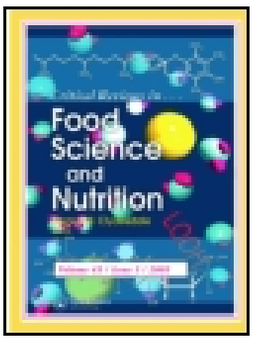

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REVIEW

Exploration on bioactive properties of quinoa protein hydrolysate and peptides: a review

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ABSTRACT

Quinoa is an excellent source of nutritional and bioactive components. Protein is considered a key nutritional advantage of quinoa grain, and many studies have highlighted the nutritional and physicochemical properties of quinoa protein. In addition, quinoa protein is a good precursor of bioactive peptides. This review focused on the biological properties of quinoa protein hydrolysate and peptides, and gave a summary of the preparation and functional test of quinoa protein hydrolysate and peptides. A combination of milling fractionation and solvent extraction is recommended for the efficient production of quinoa protein. The biological functionalities of quinoa protein hydrolysate, including antidiabetic, antihypertensive, anti-inflammatory, antioxidant activities, and so on, have been extensively investigated based on in vitro studies and limited animal models. Additionally, bioinformatics analysis, including proteolysis simulation, virtual screening, and molecular docking, provides an alternative or assistive approach for exploring the potential bioactivity of quinoa protein and peptides. Nevertheless, further research is required for industrial production of bioactive quinoa peptides, verification of health benefits in humans, and mechanism interpretation of observed effects.

KEYWORDS

Biological activity; enzymatic hydrolysis; *in silico* analysis; protein extraction

Introduction

Quinoa (*Chenopodium quinoa* Willd.), known as an ancient and underutilized crop, holds great potential to be integrated into the future agriculture and food industry. This crop has a strong tolerance to extreme climate and soil conditions, like frost, drought, and high salinity, and can be cultivated in a wide range of environments at latitudes from 1°39′N in Colombia to 42°S in Chile (from sea level to the altiplano), making it a promising crop to meet challenges of global climate change (Ruiz et al. 2014; López-Marqués et al. 2020). As a pseudocereal native to the Andean region of South America, quinoa has been now spread to North America, Europe, Asia, and Africa (Alandia et al. 2020). According to the statistical data of Food and Agriculture Organization (FAOSTAT, 2021), Bolivia and Peru are the two largest production countries of quinoa with a total yield of 67,135 and 89,775 tonnes, respectively, in the year of 2019. Meanwhile, the global market for quinoa consumption takes on an extensively expanding tendency as the result of the pursuit of healthy diets by consumers. Generally, quinoa grain is consumed similarly to cereal grains, and it is capable of fulfilling the requirement of nutrients such as protein, unsaturated fatty acids, minerals, and vitamins (Navruz-Varli and Sanlier 2016). With extensive agricultural adaptability and extraordinary nutritional properties, quinoa has attracted great interest in the fields of the agricultural industry and food processing.

In order to clarify the current research state of quinoa, bibliometric method was employed in this work. We searched publications containing the term "quinoa" in the title, abstract, or keywords from the year 1970 to 2020 via the Scopus database. Accordingly, a total of 2579 records (limited to the document type of "article or review") were obtained, which involved subject categories of "Agricultural and Biological Sciences", "Biochemistry, Genetics, and Molecular Biology", "Chemistry" and so on. The number of records grew from 5 in 1970 to 379 in 2020 (Figure 1A), and nearly half of the records were published during 2015–2020. The bibliographic data from 2015 to 2020 was transferred to VOSviewer software for data visualization (Figure 1B). As we can see from the co-occurrence network of keywords, recent studies paid special attention to some chemical compositions of quinoa, e.g., "protein", "saponins", and "starch". Protein is considered the major nutritional superiority of quinoa grain. The protein content in quinoa grain ranges from 9.15% to 21.02% (Bhargava et al. 2007; Gonzalez et al. 2012), which is highly variable impacted by genotype, agroecological conditions, and processing before consumption (Craine and Murphy 2020). Certainly, the quality of quinoa protein may be a more important aspect than the quantity. Plant-based protein is mostly known to be deficient in some essential amino acids, e.g., lysine in cereals and sulfur amino acids in legumes, which derived from a single source cannot fully meet the body's nutritional

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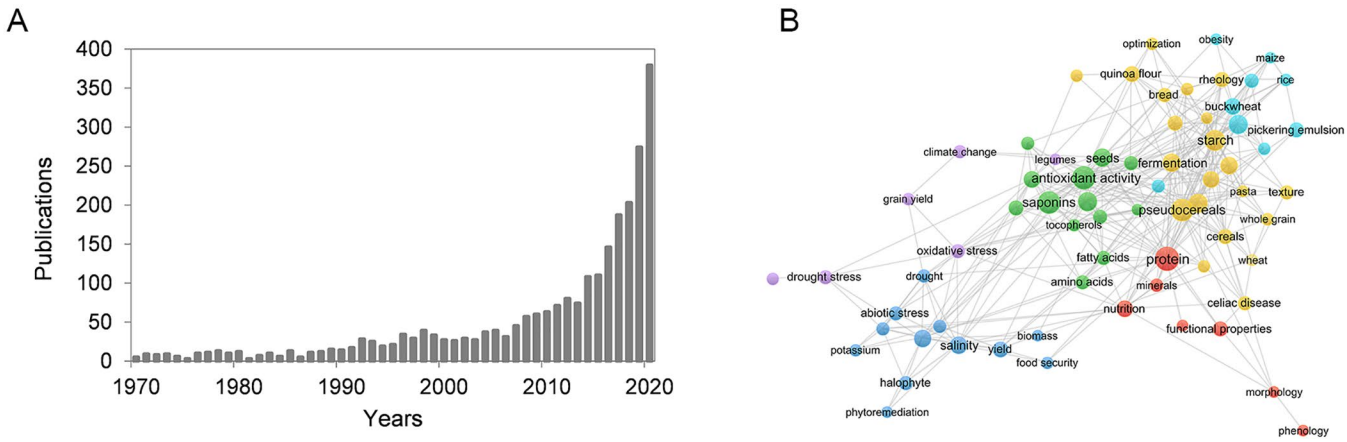

Figure 1. (A) Number of scientific publications reported by Scopus containing the term "quinoa" in the title, abstract, or keywords within the years from 1970 to 2020. (B) Co-occurrence network of keywords in bibliographic data from 2015 to 2020 via VOSviewer software. Circles with a larger size represent the keywords appeared at a higher frequency. The top five keywords with the highest frequency are "protein", "antioxidant activity", "pseudocereals", "saponins", and "starch".

requirement for amino acids. However, quinoa protein is reported to be a complete protein with a well-balanced profile of essential amino acids, and the contents of lysine and methionine in quinoa seeds are higher than in common cereals and legumes, which can be considered as a protein supplement in cereals-based diets and even the replacement of animal proteins (Abugoch James 2009; Angeli et al. 2020). The in vitro digestibility of quinoa protein is reported between 75.9% and 84.8%, being comparable to those other plant proteins such as rice, corn, and beans, though that is lower than animal protein digestibility (Nasir et al. 2015; Shi et al. 2020). Quinoa protein also has good performance in the functionality characteristics, including foam stability, emulsion stability, water/oil holding capacity, gelation property, and film-forming ability (Steffolani et al. 2016; Shen, Tang, and Li 2021). Great efforts made on the characterization and modification of quinoa protein functionality facilitate its application in the food industry, which can be used as ingredients in food formulation to exert a strong influence on processing properties of food matrixes and sensory qualities of food products (Jeske et al. 2018; Duran, Spelzini, and Boeris 2019; López-Alarcón et al. 2019; Vera et al. 2019; Bahmanyar et al. 2021).

Beyond nutritional properties and functionality properties, there is an increasing number of researches focus on the biological activities of quinoa-derived protein hydrolysate and peptides. In the classical process to explore bioactive peptides, quinoa protein is firstly hydrolyzed to generate peptides, using either enzymes or by microbial fermentation, and then the biological activity of the whole hydrolysate is evaluated. This would be followed by a series of bioactivity-guided fractionation procedures, such as membrane separation, size exclusion chromatography, and reversed-phase HPLC, so as to find the most potent peptide pool. Subsequently, mass spectrometry will be applied to determine the peptide sequences, and the bioactivity is validated by testing chemically synthesized peptides. Besides, advance in bioinformatics provides an alternative strategy for the investigation of novel bioactive peptides. *In silico* study, comprehensively utilizing the biological data and computational method, is an emerging technology for the evaluation on potential bioactivity of known protein sequences and peptides, which can be applied in the simulative hydrolysis of protein, virtual screening of potential peptides, and prediction of peptide bioactivities, etc. Quinoa protein presents a great potential to obtain bioactive peptides with multifunctional properties, such as antidiabetic, antihypertensive, anti-inflammatory, and antioxidant activity, making it suitable for applications in functional foods.

Several review articles are available now that covered the research progress of quinoa protein, typically including physicochemical, structural and functional properties (López et al. 2018; Dakhili et al. 2019). The bioactive properties of quinoa protein have been partially reviewed, like antioxidant, inhibition of angiotensin converting enzyme (ACE) and antidiabetic bioactivity (López et al. 2019; Valenzuela Zamudio and Segura Campos 2020; Morales, Miguel, and Garcés-Rimón 2021). However, there is no systematic summary and updating of studies on bioactivities of quinoa protein hydrolysate and peptides, as well as the related advances in *in silico* research. The focus of this review is to provide insight into the biological activities of quinoa protein hydrolysate and peptides, including explored by experimental assay or *in silico* approach, and summarize the current knowledge of protein fractionation and peptides generation, which are foundation work for the exploration of bioactive protein hydrolysate and peptides.

Distribution, composition and fractionation of quinoa protein

Quinoa protein is a good precursor of bioactive peptides. Research advances on the distribution, composition, and fractionation of quinoa protein could provide important information to support the production of quinoa hydrolysates and peptides.

Distribution of protein in quinoa grain

Quinoa seeds are small, which look like a disk with a diameter of 1-3 mm and a thickness of 0.5-1 mm. The major anatomical parts of quinoa seeds include a large central perisperm and a peripheral embryo (consisted of a hypocotyl-radicle axis and two cotyledons) (Burrieza, López-Fernández, and Maldonado 2014). The cells of the perisperm are full of starch granules, while protein is located mostly in the embryo (Prego, Maldonado, and Otegui 1998; Ninfali et al. 2020). D'Amico et al. (2019) performed a study in which the quinoa seeds were milled by a laboratory scale abrasive mill for 8 min by intervals of 1 min. Starting from the second minute, protein content in the kernel fraction was decreased with ongoing abrasive milling, and diminished the most after the fourth minute, which was a result of the detachment of embryo. After 8 min of milling, an extraordinarily high content of carbohydrates about 94.95% was detected in the abraded kernels, which can be regarded as a pure perisperm fraction produced by removal of the outer layers of the kernel. Consequently, these distribution characteristics of key nutritional components within the quinoa seeds will play an important role in quinoa processing and utilization.

Composition of quinoa protein

Protein is often fractionated based on its solubility in water (albumin), dilute saline (globulin), aqueous alcohol (prolamin), and dilute acid or alkali solution (glutelin). It is well acknowledged that albumin and globulin are the main protein fractions in quinoa seeds, leading to extensive research efforts on them. The review by Dakhili et al. (2019) gave a good overview of the characteristics of subunit composition and structure of quinoa albumin and globulin. However, the contents of albumin and globulin are highly variable in quinoa seeds, with reported ranges of 28.5%–43.3% and 27.9%–37%, respectively (Prakash and Pal 1998; Watanabe et al. 2003; Ruiz et al. 2016c; D'Amico et al. 2019). The discrepancy between these values can be explained by the difference in the plant material and extraction procedures. Besides, previous studies showed that globulin is partly extractable when albumin is extracted with water, because of the dissolution of salt ions present in flour (Villareal and Juliano 1981; Tu et al. 2015). And a positive relationship between the water-soluble protein yield and the mineral contents of quinoa wholemeal (R^2 = 0.88) was observed by Van de Vondel, Lambrecht, and Delcour (2020), indicating that minerals present in the quinoa flour render some globulin extractable in water. As for prolamin, it represented 0.5%–11.1% of total protein in quinoa seeds (Koziol 1992; D'Amico et al. 2019). However, these descriptions about prolamins in quinoa seeds were based on the extraction methodology but did not refer to the protein identification (Burrieza et al. 2020). Furthermore, quinoa is considered a naturally gluten-free product, and which has been confirmed by some biochemical and immunochemical evidence (Bergamo et al. 2011; Peñas et al. 2014). It certainly cannot rule out the possibility that celiac-toxic protein exists within some quinoa cultivars (Zevallos et al. 2012), and much more needs to be learned about the prolamins in quinoa seeds and the safety of quinoa for celiac patients.

Fractionation of protein from quinoa seeds

Protein fractionation is a fundamental work for the research on bioactive protein hydrolysate and peptides. According to the pertinent literature, conventional solvent extraction is the most frequently adopted method to obtain quinoa protein isolation. In this way, the preparation of protein products is achieved primarily through a series of separation steps in the aqueous extraction process, which is mainly based on the different solubility of the target object and other matrix components in solvents. On the other hand, the spatial distribution feature of nutritional components within the quinoa seeds has been fully applied in the milling fractionation for the obtainment of the protein-enriched fraction. Representative extraction procedures applied to extract protein from quinoa seeds were listed in Table 1.

Chemical fractionation based on protein solubility

Prior to the protein extraction, saponins and oil are expected to be removed from the raw materials. Saponins, located in the pericarp of quinoa seeds, are mostly removed before milling by washing or dry polishing (Suárez-Estrella et al. 2018), and petroleum ether or hexane is the most frequently employed solvent for oil removal. A Plackett-Burman design showed that seven factors affecting protein extraction yield in descending order as the rate of material and solvent, pH, NaCl concentration, temperature, time, solvent type, and particle size (Guerreo-Ochoa, Pedreschi, and Chirinos 2015). Alkali dissolution is recognized for its effectiveness in the extraction of plant protein, and it is also the most mature method in the extraction of quinoa protein. With the increase of extraction pH, protein solubility increased from 7.5 mg protein/mL at pH 8 to 12.1 mg protein/mL at pH 12 (Valenzuela et al. 2013), and protein yield showed a similar tendency (precipitated at pH 4.5), from 36.3% at pH 8 to 52.0% at pH 11 (Ruiz et al. 2016a). Although this approach is highly effective in getting most of the protein from quinoa seeds, it has some influence on protein structural and functional characteristics. Under extreme alkaline conditions above pH 10, natural protein readily undergoes a structural change, such as denaturation/aggregation/dissociation (Valenzuela et al. 2013), which may result in unfavorable influences on the digestibility of protein (Ruiz et al. 2016b).

Adding NaCl with a certain concentration had a significantly positive effect on the solubility of quinoa protein (Brinegar and Goundan 1993; Elsohaimy, Refaay, and Zaytoun 2015). Recently, a multiple-extraction procedure based on the Osborne method was proposed for mild extraction of protein from quinoa wholemeal, in which protein with high yield and little denaturation was obtained via successive extraction with water (10 min, twice) and 0.4 mol/L sodium chloride (10 min, twice) (Van de Vondel, Lambrecht, and Delcour 2020). It is speculated that this protein fraction maintains its natural characteristics and has

Table 1. Representative procedures for fractionation of protein from quinoa seeds.

Extraction method	Extraction conditions	PY or PC *	References
Solvent extraction	Milling by a hammer mill; defatting using hexane, solubilization with solvent/meal ratio (v/w) :19.6/1, pH = 9, 90 min, particle size of 500 µm; centrifugation at 4000 g and 4 °C for 30 min.	PY: 62.1%	Guerreo-Ochoa, Pedreschi, and Chirinos 2015
	Milling with a 200 µm sieve; defatting using petroleum ether; solubilization at pH 8, 9, 10, and 11, stirring for 4 h, then stored at 4 °C for 16 h; centrifugation at 6,000 g and 10 °C for 30 min; precipitation at pH 4.5; centrifugation at 13,000 g and 10 °C for 30 min.	PY: 36.3% (pH 8) −52% (pH 11)	Ruiz et al. 2016a
	Milling with a 500 µm sieve; twofold extraction with water (10 min, 150 rpm) followed by twofold extraction with 0.4 mol/l sodium chloride (10 min, 150 rpm); centrifugation at 10,000 g for 10 min.	PY: 63.5%	Van de Vondel, Lambrecht, and Delcour 2020
Dry milling	Removal of saponins by abrasion polishing; dry milling by a ball mill; grits were divided into fine (with a particle size ~200 µm), medium (~500 µm), and coarse (~1000 µm) fractions.	PC: 23.54% for medium fraction	Solaesa et al. 2020
	Milling by an experimental rice mill for 0-70 min at 10 min intervals, to obtain flours (FP10-FP70) and polished grain (PG10-PG70).	PC: 30.08%–34.85% for FQ30-FQ60	Roa-Acosta et al. 2020
	Milling by a lab scale mill with a 1.5 mm or 2 mm screen; air jet sieving with different sieves (1, 0.85, 0.63, 0.5 and 0.315 mm), 1500 Pa, 2.5 min.	PC: 23.3%–27.8% for 0.5–0.315 mm PY: 44.7%–46.8% for 0.5–0.315 mm	Opazo-Navarrete et al. 2018a
Wet milling	Seeds sieving; washing and wetting; microwaves extraction of saponins by methanol; wet milling by a roller mill (rolls gap: 0.5, 0.3, and 0.15 mm); recovering the germ-rich fraction (ASTM mesh No. 200); airflow drying; sieving (ASTM mesh No. 20, 30, 40, and 50).	PY: 46.68% for R40 PC: 35.18% for R40	Mufari, Miranda-Villa, and Calandri 2018
	Steeping in sodium bisulfite solution; milling by a plate mill; germ fraction separation by flotation in water.	No data	Ballester-Sánchez et al. 2019
Hybrid fractionation	Conditioning by spraying water and stirring (15% moisture, 20 °C, 20 h); milling by a roller mill; enriching the bran fraction by sieving (mesh of 200 µm); solubilization at pH 9 and 20 °C for 1 h, particle size of 250 µm; precipitation at pH 4; centrifugation at 15,000 g and 4 °C for 20 min.	PC: 67.93%	Föste et al. 2015
	Milling by a rotor mill with a 2 mm sieve); air classification; further milling of fine fraction by an impact mill; aqueous phase separation (0.5 M NaCl); ultrafiltration.	PY: 62% PC: 59.4%	Ruiz et al. 2016c
	Milling by a lab scale mill with a 2 mm screen; air jet sieving with different sieves (0.800, 0.630 and 0.315 mm); solubilization at pH 8, room temperature for 4 h; centrifugation at 6,000 g and 10 °C for 30 min; precipitation at pH 4.5, room temperature for 1 h; centrifugation at 13,000 g and 10 °C for 30 min.	PC: 32.0% for 0.630–0.315 mm	Opazo-Navarrete et al. 2018b

*PY, protein yield; PC, protein content.

good solubility in mild conditions, and as a result, it may have great performance in food processing. It should be noted that, under higher NaCl concentrate, protein extractability is not significantly improving, and even may be negatively affected because of the salt precipitation phenomenon. And the increasing workload of protein purification caused by NaCl should also be taken into account (Guerreo-Ochoa, Pedreschi, and Chirinos 2015). After highly dissolution of quinoa protein under alkaline or salty conditions, the supernatants are usually adjusted to pH 4.0–5.5 (Föste et al. 2015; Ruiz et al. 2016b), where quinoa protein is precipitated isoelectrically.

Physical fractionation based on protein distribution

Separation of protein-rich embryo fraction from the starchy perisperm can be achieved by dry/wet milling, sieving, and/or air classification. Humidity conditioning is considered an important step before quinoa grain milling. The proper increase of humidity (reach 15% in dry milling; reach 40%–50% in wet milling) renders the embryo more elastic, being conducive to the better separation of seed tissues (Föste et al. 2015; Mufari, Miranda-Villa, and Calandri 2018). Shear forces applied by the rotor milling or compression forces applied by the roller milling were proved to be conducive for the dissociation of the embryo from perisperm (Ruiz et al. 2016c). In order to obtain the desired fractions, the milling process requires the best choice of milling parameters which determines the particle size distribution of the milled flour. Three consecutive wet millings with gradually reduced distances (starting from 0.5 mm and then lowered to 0.3 mm and 0.15 mm) between the rollers are perfectly capable of detaching fractions by compressing the germ and disintegrating the perisperm (Mufari, Miranda-Villa, and Calandri 2018)). According to the difference of particle size, pre-milled quinoa grain can be divided into more than two fractions via sieving and/or air classification. Cut size is experimentally adjusted by the reference of particle size distributions and the yields of the resulted fractions (Pelgrom et al. 2013). Generally, the fraction with medium particle size presented a relatively high protein content compared to the coarse and fine fractions (Opazo-Navarrete et al. 2018a; Solaesa et al. 2020). The variation of protein content with particle size is not universal and it depends on the characteristics of grain structure and milling procedure. For the wet-milling procedure by Ballester-Sánchez et al. (2019), in which quinoa starch was designed as the main purpose, germ (embryo) fraction as the coproduct was separated by flotation from the pre-milled seed slurry. Additionally, protein loss during the wet milling process is almost inevitable due to the dissolution of the soluble protein in water (Opazo-Navarrete et al. 2018a; Ballester-Sánchez et al. 2019).

Overall, the milling fractionation of quinoa seeds is a feasible procedure to produce a fraction rich in protein. Milling fractionation is a physical separation process, avoiding the addition of chemicals and that influence on protein functionality. Although the quinoa embryo-enriched fraction is featured by relatively low protein purity compared to the fraction prepared via conventional solvent extraction, it is still a promising ingredient for food processing, which may help to improve the quality of food products by improving the nutritional value and modifying the textural characteristics (Mufari, Miranda-Villa, and Calandri 2018). Meanwhile, quinoa embryo-enriched fraction can be used as a starting material to obtain quinoa protein and oil, and the starch-enriched fraction obtained can be used for other purposes (Solaesa et al. 2020).

Hybrid fractionation

Currently, a hybrid physical and chemical fractionation method has been successfully applied in the isolation of protein from quinoa seeds. Briefly, a pre-fractionation is performed by the milling process, and obtained protein-enriched fraction will be used as the raw material for solvent extraction of quinoa protein. In the study of Föste et al. (2015), quinoa seeds were milled using a roller mill equipped with a 200-mesh rotating sifter to separate the protein-rich bran from starch-rich core flour. The obtained bran fraction contained higher amounts of protein (23.97%–27.78%) compared to white flour (5.86%–6.33%). Then, alkaline extraction followed by acidic precipitation was performed for the extraction of protein from the quinoa bran fraction, resulting in a protein yield of 68.0% from the bran fraction. In the study of Ruiz et al. (2016c), the protein-rich embryo fraction obtained by the similar milling fractionation procedure was further milled by an impact mill to obtain the finer power, which will facilitate the dissolution of protein during the suspension. Subsequently, quinoa flour suspensions were separated into three layers by aqueous phase separation, and consequently, the liquid layer showed a higher protein yield (40.3%) than the other two solid layers, especially when adding 0.5 M NaCl to suspensions (80.3%). Besides, ultrafiltration (3 kDa) was applied to the top aqueous phase to further increase protein purity. Finally, these successive operations provided protein extracts with a purity of 59.4% and a protein yield of 62%. In summary, combining milling fractionation and solvent extraction can greatly improve the efficiency of protein isolation.

Production of quinoa protein hydrolysate and peptides

Bioactive peptides can be released from quinoa protein as a result of in vivo gastrointestinal digestion, in vitro enzymatic hydrolysis, and fermentation processes. Gastrointestinal digestion could be able to naturally generate peptides under the action of digestive enzymes. However, very few researchers would take this way to capture bioactive peptides released from protein, since this is an unmanageable dynamic process of digestion and absorption. Therefore, in vitro simulated gastrointestinal digestion has been established and widely used by employing gastrointestinal enzymes (e.g., pepsin, trypsin, chymotrypsin, and pancreatin) for the preparation of protein hydrolysate. In the studies by Vilcacundo, Barrio, et al. (2017, Vilcacundo et al. 2018), simulated gastric fluid (containing pepsin) and intestinal fluid (containing pancreatin and porcine bile extract) were

employed for the hydrolyzation of quinoa protein concentrate, and the results suggested that peptides released during the gastroduodenal phase are more potent in antioxidative and colon cancer cell viability inhibitory activities than the gastric phase. In addition, quinoa protein hydrolysate with diverse biological activities including antihypertensive, antidiabetic, antioxidative, and antibacterial properties have reportedly been produced by enzymatic hydrolysis using alcalase, papain, bromelain (Aluko and Monu 2003; Nongonierma et al. 2015; Mudgil et al. 2019). These enzymes have specific cleavage positions on the amino acid sequences, resulting in various protein hydrolysate differed in peptide composition and biological properties. The factors to be controlled during the hydrolysis process including temperature, pH, time, and the ratio of enzyme and substrate, are known to affect the biological properties of the resulting hydrolysate. In addition, quinoa seeds germination facilitates the natural release of peptides, as well as the fermentation process of quinoa dough and quinoa yogurt beverages (Rizzello et al. 2017; Ujiroghene et al. 2019).

Protein hydrolysate is a complex mixture of free amino acids and peptides in different chain lengths. As shown in the study of Nongonierma et al. (2015), the quinoa protein hydrolysates had a significantly higher concentration of free amino groups than the corresponding protein isolate, and presented fainter bands in the high molecular mass range on the SDS-PAGE profiles. The molecular mass distribution profile of the hydrolysates further showed a reduction in the higher molecular weight (MW >10 kDa) components and an increment in the lower molecular weight (MW < 5 kDa) components. For a better understanding of the quinoa peptides responsible for observed bioactive effects, a series of bioactivity-guided fractionation processes will be carried out to reduce the compositional complexity of crude protein hydrolysate. Ultrafiltration is usually applied during the initial phase of fractionation to obtain fractions with a particular molecular weight range, followed by a bioactivity evaluation of the resulting fractions. Investigations revealed that quinoa protein hydrolysate with the lower molecular weight (MW < 5 kDa) exhibited stronger bioactivity compared to that with higher one (MW > 5 kDa) (Aluko and Monu 2003; Vilcacundo, Martínez-Villaluenga, et al. 2017). Then, the fraction with stronger bioactivity may undergo additional purification and identification to find the most potent peptides. For instance, after simulated gastrointestinal digestion of quinoa protein isolation and preliminary bioactivity screening for fractions obtained by ultrafiltration and size exclusion chromatography, quinoa peptide sequences were identified using ESI-Q-TOF-MS/MS, and three promising peptides (FHPFPR, NWFPLPR, NIFRPF) were experimentally confirmed to be remarkably effective in ACE inhibition (Guo et al. 2020b). From the quinoa yogurt beverage, bioactive quinoa peptides were identified by RP-HPLC-MS/MS, among which LAHMIVAGA and VAHPVF were promising inhibitors against α-glucosidase and ACE (Ujiroghene et al. 2019). Overall, the peptide composition has not been profiled for most of the bioactive hydrolysates from quinoa protein, and extensive studies are required to further identify the peptide sequences responsible for the observed biological effect.

Bioactivities of quinoa protein hydrolysate and peptides

Considerable progress has been made in the knowledge of bioactive properties of quinoa protein hydrolysate and peptides (Table 2). Like a lot of cereal protein-derived bioactive peptides (Gong et al. 2020), quinoa protein hydrolysate and peptides could contribute to reduce the risk associated with diseases related to cardiometabolic area by exerting antidiabetic, antihypertension, and hypolipidemic activity, provide protection against inflammatory and oxidative stress, and serve as cytotoxic compounds against microbes and cancer cells.

Antidiabetic activities

Diabetes mellitus is a chronic metabolic disorder that may lead to various diabetes-specific complications such as cardiovascular diseases, end-stage renal disease, retinopathy, and neuropathy (Harding et al. 2019). Continuous efforts have long been undertaken by researchers to search for natural bioactive components that are helpful in the prevention and management of diabetes. Food protein hydrolysate has been found to be a good candidate for the production of antidiabetic peptides, which may affect the regulation of blood glucose levels by multiple mechanisms, including the inhibition of specific enzymes such as DPP-IV, α-amylase, and α-glucosidase (Kehinde and Sharma 2020; Mudgil et al. 2020). DPP-IV plays a major role in the regulation of glucose metabolism through the inactivation of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). GIP and GLP-1 are intestinal hormones released in response to food ingestion, which are known to lower blood glucose by enhancing insulin secretion and inhibiting glucagon release (Juillerat-Jeanneret 2014). DPP-IV inhibitors exert their positive effect on glucose regulation by preventing the rapid degradation of incretin hormones, which has been developed as an effective therapeutic strategy for the treatment of diabetes. In addition, α-amylase is responsible for the decomposition of dietary carbohydrates into oligosaccharides, which are subsequently hydrolyzed into absorbable monosaccharides by α-glucosidase, thereby leading to an increase in blood glucose level (Zheng et al. 2020). The inhibitors of these enzymes could delay the carbohydrate hydrolysis and consequently glucose absorption, and thus have been used for the control of blood glucose levels in diabetic patients.

Studies have reported that quinoa protein hydrolysates prepared by in vitro enzymolysis exhibited significant antidiabetic effect by inhibiting the activity of certain enzymes (Nongonierma et al. 2015; Mudgil et al. 2020). In a study of in vitro simulated gastrointestinal digestion, the gastroduodenal digests showed enhanced DPP-IV inhibitory activity compared to the gastric digest (Vilcacundo,

Table 2. Biological activities of quinoa protein hydrolysate/peptides.

Bioactivities	Production method of quinoa protein hydrolysate/peptides	Description of bioactivity test	References
Anti-diabetic	Enzymolysis by papain and microbial papain-like enzyme	Inhibitory effect on DPP-IV	Nongonierma et al. 2015
	Simulated gastrointestinal digestion	Inhibitory effect on DPP-IV, α-amylase, and α-glucosidase	Vilcacundo, Martínez-Villaluenga, et al. 2017
	Fermented with probiotic lactic acid bacteria strains	Inhibitory effect on α-glucosidase	Ujiroghene et al. 2019
	In silico proteolysis by papain, ficin, and stem bromelain	Inhibitory effect on DPP-IV	Guo et al. 2020a
	Enzymolysis by Bromelain, chymotrypsin, and Pronase E	Inhibitory effect on DPP-IV and α-glucosidase	Mudgil et al. 2020
Antihypertension	Enzymolysis by alcalase	Inhibitory effect on ACE	Aluko and Monu 2003
	Enzymolysis	Inhibitory effect on ACE	Ravisankar et al. 2015
	In silico proteolysis by papain, ficin, and stem bromelain	Inhibitory effect on ACE	Guo et al. 2020a
	Enzymolysis by papain, pepsin, and pancreatin	Inhibitory effect on ACE	Shi et al. 2019
	Fermented with probiotic lactic acid bacteria strains	Inhibitory effect on ACE	Ujiroghene et al. 2019
	Enzymolysis by alcalase and trypsin	Inhibitory effect on ACE, lower the blood pressure level in SHRs	Zheng et al. 2019
	Simulated gastrointestinal digestion	Inhibitory effect on ACE, lower the blood pressure level in SHRs	Guo et al. 2020b
	Enzymolysis by Bromelain, chymotrypsin, and Pronase E	Inhibitory effect on ACE	Mudgil et al. 2020
Hypolipidemic	Enzymolysis by papain, pepsin, and pancreatin	Inhibit the lipid accumulation during the differentiation of 3T3-L1 cells	Shi et al. 2019
	In vivo digestion by mice model	Inhibit the reabsorption of bile acids; inhibit the expression of HMG-CoA reductase and promotes the expression of Cyp7α	Takao et al. 2005
Anti-inflammatory	Enzymolysis	Downregulate the expression of NF-κB transcription factor and its target genes, activate the PPAR-γ transcription factor in LPS-challenged HUVEC	Ravisankar et al. 2015
	Buffer extraction	Inhibitory effect on the inflammation induced by LPS in RAW264.7	Ren et al. 2017
	Enzymolysis by papain, pepsin, and pancreatin	Inhibitory effect on the inflammation induced by LPS in RAW264.7	Shi et al. 2019
	Low charge Chenopodin and high charge Chenopodin	Protection against IL-1β-induced inflammation in Caco-2 cell	Capraro et al. 2020
	Osborne's classification	Protection against IL-1β-induced inflammation in Caco-2 cell	Capraro et al. 2021
Antioxidant	Enzymolysis by alcalase	DPPH radical scavenging activity	Aluko and Monu 2003
	Enzymolysis by papain and microbial papain-like enzyme	ORAC	Nongonierma et al. 2015
	Buffer extraction	ABTS and oxygen radical scavenging activity	Ren et al. 2017
	Fermented with lactic acid bacteria	DPPH and ABTS radical scavenging activity, inhibition of linoleic acid autoxidation, protective effect on oxidative-induced stress in NCTC 2544	Rizzello et al. 2017
	Simulated gastrointestinal digestion	Inhibit lipid peroxidation in Zebrafish Larvae Model	Vilcacundo, Barrio, et al. 2017b
	Simulated gastrointestinal digestion	ORAC	Vilcacundo et al. 2018
	Enzymolysis by alcalase	ABTS, DPPH, and hydroxyl radical scavenging activity, reducing power, metal chelating activity	Li et al. 2018
	Enzymolysis by bromelain, chymotrypsin, and protease	ABTS and DPPH radical scavenging activity	Mudgil et al. 2019
	Enzymolysis by alcalase and trypsin	ABTS, Fe^{2+} chelating ability	Zheng et al. 2019
	Simulated gastrointestinal digestion	DPPH, ABTS, ORAC, inhibit ROS production in Zebrafish Embryo Model	Piñuel et al. 2019
	Enzymolysis by fungal serin protease	DPPH and ABTS radical scavenging activity	Galante et al. 2020
	In vitro gastrointestinal digestion	ABTS radical scavenging activity	Rangaswamy et al. 2021
	In vitro gastrointestinal digestion	DPPH radical scavenging activity	Capraro et al. 2021
	Enzymolysis by endopeptidase COROLASE® 7089	ABTS radical scavenging activity	Olivera-Montenegro, Best, and Gil-Saldarriaga 2021
Anti-hemolytic	Enzymolysis by bromelain, chymotrypsin, and protease	Protection against thermal destruction of human erythrocytes	Mudgil et al. 2019
Antimicrobial	Enzymolysis by bromelain, chymotrypsin, and protease	Inhibition against *Staphylococcus aureus, Salmonella typhimurium, Escherichia. coli* and *Enterobacter aerogenes*.	Mudgil et al. 2019
Anticancer	Simulated gastrointestinal digestion	Inhibitory effect on colon cancer cell viability (Caco-2, HT-29, and HCT-116)	Vilcacundo et al. 2018

Martínez-Villaluenga, et al. 2017). Furthermore, α-amylase and α-glucosidase inhibitory activities were only observed in the quinoa protein gastroduodenal digests, and the fractions containing small peptides (< 5 kDa) exerted stronger inhibition on both carbohydrate hydrolyzing enzymes. Quinoa protein hydrolysate isolated from the sprouted quinoa yogurt beverage has been reported to have antidiabetic effects by inhibiting α-glucosidase activity, and the activity was influenced by germination time and inoculants strains (Ujiroghene et al. 2019).

Antihypertension activities

Hypertension is one of the most common chronic diseases in the world and is widely known as a primary risk factor for cardiovascular disease. At present, the antihypertensive activity of quinoa protein hydrolysate and peptides was mainly characterized by in vitro ACE inhibitory effect. The enzyme ACE is involved in the production of vasoconstrictor angiotensin II in the renin-angiotensin system and degradation of vasodilator bradykinin in the kinin-kallikrein system, and thus plays a crucial role in the regulation of blood pressure (Udenigwe and Mohan 2014). Therefore, the inhibition of ACE is one of the effective strategies in the treatment of hypertension.

Many quinoa protein hydrolysates, prepared by alcalase, Bromelain, chymotrypsin, and Pronase E, have exhibited in vitro ACE inhibitory activity (Aluko and Monu 2003; Mudgil et al. 2020). In addition, antihypertensive activity has also been studied by determination of blood pressure in spontaneously hypertensive rats (SHRs), which is a widely used animal model of hypertension. In the study of Zheng et al. (2019), a novel ACE inhibitory peptide RGQVIYVL derived from quinoa bran albumin was identified, and oral administration of the peptide (100–150 mg/kg body weight) to SHRs resulted in significant decreases in both systolic blood pressure and diastolic blood pressure. A quinoa protein hydrolysate obtained by simulated gastrointestinal digestion also showed a significant antihypertensive effect on SHRs (Guo et al. 2020b). And the isolated peptides (FHPFPR, NWFPLPR, and NIFRPF) derived from the hydrolysate showed great in vitro ACE inhibitory activity. In general, hydrolysate and peptides derived from quinoa protein have significant in vitro ACE inhibitory activity and antihypertensive effect on animals, which requires to be confirmed by clinical trials.

Hypolipidemic activities

Quinoa protein could significantly reduce the plasma cholesterol level even though under a cholesterol-supplemented diet in a mice model study (Takao et al. 2005). This cholesterol-lowering effect was attributed to the bile acid-binding activity of quinoa protein and control of cholesterol synthesis and catabolism, by inhibiting the expression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and promoting the expression of cholesterol-7α-hydroxylase (Cyp7α). Besides, quinoa protein hydrolysate could inhibit lipid accumulation during the differentiation of 3T3-L1 cells, without obvious cytotoxicity, indicating a potential adipogenesis inhibitory activity of quinoa protein hydrolysate (Shi et al. 2019).

Anti-inflammatory activities

Inflammation is the protective response from the immune system that is crucial for maintaining the body's homeostasis. However, a prolonged pro-inflammatory state in chronic inflammation can lead to tissue malfunction and homeostatic imbalance (Scrivo et al. 2011). And this pro-inflammatory state has been implicated in many diseases including atherosclerosis, cardiovascular disease, cancer, diabetes, rheumatoid arthritis, and osteoporosis (Guha and Majumder 2019; Sorriento and Iaccarino 2019). Understandably, inflammation has been widely concerned by researchers, and anti-inflammatory therapy might have efficacy in the treatment and prevention of diseases associated with chronic inflammation.

Bioactive peptides have been shown to exhibit anti-inflammatory activity through the inhibition on the expression of inflammatory biomarkers and regulation of transcription factors (Majumder, Mine, and Wu 2016). Quinoa peptides were found to modulate the nuclear factor kappa B (NF-κB) pathway in lipopolysaccharides-challenged Human Umbilical Vein Endothelial Cells (HUVEC) by downregulating NF-κB and upregulating the peroxisome proliferator-activated receptor (PPAR-γ) (Ravisankar et al. 2015). Lunasin, a 43-amino-acid peptide, was detected in quinoa seeds and its effect on lipopolysaccharide-stimulated RAW264.7 macrophages were confirmed by inhibiting the production of nitric oxide, tumor necrosis factor-α, and interleukin-6 (Ren et al. 2017). According to Shi et al. (2019), quinoa protein and hydrolysate prepared by papain, pepsin, and pancreatin exhibited high anti-inflammatory activities in RAW 264.7 macrophage cells, while no significant difference was seen between the protein and hydrolysate. Chenopodin, namely 11S globulin of quinoa seeds, showed the ability to decrease NF-κB activation and interleukin 8 (IL-8) expression, protecting Caco-2 cells from the inflammatory stimulus (Capraro et al. 2020). Furthermore, quinoa seed albumin in the intact form showed higher anti-inflammatory activity than globulin fractions (Capraro et al. 2021).

Antioxidant activities

In the human body, the balance between the generation of free radicals and the endogenous antioxidant defense system has important health implications. Oxidative stress characterized by excessive production of free radicals may cause cellular damage and trigger a variety of chronic diseases. Dietary intake of antioxidants has been reported to be helpful in protecting the organism from oxidative stress and reducing the risk of chronic diseases related to oxidative stress (Neha et al. 2019; Wen et al. 2020). The plant protein-derived antioxidant peptides have attracted extensive attention for their effective prevention of oxidative stress and potential application in the food system.

The antioxidant activity of quinoa protein hydrolysate has been studied in many publications using DPPH radical scavenging assay, ABTS radical cation assay, oxygen radical absorbance capacity (ORAC) assay, iron-chelating capability, and the inhibitory activity toward lipid peroxidation. Intact quinoa protein isolates possessed a certain degree of antioxidant capacity, and its hydrolysate prepared by enzymatic hydrolysis exhibited enhanced ability to scavenge free radicals (Nongonierma et al. 2015). Hydrolysis of quinoa protein improves its antioxidant potential because of the release of low molecular mass peptides, and the differences in antioxidant capacity among various hydrolysate could be attributed to differences in enzyme specificities, degree of hydrolysis, and composition of peptides generated (Mudgil et al. 2019; Olivera-Montenegro, Best, and Gil-Saldarriaga 2021). Furthermore, fraction < 5 kDa obtained from gastroduodenal digests of quinoa protein has been found to exert higher ORAC activity than fraction >5 kDa, and more potent peptides with antioxidant activity were released during the intestinal phase (Vilcacundo et al. 2018). Additionally, the effect of ultrasound pretreatment prior to protein hydrolysis has been investigated by Li et al. (2018). Hydrolysate obtained from ultrasound-treated quinoa protein shows a greater antioxidant capacity than the non-treated hydrolysate. Further analyses proved that ultrasound treatment could induce unfolding of quinoa protein, improve the efficiency of alcalase hydrolysis, and thus facilitate the production of hydrolysate with better antioxidant activity. In addition, the antioxidant capacity of quinoa protein hydrolysate was also confirmed in the Zebrafish Larvae Model by the way of inhibiting lipid peroxidation induced by hydrogen peroxide (Vilcacundo, Barrio, et al. 2017) and reducing reactive oxygen species (ROS) formation in Zebrafish Embryos (Piñuel et al. 2019). The antioxidant peptides were also released during the fermentation of quinoa doughs (Rizzello et al. 2017). The water/salt-soluble extracts from fermented quinoa doughs showed elevated radical scavenging activity and inhibition of linoleic acid autoxidation than that from the inoculated doughs, and purified peptides fraction showed potential against oxidative stress-mediated injuries in human keratinocytes NCTC 2544. Furthermore, five peptides (IVLVQEG, TLFRPEN, VGFGI, FTLIIN, and LENSGDKKY) in the purified fraction were identified by nano-LC-ESI-MS/MS analysis, but the publication presented no validation of antioxidant activity of individual peptides. In another study, three peptides (RGQVIYVL, ASPKPSSA, and QFLLAGR) derived from the quinoa bran albumin demonstrated excellent antioxidant activity when assessed by radical scavenging activity and Fe^{2+} chelating ability (Zheng et al. 2019).

Other bioactivities

Beyond these bioactivities highlighted above, other capacities such as anti-hemolytic, anti-cancer and antimicrobial activities were also reported for quinoa protein hydrolysate or peptides. Mudgil et al. (2019) demonstrated that both the quinoa protein isolates and hydrolysate have anti-hemolytic effects on the thermal destruction of human erythrocytes. The antimicrobial activity was also evaluated in this study, and it is demonstrated that the hydrolysate shows promising antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia. coli* and *Enterobacter aerogenes*, indicating that quinoa peptides can be used for the prevention of microbial infections. Quinoa protein is also considered a source of anti-proliferative peptides. As proved in three human colorectal cancer cell lines (Caco-2, HT-29, and HCT-116), gastroduodenal digests of quinoa protein exerted effective anticancer activity, and peptides with higher molecular weight (> 5 kDa) were more potent inhibiting the cell viability than smaller molecular weight peptides (< 5 kDa) (Vilcacundo et al. 2018).

Profiles of potential bioactivity of quinoa peptides by *in silico* approaches

Discovery and characterization of bioactive peptides are conventionally realized through a series of operations, including protein extraction and hydrolysis, and peptides purification, identification, and biological test (Figure 2), as summarized above. Bioinformatics provides a more economical and time-saving method for this research work, which has been known as *in silico* approach. This is a kind of computer-assisted analysis, based on current information derived from experimented facts (Tu et al. 2018). There are a number of databases related to protein and peptides available for *in silico* analysis. National Center for Biotechnology Information (NCBI) and UniProt Knowledgebase (UniProtKB) can be adopted to acquire information of protein sequences. Many online tools can directly serve for the release of peptides from protein, such as BIOPEP-Enzyme(s) Action and PeptideCutter. The BIOPEP database has collected more than 4300 bioactive peptides with 55 different bioactivities by September 2021, which has been widely used for the evaluation of protein as a precursor of bioactive peptides. For the activity-unknown peptides, PeptideRanker is capable of predicting the potential of peptides to be bioactive. The peptides with relatively high PeptideRanker scores are considered as potential candidates with bioactivity. Besides, various online tools can be employed for the

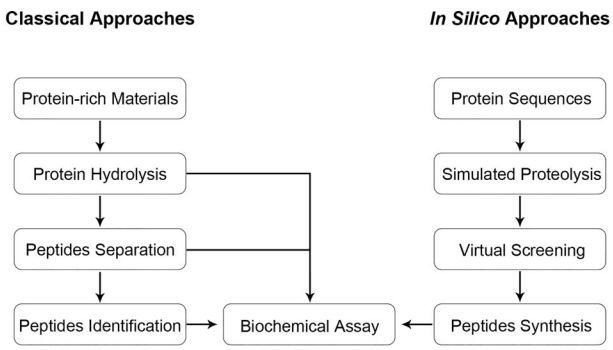

Figure 2. Classical and *in silico* approaches for screening bioactive peptides from quinoa protein.

Table 3. Profiles of potential biological activity of quinoa protein sequences shown with frequency of the occurrence of peptides with given activity performed by BIOPEP (Guo et al. 2020a).

Bioactive peptides	XP_021758596	AAS67036	XP_021770184	XP_021752233	XP_021752668
ACE inhibitor	0.3247	0.4208	0.3945	0.4168	0.393
Peptide activating ubiquitin-mediated proteolysis	0.013	0.0104	0.0063	0.013	0.0129
α-glucosidase inhibitor	0	0.0021	0	0.0022	0.0018
Anti-amnestic peptide	0.0065	0.0042	0.0063	0.0043	0.0018
Anticancer peptide	0	0.0021	0	0.0022	0
Antioxidative peptide	0.039	0.0646	0.0527	0.0302	0.0461
Calcium binding peptide	0.0065	0	0	0	0.0018
Antithrombotic peptide	0	0.0042	0.0063	0.0022	0
Bacterial permease ligand	0	0	0	0.0022	0
DPP-IV inhibitor	0.5195	0.6354	0.654	0.6609	0.6181
Embryotoxic	0	0	0.0042	0	0.0018
HMG-CoA reductase inhibitor	0	0	0.0021	0	0
Renin inhibitor	0.0325	0.0312	0.0422	0.0259	0.0258
Immunomodulating peptide	0	0.0021	0	0.0022	0.0018
CaMPDE inhibitor	0.0065	0.0083	0.0042	0.0065	0.0129
Neuropeptide	0	0.0063	0.0063	0.013	0.0148
Peptide regulating the stomach mucosal membrane activity	0.0065	0.0063	0.0084	0.0022	0
Glucose uptake stimulating peptide	0.0844	0.05	0.0633	0.0605	0.0756

prediction of physicochemical properties, toxicity, and allergenicity of the peptides, such as ProtParam, SwissADME, ToxinPred, and AllerTOP (Tu et al. 2018; Wong et al. 2021). Molecular docking is to predict the binding pose and affinity of a small molecule ligand and target protein with the help of docking tools and programs, like AutoDock, Glide, and HPEPDOCK, which have been widely used to screen bioactive peptides and illustrate their molecular mechanisms (Tu et al. 2018).

In our previous study, a comprehensive look at quinoa protein as a potential source of bioactive peptides was given using *in silico* approaches (Guo et al. 2020a). Five sequences of quinoa protein were examined for the presence of known bioactive peptides using the BIOPEP database, and an abundance of potential biological activities was found in quinoa protein (Table 3). So far, only a few roles have been validated, which encourages more studies exploring the physiological benefits of quinoa peptides. The computational approaches are also capable of screening protein sequences, enzymes, and potential bioactive peptides. Globulin in quinoa seeds was shown to be more potential to act as a precursor of bioactive peptides, especially DPP-IV and ACE inhibitors. Three commercial enzymes papain, ficin, and stem bromelain were used for *in silico* proteolysis by BIOPEP, as a result of a large number of peptides being released. The results also indicated that papain has relatively strong potential as an enzyme releasing DPP-IV and ACE inhibitory peptides from quinoa protein.

PeptideRanker has been successfully applied in the preliminary screening of bioactive quinoa peptides. In studies of Guo et al. (2020a, b), both peptides theoretically released from quinoa protein and identified in quinoa protein hydrolysate were submitted to PeptideRanker for the calculation of theoretical bioactivity, and then the peptides with high score values (> 0.8) were selected to proceed to the further study. Virtual screening of bioactive peptides can also be conducted by molecular docking, which assesses the behavior of peptides in the binding site of a target protein. In the study of Mudgil et al. (2020), molecular binding analysis of the potential peptides with three targeted enzymes (DPP-IV, α-glucosidase, and ACE) was performed using Pepsite 2.0, and the results showed that most of the peptides were capable of binding to the important sites of targeted enzymes. However, there was no experimental validation for predicted bioactivities of selected peptides in this study. Additionally, seven quinoa seed peptides were identified as nontoxic, non-allergenic, and multi-target anti-severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) peptides by *in silico* proteolysis and molecular docking (Wong et al. 2021). In HPEPDOCK analysis, these peptides had relatively low docking scores with receptors SARS-CoV-2 spike glycoprotein receptor-binding domain, main protease, and papain-like protease, which were predicted to bind to key binding/catalytic residues in the three target proteins. Of course, future experiments should be conducted to verify their inhibitory effects on three SARS-CoV-2 target proteins, bioavailability, toxicity, and allergenicity. Similarly, the quinoa peptides generated by in vitro digestion were served as a library for screening potential therapeutic targets for SARS-CoV-2 (Rangaswamy et al. 2021). The results were that the peptide NWRTVKYG from quinoa presented favorable interactions with the binding site of angiotensin-converting enzyme-2 (ACE-2), a functional receptor for the SARS-CoV-2, and the molecular dynamic simulation revealed the ability of the peptide in stabilizing the protein-peptide composite. Thus, quinoa protein was computationally identified as a good source of antiviral peptides that could serve as the

Table 4. Peptide sequences and activities obtained from *in silico* analysis.

Peptides	Bioactivity prediction	Analysis methods (tools)	Reference
FFE, DFTF, DFLM, ML, CDCP, CYTF, FSAGGLP, MLLL, MYLY, QHPHGLGALCAAPPST, PGGAR, KIVLDSDDPLFGGF, AHCGGLPY, LYNDWDLR, SGPAR, LPLLR, KPGGTAGSALPRPAHW, FE, HDSF, LR, VYML, RR, LRL, MFVPVPH, AGLR, HVASGAGPW, HVQGHPALPGVPAHW, FTVM, LLPYH, MVLP, GARR, CVLSPL, HMLH, ASNLDNPSPEGTVM, CALVGL,	Inhibition on DPP-IV, ACE, and α-glucosidase	Proteolysis by bromelain, chymotrypsin and Pronase E, LCMS Q-TOF analysis, prediction of bioactivity (PeptideRanker), molecular docking (Pepsite2)	Mudgil et al. 2020
MAF, NMF, HPF, MCG	Inhibition on ACE and DPP-IV	*In silico* proteolysis (BIOPEP), prediction of bioactivity (PeptideRanker), toxicity (ToxinPred) and solubility (Innovagen)	Guo et al. 2020a
NWRTVKYG	Inhibition on ACE-2	*In vitro* gastrointestinal digestion, nano-LC-MS/MS analysis, molecular docking (Schrödinger), molecular dynamic simulation (GROMACS)	Rangaswamy et al. 2021
PKRF, PNWKIN, AIRAMPL, PHWNIN, ERHHRGGRGRQS, TKHGGRINTL, VEDKGMMHQQRMMEKAMNI-PRMCGTMQRKCRMS	Inhibition on the SARS-CoV-2 targets spike glycoproteins, main protease, and papain-like protease	*In silico* proteolysis (BIOPEP), molecular docking (HPEPDOCK), computational alanine scanning, prediction of physicochemical properties, pharmacokinetics, druglikeness, medicinal chemistry (SwissADME), toxicity (ToxinPred) and allergenicity (AllerTOP)	Wong et al. 2021

promising candidate for the peptide-based therapeutics on SARS-CoV-2. The potential bioactive peptides explored by *in silico* approach are presented in Table 4.

In silico approach provides an alternative strategy for the investigation of novel bioactive peptides, but also has its limitations. This *in silico* analysis is feasible for known protein sequences, and it is essentially useless for the sample whose protein has not been sequenced. The products of *in silico* hydrolysis of identified protein sequences are determined by specific cleavage sites of selected enzymes, without consideration of other variables that make a large impact on the degree of hydrolysis, such as enzymatic activity, enzyme-substrate ratios, temperature, pH, and time (Tu et al. 2018). Although *in silico* approach can not totally substitute the classical approaches, it is an excellent assistive technology to provide the instruction for experiments and reduce test blindness.

Future perspectives

This article provides an overview of recently published research on the preparation and biological activity of quinoa protein hydrolysate and peptides. Chemical extraction is currently the principal method to extract protein from quinoa seeds, while milling fractionation gives a solvent-free and environment-friendly way to obtain protein fraction, although with lower purity. Investigation on the integration of these two methods and innovative approaches are encouraged to greatly improve the efficiency of protein isolation. In vitro enzymatic hydrolysis is the main method to prepare hydrolysate and peptides from quinoa protein. Exploration of enzyme combination and hydrolysis condition optimization is required for the production of various hydrolysates with different composition and biological activity. Quinoa protein hydrolysates and peptides exert some benefits for human health, including antidiabetic, antihypertension, hypolipidemic, anti-inflammatory, and antioxidant activity. *In silico* approaches are helpful to discover bioactive peptides in a cost-effective manner, and facilitate the study of the relationship between peptide structure and activity. Thus, enhancement of the prediction capability of bioinformatics tools could trigger a significant impact on the research of bioactive peptides. It can be clearly stated that the quinoa protein is a valuable source of bioactive peptides with multifunctional properties, making it a promising ingredient for functional food applications. However, further investigation is required, especially in vivo studies based on animal models and clinical experiments to further testify the bioactivities of quinoa protein hydrolysate and peptides, as well as to uncover their inner mechanisms.

Disclosure statement

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