Rochette, Claire Mouquet-Rivier, and Antonella Pasqualone. 2019. "Influence of the preparation process on the chemical composition and nutritional value of canned purée of kabuli and Apulian black chickpeas." *Heliyon* 5 (3):e01361. doi: https://doi.org/10.1016/j.heliyon.2019.e01361.

Sun, Xiang Dong, and Richard A. Holley. 2010. "High Hydrostatic Pressure Effects on the Texture of Meat and Meat Products." *Journal of Food Science* 75 (1):R17-R23. doi: 10.1111/j.1750-3841.2009.01449.x.

Sun, Yongkang, Zhongwei Wu, Bing Hu, Wei Wang, Hong Ye, Yi Sun, Xiaoqing Wang, and Xiaoxiong Zeng. 2014. "A new method for determining the relative crystallinity of chickpea starch by Fourier-transform infrared spectroscopy." *Carbohydrate Polymers* 108:153-158. doi: https://doi.org/10.1016/j.carbpol.2014.02.093.

Sundaram, Prity, Srinivasan Samineni, Sobhan B. Sajja, Chandan Roy, Suresh P. Singh, Priyanka Joshi, and Pooran M. Gaur. 2019. "Inheritance and relationships of flowering time and seed size in kabuli chickpea." *Euphytica* 215 (9):144. doi: 10.1007/s10681-019-2464-8.

Tahir, Mohammad, Nienke Lindeboom, Monica Båga, Albert Vandenberg, and Ravindra Chibbar. 2011. "Composition and correlation between major seed constituents in selected lentil (*Lens culinaris*. Medik) genotypes." *Canadian Journal of Plant Science* 91 (5):825-835. doi: 10.4141/cjps2011-010.

Tao, Aifen, Reza Keshavarz Afshar, Jinwen Huang, Yesuf Assen Mohammed, Matthew Espe, and Chengci Chen. 2017. "Variation in Yield, Starch, and Protein of Dry Pea Grown Across Montana." *Agronomy Journal* 109 (4):1491-1501. doi: https://doi.org/10.2134/agronj2016.07.0401.

Tarzi, Babak Ghiassi, Maryam Gharachorloo, Marzieh Baharinia, and Seyed Alireza Mortazavi. 2012. "The effect of germination on phenolic content and antioxidant activity of chickpea." *Iranian journal of pharmaceutical research : IJPR* 11 (4):1137-1143.

Tayade, Rupesh, Krishnanand P. Kulkarni, Hyun Jo, Jong Tae Song, and Jeong-Dong Lee. 2019. "Insight Into the Prospects for the Improvement of Seed Starch in Legume—A Review." *Frontiers in Plant Science* 10 (1213). doi: 10.3389/fpls.2019.01213.

Tayyar, S., C. O. Egesel, K. M. Gul, and H. Turhan. 2008. "The effect of autumn and spring planting time on seed yield and protein content of chickpea genotypes." *African Journal of Biotechnology* 7 (11):1655-1661.

Tayyar, Semun, CÖ Egesel, K. M. Gül, and Hakan Turhan. 2008. *The effect of autumn and spring planting time on seed yield and protein content of chickpea genotypes*. Vol. 7.

Tena, Esayas, Frehiwot Goshu, Hussein Mohamad, Melaku Tesfa, Diribu Tesfaye, and Abebech Seife. 2019. "Genotype × environment interaction by AMMI and GGE-biplot analysis for sugar yield in three crop cycles of sugarcane (Saccharum officinirum L.) clones in Ethiopia." *Cogent Food & Agriculture* 5 (1). doi: 10.1080/23311932.2019.1651925.

Thaipong, Kriengsak, Unaroj Boonprakob, Kevin Crosby, Luis Cisneros-Zevallos, and David Hawkins Byrne. 2006. "Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts." *Journal of Food Composition and Analysis* 19 (6):669-675. doi: http://dx.doi.org/10.1016/j.jfca.2006.01.003.

Thakur, B. R., and P. E. Nelson. 1998. "High-pressure processing and preservation of food." *Food Reviews International* 14 (4):427-447. doi: 10.1080/87559129809541171.

Thangwana, N. M., and J. B. O. Ogola. 2012. "Yield and yield components of chickpea (*Cicer arietinum*): Response to genotype and planting density in summer and winter sowings." *Journal of Food Agriculture & Environment* 10 (2):710-715.

Tharanathan, R. N., and S. Mahadevamma. 2003. "Grain legumes—a boon to human nutrition." *Trends in Food Science & Technology* 14 (12):507-518. doi: https://doi.org/10.1016/j.tifs.2003.07.002.

Thushan Sanjeewa, W. G., Janitha P. D. Wanasundara, Zeb Pietrasik, and Phyllis J. Shand. 2010. "Characterization of chickpea (*Cicer arietinum* L.) flours and application in low-fat pork bologna as a model system." *Food Research International* 43 (2):617-626. doi: https://doi.org/10.1016/j.foodres.2009.07.024.

Tian, Yaoqi, Dandan Li, Jianwei Zhao, Xueming Xu, and Zhengyu Jin. 2014. "Effect of high hydrostatic pressure (HHP) on slowly digestible properties of rice starches." *Food Chemistry* 152:225-229. doi: https://doi.org/10.1016/j.foodchem.2013.11.162.

Tilman, David, and Michael Clark. 2014. "Global diets link environmental sustainability and human health." *Nature* 515 (7528):518-522. doi: 10.1038/nature13959.

Tosh, Susan, and Sylvia Yada. 2010. *Dietary fibres in pulse seeds and fractions: Characterization, functional attributes, and applications*. Vol. 43.

Tovar, Juscelino, and Carmelo Melito. 1996. "Steam-Cooking and Dry Heating Produce Resistant Starch in Legumes." *Journal of Agricultural and Food Chemistry* 44 (9):2642-2645. doi: 10.1021/jf950824d.

Trejo Araya, Ximenita I., Marc Hendrickx, Bert E. Verlinden, Sandy Van Buggenhout, Nicholas J. Smale, Cynthia Stewart, and A. John Mawson. 2007. "Understanding texture changes of high pressure processed fresh carrots: A microstructural and biochemical approach." *Journal of Food Engineering* 80 (3):873-884. doi: https://doi.org/10.1016/j.jfoodeng.2006.08.014.

Tsai, P. J., and C. H. She. 2006. "Significance of phenol-protein interactions in modifying the antioxidant capacity of peas." *Journal of Agricultural and Food Chemistry* 54 (22):8491-8494. doi: 10.1021/jf061475y.

Tziva, M., S. O. Negro, A. Kalfagianni, and M. P. Hekkert. 2020. "Understanding the protein transition: The rise of plant-based meat substitutes." *Environmental Innovation and Societal Transitions* 35:217-231. doi: https://doi.org/10.1016/j.eist.2019.09.004.

UNICEF. 2020. "Malnutrition." UNICEF, accessed 02/03. https://data.unicef.org/topic/nutrition/malnutrition/.

Urbano, Gloria, Maria Lopez-Jurado, Javier Hernandez, Mar Fernandez, Maria-Carmen Moreu, Juana Frias, Concepcion Diaz-Pollan, Marin Prodanov, and Concepcion Vidal-Valverde. 1995. "Nutritional Assessment of Raw, Heated, and Germinated lentils." *Journal of agriculture and food chemistry* 43 (7):1871-1877. doi: 10.1021/jf00055a022.

USDA. 2017. "National Nutrient Database ". United States department of Agriculture. https://ndb.nal.usda.gov/ndb/foods/show/4795?manu=&fgcd=&ds=.

USDA. 2019. Chickpeas (garbanzo beans, bengal gram), mature seeds, raw. U.S. Department of Agriculture.

Vaezi, B., A. Pour-Aboughadareh, R. Mohammadi, M. Armion, A. Mehraban, T. Hossein-Pour, and M. Dorii. 2017. "GGE biplot and AMMI analysis of barley yield performance in Iran." *Cereal Research Communications Cereal Research Communications* 45 (3):500. doi: 10.1556/0806.45.2017.019.

van der Poel, A. F. B., S. Gravendeel, and H. Boer. 1991. "Effect of different processing methods on tannin content and in vitro protein digestibility of faba bean (Vicia faba L.)." *Animal Feed Science and Technology* 33 (1):49-58. doi: https://doi.org/10.1016/0377-8401(91)90045-T.

Vandemark, George, Samadhi Thavarajah, Niroshan Siva, and Dil Thavarajah. 2020. "Genotype and environment effects on prebiotic carbohydrate concentrations in kabuli chickpea cultivars and breeding lines grown in the U.S. Pacific Northwest." *Frontiers in Plant Science* 11 (112). doi: 10.3389/fpls.2020.00112.

Varol, Ihsan Serkan, Yusuf Murat Kardes, Hasan Ali Irik, Halil Kirnak, and Mahmut Kaplan. 2020. "Supplementary irrigations at different physiological growth stages of chickpea (*Cicer arietinum* L.) change grain nutritional composition." *Food Chemistry* 303:125402. doi: https://doi.org/10.1016/j.foodchem.2019.125402.

Vasishtha, H., and R. P. Srivastava. 2012. "Changes in lipids and fatty acids during soaking and germination of chickpea (*Cicer arietinum*)." *Indian Journal of Agricultural Biochemistry* 25 (1):14-19.

Vasishtha, Hina, Rajendra P. Srivastava, and Prasoon Verma. 2014. "Effect of dehusking and cooking on protein and dietary fibre of different genotypes of desi, kabuli and green type chickpeas (*Cicer arietinum*)." *Journal of food science and technology* 51 (12):4090-4095. doi: 10.1007/s13197-012-0909-3.

Verma, Arun K., Rituparna Banerjee, and B. D. Sharma. 2012. "Quality of Low Fat Chicken Nuggets: Effect of Sodium Chloride Replacement and Added Chickpea (*Cicer arietinum* L.) Hull Flour." *Asian-Australasian journal of animal sciences* 25 (2):291-298. doi: 10.5713/ajas.2011.11263.

Vidal-Valverde, Concepción, and Juana Frias. 1991. "Legume Processing Effects on Dietary Fiber Components." *Journal of Food Science* 56 (5):1350-1352. doi: 10.1111/j.1365-2621.1991.tb04770.x.

Vidal-Valverde, Concepción, Juana Frias, Isabel Sierra, Inmaculada Blazquez, Fernand Lambein, and Yu-Haey Kuo. 2002. "New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas." *European*

Food Research and Technology 215 (6):472-477. doi: 10.1007/s00217-002-0602-2.

Villarino, C. B. J., V. Jayasena, R. Coorey, S. Chakrabarti-Bell, R. Foley, K. Fanning, and S. K. Johnson. 2015. "The effects of lupin (*Lupinus angustifolius*) addition to wheat bread on its nutritional, phytochemical and bioactive composition and protein quality." *Food Research International* 76, Part 1:58-65. doi: http://dx.doi.org/10.1016/j.foodres.2014.11.046.

Wagner, J. R., and J. Gueguen. 1999. "Surface functional properties of native, acid-treated, and reduced soy glycinin. 2. Emulsifying properties." *Journal of Agricultural and Food Chemistry* 47 (6):2181-7.

Wallace, Taylor C., Robert Murray, and Kathleen M. Zelman. 2016. "The Nutritional Value and Health Benefits of Chickpeas and Hummus." *Nutrients* 8 (12):766. doi: 10.3390/nu8120766.

Wang, N., and J. K. Daun. 2004. "The chemical composition of and nutritive value of Canadian pulses."

Wang, N., D. W. Hatcher, R. T. Tyler, R. Toews, and E. J. Gawalko. 2010. "Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (Cicer arietinum L.)." *Food Research International* 43 (2):589-594. doi: https://doi.org/10.1016/j.foodres.2009.07.012.

Wang, Ning, James K Daun, and Linda J Malcolmson. 2003. "Relationship between physicochemical and cooking properties, and effects of cooking on antinutrients, of yellow field peas (*Pisum sativum*)." *Journal of the Science of Food and Agriculture* 83 (12):1228-1237. doi: doi:10.1002/jsfa.1531.

Wang, Ning, and James K. Daun. 2006. "Effects of variety and crude protein content on nutrients and anti-nutrients in lentils (*Lens culinaris*)." *Food Chemistry* 95 (3):493-502. doi: https://doi.org/10.1016/j.foodchem.2005.02.001.

Wang, Ning, David W. Hatcher, and Eugene J. Gawalko. 2008. "Effect of variety and processing on nutrients and certain anti-nutrients in field peas (*Pisum sativum*)." *Food Chemistry* 111 (1):132-138. doi: https://doi.org/10.1016/j.foodchem.2008.03.047.

Wang, Runfeng, Manu P. Gangola, Sarita Jaiswal, Pooran M. Gaur, Monica Båga, and Ravindra N. Chibbar. 2017. "Genotype, environment and their interaction influence seed quality traits in chickpea (*Cicer arietinum* L.)." *Journal of Food Composition and Analysis* 63 (Supplement C):21-27. doi: https://doi.org/10.1016/j.jfca.2017.07.025.

Wang, Shiow Y., and Wei Zheng. 2001. "Effect of Plant Growth Temperature on Antioxidant Capacity in Strawberry." *Journal of Agricultural and Food Chemistry* 49 (10):4977-4982. doi: 10.1021/jf0106244.

Wang, Shujun, Shaokang Wang, Lu Liu, Shuo Wang, and Les Copeland. 2017. "Structural Orders of Wheat Starch Do Not Determine the In Vitro Enzymatic Digestibility." *Journal of Agricultural and Food Chemistry* 65 (8):1697-1706. doi: 10.1021/acs.jafc.6b04044.

Wang, Tao, Fuli He, and Guibing Chen. 2014. "Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: A concise review." *Journal of Functional Foods* 7:101-111. doi: https://doi.org/10.1016/j.jff.2014.01.033.

Wang, X., W. Gao, J. Zhang, H. Zhang, J. Li, X. He, and H. Ma. 2010. "Subunit, amino acid composition and in vitro digestibility of protein isolates from Chinese kabuli and desi chickpea (*Cicer arietinum* L.) cultivars." *Food Research International* 43 (2):567-572. doi: http://dx.doi.org/10.1016/j.foodres.2009.07.018.

Wani, Idrees A., Dalbir S. Sogi, and Balmeet S. Gill. 2013. "Physical and cooking characteristics of black gram (*Phaseolus mungoo* L.) cultivars grown in India." *International Journal of Food Science & Technology* 48 (12):2557-2563. doi: 10.1111/ijfs.12249.

Wani, Idrees Ahmed, Dalbir Singh Sogi, and Balmeet Singh Gill. 2015. "Physico-chemical properties of acetylated starches from Indian black gram (*Phaseolus mungo* L.) cultivars." *Journal of food science and technology* 52 (7):4078-4089. doi: 10.1007/s13197-014-1480-x.

Warren, Frederick J., Michael J. Gidley, and Bernadine M. Flanagan. 2016. "Infrared spectroscopy as a tool to characterise starch ordered structure—a joint FTIR–ATR, NMR, XRD and DSC study." *Carbohydrate Polymers* 139:35-42. doi: https://doi.org/10.1016/j.carbpol.2015.11.066.

Wennberg, M., and M. Nyman. 2004. "On the possibility of using high pressure treatment to modify physico-chemical properties of dietary fibre in white cabbage (*Brassica oleracea* var. capitata)." *Innovative Food Science & Emerging Technologies* 5 (2):171-177. doi: https://doi.org/10.1016/j.ifset.2004.02.002.

Whybro, A., H. Jagger, M. Barker, and R. Eastell. 1998. "Phosphate supplementation in young men: lack of effect on calcium homeostasis and bone turnover." *Eur Journal of Clinical Nutrition* 52 (1):29-33. doi: 10.1038/sj.ejcn.1600508.

Williams, Philip C., Nakoul, Hanni, Singh, K. B. 1983. "Relationship between cooking time and some physical characteristics in chickpeas (*Cicer arietinum* L.)." *Journal of the Science of Food and Agriculture* 34 (5):492-496. doi: 10.1002/jsfa.2740340510.

Wollgast, Jan, and Elke Anklam. 2000. "Polyphenols in chocolate: is there a contribution to human health?" *Food Research International* 33 (6):449-459. doi: https://doi.org/10.1016/S0963-9969(00)00069-7.

Wood, J. A., and M. A. Grusak. 2007. *Nutritional values of chickpea*. Edited by S. S. Yadav, R Reeden, W. Chen and B. Sharma, *Chickpea breeding & management*. Waalingford, UK: CAB Int.

Wu, Gangcheng, Stuart K. Johnson, Janet F. Bornman, Sarita J. Bennett, Michael W. Clarke, Vijaya Singh, and Zhongxiang Fang. 2016. "Growth temperature and genotype both play important roles in sorghum grain phenolic composition." *Scientific Reports* 6 (1):21835. doi: 10.1038/srep21835.

Wu, Gangcheng, Stuart K. Johnson, Janet F. Bornman, Sarita J. Bennett, and Zhongxiang Fang. 2017. "Changes in whole grain polyphenols and antioxidant activity of six sorghum genotypes under different irrigation treatments." *Food Chemistry* 214:199-207. doi: https://doi.org/10.1016/j.foodchem.2016.07.089.

Wu, Gangcheng, Stuart K. Johnson, Janet F. Bornman, Sarita J. Bennett, Vijaya Singh, Azra Simic, and Zhongxiang Fang. 2016. "Effects of Genotype and Growth Temperature on the Contents of Tannin, Phytate and In Vitro Iron Availability of Sorghum Grains." *PLOS ONE* 11 (2):e0148712. doi: 10.1371/journal.pone.0148712.

Wu, Guoyao. 2009. "Amino acids: metabolism, functions, and nutrition." *Amino Acids* 37 (1):1-17. doi: 10.1007/s00726-009-0269-0.

Wu, Guoyao. 2016. "Dietary protein intake and human health." *Food & Function* 7 (3):1251-1265. doi: 10.1039/C5FO01530H.

Wu, Z., L. Song, S. Feng, Y. Liu, G. He, Y. Yioe, S. Q. Liu, and D. Huang. 2012. "Germination dramatically increases isoflavonoid content and diversity in chickpea (*Cicer arietinum* L.) seeds." *J Agric Food Chem* 60 (35):8606-15. doi: 10.1021/jf3021514.

Xia, Qiang, Liping Wang, Congcong Xu, Jun Mei, and Yunfei Li. 2017. "Effects of germination and high hydrostatic pressure processing on mineral elements, amino acids and antioxidants in vitro bioaccessibility, as well as starch digestibility in brown rice (*Oryza sativa* L.)." *Food Chemistry* 214:533-542. doi: https://doi.org/10.1016/j.foodchem.2016.07.114.

Xiao, J. B., and P. Hogger. 2015. "Dietary Polyphenols and Type 2 Diabetes: Current Insights and Future Perspectives." *Current Medicinal Chemistry* 22 (1):23-38.

Xiong, Jian, Qingyong Li, Zhenxing Shi, and Jun Ye. 2017. "Interactions between wheat starch and cellulose derivatives in short-term retrogradation: Rheology and FTIR study." *Food Research International* 100:858-863. doi: https://doi.org/10.1016/j.foodres.2017.07.061.

Xiong, Weiyan, Bin Zhang, Qiang Huang, Chao Li, Elizabeth A. Pletsch, and Xiong Fu. 2018. "Variation in the rate and extent of starch digestion is not determined by the starch structural features of cooked whole pulses." *Food Hydrocolloids* 83:340-347. doi: https://doi.org/10.1016/j.foodhyd.2018.05.022.

Xu, B.J., S.H. Yuan, and S.K.C. Chang. 2007. "Comparative Analyses of Phenolic Composition, Antioxidant Capacity, and Color of Cool Season Legumes and Other Selected Food Legumes." *Journal of Food Science* 72 (2):S167-S177. doi: https://doi.org/10.1111/j.1750-3841.2006.00261.x.

Xu, Baojun, and Sam K. C. Chang. 2008. "Effect of soaking, boiling, and steaming on total phenolic contentand antioxidant activities of cool season food legumes." *Food Chemistry* 110 (1):1-13. doi: https://doi.org/10.1016/j.foodchem.2008.01.045.

Xu, Yixiang, Arrieyana Cartier, Mary Obielodan, Krystle Jordan, Teara Hairston, Anna Shannon, and Edward Sismour. 2016. "Nutritional and anti-nutritional composition, and in vitro protein digestibility of Kabuli chickpea (*Cicer arietinum* L.) as affected by differential processing methods." *Journal of Food Measurement and Characterization* 10 (3):625-633. doi: 10.1007/s11694-016-9346-8.

Xu, Yixiang, Edward N. Sismour, Satya S. Narina, Donnica Dean, Harbans L. Bhardwaj, and Zhenxing Li. 2013. "Composition and properties of starches from Virginia-grown kabuli chickpea (*Cicer arietinum* L.) cultivars." *International Journal of Food Science & Technology* 48 (3):539-547. doi: doi:10.1111/j.1365-2621.2012.03217.x.

Xue, Zhaohui, Haichao Wen, Lijuan Zhai, Yanqing Yu, Yanni Li, Wancong Yu, Aiqing Cheng, Cen Wang, and Xiaohong Kou. 2015. "Antioxidant activity and anti-proliferative effect of a bioactive peptide from chickpea (*Cicer arietinum* L.)." *Food Research International* 77:75-81. doi: https://doi.org/10.1016/j.foodres.2015.09.027.

Yadav, Ritika B., Baljeet S. Yadav, and Nisha Dhull. 2012. "Effect of incorporation of plantain and chickpea flours on the quality characteristics of biscuits." *Journal of Food Science and Technology* 49 (2):207-213. doi: 10.1007/s13197-011-0271-x.

Yadav, Shyam S., A. K. Verma, A. H. Rizvi, D. Singh, J. Kumar, and Mitchell Andrews. 2010. "Impact of genotype × environment interactions on the relative performance of diverse groups of chickpea (*Cicer arietinum* L.) cultivars." *Archives of Agronomy and Soil Science* 56 (1):49-64. doi: 10.1080/03650340902922688.

Yalçın, İ, and C. Özarslan. 2004. "Physical Properties of Vetch Seed." *Biosystems Engineering* 88 (4):507-512. doi: https://doi.org/10.1016/j.biosystemseng.2004.04.011.

Yan, WeiKai. 2011. "GGE biplot vs. AMMI graphs for genotype-by-environment data analysis." *Journal of the Indian Society of Agricultural Statistics* 65 (2):181-193.

Yan, Weikai, Paul L. Cornelius, Jose Crossa, and L.A. Hunt. 2001. "Two Types of GGE Biplots for Analyzing Multi-Environment Trial Data." *Crop Science* 41 (3):656-663. doi: https://doi.org/10.2135/cropsci2001.413656x.

Yan, Weikai, and Istvan Rajcan. 2002. "Biplot Analysis of Test Sites and Trait Relations of Soybean in Ontario." *Crop Science* 42 (1):11-20. doi: https://doi.org/10.2135/cropsci2002.1100.

Yan, Zhaoming, Yinzhao Zhong, Yehui Duan, Qinghua Chen, and Fengna Li. 2020. "Antioxidant mechanism of tea polyphenols and its impact on health benefits." *Animal Nutrition* 6 (2):115-123. doi: https://doi.org/10.1016/j.aninu.2020.01.001.

Yildirim, M., C. Barutcular, M. Koc, H. Dizlek, A. Hossain, M. S. Islam, I. Toptas, F. Basdemir, O. Albayrak, C. Akinci, and A. El Sabagh. 2018. "Assessment of the Grain Quality of Wheat Genotypes Grown under Multiple Environments Using Gge Biplot Analysis." *Fresenius Environmental Bulletin* 27 (7):4830-4837.

Yniestra Marure, Lucero M., M. Carmen Núñez-Santiago, Edith Agama-Acevedo, and Luis A. Bello-Perez. 2019. "Starch Characterization of Improved Chickpea Varieties Grown in Mexico." *Starch - Stärke* 71 (3-4):1800139. doi: 10.1002/star.201800139.

Yordanov, D. G., and G. V. Angelova. 2010. "High Pressure Processing for Foods Preserving." *Biotechnology & Biotechnological Equipment* 24 (3):1940-1945. doi: 10.2478/V10133-010-0057-8.

Yu, Y., F. Pan, H. S. Ramaswamy, S. Zhu, L. Yu, and Q. Zhang. 2017a. "Effect of soaking and single/two cycle high pressure treatment on water absorption, color, morphology and cooked texture of brown rice." *J Food Sci Technol* 54 (6):1655-1664. doi: 10.1007/s13197-017-2598-4.

Yu, Yong, Fang Pan, H. S. Ramaswamy, Songming Zhu, Liang Yu, and Qiuting Zhang. 2017b. "Effect of soaking and single/two cycle high pressure treatment on water absorption, color, morphology and cooked texture of brown rice." *Journal of Food Science and Technology* 54 (6):1655-1664. doi: 10.1007/s13197-017-2598-4.

Yust, Marı, amp, x, M. a, Justo Pedroche, Julio Girón-Calle, Manuel Alaiz, Francisco Millán, and Javier Vioque. 2003. "Production of ace inhibitory peptides by digestion of chickpea legumin with alcalase." *Food Chemistry* 81 (3):363-369. doi: http://dx.doi.org/10.1016/S0308-8146(02)00431-4.

Yust, María del Mar, María del Carmen Millán-Linares, Juan María Alcaide-Hidalgo, Francisco Millán, and Justo Pedroche. 2012. "Hypocholesterolaemic and antioxidant activities of chickpea (*Cicer arietinum* L.) protein hydrolysates." *Journal of the Science of Food and Agriculture* 92 (9):1994-2001. doi: 10.1002/jsfa.5573.

Zavareze, Elessandra da Rosa, and Alvaro Renato Guerra Dias. 2011. "Impact of heat-moisture treatment and annealing in starches: A review." *Carbohydrate Polymers* 83 (2):317-328. doi: https://doi.org/10.1016/j.carbpol.2010.08.064.

Zewdu, A. D., and W. K. Solomon. 2007. "Moisture-Dependent Physical Properties of Tef Seed." *Biosystems Engineering* 96 (1):57-63. doi: https://doi.org/10.1016/j.biosystemseng.2006.09.008.

Zhang, Genyi, Zihua Ao, and Bruce R. Hamaker. 2006. "Slow Digestion Property of Native Cereal Starches." *Biomacromolecules* 7 (11):3252-3258. doi: 10.1021/bm060342i.

Zhang, H., M. Pala, T. Oweis, and H. Harris. 2000. "Water use and water-use efficiency of chickpea and lentil in a Mediterranean environment." *Australian Journal of Agricultural Research* 51 (2):295-304. doi: https://doi.org/10.1071/AR99059.

Zhang, Haiyan, Thava Vasanthan, and Mahinda Wettasinghe. 2007. "Enrichment of Tocopherols and Phytosterols in Canola Oil during Seed Germination." *Journal of Agricultural and Food Chemistry* 55 (2):355-359. doi: 10.1021/jf060940o.

Zhang, Hua, and Rong Tsao. 2016. "Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects." *Current Opinion in Food Science* 8:33-42. doi: https://doi.org/10.1016/j.cofs.2016.02.002.

Zhang, Zuosheng, Xiaolin Tian, Peng Wang, Hao Jiang, and Wenhao Li. 2019. "Compositional, morphological, and physicochemical properties of starches from red adzuki bean, chickpea, faba bean, and baiyue bean grown in China." *Food Science & Nutrition* 7 (8):2485-2494. doi: http://dx.doi.org/10.1002/fsn3.865.

Zhong, Liezhou, Hayder Ali, Zhongxiang Fang, Mark L. Wahlqvist, Jonathan M. Hodgson, and Stuart K. Johnson. 2020. "Lupin seed coat as a promising food ingredient: physicochemical, nutritional, antioxidant properties, and effect of genotype and environment." *International Journal of Food Science & Technology* 55 (4):1816-1824. doi: https://doi.org/10.1111/ijfs.14460.

Zhou, Zhongkai, Xiaochong Ren, Fang Wang, Jing Li, Xu Si, Ruge Cao, Rui Yang, Padraig Strappe, and Chris Blanchard. 2015. "High pressure processing manipulated buckwheat antioxidant activity, anti-adipogenic properties and starch digestibility." *Journal of Cereal Science* 66:31-36. doi: https://doi.org/10.1016/j.jcs.2015.09.002.

Zia-ul-Haq, M., S. Ahmad, M. Ahmad, S. Iqbal, and K. M. Khawar. 2009. "Effects of Cultivar and Row Spacing on Tocopherol and Sterol Composition of Chickpea (*Cicer arietinum* L) Seed Oil." *Tarim Bilimleri Dergisi-Journal of Agricultural Sciences* 15 (1):25-30.

Zia-Ul-Haq, Muhammad, Shahid Iqbal, Shakeel Ahmad, Muhammad Imran, Abdul Niaz, and M. I. Bhanger. 2007. "Nutritional and compositional study of Desi chickpea (*Cicer arietinum* L.) cultivars grown in Punjab, Pakistan." *Food Chemistry* 105 (4):1357-1363. doi: http://dx.doi.org/10.1016/j.foodchem.2007.05.004.

Zulueta, Ana, Maria J. Esteve, and Ana Frígola. 2009. "ORAC and TEAC assays comparison to measure the antioxidant capacity of food products." *Food Chemistry* 114 (1):310-316. doi: https://doi.org/10.1016/j.foodchem.2008.09.033.

APPENDICES

Appendix 1 Supplemental Materials

Table S 1. ANOVA for genotype x environment interaction for protein content

Location/ variety	Mean protein content (g/ 100 g, db)
Rainbow Kalkee	24.34a
Birchip Kalkee	24.2ab
Birchip Genesis 090	24.18ab
Rainbow PBA Monarch	23.64abc
Birchip Almaz	23.61abc
Rainbow Almaz	23.41abcd
Rainbow Genesis 090	23.37abcd
Birchip PBA Monarch	22.36bcde
Tarranyurk Genesis 090	21.99cdef
Tarranyurk PBA Monarch	21.55defg
Tarranyurk Almaz	21.01efgh
Kununurra Almaz	20.48fghi
Tarranyurk Kalkee	20.48fghi
Kununurra Genesis 090	20.06ghij
Walgett Kalkee	19.22hij
Kununurra Kalkee	19.05ij
Walgett Almaz	18.86ij
Walgett Genesis 090	18.79ij
Kununurra PBA Monarch	18.39j
Walgett PBA Monarch	18.23j

Data presented as mean. Means followed by the same alphabet in the column does not differ significantly ($p \leq 0.05$) following Tukey's post hoc test.

Table S 2. ANOVA for genotype x environment interaction for fat content

Location/ variety	Mean fat content (g/ 100 g, db)
Tarranyurk Kalkee	5.59a
Kununurra PBA Monarch	5.577a
Walgett PBA Monarch	5.552a
Birchip Almaz	5.493a
Walgett Kalkee	5.463a
Walgett Genesis 090	5.405ab
Tarranyurk Genesis 090	5.39ab
Tarranyurk PBA Monarch	5.363ab
Kununurra Kalkee	5.207abc
Birchip Genesis 090	5.123abc
Birchip PBA Monarch	5.035abc
Rainbow Genesis 090	4.999abc
Tarranyurk Almaz	4.923abcd
Rainbow Almaz	4.757bcde
Kununurra Almaz	4.559cde
Rainbow Kalkee	4.266de
Kununurra Genesis 090	4.25de
Rainbow PBA Monarch	4.192e
Birchip Kalkee	4.149e
Walgett Almaz	4.093e

Data presented as mean. Means followed by the same alphabet in the column does not differ significantly ($p \leq 0.05$) following Tukey's post hoc test.

Table S 3. ANOVA for genotype x environment interaction for ash content

Location/ variety	Mean ash content (g/ 100 g, db)
Kununurra Almaz	3.13a
Kununurra Genesis 090	3.086ab
Kununurra Kalkee	3.038ab
Kununurra PBA Monarch	3.025bc
Rainbow PBA Monarch	2.987bc
Rainbow Kalkee	2.932cd
Rainbow Almaz	2.863d
Rainbow Genesis 090	2.847d
Walgett Almaz	2.695e
Walgett PBA Monarch	2.647ef
Tarranyurk Almaz	2.572fg
Tarranyurk PBA Monarch	2.555fgh
Tarranyurk Kalkee	2.55fgh
Walgett Kalkee	2.511ghi
Walgett Genesis 090	2.465hi
Tarranyurk Genesis 090	2.464hi
Birchip PBA Monarch	2.426i
Birchip Almaz	2.284j
Birchip Kalkee	2.191jk
Birchip Genesis 090	2.175k

Data presented as mean. Means followed by the same alphabet in the column does not differ significantly ($p \leq 0.05$) following Tukey's post hoc test.

Table S 4. ANOVA for genotype x environment interaction for starch content

Location/ variety	Mean starch content (g/ 100 g, db)
Kununurra Genesis 090	31.69a
Walgett Kalkee	28.76ab
Kununurra PBA Monarch	28.16ab
Walgett PBA Monarch	27.78abc
Kununurra Kalkee	27.52abc
Kununurra Almaz	27.49abc
Birchip Almaz	26.4bcd
Walgett Genesis 090	26.11bcd
Birchip PBA Monarch	25.01bcde
Birchip Genesis 090	24.48bcde
Tarranyurk PBA Monarch	23.1cde
Tarranyurk Genesis 090	22.54de
Tarranyurk Almaz	22.44de
Tarranyurk Kalkee	21.14e
Walgett Almaz	20.45e
Rainbow Almaz	15.54f
Birchip Kalkee	15.37f
Rainbow PBA Monarch	14.51f
Rainbow Genesis 090	13.82f
Rainbow Kalkee	12.43f

Data presented as mean. Means followed by the same alphabet in the column does not differ significantly ($p \leq 0.05$) following Tukey's post hoc test.

Table S 5. ANOVA for genotype x environment interaction for total polyphenol content

Location/ variety	Mean total polyphenol content (mg GAE / 100 g sample, db)
Walgett Genesis 090	73.95a
Walgett Almaz	71.95ab
Kununurra Genesis 090	71.11abc
Tarranyurk Kalkee	70.03abcd
Kununurra PBA Monarch	66.14abcde
Walgett Kalkee	65.68bcde
Kununurra Kalkee	64.95bcde
Kununurra Almaz	64.45bcde
Rainbow Kalkee	64.14bcdef
Tarranyurk Genesis 090	63.64cdef
Rainbow Genesis 090	62.44defg
Tarranyurk Almaz	61.01efg
Walgett PBA Monarch	56.41fgh
Rainbow Almaz	54.77ghi
Tarranyurk PBA Monarch	52.45hij
Rainbow PBA Monarch	51.98hij
Birchip Almaz	46.8ijk
Birchip PBA Monarch	45.95jkl
Birchip Genesis 090	43.26kl
Birchip Kalkee	38.48l

Data presented as mean. Means followed by the same alphabet in the column does not differ significantly ($p \leq 0.05$) following Tukey's post hoc test.

Table S 6. ANOVA for genotype x environment interaction for oxygen radical antioxidant capacity (ORAC)

Location/ variety	Mean ORAC (µmol TE/ 100 g sample, db)
Walgett Kalkee	644.7a
Walgett Genesis 090	622.8ab
Kununurra Kalkee	611.9abc
Kununurra Genesis 090	584.7abcd
Kununurra PBA Monarch	561bcde
Walgett Almaz	557.9cdef
Kununurra Almaz	523.1defg
Tarranyurk Kalkee	510.3efg
Tarranyurk Genesis 090	509.4efg
Walgett PBA Monarch	506.6efg
Rainbow Kalkee	495.4fg
Rainbow Genesis 090	491.1g
Rainbow Almaz	422.3h
Tarranyurk Almaz	405.5h
Tarranyurk PBA Monarch	376hi
Rainbow PBA Monarch	329.1ij
Birchip Kalkee	321.7ij
Birchip Almaz	307.4jk
Birchip Genesis 090	285.2jk
Birchip PBA Monarch	246.4k

Data presented as mean. Means followed by the same alphabet in the column does not differ significantly ($p \leq 0.05$) following Tukey's post hoc test.

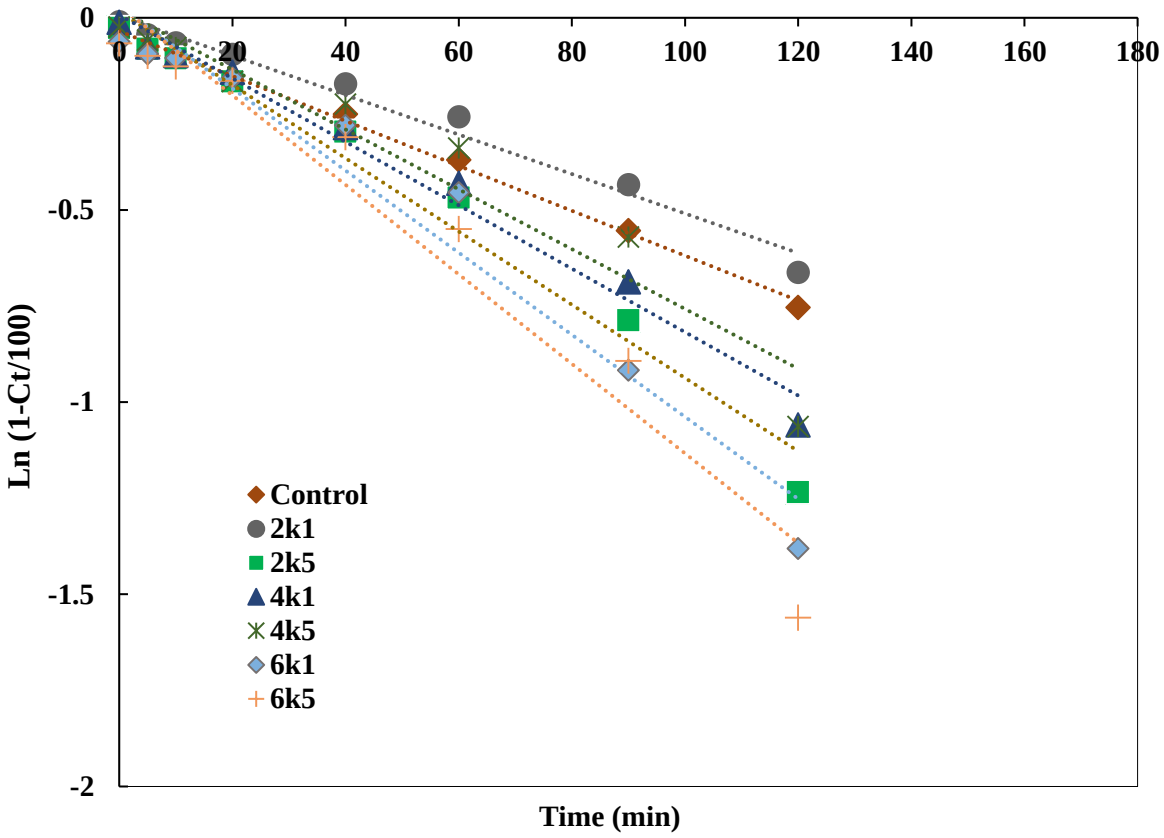

Fig S 1. Al Rabadi's First order fittings of data for starch hydrolysis in cooked and high pressure processed chickpeas.

Note: The treated chickpea samples follow the first order kinetics, as seen in good fitting (R^2 >0 .9) in the first order model.

Table S 7. Calculated digestion coefficient rate (k) values for control and HPP chickpea samples (Al-Rabadi, Gilbert, and Gidley 2009)

Sample	**k*10^{-3}**	**R^2**
Control	5.8	0.997
2k1	5.1	0.980
2k5	9.5	0.974
4k1	8.3	0.983
4k5	7.8	0.935
6k1	10.7	0.955
6k5	11.6	0.947

(Where 2K1= 200 MPa, 1min; 2K5= 200 MPa, 5 min; 4K1= 400 MPa, 1 min; 4K5= 400 MPa, 5 min; 6k1= 600 MPa, 1 min; and, 6K5 = 600 MPa, 5min)