Title: Chickpea (*Cicer arietinum* L.) protein as a prospective plant-based ingredient: a review

Running title: Chickpea protein as a food ingredient

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Abstract:

Chickpea (*Cicer arietinum* L.) is one of the most grown and consumed pulses and they are traditionally commercialized as seeds, flour, or canned foods. In the frame of alternative protein sources, chickpea emerged as a rich source of dietary proteins (17–22%) that can be dry or wet extracted. The application of chickpea proteins as food ingredients is still in early stages, where their properties and how they interact within food matrices is scarcely studied. Therefore, this review provides recent advances in processing, characteristics and applications of chickpea proteins. Nutritionally, these proteins have various biological activities, adequate levels of essential amino acids and protein digestibility. Technologically, their bland flavor, neutral taste, and light color make them suitable ingredients for new products development including noodles, breads, cookies, and sausages. Chickpea proteins and particularly hydrolysates are a promising alternative to be used more broadly as functional ingredients.

Keywords: chickpea protein, functional properties, bioactive peptides, allergy, food, encapsulation

1. Introduction

The realm of alternative proteins keeps expanding in response to the high demand for proteins that is expected to double by 2050 to cover the needs of the world population growth expected to reach 10 billion by 2050 (United Nations, 2019). Plant-based proteins are viewed as a more sustainable and healthy proteins than those deriving from animals. Moreover, the trend of flexitarianism, vegetarianism and veganism has been rising at a rapid pace due to growing awareness about environmental conservation, concerns related to animal welfare and high demand of meat alternative products (Research and Markets, 2018). Associating meat consumption with health concerns due to the use of antibiotics and hormones in livestock feed also consolidated the position of plant proteins in the market particularly those deriving from legumes and pulses (Boukid, 2020; Sofi *et al.*, 2020b). Proteins from legumes have been gaining traction as a necessity more than a choice due to their high nutritional benefits, hypo-allergenicity, gluten-free and non-genetically modified organism labels, affordability, high productivity and versatility (Nosworthy and House, 2017; Boukid *et al.*, 2019). Proteins from legumes also exhibit a wide range of techno- and bio-functional applications comparable with proteins from animal and dairy sources providing a multitude of health benefits (Sharif *et al.*, 2018; Boukid *et al.*, 2019; Glusac *et al.*, 2020). The industry of proteins from legumes keep expanding beyond pea to lupine, mung, faba and chickpea proteins (Research and Markets, 2018; Boukid *et al.*, 2019).

Chickpea (*Cicer arietinum* L.) is the third most abundantly grown pulse with global production of 15 million tons after dry beans (27 million tons) and dry peas (16 million tons) (FAO, 2020). Chickpea is the main legume crop in the diet of consumers from different parts of the world, mostly in the African and Asian countries (Sofi *et al.*, 2020b). The main chickpea seeds are Kabuli and desi varieties with wide differences in chemical composition, color, size and geographic distribution (Boukid *et al.*, 2019). Chickpeas contain from 18 to 29% protein, 4to 7% lipids and 50to 60% starch (Espinosa-Ramírez and Serna-Saldívar, 2019; Sofi *et al.*, 2020b). However, chickpea contains antinutritional factors yet pretreatments strategies as well as protein extraction ensure the removal or the reduction of these components. Chickpea protein has advantages of high production volumes, low cost, excellent balance in composition of essential amino acid, high bioavailability, and low allergenicity compared to soybeans (Wang *et al.*, 2018, 2020; Xing *et al.*, 2020). Several studies have reported biological activities in chickpea proteins including antioxidant activity, antifungal activity, antigenic activity and metal-chelating ability (Kou *et al.*, 2013; Ghribi *et al.*, 2015c). Chickpea protein is, therefore, a promising health-beneficial ingredient for new products development (Glusac *et al.*, 2020; Xing *et al.*, 2020).

Until now, the focus on chickpea ingredients was mainly attributed to chickpea flour (Cunha *et al.*, 2019; Guardado-Félix *et al.*, 2020; Xing *et al.*, 2020). In the light of the shift toward more plant proteins consumption, chickpea proteins are increasingly gaining traction as a functional, clean label, sustainable and healthy ingredient for food formulation. The addition of chickpea protein was designed to increase the content of protein and to improve both functional and sensory properties of the reformulated product (Shaabani *et al.*, 2018; Wang *et al.*, 2018; El-Sohaimy *et al.*, 2020). The successful use of chickpea protein is closely related to its physicochemical, functional, thermal, and structural properties and its interactions with the different components of the matrices (Ghribi *et al.*, 2015b; Sofi *et al.*, 2020b). Thus, the scope of this review is to provide insights into the recent advances in technologies applied for the extraction and treatments of chickpea protein as well as to address the challenges and opportunities for its applications in food development.

2. Market dynamics of chickpea proteins

The global chickpea protein ingredients market has been witnessing a sharp growth over the last few years and is projected to reach $737.8 Million by 2025 at a compound annual growth rate (CGAR) of 11.2% (Market Research Future, 2019). The drivers are the general transition of market from animal products toward plant proteins as a clean and sustainable protein source due animal welfare, human health, and environmental concerns or increasing incidences towards lactose intolerance and soy allergy. However, the key challenges toward the expansion of chickpea protein market is related to the environmental limitations leading to a rise in prices and high dependence on the imports of chickpea (Market Research Future, 2019). Chickpeas grow at the end of the rainy season, and grow on the residual soil moisture (Upadhyaya *et al.*, 2012). Most of the global production is focused on Asia-Pacific (mainly India), the Middle East, and some parts of Africa, while it is limited to Russia and US in Europe and North America (Reports Insights, 2020). In turns, Europe and North America had the highest demand and consumption of chickpea protein ingredients, which creates a wide gap between demand and supply chain (Market Research Future, 2019).

Based on type, chickpea proteins are available as concentrates, isolates, and flour, where the isolates hold the largest share (50% in 2018) as illustrated in Table 1. By category, the global chickpea protein ingredients market has been bifurcated into organic and conventional, where the conventional segment accounted for a larger market share in 2018 and estimated to reach $500.87 million by 2025 (Market Research Future, 2019). In terms of form, the solid segment dominated the market in 2018 with a value of $415 thousand and forecasted to reach the value of $535 thousand by 2023, registering a CAGR of 5.3% during the forecasting period between 2018 until 2023 (Research and Markets, 2018). Based on application, the market has been divided into food & beverages, animal feed, and others. The food & beverages segment is further

divided into dairy products, bakery and confectionery, beverages, dietary supplements, sweet and savory snacks, infant nutrition, and others. Meat substitute, dairy alternatives, processed food, and bakery industry captures the highest share of application of chickpea protein due to growing concern about the lactose intolerance and gluten sensitivity. The animal feed segment is expected to register the highest CAGR of 11.9% during the forecast period of 2019 to 2025) (Market Research Future, 2019). The global chickpea protein ingredients market has been dominated by North America and it is projected to grow by $131.92 million from 2018 to 2025. Increasing consumer preference for organic protein products and high incidences of lactose intolerance are expected to create lucrative opportunities for the vendors active in the global market (Market Research Future, 2019). The prominent players in the global chickpea protein ingredients market include Archer Daniels Midland Company (US), Nutriati, Inc. (US), Batory Foods (US), InnovoPro Ltd (Israel), Cambridge Commodities Limited (UK), AGT Food and Ingredients Inc. (Canada), Ingredion Incorporated (US), Chickplease (US), and Nutraonly (Xi'an) Nutritions Inc. (China) (Market Research Future, 2019).

Table 1

3. Processing technologies for chickpea proteins extraction

3.1. Pre-treatment

Prior to protein extraction, chickpea seeds can be subjected to soaking, splitting, dehulling, milling, defatting or/ and germination. Soaking, using hot or cold water, enable the softening of the outer layers of the seeds thereby facilitating the wet dehulling. Dry dehulling of chickpea seeds is commonly performed by air separation of the hulls from split seeds (Boye *et al.*, 2010b). Both soaking and dehulling enhance protein extraction and reduce anti-nutritional factors (Boukid *et al.*, 2019). Unlike other pulses (*e.g.* peas, lentils and beans) having a fat content up to 3%, chickpea contains a relatively high amounts of fat (4 to 7%) that affect the production of proteins (Espinosa-Ramírez and Serna-Saldívar, 2019). Defatting can be carried out using hexane and results in improving the yield and purity of proteins (Wang *et al.*, 2020). High-power sonication was also reported an efficient pretreatment to favor defatting and consequently the yield of protein isolates increase without changing peptide profile (Byanju *et al.*, 2020). For germination, chickpea seeds were cleaned, soaked in saline aqueous (at 25°C for 12 h) and then germinated (at 30 °C for 48 h), dried and milled (Serrano-Sandoval *et al.*, 2019; Sofi *et al.*, 2020a). Germination enhanced the nutritional, functional and antioxidant properties of proteins (Sofi *et al.*, 2020a).

3.2. Extraction

The most applied technique to obtain protein isolates is the alkaline-extraction or salt extraction followed by isoelectric precipitation or, ultrafiltration and ultrafiltration/diafiltration. Briefly, defatted chickpea flour is solubilized in alkaline solution (pH of 8.5-9), centrifuged and the supernatant is filtered and precipitated under acid conditions (pH of 4.5) (Papalamprou et al., 2010; Ghribi et al., 2015a). After centrifugation (11,200 g for 10 min), the recovered protein was neutralized, washed, dried until moisture content reached 3 g/100 g, and milled to produce protein isolates (~88 g/100 g proteins) (Espinosa-Ramírez and Serna-Saldívar, 2019; Sofi *et al.*, 2020a). As an alternative to isoelectric precipitation, ultrafiltration (using a 50 kDa hollowfiber membrane module) can be used to recover isolates or to further fractionate total protein into glutelin, albumin and globulin fractions through different membranes (Serrano-Sandoval *et al.*, 2019). Salt extraction also can be applied to extract isolates, where the key steps are solubilization of defatted chickpea flour in salt (e.g., potassium sulphate and sodium chloride) solution, centrifugation, dialysis and ultrafiltration (Karaca *et al.*, 2011; Hadnađev *et al.*, 2018). Finally, different drying technologies can be applied such as spray drying and freeze drying (Tontul *et al.*, 2018).

Wet milling is a hybrid process designed to produce as mainstreams starch and oil, while protein and fiber fractions are side streams. The first stage consists of soaking chickpea seeds with sulfur dioxide to increase the rate of water diffusion in the seeds (Espinosa-Ramírez and Serna-Saldívar, 2019). After soaking (50 °C for 48 h), seeds were milled, and the obtained slurry was filtered through different sieves to remove the fraction rich in fiber. The mixture of starch granules and protein was delivered in an inclined stainless-steel separation table resulting in starch settlement and protein draining due to differences in sedimentation rate. The protein rich suspension was centrifuged, neutralized, dried and defatted (Espinosa-Ramírez and Serna-Saldívar, 2019). Compared to isoelectric precipitation method, wet-milled protein isolates had higher protein recovery, protein purity, lower fat content and higher fat absorption capacity. The color of the wet-extracted isolate powder was also lighter compared to the isoelectric-extracted counterpart due to the final defatting process leading to the removal of colored fat-soluble compounds (Ghribi *et al.*, 2015a; Espinosa-Ramírez and Serna-Saldívar, 2019). The method seems suitable to obtain high-value protein, but more works are need for the up-scaling of this process and modulating the impact of the process on the functional properties of proteins particularly water holding capacity and foaming stability (Table 2).

Dry milling followed by air classification of chickpeas yielded 31% protein and 51% starch-enriched concentrates (Pelgrom *et al.*, 2015) which are comparable to a recent study (28.4% of protein- and 47.7% of starch-enriched fractions) (Xing *et al.*, 2020). The purity of protein-enriched fraction (45.3 g/100 g) was found higher than chickpea flour (21.6 g/100 g) (Pelgrom *et al.*, 2015). This method is considered a

sustainable route to prepare concentrates since the use of water and energy is minimized (Xing *et al.*, 2020), and enable the preservation of the native functional properties of proteins due to the absence of additives and extensive processing (Schutyser *et al.*, 2015). Compared to wet-milling, dry milling requires less energy and water, but protein recovery is lower (11-31%) and produces more damaged starch that can negatively impact the quality of the derived products (Espinosa-Ramírez and Serna-Saldívar, 2019). Anti-nutritional factors (e.g. phytic acid, tannins, trypsin inhibitors and raffinose) are not removed unlike wet protein extraction and thus remained in the dry-enriched fractions (Hall *et al.*, 2017; Sozer *et al.*, 2017; Xing *et al.*, 2020).

Table 2

3.3. Post-treatment

To boost the nutritional and functional attributes, chickpea protein isolate can be subjected to several physical, biochemical and physical post-treatments. A treatment with alcalase results in the improvement of protein solubility especially at pH near to the isoelectric point compared to that untreated. A small degree of hydrolysis (4%) enhanced emulsification activity and stability (Ghribi *et al.*, 2015b). The right degree of hydrolysis still requires more investigations for tailoring the functional properties of chickpea proteins. Solid state fermentation of chickpea proteins was found efficient to reduce the anti-nutritional factors (α-galactosides and phytic acid) up to 88.3–99.1%, and to increase water holding capacity and decrease foaming capacity (Xing *et al.*, 2020). Germination also increased solubility through an endogenous enzymatic activity during germination with exposed protein molecules to surface which in turn enhanced emulsifying capacity (Sofi *et al.*, 2020b). Compared to native proteins, proteins isolated from germinated chickpea seeds had higher water holding capacity probably due to the increase in soluble proteins during germination and higher oil holding capacity due to non-polar amino acids groups exposed to protein chain (Sofi *et al.*, 2020b). Enzymatic crosslinking was also used as a strategy to improve the functionality of chickpea protein. The application of transglutaminase improved both physical stability and rheological properties of protein-stabilized emulsions leading to gelation of the system (Glusac *et al.*, 2020). High ultrasound technology also enabled the increase in solubility (from 7.5 to 9.5 mg/mL), foaming capacity (62 from to 136.7%), emulsifying index (from 22.3 to 24.17 m^2/g) water holding capacity and breaking force of the heat induced chickpea protein isolates gel (from 58.4 to 80.9%) (Wang *et al.*, 2020). These changes can be attributed to increasing free sulfhydryl content, surface hydrophobicity, surface potential and decreasing particle size of chickpea proteins as function of ultrasonic time (Wang *et al.*, 2020). These results demonstrate the relationship between the structure and functional properties, and thus further studies to decipher these association might promote its application in the food industry (Wang *et al.*, 2020).

4. Characteristics of chickpea proteins

4.1. Structure and composition

Chickpea proteins contain globulins (~56 g/100 g), albumins (~12 g/100 g), glutelins (~18 g/100 g), prolamin (~3 g/100 g), and residual proteins. Among pulses, chickpea proteins have higher glutelin content (Chang *et al.*, 2011). Globulins are the main storage proteins of chickpea and they are composed of two major groups the 11S legumin (320–400 kDa) and the 7S vicilin (145–190 kDa) proteins (Yust *et al.*, 2003). Legumins are oligomeric proteins made up of six αβ subunits (54–60 kDa), where α and β chain are linked by disulfide bonds (Yust *et al.*, 2003). Vicilins are trimeric proteins that lack cysteines and thus disulfide bonds (Chang *et al.*, 2012). The albumin fraction plays an essential role in seeds because they include most of the enzymatic and metabolic proteins (Singh *et al.*, 2008). Albumins are a rich source of essential amino acids like other legume proteins particularly sulfur containing amino acids (tryptophan, threonine, and lysine), and therefore have a higher nutritive value compared to globulins (Liu *et al.*, 2008). Glutelins belong to the 11–12S globulin family; structurally glutelin is similar to globulin (Chang *et al.*, 2011). Prolamin was found in traces regardless of the variety of chickpea (Singh *et al.*, 2008).

Chickpea protein was rich in essential amino acids such as isoleucine, lysine, total aromatic amino acids and tryptophan (Alajaji and El-Adawy, 2006). Leucine (8.7% of protein) was found in highest concentration, followed by arginine (8.3% of protein) and lysine (7.2% of protein) (Iqbal *et al.*, 2006). Therefore, total aromatic amino acid content was found higher than the requirement of FAO/WHO for preschool children (8 vs 6 g/100g) (WHO/FAO/UNU, 2007). However, leucine, total sulfur amino acids, methionine, cystine, threonine and valine were first limiting amino acids (Alajaji and El-Adawy, 2006). PDCAAS (protein digestibility-corrected amino acid score) of chickpea isolates (92%) and concentrates (PDCAAS = 0.76) was higher than pea proteins (PDCAAS = 0.73) and common beans (0.63–0.68) but slightly lower that soy protein isolates as well casein (PDCAAS = 1.0) (Tavano *et al.*, 2016; Nosworthy *et al.*, 2017; Espinosa-Ramírez and Serna-Saldívar, 2019). This confirms the high nutritional value of chickpea proteins and suggest its readiness to compete with the most marketed ones, namely soy and pea proteins.

4.2. Tech-functionality

The solubility of chickpea protein isolates was found to be the lowest (2- 30%) around the isoelectric point (pH 4-6) and reached its maximum (up to 90%) at pH ranging from 1 to 3 and 8 to 12 (Boye *et al.*, 2010b; Shevkani *et al.*, 2015). The high solubility of protein isolates at alkaline and acidic pH might be due to their

lower protein denaturation (Tontul *et al.*, 2018; Sofi *et al.*, 2020b). At neutral pH (pH 7), chickpea proteins had low solubility (around 60%) unlike yellow pea and red lentil proteins (Boye *et al.*, 2010a). Water holding capacity of chickpea protein isolates was in the range of critical values (1.49-4.71g water/g protein isolate) (Tontul *et al.*, 2018). It was reported that there is no difference among different varieties of chickpea. Nevertheless, chickpea proteins had lower values than green and lentils and yellow pea proteins (Boye *et al.*, 2010b). The oil holding capacity of the chickpea protein isolates was determined to be 3.15-3.65 g oil/g protein isolate slightly high that soy proteins (1.9-2.61 g oil/g protein isolate) (Tontul *et al.*, 2018). It was reported that depending on the process of extraction, oil holding capacity significantly varied, where micellized protein isolate (2 g oil/g protein isolate) had higher value than that isoelectric precipitated protein isolate (1.7 g oil/g protein isolate) (Boye *et al.*, 2010b). Emulsion activity index and emulsion stability index of chickpea protein isolates ranged from 15.86 to 44.13 m^2/g and from 5.28 to518.63 min, respectively, depending on pH. The values were found comparable or better than that of yellow pea and soy proteins (Boye *et al.*, 2010b; Ladjal Ettoumi *et al.*, 2016; Ladjal-Ettoumi *et al.*, 2016; Tontul *et al.*, 2018; Felix *et al.*, 2019). At neutral pH, the emulsifying activity index of chickpea proteins (5.7 m^2/g) was higher than yellow pea (4.6 m^2/g) (Boye *et al.*, 2010b). Foam formation and stability of the chickpea protein isolates were determined as 30- 58% and 5-32%, respectively (Tontul *et al.*, 2018). The high range of variability can be attributed to different processing of extraction, analytical method of determining foaming properties (concentration of the solution, whipping speed, and pH). This suggests the need to standardized method to determine the properties of pulses flours and proteins and not rely on methods tailored for cereals flours. Chickpea protein had intermediate gelling properties since it forms a gel at a concentration of 14% compared to yellow pea and lentil proteins (forming a gel at 8% concentration) (Boye *et al.*, 2010b). As a function of pH, chickpea forms hard but adhesive gels at pH 2 (Tontul *et al.*, 2018).

Considering the impact of the method of extraction, both protein structure (molecular weight, particle size, zeta potential, surface hydrophobicity and free sulfhydryl content) and functional characteristics (solubility, emulsifying, foaming and gel properties) can be affected (Siddique *et al.*, 2016; Malik *et al.*, 2017; Ochoa-Rivas *et al.*, 2017). Further studies on these properties are required considering different varieties and processing (extraction methods and drying temperatures). Such studies will provide insightful information to suitably incorporate these proteins in food formulation (Wang *et al.*, 2020).

4.3. Bio-functionality

Chickpea proteins, hydrolysates and peptides have demonstrated to be a notable source of bioactive peptides with antioxidant, hypolipedimic and hypocholesterolemic activities (del Mar Yust *et al.*, 2012; Torres-Fuentes *et al.*, 2015; Gupta and Bhagyawant, 2019; Shi *et al.*, 2019). Chickpea peptides had important

antioxidant activities based on free radical scavenging activities and metal chelating abilities, anti-inflammatory potentials, anti-proliferative effects, anti-bacterial and angiotensin converting enzyme (ACE) inhibitory activities (Boschin *et al.*, 2014; Ghribi *et al.*, 2015b; Jamdar *et al.*, 2017; Mamilla and Mishra, 2017). Peptide sequences (ALEPDHR, TETWNPNHPEL, FVPH and SAEHGSLH) deriving from legumin showed copper chelating activity and antioxidant properties with the potential to inhibit the copper-mediated lipid peroxidation (Torres-Fuentes *et al.*, 2011, 2012, 2015). Albumin exhibited antioxidant activities where the peptide (RQSHFANAQP) was identified with the highest antioxidant activity (Kou *et al.*, 2013). Recently, a novel antioxidant peptide (NF2-4-1) was identified as natural antioxidant peptides for food and nutraceutical applications (Wali *et al.*, 2020) . Peptides deriving from enzymatic hydrolysis (by pepsin alcalase, flavourzyme or/and pancreatin) drastically inhibited THP-1 and Caco-2 cells proliferation (by 45 and 78%, respectively), suggesting that chickpea-derived peptides might inhibit the growth of tumors in the colon (Girón-Calle *et al.*, 2010; Gupta and Bhagyawant, 2019). The consumption of chickpea protein hydrolysates might confer a protective effect against colon carcinogenesis (Sánchez-Chino *et al.*, 2019). As hypolipemic agents, chickpea peptides were found efficient in decreasing serum total cholesterol, total triglyceride, and low-density lipoprotein cholesterol due the ability of these peptides to inhibit the activities of fatty acid synthetase and 3-hydroxy-3-methyl-glutaryl-CoA reductase and the regulation of peroxisome proliferator-activated receptors and LDL receptor expressions (Shi *et al.*, 2019). Peptides exhibited better hypocholesterolaemic activity when compared with chickpea protein isolate (del Mar Yust *et al.*, 2012). The peptide VFVRN was found to have high hypolipidemic effects (Shi *et al.*, 2019; Zhang *et al.*, 2020). Overall, these studies suggest the important potential of chickpea protein hydrolysates as bioactive ingredients as a promising center of bioactive peptides and unlock new opportunities to develop new nutraceuticals and functional foods (del Mar Yust *et al.*, 2012; Torres-Fuentes *et al.*, 2015; Gupta and Bhagyawant, 2019). The selection of the enzyme and the degree of hydrolysis require more investigation to ensure a stable and high production of bioactive peptides.

4.4. Allergenicity

Chickpea proteins are not included in the WHO/IUIS Allergen Nomenclature database (Wangorsch *et al.*, 2020). Chickpea allergy was mostly reported in specific geographic areas, the Mediterranean area and India, where the consumption of chickpea-based products is high (Cuadrado *et al.*, 2009; Verma *et al.*, 2012; Bar-El Dadon *et al.*, 2014; Wangorsch *et al.*, 2020). In India, the prevalence of chickpea allergy reached 13% (Patil *et al.*, 2001). As for symptomalogy, chickpea can cause IgE-mediated hypersensitivity reactions ranging from rhinitis to anaphylaxis (Patil *et al.*, 2001; Verma *et al.*, 2012). The symptoms after chickpea ingestion were predominantly respiratory (Patil *et al.*, 2001). Among legumes, chickpea allergy is merely studied and in most cases, it is associated with cross reactivity with other pulses mostly with allergy to lentil

(Bar-El Dadon *et al.*, 2014). Recent data also suggested the potential cross-reactivity of chickpea proteins and peanut (Wangorsch *et al.*, 2020). Globulin were found putative allergens (Verma *et al.*, 2013; Bar-El Dadon *et al.*, 2014; Wangorsch *et al.*, 2020). Albumins (2S and Pa2) were also considered to evoke allergic reactions in chickpea-sensitive individuals (Bar-El Dadon *et al.*, 2013; Verma *et al.*, 2016). Seven putative chickpea allergens (Q9SMK8, Q39450, Q9SMJ4, Q304D4, G1K3R9, G1K3S0 and O23758) were identified, where the sequences Q9SMK8, Q39450, Q9SMJ4 and Q304D4 were predicted to have cross-reactivity with the allergens Ara h 8, Gly m 4, Vig r 1 and Bet v 1 (Kulkarni *et al.*, 2013). Noteworthy, it was reported that that thermal processing such as boiling (up to 60 min) and autoclaving (1.2 and 2.6 atm, up to 30 min) can mitigate these epitopes particularly harsh autoclaving (Cuadrado *et al.*, 2009). However, allergenic vicilins, Cic PR-10 and Cic a 1.01, were found in boiled chickpeas (Wangorsch *et al.*, 2020)

5. Applications of chickpea proteins

The use of chickpea proteins as protein supplements or carrier of nutrients in food design has become increasingly attractive in the last five years. Nevertheless, the application of chickpea protein is still in its infancy stages and few applications are reported in literature compared to chickpea flour. Market request for alternative proteins is boosting to widen the application of chickpea proteins thanks to their high functional properties, low flavor profile and relative freedom from toxins and allergens (Singh *et al.*, 2008; Mokni Ghribi *et al.*, 2018). The incorporation of chickpea protein isolate (up to 10%) in gluten free noodles decreased in *vitro* starch digestibility and glycemic index (from 70.8 to 61.0) compared to rice flour-based noodles (Sofi *et al.*, 2020a). This addition enhanced the nutritional properties by increasing protein content (from 7.52 to 19.3%) and antioxidant activity (from 22.6 to 31.3%) of the noodles. Regarding cooking behavior, increased level of addition resulted in increasing cooking time (from 13.4 to 15.1 min) and decreasing cooking loss and color. From a sensory perspective, noodles prepared with 6% chickpea protein isolate showed improved overall acceptability (Sofi *et al.*, 2020a,b). Likewise, substituting durum semolina wheat with chickpea protein isolate (10%) decreased optimum cooking time but increased in cooking losses and hardness and doubled the chewiness (El-Sohaimy *et al.*, 2020). *In vitro* protein digestibility of enriched pasta was improved compared to control (from 91.89 to 95.57%). Furthermore, 10% chickpea fortified pasta recorded high acceptance scores (El-Sohaimy *et al.*, 2020). The fortification of gluten free muffins with chickpea protein isolate (0–7%) decreased the specific volume and porosity and decreased the hardness whilst decreasing browning index of crust by increasing its concentration. It can be concluded that chickpea protein can form a protein network in the gluten-free muffins with the addition of transglutaminase and xanthan, yet more investigation is needed on the formulation to boost the use of proteins and reduce starchy ingredients (Shaabani *et al.*, 2018). In bread, chickpea protein concentrate substitution (2/3 of soy substitution) increased hardness, chewiness and lowered specific loaf volume, while a 1/3 of soy

substitution did not induce negative effects on texture. These differences might be attributed to the reduction of fat content [soy (9 g/100 g w/w fat) vs chickpea (3 g/100 g fat)] particularly polar lipids contributing into the improvement of gluten-starch plasticization thereby increasing softness and specific volume of breads. This substitution (2/3 of soy by chickpea protein) also increased lightness and reduced yellowness due difference in the natural pigmentation of both proteins (Serventi *et al.*, 2013, 2018). It significantly reduced total saponin content (-60%) (Serventi *et al.*, 2013), but increased B-type saponins known as inhibitor to cholesterol micellar solubility (Serventi *et al.*, 2018). Sausages made with 5% chickpea protein concentrates had increased protein content and improved yield, and recorded similar taste score but better texture and global acceptability compared to the control (Mokni Ghribi *et al.*, 2018).

Chickpea proteins have been also used to produce protein microencapsulate for carrying nutrients in food preparations owing to their biocompatibility, non-toxicity and nutritional advantage (Ariyarathna and Nedra Karunaratne, 2015). Chickpea protein was found efficient in improving the stability of folate (vitamin B9) as confirmed by the encapsulation efficiency and loading capacity (62 and 10%, respectively) (Ariyarathna and Nedra Karunaratne, 2015). The complex chickpea protein-high methoxylated pectin improved the physical integrity and stability of emulsion buriti oil droplets, and showed a slight increase of the conjugated dienes content in all microcapsules after 6 months of storage (Moser *et al.*, 2020). This complex was reported efficient in the microencapsulation of carotenoids, where the obtained microparticles had a regular and stable morphology for protecting the carotenoids (Moser *et al.*, 2019). This suggest that chickpea protein can be used to prepare various types of microcapsules for food and drug encapsulations.

6. Conclusions

In the present animal-vegetable protein transition, isolated vegetal proteins are broadly used as functional ingredients. Considering the potential of chickpea protein as prospective alternative food ingredients, the industrial production for manufacturing chickpea protein-based products will witness a sharp growth in the future. Chickpea protein is expected to be the next generation of plant proteins owing to its functionality, hypo-allergenicity and nutritional properties. Bottom line is that chickpea protein isolate can be suitable ingredient in a wide spectrum of food products such as cereal products, meat products and meat analogues targeting high nutritional value and high-quality functional products with enhanced nutritional, physicochemical, texture and sensory attributes. Technology innovation undoubtedly will play an important role in unlocking more opportunities for applying chickpea proteins. For the future, in-depth investigations are deemed necessary to optimize protein extraction methods and conditions and to understand how processing can impact the purity, protein content, amino acid composition, tech-functionality, and bio-

functionality. Such information will be of great help in boosting chickpea protein production and commercialization, keeping in mind cost and sustainability.

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Ethics declarations

Conflict of interest

None.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

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Wangorsch, A., Kulkarni, A., Jamin, A., Spiric, J., Bräcker, J., Brockmeyer, J., Mahler, V., Blanca-López, N., Ferrer, M., Blanca, M., Torres, M., Gomez, P., Bartra, J., García-Moral, A., Goikoetxea, M.J., Vieths, S., Toda, M., Zoccatelli, G. & Scheurer, S. (2020). Identification and Characterization of IgE-Reactive Proteins and a New Allergen (Cic a 1.01) from Chickpea (*Cicer arietinum*). *Molecular Nutrition & Food Research*, **64**, 2000560. This paper gives new insignts on a new epitopes recently identified in chickpea protein