Instituto Tecnológico y de Estudios Superiores de Monterrey

Campus Monterrey

School of Engineering and Sciences

Nutraceutical properties of isolated starch, phytochemical compounds and bioactive peptides from pigmented chickpea cultivars influenced by cooking or germination process.

A dissertation presented by

MSc. Ada Keila Milán Noris.

Submitted to the
School of Engineering and Sciences
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Engineering Sciences
Major in Biotechnology

Monterrey Nuevo León, December 14th, 2017

Dedication

A mi familia con todo mi amor.

A padres por su apoyo incondicional y su ejemplo tanto profesional como personal.
A mis hermanas por sus palabras de animo y estar siempre presente a pesar de la distancia.
A mis sobrinitos por su cariño que me hace muy feliz.

Acknowledgements

Gracias al CONACYT por la beca de mi estudios doctorales (233598) y beca mixta para la realización de mi estancia doctoral en el ICTAN-CSIC en Madrid, España. Al Tecnológico de Monterrey por el apoyo económico durante mi estudios doctorales. Al grupo NutriOmics por financiar mi trabajo doctoral y por el apoyo económico para presentación de resultados en congresos nacionales e internacionales durante mi doctorado.

Al doctor Serna por aceptar guiarme durante mi formación doctoral. Le agradezco infinitamente por sus consejos, y todo el apoyo para la realización de este proyecto. Ha sido un gran honor tenerlo como asesor.

Deseo agradecer a la Dra. Janet Gutiérrez por sus consejos y su tiempo para ayudarme a mejorar día a día en mi formación como investigador. Te admiro mucho y ha sido muy satisfactorio el trabajar contigo desde mi maestría.

Muchas gracias al Dr. Julián de la Rosa por su paciencia y consejos. Tu contribución mejoro significativamente la fluidez de la primera etapa experimental de mi trabajo.

Gracias a la Dra. Cristina Martínez-Villaluenga, por recibirme en su laboratorio durante la realización de mi estancia doctoral. Te agradezco mucho tus consejos tanto profesionales como personales, fue una experiencia genial el colaborar contigo.

Gracias a los miembros de mi comité, Dr. Daniel Jacobo , Dr. Cuauhtémoc Reyes y la Dra. Arlette Santacruz por las sugerencias realizadas durante la revisión de mi trabajo doctoral. A la M.C. Janett Ontiveros y Dr. Garzón por proporcionarme la materia prima para la realización de este trabajo.

A todos los compañeros del grupo Nutriomics por el buen compañerismo dentro del grupo. En especial a Marilena, Lily, Diana, Javi, Dani chiquito, Fabiola y Nydia. A mis compañeros del doctorado johanan, mariana, carrasco y lalo por hacer mas agradables las clases y carga de trabajo durante esta etapa.

A mis amigos David, Yareli, Ricardo, Marcela, Daniela, Eslim, Leydi, Chuy, Karla, Edna, Laura, Betty, Victor, Marco, Valeria, Irasema, Raúl, Daniel y Dulce, gracias por su amistad y aligerar esta etapa de mi vida.

" Nutraceutical properties of isolated starch, phytochemical compounds and bioactive peptides from pigmented chickpea cultivars influenced by cooking or germination process "

by Ada Keila Milán-Noris.

Chickpea (*Cicer arietinum* L.) is the third most consumed pulse worldwide and a potential functional ingredient due to its nutritious composition and bioactive compounds. The aim of this investigation was to evaluate the potential of ten pigmented chickpea cultivars as ingredients in functional foods using cooking or germination to enhance bioactive compounds with health effect. The investigation was performed in five steps in order to evaluate the potential of ten chickpea cultivars differing in seed coat color (black, brown, green, red and cream).

The first approach was to evaluate chickpea flours on the techno-functionality, chemical composition and nutritional properties related to starch and protein. The colored chickpeas flours showed higher content of bioactive compounds as total phenolics (TPC), β-glucans, resistant starch and higher protein digestibility corrected amino acid score (PDCAAS) compared with the commercial chickpea Blanco Sinaloa (cream seed coat). The limiting amino acids in chickpea flours were Trp, Thr and Met+Cys, whereas PDCAAS ranged from 0.59 to 0.82. Correlation analysis showed a possible interaction between TPC and starch which influenced thermal properties and starch digestion. The principal component analysis (PCA) showed several differences among the chemical compositions, starch digestions and seed protein qualities.

Moreover, starch is the major component of chickpea seeds; therefore the wet-milled chickpea starches were studied on physicochemical, functional and *in vitro* starch digestion properties. The yield of chickpea starches ranged from 19.22 to 30.06%; total starch and amylose contents in the starches varied from 87.14 to 96.02% and 25.05 to 35.26%, respectively. Gelatinization properties (DSC, RVA) showed large differences among starches. The rapidly digestible (RDS), slowly digestible (SDS) and resistant (RS) gelatinized starch fractions varied from 56.34 to 59.15%, 33.22 to 35.43% and 6.42 to 9.22%, respectively. The predicted glycemic indexes (pGI) of native and gelatinized chickpea starches ranged from 65.52 to 66.10 and 74.39 to 75.74, respectively. A close correlation among the viscosity characteristics of isolated starches and the starch digestion fractions were found after PCA analysis. The starches properties were not dependent of the seed coat coloration of the cultivars. Overall, the results suggest that the starches of the array of chickpeas studied may hold a potential for the development of functional foods especially due to its functional properties, medium glycemic index, high SDS and RS contents.

Additionally, the fate of phytochemicals during cooking and germination was evaluated. The 10 chickpea cultivars were soaked and cooked for the phytochemical evaluation. The compounds were identified by HPLC-IT-MS and quantified by HPLC-UV-ELSD. In

the raw chickpea seeds, eleven compounds were identified among cultivars; soyasaponin βg (m/z 1068) and biochanin A (m/z 285) were the principal compounds found. The thermal process caused significant ($p \leq 0.05$) reduction in flavonoids content but only minor lost of total saponins content. Besides, the effect of the germination process on phytochemicals of four chickpea cultivars (black, cream, green and red) was evaluated. Chickpea cultivars were germinated during five day at 24ºC. Eight isoflavonoids and soyasaponin βg were identified in germinated chickpea cultivars. However, genotype showed a significant effect on the profile and content of isoflavonoids during germination process. Phytochemical content increased significantly during germination process in all chickpea cultivars.

Lastly, the anti-inflammatory effects of peptides and isoflavonoids associated to the unabsorbed digestion of protein concentrates from cooked or germinated (G.ICC5613 and Blanco Sinaloa) chickpea cultivars were investigated. The simulated gastrointestinal digestion released isoflavonoids and peptides (<10 kDa) in cooked and germinated samples with adequate capacity to reduce nitric oxide production in induced-LPS macrophages. In both cultivars, the germinated samples showed higher reduction in nitric oxide by phenolics and peptides fraction. The digests from germinated Blanco Sinaloa showed anti-inflammatory effects exerted by phenolics (IC_{50}: 0.22mg/mL) and peptides (IC_{50}: 1.92 mg protein/mL). The major phenolics were biochanin-A and formononetin. The further purification of the most active fraction produced peptides from legumin and vicilin. This is the first report of anti-inflammatory peptides from processed chickpea released by simulated gastrointestinal digestion. Overall, the results suggest that pigmented chickpea cultivars of this study showed a great potential as functional ingredients.

List of Tables

List of Figure

Contents

Chapter 1. Introduction

1.1 Chickpea (*Cicer arietinum* L).

Chickpea are classified in *Fabaceae* family, *Viviae* tribu, *Papolioniedes* subfamily, and Cicer genus; *Cicer arietinum* L is the major specie with commercial and agronomic impact. ICRISAT (*International Crops Research Institute for the Semi Arid Tropic*) had close to 9,000 chickpea germoplasm lines [1]. Chickpea is the third most important pulse by world production after bean and pea. This pulse is mainly consumed and produced in developing countries. India produced 7,700,000 tons, which represented close to 66% of worlds total production. Other high producers countries of chickpea are Australia (673 m ton), Turkey (535 m ton), Ethiopia (409 m ton) and Mexico (270 m ton). [2]. In Mexico, chickpea for human consumption is harvested in the states of Sinaloa, Sonora and Baja California Sur. In the last 10 years, the average production was 146,618 ton, from which Sinaloa produce 50.5% of Mexican production [3]. Chickpea seeds are classified according to their origin: Desi (Indian region) with a thick and pigmented seed coat, and Kabuli (Mediterranean region) with thin seed coat and cream or white pigmentation and depending of the cultivar, the seed shape can be round, wrinkled and exalbuminous [4, 5]. According to the botanical differences, the seed coat and cotyledon varies from 3 to 16% and 82 to 97% of the seed weight; thus, these relative amounts affect chemical-nutritional composition and influences functionality and therefore the use as food. The external seed coat presents some well-defined structures: hilum, micropyle and raphe, which are related to both the seed integrity and germination potential [6, 7].

1.1.1. Nutritional and nutraceutical composition.

1.1.1.1 Nutritional composition.

Chickpea is a good source of protein and carbohydrates. Also it has a good amount of vitamin (Niacin, ascorbic acid) and minerals (Ca, P, Mg, Na, Fe, K) [1, 4]. In table 1.1 are shown the chemical composition of raw chickpea.

1.1.1.1.1 Protein content and quality

Protein content in chickpea seed (dry basis) varied from 12.4% to 30.6% with average of 21.5%. The amount of protein is not different among Desi and Kabulli genotypes [4]. The main protein fractions are globulins, from which represent 56.6% of total protein. The other fractions are present with 18.1 %, (glutelins), 12% (albumin) and 2.8% (prolamins) [8]. Protein quality is determinated comparing the amino acid composition with a standard protein [9]. In chickpea the following protein quality parameters have been reported: 52 to 85 % of biological value (BV), 1.2 to 2.64 of protein efficiency ratio (PER), 76 to 92.8 of true digestibility (TD) and 87 to 92 of net protein utilization [5].

Table 1.1. Chickpea chemical composition and anti-nutritional factors (db).

Chemical composition (%) [4]	**Range**
Protein (%)	12-30
Carbohydrates (%)	50.6-70.9
Starch	37.2-50.0
Sugar	3.5-9.0
Fiber	10.6-27.3
Ash (%)	2.5-4.0
Total Fat	3.1-7.42
SFA	0.46-1.11
MUFA	0.58-1.40
PUFA	2.04-4.94
Vitamins [1] (mg/100g)	
Thiamin (B1)	0.028-0.40
Pyridoxine (B6)	0.55
Riboflavine (B2)	0.15-0.30
Niacin (B3)	1.6-2.9
Folic acid	150
Vitamin C	2.15-6.00
Vitamin K	120
Minerals [4] (mg/100g)	
Ca	105-220
Mg	115-212
Fe	4.3-7.6
Cu	0.5-1.40
Zn	2.8-6.11
Mn	1.21-4.8
Na	21-24
K	878-926
P	398
Cr	0.008
Antinutritional Factor [8]	
Phytic acid (mg/g) [8]	2.8
Phytolectins (units/g) [4].	400
Enzyme inhibitors (units/mg)	
Trypsin [8]	6.7-14.6
Chymotrypsin [8]	5.7-9.4
Amylase [8]	0.0-15.0

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

1.1.1.1.2 Carbohydrates

Carbohydrates in chickpea are more than 50% of total composition; which are divided in digestible (starch, mono- and disaccharides) and non-digestible (fiber and oligosaccharides). The amount of carbohydrates fractions varied according to Desi and Kabulli genotypes. Kabulli type usually had more soluble sugars than Desi [4]. Starch is the main source of carbon in legumes and is integrated by 2 polymers, amylose and amylopectin, in which glucose residues are linked by α (1-4) bonds and branched by α (1-6) to form a linear molecule and linkages, correspondingly. The starch is usually classified as type A in cereals, type B in tubers and type C in pulses, in which type C is an intermediate of A and B in structure and packing density; these polymorphs differ in the form that side amylopectin chains are packed in the lamella of starch granule [4, 10]. The average content of starch in chickpea seeds varied from 41 to 50 % of total content [4]. Starch can be completely digestible; however, it digestion can be affected by the amount of resistant starch (RS) in samples [11]. The starch digestibility and RS content are influenced by several factors as enzymes accessibility, starch granules structure, among others [11]. The non-digestible polysaccharides were addressed as nutraceuticals.

1.1.1.1.3 Lipids

The lipid content in chickpea seeds range from 3 to 10% (db). Chickpea had 66% PUFA, 19 % MUFA and 15 % SFA [4]. The main fatty acid are oleic, linoleic and palmitic [12].

1.1.1.1.4 Vitamins and Minerals

Chickpea are a good source of folic acid and tocopherols. Also it has riboflavin, pantothenic acid and pyridoxine. Chickpea showed good amount of iron, zinc, calcium and magnesium. Some authors have reported presence of selenium [4].

1.1.1.1.5 Anti-nutritionals factors

Although the chickpea possesses excellent nutritional attributes, it has the presence of undesirable components that limit its nutritional quality [4]. Some of these undesirable components are enzyme inhibitors, phytic acid, oligosaccharides, lectins, saponins and tannins. Anti-nutritional factors can inhibit the enzymatic activity of trypsin and chymotrypsin, or can form complexes with ions of minerals, give astringent or bitter taste, or produce undesirable gases during fermentation in the colon [13]. Nowadays, some of these compounds are also attributed beneficial health effects (Table 1.1) [14].

1.1.1.2. Nutraceutical composition

Chickpea is a good source of nutraceuticals compounds such phytosterols, saponins, isoflavones among others. Those compounds had the potential to improve human

health [4]. The table 1.2 summarized the content of bioactive compounds in raw chickpea and the possible heath effect.

1.1.1.2.1 Polyphenols

The chickpea seeds contain several phenolic compounds, in which, the isoflavones biochanin A (5,7-dihydroxy-4´-methoisoflavone) and formononetin (7-dihydroxy-4´-methoisoflavone, biochanin-B) are worthy to mention [4]. Isoflavones are a subclass of flavonoids and also called as phytoestrogens due to its estrogenic properties. These compounds occur naturally in glycosylated forms, the isoflavones are conjugated and usually esterified with malonyl or acetyl groups. In plants, the most studied are genistein and dadzein which are conjugated (acetyl glycosides or malonyl) and are hydrolyzed in the human gut into its active form, aglycones [15]. In table 1.2 are described the content and isoflavones reported in chickpea. The concentration of biochanin-A is higher in grains type Kabulli compared to Desi grains [5]. Also the content of biochanin-A than formononetin is usually higher in chickpea seed [18]. On the other hand, Konar et al. (2012) Identified and quantified the content of free and conjugated isoflavones in chickpea: Biochanin-A, daidzein, formononetin, genistein, glycitein, sissotrin (biochanin-A glycosylated), daidzin, ononine (glycosylated formononetin), genistin and glycitin [15].

Besides, the content of phenolic acids, flavonoids and anthocyanins have been reported in chickpea seeds. Sreerama et al (2010) reported the phenolic acid content in the anatomical parts of the chickpea (testa, cotyledon and embryo). Among the compounds found were the following phenolic acids: gallic, protocatechuic, hydroxybenzoic, vanillic, syringic, caffeic, chlorogenic, sinaptic, coumaric and ferulic [16]. The most abundant in all anatomical parts was ferullic acid with 159 µg/g, 60 µg/g and 42.4 µg/g in cotyledon, embryo and testa, respectively [16]. Other investigators reported the content of phenolic acids in whole grain, finding highest values of protocatechuic acid with 358.9 µg/g [17]. Xu et al (2009) reported as main phenolic chlorogenic acid [18].

The contents myricetin, kampferol and quercitin have been reported in the anatomical parts of the chickpea seeds. Kampferol was the main flavonoid found in testa, cotyledon and embryo; where the largest amount was found on the testa. On the other hand, the testa and embryo present considerable amounts of anthocyanins (cyanidin, petudin and delphinidin), whereas in the cotyledon were not detected. The main anthocyanin was cyanidin which content represented 84% of the total content of anthocyanins in the testa [16]

1.1.1.2.2 Saponins

Saponins are triterpenic glycosides structurally divided into 2 groups A (bidesmosid) and B (monodesmosidic). The chickpea contains soyasaponin I (Bb) and soyasaponin

βg (VI) [19-22], which are part of group B. Soyasaponin βg has the 2,3-dihydro-2,5-dihydroxy-6-methyl-4H pyran-4-one (DDMP) at position C22 and is the natural precursor of soyasaponin I [20]. Other saponins have been detected in chickpea as lablab saponin I, soyasaponin ag, soyasaponin II, kaikasaponin II and III [21].

1.1.1.2.3 Phytsterols

Phytosterols are compounds with a similar structure as cholesterol and contributes at structural part of membranes of plant cells. Their consumption have been related with cholesterol lowering effect [23]. Phytosterols can be found in vegetables, grains and legumes [12, 24]. The most common phytosterols are β-sitoterol, campesterol and stigmasterol have been found in chickpea [12]. In defatted chickpea flour have reported significant amounts of glycosylated forms of β-sitosterol [14]. Other researchers reported 31% of total content are glycosylated forms of phytosterols [25].

1.1.1.2. 4 Peptides

In addition to the nutritional and functional properties provided by proteins, they may also possess other properties. These properties are attributed to active peptides encoded in protein molecules that can be released during gastrointestinal digestion or by controlled hydrolysis processes using exogenous proteases. Several bioactive peptides have been studied from animal and plant sources, especially peptides associated with milk or soybean [26]. Legumin is the main storage protein in chickpea; this globulin is formed of 6 α units that bind as a trigonal antiprism by non-covalent bonds. Each α chain is attached to β chains by disulfide bonds. The α chains are attached on the outside of the molecule, where β chains constitute the hydrophobic part of the center of the protein. The approximate molecular weight of legumin is 360 kDa [27].

The principal peptides research has been focus in raw chickpea seeds. Chickpea protein isolated have been treated with alcalase in order to produce protein hydrolysates with the capacity to inhibit angiotensin I (ACEI), enzyme related to hypertension. The purification with RP-HPLC generated 6 peptides fraction with ACEI. The peptide with the highest inhibition of ACE contains 5 amino acids (Met: Asp: Phe: Leu: Ile), while the other peptides generated contain methionine and are rich in other hydrophilic amino acids [27]. In another study, the alcalase protein hydrolysates of chickpea was fractionated by gel chromatography [28] and RP-HPLC purification; which generated a peptide with good antioxidant activity and molecular weight of 717.37 Da (NRYHE) [29]. The antioxidant effect of the NRYHE peptide was studied by catalase, glutathione peroxidase and reductase activity in Caco-2 and HT-29 cells; this peptide had a positive correlation between the concentration and activity of the three enzymes studied [30].

1.1.1.2.5 Polysacharides (non digestible)

The non-digestible polysacharides are formed by oligosaccharides, fiber, resistant starch and b-glucans. Legume seed commonly had high amounts of oligosaccharides, in contrast to others seeds. α-galactosides are the most abundant carbohydrate after sucrose, chickpea presented 62% of total sugar content (mono, di and oligo-saccharides). The two main groups of oligosaccharides in chickpea are: raffinose family (raffinose: trisaccharide, stachyose: tetrasaccharide, verbascose: pentasaccharide) and galactosyl cyclitols (ciceritol)[4, 31]. The oligosaccharides can not be absorbed or degraded due to absence of α-galactosidase in humans, but are fermented by colonic bacteria and can promote the growth of *Bifidobacteria* in colon [4, 31]. Dietary fiber is a part of food that is not digested in the small intestine. It is composed of poly/oligosaccharides, lignin and other substances [32]. The fiber can be classified into soluble and insoluble, where insoluble is slowly digested in the colon and insoluble is metabolically inert and helps with bowel movement; the latter is fermented in the colon helping the growth of bacteria [4] In chickpea samples, resistant starch is 35 % of total starch and the rest can be digestible. Starch digestibility in pulses is lower than cereals [11].

Table 1.2 Bioactive compounds in raw chickpea.

Bioactive compounds: Health effect	**Group/Compounds**		**Concentration**	
Polyphenols: estrogenic [33], antioxidant[34], anti-inflammatory [35], antimicrobial [34] and anti-proliferative [36-38] .	Total phenolic content		0.5-6.8 mg CE/g 0.5-1.5 mg GAE/g	[39] [40]
	Isoflavones	Total content	3078 µg/Kg, wb	[15]
		Biochanin-A	19 to 846 µg/Kg, wb 838 to 3080 µg/100g	[15, 41] [42]
		Formononetin	1 µg/g, wb 94 to 215 µg/100g	[41] [42]
		Biochanin-A glucoside	1016 µg/Kg, wb	[15]
	Flavones	Luteolin	0.55 to 132 µg/g	[17, 43, 44]
	Flavanols	Catechin	147.49 to 1507.6 µg/g	[18, 44]
		Epicatechin	1.23 to 145.5 µg/g	[18, 44]
		Epigallocatechin	23.95 µg/g	[18]
		Epicatechin gallate	16.79 µg/g	[18]
	Phenolic acids	Total content	1285.7 µg/g	[18]
		Chlorogenic	4.62 to 197 µg/g	[16, 43, 44]
		Gallic	5.42 to 40.2 µg/g	[16, 17, 44]
		Ferulic	0.9 to 262.5 µg/g	[16, 17, 43, 44]
		Coumaric	0.43 to 161.3 µg/g	[16, 43, 44]
		Syringic	62.6 to 1947µg/g	[16, 17, 43]
		Hydroxibenzoic	10.5 to 57 µg/g	[16, 17]
		Vanillic	80.8 to 82.3 µg/g	[16, 17]
		Caffeic	51.8 µg/g	[16]
		Shikímic	89.92 mg/g	[43]
		Protocatecuic	117.9 to 358.9 µg/g	[16, 17]
		Dihidroxibenzoic	26 µg/g	[17]
		Sinapic	7.81 to 33.2 µg/g	[16, 17]

(Table continue)

Bioactive compounds: Health effect	**Group/Compounds**		**Concentration**	
Polyphenols: (cont)	Flavonols	Rutin	18 µg/g	[43]
		Quercetin	0.37 to 160.61 µg/g	[18, 44]
		Kaempherol	18.11 to 133.8 µg/g	[16, 18]
		Myricetin	32.09 to 44.3 µg/g	[16, 18]
	Anthocyanins	Total content	538.9 µg/g	[16]
		cyanidin	443.6 µg/g	[16]
		petunidin	58 µg/g	[16]
		delphinidin	37.3 µg/g	[16]
Phytosterols: Cholesterol lowering effect [45, 46], antioxidant [47, 48], anti-inflammatory [49-51] and anti-proliferative [49, 52-54].		Total content	75.32 mg/100 g	[25]
		β-sitosterol glucoside	85% glycolipids	[55]
		β-sitosterol	159.8 mg/100 g	[12]
		Campesterol	21.4 mg /100 g	[12]
		Stigmasterol	23.4 mg /100 g	[12]
Saponins: Cholesterol lowering effect [56], prebiotic [57]; cancer prevention [56].		Soyasaponin βg	711 to 1412 mg/Kg Identification	[20, 22] [19, 21]
		Soyasaponin I	688 to 761 mg/Kg Identification	[20] [19]
		Lablab saponin I	Identification	[21]
		Soyasaponin αg	Identification	[21]
		Soyasaponin II	Identification	[21]
		Kaikasaponin II-III	Identification	[21]
Carotenoids: Antioxidant, protection in age related macular degeneration, cholesterol lowering effect [58].		Total content	9.2-31.3 µg/g	[58]
		Violaxanthin	0-1.2 µg/g	[58]
		Lutein	5.3-21.5 µg/g	[58]
		Zeaxanthin	2.9-14.8 µg/g	[58]
		β -Cryptoxanthin	0-2.6 µg/g	[58]
		β-carotene	0.1-2.6 µg/g 46.3 µg/100g	[58] [4]

(Table continue)

Bioactive compounds: Health effect	**Group/Compounds**		**Concentration**	
Polysaccharides: Prebiotic [59], Cholesterol lowering effect [60, 61], prevention of metabolic syndrome [62] and cancer [63].	Resistant starch		33.9-198.5 g/Kg	[64-66]
	Oligosaccharides	Total content	6.3-8.68 %	[67]
		Raffinose	0.46-0.92 %	[67]
		stachyose	2.03-3.06%	[67]
		verbascose	0.27-0.7 %	[67]
		ciceritol	3.08-5.06%	[67]
	Fiber	Insoluble	13.9 to 20.7 g/100g	[64, 66]
		Soluble	1.3 g/100g	[66]
	β-glucans		0.2 to 0.5 %	[68, 69]
Peptides: Antioxidant (AOX) and angiotensin I activity inhibition (ACEI)[27, 30, 70].	Met-Asp-Phe-Leu-Ile (ACEI)		EC_{50} 11 µg/mL	[27]
	Met-Asp (ACEI)		EC_{50} 21 µg/mL	[27]
	Met-Phe-Asp-Leu (ACEI)		EC_{50} 13 µg/mL	[27]
	Met-Asp-Leu-Ala (ACEI)		EC_{50} 13 µg/mL	[27]
	NRYHE (AOX)		50 µg /mL	[29]
	RQSHFANAQP (AOX)		2.3/1.5 µmol/mL	[71]
	NRYHE (AOX)		0.05-0.5 mg/mL	[30]
	ALEPDHR, TETWNPNHPEL, FVPH, SAEHGSLH (AOX)		0.3 mg/mL	[72]

wb: wet basis, mg CE: mg of cathequin equivalents, mg GAE: mg of gallic acid equivalents.

1.1.2 Processing effect on chickpea composition.

Food processing usually improve the nutritional value of pulses, increasing in vitro digestibility of protein and starch close to 40 % and 98%, correspondingly [13]. Also chickpea processing can reduce or eliminate anti-nutritional factors, which are a limiting in chickpea consumption [4, 13]. The chickpea are mainly processing forms: deshulling [73], soaking [74], roasting [75], cooking [66, 76], extrusion [74], germination [41, 77] and fermentation [43, 78]. A limiting part of chickpea consumption is the content of anti-nutritional factors but this can be reduced or eliminated by chickpea processing

Table 1.3. Effect of processing on bioactive compounds

Process details	Cultivar/ genotype	Processing effect	
Thermal processing			
Roasting 3h in oven 105 ºC	cv sultano	Increased in the amount of insoluble dietary fiber, resistant starch (RS), total phenolic content (TPC) and antioxidant capacity (Aox; TPAC, TEAC)	[79]
Dry heating, autoclaving (y/n soaking)	NS	Decreased in TPC, total flavonoids (TFC) and tannins	[80]
Autoclaving with soaking	NS	Decreased in TPC, TFC, reducing power, DPPH and increased in metal chelating activity	[81]
Decorticated, cooking and soaking	cv Dwelly	Decreased in TPC	[82]
Conventional boiling, conventional steaming, pressure boiling, pressure steaming	cv Amits	Reduced in TPC, procyanidin, saponin, phytic acid and Antioxidant capacity (AOX; FRAP).	[18]
Soaking (16h at 20ºC 1:10); cooking (70ºC boiling) and dehydration (70ºC 6 h)	cv Sinaloa and cv Castellano (kabulli)	Reduced isoflavones in castellano cultivar, in contrast to sinaloa cultivar that showed stability during process.	[76]
Soaking (16h at 20ºC 1:10); cooking (70ºC boiling) and dehydration (70ºC 6 h)	cv Sinaloa and cv Castellano (kabulli)	Decreased oligosaccharides, RS content and increased total dietary fiber	[66]
Cooking 30. 60. 90 and 120 min, presoaking	cv Fardon (Desi) and cv Blanco Lechoso (Kabulli)	Conversion of soyasaponin Bg to Soyasaponin I. Loss of soyasaponin I in cooking water 2-5%	[22]
Soaking by 12 h and cooking 30 min.	cv Puglia, cv Marches, cv Mexican, cv Italian 1 and cv Italian 2	Conversion of soyasaponin Bg to soyasaponin I. Loss of soyasaponin I in cooking and soaking water.	[20]

Fermentation			
Solid state fermentation with *Rhizopus oligosporus*	cv Blanco Sinaloa (Kabulli)	Improved protein digestibility, essential amino acid content and protein quality.	[78]
Solid state fermentation *Cordyceps militaris* SN-18	NS	Increased TPC, saponin content and AOX (DPPH, ABTS, reducing power) compared with raw chickpea. Fermented sample showed protection against oxidative DNA damage. Also accumulated phenolics acids, flavonols, as shikimic acid, chlorogenic acid, rutin, daidzein, genistein and biochanin A.	[43]
Natural fermentation and with *Lactobacillus plantarum*	cv Blanco Lechoso (Kabulli)	Decresed vitamin C and glutathione. Also increased TPC and AOX (PRTC, TEAC).	[83]
Fermentation with commercial lyophilized yoghurt culture	Australian (Desi, Kabulli)	Improved protein digestibility, decreased protein content and trypsin inhibitor activity.	[84]
Germinated seed were treated with Pseudomonas (PHU 094), *Trichoderma harzianum* (THU 0816) and *Mesorhizobium* ps (RL 091) and its combinations	cv Radhey	Increased TPC, TFC, ascorbic acid, reducing power and iron chelation. The combination of 3 microbes improved the accumulation of phenolics as rutin, quercitin, shikimic, gallic, tannic, p-coumaric and ferulic acids.	[85]
Sourdough fermentation, *Lactobacillis plantarum* C48 or *Lactococcus lactis* subsp *lactis* PU1, 24 h.	Market from	Increased Y-aminobutyric acid (GABA) and free amino acids	[86]
Solid state fermentation with *Bacillus amyloluquefanciens*	NS	Increased TPC, TFA and AOX (DPPH) and produced fibronolytic enzymes with anticoagulant activity.	[87]
Solid state fermentation with *Rhizopus oligosporus.*	cv Blanco Sinaloa	Increased TPC, AOX (ORAC) and a-amylase and a-glucosidase inhibition in vitro	[88]
Extrusion			
Extrusion pilot scale twin extruder 381 rpm, 12.5 water, low temperature 120 to 90ºC and 150 to 120ºC. pretreatment at 70, 90 and 100 ºC.	NS	Non-starch polysaccharides and oligosaccharides were no drastically affected by processing. Trypsin inhibitor, phytic acid and tannins were significant affected by processing	[89]

Chickpea extrusion in a single-screw extruder at 155ºC and 240 rpm.	cv brown-icc2512, red-icc13124, black-icc3761 (Desi)	Increased TPC, Aox (ORAC), and anti-mutagenic activity by 5.3 to 9.2%, 9.9 to 12.2 and 17.5 to 21.9 %, respectively.	[90]
Extrusion in a single screw extruder, 250 rpm; barrel temperature 140ºC and 180ºC, feed moisture 18% and 22%; presoaking for 16h	Market from Egypt	Reduced anti-nutritionals as phytic acid, tannins, amylase and trypsin inhibitors and improved the protein digestion.	[74]
Extrusion in a single screw extruder, at 150.5ºC, at 190.5 rpm and 265 g/Kg of water feed.	cv Hardened Blanco Sinaloa (Kabulli)	Improved in vitro protein digestibility (21.7%) and protein efficiency ratio (36.9%)	[91]
Extrusion in a single screw extruder, at 151ºC, a 189.5 rpm. Pretreatment: dehulling and softening in 1% salt solution.	cv Fresh and hardened Blanco Sinaloa (Kabulli)	Improved in vitro protein digestibility and protein quality	[92]
Extrusion a single screw extruder at 130ºC, 200 rpm and 14% moisture feed.	cv IAC-Marrocos	Reduced total fiber and resistant starch but had a good iron bioavailability similar than home cooking chickpeas.	[93]
Extrusion a twin crew extruder at 160ºC, 500 rpm and 17% moisture feed.	NS	Reduced dietary fiber and oligosaccharides	[94]
Germination			
Germination 2 and 3 days	cv Blanco Lechoso (Kabulli)	Increased TPC, Aox (SOD-like activity, PRTC, TEAC) and vitamins (C,E) content.	[83]
Germination 30º or 40ºC for 5 days in dark	NS	Increased significantly TPC, flavonoids, phenolics acids and DPPH inhibition.	[95]
Germinated 25ºC in dark for 4 days	NS	Increased TPC, antioxidant capacity, and isoflavones (biochanin-A and formononetin).	[41]
Germination in light, dark, under ethanol stress and salt stress 25ºC for 12 days.	cv 88-1	Increased significantly isoflavone content. Biochanin A and formononetin were 154 and 130 times higher.	[96]
Germination at 25ºC for 36 to 60 hours	NS	Increased protein digestibility from 6.3 to 16.7% and starch digestibility from 8.6 to 10.5 %.	[97]

Germination with fluorescent, yellow, blue, green and red light. Also in dark and gamma irradiated at 28ºC for 0 to 120 hours.	cv NIFA-2005 (Desi)	Highest carotene value was found at 72 h with yellow light. gamma irradiated previous to processing improved protein digestibility.	[98]
Germination in dark 20ºC for 0 to 120 hours.	Market from Iran	Increased TPC and antioxidant capacity	[99]
Germination with fluorescent, yellow, blue, green and red light. Also in dark and gamma irradiated at 28ºC for 0 to 120 hours.	cv NIFA-2005 (Desi)	Green light showed the highest values on ascorbic acid during germination	[100]
Germination with Na_2SeO_3 at 28ºC	cv Peshawar (Desi)	Supplementation with 50 mg Na_2SeO_3 accumulated Se and isoflavones content. Supplementation higher than 50-125 mg inhibit sprout growing and isoflavones biosynthesis.	[101]
Germination for 24 hours	Market from India	Increased protein, thiamin, in vitro iron and calcium bioavailability. Also processing improved in vitro protein and starch digestibility	[73]
Germination with Na_2SeO_3 (0, 1 and 2 mg/100g) at 24ºC for 4 days.	cv Blanco Sinaloa (Kabulli)	Supplementation with Na_2SeO_3 (2 mg/100g) increased significantly total isoflavonoid, PAL activity and antioxidant capacity.	[102]
Germination with fluorescent, yellow, blue, green and red light. Also in dark and gamma irradiated at 28ºC for 0 to 120 hours.	cv NIFA-2005 (Desi)	The methanolic phenolics decreased at 24 and 48 h after that increased significantly until 120 h. Similar behavior occurs with phytic acid content.	[103]

NS: not specified.

1.1.3 Health effect of chickpea consumption.

Chickpea intake has been related the reduction of the risk to chronic degenerative diseases; which can be attributed to the content of soluble fiber, isoflavones, phytosterols and saponins in chickpea. The chickpea can reduce plasma glucose, insulin levels and its resistance [104, 105]. Other authors found reductions in LDL and total cholesterol [104-106]. Besides health effect related to diabetes and metabolic syndrome, chickpea can reduce risk factors in cancer [36, 107], inflammation [108, 109] and obesity [110, 111]. The ingesting of fiber from chickpea and lupine stimulate the growth of *Bifidobacterium* in colon and had beneficial effect on colon health. Also chickpea oligosaccharides modulate microbial composition in the intestine with beneficial effects [112]. Furthermore a study showed that chickpea group decreased the presences of *Clostridium histolyticum* and *Clostridum lituseburense,* pathogenic and putrefactive bacteria, compared with control group [113].

Table 1.3. Health effect of nutraceuticals found in chickpea

Details	Main effects	Nutraceutical related	
Cardiovascular			
In vitro, cholesterol micelle inhibition	Chickpea hydrolysates showed better hypocholesterolemic activity than protein isolate.	Peptides	[26]
In vivo, 10 mice by group (normal diet, high fat diet (HFD), HFD with low, medium and high doses of chickpea hydrolysate for 4 weeks.	The chickpea hydrolysates treatment decreased in a dose manner triglyceride, total cholesterol and LDL-C, also increased fecal fat excretion.	Peptides	[107]
Cancer			
In vitro, breast cancer lines (SKBr3 and MCF-7)	Chickpea sprout extract (10-60 µg/mL) showed an inhibition in cell proliferation in a dose-dependent and time-depend manner. The cell inhibition occurred via mitochondria-dependent apoptotic mechanism.	Isoflavones	[36]
In vitro, caco-2 and THP-1	Protein hydrolysates inhibited cell proliferation of caco-2 and THP-1 by 45% and 78%, respectively.	Peptides	[114]
In vitro, MCF-7 and MDA-MB-231	Chickpea peptides showed a high inhibition on MCF-7 and MDA-MB-231 cell lines with EC_{50} of 2.38 and 1.50 µmol/mL, respectively.	Peptides	[71]
In vivo, 10 mice by group (normal diet (not inoculated), high fat diet (HFD), HFD with low, medium and high doses of chickpea hydrolysate were inoculated with H-22 tumor cells and fed by 12 days.	The chickpea hydrolysates decreased tumor volume and increased the tumor inhibition rate.	Peptides	[107]
Antioxidant			
In vitro, cellular antioxidant activity in Caco-2.	Protein fractions showed antioxidants by different modes of action, as donating electrons and scavenging peroxyl-like radicals.	Peptides	[72]
In vitro, DDPH, B-carotene bleaching reducing power	Hydrolysates showed a better antioxidant activity than protein concentrate.	Peptides	[26]
In vitro, caco-2 and HT-29	The activity of three important antioxidant enzymes (catalase,	Peptides	[30]

	glutathione reductase and glutathione peroxidase) were enhanced by chickpea peptide		
In vitro, DPPH, ABTS, reducing power, and others	Chickpea peptides at different concentration showed a certain antioxidant capability and reducing activity.	Peptides	[71]
Hypertension			
In vitro, enzyme inhibition	Protein hydrolyzed fractions showed inhibition of angiotensin I converting enzyme.	Peptides	[27]
In vitro, enzymatic inhibition.	Peptides generated by simulated gastro intestinal digestion of desi chickpea showed better ACEI than other digestion methods and peptides generated in kabulli chickpea.	Peptides	[115]
Microbiota (prebiotic)			
In vivo, a group of 16 rats feed for 28 days with cooked chickpea	Bifidogenic effect	Fiber Resistant starch	[116]
In vivo, Healthy adults were supplemented with canned chickpeas by 3 weeks	The chickpea diet modulated the gut microbiota of subjects with potentially beneficial effects associated with increase of *Bifidobacterium spp* and a decreased in *Clostridium spp.*	Oligosaccharides	[113]
In vitro 3 stage fermentative system simulating the human colon	Modulation of colonic metabolome; increased *Bacteriodes/Prevotella* species.	Fiber, β-glucans, Resistant starch Oligosaccharides	[69]
Diabetes			
In vitro, 3T3-L1 pre-adipocyte cells	Isoflavones in chickpeas suppressed 3T3-L1 adipocyte differentiations, lipid accumulation and stimulated glucose uptake through the down-regulation of PPARγ, C7EBP α, aP2, LPL, UCP-2 and GLUt4 mRNA expression in a dose dependent manner.	Isoflavones	[117]
Inflammation			
In vivo, randomized cross-over clinical trial aimed to compared effects of legume-free and non soya legume based diet among type 2 diabetic patients.	The non-soya legume (pea, chickpea, beans) based diet has good effects on inflammatory markets that are associated with type 2 diabetes.	Mg Fiber	[108]
In vivo, obese subject were assigned to a	Consumption of legumes within a hypocaloric diet reduced pro-	Fiber	[109]

hypocaloric diet (control or 4 servings of legume: chickpeas, lentil) by 8 weeks	inflammatory markers as CRP and C3 and improved lipid profile in obese subjects.	Mg	
Obesity			
In vivo, Male sprague-dawley rats were fed with normal, high fat and high fat with chickpea diets by 8 weeks.	Chickpea consumption reduced triglycerides, LDL- cholesterol and improved insulin resistances and prevented postprandial hyperglycemia.	Isoflavones Unsaturated fatty acids	[110]
Metabolic syndrome			
In vivo, obese subjects 5 cup/week of pulses (chickpea, peas, beans, lentils) by 8 weeks	The pulses consumption improved glycemic control and increased HDL compared with control.	Fiber Resistant starch	[111]
In vitro, enzyme inhibition	Chickpea phenolics showed inhibition effect on α-amylase, a α-glucosidase and angiotensin I converting enzyme.	Phenolics	[16]
In vitro, enzyme inhibition	Chickpea showed inhibition effect on α-amylase, α-glucosidase and lipase.	Saponins	[118]

1.2. Meaning and relevance

Pulses are ancient plant species, which provide the main source of plant protein, specially in developing countries and are called the superfoods of the future [119]. Chickpea is the third most important pulse in the world; with a high economic importance in the Northwest of Mexico but is less popular than beans. Chickpea is considered a potential functional ingredient due to its nutritious composition [4].

There are several studies that explore the differences on compositional and techno-functional properties among kabulli and desi chickpea seeds. Although the phytochemicals dissimilarities affected by seed coat color are poorly explored in raw samples [39, 40]. Chickpea is usually processed for ingestion, in order to increase its palatability and nutritional properties. Some chickpea processing effect has been reported in phytochemicals, as saponins [20] and isoflavones [76], but only the extrusion process effect has been evaluated in pigmented chickpea. [90]. Additionally cooked chickpea intake has been associated with several health benefits [104-106], however the consumption of germinated [33, 120] or extruded [121] chickpea has been scarcely addressed. Moreover the simultaneous release of peptides and phytochemicals during digestions of germinated chickpea has not studied as others seeds [122, 123].

In contrast to other legumes, the study of phytochemicals properties in pigmented chickpea and processing effect are poorly explored. The general goal of this research studies are to generate information on chemical, nutritional and phytochemical differences among pigmented chickpea cultivars. Besides quantity the phytochemicals changes that may occur during chickpea processing. Finally evaluate the anti-inflammatory effect that can provide the digested chickpea ingredients

1.3. Thesis statement

1.3.1. General objective

The main objective of this work is to evaluate the dissimilarities among pigmented chickpea on chemical, nutritional and phytochemical characteristics. Likewise evaluate the phytochemicals changes that may occur during cooking or germination process. Lastly evaluate the anti-inflammatory effect that can provide the digested processed chickpea ingredients.

1.3.2. Hypothesis

The pigmented chickpea had more bioactive compounds with potential heath effect.

1.3.3. Specific objectives

a) Compare some physicochemical and functional properties, protein quality *(in vitro* protein digestibility and essential amino acid profile) and *in vitro* starch digestion of nine pigmented chickpea flours with a commercial Kabuli chickpea.

b) Evaluate the physicochemical, functional and digestion characteristics of isolated starches from ten chickpea cultivars differing in seed coat color (black, brown, green, red and cream), nine classified as Desi and a commercial Kabuli chickpea.

c) Investigated the effect of soaking and cooking on flavonoids and saponins content and profile in nine Desi chickpea cultivars and a commercial Kabuli chickpea.

d) Evaluate the effect of germination process on phytochemical content and profile on whole seeds of three colored seed coat chickpea and a commercial cultivar.

e) Purify and identify potential anti-inflammatory unabsorbed digested peptides from processed chickpea protein concentrate extracted from two different seeds varying in color. Also evaluate the potential anti-inflammatory of phytochemicals released in chickpea protein concentrates.

Chapter 2. Materials and Methods

2. 1. Materials

2.1.1 Chickpea seeds

Nine pigmented cultivars (Desi type) from the core collection/World Germplasm Bank of the International Crops Research Institute of Semi-Arid Tropics (ICRISAT) were grown in the Culiacan valley experiment station of the National Research Institute for Forestry, Agriculture and Livestock (INIFAP), located in Sinaloa, Mexico. The commercial Kabuli cultivar Blanco Sinaloa (used as reference) was grown at Evora Region, Sinaloa, Mexico. The chickpea seeds were harvested in April-May 2014, cleaned for removal of dockage and foreign material and stored in a double hermetic bag and a container at -20ºC until use. Also 3 Desi cultivars and Blanco Sinaloa were harvested in 2016. In table 2.1 are described some characteristic of chickpea cultivars

Table 2.1. Chickpea cultivars codification

Cultivar	Seed coat color	Type	Code	2014[a]	2016[a]
ICC 6306	Black	Desi	B.ICC6306	x	x
ICC 4418	Black	Desi	B. ICC 4418	x	
ICC 3761	Black	Desi	B. ICC 3761	x	
ICC 3512	Brown	Desi	Br.ICC3512	x	
Blanco Sinaloa	Cream	Kabulli	C.BS	x	x
ICC 3421	Cream	Desi	C.ICC3421	x	
ICC 5613	Green	Desi	G.ICC5613	x	x
ICC 14782	Red	Desi	R.ICC14782	x	x
ICC 13124	Red	Desi	R.ICC13124	x	
ICC 5383	Red	Desi	R.ICC5383	x	

[a] Year in which the cultivar was harvested.

2.1.2 Chemicals and Reagents.

Murine macrophage cell line RAW 264.7 was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). High-glucose Dulbecco's Modified Eagle's Medium (DMEM) and penicillin/streptomycin (10,000 U/mL) were purchased from Lonza Group Ltd (Madrid, Spain). Fetal bovine serum (FBS) was obtained from Hyclone (GE Healthcare, Logan, UT). Cell Titer 96® AQueous One Solution Proliferation Assay kit (MTS/PES) was supplied from Promega (Madison, WI, USA). A Quantitative Colorimetric Peptide Assay kit was from Pierce™ (Rockford, IL, USA). Cell culture

flasks and plates were obtained from Sarstedt (Nümbrecht, Germany). All other chemicals were purchased from Sigma-Aldrich (St Louis, MO) unless otherwise stated. Myricitin (≤99%), kaempferol (≤99%), biochanin A (≤99%), and soyasaponin I (≤95%) was purchased from Sigma (St. Louis, MO, USA). Methanol, acetonitrile and water (HPLC or chemical grade) were purchased from local sources.

2.2 Seed physical characterization.

The seed physical characteristics were determined as described by Heiras-Palazuelos et al. [40]. Briefly, the 1000-grain weight was determined weighing randomly picked seeds by 5 replicates. The hectoliter weight was determined using the Winchester bushel meter following the official procedure by 10 replicates [124]. Anatomical seed parts were determined by first soaking 25 whole seeds for 12 h at room temperature (≈25°C), and manually dissected into hilum, seed coat and cotyledons. These fractions were dried in a convection oven (1350 FMS, VWR, Radnor, PA, USA) set at 105°C for 24 h and weighed. The average diameter was determined after measuring 25 seeds by triplicate.

2.3. Chickpea processing

2.3.1 Milling

The chickpea whole flours were obtained from seeds by grinding in a coffee beans grinder (Krups, GX4100) and filtered through a sieve with 0.177 mm orifices. All flours were stored at -20°C in hermetic containers until analysis.

2.3.2 Starch isolation

Starches were obtained following the sodium sulfite wet milling process described by Pérez-Carrillo and Serna-Saldívar [125] with slight modifications. Briefly, the seeds were soaked for 48 h at 50ºC in a 0.2% sodium sulfite with 0.47% lactic acid (85%) solution. Then, the steep liquor was discarded and the soaked seeds were mixed with distilled water in preparation for grinding in a commercial blender (Oster, model 450-10) for 1 min at high speed. The slurry was filtered through a 100 US mesh sieve, then centrifuged at 3000 *g* for 10 min, and the resulting pellet or sediment thoroughly washed until the water and starch were color free. The starch pellet was collected and freeze-dried. After drying, the sample was weighed to calculate extraction yield, and stored until analysis. Starch yield and recovery were calculated using the following equations:

(1) Starch yield = (Isolated starch weight/grain weight) x 100.
(2) Starch recovery = (Isolated starch weight/starch content in kernels) x 100.

2.3.3 Cooking seeds.

In order to evaluate the phytochemicals in chickpea, cooked chickpeas were prepared by soaking the seeds in 10 volumes of distilled water at room temperature for 12 h. After that, water was drained and seeds were cooked in 3 volumes of boiling water for 30 min (Sagratini et al., 2013). The cooked seeds were freeze-dried, grounded, passed through a 60 US sieve and stored at -20 ºC until analysis.

2.3.4 Germination

Germinated or sprouted chickpeas were obtained as previously reported [102]. Briefly, the seeds were disinfected with 2 volumes of 0.2% sodium hypochlorite solution for 3 min and then washed 3 times with distilled water. Subsequently, the seeds were hydrated in 0.85 volumes of distilled water for 5 hours at 25ºC with constant agitation. The resulting soaked seeds were transferred onto plastic trays. The seeds were germinated at 24ºC in darkness conditions for 5 days and 80% of relative humidity. The germinated seeds were freeze-dried, ground to pass through a 60 US sieve and stored at -20 ºC until analysis

2.3.5 Protein concentration

Previous to protein concentration, the sample was defatted with hexane (1:4, w/v) in agitation at 500 rpm for 4 h, and the defatted cake dried overnight at 25 °C. The defatted samples (300 g) were suspended in water (1:10, w/v) and blended for 1 min, then adjusted at pH 8.5 with a solution 1 M NaOH and agitated at 500 rpm for 2 h. Sample was centrifuged at 3,000 x g by 10 min and the pellet extracted again using the same conditions. The clarified supernatants were pooled and adjusted to pH 4.5 with 1 M HCl. The sample was centrifuged at 3,000 x g for 10 min, and the pellet was freeze-dried and stored at -20 ºC until analysis.

Cooked chickpeas were prepared as previously describe in cooking seeds. Prior to protein isolation, the gelatinized starch in the cooked sample was hydrolyzed with α-amylase (*Bacillus subtilis*; Megazyme, Wicklow, Ireland). Sample (500 g) was suspended in water (1:6; w:v) and blended for 1 min, then shaked at 500 rpm for 30 min Thermoresistant α-amylase (10units/g sample) was added and then heated for 20 min at 95ºC. The sample was kept at 60ºC for 12 h. Then, two volumes of ethanol were added and shaked at 500 rpm for 20 min. The resulting sample was centrifuged at 3,000 *x g* for 10 min and the pellet was used as feedstock for protein extraction as previously described in germinated chickpeas. The protein concentrates were freeze-dried and stored at -20 ºC until analysis.

2. 4 Chemical composition

The chemical composition in flours and isolated starches were determined according to AOAC standard methods 925.09B, 923.03, and 960.52 for moisture, ash and protein

(Nx6.25) [126]. Total starch (AOAC 996.11) was determined using the Megazyme kit K-TSTA (Wicklow, Ireland). In isolated starches, amylose content was determined using the Megazyme kits L-AMYL (Wicklow, Ireland).

2.5 Physicochemical and functional properties

2.5.1 Color

The color of seed, flours and isolated starches of chickpea cultivars were measured by a Chroma meter Minolta CM-600 (Konica Minolta Co., Osaka, Japan) to determine color values *L** (Lightness), *a** (redness-greenness) and *b** (yellowness-blueness). These values were used to calculate whiteness (W) [127] of starch powders and hue angle (H) and chroma (C) [128] in chickpea seeds and flours, using the following equations:

(3) H= arc tan (b*/a*) x degree; if a<0 and b>0 then H= 180+ arc tan (b*/a*) x degree.
(4) $C=[a^{*2}+b^{*2}]^{1/2}$
(5) $W= 100-[(100-L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$

2.5.2 Water absorption index (WAI) and water solubility index (WSI).

WAI and WSI were determinated according with the method reported by Du et al., (2014)[129]. Briefly, 2.5 g of flour were placed in a 50 mL centrifuge tube and 30 mL of water were added. The tubes were placed in a water bath at 70ºC by 30 min, and then were centrifuged at 3000 g by 10 min. The supernatant were decanted in a pre-weight glass vial to determinate solids and the tube was weight. The supernatant were placed overnight in an oven at 105ºC. The WAI and WSI were calculated according to the following formulas (6 and 7).

(6) WAI (g/g)=Weight of sediment/ Weight of flour sample
(7) WSI (%)= (Weight of dissolved solids in supernatant/Weight of flour sample) x100

2.5.3 Swelling power, water retention capacity (WRC) and solubility.

In order to understand their potential use of chickpea starches as food ingredients, some of the functional properties related with water absorption were tested; the swelling power was expressed as the amount of water in weight of the wet sediment to the initial weight of dry starch and was determined using a method reported by Tester and Morrison [130]. The starch solubility was expressed as the amount of the dried supernatant weight in relation to the initial weight of dry starch following the method reported by Schoch [131]. Though the water retention capacity (WRC) was assessed with the method reported by Bryant and Hamaker [132] with modifications. Briefly, chickpea starch/water dispersions (10% w/v) were heated in a water bath at 95°C with vortex homogenization every 5 minutes during 20 min. The tubes were then centrifuged for 15 min at 1000 *g* and the resulting supernatants decanted. The tubes with the pellets

were allowed to drain off excess water for 10 min at a 45° angle and the differences in weight were used to calculate the WRC.

2.6 Rapid viscoamylographs

The chickpea flours or starches (3 g) were mixed with distilled water (25 g) in an aluminum canister, then heated and cooled in a Rapid Visco-Analyser (RVA model 1170, Newport Scientific, Warriewood, NSW, Australia) using the following profile: heating from 50 to 95ºC at 5°C/min; temperature held at 95°C for 7 min and then cooling from 95 to 50°C at a rate of - 6ºC/min. The viscosity values during heating, cooking and cooling were calculated using the Thermocline software (Ver. 3.15.3.347).

2.7 Thermal properties

The gelatinization characteristics of the chickpea flours and starches were determined with a differential scanning calorimeter (Diamond DSC, Perkin Elmer, Nortfolk, VA, USA). 3 mg of each starch sample was placed in a stainless steel pan, mixed with 7 mg of deionized water and hermetically sealed. Once hydrated, the sample was allowed to equilibrate at room temperature for 1 h. Samples were kept 2 min at 30ºC then heated from 30 to 95ºC at a heat rate of 10ºC/min and the temperature was held at 95°C for 1.5 min. The DSC was calibrated using indium as a standard and during the experiments an empty steel pan was used as reference. In order to examine the retrogradation effects of starch molecules on the chickpea flours, the scanned pans were cooled down to 25°C, following by storage at 4 °C for 7 days before re-scanned under the same conditions. The onset temperature (To_g), peak temperature (Tp_g), conclusion temperature (Tc_g), enthalpy of gelatinization (ΔH_g), peak temperature (Tp_r), and enthalpy of retrogradation (ΔH_r) were calculated with the Pyris software (Perkin Elmer, Norwalk, CT, USA).

2.8 Starch granule morphology

The starch granules morphology and birefringence patterns were observed with a Motic BA-210 digital microscope (Hong Kong, China). The images were recorded at the same magnification (x40) under normal and polarized light. Additionally, scanning electron micrographs were acquired with a Nova Nano Scanning electron microscope (FEI Company, Eindhoven, Netherlands). For this, the dried samples were mounted in an aluminum stub using carbon conductive tape, and then examined at an accelerating voltage of 10 kV in low vacuum mode and using a helix detector.

2.9 Attenuated total reflectance-Fourier transformed infrared spectroscopy (ATR-FTIR)

The ATR-FTIR analysis of starches were recorded on an FTIR spectrometer (Spectrum 1, Perkin Elmer) using an attenuated total reflectance (ATR) mode [133]. For each

spectrum, 20 scans were recorded at a resolution of 4 cm^{-1} at room temperature (≈25°C). The obtained spectra were baseline-corrected, normalized and deconvoluted over the range of 1200–800 cm^{-1}, with a half-width of 22 cm^{-1}, with a resolution enhancement factor of 1.5. The amplitudes of absorbance for each spectrum at 1022 and 1047 cm^{-1} were noted and the ratio 1047 cm^{-1}/1022 cm^{-1} was calculated in each sample in order to estimate the degree of crystalline to amorphous order of starches.

2.10 In vitro protein digestibility.

The multienzyme method proposed by Hsu et al., (1977) [134] was used to assess *in vitro* protein digestibility (*IVPD*). For this, 50 mL of chickpea flours (adjusted to 6.25 mg protein/mL) dispersions were prepared in hermetic containers using distilled water and their pH was adjusted to 8.0 with NaOH. To emulate common cooking procedures, these dispersions were first heated in a boiling water bath for 30 min and then their temperature decreased to reach 37°C, all with magnetic stirring. After this cooking process, a multienzyme solution, consisting of a mixture of porcine pancreatic trypsin type IX, bovine pancreatic chymotrypsin type II and type XIV protease from *Streptomyces griseus* (Sigma Chemical CO. St. Louis, MO, USA) was prepared and five milliliters aliquots of this solution were added to each protein suspension pH 8.0. The rapid drop in pH was recorded during a 10 min period using a pH meter (Orion Star A series, Thermo fisher Scientific Inc.). The *IVPD* was calculated using the following equation (8).

(8) $IVPD = 210.46 - 18.10 \times pH$

2.11 Protein nutritional parameters.

a) Amino acid profile.

In order to evaluate the amino acid profile of thermally-processed chickpea flours to emulate consumption conditions, the flours were first dispersed in water (13% w/v) under agitation for 20 min at room temperature and then immediately heated in boiling water bath (>95ºC) with constant magnetic stirring (300 rpm) for 30 min. The resulting dispersion was cooled down to room temperature and immediately freeze-dried and stored at -20°C until analysis. The amino acid profile was determined following the method 982.30 [135].

b) Amino acid score

The amino acid score (AAS) was calculated according FAO [136] procedure, using the following formula (9):

(9) **AAS**= Sample essential amino acids contents / recommended essential amino acids

c) Calculated protein efficiency ratio

The calculated protein efficiency ratio values (cPER) of chickpea flours were determined using their amino acids composition of chickpea flours based on the followed equations (10, 11 and 12) [137]:

(10) **$cPER_1$**= -0.684 + (0.456 x Leu) - (0.047 x Pro) x
(11) **$cPER_2$**= -0.468 + (0.454 x Leu) - (0.105 x Tyr)
(12) **$cPER_3$**= -1.816 + (0.435 x Met)+ (0.78 x Leu) + (0.211 x Hys) - (0.944 x Tyr)

d) PDCAAS

Protein digestibility corrected amino acid score (PDCAAS) was calculated accordingly with the following:

(13) **PDCAAS**= (Lowest individual essential amino acid score) x (*IVPD)*.

e) Predicted biological value (pBV).

The predicted biological value was calculated using the following equation (11) [138] :

(14) **pBV**=$10^{2.15}$ x $Lys^{0.41}$ x $(Phe + Tyr)^{0.60}$ x $(Met + Cys)^{0.77}$ x $Thr^{2.4}$ x $Trp^{0.21}$

Where each Amino acid symbol represents: % Amino acid of sample / % amino acid FAO pattern, if Amino acid of sample <% Amino acid FAO pattern. % Amino acid FAO pattern / % Amino acid of sample, if % Amino acid of sample > % Amino acid FAO pattern.

2.12 *In vitro* starch digestibility and Predicted glycemic index

The *in vitro* starch digestibility was determined according to the Englyst protocol [139], with modifications. Chickpea flours were hydrated (400 mg) with 25 mL of distilled water into hermetic polypropylene tubes, which were heated in a boiling water bath during 30 min to emulate cooking, after the time, the samples were cooled down to 37°C and their pH dropped to 2.5 with HCl, followed by pepsin (P7000) digestion during 30 min. After the pepsin hydrolysis, the pH was neutralized and processed. Also, starch samples were analyzed in both raw and gelatinized or cooked forms. The gelatinized starch hydrolysis was performed in order to understand the behavior in more practical cooked food systems. In this case, hermetic polypropylene tubes with the starch dispersions (100 mg in 4 mL of 0.5 M sodium acetate buffer pH 5.2) were heated in a boiling water bath with constant magnetic stirring (300 rpm) for 20 min, after they were allowed to cool at 37°C in a water bath and processed. The flours and starch samples were digested by one milliliter of an enzyme solution, consisted of a mixture of 2.5 mL of the supernatant of porcine pancreatic α-amylase (0.45 g in 4 mL of water, centrifuged at

1500 *g* for 15 min), 0.3 mL of amyloglucosidase and 0.2 mL of invertase were mixed thoroughly, and added to each test tube containing 5 glass beads (7 mm diameter), that were then incubated in a shaking water (200 strokes/min) at 37°C. Aliquots (0.1 mL) were taken at intervals and mixed with 1 mL of 80% ethanol. The hydrolyzed glucose content was measured with the glucose oxidase-peroxidase reagent. Starch classifications based on the rate of hydrolysis were: rapidly digestible (digested within 20 min) starch (RDS), slowly digestible (digested between 20 and 120 min) starch (SDS) and resistant (undigested after 120 min) starch (RS).
With the aim to estimate glycemic index of flours and starches, the hydrolysis data (from 0-180 min) from the starch digestion was used to calculate the hydrolysis index (HI), which was obtained from the area under the hydrolysis curve compared with the area obtained for the hydrolysis of a standard material (white bread) under the same conditions. The predicted glycemic index (pGI) was estimated with the equation reported by Goñi et al [140] which has a correlation coefficient of $R^2 = 0.89$, $p<0.05$:

(15) pGI = 39.71 + 0.549 (HI).

2.13 Simulated gastrointestinal digestion of protein concentrate

The chickpea concentrates from germinated and cooked chickpea were *in vitro* digested according to the method reported by Mosele et al [141]. The chickpea concentrates from processed samples were *in vitro* digested according to the method reported by Mosele *et a*l., (2015) [141]. The method consists in a simulated digestion process consisting of three sequential steps: mouth, stomach and small intestine. Briefly, 1 g of sample was suspended in phosphate buffer solution (pH 6.9) and 10 mg α-amylase (A3176, porcine pancreas, Sigma-Aldrich) supplemented for 5 min incubation at 37°C. For gastric digestion, the pH was adjusted to 2 (HCl) and 15 mg of pepsin (P7000, porcine gastric mucose, Sigma-aldrich) solution (in 1 mL 0.01N HCl) was added and shaken for e1 h at 37ºC; After that, the duodenal simulation was performed using a continuous-flow dialysis system, in which the sample (adjusted to pH 6.5) was added in a dialysis tube with 5 mL of duodenal juice (2.5 mL of bile salts (bile) and 8 g/L of pancreatin (P3292, porcine pancreas, Sigma-Aldrich) and covered by phosphate buffer solution (pH 7.4). After the programmed duodenal digestion, two fractions were collected (IN and OUT). IN is the non-absorbable fraction that reaches the colon whereas OUT represents the absorbable fraction. In this study, only the IN fraction was analyzed.

2.14 Saponins and flavoinds extraction.

Extractions were carried out as previously reported by Luthria *et al.,* (2007) [142] with some modifications. Briefly, the chickpea sample was extracted with 80% aqueous methanol (1:20 w/v) and mixed for 1 min. The solution was sonicated at 40 mHz, 135 W

for 15 min, centrifuged at 8,000 g at 4ºC in order to recover the superior phase. The extraction procedure was repeated and supernatants pooled. The samples were evaporated (45ºC) and then brought to 1 ml with 80% aqueous methanol. The dried extract of some chickpea samples was used to evaluate anti-inflammatory effect.

2.15 Total phenolic content.

Total phenolics content were determined using the Folin-Ciocalteu reagent as previously described [143]. Briefly, 100 µL of diluted extract was mixed with 625 µL of distilled water, 250 µL 7.5% (w/v) Na_2CO_3 and 25 µL Folin-Ciocalteu reagent. Samples were vortexed and incubated for 2 h at room temperature in darkness. The absorbance was measured at 739 nm using a microplate reader (Synergy HT microplate reader, BioTek Instruments). Total phenolics were quantified by external calibration using gallic acid. Samples were analyzed in triplicate and results expressed as mg of gallic acid equivalents (GAE) per g of sample (mg GAE/g).

2.16 Identification and quantification of flavonoids and saponins.

The identification of flavonoids and saponins in chickpeas cultivars and some processed samples were performed in an HPLC coupled to an ion trap (IT) mass detector (1100 series, Agilent technologies, Santa Clara, CA). Ionization was carried out using a electrospray source (ESI) at 300ºC and 4kV capillary temperature and voltage nebulizer pressure at 50 psi and nitrogen gas flow rate at 10 L/min. Range for mass scan covered from m/z 150 to 2000 and data was acquired in positive mode, as previous reported [144]. The separation was adapted for chickpea samples and achieved using an Eclipse XDB C18 column (3 mm x 150 mm, 3.5 µm; Agilent technologies, Santa Clara, CA) at flow rate 0.4 ml/min. The column temperature was 30ºC and injection volume was 2 µL. The mobile phase consisted in 0.1% of formic acid in water (solvent A) and acetonitrile (solvent B). The gradient elution was: the first 8 min increased from 0 to 10 % B, 8 to 16 min increased to 35% B, 16 to 26 min increased to 90% B and 26 to 36 min increased to 100% B.

The quantification of flavonoids and saponins in chickpeas were performed in an HPLC-DAD-ELSD. The separation was as described for HPLC-IT-MS. The injection volume was 2 µL and detection was recorded at 260 and 295 nm. The compounds were confirmed by UV spectra and retention time. Saponins were quantified using a soyasaponin I (SSI) standard curve (1-250 ppm) and reported as µg SSI eq/g. The isoflavones in chickpea were reported as Biochanin A equivalents (µg BA eq/g; 1-250 ppm).

2.17 Sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) gel electrophoresis

The protein content of samples was determined by the Detergent compatible Protein Assay (Biorad, Hercules, CA) using bovine serum albumin as standard protein. SDS-

PAGE analysis of of raw, germinated, concentrates and digestes from chickpea cultivars was performed loading 20 µg of protein/well on NuPAGE® Novex 4-12% Bis-Tris Gels (Invitrogen, Madrid, Spain). Gels were placed in an XCell-sure lock Mini-Cell and run at 200 V for 35 min under reducing conditions. NuPAGE® MES-SDS and NuPAGE® LDS (Invitrogen) were used as running and sample buffers, respectively. Gels were stained with SimplyBlue SafeStain (Invitrogen) for 1 h and distained in deionized water for 2 h. After destaining, an image of the gel was taken using a Chemdoc® XRS+ Imaging system (BioRad,). The molecular weight of poly- and oligopeptides was determined by comparison with the molecular weight marker Novex® Sharp Prestained Protein Standard (20-260 kDa) (Invitrogen).

2.18 Protein and peptide content of protein concentrates.

Soluble protein quantification was carried out in duplicate using the DC (Detergent compatible) Protein Assay (Biorad). Bovine serum albumin was used as standard at a concentration range from 0.1 to 1 mg/mL. Peptide concentration was measured by the Quantitative Colorimetric Peptide Assay kit from PierceTM (Rockford, IL, USA). Results were expressed as mg/g sample in dry weight (DW).

2.19 Anti-inflammatory activity

2.19.1. Macrophages cell culture and treatment protocol

The mouse macrophage cell line RAW 264.7 was grown in DMEM supplemented with 10% of FBS and 1% penicillin/streptomycin. Cells were plated at densities of 1×10^6 cells in 75 cm^2 tissue culture flasks and maintained at 37 °C under 5% CO_2 in a humidifier incubator until 90% of confluence. The culture medium was changed every 2 days. After a confluent monolayer appeared, subculturing cell process was carried out.

2.19.2. Determination of cell viability in macrophages cell cultures

Fifty thousand cells were plated in a 96-well plate in 200 µL volume and allowed to attach overnight in humidified 5% CO_2 incubator at 37 ºC. Cells were treated with protein isolates and digests (0.5-5 mg/mL) and phenolic extracts (0.1-0.5 mg/mL) dissolved in serum-free medium for 24 h. After treatment for indicated time, medium was removed and cell viability was determined by using the Cell Titer 96® AQueous One Solution Proliferation Assay kit (Promega, Madison, WI). Briefly, 20 µL of Cell Titer 96® solution followed by 100 µL of serum free DMEM were added. After 45 min of incubation, absorbance was read at 490 nm in a microplate reader Synergy MX (BioTek Instruments, Winooski, VT, USA). The viability was calculated considering controls (non-treated cells) as 100% viable. All experiments were performed in three independent trials with three replicates per trial.

2.19.3. Nitric oxide quantification in macrophages culture medium

Anti-inflammatory activity was investigated through determination of NO production. Nitrite accumulation, and indicator of NO synthesis, was measured in the macrophages culture medium by the Griess reaction according to a previously described method [145]. Briefly, 100 µL of medium were plated in 96-well plate and an equal amount of Griess reagent constituted by 1% (w/v) sulfanil amide and 0.1% (w/v) N-1-(naphthyl) ethylenediamine-diHCl in 2.5% (v/v) H_3PO_4, was added. The plate was incubated for 15 min and the absorbance measured at 550 nm in a microplate reader (BioTek Instruments). The amount of NO was calculated using a sodium nitrite standard curve (0-10 µg/mL). All experiments were performed in three independent trials with three replicates per trial.

2.20 Purification of peptides by preparative RP-HPLC

Further peptide purification of the most active chickpea digest was performed by preparative RP-HPLC. Peptides separation was performed on a HPLC system (Waters, Mildford, MA, USA) equipped with four pumps, a pump controller (Binary Gradient Module 2545), a System Fluidic Organizer, an autosampler (Sample Manager 2767) and a diode array detector (module 2998). The data-processing software was Empower 2 (Waters). A 250 x 21.5 mm Hi-Pore 318 reverse phase column (BioRad) was used. The peptide fractions were dissolved in solvent A at concentration of 100 mg/mL, and the injection volume was 1500 µL. Fractions were eluted at a flow rate of 10 mL/min, in isocratic with solvent A (water:TFA 1000:1 v/v) for 10 min followed by a linear gradient of solvent B (acetonitrile:trifluoroacetic acid (TFA) 1000:0,8 v/v) in A going from 5% to 45% B in 15 min. Each chromatographic run was repeated 3 times and the fractions were collected automatically with a fraction collector (Sample Manager 2767). The collection times for both fractions were: F1 (1.5-4.5 min), F2 (7.5-8.5 min), F3 (18-21min) and F4 (24-24.5 min). The collected fractions were pooled, freeze-dried and stored at -20 °C until further analysis. Quantification of peptides in each fraction was performed by a colorimetric assay using a peptide colorimetric assay kit according to the manufacturer's protocol.

2.21 Peptide identification by nanoUPLC–ESI-MS/MS.

For peptide identification, sample separation was carried out on an Easy-nLC 1000 nano system (Thermo Scientific). For each analysis, the sample was loaded into a precolumn Acclaim PepMap 100 (Thermo Scientific) and eluted in a RSLC PepMap C18 (15 cm x 75 µm x 3 µm; Thermo Scientific). The mobile phase flow rate was 300 nL/min using 0.1% formic acid in water (solvent A) and 0.1% formic acid and 100% acetonitrile (solvent B). The gradient profile was set as follows: 5%–35% solvent B for 100 min, 35%-100% solvent B for 10 min, 100% solvent B for 20min. Four microliters of each sample was injected. MS analysis was performed using a Q Exactive mass spectrometer (Thermo Scientific). For ionization, 2000 V of liquid junction voltage and

270 °C capillary temperature was used. The full scan method employed a m/z 400–1500 mass selection, an Orbitrap resolution of 70,000 (at m/z 200), a target automatic gain control (AGC) value of 3e6, and maximum injection times of 100 ms. After the survey scan, the 15 most intense precursor ions were selected for MS/MS fragmentation. Fragmentation was performed with a normalized collision energy of 27 eV and MS/MS scans were acquired with a starting mass of m/z 100, AGC target was 2e5, resolution of 17,500 (at m/z 200), intensity threshold of 8e3, isolation window of 2 m/z units and maximum IT was 100 ms. Charge state screening was enabled to reject unassigned, singly charged, and equal or more than seven protonated ions. A dynamic exclusion time of 20s was used to discriminate against previously selected ions. MS data were analyzed with Proteome Discoverer (version1.4.1.14) (Thermo) using standardized workflows. Mass spectra *.raw files were searched against files were searched against Uniprot *Cicer arietinium* database (29535 sequences protein entries) using SEQUEST search engine. Precursor and fragment mass tolerance were set to 10 ppm and 0.02 Da, respectively, allowing 2 missed cleavages, carbamidomethylation of cysteines as a fixed modification, methionine oxidation as a variable modification. Identified peptides were filtered using Percolator algorithm 9 (1) with a q-value threshold of 0.01.

2.22 Statistical analysis

All statistical analyses were performed with the use of JMP 13 software from SAS institute (Cary, NC, USA). All experiments and procedures were performed in triplicate, unless otherwise specified. The results were reported as mean $\pm$ standard error mean (sem) or standard deviation (SD). Data was analyzed by one-way ANOVA followed by Tukey test to detect differences among chickpea cultivars. A p-value>0.05 was considered significant. Two way ANOVA with interaction were performed in germination data in order to detect differences caused by genotype, germination time and its interactions. Correlation coefficients were obtained by multivariate analysis. Principal component analysis (PCA) was carried out in some experimental sections in order to visualize similarities and differences among the variables. PCA in flours was performed on color, chemical and protein and starch digestion properties. Also a clustering analysis was performed on the same variables to evaluate similarities among samples, a K-means clustering method analysis was used to determinate the number of groups. In starches, a PCA was carried out to visualize similarities and differences among chickpea starches in terms of functionality and digestibility parameters.

Chapter 3. Results and Discussion

3.1 Techno-functional and nutritional characterization of chickpea flours varying seed coat color.

3.1.1 Chemical composition on chickpea flours.

The chemical composition of chickpea flours varied significantly ($p<0.05$) among cultivars. The protein content (Table 1) fluctuated from 20.66 to 24.83 g/100g, being R.ICC13124 and B.ICC3761 the flours with the lowest and highest values, respectively. The differences in protein content are mainly attributed to genetic background of the cultivars [146]. The ash and lipid contents in chickpea flours ranged from 2.50 to 3.27 g/100g and 2.77 to 5.02 g/100g, respectively. These values agreed with previous reports [40, 146]. The G.ICC5613 chickpea flour showed the highest content in starch (46.23 g/100g) and B.ICC3761 the lowest (35.78 g/100g). Others authors have reported similar starch contents (up to 45 g/100g) in chickpea flours [129, 147]. Dietary fiber in chickpea flours was determined as total and subdivided as insoluble (IDF) and soluble (SDF); however, no significant differences were detected among chickpea samples. IDF and SDF varied from 5.35 (B.ICC4418) to 5.99 g/100g (B.ICC6306) and 4.01 g/100g (B.ICC6306) to 5.02 (C.BS), respectively. C.BS flour (10.52 g/100g) contained the highest total dietary fiber whereas B.ICC4418 the lowest (9.71 g/100g). Higher values of TDF ranging from 18 to 22 g/100g have been reported by others [4, 147].

The β-glucans contents in chickpea flours varied significantly ($p<0.05$) from 0.96 to 2.42 g/100g, the control C.BS flour had the lowest whereas the Br.ICC3512 the highest. Lower values have been reported in chickpea (0.2 and 0.9%) [68, 69]. In this study, Desi cultivars with seed coats red, black, green and brown showed higher values of β-glucans and also had higher amounts of seed coats (9.9-13.6%) compared to the seed coats associated to the control C.BS seeds (3.6-4.1%) [148]. Thus, a strong positive correlation ($r=0.8258$, $p=0.0032$) between β-glucans and seed coat percentage was observed clearly indicating that the β-glucans are associated to the cell walls of the fibrous rich testa. Wood et al. (2011) concluded that Desi chickpeas contain higher β-glucans in the outer palisade layer of the seed coats compared to the Kabulli seed coats. The chickpea flours showed total phenolic content (TPC) from 236.58 (C.BS) to 444.41 (B.ICC3761) µg GAE/g. Similar TPC values have been reported in colored chickpea (260-370 µg GAE/g) [40]. In contrast, higher values of TPC (1.5 to 6.8 mg catechin Eq/g) have been reported in other colored chickpeas [39]. The only Kabulli cultivar (C.BS) studied herein contained significant lower TPC compared to the experimental Desi cultivars agreeing with results previously discussed by others [39, 40]. The seed physical properties, genetic background and seed coat color affected TPC differences observed among the chickpea samples as previously documented by other authors [39].

Table 3.1.1 Chemical, dietary fiber and total phenolic compositions of raw chickpea flours (db).

Cultivar	Protein (g/100g)	Lipids (g/100g)	Ash (g/100g)	Total starch (g/100g)	Fiber (g/100g)			β-glucans (g/100g)	TPC (µg GAE/g)
					IDF	SDF	Total		
C.BS^{A}	21.50±0.29^{e}	3.03±0.02^{g}	2.50±0.01^{d}	38.27±0.25^{def}	5.50±0.24^{a}	5.02±0.08^{a}	10.52±0.48^{a}	0.96±0.00^{i}	236.58±2.85^{f}
B.ICC6306^{B}	23.73±0.23^{bcd}	3.48±0.03^{de}	2.71±0.00^{cd}	40.28±0.77^{cde}	5.99±0.15^{a}	4.01±0.21^{a}	10.00±0.11^{a}	2.26±0.00^{b}	351.60±0.99^{c}
B.ICC4418^{B}	24.13±0.09^{ab}	3.40±0.02^{ef}	3.02±0.07^{abc}	37.00±0.74^{ef}	5.35±0.49^{a}	4.36±0.29^{a}	9.71±0.06^{a}	2.26±0.00^{b}	402.60±1.15^{b}
B.ICC3761^{B}	24.83±0.08^{a}	2.89±0.03^{gh}	3.15±0.08^{ab}	35.78±1.08^{f}	5.37±0.23^{a}	4.80±0.50^{a}	10.16±0.05^{a}	2.22±0.01^{c}	444.41±2.02^{a}
Br.ICC3512^{B}	23.17±0.30^{cd}	3.31±0.04^{f}	2.80±0.10^{d}	43.42±1.06^{abc}	5.97±0.30^{a}	4.50±0.16^{a}	10.47±0.46^{a}	2.42±0.00^{a}	241.25±1.76^{f}
C.ICC3421^{B}	23.85±0.14^{bc}	5.02±0.06^{a}	3.27±0.13^{a}	42.00±1.28^{bcd}	5.44±0.11^{a}	4.71±0.40^{a}	10.15±0.40^{a}	1.02±0.01^{h}	344.68±7.20^{c}
G.ICC5613^{B}	22.88±0.04^{d}	2.77±0.02^{h}	3.02±0.05^{abc}	46.23±0.74^{a}	5.51±0.01^{a}	4.82±0.06^{a}	10.33±0.39^{a}	1.58±0.00^{e}	276.68±3.05^{e}
R.ICC14782^{B}	20.74±0.16^{f}	4.50±0.03^{b}	2.75±0.02^{cd}	42.97±0.50^{abc}	5.42±0.16^{a}	4.15±0.47^{a}	9.74±0.10^{a}	1.44±0.00^{g}	329.09±1.73^{d}
R.ICC13124^{B}	20.66±0.50^{f}	4.20±0.04^{c}	2.85±0.01^{bcd}	44.90±0.46^{ab}	5.59±0.04^{a}	4.34±0.33^{a}	9.76±0.17^{a}	1.48±0.00^{f}	328.94±1.52^{d}
R.ICC5383^{B}	23.27±0.18^{cd}	3.61±0.02^{d}	2.85±0.04^{bcd}	39.79±0.31^{cde}	5.52±0.27^{a}	4.51±0.05^{a}	10.30±0.16^{a}	1.70±0.01^{d}	359.29±3.45^{c}

IDF: insoluble dietary fiber, SDF: soluble dietary fiber. TPC: total phenolic content, BS: cultivar Blanco Sinaloa 92, B: Black color, Br: Brown color, C: Cream color, G: Green color, R. Red color. ^{A} Kabuli seeds. ^{B} Desi seeds. Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p<0.05$).

3.1.2 Techno-functional properties of chickpea flours

3.1.2.1 Physicochemical properties

The color parameters (L, a, b, chroma and hue angle) in chickpea flours are reported in Table 2. The color parameters varied significant among flours. The L* values ranged from 73.13 (G.ICC5613) to 83.72(R.ICC8383), which are in the expected range among light and pale flours. The a* and b* values ranged from -3.33 (G.ICC5613) to 2.40 (R.ICC13124) and 15.84 (B.ICC3761) to 23.96 (R.ICC14782), respectively. The flours from seed colored chickpeas showed a yellow color except for G.ICC5613, which is the only cultivar, which also have green colored cotyledons. This correspond with the hue angle values (H) of 83.85º to 97.24º; in which H=90º(yellow) and H= 180º (bluish-green)[128]. The chroma (C) values ranged from 15.86 (B.ICC3761) to 24.08 (R.ICC14782), which indicate the saturation color of the samples.

The Water Absorption Index (WAI) and Water solubility index (WSI) are summarized in Table 2. WAI showed significant differences among cultivars and varied from 3.94% (C.ICC3421) to 4.74%(B.ICC4418). WAI is usually related with starch-water interaction but pulse flours have diverse components that can induce different interactions with water, no only the water absorption and swelling of starch. Likewise WAI have been associated with gelation capacity of starch and protein on flours [129, 149]. WSI varied significant among chickpea flours, the B.ICC4418 flour (17.51%) showed the lowest value whereas the R.ICC13124 (28.20%) the highest value. Similar WSI values have been reported in chickpea flours analyzed by others [129].

3.1.2.2 Flour pasting properties.

The pasting properties of chickpea flours are summarized in Table 3. The pasting temperature (PT) in chickpea flours varied significantly ($p<0.05$) from 76.35 ºC (C.BS) to 81.25 ºC (C.ICC3421). PT is an indicator of the minimum temperature needed to cook the flour. PT of chickpea flours showed strong negative correlation to WAI (*r=-0.8303,p=0.0029*) and positive with protein content (*r=0.6785,p=0.0310*). The viscosity parameters during pasting have been related to swollen granules properties and the swollen material that leached out from the granules [146]. The C.ICC3421 flour showed the lowest values of peak (958cP), through (879 cP) and final viscosity (1141 cP), while the R.ICC14782 flour showed the highest viscosities, 1265 cP, 1226 cP and 1541cP, respectively. The setback viscosity (SBV) shows the tendency to retrogradation o syneresis of the flour during cooling of the cooked paste [146, 149]. The SBV in chickpea flour varied significantly ($p<0.05$) from 260 to 238 cP, in which the Br.ICC3512 flour was the lowest and G.ICC5613 the highest. The breakdown viscosity (BV) determines the stability of the paste by the disintegration of swollen granules during shearing [146]. Relatively low BV values relate to more paste stability, which could be correlated with seed matrix (Chung et al., 2008). BV was higher in C.ICC3421 flour and

Table 3.1.2. Physicochemical properties of chickpea flours.

Cultivar	Color					WSI (%)	WAI (g/g)
	L*	a*	b*	H	C		
C.BS[A]	82.57±0.01[b]	1.32±0.01[f]	18.06±0.02[g]	85.83±0.02[c]	18.10±0.02[h]	27.37±0.11[b]	4.73±0.03[ab]
B.ICC6306[B]	79.33±0.06[de]	2.06±0.01[c]	22.71±0.01[b]	84.80±0.02[e]	22.80±0.01[b]	27.41±0.11[b]	4.08±0.05[de]
B.ICC4418[B]	76.96±0.25[f]	1.50±0.01[e]	19.88±0.03[f]	85.68±0.02[d]	19.93±0.03[g]	17.51±0.07[f]	4.74±0.12[a]
B.ICC3761[B]	77.09±0.21[f]	0.76±0.01[g]	15.84±0.10[h]	87.24±0.04[b]	15.86±0.10[i]	27.49±0.11[b]	4.52±0.08[abcd]
Br.ICC3512[B]	78.43±0.12[e]	2.33±0.01[b]	21.54±0.02[d]	83.82±0.02[h]	21.67±0.02[e]	22.90±0.09[c]	4.20±0.03[cde]
C.ICC3421[B]	80.80±0.02[c]	2.07±0.01[c]	20.59±0.02[e]	84.26±0.02[fg]	20.69±0.02[f]	21.58±0.09[e]	3.92±0.10[e]
G.ICC5613[B]	73.13±0.51[g]	-3.33±0.02[h]	21.66±0.07[d]	89.74±0.07[a]	21.91±0.07[d]	27.33±0.11[b]	4.29±0.16[bcde]
R.ICC14782[B]	80.20±0.01[cd]	2.42±0.00[a]	23.96±0.01[a]	84.22±0.01[g]	24.08±0.01[a]	22.83±0.09[cd]	4.71±0.00[ab]
R.ICC13124[B]	80.79±0.12[c]	2.40±0.01[a]	22.33±0.01[c]	83.85±0.01[h]	22.46±0.01[c]	28.20±0.11[a]	4.55±0.05[abc]
R.ICC5383[B]	83.72±0.01[a]	1.77±0.01[d]	17.98±0-01[g]	84.36±0.02[f]	18.07±0.01[h]	22.33±0.09[d]	4.57±0.03[abc]

H: hue angle, C: Chroma, WSI, Water solubility index. WAI, Water absorption index, BS: cultivar Blanco Sinaloa 92, B: Black color, Br: Brown color, C: Cream color, G: Green color, R. Red color. [A] Kabuli seeds. [B] Desi seeds. Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p<0.05$).

Table 3.1.3. Pasting properties of chickpea flours.

Cultivar	Pasting temperature (ºC)	Viscosity (cP)				
		Peak	Through	Final	Setback	Breakdown
C.BS[A]	76.35 ±0.04^{g}	1041 ±18^{ef}	986 ±17de	1253 ±14de	267 ±4^{ef}	55 ±0^{c}
B.ICC6306[B]	80.40 ±0.02^{b}	1185 ±20^{abc}	1135 ±16^{abc}	1425 ±16^{b}	290 ±5^{cde}	50 ±0^{d}
B.ICC4418[B]	79.65 ±0.05^{c}	979 ±16^{f}	918 ±15^{ef}	1195 ±13^{ef}	277 ±4^{def}	61 ±1^{b}
B.ICC3761[B]	78.75 ±0.05^{d}	1085 ±18de	1009 ±17de	1288 ±14^{cd}	279 ±4^{f}	76 ±1^{a}
Br.ICC3512[B]	80.45 ±0.05^{b}	1204 ±20^{ab}	1171 ±20^{ab}	1430 ±16^{b}	260 ±4^{f}	33 ±0^{f}
C.ICC3421[B]	81.25 ±0.02^{a}	958 ±16^{f}	879 ±15^{f}	1141 ±13^{f}	262 ±4^{f}	79 ±1^{a}
G.ICC5613[B]	79.65 ±0.07^{c}	1038 ±17^{ef}	985 ±17de	1313 ±15^{cd}	328 ±5^{a}	53 ±0^{cd}
R.ICC14782[B]	77.25 ±0.07^{f}	1265 ±21^{a}	1226 ±21^{a}	1541 ±17^{a}	315 ±5^{abc}	39 ±0^{e}
R.ICC13124[B]	78.10 ±0.05^{e}	1149 ±19^{bcd}	1107 ±19^{bc}	1431 ±16^{b}	324 ±5^{ab}	42 ±0^{e}
R.ICC5383[B]	78.75 ±0.07^{d}	1105 ±19^{cde}	1045 ±18^{cd}	1347 ±15^{c}	302 ±5^{bcd}	60 ±1^{b}

BS: cultivar Blanco Sinaloa 92, B: Black color, Br: Brown color, C: Cream color, G: Green color, R. Red color. [A] Kabuli seeds [B] Desi seeds. Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p<0.05$).

lower in the Br.ICC3512 counterpart. The BV showed a positive correlation with protein (*r=0.6501, p=0.0418*) and ash contents (*r=0.6727,p=0.0330*).

3.1.2.3. Flours thermal properties

The gelatinization and retrogradation properties of chickpea flours are depicted in Table 4. The onset (To_g), peak (Tp_g) and conclusion (Tc_g) and gelatinization range (ΔT_g) temperatures varied significantly ($p<0.05$) among chickpea flours. The C.ICC3421 flour required the highest To_g (82.42ºC), while the B.ICC4418 the highest Tp_g (87.77ºC) and Tc_g (90.26ºC) in contrast to the control C.BS flour that showed the lowest To_g (77.35ºC), Tp_g (80.16ºC) and Tc_g (83.76ºC) values. These results agree with previous publications in which kabulli chickpeas had lower values of gelatinization temperatures compared to Desi types [149, 150]. The PT values were positive correlated with gelatinization, To_g (*r=0.8826, p=0.0007*), Tp_g (*r=0.7751,p=0.0085*) and Tc_g (*r=0.8525, p=0.0017*) temperatures. Only To_g was negative correlated with WAI (*r=-0.8296, p=0.0030*). On the other hand, β-glucan contents showed a positive correlation with Tp_g (*r=0.7845,p=0.0072*) whereas protein contents a positive correlation with both Tp_g (*r=0.7356,p=0.0153*) and Tc_g (*r=0.7249, p=0.0177*). Several authors (Chung et al., 2008; Kaur & Singh, 2005) have concluded that the protein content and starch structure influences the gelatinization temperatures of different pulse flours. The enthalpy of gelatinization (ΔH_g) ranged from 6.28 to 7.71 J/g but was not statistically different among chickpea flours. ΔH_g showed a correlation with TPC (*r=0.7689, p=0.0093*) and TDF (*r=-0.6668, p=0.0352*). According to (Li & Zhu, 2017) the thermal properties of flours are affected by interactions among dietary fiber, water and starch or by interactions of phenolic compounds with starch through hydrogen bonding [151]. The flours retrogradation was determined after sample storage at 4ºC for 7 days. The peak temperature (Tp_r) of retrograded flours ranged from 60.92ºC (Br.ICC3512) to 76ºC (R.ICC5383). The enthalpy of retrogradation (ΔH_r) was not statistically different among chickpea flours and ranged from 3.51 to 4.41 J/g. As expected, the retrogradation thermal parameters Tp_r and ΔH_r were lower compared with gelatinization parameters (Tp_g; ΔH_g). The ΔH_r was correlated with SBV (*r=0.6663, p=0.0354*), which is closely related to retrogradation and the behavior during storage.

3.1.3 Essential amino acid content in cooked chickpea flours

The amino acid profiles and amino acid scores of the cooked chickpea flours are reported in Table 5. The individual amino acid contents showed significant variations among cultivars. For instance, the contents of His (2.2-2.4 g/100g protein), Lys (6.16-7.46 g/100g protein), Leu (6.60-7.71 g/100g protein), Ile (3.77-4.46g/100g protein), Phe+Tyr (7.54-8.26 g/100g protein) and Val (3.93-4.56 g/100g protein) were above the FAO recommendations for preschool children in all samples ([136]. According to [4], the

Table 3.1.4. Effect of chickpea type on gelatinization and retrogradation of flours.

Cultivar	Gelatinization					Retrogradation	
	To_g (°C)	Tp_g (°C)	Tf_g (°C)	ΔT_g (°C)	ΔH_g (J/g)	Tp_r (°C)	ΔH_r (J/g)
C.BS[A]	77.35+0.39^{e}	80.16±0.24^{d}	83.76±0.36^{c}	6.41±0.54^{bc}	6.28±0.49^{a}	64.85±0.19^{c}	3.94±0.40^{a}
B.ICC6306[B]	81.26+0.22^{ab}	86.36±0.53^{ab}	90.21±0.27^{a}	8.95±0.31^{a}	6.43±0.30^{a}	67.58±0.09^{b}	4.36±0.38^{a}
B.ICC4418[B]	80.42+0.54^{bc}	87.77±0.07^{a}	90.26±0.55^{a}	9.84±0.45^{a}	7.26±0.49^{a}	64.05±0.28^{cd}	4.24±0.09^{a}
B.ICC3761[B]	78.26+0.36^{de}	86.56±0.42^{ab}	87.42±0.05^{b}	9.16±0.00^{a}	7.71±0.52^{a}	64.94±0.54^{c}	3.77±0.25^{a}
Br.ICC3512[B]	81.15+0.47^{ab}	87.75±0.00^{a}	89.33±0.37^{a}	8.18±0.14^{ab}	6.39±0.54^{a}	60.92±0.27^{e}	3.71±0.42^{a}
C.ICC3421[B]	82.42+0.05^{a}	85.33±0.49^{b}	88.66±0.23^{ab}	6.24±0.47^{bcd}	6.67±0.57^{a}	62.63±0.40^{d}	3.51±0.46^{a}
G.ICC5613[B]	81.25+0.51^{ab}	86.98±0.57^{ab}	89.96±0.31^{a}	8.71±0.47^{a}	6.39±0.28^{a}	63.98±0.10^{cd}	4.21±0.40^{a}
R.ICC14782[B]	78.56+0.50^{cde}	82.41±0.05^{c}	84.51±0.46^{c}	5.95±0.38^{cd}	7.25±0.53^{a}	63.23±0.10^{d}	4.10±0.42^{a}
R.ICC13124[B]	80.21+0.12^{bcd}	82.26±0.31^{c}	84.33±0.42^{c}	4.12±0.52^{d}	7.45±0.45^{a}	63.16±0.57^{d}	4.41±0.50^{a}
R.ICC5383[B]	80.11+0.35^{bcd}	83.11±0.01^{c}	85.26±0.23^{c}	5.15±0.49^{cd}	7.48±0.01^{a}	76.00±0.13^{a}	3.86±0.46^{a}

To_g: onset temperature, Tp_g, peak temperature, Tc_g: conclusion temperature, ΔT_g: gelatinization range (Tc-To), ΔH_g: enthalpy of gelatinization, Tp_r, peak temperature, ΔH_r: enthalpy of retrogradation, BS: cultivar Blanco Sinaloa 92, B: Black color, Br: Brown color, C: Cream color, G: Green color, R. Red color. [A] Kabuli seeds. [B]Desi seeds. Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p<0.05$).

Table 3.1.5. Effect of chickpea type on essential amino acids and amino acid score of cooked flours.

Cultivar	Essential amino acids (g/100g protein)									Total EAA (33.9)[C]
	His (1.90)[C]	Lys (5.80)[C]	Leu (6.60)[C]	Ile (2.80)[C]	Val (3.50)[C]	Phe+Tyr (6.30)[C]	Thr (3.40)[C,]	Trp (1.10)[C,]	Met+Cys (2.50)[C,]	
C.BS[A]	2.2±0.01^{f}	6.1±0.02^{f}	6.6±0.02^{e}	3.7±0.01^{f}	3.9±0.01^{e}	7.5±0.02^{f}	2.9±0.01^{f}	0.8±0.00^{e}	2.3±0.01^{f}	36.38±0.09^{f}
B.ICC6306[B]	2.4±0.02^{bcd}	6.6±0.04^{e}	6.9±0.05^{d}	4.0±0.03^{e}	4.1±0.03^{d}	7.7±0.05^{ef}	3.1±0.02^{e}	0.9±0.01^{cd}	2.7±0.02^{e}	38.86±0.26^{e}
B.ICC4418[B]	2.4±0.01^{bcde}	6.9±0.03^{cd}	7.2±0.03^{bc}	4.1±0.02^{bcd}	4.3±0.02^{bc}	8.1±0.03^{ab}	3.2±0.01^{de}	0.8±0.00^{cd}	2.8±0.01^{e}	40.16±0.16^{bcd}
B.ICC3761[B]	2.4±0.01^{e}	6.8±0.02^{cd}	7.2±0.02^{bc}	4.1±0.01^{cde}	4.1±0.01^{cd}	8.0±0.02^{bcd}	3.3±0.01^{cd}	0.8±0.00^{e}	2.8±0.01^{de}	39.76±0.11^{cde}
Br.ICC3512[B]	2.4±0.03^{bcde}	6.8±0.07^{d}	7.1±0.08^{cd}	4.1±0.05^{cde}	3.9±0.04^{e}	7.8±0.09^{de}	3.2±0.04^{de}	0.8±0.01^{d}	2.9±0.03^{c}	39.40±0.43^{de}
C.ICC3421[B]	2.4±0.00^{bc}	6.6±0.01^{e}	6.9±0.01^{d}	4.0±0.01^{de}	4.1±0.01^{d}	7.8±0.01^{de}	3.2±0.01^{de}	1.0±0.00^{b}	2.8±0.00^{e}	39.22±0.06^{de}
G.ICC5613[B]	2.6±0.01^{a}	7.4±0.03^{a}	7.7±0.04^{a}	4.4±0.02^{a}	4.5±0.02^{a}	8.2±0.04^{a}	3.6±0.02^{a}	0.9±0.00^{cd}	3.2±0.02^{a}	42.80±0.20^{a}
R.ICC14782[B]	2.4±0.01^{b}	7.1±0.02^{b}	7.4±0.02^{b}	4.2±0.01^{b}	4.3±0.01^{b}	8.1±0.02^{abc}	3.5±0.01^{b}	0.9±0.00^{c}	2.9±0.01^{c}	41.17±0.11^{b}
R.ICC13124[B]	2.4±0.01^{de}	6.9±0.03^{cd}	7.3±0.03^{bc}	4.1±0.02^{bcd}	4.2±0.02^{bcd}	7.7±0.04^{e}	3.3±0.02^{cd}	0.9±0.00^{b}	2.8±0.01^{cd}	40.07±0.19^{bcd}
R.ICC5383[B]	2.4±0.02^{cde}	7.0±0.06^{bc}	7.3±0.06^{xbc}	4.1±0.04^{bc}	4.2±0.04^{bc}	7.9±0.07^{cde}	3.3±0.03^{c}	1.1±0.01^{a}	3.0±0.03^{b}	40.68±0.36^{bc}

Cultivar	His Score	Lys Score	Leu Score	Ile Score	Val Score	Phe+Tyr Score	Thr Score	Trp Score	Met+Cys Score	AAS
C.BS[A]	117.28	106.18	100.06	134.57	112.29	119.62	86.99	**73.66**	95.62	107.32±0.27^{f}
B.ICC6306[B]	129.75	114.38	105.95	144.08	119.10	123.09	93.60	**81.50**	109.37	114.64±0.77^{e}
B.ICC4418[B]	127.97	119.31	109.81	148.62	122.91	129.14	96.27	**80.76**	112.22	118.48±0.47^{bcd}
B.ICC3761[B]	126.09	118.36	109.59	146.44	119.79	127.24	97.56	**75.39**	112.41	117.29±0.32^{cde}
Br.ICC3512[B]	127.32	117.91	108.55	147.87	112.98	124.06	95.78	**80.36**	117.23	116.24±1.27^{de}
C.ICC3421[B]	130.33	114.31	105.90	145.50	118.68	124.26	96.33	**90.78**	111.84	115.70±0.18^{de}
G.ICC5613[B]	137.01	128.61	116.81	159.12	130.16	131.11	106.01	**81.92**	130.16	126.26±0.59^{a}
R.ICC14782[B]	130.91	123.40	112.29	152.28	124.73	128.92	103.01	**83.06**	117.77	121.44±0.33^{b}
R.ICC13124[B]	126.34	119.94	110.60	148.72	121.77	123.64	97.98	**89.07**	115.61	118.21±0.55^{bcd}
R.ICC5383[B]	126.84	121.32	111.00	149.76	122.56	125.47	**99.23**	100.78	121.46	120.00±1.05^{bc}

EAA: Essential amino acids, AAS: Amino acid score, BS: cultivar Blanco Sinaloa 92, B: Black color, Br: Brown color, C: Cream color, G: Green color, R. Red color. [A] Kabuli seeds. [B] Desi seeds. [C] Recommended value by FAO. Values underlined represent limiting amino acid and bold the principal. Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p<0.05$).

sulphur containing amino acids (met+cys) are the most limiting in chickpeas. Results herein demonstrate that only the C-BS control flour had a slight deficiency of met+cys (95%) and the main limiting amino acids were Thr and Trp. These results agree with [152] who also identified these three amino acids as the most limiting. In nine of the chickpea flours, the most limiting amino acid was Trp (73-90%) except the R.ICC5383 flour, which was Thr (99%). Amino acid composition is very important in the nutritional quality of proteins. The total content of essential amino acid in chickpea flours varied form 36.38 (C.BS) to 42.80 (G.ICC5613), which corresponded with values previously reported for chickpea flours [78, 152]. In cooked flours, the AAS varied from 107.3 (C.BS) to 126.3 (G.ICC5613).

3.1.4 Protein quality of cooked flours

The *IVPD* in cooked chickpea flours (Table 6) varied significant among cultivars. The R.ICC13124 flour had the highest value (82.44%) whereas the B.ICC6206 the lowest (76.26%). *IVPD* values higher than 80% have been reported in processed chickpea [78, 153]. *IVPD* showed a negative correlation with β-glucans content (*r=-0.7394, p=0.0145*) and IDF (*r=-0.6528, p=0.0107*) indicating that these fiber components interfered with the biocatalytic action of pepsin and/or trypsin, chymotrypsin and peptidases. The nutritional parameters of cooked chickpea flours (Table 6) were estimated using their amino acid composition and *IVPD*. The relation of essential amino acids versus total amino acid content (%EAA/TAA) varied significant among cultivars, R.ICC13124 flour (45.44) showed the higher value of this parameter, while the C.BS flour (43.13) the lowest. The predicted biological value (pBV) estimated the amount of the ingested protein that is incorporated in the organism. The highest value of pBV was found in G.ICC5613 flour (60.20) whereas the C.BS flour had the lowest (25.26). The *in vivo* PER is an important method to determine overall protein quality. The in vitro calculated PER values have a good correlation versus real PER values [137]. In chickpea cooked flours, the cPER values were higher than 2, which are observed in foods with high protein quality. The highest and lowest or worst cPERs were observed in the G.ICC5613 and C.BS chickpea flours respectively. Other authors have reported cPER values in raw and fermented chickpeas of 1.54 and 2.21, respectively [78].

Nowadays, PCDAAS is the most recommended theoretical parameter to determine food protein quality. It is calculated based in the limiting AAS according to the FAO recommendations for 2-5 year old child and *IVPD*. The highest PDCAAS value is 1, which will provide 100% of the essential amino acids required by a two year old preschool children [136]. PDCAAS must be above 0.6 to meet amino acids needs [78]. The PDCAAS in chickpea flours varied significantly among cultivars, from 0.59 (C.BS) to 0.82 (R.ICC5383). PDCAAS values have been previously reported for raw (0.78) [78] and heated chickpea flour (0.28-0.61) using different protein digestion methods [154].

Table 3.1.6. Effect of chickpea type o*n vitro* protein digestibility and protein quality of cooked flours.

Cultivar	*IVPD*	%EAA/TAA	pBV	cPer$_1$	cPer$_2$	cPer$_3$	PDCAAS
C.BS^{A}	80.63±0.40^{bcd}	43.13±0.01^{h}	25.26±0.28^{f}	2.17±0.01^{e}	2.29±0.01^{e}	2.17±0.00^{e}	0.59±0.00^{fg}
B.ICC6306^{B}	76.26±0.06^{g}	43.92±0.01^{g}	35.81±1.06^{e}	2.33±0.02^{d}	2.46±0.02^{d}	2.52±0.03^{d}	0.62±0.00^{def}
B.ICC4418^{B}	78.43±0.47^{ef}	44.35±0.01^{d}	40.82±0.71^{de}	2.44±0.01^{bc}	2.55±0.01^{bc}	2.53±0.01^{d}	0.63±0.01^{de}
B.ICC3761^{B}	78.25±0.17^{f}	44.05±0.01^{f}	41.09±0.48^{de}	2.44±0.01^{bc}	2.56±0.01^{bc}	2.63±0.01^{bcd}	0.59±0.00^{g}
Br.ICC3512^{B}	75.43±0.26^{g}	44.16±0.01^{e}	40.53±1.94^{de}	2.41±0.03^{cd}	2.53±0.03^{cd}	2.58±0.06^{bcd}	0.61±0.01^{efg}
C.ICC3421^{B}	80.19±0.45^{cde}	43.94±0.01^{g}	40.11±0.27^{de}	2.33±0.00^{d}	2.46±0.00^{d}	2.56±0.00^{cd}	0.73±0.01^{b}
G.ICC5613^{B}	79.45±0.51^{def}	44.79±0.01^{c}	60.20±1.23^{a}	2.65±0.02^{a}	2.76±0.01^{a}	3.01±0.02^{a}	0.65±0.01^{cd}
R.ICC14782^{B}	81.36±0.53^{abc}	44.93±0.01^{b}	50.78±0.60^{b}	2.52±0.01^{b}	2.62±0.01^{b}	2.69±0.02^{bc}	0.68±0.01^{c}
R.ICC13124^{B}	82.44±0.18^{a}	45.44±0.01^{a}	43.40±0.88^{cd}	2.47±0.01^{bc}	2.58±0.01^{bc}	2.64±0.02^{bcd}	0.73±0.01^{b}
R.ICC5383^{B}	82.19±0.00^{ab}	44.80±0.01^{c}	48.41±1.87^{bc}	2.48±0.03^{bc}	2.59±0.03^{bc}	2.69±0.04^{b}	0.82±0.01^{a}

IVPD: in vitro protein digestibility, %EAA/TAA; Essential amino acid/Total amino acids, pBV: predicted biological value, cPER: calculated protein efficiency ratio, PDCAAS: Protein digestibility corrected amino acid score, BS: cultivar Blanco Sinaloa 92, B: Black color, Br: Brown color, C: Cream color, G: Green color, R. Red color. ^{A} Kabuli seeds, ^{B} Desi seeds. Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p<0.05$).

3.1.5 Starch digestion fractions and glycemic index of cooked flours.

The rapidly digestible (RDS), slowly digestible (SDS) and resistant starch (RS) fractions in cooked chickpea flours are reported in Table 7. The RDS ranged from 47.3 (R.ICC14782) to 67.17 % (C.BS) in cooked flours, the higher RDS can be related to the increase of available starch molecules after the cooking process. The SDS fraction ranged from 10.11 to 23.36% in the cooked chickpea flours. Interestingly, the RS fraction was highest in B.ICC4418 (37.52%) and lowest in the C.BS (22.68%). Other authors had reported RS amounts of 19 to 23 % in cooked chickpea flours [66, 147]. Cooked chickpea flours had high amounts of RS that is known to act as prebiotic [68, 69]. Also, RS fraction in chickpea flours correlated positive with TPC (*r=0.7649, p=0.0099*) and β-glucans contents (*r=0.7347,p=0.0155*). Some studies have reported that starch digestion is diminished by interactions with phenolics and enzymes such as amylases [151]. Moreover, the HI and pGI ranged from 81.52 (B.ICC4418) to 88.96 (C.BS) and from 78.47 (B.ICC4418) to 84.88 (C.BS), respectively. These values were higher compared to their corresponding pGI values assayed in refined cooked starches [148]. According to the observed pGI values, cooked chickpea flours can be classified as high glycemic impact foods [155].

3.1.6 Principal components and cluster analysis

The PCA plots provided an overview of interrelationships between the measured properties (Figure 1A), and the differences and similarities among the chickpea flours (Figure 1B). The first and second components (PC1 and PC2) accounted for an accumulative variance of 73.4%. The loading plot (Figure 1A) of PCA contains the correlations among nutritional properties and chemical attributes of flours. PC1 accounted 43.9% of the accumulated variance, in which the main contributors factors were TAAE, %EAA/TAA, AAS, pBV, cPER, PDCAAS, RDS, SDS and TDF. Meanwhile, TPC, *IVPD*, RS, HI, pGI, protein, starch and β-glucans contents (b-glu) were the main factors in PC2 (29.5%). Cluster analyses of PC1 and PC2 formed five groups according to similarities among the chickpea flours and the variables evaluated (Figure 1B). Group 1 was comprised only of the G.ICC5313 flour, which had the highest starch and protein quality (cPER, AAS, pBV and TEAA). Group 2 was formed with the red seed coat chickpea flours (R.ICC14782, R.ICC13124 and R.ICC5383); this group had the highest *IVPD* values and high values of SDS fraction. On the other hand B.ICC4418 and B.ICC3761 chickpea flours, which constituted group 3, showed the highest values of TPC, Protein, RS and relatively lower values of S and pGI. Group 4, which clustered B.ICC6306, Br.ICC3761 and C.ICC3421 flours showed negative and low scores in PC1 and PC2. Finally, Group 5 which was formed by the only Kabulli chickpea of this study, C.BS, had the highest RDS, pGI and TDF, but the lowest values protein quality, TPC, b-glu and RS values.

Table 3.1.7. Effect of chickpea type on starch digestibility and predicted glycemic index in cooked flours.

Cultivar	Digestion fractions (% Starch content)			HI	pGI
	RDS	SDS	RS		
C.BS[A]	67.17±0.50[a]	10.15±0.43[f]	22.68±0.34[g]	88.96±0.40[a]	84.88±0.55[a]
B.ICC6306[B]	54.31±0.23[c]	14.14±0.20[e]	31.55±0.13[cd]	84.51±0.34[b]	81.05±0.19[cd]
B.ICC4418[B]	50.29±0.42[e]	12.19±0.15[e]	37.52±0.13[a]	81.52±0.13[d]	78.47±0.36[e]
B.ICC3761[B]	52.26±0.06[d]	11.99±0.32[d]	35.75±0.40[b]	82.41±0.24[cd]	79.23±0.01[de]
Br.ICC3512[B]	57.31±0.24[b]	10.11±0.07[f]	32.58±0.01[c]	84.00±0.17[bc]	80.6±0.05[cd]
C.ICC3421[B]	50.61±0.35[e]	19.17±0.17[b]	30.22±0.10[de]	85.18±0.55[b]	81.62±0.39[c]
G.ICC5613[B]	58.33±0.06[b]	16.52±0.27[c]	25.15±0.13[f]	87.72±0.45[a]	83.82±0.53[ab]
R.ICC14782[B]	47.32±0.19[f]	23.26±0.17[a]	29.42±0.06[e]	85.58±0.42[b]	81.97±0.53[bc]
R.ICC13124[B]	50.32±0.06[e]	19.25±0.51[b]	30.43±0.48[de]	85.07±0.00[b]	81.53±0.34[c]
R.ICC5383[B]	50.23±0.02[e]	17.29±0.07[c]	32.48±0.35[c]	84.05±0.46[bc]	80.65±0.20[cd]

RDS: Rapidly digestible starch, SDS: Slowly digestible starch, RS: Resistant starch, HI: Hydrolysis index (estimated from a 100% digestible starch from White bread), pGI: predicted glycemic index, BS: cultivar Blanco Sinaloa 92, B: Black color, Br: Brown color, C: Cream color, G: Green color, R. Red color, [A] Kabulli seeds. [B] Desi seeds. Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p<0.05$).

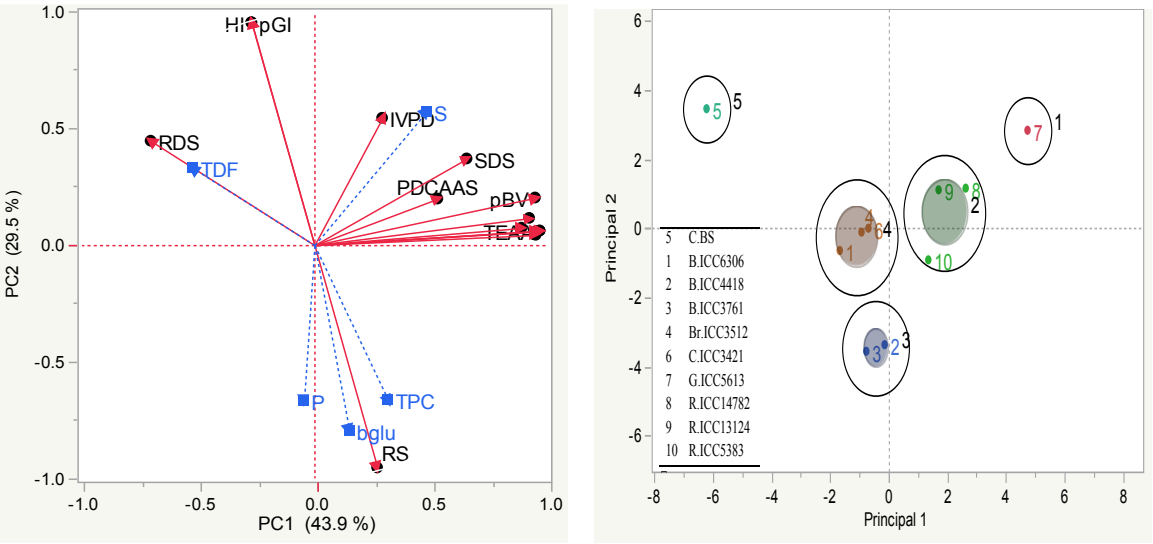


Figure 3.1.1. Principal components and cluster analysis: (A) loading plot of PCs describing variation among chickpea flours properties and (B) cluster and score plot of overall variation on PCs on chickpea flours.

TDF: Total dietary fiber, b-glu: β-glucans content, TPC: total phenolic content, P: protein content, S: starch content, %EAA/TAA; Essential amino acid/Total amino acids, AAS: Amino acid score, pBV: predicted biological value, cPER: calculated protein efficiency ratio, PDCAAS: protein digestibility corrected amino acid score, *IVPD*: in vitro protein digestion. RDS: Rapidly digestible starch, SDS: Slowly digestible starch, RS: Resistant starch, HI: Hydrolysis index, pGI: predicted glycemic index, BS: cultivar Blanco Sinaloa 92, B: Black color, BR: Brown color, C: Cream color, G: Green color, R. Red color.

3.2 Physicochemical, functional properties and digestion of isolated starches from pigmented chickpea (Cicer arietinum L.) cultivars.

3.2.1. Chickpea seeds physical characterization

Significant differences were found in 1000-grain weight between the Kabuli type (C.BS) (666.30 g) and all the Desi types (≤352.67 g) (Table 3.2.1). The apparent density measured with the test weight showed the opposite trend, where cultivars ranged from 67.62 (C.BS) to ≥74.12 kg/hL. The diameter of the chickpea seeds ranged from 7.12 (B.ICC4418) to 12.22 mm (C.BS). The amount of seed coat in the array of chickpeas ranged from 3.64% (C.BS) to 13.62% (green cultivar G.ICC5613). The amounts of cotyledon and hilum ranged from 84.80 (G.ICC5613) to 95.66% (C.BS) and 0.68 (C.BS) to 2.01% (B.ICC4418) of the total seed weight, respectively. These results agreed with previous reports that showed important size differences when Desi and Kabuli cultivars were compared mainly due to their different genetic background [5, 7] .

3.2.2 Chemical composition of chickpea starches

The principles of the sulfur dioxide wet milling process used for maize were followed to obtain chickpea starches. The starch content in raw seeds ranged from 35.78 (B.ICC3761) to 46.23% (G.ICC5613). The calculated starch yield and recovery of the ten different chickpea starches ranged from 19.22 (B.ICC4418) to 30.06% (C.ICC3421) and 43.93 (Br.ICC3512) to 71.69 % (C.BS), respectively (Table 3.2.2). Both parameters showed a negative strong correlation with the amount of seed coat (r=-0.9386, p<0.0001) and (r=-0.9235, p<0.0001), respectively. One of the most relevant characteristics of starches intended for food applications is their purity. It is difficult to obtain pure starches from pulses because of their high protein and fiber contents present in cell walls and the strong interaction between proteins and starch granules. Moreover, the high amounts of fiber tend to co-sediment with the dense starch fraction during the extraction protocols [156-158]. Results indicated that the refined starches contained relatively low amounts of protein (≤0.54%) and ash (≤0.07%) indicating the effectiveness of the sulfur dioxide wet-milling procedure. The total starch contents ranged from 87.14% (C.BS) to 96.02% (R.ICC14782). These results agree with purity values previously reported in chickpea starches (87-94%) [156, 158]. One of the main characteristics of legume starches is that they contain relative high amounts of amylose. The amounts of this molecule varied from 25.05% (C.ICC3421) to 35.26% (R.ICC13124) and interestingly there were not significant differences when the Desi and Kabuli types were compared. These results are in agreement with previous reported amylose contents (20.7 to 35%) in chickpea starches reported by other authors [156, 157, 159].

Table 3.2.1. Physical properties of pigmented chickpea seeds.

				Anatomical seed parts		
Cultivar	**1000 GW (g)**	**HW (kg/hL)**	**MD (mm)**	**Seed coat (%)**	**Cotyledons (%)**	**Hilum (%)**
C.BS[A]	666.30±14.05^{a}	67.62±1.28^{d}	12.22±0.22^{a}	3.64±0.06^{e}	95.66±0.08^{a}	0.68±0.01^{e}
B.ICC6306[B]	290.65±3.43^{c}	77.78±0.78^{ab}	9.42±0.10^{b}	10.95±0.06^{c}	87.93±0.07^{c}	1.12±0.07^{cd}
B.ICC4418[B]	124.86±3.92^{f}	79.52±0.74^{a}	7.12±0.11^{d}	13.04±0.19^{ab}	84.96±0.13^{e}	2.01±0.05^{a}
B.ICC3761[B]	121.46±1.18^{f}	78.64±0.66^{a}	7.52±0.09^{d}	13.28±0.24^{a}	84.94±0.25^{e}	1.74±0.04^{ab}
Br.ICC3512[B]	198.63±0.76^{de}	77.24±0.53^{ab}	8.28±0.10^{c}	13.13±0.24^{ab}	85.57±0.26^{de}	1.28±0.05^{c}
C.ICC3421[B]	196.68±1.82^{de}	78.71±0.34^{a}	8.32±0.07^{c}	4.11±0.07^{e}	95.13±0.08^{a}	0.74±0.03^{e}
G.ICC5613[B]	181.27±1.71^{e}	74.12±0.35^{c}	8.48±0.07^{c}	13.62±0.22^{a}	84.80+0.25^{e}	1.57±0.06^{b}
R.ICC14782[B]	191.29±1.34^{e}	78.16±0.51^{a}	9.64±0.07^{b}	9.91±0.34^{c}	88.99±0.37^{b}	1.09±0.02^{cd}
R.ICC13124[B]	352.67±2.50^{b}	75.00±0.31^{bc}	8.18±0.12^{c}	10.84±0.22^{cd}	88.21±0.24^{bc}	0.94±0.03^{de}
R.ICC5383[B]	218.42±1.88^{d}	77.69±0.17^{ab}	8.52±0.08^{c}	12.17±0.10^{b}	86.23±0.05^{d}	1.59±0.05^{b}

GW:grain weight, HW: hectoliter weight, MD: diameter, BS: cultivar Blanco Sinaloa 92, B: Black color, BR: Brown color, C: Cream color, G: Green color, R. Red color. [A] Kabulli seeds. [B] Desi seeds. Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p<0.05$).

Table 3.2.2 Wet milling yields and chemical composition of isolated starches from pigmented chickpea cultivars (dwb).

Cultivar	Grain starch (%)	Yield (%)	Recovery (%)	Protein (%)	Ash (%)	Starch (%)	Amylose (%)
C.BS[A]	38.27±0.25^{def}	27.42±1.01^{ab}	71.69±2.71^{a}	0.54±0.02^{a}	0.06±0.00^{ab}	87.14±0.43^{d}	30.19±0.12^{c}
B.ICC6306[B]	40.28±0.77^{cde}	21.52±3.05^{bc}	53.41±7.16^{bc}	0.36±0.01^{def}	0.06±0.00^{ab}	95.83±1.36^{a}	26.66±0.09^{ef}
B.ICC4418[B]	37.00±0.74^{ef}	19.22±0.33^{c}	51.18±0.72^{bc}	0.26±0.00^{gh}	0.04±0.00^{ab}	91.03±1.01^{bc}	27.53±0.12^{de}
B.ICC3761[B]	35.78±1.08^{f}	19.95±1.47^{bc}	55.46±3.79^{abc}	0.43±0.00^{cde}	0.06±0.00^{ab}	92.71±0.55^{ab}	28.46±0.10^{d}
Br.ICC3512[B]	43.42±1.06^{abc}	19.53±2.04^{c}	43.93± 3.93^{c}	0.51±0.01^{abc}	0.05±0.00^{ab}	90.12±0.58^{bcd}	25.99±0.06^{fg}
C.ICC3421[B]	42.00±1.28^{bcd}	30.06±1.02^{a}	69.80±2.15^{ab}	0.35±0.01^{ef}	0.07±0.00^{ab}	89.59±1.03^{bcd}	25.05±0.37^{g}
G.ICC5613[B]	46.23±0.74^{a}	21.62±1.39^{bc}	45.90±3.16^{c}	0.24±0.00^{h}	0.03±0.00^{b}	90.54±0.83^{bcd}	26.01±0.20^{fg}
R.ICC14782[B]	42.97±0.50^{abc}	24.26±0.81^{abc}	56.68±2.03^{c}	0.44±0.02^{bcd}	0.05±0.00^{ab}	96.02±1.31^{a}	33.26±0.26^{b}
R.ICC13124[B]	44.90±0.46^{ab}	23.28±0.19^{abc}	51.66±0.57^{abc}	0.34±0.00^{fg}	0.06±0.00^{ab}	89.67±0.65^{bcd}	27.29±0.38^{de}
R.ICC5383[B]	39.79±0.31^{cde}	22.35±1.83^{abc}	55.90±4.50^{abc}	0.52±0.00^{ab}	0.07±0.00^{a}	87.99±0.73^{cd}	35.26±0.32^{a}

BS: cultivar Blanco Sinaloa 92, B: Black color, BR: Brown color, C: Cream color, G: Green color, R. Red color. [A] Kabulli seeds. [B] Desi seeds. Values are means ± SEM (standard error mean). Values with different letter(s) in every column are significantly different ($p<0.05$).

3.2.3 Granule morphology of chickpea starches.

Starch granules micrographs are depicted in Figure 3.1. The starches showed lenticular shaped granules, which varied in size. However, all starches showed smooth surfaces, similar to those reported by Miao et al [160]. Some of the starch granules showed a central depression, that when observed under polarized light, depicted the characteristic center-hollowed birefringence pattern, which in the case of legume starches is related with their particular shape and could be related with the granule architecture developed during biosynthesis [157-161].

3.2.4 Color of chickpea starches.

The color of isolated starch is an important quality parameter, in which clean white is desirable [127]. The *a** and *b** values in the starches ranged from -0.91 (B.ICC3761) to -2.10 (R.ICC14782) and 2.45 (C.ICC3421) to 5.42 (R.ICC14782), respectively, which implies that samples showed small traces of residual pigments (Table 3.2.3). However, the calculated whiteness of chickpea starches ranged from 84.83 (B.ICC3761) to 91.99 (Br.ICC3512) respectively, which are similar to the reported by Uriarte-Aceves et al [127].

3.2.5 Functional properties of chickpea starches

The swelling power of starch granules is affected by both inter and intra granular interactions with water. This phenomenon occurs concurrently with the loss of birefringence and precedes solubilization [162]. The swelling power of the array of chickpea starches ranged from 12.00 to 14.62 g/g for G.ICC5613 and R.ICC5383 samples, respectively (Table 3.2.3). This important feature showed a positive strong correlation with amylose (r=0.9262, p=0.0001) and a non-significant difference with starch solubility (11.19-13.12%). The water retention capacity (WRC) values that are directly related to starch chain length and water molecules interaction varied from 83.21 (C.ICC3421) to 91.26% (R.ICC5383) and were similar to values (77-92%) reported by other authors [157, 160].

3.2.6 Pasting profiles of chickpea starches.

The RVA pasting profiles show the changes occurred due to starch gelatinization as a consequence of heating in excess water under constant shear stress [162]. The starch pasting temperatures ranged from 70.80 to 76.45ºC for Br.ICC3512 and R.ICC14782, respectively (Table 3.2.4); that according to other studies could be related with starch granule size distribution and its molecular characteristics [7, 158, 163]. In a general way, the viscous behavior of pulse starches reflects their particular molecular conformation, in which longer amylopectin chains promote higher peak viscosities (PV) whereas the amount and particular amylose characteristics help to generate higher final

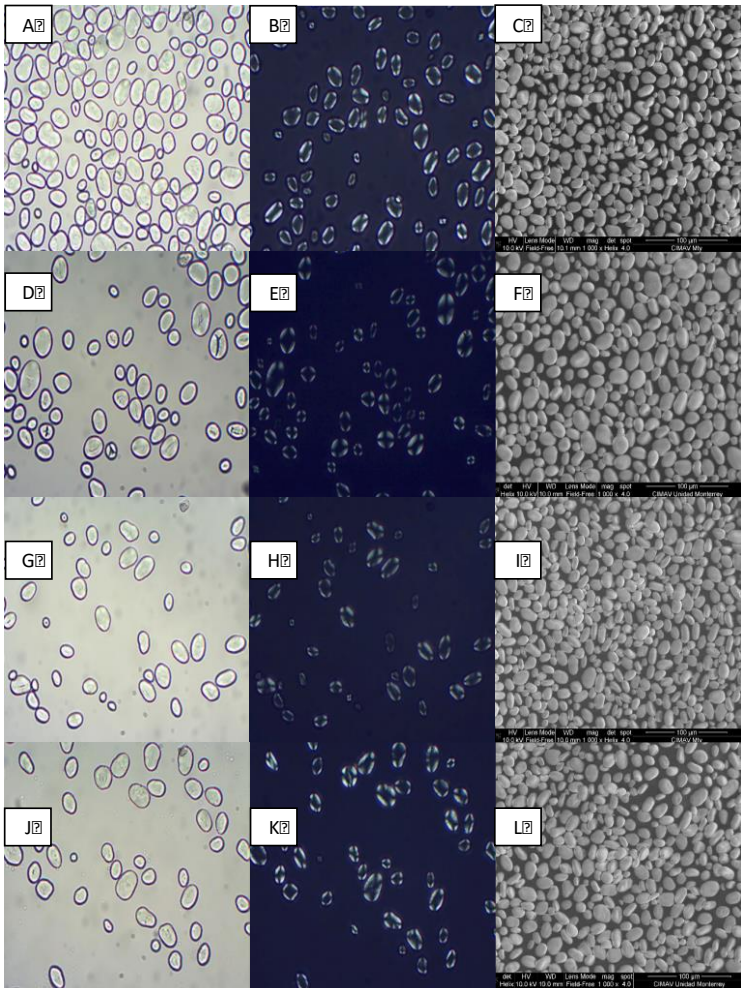


Figure 3.2,1. Microscopy analysis of starch granules, from left to right normal light (x40), polarized light (x40), and scanning electron micrograph (x1000). A, B and C, B.ICC3761 (black colored). D, E and F, C.BS. (cream colored) G, H and I, G.ICC5613 (green colored) and J, K and L, R.ICC5383 (red colored).

Table 3.2.3. Physicochemical properties of isolated starch from pigmented chickpea.

Cultivar	Starch color			Whiteness	Swelling power (g/g)	Solubility (%)	Water retention capacity (%)
	L*	a*	b*				
C.BS[A]	88.41±0.03[d]	-1.80±0.00[e]	2.49±0.00[h]	88.01±0.03[c]	13.11±0.17[bc]	12.06±0.31[a]	89.95±0.32[ab]
B.ICC6306[B]	86.75±0.09[f]	-1.47±0.00[b]	2.81±0.01[f]	86.38±0.09[e]	12.27±0.21[bc]	12.63±0.56[a]	89.95±0.37[ab]
B.ICC4418[B]	87.50±0.04[e]	-1.62±0.00[c]	2.67±0.01[g]	87.12±0.04[d]	12.54±0.40[bc]	11.67±0.57[a]	86.36±0.18[c]
B.ICC3761[B]	84.83±0.09[h]	-0.91±0.00[a]	2.17±0.00[j]	84.65±0.08[h]	12.79±0.24[bc]	11.97±0.49[a]	88.48±0.21[b]
Br.ICC3512[B]	91.99±0.12[a]	-2.05±0.00[g]	3.50±0.02[e]	91.99±0.11[a]	12.36±0.05[bc]	13.00±0.50[a]	89.36±0.56[b]
C.ICC3421[B]	91.06±0.08[b]	-1.79±0.00[e]	2.45±0.01[i]	90.56±0.08[b]	12.36±0.06[bc]	12.46±0.21[a]	83.21±0.43[d]
G.ICC5613[B]	85.80±0.03[g]	-1.69±0.00[d]	3.94±0.01[d]	85.17±0.03[g]	12.00±0.03[c]	12.36±0.43[a]	84.17±0.32[d]
R.ICC14782[B]	89.49±0.05[c]	-2.10±0.01[h]	5.42±0.01[a]	88.00±0.05[c]	13.29±0.38[b]	11.19±0.43[a]	86.46±0.20[c]
R.ICC13124[B]	88.29±0.04[d]	-1.90±0.00[f]	5.28±0.00[c]	87.02±0.03[d]	12.06±0.25[c]	12.21±0.50[a]	88.42±0.24[b]
R.ICC5383[B]	87.27±0.02[e]	-1.89±0.00[f]	5.34±0.01[b]	86.07±0.02[f]	14.62±0.20[a]	13.12±0.38[a]	91.26±0.15[a]

BS: cultivar Blanco Sinaloa 92, B: Black color, BR: Brown color, C: Cream color, G: Green color, R. Red color. [A] Kabulli seeds. [B] Desi seeds. Values are means ± SEM (standard error mean). Values with different letter(s) in every column are significantly different ($p<0.05$). Whiteness: $100-[(100-L^*)^2+(a^*)^2+(b^*)^2]^{1/2}$

Table 3.2.4. Rapid viscosity profiles of isolated chickpea starches.

Cultivar	Pasting temperature (ºC)	Viscosity (cP)			
		Peak	Through	Final	Setback
C.BS[A]	73.35 ± 0.01^{f}	5490 ± 15.84^{g}	3841 ± 17.73^{g}	9455 ± 43.67^{c}	5614± 16.20^{b}
B.ICC6306[B]	74.90 ± 0.01^{d}	6162 ± 17.78^{e}	4823 ± 22.27^{b}	8604 ± 39.74^{d}	3781± 10.91^{g}
B.ICC4418[B]	74.05 ± 0.02^{e}	6277 ± 18.12^{d}	4627± 21.37^{c}	8682 ± 40.10^{d}	4055 ± 11.70^{f}
B.ICC3761[B]	73.35 ± 0.01^{f}	6535 ± 18.86^{c}	4614 ± 21.31^{c}	9336 ± 43.12^{c}	4722± 13.63^{d}
Br.ICC3512[B]	76.45 ± 0.02^{a}	5914 ± 17.07^{f}	4413 ± 20.38^{d}	7973 ± 36.82^{f}	3560± 11.27^{h}
C.ICC3421[B]	76.35 ± 0.02^{b}	5270 ± 15.21^{i}	4152 ± 19.17^{e}	8258 ± 38.14^{e}	4106± 11.85^{f}
G.ICC5613[B]	75.60 ± 0.01^{c}	5406 ± 15.60^{gh}	4121 ± 19.83^{e}	8512 ± 39.31^{d}	4391± 12.67^{e}
R.ICC14782[B]	70.80 ± 0.02^{g}	7538 ± 21.76^{b}	4582 ± 21.16^{c}	10123 ± 46.75^{b}	5541 ± 15.99^{c}
R.ICC13124[B]	75.65 ± 0.01^{c}	5333 ± 15.39^{hi}	4000 ± 18.47^{f}	8676 ± 40.07^{d}	4676 ± 13.49^{d}
R.ICC5383[B]	74.00 ± 0.02^{e}	8485 ± 24.49^{a}	6264 ± 28.93^{a}	13158 ± 60.77^{a}	6894 ± 15.90^{a}

BS: cultivar Blanco Sinaloa 92, B: Black color, BR: Brown color, C: Cream color, G: Green color, R. Red color. [A] Kabulli seeds. [B] Desi seeds. Values are means ± SEM (standard error mean). Values with different letter(s) in every column are significantly different (p<0.05).

viscosities (FV)[130, 156, 158, 159]. Results herein indicated that PV ranged from 5270 to 8485 cP whereas FV from 7973 to 13158 cP, that showed strong positive correlations with amylose content (r=0.8667, p=0.0012 and r=0.9213, p=0.0002) and with swelling power (r=0.8601, p=0.0012 and r=0.9548, p<0.0001). Others authors have shown that the differences on molecular weight (MW) as well as chain length distribution of both amylose and amylopectin may have a significant effect on starch pasting formation, thermal stability and may slow its digestion rate and pGI [159, 161, 164].

3.2.7 Thermal properties of chickpea starches

In this study, the onset temperature (*To*) in chickpea starches varied from 61.93 to 68.94ºC (R.ICC13124-G.ICC5613) whereas the peak (*Tp*) and conclusion temperatures (*Tc*) varied from 66.4 to 72.57ºC (R.ICC13124 and G.ICC5613) and 73.34 to 78.5ºC (R.ICC14782 to B.ICC4418), respectively (Table 3.2.5). These parameters had a positive correlation with pasting temperature (r=0.779, p=0.007) and negative with amylose content (r=-0.697, p=0.02). In legumes, the differences in gelatinization temperature may be attributed to dissimilarities in the amylopectin double helix arrangement, and to the size and complexing of amylose molecules within the starch granules, that together influence the internal crystallinity [130, 158, 163]. The DSC gelatinization range in chickpea starches varied from 8.73 to 15.12ºC for C.BS and R.ICC13124, respectively. A broader range is indicative of heterogeneous crystallites with varying stability within the crystalline domains of the starch granule [130]. The gelatinization enthalpies ranged from 8.82 to 10.68 J/g, which agree within values previously reported [157, 165]. Other authors have related the relative low enthalpy values of pulses with the molecular conformation and arrangement of their amylopectin structural arrangement, in particular with the double helices and to the presence of long and partially branched amylose molecules, that tends to decrease granular swelling and crystalline melting during gelatinization [133, 156, 164].

3.2.8 ATR-FTIR analysis of chickpea starches

It is known that the IR absorbance bands at 1047 cm^{-1} and 1022 cm^{-1} are sensitive to crystalline/ordered and amorphous structures within the starch granules [161]. The crystalline and amorphous zones ranged from 0.54 to 0.70 and 0.84 to 0.90, respectively, and statistical differences were not detected among the array of chickpea starches (Table 3.2.5). Other studies have calculated the ratio of 1047 cm^{-1}/1022 cm^{-1} bands, that are related with the amount of crystalline to amorphous (C/A) domains in starch [161]. The ratio of C/A domains in chickpea starches ranged from 0.60 to 0.79 for C.BS and G.ICC5613, respectively. Similar ratios have been reported previously in chickpea starches, which commonly are correlated with high amylose contents [158, 161].

Table 3.2.5. Thermal properties and ATR- FTIR crystalline proportion of isolated chickpea starches.

Cultivar	Gelatinization temperatures					ATR- FTIR		
	To (°C)	Tp (°C)	Tc (°C)	ΔT (°C)	ΔH (J/g)	Crystalline (1024 cm^{-1})	Amorphous (1044cm^{-1})	Ratio (C/A)
C.BS[A]	68.10±0.19^{ab}	71.78±0.52^{a}	76.83±0.69^{a}	8.73±0.50^{d}	10.18±0.23^{ab}	0.58±0.01^{a}	0.96±0.00^{a}	0.60±0.01^{b}
B.ICC6306[B]	67.14±0.08^{ab}	71.12±0.23^{a}	77.35±0.63^{a}	10.21±0.43^{d}	9.21±0.09^{de}	0.63±0.02^{a}	0.89±0.03^{a}	0.71±0.03^{ab}
B.ICC4418[B]	68.56±0.22^{ab}	72.43±0.49^{a}	78.50±1.02^{a}	9.94±0.79^{d}	10.68±0.17^{a}	0.58±0.04^{a}	0.93±0.02^{a}	0.62±0.04^{b}
B.ICC3761[B]	63.72±0.42^{cd}	68.00±0.11^{bc}	78.00±0.36^{a}	14.28±0.05^{a}	9.29±0.08^{cde}	0.54±0.05^{a}	0.84±0.04^{a}	0.64±0.03^{b}
Br.ICC3512[B]	67.79±0.57^{ab}	71.96±0.95^{a}	78.23±0.86^{a}	10.44±0.28^{cd}	9.13±0.15^{de}	0.56±0.02^{a}	0.87±0.03^{a}	0.64±0.02^{b}
C.ICC3421[B]	66.00±0.13^{ab}	70.80±0.15^{ab}	78.27±0.30^{a}	12.28±0.16^{bc}	9.67±0.11^{bcd}	0.58±0.04^{a}	0.92±0.02^{a}	0.62±0.01^{b}
G.ICC5613[B]	68.94±0.37^{a}	72.57±0.22^{a}	78.00±0.40^{a}	9.06±0.03^{d}	8.82±0.11^{e}	0.70±0.04^{a}	0.88±0.03^{a}	0.79±0.02^{a}
R.ICC14782[B]	64.00±0.75^{cd}	66.69±0.17^{c}	73.34±0.98^{b}	9.34±0.23^{d}	9.55±0.02^{bcd}	0.59±0.03^{a}	0.92±0.01^{a}	0.64±0.01^{b}
R.ICC13124[B]	61.93±0.93^{d}	66.47±0.16^{c}	77.05±0.64^{a}	15.12±0.28^{a}	9.91±0.00^{bc}	0.67±0.04^{a}	0.90±0.01^{a}	0.74+0.02^{ab}
R.ICC5383[B]	67.75±0.69^{ab}	71.55±1.35^{a}	76.75±0.09^{a}	9.00±0.59^{d}	9.98±0.12^{b}	0.59±0.02^{a}	0.88±0.01^{a}	0.67±0.04^{ab}

To: onset temperature, Tp, peak temperature, Tc: conclusion temperature, ΔT: gelatinization range (Tc-To), ΔH: enthalpy of gelatinization, BS: cultivar Blanco Sinaloa 92, B: Black color, BR: Brown color, C: Cream color, G: Green color, R. Red color. [A] Kabulli seeds. [B] Desi seeds. Values are means ± SEM. Values with different letter(s) in every column are significantly different (p<0.05).

3.2.9 *In vitro* starch digestibility and predicted glycemic index of chickpea starches

The rapidly digestible (RDS), slowly digestible (SDS) and resistant (RS) fractions of raw and gelatinized chickpea starches are reported in Table 3.2.6. The RDS fraction in raw starch ranged from 17.26 to 29.90% for the R.ICC14782 and R.ICC13124 starches, respectively. On the other hand, the SDS, which is considered the most desirable form of dietary starch because it is not completely degraded in the small intestine and therefore releases glucose at a slower rate [164], ranged from 27.42 to 36.26% for the R.ICC13124 and R.ICCC5383 starches, respectively. Moreover, the RS fraction was highest in R.ICC14782 (52.56%) and lowest in the R.ICC13124 (42.68%). These results are comparable with other studies that showed a relative high amount of RS in raw legume starches that could promote health benefits [156, 160]. However, the consumption of these pulses, as well as the vast majority of starchy foods is in cooked form, due to thermal processes greatly increase the amount of available starch molecules, and contribute to a larger caloric output when consumed [139, 166]. In cooked starches we found RDS contents up to 59.15%, which is related with the changes in molecule availability due to gelatinization of starch granules, nevertheless, the array of cooked starches showed SDS and RS contents from 33.22 to 35.35% and 6.42 to 9.22%, respectively. Our results are similar with other studies that showed a relative high amount of RS in cooked legume starches, that after their digestion could promote health benefits especially in terms of glycemic index and activation of microbiota due to their prebiotic effects [161, 167]. The RS fraction in cooked chickpea starches was highly positive correlated with amylose content (*r=0.8103, p=0.0045*), swelling power (*r=0.7313, p=0.0162*) and RVA viscosities (final, *r=0.7432, p=0.138*; setback, *r=0.8421, p=0.0022)*. Previous research have related the low digestion rates of legume starches to differences on their granular structures as well of their molecular conformations, in which the amylose/amylopectin ratio, degree of crystallinity and type of crystalline polymorphs have been related with these characteristics [156, 157, 163].

Moreover, the HI and pGI were estimated in order to obtain more information about the digestion performance of chickpea starches. For raw starches, HI ranged from 46.63 (R.ICC5383) to 48.08 (B.ICC4418), while pGI from 65.30 (R.ICC5383) to 66.10 (B.ICC4418). Thus, these starches can be classified as medium glycemic impact [155]. Additionally, negative correlations between pGI in raw starch samples with amylose contents (r=-0.6860, p=0.0282), and final viscosities (r=-0.6418, p=0.045) were found. These significant correlations are attributed to the amylose molecular interactions within the starch granules [162, 164]. Interestingly, when starches were cooked they increase their HI from 62.11 to 65.63, which resulted in higher pGI, 73.80 (B.ICC4418) to 75.74 (C.ICC3421).

Table 3.2.6. Starch digestion fractions of raw and gelatinized starches from pigmented chickpea varieties.

Cultivar	Raw starch					Gelatinized starch				
	Digestion fractions					Digestion fractions				
	RDS (%)	SDS (%)	RS (%)	HI	PGI	RDS (%)	SDS (%)	RS (%)	HI	PGI
C.BS[A]	17.6±0.43^{ef}	31.1±0.12^{cd}	51.2±0.38^{ab}	47.2±0.15^{a}	65.6±0.21^{abc}	56.8±0.44^{bc}	34.7±0.49^{ab}	8.4±0.29^{ab}	63.4±0.31^{cd}	74.5±0.18^{cd}
B.ICC6306[B]	20.5±0.07^{c}	29.5±0.25^{e}	49.9±0.15^{b}	47.7±0.24^{a}	65.9±0.05^{ab}	59.1±0.50^{a}	34.4±0.32^{ab}	6.4±0.44^{c}	64.2±0.18^{abc}	74.9±0.09^{bc}
B.ICC4418[B]	18.5±0.16^{de}	31.2±0.24^{c}	50.1±0.57^{b}	48.0±0.44^{a}	66.1±0.03^{a}	56.9±0.57^{bc}	35.0±0.33^{ab}	8.0±0.36^{abc}	62.1±0.32^{d}	73.8±0.18^{e}
B.ICC3761[B]	18.9±0.08^{d}	30.0±0.15^{e}	51.1±0.19^{ab}	47.7±0.20^{a}	65.9±0.12^{ab}	56.7±0.38^{bc}	34.8+0.44^{ab}	8.3±0.47^{ab}	64.2±0.44^{abc}	74.9±0.08^{bc}
Br.ICC3512[B]	18.5±0.26^{de}	34.1±0.08^{b}	47.2±0.31 ^{c}	47.4±0.20^{a}	65.7±0.04^{abc}	58.1±0.39^{abc}	35.4±0.46^{a}	6.4±0.13^{c}	63.1±0.30^{cd}	74.3±0.07^{d}
C.ICC3421[B]	21.4±0.19^{bc}	33.2±0.18^{b}	45.2±0.10^{d}	47.0±0.10^{a}	65.5±0.11^{bc}	59.2±0.47^{a}	33.4±0.47^{ab}	7.2±0.40^{bc}	65.6±0.18^{a}	75.7±0.09^{a}
G.ICC5613[B]	22.2±0.32^{b}	31.1±0.05^{c}	46.6±0.19^{cd}	47.9±0.24^{a}	66.0±0.07^{a}	58.6±0.33^{ab}	33.2±0.39^{b}	8.1±0.36^{abc}	64.9±0.39^{ab}	75.3±0.05^{ab}
R.ICC14782[B]	17.2±0.13^{f}	30.1±0.35^{de}	52.5±0.06^{a}	46.6 ±0.38^{a}	65.3±0.05^{c}	56.3±0.06^{c}	34.5±0.49^{ab}	9.1±0.32^{a}	65.3±0.21^{a}	75.5±0.11^{a}
R.ICC13124[B]	29.9±0.08^{a}	27.4±0.24^{f}	42.6±0.32^{e}	47.9±0.18 ^{a}	66.0±0.12^{a}	58.0±0.45^{abc}	34.7±0.25^{ab}	7.2±0.38^{bc}	63.6±0.14^{bc}	74.6±0.04^{cd}
R.ICC5383[B]	18.4±0.38^{def}	36.2±0.20^{a}	45.2±0.20^{d}	46.6±0.55^{a}	65.3±0.08^{c}	58.0±0.44^{abc}	35.3±0.38^{a}	9.2±0.33^{a}	65.3±0.25^{a}	75.5±0.09^{a}

RDS: Rapidly digestible starch, SDS: Slowly digestible starch, RS: Resistant starch, HI: Hydrolysis index (estimated from a 100% digestible starch from White bread), pGI: predicted glycemic index (pGI= 39.71 + 0.549HI), BS: cultivar Blanco Sinaloa 92, B: Black color, BR: Brown color, C: Cream color, G: Green color, R. Red color, [A] Kabulli seeds. [B] Desi seeds. Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p<0.05$).

3.2.10 Principal component analysis (PCA) of chickpea starches.

The PCA plots provide an overview of the similarities and differences between chickpea starches, as well as the interrelationships among the measured properties. The distance between the positions of any two cultivars on the score plot (Figure 3.2.2A) is directly proportional to the degree of difference or similarity between them. Regarding to seed coat coloration, no clear tendency was observed among the studied samples. The first and second components (PC1 and PC2) accounted for an accumulative variance of 82.64%. In the same sense, the loading plot (Figure 3.2.2B) of PC's provide information about the correlations between some viscous and digestion properties, in this figure, the properties (represented by lines) that lie close to each other on the plot are positively correlated; whereas, those with lines going in opposite directions are negatively correlated. The principal contributors in PC1 variation were the parameters related with granule swelling and viscosity that were closely related to amylose contents and the RS fractions in gelatinized starches. Such relationships have been previously reported and are distinctive characteristics of pulse starches [158, 162]

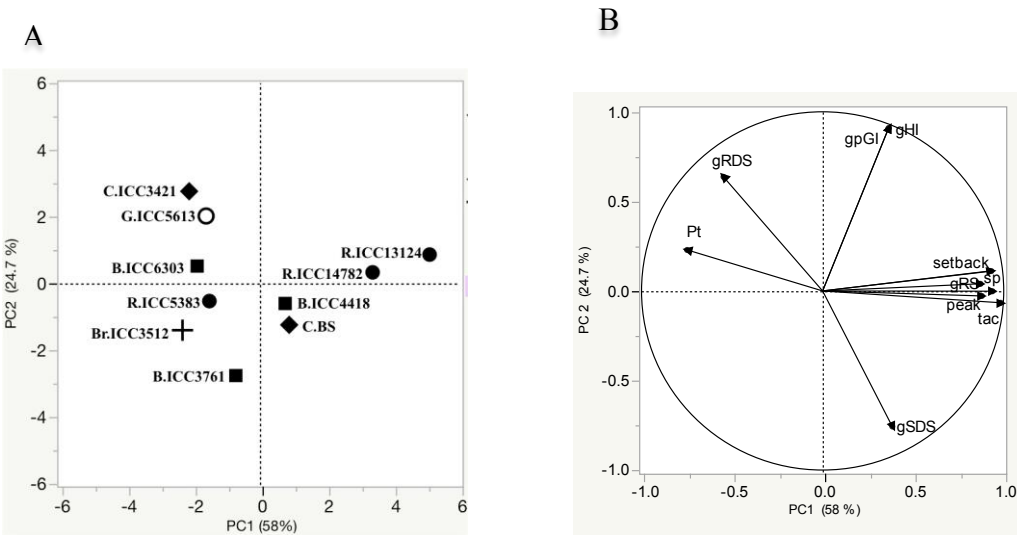


Figure 3.2.2 Principal component analysis: score plot (A) describing overall variation in the first (PC1) and second component (PC2) in chickpea starches and loading plot (B) of PC1 and PC2 describing variation among properties of chickpea starches.
Pt: pasting temperature, tac: total amylose content, sp: swelling power, peak, final and setback: RVA viscosities, gRDS: rapidly digestible starch, gSDS: slowly digestible starch gRS: resistant starch, gHI: Hydrolysis index gpGI: predicted glycemic index in gelatinized starches. BS: cultivar Blanco Sinaloa 92, B: Black color, BR: Brown color, C: Cream color, G: Green color, R. Red color.

3.3 Effect of soaking and cooking on flavonoids and saponins content of seed coat colored chickpea genotypes.

3.3.1 Identification of flavonoids and saponins in chickpea cultivars.

The identification of compounds in chickpea cultivars was conducted using the mass spectra data, uv-spectra and retention time (RT) of the chromatographic conditions. Among the samples analyzed five flavonoid, three isoflavones and three saponins were identified (Table 3.3.1). Figure 1 showed the representative compounds found in chickpea samples. The saponins identification was achieved compared retention time of ELSD chromatograms (Figure 2) and MS spectra (Figure 3). Soyasaponin I showed the presence of characteristic ions at 943.6 m/z [$M+H^+$], 965.6 m/z [$M+Na^+$], 797.6 m/z [$M-Rha+H^+$] and 925.6 m/z [$M-H_2O$][19, 21]. Soyasaponin βg and lablab saponin I had a DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H pyran-4-one) in their structure (C22) showing absorbance at 295 nm. Soyasaponin bg exhibited the presence of major ions at 1069.7 m/z [$M+H^+$], 1091.7 m/z [$M+Na^+$] and 923.6 m/z [M-Rha] as previously reported. The presence of lablab saponin I in chickpea has been only reported by Mekky et al 2015 [21], this compound showed major ions at 1083.7 m/z [$M+H^+$] and 1105.7 m/z [$M+Na^+$], and fragments ions at 937 m/z [M-Rha].

3.3.2 flavonoids content and processing effect in chickpea cultivars.

In chickpea cultivars five flavonoids were identified three Kaempferol isomers and two myricetin isomers. Flavonoids in chickpea cultivars were not quantify in raw samples. Also these compounds were not detected in soaking and cooked samples.

3.3.3. Isoflavones content and processing effect in chickpea cultivars.

The isoflavones (or phytoestrogens) are an important phenolic compound that usually found in legumes [4, 15]. The principal isoflavones found in chickpea cultivars were biochanin A glycoside, biochanin-A malonyl glycoside and biochanin A, the content in raw samples ranged from 1.14 to 18.47 µg/g, 0.18 to 24.15 µg/g and 0.59 to 7.25 µg/g, respectively (Table 3.3.2). The total isoflavone content were highest in (R.14782). However, the cooking process reduced the content of this compounds, converting the glycosides forms of biochanin-A in free forms. The biochanin-A was previously report in chickpea samples, our results agree with values reported by Wu et al [41]. Also Konar et al, 2012 [15] reported biochanin-A and biochanin-A glycoside but they found less content of this compounds compared with the studied chickpea cultivars.

3.3.4 Saponins content and processing effect in chickpea cultivars.

In all chickpea cultivars, Soyasaponin βg were identified as the most abundant saponin representing more than 80% of total content except by green (G.ICC5613) cultivar (64%), and the content ranged from 351-1707 µg/g (C.BS-R.ICC13124) (Table 3.4.4). Besides, Soyasaponin I was found in all samples in less than 10% except for green cultivar (G.ICC5613) (24%). The soyasaponin βg content in chickpea cultivars agree with previous reported but this chickpea samples had

nearby 2.3-20 fold less soyasaponin I as reported [20, 22]. Likewise, Lablab saponin I have been previously identify in chickpea but not quantified, however the chickpeas cultivars presented low (10%) amounts of this saponin.

The cooking process caused that Soyasaponin I content increased (304-1091%) in all chickpea samples (Table 3.3.3), also Soyasaponin βg content increased (84-178%). Nevertheless, lablab saponin I was not detected in cooked chickpea. The total saponins content in cooked chickpea cultivars were higher than previous reported (682-1583 μg/g)[20, 22] except for C.BS cultivar, which had 11 fold less than R.ICC14782 cultivar. The soyasaponin I content increased after cooking process in all chickpea cultivars as previous studies reported [20, 22]. It was assumed that this increase was caused by hydrolysis of DDMP of soyasaponin βg during cooking [20]. However, in the chickpea cultivars only four (C.BS, G.ICC5613, Br.ICC3512 and B.ICC6306) cooked samples had less soyasaponin βg content than in raw samples, and the others samples increased their soyasaponin βg after cooking by 16-130 % (R.ICC13124-B.ICC3761). Perhaps the saponins are present in the fiber o in anatomical seed part that the temperature reached during cooking help to their release [4]. The soaked seed was also analyzed (data not shown), the soaked samples had similar profile as raw samples, hence the convertion of soyasaponin I only happen after thermal process. Also the soaking and cooking water were analyzed for leaching saponins however saponin content were not detected in samples as others researchers [20, 22].

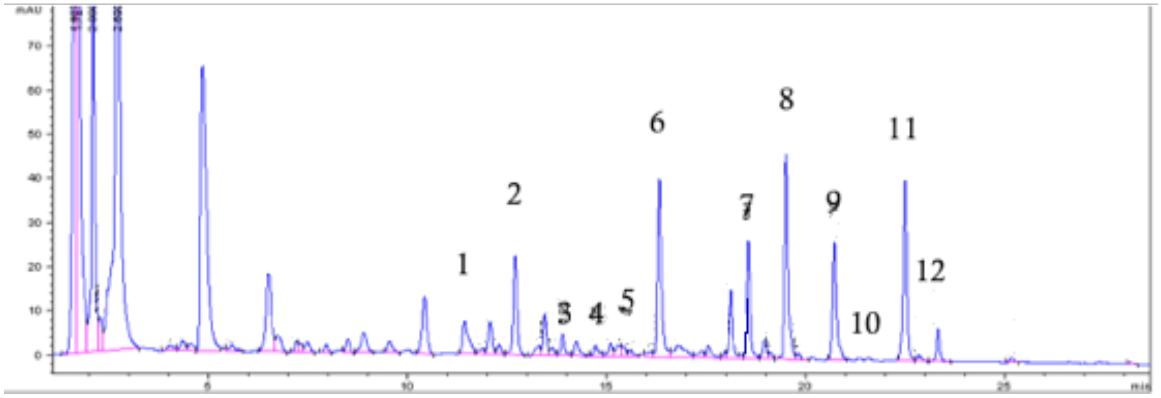


Figure 3.3.1. Representative HPLC chromatogram (260nm) of flavonoids, isoflavonoids and saponins of raw R.ICC14872 chickpea cultivar. Tentative list of compounds is listed in table 1.

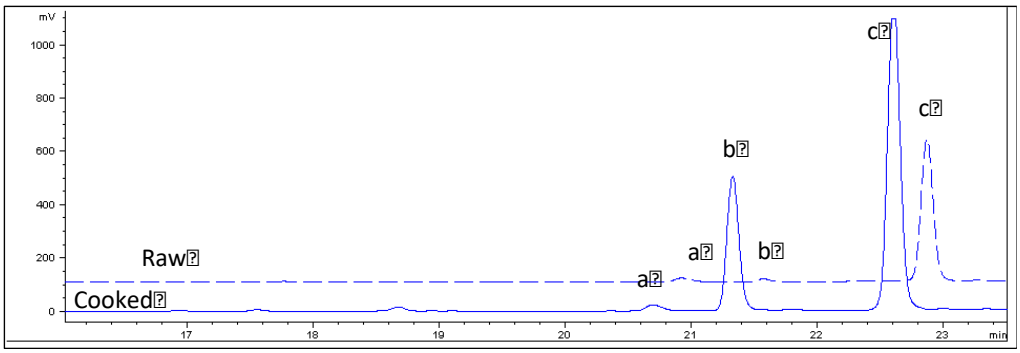


Figure 3.3.2. Representative HPLC chromatogram (ELSD) of saponins of raw and cooked R.ICC14872 chickpea cultivar. Lablab saponin I, b. Soyasaponin I, and Soyasaponin βg

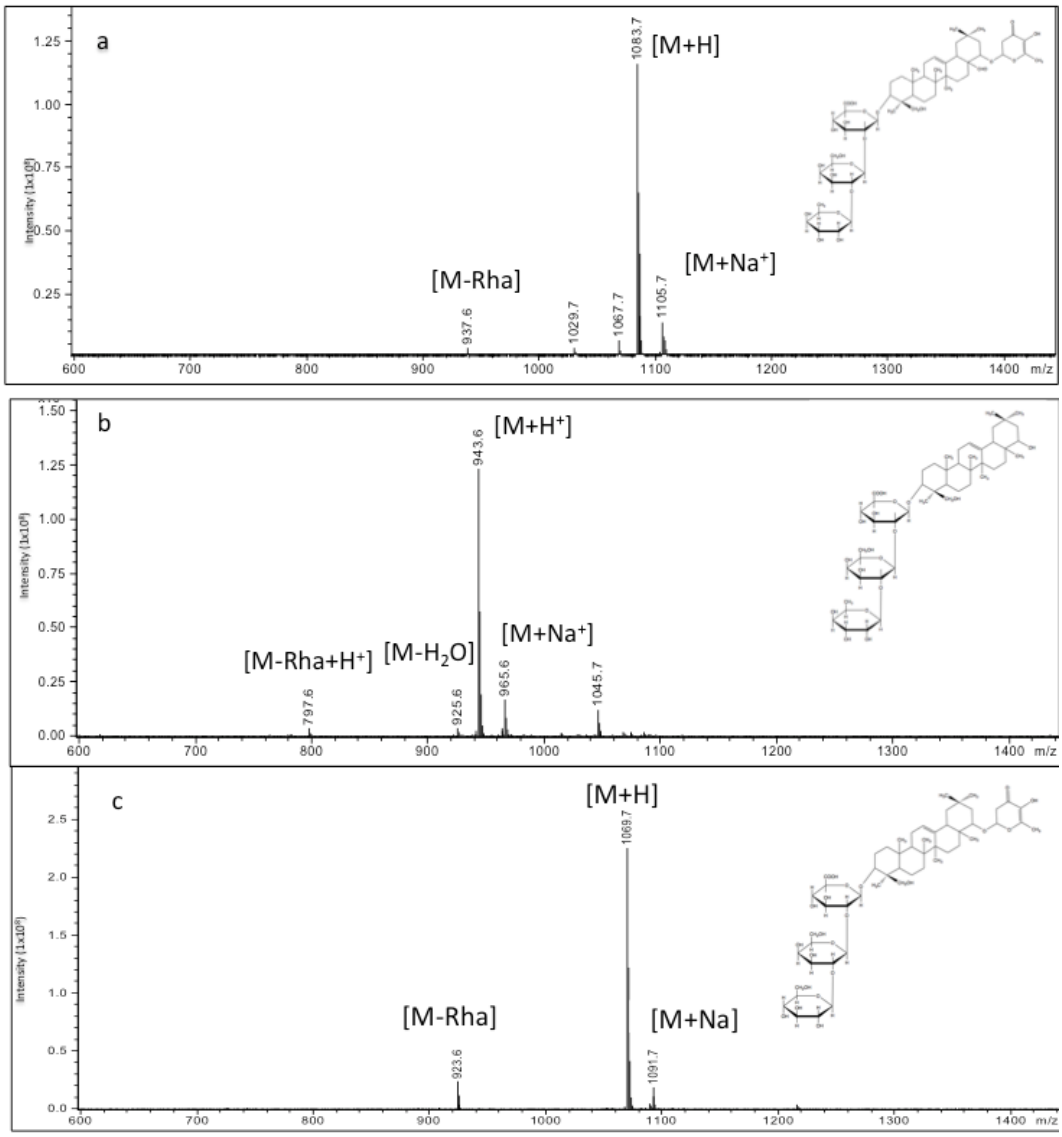


Figure 3.3.3. Positive ion mass spectra of a. Lablab saponin I, b. Soyasaponin I, and Soyasaponin βg found in R.ICC14872 chickpea cultivar.

Table 3.3.1. Phenolic compounds and saponins identified in raw chickpea cultivars according to retention time (RT), UV absorption and mass spectra data.

Peak	RT (min)	UV_{max} (nm)	Ions $[M+H]^+$	Fragment ions (m/z)	Compound	Cultivars										
						C.BS[A]	B.ICC6306[B]	B.ICC4418[B]	B.ICC3761[B]	Br.ICC3512[B]	C.ICC3421[B]	G.ICC5613[B]	R.ICC14782[B]	R.ICC13124[B]	R.ICC5383[B]	
	Flavonoids															
1	11.42	260, 354	821.5	319.3, 515.4	Myricetin derivative	+	-	-	-	+	-	+	+	-	-	
2	12.69	265, 343	627.3	319.3, 481.2	Myricitin 3-O-β-D-Galactopyranoside, 3'-O-α-L-rhamnopyranoside	-	-	+	+	+	-	+	+	+	+	
3	13.88	258, 349	727.3	287.1, 595.3	Kaempferol 3-O-lathyroside-7-O-a-Lrhamnopyranoside	-	+	+	+	+	-	+	+	+	+	[21]
4	14.70	264, 348	744.2	287.1	Kaempferol 3-**O**-**b**-D-apiofuranosyl-(1/ 2)-**b**-Dglucopyranoside- 4**0**-**O**-**b**-D-glucopyranoside	-	+	+	+	+	-	-	+	+	+	[21]
5	15.06	265, 345	772.9	287.1	Kaempferol derivative	-	+	-	+	+	-	-	+	+	+	
	Isoflavones															
7	18.56	261	447.2,	285.2	Biochanin A glycoside	+	+	+	-	+	+	+	+	+	+	[41]
8	19.52	260	533.2,	285.2	Biochanin A malonyl glycoside	+	+	+	+	+	+	+	+	+	+	[41]
12	23.32	261	285.2		Biochanin A	+	+	+	-	+	+	+	+	+	+	[41]
	Saponins															
9	20.72	297	1083.7,	937.6, 1105.7	Lablab saponin I	+	+	+	+	+	+	+	+	+	+	[19, 21]
10	21.33	nd	943.6,	797.6, 1045	Soyasaponin (I) Bb[19][19][19][19]	+	+	+	+	+	+	+	+	+	+	[19]
11	22.44	296	1069.7,	1091.7, 923.6	Soyasaponin (V) βg	+	+	+	+	+	+	+	+	+	+	[19]

[A] Kabuli seeds. [B] Desi seeds. BS: cultivar Blanco Sinaloa 92. B: Black color. BR: Brown color. C: Cream color. G: Green color. R: Red color.

Table 3.3.2. Isoflavones content (µg/g d.b) in raw and cooked chickpea cultivars.

Isoflavones	Cultivars	Raw	Cooked
Biochanin-A glycoside	C.BS[A]	5.34±0.0[a]	5.02±0.0[b]
	B.ICC6306[B]	4.58±0.0[a]	2.47±0.0[i]
	B.ICC4418[B]	2.24±0.0[a]	2.30±0.0[f]
	B.ICC3761[B]	**N.D.**	0.31±0.0[e]
	Br.ICC3512[B]	3.16±0.0[a]	4.36±0.0[c]
	C.ICC3421[B]	1.14±0.0[a]	3.79±0.0[d]
	G.ICC5613[B]	18.47±0.0[a]	1.83±0.0[g]
	R.ICC14782[B]	10.20±0.0[a]	11.38±0.0[a]
	R.ICC13124[B]	1.61±0.0[a]	2.26±0.0[f]
	R.ICC5383[B]	2.62±0.0[a]	1.11±0.0[h]
Biochanin-A malonyl glycoside	C.BS[A]	1.76±0.0[a]	5.36±0.0[b]
	B.ICC6306[B]	7.68±0.0[a]	3.18±0.0[i]
	B.ICC4418[B]	6.48±0.0[a]	3.19±0.0[e]
	B.ICC3761[B]	1.02±0.0[a]	0.49±0.0[e]
	Br.ICC3512[B]	8.94±0.0[a]	5.04±0.0[c]
	C.ICC3421[B]	5.10±0.0[a]	4.29±0.0[d]
	G.ICC5613[B]	5.34±0.0[a]	2.38±0.0[g]
	R.ICC14782[B]	24.15±0.0[a]	10.70±0.0[a]
	R.ICC13124[B]	4.44±0.0[a]	2.66±0.0[f]
	R.ICC5383[B]	0.18±0.0[a]	1.62±0.0[h]
Biochanin-A	C.BS[A]	2.54±0.0[a]	1.60±0.0[a]
	B.ICC6306[B]	0.59±0.0[a]	0.47±0.0[a]
	B.ICC4418[B]	4.26±0.0[a]	0.48±0.0[a]
	B.ICC3761[B]	**N.D.**	0.00±0.0[a]
	Br.ICC3512[B]	5.61±0.0[a]	1.01±0.0[a]
	C.ICC3421[B]	7.25±0.0[a]	0.55±0.0[a]
	G.ICC5613[B]	3.41±0.0[a]	0.80±0.0[a]
	R.ICC14782[B]	2.34±0.0[a]	5.68±0.0[a]
	R.ICC13124[B]	3.82±0.0[a]	1.23±0.0[a]
	R.ICC5383[B]	2.38±0.0[a]	0.53±0.0[a]
Total	C.BS[A]	9.64±0.0[a]	11.98±0.0[a]
	B.ICC6306[B]	12.84±0.0[a]	6.12±0.0[a]
	B.ICC4418[B]	12.97±0.0[a]	5.96±0.0[a]
	B.ICC3761[B]	0.84±0.0[a]	0.80±0.0[a]
	Br.ICC3512[B]	17.71±0.0[a]	10.42±0.0[a]
	C.ICC3421[B]	13.49±0.0[a]	8.63±0.0[a]
	G.ICC5613[B]	27.21±0.0[a]	5.00±0.0[a]
	R.ICC14782[B]	38.18±0.0[a]	27.76±0.0[a]
	R.ICC13124[B]	8.39±0.0[a]	6.15±0.0[a]
	R.ICC5383[B]	5.17±0.0[a]	3.25±0.0[a]

[A] Kabuli seeds. [B] Desi seeds. N.D.: not detected. d.b: dry basis. BS: cultivar Blanco Sinaloa 92. B: Black color. BR: Brown color. C: Cream color. G: Green color. R: Red color. Values are means ± SEM (standard error mean). Values with different letter(s) in every column are significantly different by compound ($p<0.05$).

Table 3.3.3. Saponins content (µg/g d.b.) in raw and cooked chickpea cultivars.

Compound	Cultivars	Raw	Cooked
Lablab saponin I	C.BS[A]	44 ± 1[h]	**N.D.**
	B.ICC6306[B]	64 ± 2[g]	**N.D.**
	B.ICC4418[B]	161 ± 3[cd]	**N.D.**
	B.ICC3761[B]	132 ± 2[ef]	**N.D.**
	Br.ICC3512[B]	136 ± 6[ef]	**N.D.**
	C.ICC3421[B]	120 ± 3[f]	**N.D.**
	G.ICC5613[B]	164 ± 1[c]	**N.D.**
	R.ICC14782[B]	248 ± 2[a]	**N.D.**
	R.ICC13124[B]	201 ± 2[b]	**N.D.**
	R.ICC5383[B]	146 ± 3[de]	**N.D.**
Soyasaponin I	C.BS[A]	57 ± 1[f]	154 ± 6[g]
	B.ICC6306[B]	64 ± 3[ef]	246 ± 11[fg]
	B.ICC4418[B]	136 ± 3[cd]	918 ± 43[e]
	B.ICC3761[B]	138 ± 3[cd]	1188 ± 36[bc]
	Br.ICC3512[B]	82 ± 1[a]	326 ± 19[f]
	C.ICC3421[B]	117 ± 2[d]	999 ± 38[de]
	G.ICC5613[B]	323 ± 7[a]	1086 ± 49[cd]
	R.ICC14782[B]	141 ± 3[c]	1467 ± 18[a]
	R.ICC13124[B]	126 ± 2[cd]	1056 ± 20[cde]
	R.ICC5383[B]	165 ± 8[b]	1291 ± 23[b]
Soyasaponin βg	C.BS[A]	351 ± 14[f]	236 ± 11[g]
	B.ICC6306[B]	483 ± 5[ef]	478 ± 21[f]
	B.ICC4418[B]	1118 ± 8[c]	1627 ± 41[d]
	B.ICC3761[B]	978 ± 2[cd]	2251 ± 28[b]
	Br.ICC3512[B]	636 ± 6[e]	628 ± 24[f]
	C.ICC3421[B]	895 ± 5[e]	1665 ± 52[d]
	G.ICC5613[B]	929 ± 7[d]	895 ± 28[e]
	R.ICC14782[B]	1682 ± 4[a]	2836 ± 45[a]
	R.ICC13124[B]	1707 ± 4[a]	1995 ± 57[c]
	R.ICC5383[B]	1400 ± 3[b]	1804 ± 51[cd]
Total	C.BS[A]	453 ± 14[f]	391 ± 4[i]
	B.ICC6306[B]	611 ± 16[f]	725 ± 9[h]
	B.ICC4418[B]	1417 ± 38[c]	2546 ± 1[e]
	B.ICC3761[B]	1248 ± 15[cd]	3439 ± 8[b]
	Br.ICC3512[B]	855 ± 23[e]	954 ± 5[g]
	C.ICC3421[B]	1133 ± 22[d]	2665 ± 13[d]
	G.ICC5613[B]	1416 ± 33[c]	1981 ± 20[f]
	R.ICC14782[B]	2072 ± 54[a]	4304 ± 27[a]
	R.ICC13124[B]	2035 ± 48[a]	3052 ± 37[c]
	R.ICC5383[B]	1713 ± 45[b]	3095 ± 28[c]

[A] Kabuli seeds. [B] Desi seeds. N.D.: not detected. d.b: dry basis. BS: cultivar Blanco Sinaloa 92. B: Black color. BR: Brown color. C: Cream color. G: Green color. R: Red color. Values are means ± SEM (standard error mean). Values with different letter(s) in every column are significantly different by compound ($p<0.05$).

3.4 Effect of the germination process in physical characteristics and phytochemical content in four chickpea cultivars.

3.4.1 Physical changes during the germination process in chickpea cultivars.

Seed germination starts with the imbibition of water through and is completed by radicle protuberance through the tissues adjacent the embryo [6]. In table 3.4.1, the germination percentage (G%) in cultivar B.ICC6303 was significant low ($p>0.05$) at day 2, by it increased favorably in the consecutive days. After day 3, the 91% of seeds showed the presence of sprout in all cultivars. The permeability of seed coat has an important role during the germination process of legumes by regulating water uptake [6]. The seed coat of cultivars B.ICC6303 and R.ICC14782 may have low permeability causing delaying in germination. The sprout length in day 5 increased 2-3 times compared with day 2, except in cultivar R.ICC14782, which had a mean sprout length of 2.15 cm at day 3 to 5. Also the sprout % increased 4 times in all cultivars. Similar results have been reported in black beans germination [168]. The seed coat % was not affect during germination process. Meanwhile cotyledon % showed slight reductions.

3.4.2 Total phenolic content changes during germination

The changes in total phenolic content (TPC) during germination process in chickpea cultivars are shown in Figure 3.4.1. Significant differences among chickpea cultivars were found after day 2. In day 2 and 3, C.BS cultivar showed highest TPC but in day 4 and 5, TPC in G.ICC5613 cultivar increased dramatically. It increased 4 fold compared with day 1. In day 5, G.ICC5613 cultivar had almost 2 fold TPC than the others cultivars. Other researchers have reported significant TPC increased during chickpea germination [41].

3.4.3 Effect of the germination process on phytochemical content in chickpea cultivars

The Figure 3.4.2 depicted comparative chromatograms of phytochemical profile in the four chickpea cultivars after five germination days at 260 nm. The mass spectra data, UV absorption maxima and retention time (RT) of the chromatographic conditions were used to in order to identify the compounds in chickpea cultivars. The m/z values were matched with previous known phytochemicals in germinated chickpea [19, 41, 102]. Eight isoflavonoids (isoformononetin glycosde, formononetin malonyl glycoside, biochanin A glycoside, biochanin A malonyl glycoside, Pseudobaptigenin, formononetin, 5-hydropseudobaptigenin and biochanin A) and one saponin (soyasaponin βg) were detected in chickpea samples (Table 3.4.2).

The total isoflavonoid content in chickpea seeds was reached after 5 germination days in all cultivars. Total isoflavonoid content (TIFC) ranged from 1103 µg/g (R.ICC14782) to 8977 µg/g (G.ICC5613). The TIFC in chickpea cultivars varied from

18 to 225 fold during germination time. Moreover, the main isoflavones after five germination days were formononetin (Peak 6), biochanin-A (Peak 8) and its malonyl glycosides (Peak 2 and 4). This four compound represented up to 90% of TIFC but diverge profile was found among cultivars. Formononetin malonyl glycoside was the major isoflavonoid found in C.BS (36%) and B.ICC6306 (41%). However, formononetin and biochanin A malonyl glycoside were the main isoflavonoids in G.ICC5613 (46%) and R.ICC14782 (29%), respectively. According with other studies of chickpea germination the major isoflavonoids are biochanin A and formononetin aglycones [41, 96], however only G.ICC5613 showed that tendency.

The soyasaponin βg was the only saponin found in germinated chickpea samples (Table 3.4.2), which agree with the fact that represented up to 80 % total saponin content in raw seeds. In C.BS and G.ICC5613 cultivars, the soyasaponin βg increased during the germination process although the content in G.ICC5613 is almost 4 times higher than C.BS. In B.ICC6306 was not significant variation in content during germination process. Similar behavior was found in R.ICC14782 although in day 5 increased 60% saponin content. The germination process decreased saponin content in some pulses [168, 169]. However others seeds showed increased content of saponins as alfalfa [170] and huazontle [171].

In table 3.4.4 are shown the main effects and interactions of genotype, germination time and their interaction. In which, % of germination, sprout length, TPC, TIFC and saponins content showed a significant effect ($p<0.0001$) in the 2 variables and their interaction, whereas % of anatomical parts showed different effects. Sprout % was only had effect by genotype and cotyledon % had not significant effect on the variables interaction.

Table 3.4.1. Change of physical properties of chickpea cultivars during the germination process.

Properties		Germination Day				
	Cultivar	1	2	3	4	5
Germination (%)	C.BS^{X}	n.d.	$93.13\pm1.97^{a}_{A}$	$94.85\pm0.13^{a}_{B}$	$91.91\pm0.25^{a}_{B}$	$80.01\pm0.84^{b}_{B}$
	B.ICC6306^{Y}	n.d.	$61.86+0.93^{b}_{C}$	$94.38\pm0.11^{a}_{B}$	$92.13\pm0.22^{a}_{AB}$	$93.65\pm0.32^{a}_{A}$
	G.ICC5613^{Y}	n.d.	$90.11\pm4.06^{a}_{AB}$	$98.88\pm0.21^{a}_{A}$	$96.08\pm0.27^{a}_{A}$	$93.27\pm0.62^{a}_{A}$
	R.ICC14782^{Y}	n.d.	$71.27\pm4.76^{b}_{BC}$	$91.47\pm0.73^{a}_{C}$	$88.44\pm1.34^{a}_{B}$	$89.37\pm1.87^{a}_{A}$
Sprout length (cm)	C.BS^{X}	n.d.	$2.25\pm0.21^{c}_{A}$	$4.04\pm0.16^{b}_{A}$	$4.80\pm0.24^{ab}_{A}$	$5.40+0.14^{a}_{A}$
	B.ICC6306^{Y}	n.d.	$0.85\pm0.08^{c}_{B}$	$1.31\pm0.10^{c}_{C}$	$2.13\pm0.21^{b}_{B}$	$3.00+0.17^{a}_{B}$
	G.ICC5613^{Y}	n.d.	$1.12\pm0.03^{b}_{B}$	$1.63\pm0.13^{b}_{BC}$	$2.20\pm0.07^{a}_{B}$	$2.35\pm0.22^{a}_{BC}$
	R.ICC14782^{Y}	n.d.	$1.16\pm0.09^{b}_{B}$	$2.15\pm0.16^{a}_{B}$	$2.15\pm0.14^{a}_{B}$	$2.15\pm0.13^{a}_{C}$
Sprout (%)	C.BS^{X}	$0.72\pm0.04^{b}_{B}$	$1.83\pm0.06^{b}_{B}$	$3.29\pm0.05^{ab}_{A}$	$4.68\pm0.29^{a}_{A}$	$4.90\pm0.96^{a}_{A}$
	B.ICC6306^{Y}	$1.14\pm0.00^{a}_{B}$	$1.55\pm0.00^{a}_{B}$	$2.13\pm0.10^{a}_{A}$	$3.33\pm0.55^{a}_{A}$	$3.43\pm0.78^{a}_{A}$
	G.ICC5613^{Y}	$1.63\pm0.17^{c}_{A}$	$2.49\pm0.11^{bc}_{A}$	$2.94\pm0.37^{b}_{A}$	$3.02\pm0.07^{ab}_{A}$	$4.23\pm0.22^{a}_{A}$
	R.ICC14782^{Y}	$0.92\pm0.05^{b}_{B}$	$2.10\pm0.14^{ab}_{AB}$	$2.64\pm0.25^{ab}_{A}$	$3.05\pm0.69^{a}_{A}$	$3.41\pm0.07^{a}_{A}$
Seed coat (%)	C.BS^{X}	$4.53\pm0.16^{a}_{C}$	$4.31\pm0.00^{a}_{C}$	$4.45\pm0.32^{a}_{C}$	$4.22\pm0.02^{a}_{C}$	$4.28\pm0.15^{a}_{C}$
	B.ICC6306^{Y}	$12.64\pm0.77^{a}_{B}$	$11.85\pm0.22^{a}_{B}$	$12.28\pm0.42^{a}_{B}$	$11.56\pm0.49^{a}_{B}$	$12.43\pm0.17^{a}_{B}$
	G.ICC5613^{Y}	$16.72\pm0.10^{a}_{A}$	$16.28\pm0.62^{a}_{A}$	$16.72\pm0.53^{a}_{A}$	$15.63\pm0.15^{a}_{A}$	$16.79\pm0.69^{a}_{A}$
	R.ICC14782^{Y}	$11.87\pm0.10^{a}_{B}$	$12.41\pm0.06^{a}_{B}$	$11.33\pm0.34^{a}_{B}$	$12.36\pm0.06^{a}_{B}$	$11.93\pm0.32^{a}_{B}$
Cotyledon (%)	C.BS^{X}	$94.75\pm0.20^{a}_{A}$	$93.86\pm0.07^{ab}_{A}$	$92.26\pm0.27^{bc}_{A}$	$91.09\pm0.27^{c}_{A}$	90.81 ± 0.81^{cA}
	B.ICC6306^{Y}	$86.22\pm0.76^{a}_{B}$	$86.60\pm0.22^{a}_{B}$	$85.59\pm0.32^{a}_{B}$	$85.11\pm0.05^{a}_{B}$	84.14 ± 0.96^{aB}
	G.ICC5613^{Y}	$81.60\pm0.04^{a}_{C}$	$81.22\pm0.74^{ab}_{C}$	$80.33\pm0.15^{ab}_{C}$	81.35 ± 0.07^{aC}	78.98 ± 0.47^{bC}
	R.ICC14782^{Y}	$87.20\pm0.05^{a}_{B}$	$85.48\pm0.20^{ab}_{B}$	$86.02\pm0.60^{ab}_{B}$	84.59 ± 0.54^{bB}	84.66 ± 0.40^{bB}

n.d. not determinate. BS: cultivar Blanco Sinaloa 92, B: Black color, C: Cream color, G: Green color, R. Red color, ^{X} Kabulli seeds. ^{Y} Desi seeds. Values are means ± SEM. Values with different uppercase letter(s) in every line are significantly different ($p<0.05$). Values with different lowercase letter(s) in every column are significantly different ($p<0.05$) by physical property.

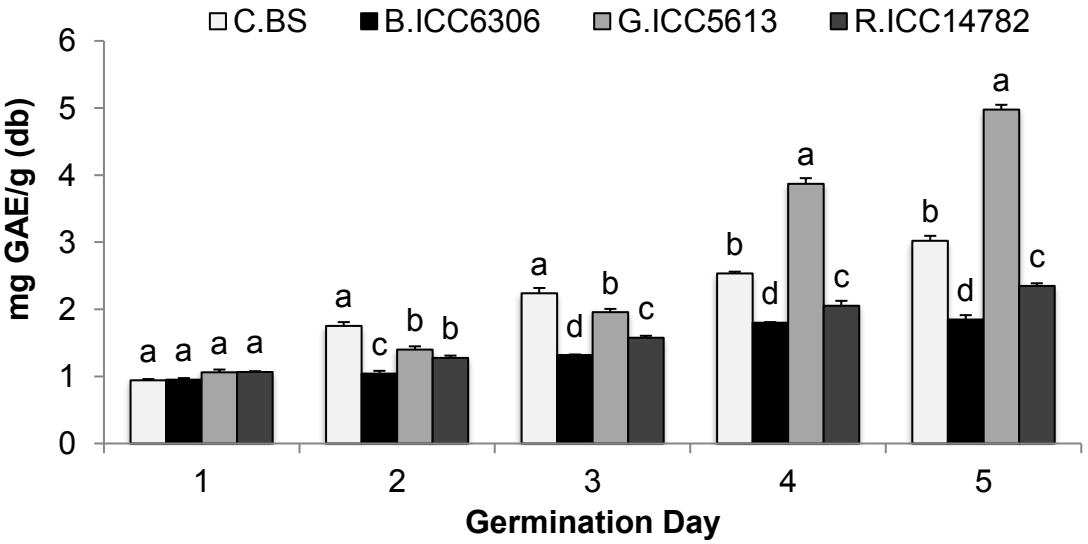


Figure 3.4.1. Effect of the germination process in total phenolics content on chickpea cultivars. Values are means ± SEM. Values with different letter(s) in each germination day are significantly different ($p<0.05$). BS: cultivar Blanco Sinaloa 92, B: Black color, C: Cream color, G: Green color, R. Red color.

Table 3.4.2. Isoflavonoids characterized germinated chickpea cultivars according to retention time (RT), UV absorption and mass spectra data.

Peak	UV_{max} (nm)	Ions $[M+H]^+$	Compound	Reference
1	259	431	Isoformononetin glycoside	[41, 102]
2	249	531	Formononetin malonyl glycoside	[41, 102]
3	261	447	Biochanin A glycoside	[41, 102]
4	260,^{288sh}	533	Biochanin A malonyl glycoside	[41, 102]
5	241,^{295sh}	283	Pseudobaptigenin	[41, 102]
6	249	269	Formononetin	[41, 102]
7	260,^{293sh}	299	5-hydroxypseudobaptigenin	[41, 102]
8	261	285	Biochanin A	[41, 102]
S	296	1069	Soyasaponin βg	[19]

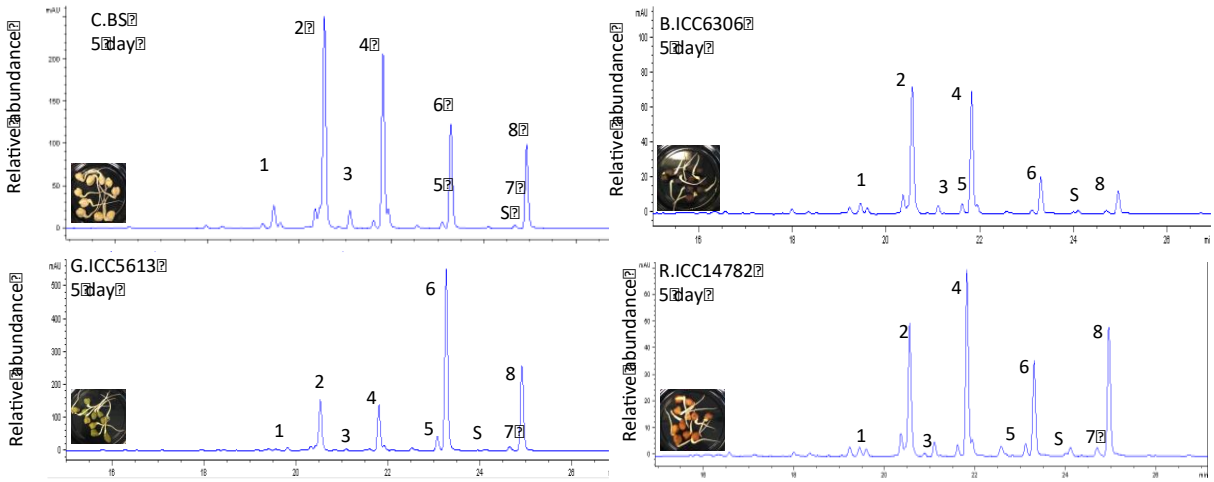


Figure 3.4.2. Comparative HPLC chromatograms (260nm) of isoflavonoids between chickpea cultivars at day 5. Tentative list of compounds is listed in table 2. BS: cultivar Blanco Sinaloa 92, B: Black color, C: Cream color, G: Green color, R. Red color.

Table 3.4.3. Effect of the germination process in the content of isoflavonoids (µg/g d.b) in chickpea cultivars.

Compound	Cultivar	Germination day				
		1	2	3	4	5
1	C.BS^{X}	N.D.	73.10±0.49$^{c}_{A}$	106.19±1.88$^{a}_{A}$	94.82±2.11$^{b}_{A}$	109.44±0.71$^{a}_{A}$
	B.ICC6306^{Y}	N.D.	N.D.	57.17±1.24$^{a}_{B}$	17.04±1.64$^{c}_{C}$	22.83±0.93$^{b}_{C}$
	G.ICC5613^{Y}	N.D.	15.49±0.50$^{c}_{C}$	63.53±0.02$^{a}_{B}$	34.63±3.11$^{b}_{B}$	58.62±3.20$^{a}_{B}$
	R.ICC14782^{Y}	N.D.	25.54±2.90$^{b}_{B}$	33.97±0.80$^{ab}_{C}$	34.72±0.63$^{a}_{B}$	26.30±1.40$^{ab}_{C}$
2	C.BS^{X}	74.26±0.11$^{e}_{A}$	502.11±32.73$^{d}_{A}$	854.63±2.51$^{b}_{A}$	713.08±20.55$^{c}_{A}$	1041.41±21.44$^{a}_{B}$
	B.ICC6306^{Y}	9.94±1.11$^{e}_{B}$	14.18±1.41$^{c}_{C}$	177.25±8.97$^{b}_{B}$	129.76±6.54$^{b}_{D}$	453.22±16.93 $^{a}_{C}$
	G.ICC5613^{Y}	6.77±0.28$^{e}_{B}$	156.87±8.13$^{d}_{B}$	836.80±4.65$^{b}_{A}$	575.75±15.81$^{c}_{B}$	1302.80±12.49$^{a}_{A}$
	R.ICC14782^{Y}	N.D.	120.70±4.92$^{c}_{B}$	176.71±2.32$^{bc}_{B}$	290.96±11.23$^{ab}_{C}$	393.41±54.67$^{a}_{C}$
3	C.BS^{X}	N.D.	65.69±1.11$^{b}_{A}$	76.75±1.23$^{a}_{A}$	83.89±2.65$^{a}_{A}$	80.77±0.59$^{a}_{A}$
	B.ICC6306^{Y}	N.D.	7.29±0.34$^{d}_{D}$	35.00±0.5$^{a}_{C}$	18.24±0.57$^{c}_{D}$	23.70±1.74$^{b}_{C}$
	G.ICC5613^{Y}	N.D.	25.22±1.00$^{d}_{C}$	49.07±1.73$^{a}_{A}$	41.85±4.13$^{ab}_{C}$	35.08±2.59$^{bc}_{B}$
	R.ICC14782^{Y}	N.D.	43.75±5.41$^{ab}_{B}$	37.52±1.89$^{b}_{C}$	56.86±0.50$^{a}_{B}$	36.92±0.31$^{b}_{B}$
4	C.BS^{X}	59.39±4.41$^{d}_{A}$	255.73±14.73$^{c}_{A}$	506.22±0.70$^{b}_{B}$	568.17±22.32$^{b}_{A}$	716.42±17.06$^{a}_{B}$
	B.ICC6306^{Y}	N.D.	46.53±0.39$^{c}_{C}$	154.55±2.27$^{b}_{D}$	135.44±4.46$^{b}_{C}$	375.82±12.87$^{a}_{C}$
	G.ICC5613^{Y}	24.08±0.9$^{e}_{B}$	160.19±10.03$^{d}_{B}$	704.55±6.49$^{b}_{A}$	543.66±19.24$^{c}_{A}$	949.17±16.65$^{a}_{A}$
	R.ICC14782^{Y}	73.41+6.27$^{c}_{A}$	149.62±16.73$^{c}_{B}$	189.43±6.04$^{bc}_{C}$	340.14±2.46$^{ab}_{B}$	471.84±63.91$^{a}_{C}$
5	C.BS^{X}	N.D.	N.D.	13.31±0.11$^{c}_{B}$	14.74±0.08$^{b}_{B}$	17.20±0.43$^{a}_{B}$
	B.ICC6306^{Y}	N.D.	9.73±0.21$^{a}_{C}$	7.52±2.20$^{a}_{B}$	8.84±2.31$^{a}_{B}$	11.25±0.64$^{a}_{B}$
	G.ICC5613^{Y}	N.D.	19.41±0.70$^{c}_{B}$	86.59±3.50$^{b}_{A}$	71.91±6.94$^{b}_{A}$	305.54±2.93$^{a}_{A}$
	R.ICC14782^{Y}	N.D.	24.42±1.54$^{a}_{A}$	19.15±3.24$^{a}_{B}$	12.92±2.70$^{a}_{B}$	27.46±13.95$^{a}_{B}$
6	C.BS^{X}	12.14±0.43$^{d}_{A}$	133.34±3.50$^{c}_{A}$	297.36±4.45$^{b}_{B}$	445.90±25.52$^{a}_{B}$	471.05±6.17$^{a}_{B}$
	B.ICC6306^{Y}	N.D.	N.D.	60.57±1.97$^{b}_{C}$	24.35±0.40$^{c}_{C}$	117.43±4.64$^{a}_{B}$
	G.ICC5613^{Y}	N.D.	155.36±27.6$^{c}_{A}$	1394.65±70.11$^{b}_{A}$	1676.08±118.78$^{b}_{A}$	4198.79±128.67$^{a}_{A}$
	R.ICC14782^{Y}	N.D.	44.58±6.28$^{b}_{B}$	56.99±1.18$^{b}_{C}$	243.60±3.35$^{a}_{BC}$	255.08±27.40$^{a}_{B}$
7	C.BS^{X}	N.D.	9.62±0.06$^{b}_{B}$	10.39±0.06$^{b}_{B}$	14.41±0.63$^{a}_{B}$	15.68±0.31$^{a}_{B}$
	B.ICC6306^{Y}	N.D.	N.D.	9.49±0.27$^{b}_{B}$	10.44±0.05$^{b}_{B}$	12.40±0.55$^{a}_{B}$
	G.ICC5613^{Y}	N.D.	11.76±0.23$^{c}_{A}$	39.98±2.02$^{b}_{A}$	44.85±4.45$^{b}_{A}$	95.52±2.51$^{a}_{A}$
	R.ICC14782^{Y}	N.D.	11.84±0.58$^{b}_{A}$	13.39±0.22$^{b}_{B}$	18.28±0.81$^{ab}_{B}$	24.85±4.36$^{a}_{B}$
8	C.BS^{X}	9.65±0.7$^{d}_{A}$	130.38±1.5$^{c}_{AB}$	266.19±2.8$^{b}_{B}$	432.58±29.6$^{a}_{B}$	394.41±6.2$^{a}_{B}$
	B.ICC6306^{Y}	8.26±0.9$^{d}_{A}$	6.40±0.2$^{d}_{C}$	48.15±0.4$^{b}_{D}$	21.88±0.1$^{c}_{C}$	75.51±3.7$^{a}_{C}$
	G.ICC5613^{Y}	9.03±2.1$^{c}_{A}$	168.37±25.5$^{c}_{A}$	1219.76±6.64$^{b}_{A}$	1310.87±107.8$^{b}_{A}$	2032.09±56.7$^{a}_{A}$
	R.ICC14782^{Y}	8.04±1.7$^{b}_{A}$	71.56±6.1$^{b}_{BC}$	100.77±11.8$^{b}_{C}$	269.41±7.3$^{a}_{BC}$	379.94±45.2$^{a}_{B}$
Total	C.BS^{X}	155.44±4.3$^{d}_{A}$	1169.97±54.04$^{c}_{A}$	2131.04±4.85$^{b}_{B}$	2367.59±103.47$^{b}_{B}$	2846.40±52.93$^{a}_{B}$
	B.ICC6306^{Y}	18.1±2.0$^{d}_{D}$	84,12±0.23$^{d}_{C}$	549.72±5.00$^{c}_{C}$	366.01±6.05$^{b}_{D}$	1103.15±59.7$^{a}_{C}$
	G.ICC5613^{Y}	39.88±3.29$^{c}_{C}$	712.67±71.28$^{c}_{B}$	4394.94±80.87$^{b}_{A}$	4299.61±273.98$^{b}_{A}$	8977.59±214.18$^{a}_{A}$
	R.ICC14782^{Y}	81.46±4.59$^{b}_{B}$	492.01±44.47$^{b}_{B}$	627.92±3.01$^{b}_{C}$	1266.90±19.99$^{a}_{C}$	1615.82±211.21$^{a}_{C}$

N.D. Not detected. BS: cultivar Blanco Sinaloa 92, B: Black color, C: Cream color, G: Green color, R. Red color, ^{X} Kabulli seeds. ^{Y} Desi seeds. Values are means ± SEM. Values with different uppercase letter(s) in every line are significantly different (p>0.05). Values with different lowercase letter(s) in every column are significantly different (p>0.05) by compound.

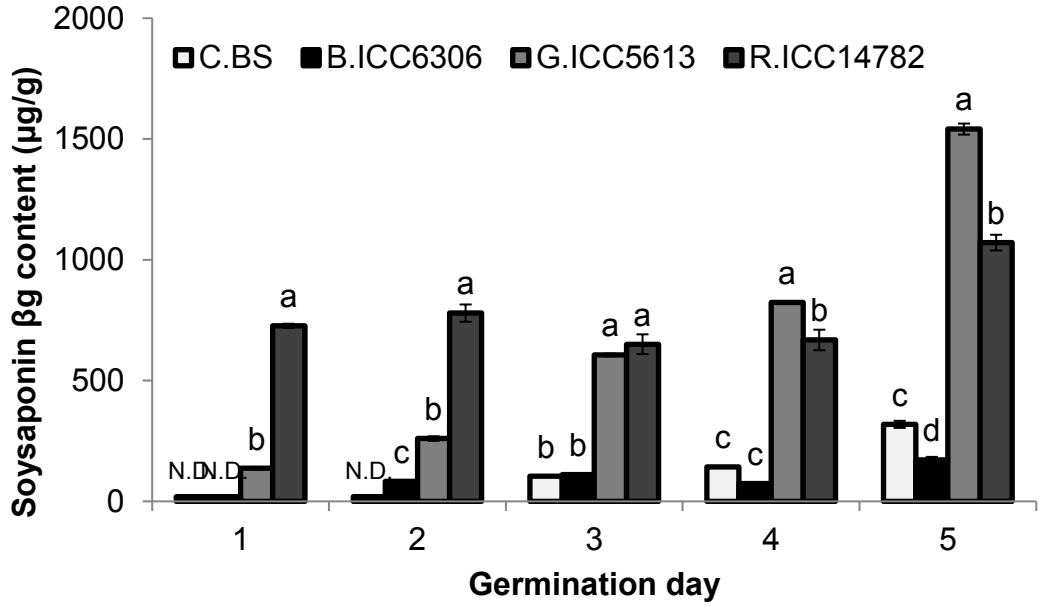


Figure 3.4.3. Effect of the germination process in soyasaponin βg content on chickpea cultivars. Values are means ± SEM. Values with different letter(s) in each germination day are significantly different ($p<0.05$). BS: cultivar Blanco Sinaloa 92, B: Black color, C: Cream color, G: Green color, R. Red color. N.D. Not detected.

Table 3.4.4. Main and interaction effects of genotype, germination time and their interaction on physical properties, total phenolics, total isoflavonoids and saponin content in chickpea cultivars.

Parameter	Genotype	Germination time	Interaction
% Germination	****	****	****
Sprout Length	****	****	****
% Sprout	***	****	*
% Seed coat	*	NS	NS
% Cotyledon	****	****	NS
TPC	****	****	****
TIFC	****	****	****
Saponin	****	****	****

* p<0.05,** p<0.01, ***p<0.001, ****p<0.0001.
TPC,total phenolic content, TIFC, total isoflavonoids content.

3.5. Purification and identification of anti-inflammatory peptides and isoflavonoids from non-absorbed protein concentrates from cooked or germinated chickpea cultivars

3.5.1 Effect of processing and digestion on total phenolics, soluble protein and peptides content in chickpea cultivars

Total phenolic content (TPC) varied significant in Green (GC) and Blanco Sinaloa (BS) cultivars during processing, protein concentration and digestion (Table 1). Chickpea germination is an inexpensive process that increases the nutritional value and isoflavonoids content [41]. This process increased 9-fold (BSg) and 6-fold (GCg) the TPC in chickpea compared with raw counterparts. TPC in GCg was significant higher than BSg due to difference of genotype and seed coat pigmentation [39]. The protein concentration by alkaline and acid conditions also showed an effect on TPC in chickpea samples. Germinated protein concentrates increased 20 % (cGg) and 80 % (cBSg) in TPC compared with germinated counterparts. Others authors [122] had reported higher amounts of TPC during protein concentration of raw chickpea. Protein concentrates from cooked chickpea (cBSc, cGCc) from both cultivars had similar TPC values compared with raw counterparts (Table 1); this could be to a reduction in TPC during cooking as previously reported [76] and/or an increased of TPC during protein concentration [122].

The simulated gastrointestinal digestion of germinated chickpea concentrates increased TPC release close to 2.5 fold compared with germinated concentrates (cBSg, cGCg) in both cultivars, in which, dBSg showed the highest release of TPC (44 fold) by digestion compared with the raw sample. Besides, the digestion released significant TPC from cooked concentrates increasing 17-fold in dBSc and 9.8-fold in dGCc compared with its concentrates counterparts (cBSc, cGCc). The protein concentrates from cooked seed had denatured proteins and gelatinizated starch that during cooking and protein concentration may formed phenolic-protein-starch interaction that interfered with phenolic quantification released after digestion [151].

Soluble protein content (Table 1) showed no significant differences by the germination process in both chickpea cultivars (BSg, GCg). It is known that soluble protein usually increases after germination due to the intrinsic synthesis of proteases [172]. The protein concentration by alkaline and acid conditions increased soluble protein 2-fold in processed Blanco Sinaloa (cBSc, cBSg) and 4-fold in the processed green cultivar (cGc, cGg) compared with raw samples. The simulated gastrointestinal digestion of processed chickpea concentrates increased soluble protein, similar as reported by others protein concentrates during enzymatic digestion [172]. In contrast with the other

Table 3.5.1. Total phenolic, soluble protein and peptides (< 10 kDa) contents in raw, germinated, concentrates and digested-concentrates from Blanco Sinaloa and Green "ICC5613" chickpea cultivars.

		Code	Total phenolics (mg GAE/g)	Soluble protein (mg/g)	Peptides < 10 kDa (mg/g)
Flour	**R**	**BSr**	0.26±0.02^{h}	187.73±22.33^{def}	Nd
		GCr	0.46±0.04^{h}	120.84±3.43^{f}	Nd
	G	**BSg**	2.53±0.06^{f}	250.73±21.01^{de}	Nd
		GCg	3.11±0.04^{g}	152.12±12.72^{ef}	Nd
PC	**C**	**cBSc**	0.42±0.00^{h}	398.05±18.58^{c}	26.85±1.91^{d}
		cGCc	0.46±0.00^{h}	488.14±62.60^{abc}	26.20±0.38^{d}
	G	**cBSg**	4.64±0.03^{d}	453.68±45.31^{bc}	28.87±1.52^{d}
		cGCg	3.98±0.03^{e}	515.95±28.45^{ab}	25.05±2.11^{d}
uadC	**C**	**dBSc**	7.43±0.19^{c}	455.76±29.74^{bc}	345.75±25.89^{b}
		dGCc	4.54±0.03^{d}	262.53±20.49^{d}	195.58±5.84^{c}
	G	**dBSg**	11.68±0.30^{a}	590.02±56.44^{a}	469.29±24.36^{a}
		dGCg	9.94±0.22^{b}	564.85±46.81^{a}	441.17±22.77^{a}

Values are means±SD. Means with different letter are statically different ($p>0.05$) in the same column. Nd: not determinated, mg GAE/g: milligrams Gallic acid equivalents/grams of sample, R: Raw, C: Cooked, G. Germinated. PC: protein concentrates, uadC: un absorbed digested protein concentrates, BSr: Blanco Sinaloa cultivar raw, GCr: Green cultivar "ICC5613" raw, BSg: Blanco Sinaloa germinated seed, GCg: Cultivar "ICC5613" germinated seed, cBSc: Protein concentrate from BS cooked seed, cGCc: Protein concentrate from GC cooked seed, cGCg: Protein concentrate from GC germinated seed, cBSg: Protein concentrate from BS germinated seed, dGCc: un absorbed digested protein concentrate from GC cooked seed, dGCg: un absorbed digested protein concentrate from GC germinated seed, dBSc: un absorbed digested protein concentrate from BS cooked seed, dBSg: un absorbed digested protein concentrate from BS germinated seed.

digested samples, the cooked digested green cultivar (dGCc) decreased (46.22%) in soluble protein content compared with the non-digested concentrate (cGCc).

Peptides content (<10 kDa) in chickpea concentrates increased (7-16 fold) significantly after simulated gastrointestinal digestion. The peptide content (Table 1) in digested concentrates was not statically different in germinated chickpea cultivars but it was significantly lower in cooked green digested concentrate (195.58 mg/g) compared with cooked dBSc (345.75 mg/g).

3.5.2 SDS-PAGE electrophoretic protein profile of chickpea samples.

The protein patterns of chickpea samples are shown in Figure 1a. The raw samples showed a molecular weight distribution in the range of 15 kDa to 120 kDa, similar as reported by other authors [173, 174]. The germinated samples showed a similar profile except for the reduction of the band (a) with molecular weight between 110 and 160 kDa. Also, the main protein bands in raw and germinated chickpea were present between 50 and 60 KDa (e) and 35 and 40 kDa (f), but in the cooked samples the major protein band was at ≈22 kDa (m). Major chickpea proteins at ranges of 34.8-40.2 and 20.7 and 27.2 kDa have been reported [173]. The digestion of germinated and cooked chickpea protein concentrates showed the degradation of protein bands to fractions with less than 15 kDa.

3.5.3 Effect of processing and digestion of chickpea cultivars on flavonoid content.

In Figure 1b are shown the representative chromatographs of isoflavonoids in chickpea samples. Ten isoflavonoids (peak 1: Isoformononetin glycoside, peak 2: Formononetin malonyl glycoside, peak 3: Biochanin-A glycoside, peak 4: Biochanin-A malonyl glycoside, peak 5: isoflavonoid derivative, peak 6: Pseudobaptigenin, peak 7: Formononetin, peak 8: isoflavonoid derivative,, peak 9: 5-hydroxypseudobaptigenin, peak 10: Biochanin-A) were identified in chickpea samples. The isoflavonoid content in chickpea samples is depicted in Table 2. In the raw samples, only biochanin-A and its glycosides were detected. The main isoflavonoid in both cultivars was biochanin-A malonyl glycoside with concentrations of 18 µg/g (BSr) and 92 µg/g (GCr) for the white and green seeds cultivars respectively. The total isoflavonoid content (TIFC) in germinated samples increased 51 (BSg) and 77 (GCg) fold compared with raw counterparts, respectively. Biochanin- A, formononetin and its glycosylated forms were the main isoflavonoids identified in germinated chickpea samples. In GCg the main isoflavonoid was formononetin with 48.6% of TIFC, as in BSg was formononetin malonyl glycoside (41.48 % of TIFC). Besides TIFC, the isoflavone profile showed several differences due to chickpea genotype both in raw and germinated samples.

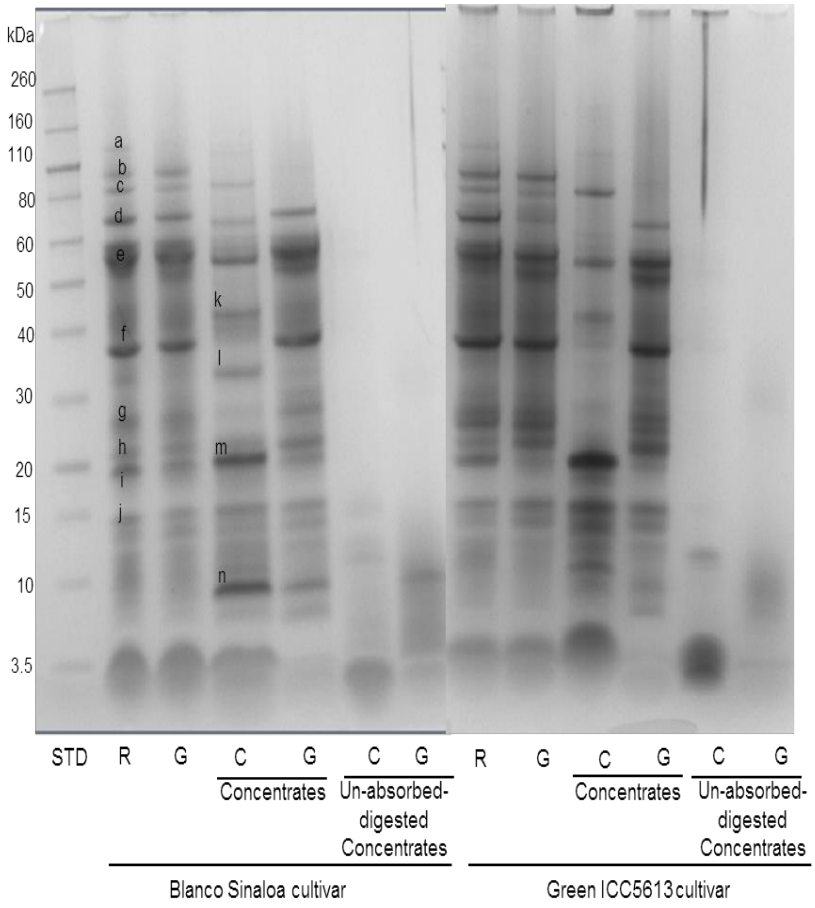
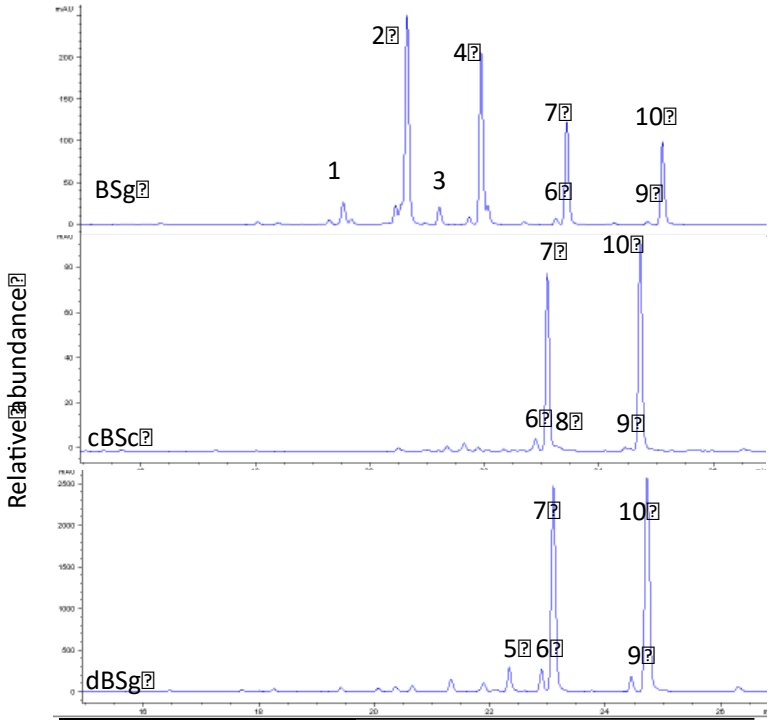


Figure 3.5.1. Electrophoretic profile of chickpea samples (SDS-PAGE). R: Raw, C: Cooked, G. Germinated. STD: Novex sharp unstained protein standard (life technologies, Madrid, Spain).



Peak	UV_{max} (nm)	Ions $[M+H]^+$	Compound
1	259	431	Isoformononetin glycoside
2	249	531	Formononetin malonyl glycoside
3	261	447	Biochanin A glycoside
4	260	533	Biochanin A malonyl glycoside
5	260	464	Isoflavonoid derivative
6	241	283	Pseudobaptigenin
7	249	269	Formononetin
8	260	207	Isoflavonoid derivative
9	260	299	5-hydroxypseudobaptigenin
10	261	285	Biochanin A

Figure 3.5.2. Representative chromatograms of chickpea germinated (BSg), protein concentrate (cBSc) and digested concentrate (dBSg) from Blanco Sinaloa at 260 nm. R: Raw, C: Cooked, G. Germinated. STD: Novex sharp unstained protein standard (life technologies, Madrid, Spain). BSg: Blanco Sinaloa germinated seed, cBSc: Protein concentrate from BS cooked seed, dBSg: un-absorbed digested protein concentrate from BS germinated seed.

Table 3.5.2. Isoflavonoids content (µg/g) in raw, germinated, concentrates and un-absorbed digested-concentrates from Blanco Sinaloa and Green Cultivar "ICC5613" chickpea cultivars.

		Peak										
		1	2	3	4	5	6	7	8	9	10	Total
Flour	**BSr**	N.D.	N.D.	18±0^{c}	8±2^{c}	N.D.	N.D.	N.D.	N.D.	N.D.	12±0^{f}	48±1^{d}
	GCr	N.D.	N.D.	92±5^{b}	26±0^{c}	N.D.	N.D.	N.D.	N.D.	N.D.	17±1^{f}	135±4^{d}
	BSg	163±0	1545±27^{a}	120±0^{a}	1075±25^{a}	N.D.	N.D.	226±3^{d}	N.D.	N.D.	592±2^{e}	3724±59^{c}
	GCg	N.D.	981±15^{b}	N.D.	781±55^{b}	N.D.	238±5^{b}	3385±121^{a}	N.D.	N.D.	1565±54^{d}	6953±140^{a}
PC	**cBSc**	N.D.	N.D.	N.D.	N.D.	Tr.	7±0^{e}	102±12^{d}	3±0^{b}	Tr.	119±23^{f}	233±36^{d}
	cGCc	N.D.	N.D.	N.D.	N.D.	Tr.	4±0^{e}	19±1^{d}	7±0^{a}	Tr.	18±0^{f}	50±1^{d}
	cBSg	N.D.	N.D.	N.D.	N.D.	138±5^{a}	118±8^{c}	1366±34^{c}	N.D.	85±5^{b}	1706±8^{cd}	3414±65^{c}
	cGCg	N.D.	N.D.	N.D.	N.D.	77±2^{b}	72±6^{d}	1359±124^{c}	N.D.	66±10^{b}	1802±122^{c}	3379±265^{c}
uadC	**dBSc**	N.D.	N.D.	N.D.	N.D.	Tr.	3±0^{e}	10±0^{d}	3±0^{b}	2±0^{c}	61±1^{f}	81±3^{d}
	dGCc	N.D.	N.D.	N.D.	N.D.	Tr.	4±0^{e}	6±0^{d}	6±1^{a}	3±0^{c}	12±1^{f}	33±3^{d}
	dBSg	N.D.	N.D.	N.D.	N.D.	67±0^{c}	241±37^{b}	3263±133^{a}	N.D.	137±10^{a}	3457±43^{a}	7167±225^{a}
	dGCg	N.D.	N.D.	N.D.	N.D.	126±4^{a}	287±13^{a}	2840±54^{b}	N.D.	140±5^{a}	3032±27^{b}	6427±104^{b}

Values are Mean±SD. Means with different letter are statically different ($p>0.05$) in the same column. Peak compounds: 1: Isoformononetin glycoside, 2: Formononetin malonyl glycoside, 3: Biochanin-A glycoside, 4: Biochanin-A malonyl glycoside, 5: isoflavonoid derivative, 6: Pseudobaptigenin, 7: Formonetin, 8: isoflavonoid derivative, 9: 5-hydroxypseudobaptigenin, 10: Biochanin-A. $^{N.D}$: not detected, ^{Tr}: Traces, R: Raw, C: Cooked, G. Germinated. PC: protein concentrates, uadC: un absorbed digested protein concentrates, BSr: Blanco Sinaloa cultivar raw, GCr: Green cultivar "ICC5613" raw, BSg: Blanco Sinaloa germinated seed, GCg: Cultivar "ICC5613" germinated seed, cBSc: Protein concentrate from BS cooked seed, cGCc: Protein concentrate from GC cooked seed, cGCg: Protein concentrate from GC germinated seed, cBSg: Protein concentrate from BS germinated seed, dGCc: un absorbed digested protein concentrate from GC cooked seed, dGCg: un absorbed digested protein concentrate from GC germinated seed, dBSc: un absorbed digested protein concentrate from BS cooked seed, dBSg: un absorbed digested protein concentrate from BS germinated seed.

Chickpea protein concentrates have been studied as hidden isoflavone sources [122]. In this study, protein concentration slightly increased TPC in both germinated chickpea cultivars. In contrast, TIFC values diminished due to protein concentration with higher loses observed in cGCg (50% less formononetin and TIFC). The protein concentration by alkaline and acid precipitation caused an increase in aglycones isoflavones in all chickpea protein concentrates as previously reported in soybean protein isolates [175]. Moreover, the isoflavonoid content was significant reduced after protein concentration process from cooked seed (cGCc), Contrasting, protein concentrate from cooked seeds (cBSc) increased its biochanin-A content by almost 10 times by protein concentration compared with raw flour. Despite TIFC losses, chickpea concentrates from germinated and cooked chickpeas contained good amounts of biochanin-A and formononetin. These results generate more evidence of co-precipitation of phenolics during protein concentration/isolation of legumes [123].

The simulated gastrointestinal digestion of protein concentrates showed the simultaneous liberation of peptides and TIFC. Although digestion of chickpea concentrates from cooked seeds showed a reduction in TIFC in both cultivars. In concentrates from germinated seeds, digestion increased formononetin, biochanin-A and TIFC close to 2 times from its respectively undigested concentrates. Some researchers have suggested that 5 to 20 % of TPC in legumes can be absorbed [176]. In this study, only the IN fraction (un-absorbed) was analyzed, suggesting that a high portion of phenolics were not absorbed in the intestine, and therefore tentatively reach the large intestine where they can be further metabolized by the microbiota.

3.5.4. Anti-inflammatory potential of phenolics and peptides from processed chickpea cultivars.

The anti-inflammatory potential of chickpea samples was measured according to the inhibition of nitric oxide production in activated LPS macrophages. The nitric oxide inhibition (NOI) was quantified in relation to phenolic extract of chickpea samples (Figure 2 a,b,e) and peptides content (Figure 2, d,e,f). Cell treatments with chickpea samples did not affect RAW 264.7 macrophages cell viability (data not shown).

3.5.4.1 Anti-inflammatory effect of phenolics from chickpea samples

The phenolic extracts of chickpea samples at 0.5 mg/ml exhibited NOI from 10 to 72 % (Figure 2a). Germination process increased the release of phytochemicals that exerted an enhanced effect on NOI compared with raw counterparts, particularly in the green cultivar, which increased 13 % ($p<0.05$) the NOI. The anti-inflammatory effect seems to be more favorable in raw and germinated colored chickpea (GC) and related to TPC as previously observed in pigmented common beans [177]. Moreover, the protein concentration process of cooked seeds caused low increment in NOI compared with

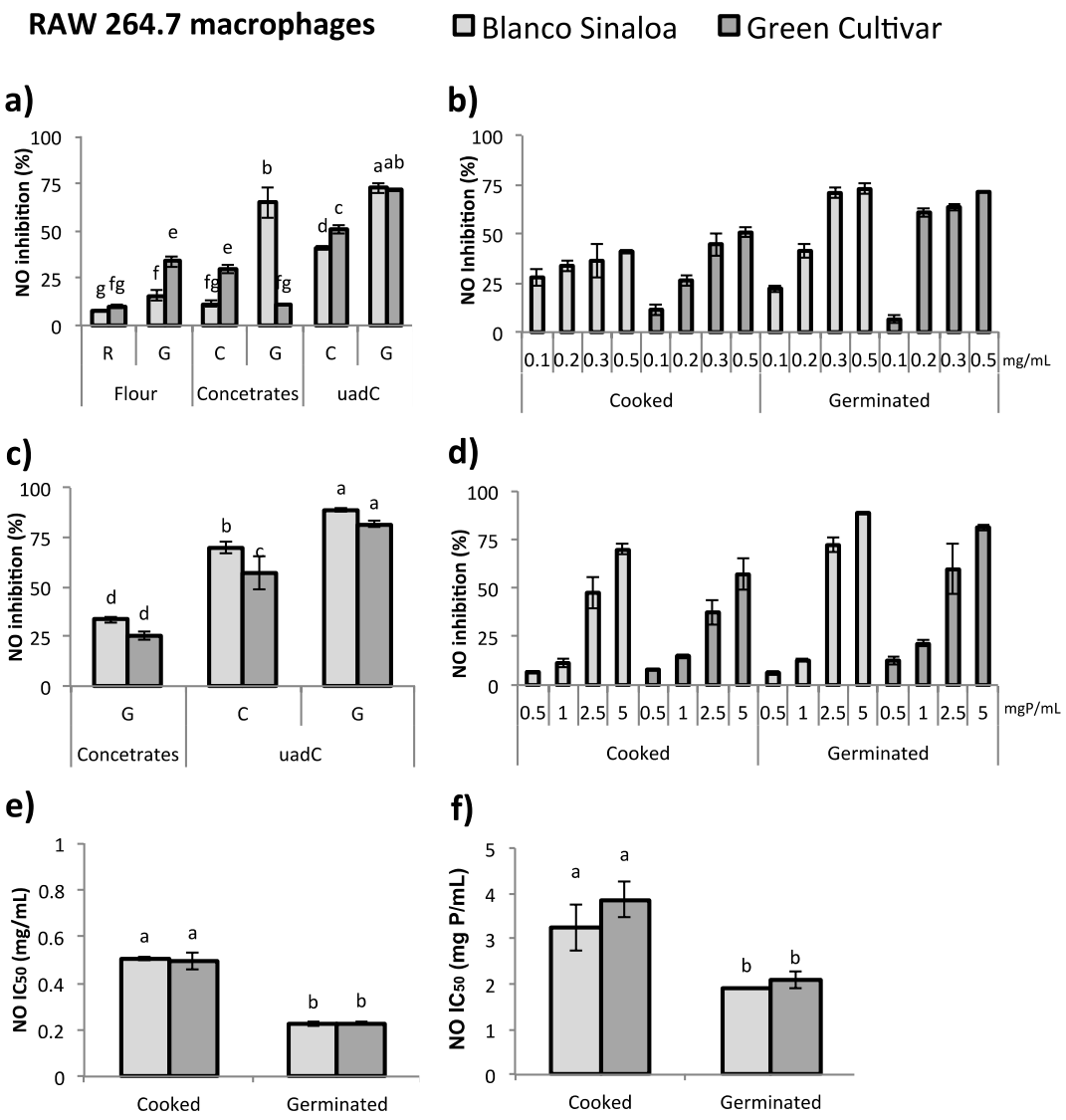


Figure 3.5.3. Effect of phenolics and peptides of processed Blanco Sinaloa and Green "ICC5613" chickpea cultivars on NO inhibition in LPS-induced RAW 264.7 macrophages. a) Effect on NO inhibition at 0.5 mg/mL of phenolic extract of chickpea samples, b) Dose-dependent effect on NO inhibition of phenolics extracts of un-absorbed-digested-concentrates, c) IC_{50} of phenolics extracts of un-absorbed-digested-concentrates chickpea samples on NO inhibition, d) Effect on NO inhibition at 5 mg P/mL of peptides of chickpea samples, e) Dose-dependent effect of peptides on NO inhibition un-absorbed-digested-concentrates chickpea samples, f) IC_{50} of peptides in digested-concentrates chickpea samples on NO inhibition. Mean±SD. Means with different letter are significant different ($p>0.05$) according to the dose. All treatment induced with 1µg/mL LPS. mg P: mg protein. uadC: un absorbed digested concentrate. R: Raw, C: Cooked, G: germinated.

raw flours. Also concentrates from germinated Blanco Sinaloa caused a significant increase ($p<0.05$) with almost 40 % of NOI more than BS germinated flours.

The simulated gastrointestinal digestion released phenolics from cooked concentrates that caused different ($p<0.05$) NOI of BS (40.01%) and GC (50.7%) when applied at same doses of 0.5 mg/ml (Figure 2a). Besides, the unabsorbed digested concentrates (uadC) obtained from germinated sprouts showed not difference ($p>0.05$) in NOI among cultivars with 72.1 % (BS) and 71.3 % (GC). Furthermore, the unabsorbed fraction showed a dose-depend effect of the phenolics extracted from processed chickpea concentrates (Figure 2b). Also, the NOI of phenolics extracts of chickpea samples was positive correlated with TPC(*r=0.8358, p<0.0001*), formononetin (*r=0.5935, p<0.0419*), and biochanin-A contents (*r=0.6742, p<0.0162)*, Likewise NOI showed a relation with peptides content (*r=0.7108,p=0.0041*). The uadC samples had a high content of biochanin-A and formononetin, which may play an important role in the anti-inflammatory effect of these samples; both isoflavones have previously shown anti-inflammatory effects. Biochanin-A attenuated the nitric oxide production and pro-inflammatory cytokines in LPS-stimulated RAW 264.7 macrophages. Furthermore, it also inhibited the gene expression of iNOS by blocking p38 MAPK phosphorylation and NF-KB pathway [178]. Likewise, formononetin have shown anti-inflammatory effects on LPS-induced acute lung injury by inhibiting cytokines (TNF-a and IL- 6) production and increasing PPAR-gamma production which inhibits production of inflammatory cytokines in monocytes [179]. Moreover, both polyphenols and isoflavones are known to suppress intestinal inflammation [180]. Subsequently, the bioactive compounds of uadC fractions that presumably reach the large intestine may exert anti-inflammatory effects in intestinal cells.

3.5.4.2 Anti-inflammatory effects of peptides from chickpea samples.

The anti-inflammatory effects of peptide fractions (<10 kDa) obtained by ultrafiltration from chickpea concentrates and digestion samples were analyzed (Figure 2c). The peptides from protein concentrates obtained from cooked chickpeas were not assayed due to their poor solubility and tendency to precipitate. The peptides obtained after digestion of protein concentrates from germinated samples reduced the nitric oxide production by 33.64% (BS) and 25.60% (GC) at 5 mg protein/mL. Interestingly, the digestion of concentrates released peptides that increased the NOI up to 50%. UadC from germinated (BS and GC) chickpeas exerted up to 80% NOI at 5 mg protein/mL with not significant differences ($p<0.05$) among cultivars. Likewise, in cooked samples, BS showed a significant ($p<0.05$) higher NOI effect (69.70%) compared to the GC cooked (57.04%) counterpart. The peptides from un-absorbed digested protein concentrate fractions showed a dose-depend effect from 0.5 to 5 mg protein/mL (Figure 2d). The NOI on chickpea samples were positive correlated with peptides content

(*r=0.9490, p=0.0001*) and TPC (*r=0.9458, p=0.0001*). Also, NOI had a weak relation with biochanin-A content (*r=0.6012, p=0.0387*). In contrast with other studies, the chickpea concentrates were not washed with organic solvents [181] in order to remove phenolics previous to the NOI assay. Therefore, the anti-inflammatory effects observed herein could be a synergy between peptides and isoflavonoids. The anti-inflammatory effects of peptides from cooked or germinated chickpeas have not been previously reported although several investigators [30, 71] reported some bioactive activities in peptides obtained from raw chickpeas.

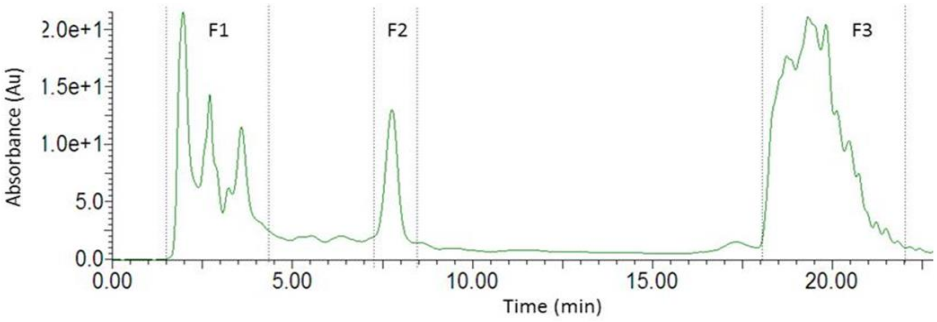
3.5.4.3 IC_{50}

The IC_{50} of NOI of unabsorbed digested processed chickpea concentrates as affected by phenolics and peptides are depicted in Figures 1c and 1f correspondingly. The IC_{50} values in both cultivars were significant lower in germinated seeds than cooked samples (Figure 2e and 2f). Peptides from germinated BS had the lowest IC_{50} values (Figure 2f) with 1.92 mg protein/mL and phenolic compounds (Figure 2e) with 0.22 mg/mL although these values were not statistically different when compared to IC_{50} values observed in the germinated green cultivar sample.

3.5.5 Identification of potential anti-inflammatory peptides.

In order to identify potential peptides with anti-inflammatory effects, the unabsorbed digested germinated protein concentrate, dBSg, was selected and fractioned by semi-preparative RP-HPLC. The sample was separated in 3 fractions named as F1, F2 and F3 (Figure 3). The 3 fractions were collected and evaluated for peptides contents and anti-inflammatory effects. The F3 fraction showed higher concentrations of peptides (969 µg/mg) and lower NOI IC_{50} (90 µg/mL) compared to the F1 fraction.

The F3 was selected for detection of potential anti-inflammatory peptides by MS/MS identification. Table 3 shows the most abundant sequences obtained from the chickpea samples. The most abundant peptides in F3 were fragments from legumin (A0A1S2XTK6) and vicilin (A0A1S2Y08; A0A1S2XQ88). Recently, Ribeiro et al [182] found that soaked and cooked chickpea storage proteins 7S (vicillin) and 11S (legumin) that resist simulated digestion, exert bioactive functions. However, up to date, there are not reported peptide sequences from processed chickpeas remaining after simulated gastrointestinal digestion.



	Fractions		
	F1	**F2**	**F3**
Peptides (µg/mg)	162.20±8.23	179.67±12.49	969.06±23.78
Yield (%)	87.24	4.96	7.79
IC_{50} (µg/mL)	161.4±40	> 1000	90.7±20

Figure 3.5.4. Chromatogram of germinated Blanco Sinaloa chickpea digests fractions with their yield and contents of peptides and their corresponding anti-inflammatory effects**.**

Table 3.5.3. Identification of remaining sequences of peptides by HPLC-MS/MS of Blanco Sinaloa chickpeas (dBSg) subjected to simulated digestion.

Source protein	Accession number	Sequence	Fragment	MH^+Da
Legumin	A0A1S2XTK6	SEGGLIETWNPSNK	47-60	1531.73
		NEDEEKGAIVKVK	238-250	1458.77
		VKGGLSIITPPEKEPR	249-264	1720.99
		GGLSIITPPEKEPR	251.264	1493.83
		GGLSIITPPEKEPRQK	251-266	1749.98
		LHQNIGSSSSPDIYNPQAGR	324-343	2141.03
		LHQNIGSSSSPDIYNPQAGRIK	324-345	2382.21
Vicilin	A0A1S2Y087	GRRGSEESEEGDAIVK	343-358	1718.82
		RGSEESEEGDAIVK	345-358	1505.70
		GSEESEEGDAIVK	356-358	1349.60
		SRNPIYSNKFGK	390-401	1410.75
	A0A1S2XQ8	ISREQIEELSKNAK	221-234	1644.89
		QQSQETDVIVK	211-220	1274.66
		EQIEELSKNAK	224-234	1288.67
Provicilin	Q304D4	ISREQIEELSKNAK	221-234	1644.89
		VLLEEQEQKPK	186-196	1340.74
		EQIEELSKNAK	224-234	1288.67
	A0A1S2XYZ0	SPIYSNKFGK	375-384	1140.60
P24 oleosin isoform A	A0A1S2XJM3	DVGQKTKEVGQDIQAK	170-185	1743.92
Sucrose-binding protein	A0A1S2XVJ8	IFKISKEDVHGLAPK	259-273	1681.96
Legumin A2	A0A1S3E0V2	HIVDKLQGRDEDEEK	166-180	1810.89
Globulin-1 S allele	A0A1S2YZ56	SREEETTEWEEEVAK	596-610	1851.82
Legumin J	A0A1S2XVG1	FSGNRGPLVQPR	516-527	1327.72
Adenosylhomocysteinase	A0A1S2YCQ2	IVGVSEETTTGVK	199-211	1319.70

General conclusions

The chemical, physicochemical, pasting and thermal properties studied showed wide variations among the set of pigmented chickpea flours. Pigmented chickpea flours contained significant amounts of protein and starch with good nutritional profiles. Furthermore, the Desi pigmented chickpeas were good sources of bioactive compounds like TPC, RS and β-glucans. The chemical composition of chickpea flours affected the techno-functional properties and the digestion of both protein and starch. Thus, these pigmented chickpea cultivars have potential as novel functional ingredients for development of foods.

Isolated chickpea starches from different chickpea seed cultivars were obtained with relative high purity and light color. Some of their physicochemical and functional characteristics related with water uptake were correlated with amylose contents as well as to thermal and pasting characteristics. The refined chickpea starches showed relatively low RDS and high levels of SDS and RS in both raw and cooked, which promoted moderated pGI. Therefore, these starches have potential as functional ingredients for development of new foods.

The cooking process showed an important effect on flavonoids and saponins content of pigmented chickpea. Moreover in raw and cooked pigmented chickpea had more saponins than the commercial chickpea, and this compounds showed an increase and stability after cooking. Hence, these chickpea cultivars have potential as functional ingredients for development of food. Meanwhile germination process increased isoflavones content in chickpea seeds. Significant differences in content and profile of isoflavones were found among cultivars. Besides, some cultivars increased the saponin content.

Peptides from cooked or germinated chickpeas after a simulated gastrointestinal digestion showed inhibition on nitric oxide on LPS-induced macrophages. Besides, chickpea processing caused significant changes in isoflavonoids and bioactivity. Therefore, processed chickpeas have shown anti-inflammatory effects and support the positive health effects related with its consumption.

Further studies are needed to evaluate the utilization of flours from germinated or cooked pigmented chickpea; isolated starches or protein concentrates in the development of functional foods.

Literature cited

1. Chavan JK, Kadam SS, Salunkhe DK: **Biochemestry and technology of chickpea *(Cicer arietinum* L) seeds**. *Crc Critical Reviews in Food Science and Nutrition* 1986, **25**(2):107-158.
2. FAOSTAT: **Chickpea world production**. In.; 2013. Available from: http://faostat.fao.org/site/339/default.aspx.
3. SIAP-SAGARPA: **Chickpea production in Mexico**. In.; 2012. Available from: http://www.siap.gob.mx/cierre-de-la-produccion-agricola-por-estado/.
4. Jukanti AK, Gaur PM, Gowda CLL, Chibbar RN: **Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review**. *British Journal of Nutrition* 2012, **108**(SupplementS1):S11-S26.
5. Kadam SS DS,JN: **Seed Structure and Composition.** In: *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization.* Boca Raton, FL. EUA. : CRC Press, Inc.; 1989.
6. Smýkal P, Vernoud V, Blair MW, Soukup A, Thompson RD: **The role of the testa during development and in establishment of dormancy of the legume seed**. *Frontiers in Plant Science* 2014, **5**.
7. Wood JA, Knights EJ, Choct M: **Morphology of Chickpea Seeds (*Cicer arietinum* L.): Comparison of desi and kabuli Types**. *International Journal of Plant Sciences* 2011, **172**(5):632-643.
8. Singh U, Jambunathan R: **Studies on Desi and Kabull Chickpea (*Cicer arietinum* L.) Cultivars: Levels of Protease Inhibitors, Levels of Polyphenolic Compounds and *in vitro* Protein Digestibility**. *Journal of Food Science* 1981, **46**(5):1364-1367.
9. FAO/WHO: **Protein quality evaluation, Food and agricultural organization of the United Nations.** In. Rome: FAO/WHO; 1991.
10. Sun Y, Ye H, Hu B, Wang W, Lei S, Wang X, Zhou L, Zeng X: **Changes in crystal structure of chickpea starch samples during processing treatments: An X-ray diffraction and starch moisture analysis study**. *Carbohydrate polymers* 2015, **121**(0):169-174.
11. Rebello CJ, Greenway FL, Finley JW: **Whole Grains and Pulses: A Comparison of the Nutritional and Health Benefits**. *Journal of agricultural and food chemistry* 2014, **62**(29):7029-7049.
12. Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM: **Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes**. *Plant Foods Hum Nutr* 2007, **62**(3):85-91.
13. Muzquiz M, Varela A, Burbano C, Cuadrado C, Guillamón E, Pedrosa M: **Bioactive compounds in legumes: pronutritive and antinutritive actions. Implications for nutrition and health**. *Phytochem Rev* 2012, **11**(2-3):227-244.
14. Rochfort S, Panozzo J: **Phytochemicals for health, the role of pulses**. *Journal of agricultural and food chemistry* 2007, **55**(20):7981-7994.
15. Konar N, Poyrazoğlu ES, Demir K, Artik N: **Determination of conjugated and free isoflavones in some legumes by LC–MS/MS**. *Journal of Food Composition and Analysis* 2012, **25**(2):173-178.
16. Sreerama YN, Sashikala VB, Pratape VM: **Variability in the Distribution of Phenolic Compounds in Milled Fractions of Chickpea and Horse Gram: Evaluation of Their Antioxidant Properties**. *Journal of agricultural and food chemistry* 2010, **58**(14):8322-8330.
17. Hithamani G, Srinivasan K: **Bioaccessibility of Polyphenols from Wheat (*Triticum aestivum*), Sorghum (*Sorghum bicolor*), Green Gram (*Vigna radiata*), and Chickpea (*Cicer arietinum*) as Influenced by Domestic Food Processing**. *Journal of agricultural and food chemistry* 2014, **62**(46):11170-11179.
18. Xu B, Chang SKC: **Phytochemical Profiles and Health-Promoting Effects of Cool-Season Food Legumes As Influenced by Thermal Processing**. *Journal of agricultural and food chemistry* 2009, **57**(22):10718-10731.
19. Ha TJ, Lee BW, Park KH, Jeong SH, Kim H-T, Ko J-M, Baek I-Y, Lee JH: **Rapid characterisation and comparison of saponin profiles in the seeds of Korean Leguminous species using ultra performance liquid chromatography with photodiode array detector and electrospray ionisation/mass spectrometry (UPLC-PDA-ESI/MS) analysis**. *Food Chemistry* 2014, **146**:270-277.
20. Sagratini G, Caprioli G, Maggi F, Font G, Giardinà D, Mañes J, Meca G, Ricciutelli M, Sirocchi V, Torregiani E *et al*: **Determination of Soyasaponins I and βg in Raw and Cooked Legumes by Solid Phase Extraction (SPE) Coupled to Liquid Chromatography (LC)–Mass Spectrometry (MS) and Assessment of Their Bioaccessibility by an *in Vitro* Digestion Model**. *Journal of agricultural and food chemistry* 2013, **61**(8):1702-1709.
21. Mekky RH, Contreras MdM, El-Gindi MR, Abdel-Monem AR, Abdel-Sattar E, Segura-Carretero A: **Profiling of phenolic and other compounds from Egyptian cultivars of chickpea (*Cicer arietinum* L.) and antioxidant activity: a comparative study**. *RSC Advances* 2015, **5**(23):17751-17767.
22. Ruiz RG, Price KR, Arthur AE, Rose ME, Rhodes MJC, Fenwick RG: **Effect of soaking and cooking on the saponin content and composition of chickpeas (*Cicer arietinum)* and lentils (*Lens culinaris*)**. *Journal of agricultural and food chemistry* 1996, **44**(6):1526-1530.
23. Moreau RA, Whitaker BD, Hicks KB: **Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses**. *Progress in Lipid Research* 2002, **41**(6):457-500.
24. Piironen V, Toivo J, Puupponen-Pimia R, Lampi AM: **Plant sterols in vegetables, fruits and berries**. *Journal of the Science of Food and Agriculture* 2003, **83**(4):330-337.
25. Nyström L, Schär A, Lampi A-M: **Steryl glycosides and acylated steryl glycosides in plant foods reflect unique sterol patterns**. *European Journal of Lipid Science and Technology* 2012, **114**(6):656-669.
26. Yust MdM, Millan-Linares MdC, Alcaide-Hidalgo JM, Millan F, Pedroche J: **Hypocholesterolaemic and antioxidant activities of chickpea (*Cicer arietinum* L.) protein hydrolysates**. *J Sci Food Agr* 2012, **92**(9):1994-2001.
27. Yust M, amp, x, a M, Pedroche J, Girón-Calle J, Alaiz M, Millán F, Vioque J: **Production of ace inhibitory peptides by digestion of chickpea legumin with alcalase**. *Food Chemistry* 2003, **81**(3):363-369.
28. Li YH, Jiang B, Zhang T, Mu WM, Liu J: **Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH)**. *Food Chemistry* 2008, **106**(2):444-450.

29. Zhang T, Li Y, Miao M, Jiang B: **Purification and characterisation of a new antioxidant peptide from chickpea (*Cicer arietium* L.) protein hydrolysates**. *Food Chemistry* 2011, **128**(1):28-33.
30. Guo Y, Zhang T, Jiang B, Miao M, Mu W: **The effects of an antioxidative pentapeptide derived from chickpea protein hydrolysates on oxidative stress in Caco-2 and HT-29 cell lines**. *Journal of Functional Foods* 2014, **7**(0):719-726.
31. Bar-El Dadon S, Abbo S, Reifen R: **Leveraging traditional crops for better nutrition and health - The case of chickpea**. *Trends in Food Science & Technology* 2017, **64**:39-47.
32. DeVries JW, United States of America AAoCC: **The definition of dietary fiber**. *Cereal Foods World* 2001, **46**(3):112-114.
33. HaiRong M, HuaBo W, Zhen C, Yi Y, ZhengHua W, Madina H, Xu C, Haji Akber A: **The Estrogenic Activity of Isoflavones Extracted from Chickpea *Cicer arietinum* L Sprouts *in Vitro***. *Phytotherapy Research* 2013, **27**(8):1237-1242.
34. Heinonen M: **Antioxidant activity and antimicrobial effect of berry phenolics - a Finnish perspective**. *Molecular Nutrition & Food Research* 2007, **51**(6):684-691.
35. Hamalainen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E: **Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappa B activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappa B activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages**. *Mediators of Inflammation* 2007.
36. Chen H, Ma H-R, Gao Y-H, Zhang X, Habasi M, Hu R, Aisa HA: **Isoflavones Extracted from Chickpea *Cicer arietinum* L. Sprouts Induce Mitochondria-Dependent Apoptosis in Human Breast Cancer Cells**. *Phytotherapy Research* 2015, **29**(2):210-219.
37. Del Rio D, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Crozier A: **Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases**. *Antioxidants & Redox Signaling* 2013, **18**(14):1818-1892.
38. Crozier A, Jaganath IB, Clifford MN: **Dietary phenolics: chemistry, bioavailability and effects on health**. *Natural Product Reports* 2009, **26**(8):1001-1043.
39. Segev A, Badani H, Kapulnik Y, Shomer I, Oren-Shamir M, Galili S: **Determination of Polyphenols, Flavonoids, and Antioxidant Capacity in Colored Chickpea (*Cicer arietinum* L.)**. *Journal of Food Science* 2010, **75**(2):S115-S119.
40. Heiras-Palazuelos MJ, Ochoa-Lugo MI, Gutiérrez-Dorado R, López-Valenzuela JA, Mora-Rochín S, Milán-Carrillo J, Garzón-Tiznado JA, Reyes-Moreno C: **Technological properties, antioxidant activity and total phenolic and flavonoid content of pigmented chickpea (*Cicer arietinum* L.) cultivars**. *International journal of food sciences and nutrition* 2013, **64**(1):69-76.
41. Wu Z, Song L, Feng S, Liu Y, He G, Yioe Y, Liu SQ, Huang D: **Germination Dramatically Increases Isoflavonoid Content and Diversity in Chickpea (*Cicer arietinum* L.) Seeds**. *J Agric Food Chem* 2012, **60**(35):8606-8615.
42. Mazur WM, Duke JA, Wähälä K, Rasku S, Adlercreutz H: **Isoflavonoids and Lignans in Legumes: Nutritional and Health Aspects in Humans 1**. *The Journal of nutritional biochemistry* 1998, **9**(4):193-200.
43. Xiao Y, Xing G, Rui X, Li W, Chen X, Jiang M, Dong M: **Enhancement of the antioxidant capacity of chickpeas by solid state fermentation with Cordyceps militaris SN-18**. *Journal of Functional Foods* 2014, **10**(0):210-222.
44. Fratianni F, Cardinale F, Cozzolino A, Granese T, Albanese D, Di Matteo M, Zaccardelli M, Coppola R, Nazzaro F: **Polyphenol composition and antioxidant activity of different grass pea (*Lathyrus sativus*), lentils (*Lens culinaris*), and chickpea (*Cicer arietinum*) ecotypes of the Campania region (Southern Italy)**. *Journal of Functional Foods* 2014, **7**:551-557.
45. Vivancos M, Moreno JJ: **Effect of resveratrol, tyrosol and beta-sitosterol on oxidised low-density lipoprotein-stimulated oxidative stress, arachidonic acid release and prostaglandin E2 synthesis by RAW 264.7 macrophages**. *The British journal of nutrition* 2008, **99**(6):1199-1207.
46. Choi S, Kim KW, Choi JS, Han ST, Park YI, Lee SK, Kim JS, Chung MH: **Angiogenic activity of beta-sitosterol in the ischaemia/reperfusion-damaged brain of Mongolian gerbil**. *Planta Med* 2002, **68**(4):330-335.
47. Wang T, Hicks K, Moreau R: **Antioxidant activity of phytosterols, oryzanol, and other phytosterol conjugates**. *J Amer Oil Chem Soc* 2002, **79**(12):1201-1206.
48. Nystrom L, Makinen M, Lampi AM, Piironen V: **Antioxidant activity of steryl ferulate extracts from rye and wheat bran**. *Journal of agricultural and food chemistry* 2005, **53**(7):2503-2510.
49. Hu Y, Xiong L, Huang W, Cai H, Luo Y, Zhang Y, Lu B: **Anti-inflammatory effect and prostate gene expression profiling of steryl ferulate on experimental rats with non-bacterial prostatitis**. *Food & Function* 2014, **5**(6):1150-1159.
50. Islam MS, Murata T, Fujisawa M, Nagasaka R, Ushio H, Bari AM, Hori M, Ozaki H: **Anti-inflammatory effects of phytosteryl ferulates in colitis induced by dextran sulphate sodium in mice**. *British journal of pharmacology* 2008, **154**(4):812-824.
51. Park EH, Kahng JH, Lee SH, Shin KH: **An anti-inflammatory principle from cactus**. *Fitoterapia* 2001, **72**(3):288-290.
52. Kim SP, Kang MY, Nam SH, Friedman M: **Dietary rice bran component γ-oryzanol inhibits tumor growth in tumor-bearing mice**. *Molecular Nutrition & Food Research* 2012, **56**(6):935-944.
53. Paniagua-Perez R, Madrigal-Bujaidar E, Reyes-Cadena S, Alvarez-Gonzalez I, Sanchez-Chapul L, Perez-Gallaga J, Hernandez N, Flores-Mondragon G, Velasco O: **Cell protection induced by beta-sitosterol: inhibition of genotoxic damage, stimulation of lymphocyte production, and determination of its antioxidant capacity**. *Arch Toxicol* 2008, **82**(9):615-622.
54. Li CR, Zhou Z, Lin RX, Zhu D, Sun YN, Tian LL, Li L, Gao Y, Wang SQ: **beta-sitosterol decreases irradiation-induced thymocyte early damage by regulation of the intracellular redox balance and maintenance of mitochondrial membrane stability**. *J Cell Biochem* 2007, **102**(3):748-758.
55. Sanchez-Vioque R, Clemente A, Vioque J, Bautista J, Millan F: **Polar lipids of defatted chickpea (*Cicer arietinum* L.) flour and protein isolates**. *Food Chemistry* 1998, **63**(3):357-361.
56. Guclu-Ustundag O, Mazza G: **Saponins: Properties, applications and processing**. *Critical reviews in food science and nutrition* 2007, **47**(3):231-258.

57. Chen L, Tai WCS, Hsiao WLW: **Dietary saponins from four popular herbal tea exert prebiotic-like effects on gut microbiota in C57BL/6 mice**. *Journal of Functional Foods* 2015, **17**:892-902.
58. Ashokkumar K, Diapari M, Jha AB, Tar'an B, Arganosa G, Warkentin TD: **Genetic diversity of nutritionally important carotenoids in 94 pea and 121 chickpea accessions**. *Journal of Food Composition and Analysis* 2015, **43**:49-60.
59. Al-Sheraji SH, Ismail A, Manap MY, Mustafa S, Yusof RM, Hassan FA: **Prebiotics as functional foods: A review**. *Journal of Functional Foods* 2013, **5**(4):1542-1553.
60. Chen Z-Y, Jiao R, Ma KY: **Cholesterol-lowering nutraceuticals and functional foods**. *Journal of agricultural and food chemistry* 2008, **56**(19):8761-8773.
61. Threapleton DE, Greenwood DC, Evans CEL, Cleghorn CL, Nykjaer C, Woodhead C, Cade JE, Gale CP, Burley VJ: **Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis**. *Bmj-British Medical Journal* 2013, **347**.
62. Papathanasopoulos A, Camilleri M: **Dietary Fiber Supplements: Effects in Obesity and Metabolic Syndrome and Relationship to Gastrointestinal Functions**. *Gastroenterology* 2010, **138**(1):65-72.
63. Harris PJ, Ferguson LR: **Dietary fiber - its composition and role in protection against colorectal-cancer**. *Mutation Research* 1993, **290**(1):97-110.
64. de Almeida Costa GE, da Silva Queiroz-Monici K, Pissini Machado Reis SM, de Oliveira AC: **Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes**. *Food Chemistry* 2006, **94**(3):327-330.
65. Silva-Cristobal L, Osorio-Díaz P, Tovar J, Bello-Pérez LA: **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* 2010, **8**(1):7-14.
66. Aguilera Y, Benítez V, Mollá E, Esteban R, Martín-Cabrejas M: **Influence of Dehydration Process in Castellano Chickpea: Changes in Bioactive Carbohydrates and Functional Properties**. *Plant Foods for Human Nutrition* 2011, **66**(4):391-400.
67. Xiaoli X, Liyi Y, Shuang H, Wei L, Yi S, Hao M, Jusong Z, Xiaoxiong Z: **Determination of oligosaccharide contents in 19 cultivars of chickpea *(Cicer arietinum* L) seeds by high performance liquid chromatography**. *Food Chemistry* 2008, **111**(1):215-219.
68. Genç H, Özdemir M, Demirbaş A: **Analysis of mixed-linked (1→3), (1→4)-β-d-glucans in cereal grains from Turkey**. *Food Chemistry* 2001, **73**(2):221-224.
69. Maccaferri S, Klinder A, Cacciatore S, Chitarrari R, Honda H, Luchinat C, Bertini I, Carnevali P, Gibson GR, Brigidi P *et al*: ***In vitro* fermentation of potential prebiotic flours from natural sources: Impact on the human colonic microbiota and metabolome**. *Molecular Nutrition & Food Research* 2012, **56**(8):1342-1352.
70. López-Barrios L, Gutiérrez-Uribe JA, Serna-Saldívar SO: **Bioactive Peptides and Hydrolysates from Pulses and Their Potential Use as Functional Ingredients**. *Journal of Food Science* 2014, **79**(3):R273-R283.
71. Xue Z, Wen H, Zhai L, Yu Y, Li Y, Yu W, Cheng A, Wang C, Kou X: **Antioxidant activity and anti-proliferative effect of a bioactive peptide from chickpea (*Cicer arietinum* L.)**. *Food Research International* 2015, **77, Part 2**:75-81.
72. Torres-Fuentes C, Contreras MdM, Recio I, Alaiz M, Vioque J: **Identification and characterization of antioxidant peptides from chickpea protein hydrolysates**. *Food Chemistry* 2015, **180**:194-202.
73. Ghavidel RA, Prakash J: **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 Sci Technol* 2007, **40**(7):1292-1299.
74. Abd El-Hady EA, Habiba RA: **Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds**. *LWT - Food Science and Technology* 2003, **36**(3):285-293.
75. Kaur M, Singh N, Sodhi NS: **Physicochemical, cooking, textural and roasting characteristics of chickpea (Cicer arietinum L.) cultivars**. *J Food Eng* 2005, **69**(4):511-517.
76. Aguilera Y, Duenas M, Estrella I, Hernandez T, Benitez V, Maria Esteban R, Martin-Cabrejas MA: **Phenolic Profile and Antioxidant Capacity of Chickpeas (*Cicer arietinum* L.) as Affected by a Dehydration Process**. *Plant Foods for Human Nutrition* 2011, **66**(2):187-195.
77. Martín-Cabrejas MA, Díaz MF, Aguilera Y, Benítez V, Mollá E, Esteban RM: **Influence of germination on the soluble carbohydrates and dietary fibre fractions in non-conventional legumes**. *Food Chemistry* 2008, **107**(3):1045-1052.
78. Angulo-Bejarano PI, Verdugo-Montoya NM, Cuevas-Rodriguez EO, Milan-Carrillo J, Mora-Escobedo R, Lopez-Valenzuela JA, Garzon-Tiznado JA, Reyes-Moreno C: **Tempeh flour from chickpea (*Cicer arietinum* L.) nutritional and physicochemical properties**. *Food Chemistry* 2008, **106**(1):106-112.
79. Fares C, Menga V: **Effects of toasting on the carbohydrate profile and antioxidant properties of chickpea *(Cicer arietinum* L.) flour added to durum wheat pasta**. *Food Chemistry* 2012, **131**(4):1140-1148.
80. Nithiyanantham S, Selvakumar S, Siddhuraju P: **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* 2012, **27**(1):52-60.
81. Gujral HS, Sharma P, Gupta N, Wani AA: **Antioxidant properties of legumes and their morphological fractions as affected by cooking**. *Food Science and Biotechnology* 2013, **22**(1):187-194.
82. Han H, Baik B-K: **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* 2008, **43**(11):1971-1978.
83. Fernandez-Orozco R, Frias J, Zielinski H, Muñoz R, Piskula MK, Kozlowska H, Vidal-Valverde C: **Evaluation of bioprocesses to improve the antioxidant properties of chickpeas**. *LWT - Food Science and Technology* 2009, **42**(4):885-892.
84. Chandra-Hioe MV, Wong CHM, Arcot J: **The Potential Use of Fermented Chickpea and Faba Bean Flour as Food Ingredients**. *Plant Foods for Human Nutrition* 2016, **71**(1):90-95.

85. Singh A, Jain A, Sarma BK, Upadhyay RS, Singh HB: **Beneficial compatible microbes enhance antioxidants in chickpea edible parts through synergistic interactions**. *LWT - Food Science and Technology* 2014, **56**(2):390-397.
86. Coda R, Rizzello CG, Gobbetti M: **Use of sourdough fermentation and pseudo-cereals and leguminous flours for the making of a functional bread enriched of gamma-aminobutyric acid (GABA)**. *International Journal of Food Microbiology* 2010, **137**(2-3):236-245.
87. Wei XT, Luo MF, Xu L, Zhang YW, Lin X, Kong P, Liu HZ: **Production of Fibrinolytic Enzyme from *Bacillus amyloliquefaciens* by Fermentation of Chickpeas, with the Evaluation of the Anticoagulant and Antioxidant Properties of Chickpeas**. *Journal of Agricultural and Food Chemistry* 2011, **59**(8):3957-3963.
88. Sánchez-Magaña LM, Cuevas-Rodríguez EO, Gutiérrez-Dorado R, Ayala-Rodríguez AE, Valdez-Ortiz A, Milán-Carrillo J, Reyes-Moreno C: **Solid-state bioconversion of chickpea (*Cicer arietinum* L.) by *Rhizopus oligosporus* to improve total phenolic content, antioxidant activity and hypoglycemic functionality**. *International Journal of Food Sciences and Nutrition* 2014, **65**(5):558-564.
89. Adamidou S, Nengas I, Grigorakis K, Nikolopoulou D, Jauncey K: **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 Journal* 2010, **88**(1):80-86.
90. Garzon-Tiznado JA, Heiras Palazuelos MdJ, Espinoza Moreno RJ, Cano Campos M, Delgado Vargas F, Gutierrez Dorado R, Milan Carrillo J, Reyes Moreno C: **Antioxidant and antimutagenic activities of optimized extruded desi chickpea (*Cicer arietinum* L) flours**. *Journal of Pharmacy and Nutrition Sciences* 2013, **3**(1):38-47.
91. Milán-Carrillo J, Reyes-Moreno C, Camacho-Hernández IL, Rouzaud-Sandez O: **Optimisation of extrusion process to transform hardened chickpeas (*Cicer arietinum* L) into a useful product**. *Journal of the Science of Food and Agriculture* 2002, **82**(14):1718-1728.
92. Milán-Carrillo J, Reyes-Moreno C, Armienta-Rodelo E, Carábez-Trejo A, Mora-Escobedo R: **Physicochemical and Nutritional Characteristics of Extruded Flours from Fresh and Hardened Chickpeas (*Cicer arietinum* L)**. *LWT - Food Science and Technology* 2000, **33**(2):117-123.
93. Poltronieri F, Arêas JAG, Colli C: **Extrusion and iron bioavailability in chickpea (*Cicer arietinum* L.)**. *Food Chemistry* 2000, **70**(2):175-180.
94. Berrios JD, Morales P, Camara M, Sanchez-Mata MC: **Carbohydrate composition of raw and extruded pulse flours**. *Food Research International* 2010, **43**(2):531-536.
95. Mamilla RK, Mishra VK: **Effect of germination on antioxidant and ACE inhibitory activities of legumes**. *Lwt-Food Science and Technology* 2017, **75**:51-58.
96. Gao Y, Yao Y, Zhu Y, Ren G: **Isoflavone Content and Composition in Chickpea (*Cicer arietinum* L.) Sprouts Germinated under Different Conditions**. *Journal of Agricultural and Food Chemistry* 2015, **63**(10):2701-2707.
97. Uppal V, Bains K: **Effect of germination periods and hydrothermal treatments on *in vitro* protein and starch digestibility of germinated legumes**. *Journal of food science and technology* 2012, **49**(2):184-191.
98. Khattak AB, Zeb A, Bibi N: **Impact of germination time and type of illumination on carotenoidcontent, protein solubility and *in vitro* protein digestibility of chickpea (*Cicer arietinum* L.) sprouts**. *Food Chemistry* 2008, **109**(4):797-801.
99. Gharachorloo M, Ghiassi Tarzi B, Baharinia M: **The Effect of Germination on Phenolic Compounds and Antioxidant Activity of Pulses**. *Journal of the American Oil Chemists' Society* 2013, **90**(3):407-411.
100. Khattak AB, Zeb A, Khan M, Bibi N, Ihsanullah, Khattak MS: **Influence of germination techniques on sprout yield, biosynthesis of ascorbic acid and cooking ability, in chickpea (*Cicer arietinum* L.)**. *Food Chemistry* 2007, **103**(1):115-120.
101. Zhang L, Li Q, Yang X, Xia Z: **Effects of Sodium Selenite and Germination on the Sprouting of Chickpeas (*Cicer arietinum* L.) and Its Content of Selenium, Formononetin and Biochanin A in the Sprouts**. *Biological Trace Element Research* 2012, **146**(3):376-380.
102. Guardado-Félix D, Serna-Saldivar SO, Cuevas-Rodríguez EO, Jacobo-Velázquez DA, Gutiérrez-Uribe JA: **Effect of sodium selenite on isoflavonoid contents and antioxidant capacity of chickpea (*Cicer arietinum* L.) sprouts**. *Food Chemistry* 2017, **226**:69-74.
103. Khattak AB, Zeb A, Bibi N, Khalil SA, Khattak MS: **Influence of germination techniques on phytic acid and polyphenols content of chickpea (*Cicer arietinum* L.) sprouts**. *Food Chemistry* 2007, **104**(3):1074-1079.
104. Udahogora M: **Health Benefits and Bioactive Compounds in Field Peas, Faba Beans, and Chickpeas**. In: *Cereals and Pulses.* Wiley-Blackwell; 2012: 199-215.
105. Nestel P, Cehun M, Chronopoulos A: **Effects of long-term consumption and single meals of chickpeas on plasma glucose, insulin, and triacylglycerol concentrations**. *American Journal of Clinical Nutrition* 2004, **79**(3):390-395.
106. Pittaway JK, Robertson AK, Ball MJ: **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* 2008, **108**(6):1009-1013.
107. Xue Z, Gao J, Zhang Z, Yu W, Wang H, Kou X: **Antihyperlipidemic and Antitumor Effects of Chickpea Albumin Hydrolysate**. *Plant Foods for Human Nutrition* 2012, **67**(4):393-400.
108. Hosseinpour-Niazi S, Mirmiran P, Fallah-Ghohroudi A, Azizi F: **Non-soya legume-based therapeutic lifestyle change diet reduces inflammatory status in diabetic patients: a randomised cross-over clinical trial**. *British Journal of Nutrition* 2015, **114**(2):213-219.
109. Hermsdorff HHM, Angeles Zulet M, Abete I, Alfredo Martinez J: **A legume-based hypocaloric diet reduces proinflammatory status and improves metabolic features in overweight/obese subjects**. *Eur J Nutr* 2011, **50**(1):61-69.
110. Yang Y, Zhou L, Gu Y, Zhang Y, Tang J, Li F, Shang W, Jiang B, Yue X, Chen M: **Dietary chickpeas reverse visceral adiposity, dyslipidaemia and insulin resistance in rats induced by a chronic high-fat diet**. *British Journal of Nutrition* 2007, **98**(4):720-726.

111. Mollard RC, Luhovyy BL, Panahi S, Nunez M, Hanley A, Anderson GH: **Regular consumption of pulses for 8 weeks reduces metabolic syndrome risk factors in overweight and obese adults**. *British Journal of Nutrition* 2012, **108**:S111-S122.
112. Dwivedi S, Sahrawat K, Puppala N, Ortiz R: **Plant prebiotics and human health: Biotechnology to breed prebiotic-rich nutritious food crops**. *Electronic Journal of Biotechnology* 2014, **17**(5):238-245.
113. Fernando WMU, Hill JE, Zello GA, Tyler RT, Dahl WJ, Van Kessel AG: **Diets supplemented with chickpea or its main oligosaccharide component raffinose modify faecal microbial composition in healthy adults**. *Beneficial Microbes* 2010, **1**(2):197-207.
114. Girón-Calle J, Alaiz M, Vioque J: **Effect of chickpea protein hydrolysates on cell proliferation and *in vitro* bioavailability**. *Food Research International* 2010, **43**(5):1365-1370.
115. Barbana C, Boye JI: **Angiotensin I-converting enzyme inhibitory activity of chickpea and pea protein hydrolysates**. *Food Research International* 2010, **43**(6):1642-1649.
116. Queiroz-Monici KdS, Costa GEA, da Silva N, Reis SMPM, de Oliveira AC: **Bifidogenic effect of dietary fiber and resistant starch from leguminous on the intestinal microbiota of rats**. *Nutrition* 2005, **21**(5):602-608.
117. Gao Y, Yao Y, Zhu Y, Ren G: **Isoflavones in Chickpeas Inhibit Adipocyte Differentiation and Prevent Insulin Resistance in 3T3-L1 Cells**. *Journal of Agricultural and Food Chemistry* 2015, **63**(44):9696-9703.
118. Ercan P, El SN: **Inhibitory effects of chickpea and Tribulus terrestris on lipase, α-amylase and α-glucosidase**. *Food Chemistry* 2016, **205**(Supplement C):163-169.
119. FAO: **Pulses: Nutritious seeds for a sustainable future**. In.: FAO; 2016.
120. Ma H-r, Wang J, Qi H-x, Gao Y-h, Pang L-j, Yang Y, Wang Z-h, Duan M-j, Chen H, Cao X *et al*: **Assessment of the estrogenic activities of chickpea (*Cicer arietinum* L) sprout isoflavone extract in ovariectomized rats**. *Acta Pharmacologica Sinica* 2013, **34**(3):380-386.
121. Johnson SK, Thomas SJ, Hall RS: **Palatability and glucose, insulin and satiety responses of chickpea flour and extruded chickpea flour bread eaten as part of a breakfast**. *European Journal of Clinical Nutrition* 2005, **59**(2):169-176.
122. Megías C, Cortés-Giraldo I, Alaiz M, Vioque J, Girón-Calle J: **Isoflavones in chickpea (*Cicer arietinum*) protein concentrates**. *Journal of Functional Foods* 2016, **21**:186-192.
123. Garcia-Mora P, Frias J, Peñas E, Zieliński H, Giménez-Bastida JA, Wiczkowski W, Zielińska D, Martínez-Villaluenga C: **Simultaneous release of peptides and phenolics with antioxidant, ACE-inhibitory and anti-inflammatory activities from pinto bean (*Phaseolus vulgaris* L. var. pinto) proteins by subtilisins**. *Journal of Functional Foods* 2015, **18, Part A**:319-332.
124. AACCInternational.: **Approved Methods of Analysis. 11th Ed. Method 55-10.01. Test Weight per Bushel**.
125. Pérez-Carrillo E, Serna-Saldívar SO: **Cell Wall Degrading Enzymes and Proteases Improve Starch Yields of Sorghum and Maize**. *Starch - Stärke* 2006, **58**(7):338-344.
126. AOAC: **Official Methods of Analysis AOAC International, 16th edn**, vol. 16th edn. Arlington, VA; 1990.
127. Uriarte-Aceves PM, Cuevas-Rodríguez EO, Gutiérrez-Dorado R, Mora-Rochín S, Reyes-Moreno C, Puangpraphant S, Milán-Carrillo J: **Physical, Compositional, and Wet-Milling Characteristics of Mexican Blue Maize (*Zea mays* L.) Landrace**. *Cereal Chemistry Journal* 2015, **92**(5):491-496.
128. McGuire RG: **Reporting of Objective Color Measurements**. *HortScience* 1992, **27**(12):1254-1255.
129. Du S-k, Jiang H, Yu X, Jane J-l: **Physicochemical and functional properties of whole legume flour**. *LWT - Food Science and Technology* 2014, **55**(1):308-313.
130. Tester RF, Morrison, W. R. : **Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids.** *Cereal Chemistry* 1990, **67**:551-559.
131. Schoch TJ: **Swelling power and solubility of granular starches**. In: *Methods in Carbohydrate Chemistry*. 1964: 106-108.
132. Bryant CM HB: **Effect of lime on gelatinization of corn flour and starch**. *Cereal Chemistry* 1997, **74**:171-175.
133. Sevenou O, Hill SE, Farhat IA, Mitchell JR: **Organisation of the external region of the starch granule as determined by infrared spectroscopy**. *International Journal of Biological Macromolecules* 2002, **31**(1–3):79-85.
134. Hsu HW VD, Satterlee LD, Miller GA. : **A multienzyme technique for estimating protein digestibility.** *J Food Sci* 1977, **42**:1269-1273.
135. AOAC: **Official Method 982.30 E(a), chp. 45.3.05, acid hydrolysis.** . In: *Official Methods of Analysis.* 23rd ed. edn. Gaithersburg, MD.. Association of Official Analytical Chemists,; 2006.
136. FAO/WHO/UNU: **Protein and amino acid requirements in human nutrition**, vol. 935: World Health Organization; 2007.
137. Alsmeyer RH, Cunningham, A. E., & Happich, M. L.: **Equations predict PER from amino acid analysis**. *Food Technology,* 1974, **28**:34-38.
138. Morup IK, & Olesen, E. S.: **New method for prediction of protein value from essential amino acid pattern.** *Nutritional Report International,* 1976, **13**:355e365.
139. Englyst HN, Kingman SM, Cummings JH: **Classification and measurement of nutritionally important starch fractions**. *European Journal of Clinical Nutrition* 1992, **46**:S33-S50.
140. Goñi I, Garcia-Alonso A, Saura-Calixto F: **A starch hydrolysis procedure to estimate glycemic index**. *Nutrition Research* 1997, **17**(3):427-437.
141. Mosele JI, Macià A, Romero M-P, Motilva M-J, Rubió L: **Application of *in vitro* gastrointestinal digestion and colonic fermentation models to pomegranate products (juice, pulp and peel extract) to study the stability and catabolism of phenolic compounds**. *Journal of Functional Foods* 2015, **14**:529-540.
142. Luthria DL, Biswas R, Natarajan S: **Comparison of extraction solvents and techniques used for the assay of isoflavones from soybean**. *Food Chemistry* 2007, **105**(1):325-333.
143. Vinson JA, Proch J, Bose P: **Determination of quantity and quality of polyphenol antioxidants in foods and beverages**. *methods enzymol* 2001, **335**:103-114.

144. Leal-Díaz AM, Santos-Zea L, Martínez-Escobedo HC, Guajardo-Flores D, Gutiérrez-Uribe JA, Serna-Saldivar SO: **Effect of Agave americana and Agave salmiana Ripeness on Saponin Content from Aguamiel (*Agave* Sap)**. *Journal of agricultural and food chemistry* 2015, **63**(15):3924-3930.
145. Martinez-Villaluenga C, Dia VP, Berhow M, Bringe NA, Gonzalez de Mejia E: **Protein hydrolysates from beta-conglycinin enriched soybean genotypes inhibit lipid accumulation and inflammation *in vitro***. *Molecular nutrition & food research* 2009, **53**(8):1007-1018.
146. Singh N, Kaur S, Isono N, Noda T: **Genotypic diversity in physico-chemical, pasting and gel textural properties of chickpea (*Cicer arietinum* L.)**. *Food Chemistry* 2010, **122**(1):65-73.
147. Aguilera Y, Martín-Cabrejas MA, Benítez V, Mollá E, López-Andréu FJ, Esteban RM: **Changes in carbohydrate fraction during dehydration process of common legumes**. *Journal of Food Composition and Analysis* 2009, **22**(7–8):678-683.
148. Milán-Noris AK, de la Rosa-Millán J, Reyes-Moreno C, Serna-Saldivar SO: **Physicochemical, functional properties, and digestion of isolated starches from pigmented chickpea (*Cicer arietinum* L.) cultivars**. *Starch - Stärke* 2017, **69**(5-6):n/a-n/a.
149. Kaur M, Singh N: **Studies on functional, thermal and pasting properties of flours from different chickpea (*Cicer arietinum* L.) cultivars**. *Food Chemistry* 2005, **91**(3):403-411.
150. Chung H-J, Liu Q, Hoover R, Warkentin TD, Vandenberg B: ***In vitro* starch digestibility, expected glycemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars**. *Food Chemistry* 2008, **111**(2):316-321.
151. Zhu F: **Interactions between starch and phenolic compound**. *Trends in Food Science & Technology* 2015, **43**(2):129-143.
152. Iqbal A, Khalil IA, Ateeq N, Khan MS: **Nutritional quality of important food legumes**. *Food Chemistry* 2006, **97**(2):331-335.
153. Clemente A, Sánchez-Vioque R, Vioque J, Bautista J, Millán F: **Effect of cooking on protein quality of chickpea (*Cicer arietinum*) seeds**. *Food Chemistry* 1998, **62**(1):1-6.
154. Tavano OL, Neves VA, da Silva Júnior SI: ***In vitro* versus *in vivo* protein digestibility techniques for calculating PDCAAS (protein digestibility-corrected amino acid score) applied to chickpea fractions**. *Food Research International* 2016, **89**(Part 1):756-763.
155. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV: **Glycemic index of foods: a physiological basis for carbohydrate exchange**. *The American Journal of Clinical Nutrition* 1981, **34**(3):362-366.
156. Huang J, Schols HA, van Soest JJG, Jin Z, Sulmann E, Voragen AGJ: **Physicochemical properties and amylopectin chain profiles of cowpea, chickpea and yellow pea starches**. *Food Chemistry* 2007, **101**(4):1338-1345.
157. Singh N, Singh Sandhu K, Kaur M: **Characterization of starches separated from Indian chickpea (*Cicer arietinum* L.) cultivars**. *J Food Eng* 2004, **63**(4):441-449.
158. Chung H-J, Liu Q, Donner E, Hoover R, Warkentin TD, Vandenberg B: **Composition, molecular structure, properties, and *in vitro* digestibility of starches from newly released canadian pulse cultivars**. *Cereal Chemistry Journal* 2008, **85**(4):471-479.
159. Hoover R, Ratnayake WS: **Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada**. *Food Chemistry* 2002, **78**(4):489-498.
160. Miao M, Zhang T, Jiang B: **Characterisations of kabuli and desi chickpea starches cultivated in China**. *Food Chemistry* 2009, **113**(4):1025-1032.
161. Chung H-J, Liu Q, Hoover R: **Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches**. *Carbohydrate polymers* 2009, **75**(3):436-447.
162. Hoover R, Hughes T, Chung HJ, Liu Q: **Composition, molecular structure, properties, and modification of pulse starches: A review**. *Food Research International* 2010, **43**(2):399-413.
163. Xu Y, Sismour EN, Narina SS, Dean D, Bhardwaj HL, Li Z: **Composition and properties of starches from Virginia-grown kabuli chickpea (*Cicer arietinum* L.) cultivars**. *International Journal of Food Science & Technology* 2013, **48**(3):539-547.
164. Sandhu KS, Lim S-T: **Digestibility of legume starches as influenced by their physical and structural properties**. *Carbohydrate polymers* 2008, **71**(2):245-252.
165. Edwards CH, Warren FJ, Campbell GM, Gaisford S, Royall PG, Butterworth PJ, Ellis PR: **A study of starch gelatinisation behaviour in hydrothermally-processed plant food tissues and implications for *in vitro* digestibility**. *Food & Function* 2015, **6**(12):3634-3641.
166. Granfeldt Y BI, Drews A , Tovar J: **An *in vitro* procedure based on chewing to predict metabolic response to starch in cereal and legume products.** *European Journal of Clinical Nutrition* 1992, **46**(9):649-660.
167. Zhang H, Yin L, Zheng Y, Shen J: **Rheological, textural, and enzymatic hydrolysis properties of chickpea starch from a Chinese cultivar**. *Food Hydrocolloids* 2016, **54**:23-29.
168. Guajardo-Flores D, García-Patiño M, Serna-Guerrero D, Gutiérrez-Uribe JA, Serna-Saldívar SO: **Characterization and quantification of saponins and flavonoids in sprouts, seed coats and cotyledons of germinated black beans**. *Food Chemistry* 2012, **134**(3):1312-1319.
169. Singh B, Singh JP, Singh N, Kaur A: **Saponins in pulses and their health promoting activities: A review**. *Food Chemistry* 2017, **233**(Supplement C):540-549.
170. Oleszek WA: **Composition and Quantitation of Saponins in Alfalfa (Medicago sativa L.) Seedlings**. *Journal of Agricultural and Food Chemistry* 1998, **46**(3):960-962.
171. Lazo-Vélez MA, Guajardo-Flores D, Mata-Ramírez D, Gutiérrez-Uribe JA, Serna-Saldivar SO: **Characterization and Quantitation of Triterpenoid Saponins in Raw and Sprouted Chenopodium berlandieri spp. (Huauzontle) Grains Subjected to Germination with or without Selenium Stress Conditions**. *Journal of Food Science* 2016, **81**(1):C19-C26.

172. de Souza Rocha T, Hernandez LMR, Chang YK, de Mejía EG: **Impact of germination and enzymatic hydrolysis of cowpea bean (Vigna unguiculata) on the generation of peptides capable of inhibiting dipeptidyl peptidase IV**. *Food Research International* 2014, **64**:799-809.
173. Wang X, Gao W, Zhang J, Zhang H, Li J, He X, Ma H: **Subunit, amino acid composition and *in vitro* digestibility of protein isolates from Chinese kabuli and desi chickpea (*Cicer arietinum* L.) cultivars**. *Food Research International* 2010, **43**(2):567-572.
174. Chang Y-W, Alli I, Molina AT, Konishi Y, Boye JI: **Isolation and Characterization of Chickpea (Cicer arietinum L.) Seed Protein Fractions**. *Food and Bioprocess Technology* 2012, **5**(2):618-625.
175. Lopes Barbosa AC, Lajolo FM, Genovese MI: **Influence of temperature, pH and ionic strength on the production of isoflavone-rich soy protein isolates**. *Food Chemistry* 2006, **98**(4):757-766.
176. Chen PX, Dupuis JH, Marcone MF, Pauls PK, Liu R, Liu Q, Tang Y, Zhang B, Tsao R: **Physicochemical Properties and *in Vitro* Digestibility of Cooked Regular and Nondarkening Cranberry Beans (Phaseolus vulgaris L.) and Their Effects on Bioaccessibility, Phenolic Composition, and Antioxidant Activity**. *Journal of Agricultural and Food Chemistry* 2015, **63**(48):10448-10458.
177. García-Lafuente A, Moro C, Manchón N, Gonzalo-Ruiz A, Villares A, Guillamón E, Rostagno M, Mateo-Vivaracho L: ***In vitro* anti-inflammatory activity of phenolic rich extracts from white and red common beans**. *Food Chemistry* 2014, **161**(Supplement C):216-223.
178. Kole L, Giri B, Manna SK, Pal B, Ghosh S: **Biochanin-A, an isoflavon, showed anti-proliferative and anti-inflammatory activities through the inhibition of iNOS expression, p38-MAPK and ATF-2 phosphorylation and blocking NFκB nuclear translocation**. *European Journal of Pharmacology* 2011, **653**(1):8-15.
179. Ma Z, Ji W, Fu Q, Ma S: **Formononetin Inhibited the Inflammation of LPS-Induced Acute Lung Injury in Mice Associated with Induction of PPAR Gamma Expression**. *Inflammation* 2013, **36**(6):1560-1566.
180. Shimizu M: **Multifunctions of dietary polyphenols in the regulation of intestinal inflammation**. *Journal of Food and Drug Analysis* 2017, **25**(1):93-99.
181. González-Montoya M, Hernández-Ledesma B, Silván JM, Mora-Escobedo R, Martínez-Villaluenga C: **Peptides derived from *in vitro* gastrointestinal digestion of germinated soybean proteins inhibit human colon cancer cells proliferation and inflammation**. *Food Chemistry* 2018, **242**(Supplement C):75-82.
182. Ribeiro IC, Leclercq CC, Simões N, Toureiro A, Duarte I, Freire JB, Chaves MM, Renaut J, Pinheiro C: **Identification of chickpea seed proteins resistant to simulated *in vitro* human digestion**. *Journal of Proteomics* 2017.

Curriculum Vitae

Ada Keila Milán Noris
May 5, 1983.
Culiacán de Rosales, Sinaloa, México

Education

B.S. in Biochemical Engineering. Faculty of Chemical Biological Sciences. Autonomous University of Sinaloa. August 2001 to June 2006.

M.S.c in Biotechnology. Monterrey institute technology and higher education (ITESM). August 2009 to May 2011.
PhD studies in Biotechnology started in January, 2014 to December, 2017. Visiting scholar in DCCS-ICTAN-CSIC. Madrid, Spain. February to July 2017.

Publication (PhD Thesis)

Milán Noris, A.K, De la Rosa-Millán, J, Serna-Saldivar, S. **Physicochemical, functional propierties and digestion of isolated starch from pigmented chickpea (*Cicer arietinum* L.) cultivars.** Starch/Stärke, **2016**; 68: 1-11. DOI 10.1002/star.201600152

Academic meeting (PhD Thesis)

Oral presentation

Effect of cooking on saponins content in pigmented chickpea. Milán-Noris, A.K. Gutiérrez-Uribe, J.A. Serna-Saldivar, S.O. 251st American Chemical Society National Meeting. San Diego, California, USA. March 13-17 ,2016.

Caracterización fisicoquímica, funcional y de digestibilidad de almidones de garbanzo pigmentado. Milán Noris, A.K, De la Rosa-Millán, J, Serna-Saldivar, S. 2do. Congreso internacional de alimentos funcionales y nutracéuticos. Querétaro, México. June 22-24, 2016.

Identificación y Cuantificación de Saponinas en Garbanzo Pigmentado. Milán-Noris, A.K. Gutiérrez-Uribe, J.A. Serna-Saldivar, S.O. 2do. Congreso internacional de alimentos funcionales y nutracéuticos. Querétaro, México. June 22-24, 2016.

Poster presentation

Saponins quantification in pigmented chickpea cultivars. Milán-Noris, A.K. Gutiérrez-Uribe, J.A. Serna-Saldivar, S.O. 250st American Chemical Society National Meeting, Boston, Massachusetts, USA. August 16-20, 2015.

***Characterization, functionality and in vitro digestion of refined starches from ten chickpea cultivars*.** Milán Noris, A.K, De la Rosa-Millán, J, Serna-Saldivar, S. The 2016 AACC International Annual Meeting. Savannah, Georgia, USA. October 23-26, 2016

RESEARCH ARTICLE

Physicochemical, functional properties, and digestion of isolated starches from pigmented chickpea (*Cicer arietinum* L.) cultivars

Ada Keila Milán-Noris[1], *Julián de la Rosa-Millán*[2], *Cuauhutémoc Reyes-Moreno*[3] *and Sergio O. Serna-Saldivar*[1,2]

[1] Centro de Biotecnología-FEMSA, Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Monterrey, Nuevo Leon, Mexico
[2] Centro de Investigación y Desarrollo de Proteínas (CIDPRO), Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Monterrey, Nuevo Leon, Mexico
[3] Doctorado en Biotecnología (Programa Regional del Noroeste para el Doctorado en Biotecnología), Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Ciudad Universitaria, Sinaloa, Mexico

Received: May 4, 2016
Revised: July 20, 2016
Accepted: July 21, 2016

This research was undertaken to study physicochemical, functional, and in vitro starch digestion properties of wet-milled chickpea starches obtained from an array of ten cultivars differing in seed coat color (black, brown, green, red, and cream). The yield of chickpea starches ranged from 19.22 to 30.06%, in which resulting starches varied in total starch and amylose contents from 87.14 to 96.02% and 25.05 to 35.26%, respectively. The DSC transition temperatures T_o, T_p, and T_c and gelatinization enthalpy ranged from 61.93 to 68.94°C, 66.47 to 72.57°C, 73.34 to 78.50°C, and 8.82 to 10.68 J/g, respectively, indicating large differences among the starches. Scanning electron micrographs of the starches showed lenticular-shaped granules with a smooth surface and different granule sizes. The rapidly digestible (RDS), slowly digestible (SDS), and resistant (RS) gelatinized starch fractions varied from 56.34 to 59.15%, 33.22 to 35.43%, and 6.42 to 9.22%, respectively. The predicted glycemic indexes (pGI) of native and gelatinized chickpea starches ranged from 65.52 to 66.10 and 74.39 to 75.74, respectively. A close correlation among the viscosity characteristics of isolated starches and the starch digestion fractions was found after PCA analysis. The properties of the starches were not dependent on the seed-coat coloration of the cultivars. Overall, the results suggest that the starches of the array of chickpeas studied may hold potential for the development of functional foods especially due to its functional properties, medium glycemic index, high SDS, and RS contents.

Keywords:
Chickpea / Functional characteristics / Starch digestion / Starch isolation

1 Introduction

Chickpea (*Cicer arietinum* L.) is the third most consumed pulse worldwide, after common beans and peas, due to its drought resistance and comparatively lower agronomic and economic inputs required for its production. In 2014, the northwest of México produced 171,665 ton, that represent 1.20% of the chickpea in the world [1]. There are two types of chickpeas that are classified according to its origin: Desi (Indian region) with a thick and pigmented seed coat, and Kabuli (Mediterranean region) with thin seed coat and cream or white pigmentation and depending of the cultivar, the seed shape can be round, wrinkled, and exalbuminous [2, 3]. According to the botanical differences, the seed coat and cotyledon varies from 3 to 16% and 82 to 97% of the seed weight; thus, these relative amounts affect chemical-nutritional composition and influences functionality and, therefore, the use as food. The external seed coat presents some well-defined structures: hilum, micropyle,

Correspondence: Dr. Sergio O. Serna-Saldivar, Centro de Investigación y Desarrollo de Proteínas (CIDPRO), Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, CP 64849, Monterrey, N.L. México
E-mail: sserna@itesm.mx
Fax: 52-81-83284136

Abbreviations: ATR, attenuated total reflectance; **FTIR,** Fourier transformed infrared spectroscopy; **PCA,** Principal component analysis

Colour Online: See the article online to view figures in colour.

and raphe, which are related to both the seed integrity and germination potential [4, 5].

From the nutritional perspective, chickpea seeds are a good source of high quality and digestible protein, non-fibrous carbohydrates (starch and oligosaccharides), insoluble and soluble dietary fibers, vitamins, and minerals [2, 6]. It is well known that the starch in pulses is digested in lower extent compared to counterparts isolated from cereals or tubers. Its relatively lower digestibility is reflected in the low glycemic index [7] attributed to its granular morphology, the absence of pores on the surface, as well to its molecular composition, in particular the amylose amount and structure, as well as the amylopectin conformation [8]. Nowadays, pigmented chickpea varieties have received particular attention due to their phenolic composition and antioxidant properties [9, 10]. However, there is scarce information about the starch associated to different pigmented seed cultivars and how they differ in composition, morphology, pasting, thermal, functional, and digestion characteristics [11]. Hence, the aim of this work was to evaluate the physicochemical, functional, and digestion characteristics of isolated starches from ten chickpea cultivars differing in seed coat color (black, brown, green, red, and cream), nine classified as Desi and a commercial Kabuli chickpea.

2 Materials and methods

2.1 Chickpea seed cultivars

Nine pigmented cultivars (Desi type) from the core collection/World Germplasm Bank of the International Crops Research Institute of Semi-Arid Tropics (ICRISAT) were grown in the Culiacan valley experiment station of the National Research Institute for Forestry, Agriculture and Livestock (INIFAP), located in Sinaloa, Mexico. The commercial Kabuli cultivar Blanco Sinaloa (used as reference) was grown at Evora Region, Sinaloa, Mexico. The chickpea seeds were harvested in April–May 2014, cleaned for removal of dockage and foreign material and stored at −20°C until analysis.

2.2 Seed physical characterization

The seed physical characteristics were determined as described by Heiras-Palazuelos et al. [9]. Briefly, the 1000-grain weight was determined weighing randomly picked seeds by five replicates. The hectoliter weight was determined using the Winchester bushel meter following the official procedure by 10 replicates [12]. Anatomical seed parts were determined by first soaking 25 whole seeds for 12 h at room temperature (≈25°C), and manually dissected into hilum, seed coat, and cotyledons. These fractions were dried in a convection oven (1350FMS, VWR, Radnor, PA, USA) set at 105°C for 24 h and weighed. The average diameter was determined after measuring 25 seeds by triplicate.

2.3 Wet-milling starch isolation

Starches were obtained following the sodium sulfite wet milling process described by Pérez-Carrillo and Serna-Saldívar [13] with slight modifications. Briefly, the seeds were soaked for 48 h at 50°C in a 0.2% sodium sulfite with 0.47% lactic acid (85%) solution. Then, the steep liquor was discarded and the soaked seeds were mixed with distilled water in preparation for grinding in a commercial blender (Oster, model 450–10) for 1 min at high speed. The slurry was filtered through a 100 US mesh sieve, then centrifuged at 3000×*g* for 10 min, and the resulting pellet or sediment thoroughly washed until the water and starch were color free. The starch pellet was collected and freeze-dried. After drying, the sample was weighed to calculate extraction yield, and stored until analysis. Starch yield and recovery were calculated using the following equations:

$$\text{Starch yield} = (\text{Isolated starch weight}/\text{grain weight}) \times 100 \quad (1)$$

$$\text{Starch recovery} = (\text{Isolated starch weight}/\text{starch content in kernels}) \times 100 \quad (2)$$

2.4 Starch granule morphology

The starch granules morphology and birefringence patterns were observed with a Motic BA-210 digital microscope (Hong Kong, China). The images were recorded at the same magnification (×40) under normal and polarized light. Additionally, scanning electron micrographs were acquired with a Nova Nano Scanning electron microscope (FEI Company, Eindhoven, The Netherlands). For this, the dried samples were mounted in an aluminum stub using carbon conductive tape, and then examined at an accelerating voltage of 10 kV in low vacuum mode and using a helix detector.

2.5 Starch color characteristics

The color of dehydrated starch powders were measured by a Chroma meter Minolta CM-600 (Konica Minolta Co., Osaka, Japan) to determine color values L^* (Lightness), a^* (redness–greenness), and b^* (yellowness–blueness). The whiteness (W) of starch powders was determined using

the following equation [14]:

$$W = 100 - \left[(100 - L^*)^2 + (a^*)^2 + (b^*)^2\right]^{\frac{1}{2}} \quad (3)$$

2.6 Chemical and functional characteristics of isolated starches

The chemical composition was determined according to AOAC standard methods 925.09B, 923.03, and 960.52 for moisture, ash, and protein (Nx6.25) [15]. Total starch (AOAC 996.11) and amylose contents were determined using the Megazyme kits K-TSTA and L-AMYL (Wicklow, Ireland). All analyses were done in triplicate. In order to understand their potential use as food ingredients, some of the functional properties related with water absorption were tested; the swelling power was expressed as the amount of water in weight of the wet sediment to the initial weight of dry starch and was determined using a method reported by Tester and Morrison [16]. The starch solubility was expressed as the amount of the dried supernatant weight in relation to the initial weight of dry starch following the method reported by Schoch [17]. Though the water retention capacity (WRC) was assessed with the method reported by Bryant and Hamaker [18] with modifications. Briefly, chickpea starch/water dispersions (10% w/v) were heated in a water bath at 95°C with vortex homogenization every 5 min during 20 min The tubes were then centrifuged for 15 min at 1000×*g* and the resulting supernatants decanted. The tubes with the pellets were allowed to drain off excess water for 10 min at a 45° angle and the differences in weight were used to calculate the WRC.

2.7 Rapid viscoamylographs of isolated starches

The chickpea starches (3 g) were mixed with distilled water (25 g) in an aluminum canister, then heated and cooled in a Rapid Visco-Analyser (RVA model 1170, Newport Scientific, Warriewood, NSW, Australia) using the following profile: heating from 50 to 95°C at 5°C/min; temperature held at 95°C for 7 min and then cooling from 95 to 50°C at a rate of −6°C/min. The viscosity values during heating, cooking and cooling were calculated using the Thermocline software (Ver. 3.15.3.347).

2.8 Starch thermal properties

The gelatinization characteristics of the starches were determined with a differential scanning calorimeter (Diamond DSC, Perkin Elmer, Nortfolk, VA, USA). About 3 mg of each starch sample was placed in a stainless-steel pan, mixed with 7 mg of deionized water, and hermetically sealed. Once hydrated, the sample was allowed to equilibrate at room temperature for 1 h. Samples were kept 2 min at 30°C then heated from 30 to 95°C at a heat rate of 10°C/min and the temperature was held at 95°C for 1.5 min. The DSC was calibrated using indium as a standard and during the experiments an empty steel pan was used as reference. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy of gelatinization (ΔH) were calculated with the Pyris software.

2.9 Attenuated total reflectance-Fourier transformed infrared spectroscopy (ATR-FTIR)

The ATR-FTIR analysis of starches were recorded on an FTIR spectrometer (Spectrum 1, Perkin Elmer) using an attenuated total reflectance (ATR) mode [19]. For each spectrum, 20 scans were recorded at a resolution of 4 cm^{-1} at room temperature (≈25°C). The obtained spectra were baseline-corrected, normalized, and deconvoluted over the range of 1200–800 cm^{-1}, with a half-width of 22 cm^{-1}, with a resolution enhancement factor of 1.5. The amplitudes of absorbance for each spectrum at 1022 and 1047 cm^{-1} were noted and the ratio 1047 cm^{-1}:1022 cm^{-1} was calculated in each sample in order to estimate the degree of crystalline to amorphous order of starches.

2.10 In vitro starch digestibility

The in vitro starch digestibility was determined according to the Englyst et al. [20] protocol, with modifications. Starch samples were analyzed in both raw and gelatinized or cooked forms. The gelatinized starch hydrolysis was performed in order to understand the behavior in more practical cooked food systems. In this case, hermetic polypropylene tubes with the starch dispersions (100 mg in 4 mL of 0.5 M sodium acetate buffer pH 5.2) were heated in a boiling water bath with constant magnetic stirring (300 rpm) for 20 min, after they were allowed to cool at 37°C in a water bath and processed as follows. One milliliter of an enzyme solution, consisted of a mixture of 2.5 mL of the supernatant of porcine pancreatic α-amylase (0.45 g in 4 mL of water, centrifuged at 1500×*g* for 15 min), 0.3 mL of amyloglucosidase and 0.2 mL of invertase were mixed thoroughly, and added to each test tube containing five glass beads (7 mm diameter), that were then incubated in a shaking water (200 strokes/min) at 37°C. Aliquots (0.1 mL) were taken at intervals and mixed with 1 mL of 80% ethanol. The hydrolyzed glucose content was measured with the glucose oxidase–peroxidase reagent. Starch classifications based on the rate of hydrolysis were rapidly digestible (digested within 20 min) starch (RDS), slowly digestible (digested between 20 and 120 min) starch (SDS), and resistant (undigested after 120 min) starch (RS).

2.11 Predicted glycemic index

With the aim to estimate glycemic index of starches, the hydrolysis data (from 0 to 180 min) from the previous

Table 1. Physical properties of pigmented chickpea seeds

				Anatomical seed parts		
Cultivar	1000 GW (g)	HW (kg/hL)	MD (mm)	Seed coat (%)	Cotyledons (%)	Hilum (%)
B.ICC6306[a)]	290.65 ± 3.43^{c}	77.78 ± 0.78^{ab}	9.42 ± 0.10^{b}	10.95 ± 0.06^{c}	87.93 ± 0.07^{c}	1.12 ± 0.07^{cd}
B.ICC4418[a)]	124.86 ± 3.92^{f}	79.52 ± 0.74^{a}	7.12 ± 0.11^{d}	13.04 ± 0.19^{ab}	84.96 ± 0.13^{e}	2.01 ± 0.05^{a}
B.ICC3761[a)]	121.46 ± 1.18^{f}	78.64 ± 0.66^{a}	7.52 ± 0.09^{d}	13.28 ± 0.24^{a}	84.94 ± 0.25^{e}	1.74 ± 0.04^{ab}
Br.ICC3512[a)]	198.63 ± 0.76^{de}	77.24 ± 0.53^{ab}	8.28 ± 0.10^{c}	13.13 ± 0.24^{ab}	85.57 ± 0.26^{de}	1.28 ± 0.05^{c}
C.BS[b)]	666.30 ± 14.05^{a}	67.62 ± 1.28^{d}	12.22 ± 0.22^{a}	3.64 ± 0.06^{e}	95.66 ± 0.08^{a}	0.68 ± 0.01^{e}
C.ICC3421[a)]	196.68 ± 1.82^{de}	78.71 ± 0.34^{a}	8.32 ± 0.07^{c}	4.11 ± 0.07^{e}	95.13 ± 0.08^{a}	0.74 ± 0.03^{e}
G.ICC5613[a)]	181.27 ± 1.71^{e}	74.12 ± 0.35^{c}	8.48 ± 0.07^{c}	13.62 ± 0.22^{a}	$84.80 + 0.25^{e}$	1.57 ± 0.06^{b}
R.ICC14782[a)]	191.29 ± 1.34^{e}	78.16 ± 0.51^{a}	9.64 ± 0.07^{b}	9.91 ± 0.34^{c}	88.99 ± 0.37^{b}	1.09 ± 0.02^{cd}
R.ICC13124[a)]	352.67 ± 2.50^{b}	75.00 ± 0.31^{bc}	8.18 ± 0.12^{c}	10.84 ± 0.22^{cd}	88.21 ± 0.24^{bc}	0.94 ± 0.03^{de}
R.ICC5383[a)]	218.42 ± 1.88^{d}	77.69 ± 0.17^{ab}	8.52 ± 0.08^{c}	12.17 ± 0.10^{b}	86.23 ± 0.05^{d}	1.59 ± 0.05^{b}

GW, grain weight; HW, hectoliter weight; MD, diameter; BS, cultivar Blanco Sinaloa 92; B, black color; Br, brown color; C, cream color; G, green color; R, red color.
Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p < 0.05$).
a) Desi seeds.
b) Kabuli seeds.

protocol was used to calculate the hydrolysis index (HI), which was obtained from the area under the hydrolysis curve compared with the area obtained for the hydrolysis of a standard material (white bread) under the same conditions. The predicted glycemic index (pGI) was estimated with the equation reported by Goñi, García-Alonso and Saura-Calixto [21] which has a correlation coefficient of $R^2 = 0.89$, $p < 0.05$:

$$\text{pGI} = 39.71 + 0.549\,(\text{HI}) \quad (4)$$

2.12 Statistical analysis

Data were analyzed by one-way ANOVA followed by Tukey test to detect differences among chickpea varieties. A p-value < 0.05 was considered significant. Correlation coefficients were obtained by multivariate analysis. A principal component analysis (PCA) was carried out to visualize similarities and differences among chickpea starches in terms of functional and digestibility parameters. All statistical analyses were performed with the use of JMP 12 software from the SAS institute (Cary, NC, USA). All experiments and procedures were performed in triplicate, unless otherwise specified.

3 Results and discussion

3.1 Chickpea seeds physical characterization

Significant differences were found in 1000-grain weight between the Kabuli type (C.BS) (666.30 g) and all the Desi types (≤352.67 g) (Table 1). The apparent density measured with the test weight showed the opposite trend, where

Table 2. Wet-milling yields and chemical composition of isolated starches from pigmented chickpea cultivars (dwb)

Cultivar	Grain starch (%)	Yield (%)	Recovery (%)	Protein (%)	Ash (%)	Starch (%)	Amylose (%)
B.ICC6306[a)]	40.28 ± 0.77^{cde}	21.52 ± 3.05^{bc}	53.41 ± 7.16^{bc}	0.36 ± 0.01^{def}	0.06 ± 0.00^{ab}	95.83 ± 1.36^{a}	26.66 ± 0.09^{ef}
B.ICC4418[a)]	37.00 ± 0.74^{ef}	19.22 ± 0.33^{c}	51.18 ± 0.72^{bc}	0.26 ± 0.00^{gh}	0.04 ± 0.00^{ab}	91.03 ± 1.01^{bc}	27.53 ± 0.12^{de}
B.ICC3761[a)]	35.78 ± 1.08^{f}	19.95 ± 1.47^{bc}	55.46 ± 3.79^{abc}	0.43 ± 0.00^{cde}	0.06 ± 0.00^{ab}	92.71 ± 0.55^{ab}	28.46 ± 0.10^{d}
Br.ICC3512[a)]	43.42 ± 1.06^{abc}	19.53 ± 2.04^{c}	43.93 ± 3.93^{c}	0.51 ± 0.01^{abc}	0.05 ± 0.00^{ab}	90.12 ± 0.58^{bcd}	25.99 ± 0.06^{fg}
C.BS[b)]	38.27 ± 0.25^{def}	27.42 ± 1.01^{ab}	71.69 ± 2.71^{a}	0.54 ± 0.02^{a}	0.06 ± 0.00^{ab}	87.14 ± 0.43^{d}	30.19 ± 0.12^{c}
C.ICC3421[a)]	42.00 ± 1.28^{bcd}	30.06 ± 1.02^{a}	69.80 ± 2.15^{ab}	0.35 ± 0.01^{ef}	0.07 ± 0.00^{ab}	89.59 ± 1.03^{bcd}	25.05 ± 0.37^{g}
G.ICC5613[a)]	46.23 ± 0.74^{a}	21.62 ± 1.39^{bc}	45.90 ± 3.16^{c}	0.24 ± 0.00^{h}	0.03 ± 0.00^{b}	90.54 ± 0.83^{bcd}	26.01 ± 0.20^{fg}
R.ICC14782[a)]	42.97 ± 0.50^{abc}	24.26 ± 0.81^{abc}	56.68 ± 2.03^{c}	0.44 ± 0.02^{bcd}	0.05 ± 0.00^{ab}	96.02 ± 1.31^{a}	33.26 ± 0.26^{b}
R.ICC13124[a)]	44.90 ± 0.46^{ab}	23.28 ± 0.19^{abc}	51.66 ± 0.57^{abc}	0.34 ± 0.00^{fg}	0.06 ± 0.00^{ab}	89.67 ± 0.65^{bcd}	27.29 ± 0.38^{de}
R.ICC5383[a)]	39.79 ± 0.31^{cde}	22.35 ± 1.83^{abc}	55.90 ± 4.50^{abc}	0.52 ± 0.00^{ab}	0.07 ± 0.00^{a}	87.99 ± 0.73^{cd}	35.26 ± 0.32^{a}

BS, cultivar Blanco Sinaloa 92; B, black color; Br, brown color; C, cream color; G, green color; R, red color.
Values are means ± SEM (standard error mean). Values with different letter(s) in every column are significantly different ($p < 0.05$).
a) Desi seeds.
b) Kabuli seeds.

cultivars ranged from 67.62 (C.BS) to ≥74.12 kg/hL. The diameter of the chickpea seeds ranged from 7.12 (B. ICC4418) to 12.22 mm (C.BS). The amount of seed coat in the array of chickpeas ranged from 3.64% (C.BS) to 13.62% (green cultivar G.ICC5613). The amounts of cotyledon and hilum ranged from 84.80 (G.ICC5613) to 95.66% (C.BS) and 0.68 (C.BS) to 2.01% (B.ICC4418) of the total seed weight, respectively. These results agreed with previous reports that showed important size differences when Desi and Kabuli cultivars were compared mainly due to their different genetic background [3, 5].

3.2 Chemical composition of chickpea starches

The principles of the sulfur dioxide wet-milling process used for maize were followed to obtain chickpea starches. The starch content in raw seeds ranged from 35.78 (B.ICC3761) to 46.23% (G.ICC5613). The calculated starch yield and recovery of the ten different chickpea starches ranged from 19.22 (B.ICC4418) to 30.06% (C.ICC3421) and 43.93 (Br.ICC3512) to 71.69% (C.BS), respectively (Table 2). Both parameters showed a negative strong correlation with the amount of seed coat ($r = -0.9386$, $p < 0.0001$) and

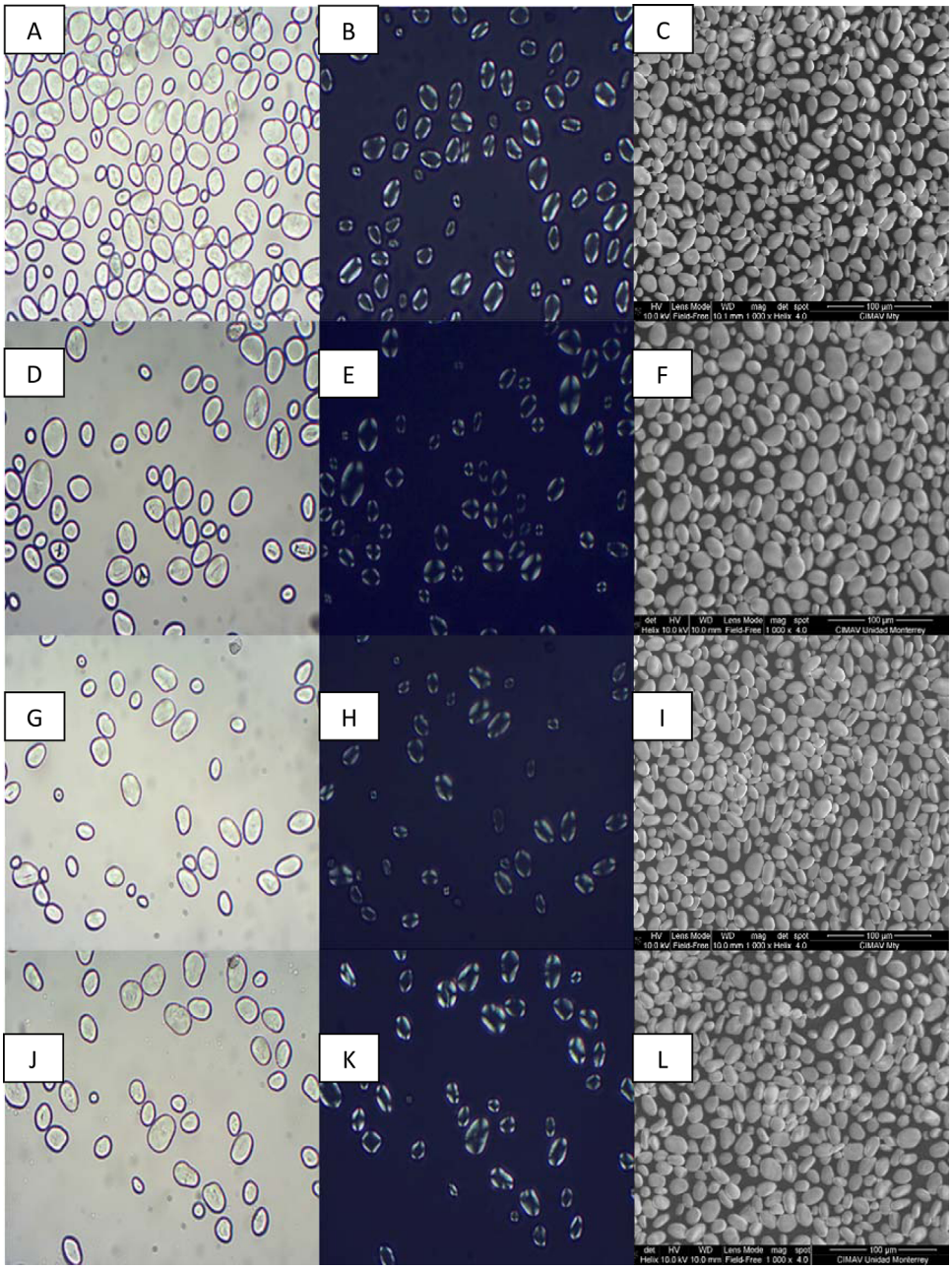


Figure 1. Microscopy analysis of starch granules, from left to right normal light (×40), polarized light (×40), and scanning electron micrograph (×1000). A–C, B.ICC3761 (black colored); D–F, C.BS (cream colored); G–I, G.ICC5613 (green colored); and J–L, R.ICC5383 (red colored).

($r = -0.9235$, $p < 0.0001$), respectively. One of the most relevant characteristics of starches intended for food applications is their purity. It is difficult to obtain pure starches from pulses because of their high protein and fiber contents present in cell walls and the strong interaction between proteins and starch granules. Moreover, the high amounts of fiber tend to co-sediment with the dense starch fraction during the extraction protocols [22–24]. The results indicated that the refined starches contained relatively low amounts of protein ($\leq$0.54%) and ash ($\leq$0.07%) indicating the effectiveness of the sulfur dioxide wet-milling procedure. The total starch contents ranged from 87.14 (C.BS) to 96.02% (R.ICC14782). These results agree with purity values previously reported in chickpea starches (87–94%) [22, 24]. One of the main characteristics of legume starches is that they contain relative high amounts of amylose. The amounts of this molecule varied from 25.05 (C.ICC3421) to 35.26% (R.ICC13124) and interestingly there were not significant differences when the Desi and Kabuli types were compared. These results are in agreement with previous reported amylose contents (20.7–35%) in chickpea starches reported by other authors [22, 23, 25].

3.3 Granule morphology of chickpea starches

Starch granules micrographs are depicted in Fig. 1. The starches showed lenticular shaped granules, which varied in size. However, all starches showed smooth surfaces, similar to those reported by Miao et al. [26]. Some of the starch granules showed a central depression, that when observed under polarized light, depicted the characteristic center-hollowed birefringence pattern, which in the case of legume starches is related with their particular shape and could be related with the granule architecture developed during biosynthesis [23–27].

3.4 Color of chickpea starches

The color of isolated starch is an important quality parameter, in which clean white is desirable [14]. The a^* and b^* values in the starches ranged from −0.91 (B.ICC3761) to −2.10 (R.ICC14782) and 2.45 (C.ICC3421) to 5.42 (R.ICC14782), respectively, which implies that samples showed small traces of residual pigments (Table 3). However, the calculated whiteness of chickpea starches ranged from 84.83 (B.ICC3761) to 91.99 (Br.ICC3512), respectively, which are similar to the reported by Uriarte-Aceves et al. [14].

3.5 Functional properties of chickpea starches

The swelling power of starch granules is affected by both inter and intra granular interactions with water. This phenomenon occurs concurrently with the loss of birefringence and precedes solubilization [8]. The swelling power of the array of chickpea starches ranged from 12.00 to 14.62 g/g for G.ICC5613 and R.ICC5383 samples, respectively (Table 3). This important feature showed a positive strong correlation with amylose ($r = 0.9262$, $p = 0.0001$) and a non-significant difference with starch solubility (11.19–13.12%). The water retention capacity (WRC) values that are directly related to starch chain length and water molecules interaction varied from 83.21 (C.ICC3421) to 91.26% (R.ICC5383) and were similar to values (77–92%) reported by other authors [23, 26].

Table 3. Physicochemical properties of isolated starch from pigmented chickpea

	Starch color						
Cultivar	L^*	a^*	b^*	Whiteness	Swelling power (g/g)	Solubility (%)	Water retention capacity (%)
B.ICC6306[a)]	86.75 ± 0.09^{f}	−1.47 ± 0.00^{b}	2.81 ± 0.01^{f}	86.38 ± 0.09^{e}	12.27 ± 0.21^{bc}	12.63 ± 0.56^{a}	89.95 ± 0.37^{ab}
B.ICC4418[a)]	87.50 ± 0.04^{e}	−1.62 ± 0.00^{c}	2.67 ± 0.01^{g}	87.12 ± 0.04^{d}	12.54 ± 0.40^{bc}	11.67 ± 0.57^{a}	86.36 ± 0.18^{c}
B.ICC3761[a)]	84.83 ± 0.09^{h}	−0.91 ± 0.00^{a}	2.17 ± 0.00^{j}	84.65 ± 0.08^{h}	12.79 ± 0.24^{bc}	11.97 ± 0.49^{a}	88.48 ± 0.21^{b}
Br.ICC3512[a)]	91.99 ± 0.12^{a}	−2.05 ± 0.00^{g}	3.50 ± 0.02^{e}	91.99 ± 0.11^{a}	12.36 ± 0.05^{bc}	13.00 ± 0.50^{a}	89.36 ± 0.56^{b}
C.BS[b)]	88.41 ± 0.03^{d}	−1.80 ± 0.00^{e}	2.49 ± 0.00^{h}	88.01 ± 0.03^{c}	13.11 ± 0.17^{bc}	12.06 ± 0.31^{a}	89.95 ± 0.32^{ab}
C.ICC3421[a)]	91.06 ± 0.08^{b}	−1.79 ± 0.00^{e}	2.45 ± 0.01^{i}	90.56 ± 0.08^{b}	12.36 ± 0.06^{bc}	12.46 ± 0.21^{a}	83.21 ± 0.43^{d}
G.ICC5613[a)]	85.80 ± 0.03^{g}	−1.69 ± 0.00^{d}	3.94 ± 0.01^{d}	85.17 ± 0.03^{g}	12.00 ± 0.03^{c}	12.36 ± 0.43^{a}	84.17 ± 0.32^{d}
R.ICC14782[a)]	89.49 ± 0.05^{c}	−2.10 ± 0.01^{h}	5.42 ± 0.01^{a}	88.00 ± 0.05^{c}	13.29 ± 0.38^{b}	11.19 ± 0.43^{a}	86.46 ± 0.20^{c}
R.ICC13124[a)]	88.29 ± 0.04^{d}	−1.90 ± 0.00^{f}	5.28 ± 0.00^{c}	87.02 ± 0.03^{d}	12.06 ± 0.25^{c}	12.21 ± 0.50^{a}	88.42 ± 0.24^{b}
R.ICC5383[a)]	87.27 ± 0.02^{e}	−1.89 ± 0.00^{f}	5.34 ± 0.01^{b}	86.07 ± 0.02^{f}	14.62 ± 0.20^{a}	13.12 ± 0.38^{a}	91.26 ± 0.15^{a}

BS, cultivar Blanco Sinaloa 92; B, black color; Br, brown color; C, cream color; G, green color; R, red color.
Values are means ± SEM (standard error mean). Values with different letter(s) in every column are significantly different ($p < 0.05$). Whiteness: $100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$.
a) Desi seeds.
b) Kabuli seeds.

Table 4. Rapid viscosity profiles of isolated chickpea starches

		Viscosity (cP)			
Cultivar	Pasting temperature (°C)	Peak	Through	Final	Setback
B.ICC6306[a)]	74.90 ± 0.01[d]	6162 ± 17.78[e]	4823 ± 22.27[b]	8604 ± 39.74[d]	3781 ± 10.91[g]
B.ICC4418[a)]	74.05 ± 0.02[e]	6277 ± 18.12[d]	4627 ± 21.37[c]	8682 ± 40.10[d]	4055 ± 11.70[f]
B.ICC3761[a)]	73.35 ± 0.01[f]	6535 ± 18.86[c]	4614 ± 21.31[c]	9336 ± 43.12[c]	4722 ± 13.63[d]
Br.ICC3512[a)]	76.45 ± 0.02[a]	5914 ± 17.07[f]	4413 ± 20.38[d]	7973 ± 36.82[f]	3560 ± 11.27[h]
C.BS[b)]	73.35 ± 0.01[f]	5490 ± 15.84[g]	3841 ± 17.73[g]	9455 ± 43.67[c]	5614 ± 16.20[b]
C.ICC3421[a)]	76.35 ± 0.02[b]	5270 ± 15.21[i]	4152 ± 19.17[e]	8258 ± 38.14[e]	4106 ± 11.85[f]
G.ICC5613[a)]	75.60 ± 0.01[c]	5406 ± 15.60[gh]	4121 ± 19.83[e]	8512 ± 39.31[d]	4391 ± 12.67[e]
R.ICC14782[a)]	70.80 ± 0.02[g]	7538 ± 21.76[b]	4582 ± 21.16[c]	10123 ± 46.75[b]	5541 ± 15.99[c]
R.ICC13124[a)]	75.65 ± 0.01[c]	5333 ± 15.39[hi]	4000 ± 18.47[f]	8676 ± 40.07[d]	4676 ± 13.49[d]
R.ICC5383[a)]	74.00 ± 0.02[e]	8485 ± 24.49[a]	6264 ± 28.93[a]	13158 ± 60.77[a]	6894 ± 15.90[a]

BS, cultivar Blanco Sinaloa 92; B, black color; Br, brown color; C, cream color; G, green color; R, red color.
Values are means ± SEM (standard error mean). Values with different letter(s) in every column are significantly different ($p < 0.05$).
a) Desi seeds.
b) Kabuli seeds.

3.6 Pasting profiles of chickpea starches

The RVA pasting profiles show the changes occurred due to starch gelatinization as a consequence of heating in excess water under constant shear stress [8]. The starch pasting temperatures ranged from 70.80 to 76.45°C for Br.ICC3512 and R.ICC14782, respectively (Table 4); that according to other studies could be related with starch granule size distribution and its molecular characteristics [5, 6, 24]. In a general way, the viscous behavior of pulse starches reflects their particular molecular conformation, in which longer amylopectin chains promote higher peak viscosities (PV) whereas the amount and particular amylose characteristics help to generate higher final viscosities (FV)[16, 22, 24, 25]. Results herein indicated that PV ranged from 5270 to 8485 cP whereas FV from 7973 to 13158 cP, that showed strong positive correlations with amylose content ($r = 0.8667$, $p = 0.0012$ and $r = 0.9213$, $p = 0.0002$) and with swelling power ($r = 0.8601$, $p = 0.0012$ and $r = 0.9548$, $p < 0.0001$). Others authors have shown that the differences on molecular weight (MW) as well as chain length distribution of both amylose and amylopectin may have a significant effect on starch pasting formation, thermal stability and may slow its digestion rate and pGI [25, 27, 28].

Table 5. Thermal properties and ATR- FTIR crystalline proportion of isolated chickpea starches

	Gelatinization temperatures					ATR- FTIR		
Cultivar	T_o (°C)	T_p (°C)	T_c (°C)	ΔT (°C)	ΔH (J/g)	Crystalline (1024 cm^{-1})	Amorphous (1044 cm^{-1})	Ratio (C/A)
B.ICC6306[a)]	67.14 ± 0.08[ab]	71.12 ± 0.23[a]	77.35 ± 0.63[a]	10.21 ± 0.43[d]	9.21 ± 0.09[de]	0.63 ± 0.02[a]	0.89 ± 0.03[a]	0.71 ± 0.03[ab]
B.ICC4418[a)]	68.56 ± 0.22[ab]	72.43 ± 0.49[a]	78.50 ± 1.02[a]	9.94 ± 0.79[d]	10.68 ± 0.17[a]	0.58 ± 0.04[a]	0.93 ± 0.02[a]	0.62 ± 0.04[b]
B.ICC3761[a)]	63.72 ± 0.42[cd]	68.00 ± 0.11[bc]	78.00 ± 0.36[a]	14.28 ± 0.05[a]	9.29 ± 0.08[cde]	0.54 ± 0.05[a]	0.84 ± 0.04[a]	0.64 ± 0.03[b]
Br.ICC3512[a)]	67.79 ± 0.57[ab]	71.96 ± 0.95[a]	78.23 ± 0.86[a]	10.44 ± 0.28[cd]	9.13 ± 0.15[de]	0.56 ± 0.02[a]	0.87 ± 0.03[a]	0.64 ± 0.02[b]
C.BS[b)]	68.10 ± 0.19[ab]	71.78 ± 0.52[a]	76.83 ± 0.69[a]	8.73 ± 0.50[d]	10.18 ± 0.23[ab]	0.58 ± 0.01[a]	0.96 ± 0.00[a]	0.60 ± 0.01[b]
C.ICC3421[a)]	66.00 ± 0.13[ab]	70.80 ± 0.15[ab]	78.27 ± 0.30[a]	12.28 ± 0.16[bc]	9.67 ± 0.11[bcd]	0.58 ± 0.04[a]	0.92 ± 0.02[a]	0.62 ± 0.01[b]
G.ICC5613[a)]	68.94 ± 0.37[a]	72.57 ± 0.22[a]	78.00 ± 0.40[a]	9.06 ± 0.03[d]	8.82 ± 0.11[e]	0.70 ± 0.04[a]	0.88 ± 0.03[a]	0.79 ± 0.02[a]
R.ICC14782[a)]	64.00 ± 0.75[cd]	66.69 ± 0.17[c]	73.34 ± 0.98[b]	9.34 ± 0.23[d]	9.55 ± 0.02[bcd]	0.59 ± 0.03[a]	0.92 ± 0.01[a]	0.64 ± 0.01[b]
R.ICC13124[a)]	61.93 ± 0.93[d]	66.47 ± 0.16[c]	77.05 ± 0.64[a]	15.12 ± 0.28[a]	9.91 ± 0.00[bc]	0.67 ± 0.04[a]	0.90 ± 0.01[a]	0.74 + 0.02[ab]
R.ICC5383[a)]	67.75 ± 0.69[ab]	71.55 ± 1.35[a]	76.75 ± 0.09[a]	9.00 ± 0.59[d]	9.98 ± 0.12[b]	0.59 ± 0.02[a]	0.88 ± 0.01[a]	0.67 ± 0.04[ab]

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔT, gelatinization range ($T_c - T_o$); ΔH, enthalpy of gelatinization; BS, cultivar Blanco Sinaloa 92; B, black color; Br, brown color; C, cream color; G, green color; R, red color.
Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p < 0.05$).
a) Desi seeds.
b) Kabuli seeds.

3.7 Thermal properties of chickpea starches

In this study, the onset temperature (T_o) in chickpea starches varied from 61.93 to 68.94°C (R.ICC13124-G.ICC5613) whereas the peak (T_p) and conclusion temperatures (T_c) varied from 66.4 to 72.57°C (R.ICC13124 and G.ICC5613) and 73.34 to 78.5°C (R.ICC14782 to B.ICC4418), respectively (Table 5). These parameters had a positive correlation with pasting temperature ($r = 0.779$, $p = 0.007$) and negative with amylose content ($r = -0.697$, $p = 0.02$). In legumes, the differences in gelatinization temperature may be attributed to dissimilarities in the amylopectin double helix arrangement, and to the size and complexing of amylose molecules within the starch granules, that together influence the internal crystallinity [6, 16, 24]. The DSC gelatinization range in chickpea starches varied from 8.73 to 15.12°C for C. BS and R.ICC13124, respectively. A broader range is indicative of heterogeneous crystallites with varying stability within the crystalline domains of the starch granule [16]. The gelatinization enthalpies ranged from 8.82 to 10.68 J/g, which agree within values previously reported [23, 29]. Other authors have related the relative low enthalpy values of pulses with the molecular conformation and arrangement of their amylopectin structural arrangement, in particular with the double helices and to the presence of long and partially branched amylose molecules, that tends to decrease granular swelling and crystalline melting during gelatinization [19, 22, 28].

3.8 ATR-FTIR analysis of chickpea starches

It is known that the IR absorbance bands at 1047 cm^{-1} and 1022 cm^{-1} are sensitive to crystalline/ordered and amorphous structures within the starch granules [27]. The crystalline and amorphous zones ranged from 0.54 to 0.70 and 0.84 to 0.90, respectively, and statistical differences were not detected among the array of chickpea starches (Table 5). Other studies have calculated the ratio of 1047 cm^{-1}:1022 cm^{-1} bands, that are related with the amount of crystalline to amorphous (C/A) domains in starch [27]. The ratio of C/A domains in chickpea starches ranged from 0.60 to 0.79 for C.BS and G.ICC5613, respectively. Similar ratios have been reported previously in chickpea starches, which commonly are correlated with high amylose contents [24, 27].

3.9 In vitro starch digestibility and predicted glycemic index of chickpea starches

The rapidly digestible (RDS), slowly digestible (SDS) and resistant (RS) fractions of raw and gelatinized chickpea starches are reported in Table 6. The RDS fraction in raw starch ranged from 17.26 to 29.90% for the R.ICC14782 and R.ICC13124 starches, respectively. On the other hand, the

Table 6. Starch digestion fractions of raw and gelatinized starches from pigmented chickpea varieties

	Raw starch					Gelatinized starch				
	Digestion fractions					Digestion fractions				
Cultivar	RDS (%)	SDS (%)	RS (%)	HI	PGI	RDS (%)	SDS (%)	RS (%)	HI	PGI
B.ICC6306[b]	20.51 ± 0.07^{c}	29.56 ± 0.25^{a}	49.93 ± 0.15^{b}	47.77 ± 0.24^{a}	65.93 ± 0.05^{ab}	59.15 ± 0.50^{a}	34.43 ± 0.32^{ab}	6.42 ± 0.44^{c}	64.21 ± 0.18^{abc}	74.96 ± 0.09^{bc}
B.ICC4418[a]	18.56 ± 0.16^{de}	31.26 ± 0.24^{c}	50.18 ± 0.57^{b}	48.08 ± 0.44^{a}	66.10 ± 0.03^{a}	56.92 ± 0.57^{bc}	35.06 ± 0.33^{ab}	8.02 ± 0.36^{abc}	62.11 ± 0.32^{d}	73.80 ± 0.18^{e}
B.ICC3761[a]	18.99 ± 0.08^{d}	30.00 ± 0.15^{a}	51.11 ± 0.19^{ab}	47.77 ± 0.20^{a}	65.93 ± 0.12^{ab}	56.78 ± 0.38^{bc}	34.86 ± 0.44^{ab}	8.36 ± 0.47^{ab}	64.26 ± 0.44^{abc}	74.98 ± 0.08^{bc}
Br.ICC3512[a]	18.57 ± 0.26^{de}	34.14 ± 0.08^{b}	47.29 ± 0.31^{c}	47.46 ± 0.20^{a}	65.76 ± 0.04^{abc}	58.12 ± 0.39^{abc}	35.43 ± 0.46^{a}	6.45 ± 0.13^{c}	63.17 ± 0.30^{cd}	74.39 ± 0.07^{d}
C.BS[b]	17.60 ± 0.43^{ef}	31.14 ± 0.12^{cd}	51.26 ± 0.38^{ab}	47.26 ± 0.15^{a}	65.65 ± 0.21^{abc}	56.86 ± 0.44^{bc}	34.73 ± 0.49^{ab}	8.41 ± 0.29^{ab}	63.42 ± 0.31^{cd}	74.52 ± 0.18^{cd}
C.ICC3421[a]	21.47 ± 0.19^{bc}	33.26 ± 0.18^{b}	45.27 ± 0.10^{d}	47.02 ± 0.10^{a}	65.52 ± 0.11^{bc}	59.28 ± 0.47^{a}	33.46 ± 0.47^{ab}	7.26 ± 0.40^{bc}	65.63 ± 0.18^{a}	75.74 ± 0.09^{a}
G.ICC5613[a]	22.20 ± 0.32^{b}	31.16 ± 0.05^{c}	46.64 ± 0.19^{cd}	47.99 ± 0.24^{a}	66.05 ± 0.07^{a}	58.67 ± 0.33^{ab}	33.22 ± 0.39^{b}	8.11 ± 0.36^{abc}	64.99 ± 0.39^{ab}	75.38 ± 0.05^{ab}
R.ICC14782[a]	17.26 ± 0.13^{f}	30.11 ± 0.35^{de}	52.56 ± 0.06^{a}	46.66 ± 0.38^{a}	65.32 ± 0.05^{c}	56.34 ± 0.06^{c}	34.55 ± 0.49^{ab}	9.11 ± 0.32^{a}	65.35 ± 0.21^{a}	75.58 ± 0.11^{a}
R.ICC13124[a]	29.90 ± 0.08^{a}	27.42 ± 0.24^{f}	42.68 ± 0.32^{e}	47.97 ± 0.18^{a}	66.04 ± 0.12^{a}	58.00 ± 0.45^{abc}	34.77 ± 0.25^{ab}	7.23 ± 0.38^{bc}	63.63 ± 0.14^{bc}	74.64 ± 0.04^{cd}
R.ICC5383[a]	18.48 ± 0.38^{def}	36.26 ± 0.20^{a}	45.26 ± 0.20^{d}	46.63 ± 0.55^{a}	65.30 ± 0.08^{c}	58.00 ± 0.44^{abc}	35.35 ± 0.38^{a}	9.22 ± 0.33^{a}	65.32 ± 0.25^{a}	75.57 ± 0.09^{a}

RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; HI, hydrolysis index (estimated from a 100% digestible starch from White bread); pGI, predicted glycemic index (pGI = 39.71 + 0.549HI); BS, cultivar Blanco Sinaloa 92; B, black color; Br, brown color; C, cream color; G, green color; R, red color.
Values are means ± SEM. Values with different letter(s) in every column are significantly different ($p < 0.05$).
a) Desi seeds.
b) Kabulli seeds.

SDS, which is considered the most desirable form of dietary starch because it is not completely degraded in the small intestine and, therefore, releases glucose at a slower rate [28], ranged from 27.42 to 36.26% for the R.ICC13124 and R.ICCC5383 starches, respectively. Moreover, the RS fraction was highest in R.ICC14782 (52.56%) and lowest in the R.ICC13124 (42.68%). These results are comparable with other studies that showed a relative high amount of RS in raw legume starches that could promote health benefits [22, 26]. However, the consumption of these pulses, as well as the vast majority of starchy foods is in cooked form, due to thermal processes greatly increase the amount of available starch molecules, and contribute to a larger caloric output when consumed [7, 20]. In cooked starches, we found RDS contents up to 59.15%, which is related with the changes in molecule availability due to gelatinization of starch granules, nevertheless, the array of cooked starches showed SDS and RS contents from 33.22 to 35.35% and 6.42 to 9.22%, respectively. Our results are similar with other studies that showed a relative high amount of RS in cooked legume starches, that after their digestion could promote health benefits especially in terms of glycemic index and activation of microbiota due to their prebiotic effects [27, 30]. The RS fraction in cooked chickpea starches was highly positive correlated with amylose content ($r = 0.8103$, $p = 0.0045$), swelling power ($r = 0.7313$, $p = 0.0162$) and RVA viscosities (final, $r = 0.7432$, $p = 0.138$; setback, $r = 0.8421$, $p = 0.0022$). Previous research have related the low digestion rates of legume starches to differences on their granular structures as well of their molecular conformations, in which the amylose/amylopectin ratio, degree of crystallinity and type of crystalline polymorphs have been related with these characteristics [6, 22, 23].

Moreover, the HI and pGI were estimated in order to obtain more information about the digestion performance of chickpea starches. For raw starches, HI ranged from 46.63 (R.ICC5383) to 48.08 (B.ICC4418), while pGI from 65.30 (R. ICC5383) to 66.10 (B.ICC4418). Thus, these starches can be classified as medium glycemic impact [31]. Additionally, negative correlations between pGI in raw starch samples with amylose contents ($r = -0.6860$, $p = 0.0282$), and final viscosities ($r = -0.6418$, $p = 0.045$) were found. These significant correlations are attributed to the amylose molecular interactions within the starch granules [8, 28]. Interestingly, when starches were cooked they increase their HI from 62.11 to 65.63, which resulted in higher pGI, 73.80 (B.ICC4418) to 75.74 (C.ICC3421).

3.10 Principal component analysis (PCA)

The PCA plots provide an overview of the similarities and differences between chickpea starches, as well as the interrelationships among the measured properties. The distance between the positions of any two cultivars on the score plot (Fig. 2A) is directly proportional to the degree of difference or similarity between them. Regarding the seed-coat coloration, no clear tendency was observed among the studied samples. The first and second components (PC1 and PC2) accounted for an accumulative variance of 82.64%. In the same sense, the loading plot (Fig. 2B) of PC's provide

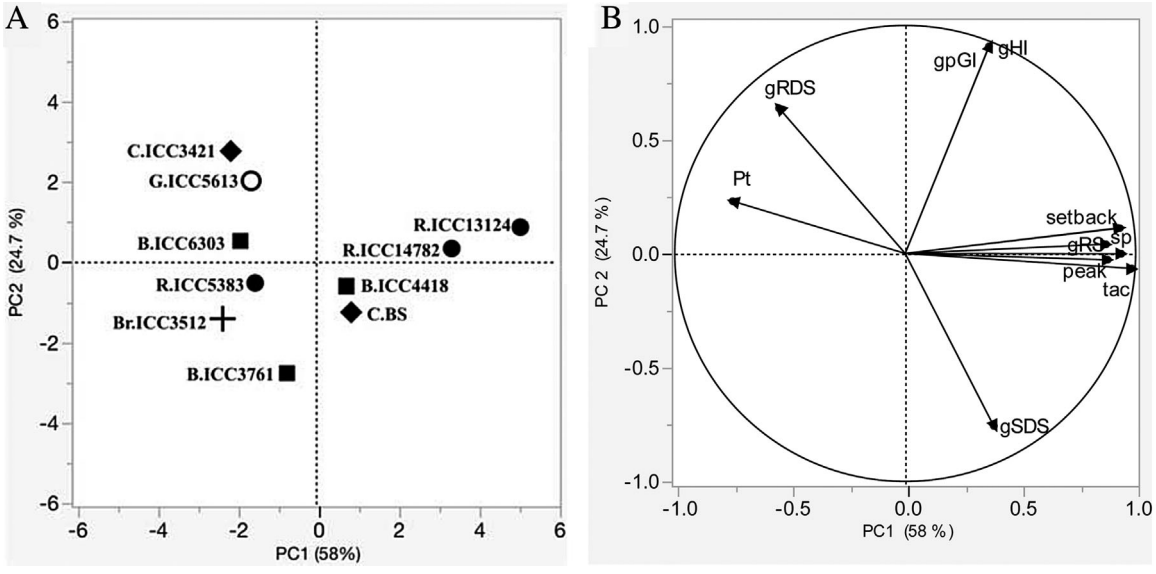


Figure 2. Principal component analysis: score plot (A) describing overall variation in the first (PC1) and second component (PC2) in chickpea starches and loading plot (B) of PC1 and PC2 describing variation among properties of chickpea starches. Pt, pasting temperature; tac, total amylose content; sp, swelling power; peak, final and setback, RVA viscosities; gRDS, rapidly digestible starch; gSDS, slowly digestible starch; gRS, resistant starch; gHI, Hydrolysis index; gpGI, predicted glycemic index in gelatinized starches; BS, cultivar Blanco Sinaloa 92; B, black color; Br, brown color; C, cream color; G, green color; R, red color.

information about the correlations between some viscous and digestion properties, in this figure, the properties (represented by lines) that lie close to each other on the plot are positively correlated; whereas, those with lines going in opposite directions are negatively correlated. The principal contributors in PC1 variation were the parameters related with granule swelling and viscosity that were closely related to amylose contents and the RS fractions in gelatinized starches. Such relationships have been previously reported and are distinctive characteristics of pulse starches [8, 24].

4 Conclusions

Isolated chickpea starches from different chickpea seed cultivars were obtained with relative high purity and light color. Some of their physicochemical and functional characteristics related with water uptake were correlated with amylose contents as well as to thermal and pasting characteristics. The refined chickpea starches showed relatively low RDS and high levels of SDS and RS in both raw and cooked, which promoted moderated pGI. Therefore, these starches have potential as functional ingredients for development of new foods. Further studies related with the amylopectin fine structure are undergoing in order to understand at a deeper level the interactions promoted by the molecular characteristics of these starches.

This research was supported by NutriOmics group funds from Tecnológico de Monterrey and Consejo Nacional de Ciencia y Tecnología (CONACYT). A.K. Milán-Noris acknowledges CONACYT for the scholarship for PhD studies (233598).

Conflicts of interest: All authors of this research paper declare no conflict of interest.

5 References

[1] FAO, FAOSTAT, Chickpea world production in 2014. http://faostat3.fao.org/home/E; accessed: April 21, 2016.

[2] Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., Chibbar, R. N., Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): A review. *Br. J. Nutr*. 2012, *108*, S11–S26.

[3] Kadam, S. S., D. S. y. J. N., *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization*. CRC Press, Inc, Boca Raton, FL, USA 1989.

[4] Smýkal, P., Vernoud, V., Blair, M. W., Soukup, A., Thompson, R. D., The role of the testa during development and in establishment of dormancy of the legume seed. *Front. Plant Sci*. 2014, *5*.

[5] Wood, J. A., Knights, E. J., Choct, M., Morphology of chickpea seeds (*Cicer arietinum* L.): Comparison of desi and kabuli types. *Int. J. Plant Sci*. 2011, *172*, 632–643.

[6] Xu, Y., Sismour, E. N., Narina, S. S., Dean, D., et al., Composition and properties of starches from Virginia-grown kabuli chickpea (*Cicer arietinum* L.) cultivars. *Int. J. Food Sci. Technol*. 2013, *48*, 539–547.

[7] Granfeldt, Y., Bjorck, I., Drews, A., Tovar, J., An in vitro procedure based on chewing to predict metabolic response to starch in cereal and legume products. *Eur. J. Clin. Nutr*. 1992, *46*, 649–660.

[8] Hoover, R., Hughes, T., Chung, H. J., Liu, Q., Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Res. Int*. 2010, *43*, 399–413.

[9] Heiras-Palazuelos, M. J., Ochoa-Lugo, M. I., Gutiérrez-Dorado, R., López-Valenzuela, J. A., et al., Technological properties, antioxidant activity and total phenolic and flavonoid content of pigmented chickpea (*Cicer arietinum* L.) cultivars. *Int. J. Food Sci. Nutr*. 2013, *64*, 69–76.

[10] Segev, A., Badani, H., Kapulnik, Y., Shomer, I., et al., Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (*Cicer arietinum* L.). *J. Food Sci*. 2010, *75*, S115–S119.

[11] Agama-Acevedo, E., de la Rosa, A. P. B., Méndez-Montealvo, G., Bello-Pérez, L. A., Physicochemical and biochemical characterization of starch granules isolated of pigmented maize hybrids. *Starch/Stärke* 2008, *60*, 433–441.

[12] AACC International, *Approved Methods of Analysis, 11th Ed. Method 55-10.01. Test Weight per Bushel*. American Association of Cereal Chemists International, St. Paul, MN, USA.

[13] Pérez-Carrillo, E., Serna-Saldívar, S. O., Cell wall degrading enzymes and proteases improve starch yields of sorghum and maize. *Starch/Stärke* 2006, *58*, 338–344.

[14] Uriarte-Aceves, P. M., Cuevas-Rodríguez, E. O., Gutiérrez-Dorado, R., Mora-Rochín, S., et al., Physical, compositional, and wet-milling characteristics of Mexican blue maize (*Zea mays* L.) landrace. *Cereal Chem. J*. 2015, *92*, 491–496.

[15] AOAC, *Official Methods of Analysis, 16th edn*, Association of Official Analytical Chemists, Washington, DC 1990.

[16] Tester, R. F., Morrison, W. R., Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids. *Cereal Chem*. 1990, *67*, 551–559.

[17] Schoch, T. J., in Whistler, R. L. (Ed.), *Methods in Carbohydrate Chemistry*, Wiley, New York 1964, pp. 106–108.

[18] Bryant, C. M., Hamaker B. R., Effect of lime on gelatinization of corn flour and starch. *Cereal Chem*. 1997, *74*, 171–175.

[19] Sevenou, O., Hill, S. E., Farhat, I. A., Mitchell, J. R., Organisation of the external region of the starch granule as determined by infrared spectroscopy. *Int. J. Biol. Macromol*. 2002, *31*, 79–85.

[20] Englyst, H. N., Kingman, S. M., Cummings, J. H., Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr*. 1992, *46*, S33–S50.

[21] Goñi, I., Garcia-Alonso, A., Saura-Calixto, F., A starch hydrolysis procedure to estimate glycemic index. *Nutr. Res*. 1997, *17*, 427–437.

[22] Huang, J., Schols, H. A., van Soest, J. J. G., Jin, Z., et al., Physicochemical properties and amylopectin chain profiles of cowpea, chickpea and yellow pea starches. *Food Chem*. 2007, *101*, 1338–1345.

[23] Singh, N., Singh Sandhu, K., Kaur, M., Characterization of starches separated from Indian chickpea (*Cicer arietinum* L.) cultivars. *J. Food Eng*. 2004, *63*, 441–449.

[24] Chung, H.-J., Liu, Q., Donner, E., Hoover, R., et al., Composition, molecular structure, properties, and in vitro digestibility of starches from newly released canadian pulse cultivars. *Cereal Chem. J*. 2008, *85*, 471–479.

[25] Hoover, R., Ratnayake, W. S., Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. *Food Chem*. 2002, *78*, 489–498.

[26] Miao, M., Zhang, T., Jiang, B., Characterisations of kabuli and desi chickpea starches cultivated in China. *Food Chem*. 2009, *113*, 1025–1032.

[27] Chung, H.-J., Liu, Q., Hoover, R., Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. *Carbohydr. Polym*. 2009, *75*, 436–447.

[28] Sandhu, K. S., Lim, S.-T., Digestibility of legume starches as influenced by their physical and structural properties. *Carbohydr. Polym*. 2008, *71*, 245–252.

[29] Edwards, C. H., Warren, F. J., Campbell, G. M., Gaisford, S., et al., A study of starch gelatinisation behaviour in hydrothermally-processed plant food tissues and implications for in vitro digestibility. *Food Funct*. 2015, *6*, 3634–3641.

[30] Zhang, H., Yin, L., Zheng, Y., Shen, J., Rheological, textural, and enzymatic hydrolysis properties of chickpea starch from a Chinese cultivar. *Food Hydrocolloids* 2016, *54*, 23–29.

[31] Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., et al., Glycemic index of foods: A physiological basis for carbohydrate exchange. *Am. J. Clin. Nutr*. 1981, *34*, 362–366.

This document was typed in using Microsoft Word by Milán-Noris, A.K.