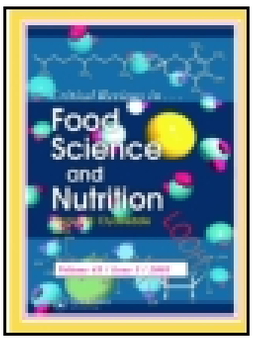

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Jian Jin , Ikenna C. Ohanenye & Chibuike C. Udenigwe (2020): Buckwheat proteins: functionality, safety, bioactivity, and prospects as alternative plant-based proteins in the food industry, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2020.1847027

To link to this article: https://doi.org/10.1080/10408398.2020.1847027

Published online: 16 Nov 2020.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2020.1847027

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REVIEW

Buckwheat proteins: functionality, safety, bioactivity, and prospects as alternative plant-based proteins in the food industry

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ABSTRACT

The need for protein in human nutrition is rapidly increasing because of the increasing world population and consumer preference for high-protein foods. Plant proteins are gaining attention as sustainable means of meeting the global protein need due to their lower carbon footprint. Nonetheless, the food industry has neglected or underutilized many plant proteins, including buckwheat protein. Buckwheat is a pseudocereal and its groats contain beneficial components such as proteins, dietary fiber, vitamins, and bioactive polyphenols. The protein quality of buckwheat seeds varies between the tartary and common buckwheat types; both are gluten-free and contain considerable amount of indispensable amino acids. This review provides a detailed discussion on the profile, amino acid composition, digestibility, allergenicity, functional properties, and bioactivity of buckwheat proteins. Prospects of processing buckwheat for improving protein digestibility and deactivating allergenic epitopes were also discussed. Based on the literature, buckwheat protein has a tremendous potential for utilization in structuring food products and developing peptide-based functional foods for disease prevention. Future research should develop new processing technologies for further improvement of the quality and functional properties of buckwheat protein in order to facilitate its utilization as an alternative plant-based protein toward meeting the global protein supply.

KEYWORDS

Alternative protein; bioactivity; buckwheat; digestibility; functional properties; protein

Introduction

Buckwheat ("Qiao" in Mandarin) is mostly cultivated in China and some European countries. With the global production of 3,827,748 tons in 2017, Russia contributed 1,524,280 tons whereas China produced 1,447,292 tons of buckwheat; followed by Ukraine (180,440 tons), France (127,406 tons), Kazakhstan (120,379 tons) and Poland (113,113 tons) (FAOSTAT 2018). Buckwheat is categorized into two types: common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*), with each species differing in phenotype including in terms of their nutritional and phytochemical compositions. On a dry weight basis, buckwheat contains 8–18% protein (Bejosano and Corke 1999; Škrabanja and Kreft 2016), with differences reported among different species, common vs. tartary, wild vs. cultivated, and diploid vs. tetraploid buckwheat (Guo, Chen, et al. 2007). Moreover, the method used for processing influences the total protein content of buckwheat protein isolate, with defatting and mechanical treatments resulting in higher protein content (~80%) compared to ultrasound pretreatment (65%) (Tang 2007). These treatments can disrupt the rigid plant cell structures thereby releasing the proteins from their complexes with non-protein components. For instance, removal of polyphenols in buckwheat flour using organic solvents significantly increased the protein content of buckwheat protein isolate from 88% (control) to ~95% (Tang, Wang, et al. 2009).

Albumin, globulin and glutelin are known to be the main fractions of buckwheat protein; however, one study found that the amounts of glutelin (~14%) and prolamin (~10%) were higher in tartary buckwheat than the amount of globulin (~8%), with albumin making up the majority (~44%) of the protein (Guo and Yao 2006). These differences may have resulted from the protein extraction methods, particle properties, sources and species of the grain, as well as physical part of buckwheat groats used in the studies. For the latter, a recent study found that the buckwheat protein contents decreased from the exterior to the interior parts of the groats (Chen, Chen, et al. 2019). This implies that the buckwheat bran flour contains more proteins than the endospermic fractions. In addition to the well-known seed storage proteins, a recent shotgun proteomic analysis resulted in the identification of 3,363 proteins in tartary buckwheat seeds belonging to a range of functional protein groups, such as enzymatic, regulatory, structural, and transport proteins (Wang, Xiao, et al. 2019).

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The molecular weight of the protein fractions provides important information for their utilization in food product development. Under non-reducing condition, major SDS-PAGE bands of buckwheat proteins were found at 41 kDa (proposed to be albumin) and 56–69 kDa (proposed to be legumin-type globulins), and minor bands were found at 57–58 kDa (proposed to be vicilin-type proteins), 26–36 kDa and 14–23 kDa (Radović, Maksimović, and Varkonji-Gašić 1996; Ma and Xiong 2009). Under reducing condition, the 56–69 kDa bands dissociated into low-molecular weight subunits whereas the 41 kDa and minor bands remained intact (Radović, Maksimović, and Varkonji-Gašić 1996; Guo and Yao 2006). Thus, the major globulin fraction was proposed to compose of acidic and basic subunits that are connected through disulfide linkages (Radović, Maksimović, and Varkonji-Gašić 1996).

Amino acid composition is an important factor for determining the nutritional quality of proteins. The protein content of buckwheat is relatively lower than that of common plant sources, such as legumes. Nonetheless, the amino acid score of buckwheat protein is 100 and its content of indispensable amino acid is comparable to the FAO/WHO suggested amino acid requirements for both children and adults (FAO 2011). Buckwheat protein is rich in lysine (Wijngaard and Arendt 2006; Zhou et al. 2015), which is the most limiting amino acid in cereal proteins and cereal-based diets. Buckwheat protein contains a low amount of prolamins and lacks gluten; thus, it can be used for developing gluten-free food products for consumers with gluten sensitivity. In contrast to cereal proteins, leucine is the limiting amino acid in buckwheat proteins. Thus, buckwheat protein can complement the amino acid profile of other cereal proteins when used together in food products. Moreover, plant breeding and genetic modification can enhance the quality of buckwheat proteins.

Many studies on buckwheat focused on utilizing the proteins to prepare nutraceuticals with beneficial effects on human health, especially in disease prevention. For nutritional and product development purposes, buckwheat proteins are underutilized in the food industry possibly because they are perceived to have lower nutritional quality, or because of insufficient data on their functional properties when compared to widely utilized proteins such as soybean proteins. In 2018, over 820 million people in the world were faced with hunger (FAO, IFAD, UNICEF, WFP and WHO 2019). In addition to lack of food, "hunger" encompasses food insecurity and related health issues. The need for protein supply is increasing rapidly because of the burgeoning world population and consumer preference for high-protein foods (Berryman et al. 2018). This review provides a comprehensive discussion on the quality (amino acid composition and digestibility), allergenicity, functional properties, and biological activities of buckwheat protein, and its potential utilization in the food industry as alternative plant-based proteins.

Digestibility of buckwheat proteins

Digestibility is a key indicator of dietary protein quality because proteins are mainly absorbed in the form of dipeptides and tripeptides resulting from digestion by gastro-pancreatic proteases (Silk, Grimble, and Rees 1985). The digestibility of buckwheat protein is ~80%, which is lower than the digestibility of animal proteins such as hemoglobin and ovalbumin (Ikeda and Kishida 1993) but comparable to or higher than the digestibility of cereal proteins (e.g., sorghum, 55–59%; maize, 66–75%; rice bran, 89%; wheat germ, 77–93%) (Duodu et al. 2002; Han, Chee, and Cho 2015; Hassan et al. 2010; Jurkovic and Colic 1993; Yousif and El Tinay 2001; Yousif and El Tinay 2000). Thus, buckwheat protein may not be completely bioavailable after digestion despite its balanced indispensable amino acid composition. The relatively low digestibility is attributable to the molecular structure of buckwheat proteins and the presence of anti-nutritional factors in the grain flour and protein isolates.

Effects of molecular structure characteristics on buckwheat protein digestibility

Buckwheat albumins with molecular weights of 24–-67 kDa were digested more rapidly with pepsin than that with ~14.3 kDa molecular weight, even after prolonged incubation (Ikeda and Kishida 1993). The authors suggested that the state of the protein surface might have influenced digestibility because of differences in the amounts of free sulfhydryl group present. Nonetheless, digestibility did not appear to depend on the protein hydrophobicity. The ~14 kDa buckwheat protein that was resistant to pepsin was digested extensively by pancreatic proteases (Ma and Xiong 2009). The resistance to peptic digestion might be due to the steric hindrance imposed by the hexameric structure of the proteins, with subunits stabilized by disulfide bonds. Besides, the processing method used for pretreatment and isolation may influence the molecular conformation of the proteins, thereby enhancing or decreasing their digestibility. For instance, rice proteins prepared by enzyme-assisted microfluidization (contained glutelin) had higher digestibility than the protein prepared by alkaline extraction (contained prolamin and globulin) (Xia et al. 2012). Alkaline extraction is the most popular method for isolating buckwheat proteins; this method can result in the formation of lysinoalanine residues in proteins, which is resistant to enzymatic hydrolysis. As stabilization of protein conformational states may lead to decrease in digestibility (Markus 1965), future studies are needed to delineate how the differential structures of individual buckwheat protein molecules influence the combined digestibility of the protein mixture utilized in food product development.

Effects of anti-nutritional factors on buckwheat protein digestibility

Protease inhibitors, tannins, phytic acid and saponins are the major antinutritional factors in buckwheat that can decrease protein digestibility in the digestive tract (Ahmed et al. 2014; Deng et al. 2015; Zhang et al. 2015). Protease inhibitors, especially trypsin inhibitors, which make up 5–10% of the water-soluble proteins (Wijngaard and Arendt

2006), can compete for the active site of trypsin. Such binding will make the enzyme inaccessible to the protein recognition and cleavage sites. Functionally relevant interaction between phenolic compounds and proteins is well established (Arts et al. 2002; Sivam et al. 2013; Skrabanja, Laerke, and Kreft 2000). For instance, hydrothermal-induced interaction between polyphenols and proteins reduced the digestibility of buckwheat proteins in the small and large intestines (Skrabanja, Laerke, and Kreft 2000). Tannin binds with proteins to form insoluble tannin-protein complexes that are stable at the gastric and intestinal pH, and in the presence of digestive proteases (Fuller 1989; Riedl and Hagerman 2001). Phytic acid can also form complexes with both proteins and proteases (Boye, Wijesinha-Bettoni, and Burlingame 2012), or compete for metal cofactors of brush border peptidases (Joye 2019), thereby inhibiting proteolysis. Polyphenols in buckwheat husks were reported to reduce the true digestibility of buckwheat proteins (Skrabanja, Laerke, and Kreft 2000). However, it is not clear if the polyphenols reduce digestibility by forming indigestible buckwheat protein-polyphenol complexes, or by inhibiting the digestive proteases directly. For the latter, interaction of phenolic compounds with digestive proteases can destabilize or stabilize the enzyme structure resulting in changes in enzymatic activity and protein digestibility (Cirkovic Velickovic and Stanic-Vucinic 2018).

Potential processing methods for improving buckwheat protein digestibility

In view of the effect caused by the presence of antinutritional factors or the molecular structure of proteins, several studies have focused on developing methods for improving plant protein digestibility. Processing methods used to achieve this goal include bioprocessing (e.g., germination and fermentation), physical processing, and enzymatic hydrolysis or enzymolysis.

Germination

Germination has successfully lowered the amount of antinutritional factors, such as phytate, in cereals thereby increasing the content of soluble proteins and improving their digestibility. For instance, 72-h germination of buckwheat increased the crude protein content by 7% (Zhang et al. 2015). This effect may be because of higher rate of protein biosynthesis than degradation (Hübner and Arendt 2010), the polyphenol-protein complex degradation during germination, or the elevated activity of seed proteolytic enzymes, which can predigest and thus increase the amounts of the soluble protein components. Although an increase in protease activity was not detected, Hübner and Arendt (2010) reported a decrease in some protein bands (31–45 kDa) with an increase in low molecular weight bands at 6–17 kDa after 144 h of buckwheat germination. Furthermore, the germination-induced protein degradation differs depending on the protein fraction. Germination was reported to completely degrade only the globulin and glutelin fractions of buckwheat due to increased protease activity, whereas new bands were detected in the albumin and prolamin fractions possibly due to enzyme biosynthesis (Phiarais, Schehl, and Arendt 2008). The overall pre-digestion effect can increase the protease accessibility and digestibility of buckwheat protein. Conversely, germination for 7 days decreased the protein content and increased the amino acid content of tartary buckwheat sprouts (Yiming et al. 2015). Thus, the germination condition needs to be optimized to effectively release the proteins from the seed structure without complete degradation. The enzymes controlling seed germination may also degrade antinutritional factors. For instance, germination resulted in elimination of the phytic acid and trypsin inhibitor present in buckwheat seeds (Ikeda et al. 1984; Zhang et al. 2015). As sprouted buckwheat is popular among consumers, it is worth studying extensively the potential of using the germination process to improve buckwheat protein quality, including digestibility and indispensable amino acid profile.

Fermentation

Microbial fermentation is an effective way of improving the digestibility of proteins. This is based on its effectiveness in removing antinutritional factors, phytate and trypsin inhibitor (Fagbemi, Oshodi, and Ipinmoroti 2005), and in increasing the content of soluble proteins due to the release of low molecular weight polypeptides. Fermentation has been used to increase the protein digestibility of cereals, such as sorghum (Ogodo et al. 2019) and wheat (Rizzello et al. 2010). This bioprocess has also been used to improve the protein digestibility of buckwheat products. Solid-state fermentation with *Rhizopus oligosporus* increased the protein digestibility of buckwheat grout from 39.9% (control) to 55.6%, with concomitant increase in the content of indispensable amino acids Cys + Met and Phe + Tyr (Starzyńska-Janiszewska et al. 2016). Functional mechanisms underlying these effects have not been reported, but it is possible that the microbial enzymatic activity altered the grout microstructure to release the proteins or directly predigested the proteins to enhance their digestibility, as reported for legumes (Ohanenye et al. 2020). Therefore, there is a tremendous opportunity in exploring the effect of the microbial enzymatic machinery during submerged or solid-sate fermentation on the digestibility, bioaccessibility and bioavailability of buckwheat proteins.

Physical processing

Physical processing is a comprehensive concept involving the use of several conventional and emerging technologies. The conventional processing technologies include size reduction (Joye 2019), fractionation (Chen, Luo, et al. 2019), cooking (Liu, Zheng, and Chen 2019), dehydration (Martín-Cabrejas et al. 2009), toasting, and extrusion (Nosworthy et al. 2017). Emerging processing technologies include microwave (Deng et al. 2015; Liu, Zheng, and Chen 2019), ultrasound (Zhang et al. 2018), high hydrostatic pressure (Deng et al. 2015; Liu, Zheng, and Chen 2019), and cold

Table 1. Buckwheat meals and their associated allergic responses.

Age (years)	Gender	Type of food consumed	IgE	Allergenic properties (reactions)	References
8	F	Noodles ("zaru soba")	2.8 kU/mL	Ulceration, necrosis, hemorrhage	(Noma et al. 2001)
40	F	Wheat burger	3.84 kU/L	Heat sensation (neck), swollen hands and urticaria	(Wüthrich and Trojan 1995)
27	F	Pancakes ("poffertjes")	2.7 kU/L	Flush, nausea and vomiting	(Maat-Bleeker and Stapel 1998)
23	F	Pancakes ("poffertjes")	16 kU/L	Cyanosis and unconsciousness	(Maat-Bleeker and Stapel 1998)
32	F	Porridge	>100 kU/L	Urticaria, and an acute exacerbation of asthma	(Wang et al. 2006)
27	F	Muesli bar	3.29 kU/L	Urticaria, nausea, and mild laryngeal edema	(Schiffner et al. 2001)
36	M	Cake	>100 kU/L	Nausea, vomiting, urticaria, dyspnea, and dizziness	(Stember 2006)
7	M	Buckwheat mixed buffet	51.9 kU/L	Angioedema, sneezing, a dry cough, vomiting and urticaria	(Varga et al. 2011)

plasma (Sadhu et al. 2017). The increase in buckwheat protein digestibility achieved with boiling, high hydrostatic pressure and microwave treatments was possibly associated with the deactivation of antinutritional factors (Deng et al. 2015). However, the digestibility of polyphenol-rich buckwheat protein slightly decreased after autoclaving (Chen et al. 2019). This is attributable to the formation of polyphenol-protein complexes during hydrothermal processing (Skrabanja, Laerke, and Kreft 2000). The basic principles underlying the physical processing include: (1) direct inactivation of antinutritional factors or disassembly of their complexes with proteins; (2) increase in the contact area between proteins and proteases; and (3) change in the protein molecular conformation. As buckwheat protein-polyphenol interactions are stabilized by hydrophobic, hydrogen and covalent bonds, physical treatments can disrupt the complexes to expose the proteins to digestive proteases. Recently, sonication at 20 kHz and 60% amplitude for 10 min was reported to increase the in vitro digestibility of buckwheat protein isolate from 41.4% (control) to 58.2%; this effect was attributed to protein unfolding, breakage of intermolecular crosslinks, and alteration of the protein secondary structure (Jin et al. 2020). However, protein unfolding due to the treatments can expose the hydrophobic core, which can facilitate protein aggregation and formation of indigestible complexes through hydrophobic interactions. Therefore, a combination of the processing technologies may be useful for optimizing buckwheat protein digestibility, but care should be taken to ensure that the downstream processes are cost-effective and sustainable.

Enzymolysis

Enzymatic hydrolysis is widely used as a processing method for the modification of protein functionality and production of bioactive peptides (Clemente 2000; McCarthy, O'Callaghan, and O'Brien 2013; Udenigwe and Aluko 2012; Jin et al. 2015). This method can improve the digestibility of buckwheat proteins since enzymolysis can simultaneously degrade antinutritional factors (e.g., phytate deactivation by phytase) and transform bound or insoluble proteins into soluble polypeptides. Enzymatic protein hydrolysates are suitable sources of proteins for human nutrition because they contain many small peptides, especially dipeptides and tripeptides, which can be absorbed more effectively than larger polypeptides and free amino acids (Clemente 2000; Manninen 2009; Roberts et al. 1999). Another approach is to prepare buckwheat protein isolates by enzymatically removing starch in the flour to release proteins from starch-protein complexes. A recent study however reported a decrease in the digestibility of proteins prepared after treatment of buckwheat flour with α-amylase and amyloglucosidase; this effect was attributed to the higher amount of polyphenols found in the protein sample (Chen, Luo, et al. 2019). Given the potential, future studies should consider using the diverse cleavage specificities of commercial enzymes and emerging hydrolysis approaches (e.g., ultrasound- and microwave-assisted) in enhancing buckwheat protein digestibility.

Safety of buckwheat protein

Although plant proteins are projected as more sustainable and environmentally friendly alternatives to animal proteins, plant protein allergy is still a concern for the food industry and some consumers. Allergenic proteins are present in some commonly consumed legumes (e.g., soybean) and cereals (e.g., wheat). In some individuals, buckwheat proteins present symptoms of allergic reactions such as asthma, hives, wheezing, anaphylactic shock and skin disorder (Wieslander and Norbäck 2001), as shown in **Table 1**. Moreover, health conditions associated with celiac disease are due to responses to gliadin from the consumption of foods made with gluten-containing ingredients, e.g., wheat, barley and rye (Bai et al. 2013; Janssen et al. 2017). The prevalence of celiac disease and gluten-intolerance cases has been on the rise (Lohi et al. 2007), thus necessitating a shift toward gluten-free food products for susceptible consumers.

To date, all isolated plant allergens are reported to be proteins, and the allergenic epitopes are classified as linear or conformational (Ivanciuc et al. 2009). Three allergenic proteins, including a trypsin inhibitor, were isolated from buckwheat seeds (Yano et al. 1989). The molecular weight of the buckwheat allergenic proteins ranged from 8–9 kDa; the protease inhibitors were heat-resistant as they retained their activity after boiling for 1 h. Later, two protease inhibitors known as BWI-1 and BWI-2b were identified in buckwheat and found to possess IgE-binding activity (Park et al. 1997). Furthermore, four possible allergenic proteins with molecular weights of 9 kDa, 16 kDa, 24 kDa and 29 kDa (Park et al. 2000), and an allergenic 2S albumin with a molecular weight of 10 kDa were reported to occur in buckwheat seeds (Matsumoto et al. 2004). The major protein allergens identified in tartary buckwheat are different and include Fag t 2 and Fag t 3 with molecular weights of 16 kDa and 56 kDa, respectively (Zhu 2016). Fag t 2 is a heat-resistant and pepsin-resistant 2S albumin protein. Fag t 3 however is a legume-like 11S storage protein and its allergenicity was

reported to be reduced substantially by heat treatment or glycation (Chen et al. 2011; Yang et al. 2013; Zhang et al. 2008). Such covalent modification of proteins can cause a steric hindrance in the recognition and binding sites of the epitopes, thereby limiting the binding of the immunoglobulin paratope. Germination was also found to degrade and eliminate some protein bands associated with buckwheat allergies (Hübner and Arendt 2010). In addition, the reduction of IgE reactivity correlated with enzymatic hydrolysis using papain and high-pressure treatment, which changed the secondary structure of buckwheat proteins (Lee et al. 2016). It is likely that this effect was associated with alteration of the conformational epitopes of the buckwheat proteins. The finding shows the potential to reduce the allergenic properties of buckwheat protein using emerging processing technologies, which also include ultrasound, microwave and cold plasma, as observed for other food allergens (Okolie, Aryee, and Udenigwe 2018).

Unlike major cereals (e.g., wheat, barley and rye) and legumes (e.g., soybean and pea), there is a small number of reports on health issues associated with buckwheat consumption (Table 1). Besides, the endosperm was found to contain undetectable amount of the low molecular weight proteins associated with buckwheat allergy when compared to the embryo (Ličen and Kreft 2005). Thus, fractionation of buckwheat can yield low allergenic protein components that can be used as alternatives to cereal proteins in food product development. Given its potential allergenicity in some individuals, there is still a need for further investigation into the safety, health risks and processing-induced deactivation of the allergenic proteins in buckwheat.

Functional properties of buckwheat protein

The solubility, emulsification, water- and fat-holding, and foaming capacities are the major functional properties of proteins that determine their use in food product development. Buckwheat is not a major source of protein in the food industry but it has potential for food application, as the protein fraction is gluten-free and possesses functional properties comparable to those of some commonly utilized food proteins. For instance, buckwheat protein concentrates obtained by alkaline extraction were more soluble in water than soy proteins and bovine casein at acidic pH, and less soluble than only bovine casein at neutral and alkaline pH (Bejosano and Corke 1999; Tomotake et al. 2002). Moreover, buckwheat protein concentrate demonstrated poor foaming ability compared to soy protein (Bejosano and Corke 1999). Nonetheless, the globulin fraction of buckwheat protein demonstrated significantly higher foamability and foam stability at 30 and 60 min compared to a commercial soy protein product (Choi and Ma 2006). This demonstrates the beneficial role of fractionation in enhancing the protein functionality.

Protein emulsification depends on its solubility, surface hydrophobicity, surface charge and molecular conformation. Emulsification ensures the retention of fat and flavor and the maintenance of consistent texture in food products. Buckwheat protein concentrate and the globulin fraction demonstrated higher emulsifying activity than soy protein products; this may be because of the high buckwheat protein solubility near neutral pH (Bejosano and Corke 1999; Choi and Ma 2006). Conversely, the emulsion stability and viscosity of buckwheat protein isolate were low at pH 7–10 when compared to soy protein and bovine casein (Tomotake et al. 2002). This result may be because of the differences in the structure of proteins in the samples, or the presence of interfering non-protein components in the buckwheat protein product. Covalent modification of buckwheat protein by glycation with several sugars significantly increased its emulsification property because of the enhanced surface activity of the conjugates (Guo and Xiong 2013; Xue et al. 2017). Furthermore, buckwheat protein and its globulin fraction had lower water holding capacity than soy protein and a similar or higher fat absorption capacity than soy protein and casein (Tomotake et al. 2002; Choi and Ma 2006). The difference in fat absorption capacity could be due to differences in the apolar amino acid content, protein sizes of buckwheat and soy proteins, or amount of lipids in the protein products. These findings show that buckwheat proteins have some potential but further studies are needed to comprehensively characterize their functional properties and assess their future application as alternative plant-based proteins in food product development.

Bioactivity of buckwheat protein and bioactive peptides

The bioactivity of food protein-derived peptides is an important topic in the functional food, nutraceutical and cosmeceutical industries. This is because of the multifunctional health-promoting and disease-preventing properties of the peptides. Buckwheat protein is a valuable raw material for generating bioactive peptides because of its diverse protein molecular composition. To date, the biological activities reported for buckwheat peptides include cholesterol-lowering, antihypertensive, antimicrobial, antioxidative, and antitumor activities (Tables 2 and 3).

Cholesterol-lowering activity of buckwheat proteins

Cholesterol is an indispensable biomolecule as a crucial structural component of cells and the precursor for endogenous synthesis of important compounds. However, abnormal levels of total and LDL cholesterol result in atherosclerosis, which may lead to coronary heart disease (Sharrett et al. 2001; Zhang et al. 2017). Therefore, dietary factors that lower endogenous cholesterol level play important roles in cardiovascular disease prevention. Some food proteins, hydrolysates and peptides are known to have hypocholesterolemic properties based on in vitro, cellular, animal and human studies (Boachie, Yao, and Udenigwe 2018; Udenigwe and Rouvinen-Watt 2015).

Dietary buckwheat protein regulates cholesterol metabolism. For instance, rats fed with diets containing buckwheat protein showed lower levels of plasma and hepatic

Table 2. Bioactivities of buckwheat-derived peptides.

Activities	Sequence	IC_{50}	Reference
Antioxidant	WPL	n.d.	(Ma et al. 2010)
	VPW	n.d.	
	VFPW	n.d.	
	PW	n.d.	
ACE inhibitory	Protein fraction	0.36 mg /mL	(Li et al. 2002)
	FY	25 μM	
	AY	100 μM	
	LF	126 μM	
	YV	580 μM	
	VK	13 μM	
	YQ	628 μM	
	YQY	4 μM	
	PSY	16 μM	
	LGI	29 μM	
	ITF	49 μM	
	INSQ	36 μM	
	GPP	6.25 μg/mL	(Ma et al. 2006)
	DVWY	0.69 mM	(Koyama et al. 2014; Koyama et al. 2013)
	FDART	1.9 mM	(Koyama et al. 2014; Koyama et al. 2013)
	FQ	7.4 mM	
	VAE	55.9 mM	
	VVG	39.6 mM	
	WTFR	6.7 mM	(Koyama et al. 2013)
Antimicrobial	AQCGAQGGGATCPGGLCCSQ WGWCGSTPKYCGAGCQSNCK (Fa-AMP1)	11 ~ 36 μg/mL	(Fujimura et al. 2003)
	AQCGAQGGGATCPGGLCCSQW GWCGSTPKYCGAGCQSNCR (Fa-AMP2)	11 ~ 36 μg/mL	(Fujimura et al. 2003)
	SEKPQQELEECQNVCRMKR WSTEMVHRCEKKCEEKFERQQR (BWI-2c)		(Oparin et al. 2012)
	N-terminal: AQXGAQGGGAT (FEAP)	79.9 μg/mL against spore germination 236.7 μg/mL against mycelial growth in *Botrytis cinerea*	(Wang, Yuan, et al. 2019)
	N-terminal: AQCGAQGGGATCPGG	35 μM against *Fusarium oxysporum* 40 μM against *Mycosphaerella arachidicola*	(Leung and Ng 2007)

cholesterol, hepatic triglyceride, and fat pad weight compared to rats that received control diets (Kayashita, Shimaoka, et al. 1996; Kayashita, Shimaoka, and Nakajyoh 1995). The effect of buckwheat protein on endogenous cholesterol levels was probably due to elevated fecal excretion of sterols associated with the low digestibility of some of the proteins (Kayashita et al. 1997). Indigestible dietary proteins and polypeptides can bind and form insoluble complexes with bile acids in the colon, thereby decreasing enterohepatic circulation and increasing excretion of the steroid acids, with concomitant increase in hepatic catabolism of cholesterol (Boachie, Yao, and Udenigwe 2018). Hamsters fed with a buckwheat protein diet had markedly suppressed gallstone formation with decreased cholesterol level in the gallbladder, plasma and liver; this resulted from upregulation of hepatic utilization of cholesterol to form bile acids and subsequent excretion in the feces (Tomotake et al. 2000; Tomotake et al. 2007; Tomotake et al. 2006). Moreover, a study in vitro showed that 83% of cholesterol was bound directly to an insoluble buckwheat protein fraction, leading to a reduction in cholesterol transport into intestinal monolayer cells (Metzger, Barnes, and Reed 2007). This type of bioactivity can be beneficial in reducing the intestinal absorption of dietary cholesterol in vivo (Udenigwe and Rouvinen-Watt 2015). In a more recent study, Zhang et al. (2017) reported that tartary buckwheat protein fed to hamster led to a better effect in lowering plasma cholesterol than the activity of cholestyramine, a bile acid sequestrant. This activity was mainly associated with fecal bile acid excretion through increased expression of hepatic gene of the enzyme that converts cholesterol to bile acids, and by inhibiting dietary cholesterol absorption through decreased expression of intestinal genes of proteins for transporting dietary cholesterol (Zhang et al. 2017).

In these studies, the reported endogenous cholesterol-lowering activities were ascribed to buckwheat proteins. However, gastrointestinal proteases digest dietary proteins to produce short and long peptide fragments and amino acids. Thus, it is possible that poorly digestible buckwheat proteins are involved in direct binding and fecal excretion of the sterols. Conversely, the effects on gene expression are likely due to buckwheat peptides released during in vivo digestion, but the bioavailability of these peptides in intestinal and hepatic tissues as well as effects in reducing endogenous cholesterol level need to be established.

Blood pressure controlling enzyme inhibitory activity of buckwheat peptides

Angiotensin converting enzyme (ACE) is a popular molecular target for developing antihypertensive agents. ACE catalyzes the processes of producing a vasoconstrictor (angiotensin II) and degrading a vasodilator (bradykinin) in the vascular endothelium. Thus, ACE inhibition is beneficial in preventing hypertension. Food proteins are well known precursors of ACE-inhibiting peptides and many of the peptides reduce blood pressure in hypertensive animals and

Table 3. Biological *activities* of buckwheat protein and peptides in different animal and cellular models.

Sample	Source/treatment	Model	Dosage/condition	Outcome	Reference
Buckwheat protein extract		Male Sprague-Dawley rats (3-wk old)	Fed as protein source for 3 weeks	Total cholesterol, cholesterol in liver was lower, HDL triglyceride in liver was higher than those fed with soy protein isolate and casein	(Kayashita, Shimaoka, and Nakajyoh 1995)
		Female Sprague-Dawley rats (4-wk old)	Fed as protein source for 61 days	Retard 7,12-Dimethylbenz[α] anthracene-induced mammary carcinogenesis	(Kayashita, Shimaoka, Nakajoh, Kishida, et al. 1999)
		Male Sprague-Dawley rats	Fed as protein source for 3 weeks	Hepatic triglyceride concentration was lower than those fed with casein	(Kayashita, Shimaoka, et al. 1996)
			Fed as protein source for 3 weeks	Suppressed growth depression	(Kayashita, Nagai, et al. 1996)
Buckwheat protein product		Male Sprague-Dawley rats (3 wk old)	Fed as protein source (20%) for 3 weeks	Lowered plasma cholesterol	(Kayashita et al. 1997)
Digested buckwheat protein	Porcine trypsin digestion (10 g/kg, temperature = 45 °C, time = 5 h, pH =8.0)	Male Sprague-Dawley rats (3-week old)	Fed as protein source (20%) for 2 weeks	Plasma cholesterol lower compared to rats fed casein, but higher in those fed with undigested buckwheat protein	(Kayashita et al. 1997)
Low-molecular-weight fraction (LMF) and high-molecular-weight fraction (HMF)			Fed as protein source (20%) for 3 weeks	Rats fed with LMF or HMF showed higher total cholesterol than those fed with buckwheat protein	
High protein buckwheat flour and buckwheat protein extract		Male Sprague-Dawley rats (70 g)	Fed as protein source (net protein content 20%) for 10 days	Flour and protein caused 33% and 31% decrease in serum cholesterol of rats fed cholesterol-enriched (0.5%) diets, resp. Flour suppressed adipose tissue weight and hepatic activity of fatty acid synthase in rats fed cholesterol-free diet	(Tomotake et al. 2006)
		Male Sprague-Dawley rats (28 g)	Fed as protein source (net protein content 20%) for 27 days	PBF and BWP suppressed gallstone formation in mice fed cholesterol-enriched (1%) diet	
Buckwheat protein product		Male Golden Syrian hamsters (63–83 g)	Fed as protein source (net protein content 20%) + 0.5 % cholesterol for 2 weeks	Suppressed gallstone formation and plasma cholesterol	(Tomotake et al. 2000)
Buckwheat protein extract		Male Wistar rats (70 g)	Fed as protein source (net protein content 20%) for 5 weeks	Muscle hypertrophy, reduced body fat	(Kayashita, Shimaoka, Nakajoh, Kondoh, et al. 1999)
Buckwheat Protein Extract		Male Sprague-Dawley rats (130–148 g)	Fed as protein source (net protein content 20%) for 8 days	Alleviated atropine-induced constipation	(Kayashita, Shimaoka, Yamazaki, et al. 1995)
Tartary buckwheat protein		Male C57BL/6 mice (4-week old)	Fed high-fat (27.3%) diet with BWP (20%) for 6 weeks	Inhibition of inflammation and prevention of hypercholesterolemia	(Zhou et al. 2018)
Tartary buckwheat protein		Male hamsters	Fed as protein source (net protein content 24%) for 6 weeks	Reduction of plasma total cholesterol	(Zhang et al. 2017)
Tartary buckwheat protein (TBP) and common buckwheat protein (BWP)		Male Sprague-Dawley rats (70 g)	Fed as protein source (20% net protein) for 13 days	BWP and TBP caused 32% and 25% reductions in serum cholesterol, respectively.	(Tomotake et al. 2007)
Tartary buckwheat protein (TBP) and common buckwheat protein (BWP)		Male ddY mice (28 g)	Fed as protein source (20% net protein) for 27 days	BWP and TBP caused 62% and 43% reductions in the lithogenic index, respectively.	(Tomotake et al. 2007)
DVWY, FDART, FQ, VAE, VVG, WTFR	Isolated from fermented buckwheat sprouts	Male spontaneously hypertensive rats (SHRs) (10–13 week old)	A single oral dose of 10 mg/kg body weight	6 h after administration: DVWY, FDART, FQ, VAE, WTFR decreased Systolic Blood Pressure by 28.3, 24.7, 27.3, 15.8, 20.1 and 21.1 mmHg, respectively. Decreased diastolic blood pressure by 19.2, 16.9, 18.6,	(Koyama et al. 2014)

(*continued*)

Table 3. Continued.

Sample	Source/treatment	Model	Dosage/condition	Outcome	Reference
				15.8, 17.0 and 16.6 mmHg, respectively.	
Buckwheat digestion	Hydrolysate from pepsin (pH 1.2, 4 h, 37 °C), followed by chymotrypsin and trypsin (pH 6.8, 4 h, 37 °C)	Male SHR (15-week old)	A single oral administration of 100 mg/kg of body weight	SBP decreased by 27.0, 29.9 mmHg after 2 and 4 h post administration, respectively.	(Li et al. 2002)
water-soluble buckwheat protein		Hepatic HepG2 cell culture		Antioxidative and hypocholesterolemic activities	(Zhou et al. 2013)
TBWSP31		Breast cancer (Bcap37) cell culture	Incubation with 10 μg/mL of TBWSP31 for 48 h	Anti-tumor activity	(Guo et al. 2010)
Buckwheat antifungal protein	Isolated from buckwheat (*Fagopyrum esculentum*) seeds	L1210 (leukemia), HepG2 (hepatoma), MCF 7 (breast cancer), and WRL68 (liver embryonic) cell cultures		IC_{50} values against proliferation of L1210, MCF-7, HepG2, WRL 68, and HIV-1 reverse transcriptase were 33, 4, 25, 37, and 5.5 μM, respectively.	(Leung and Ng 2007)

humans (Cadée et al. 2007; Koyama et al. 2014; Seppo et al. 2003; Tavares et al. 2012). Buckwheat protein has been used to produce ACE inhibitors. For instance, intact buckwheat protein and protein hydrolysate obtained from in vitro digestion using pepsin + chymotrypsin + trypsin inhibited ACE activity with IC_{50} (half-maximal inhibitory concentration) values of 3 and 0.14 mg/mL, respectively. This means that enzymolysis increased the bioactivity of buckwheat protein by 21 folds. Moreover, single oral intake (100 mg/kg body weight) of buckwheat protein hydrolysate produced with gastrointestinal proteases effectively lowered the systolic blood pressure of spontaneously hypertensive rats by 27.0 and 29.9 mmHg after 2 and 4 h post consumption, respectively (Li et al. 2002). An ACE-inhibiting tripeptide (GPP) with an IC_{50} of 6.25 μg/mL was identified in alkali-extracted buckwheat protein product (Ma et al. 2006). Koyama et al. also identified six peptides with 2–5 amino acid residues from buckwheat sprouts fermented with *Lactobacillus plantarum* (Koyama et al. 2013). Single oral dose (0.1 mg/kg body weight) of each of the six peptides to spontaneously hypertensive rats significantly lowered systolic blood pressure, with the most potent activity observed for tetrapeptide DVWY (-54.9 mmHg at 3 to 6 h), dipeptide FQ (-33.6 mmHg at 9 h), and tripeptide VVG (-28.3 mmHg at 9 h). Notably, some of the buckwheat antihypertensive peptides (DVWY, VAE and WTFR) were novel. Considering the promising results, future studies should evaluate the molecular mechanisms of the buckwheat peptides in the vascular endothelium as well as their antihypertensive effects in hypertensive human subjects.

Antimicrobial peptides derived from buckwheat protein

Because of the cultivation conditions, buckwheat has developed advanced strategies for injury repair and defense against external environmental factors (Zhou et al. 2011). As a result, some buckwheat peptides have antimicrobial properties. For instance, two peptides (Fa-AMP_1 and Fa-AMP_2; also referred to as Fα-AMP_1 and Fα-AMP_2, respectively) derived from common buckwheat seeds have antimicrobial properties; both peptides contain 40 amino acid residues and four disulfide linkages (Fujimura et al. 2003). The primary sequences of the peptides are similar as they have several Cys and Gly residues, 37% hydrophobic residue content, and a net charge of +2. However, they differ in their C-terminal residues, with Lys in Fα-AMP_1 and Arg in Fα-AMP_2. Because of the structural similarity, the buckwheat peptides have similar antimicrobial activities against plant pathogenic fungi and bacteria with IC_{50} values of 11–36 μg/mL (Fujimura et al. 2003). Moreover, a buckwheat polypeptide with a molecular weight of ~4 kDa was found to inhibit the growth of fungal plant pathogens, *Fusarium oxysporum* (IC_{50} of 35 μM) and *Mycosphaerella arachidicola* (IC_{50} of 40 μM) (Leung and Ng 2007). Notably, the peptide retained its antifungal activity at 70 °C and pH 1–13, suggesting its potential stability under a range of food matrix and processing conditions.

Furthermore, a trypsin inhibitor (BWI-2c) isolated from buckwheat was classified as a plant defense peptide because of its structural similarity to the Fα-AMP class of peptides; the peptide contains 41 amino acid residues and two intramolecular disulfide bridges that stabilize its structure (Oparin et al. 2012; Zhou et al. 2015). Furthermore, an 11-kDa buckwheat protein that was found to be antifungal (Wang, Yuan, et al. 2019) shared a similar N-terminal sequence (AQXGAQGGGAT) with the sequence of Fα-AMP_1 and Fα-AMP_2. The heat-stable and acid-resistant 11-kDa peptide inhibited the spore germination and mycelial growth of plant pathogen, *Botrytis cinerea* with IC_{50} values of 79.9 and 236.7 μg/mL, respectively (Wang, Xiao, et al. 2019). Despite these prospects, buckwheat peptides have yet to be utilized as antimicrobial agents in plant protection, food preservation, or active food packaging.

Antioxidative peptides from buckwheat protein

Several food-derived peptides are antioxidants and can be utilized in controlling oxidation in the food product matrix,

or in preventing oxidative stress and related diseases in humans (Nwachukwu and Aluko 2019). Hydrolysate of common buckwheat protein isolates produced with Alcalase exhibited strong antioxidant properties in different in vitro oxidative models (Tang, Peng, et al. 2009); however, the peptide sequences responsible for these activities are unknown. In vitro digestion of buckwheat protein using pepsin + pancreatin also resulted in an antioxidative product with free radical scavenging, reducing and prooxidant ferric ion chelating capacities (Ma and Xiong 2009; Ma et al. 2010). Six buckwheat protein hydrolysate fractions in these studies contained short chain Pro and Trp-containing peptides WPL, VPW, VFPW, PW, and free tryptophan; the tetrapeptide VFPW was the most effective radical scavenger of the peptides. In addition to the radical-trapping aromatic ring of Phe (F) residue, hydrophobic amino acid residues (V, L) in the sequences are thought to also contribute to the antioxidative capacity of peptides through unknown mechanisms (Udenigwe and Aluko 2011).

Antitumor peptides from buckwheat protein

Some food-derived peptides possess antitumor activities in cell cultures and animal models *via* multiple mechanisms (Rajendran et al. 2017), although there is a dearth of human clinical studies to substantiate these findings. Some of the anticancer peptides were derived from protein hydrolysates whereas others exist as natural polypeptides. For instance, a 57-kDa protein ($TBWSP_{31}$) from tartary buckwheat was reported to have antitumor properties (Guo, Zhu, et al. 2007). $TBWSP_{31}$ inhibited the proliferation of Bcap37 (human breast cancer cell) with IC_{50} of 43.3 and 19.7 μg/mL at 2 and 3 days post treatment, respectively. $TBWSP_{31}$ also suppressed the proliferation of other cancer cell lines with IC_{50} values of 33 µM (Hep G2; hepatoma), 4 µM (L1210; leukemia), 25 µM (MCF-7; breast cancer), and 37 µM (WRL 68; lymphocytic leukemia) (Leung and Ng 2007). Considering its large molecular size, the antitumor buckwheat peptide would not be biostable and bioavailable to exert its effects at target tissues in vivo when consumed orally. Nonetheless, the buckwheat peptide pharmacophores may retain their antitumor activity post-hydrolysis in the digestive tract, but this needs to be investigated using appropriate models.

Other applications of buckwheat protein

Buckwheat proteins have been explored for utilization in biocatalysis and as biomaterials. For instance, Nagaoka and Kayahara used immobilized water-soluble buckwheat proteins as biocatalysts for synthesizing optically active alcohols; the proteins showed promising features including reusability without decrease in yield or optical purity of products during the process of bioconversion (Nagaoka and Kayahara 2000). Wang et al. also prepared a biodegradable film using proteins derived from buckwheat distiller's dried grains and cellulose; the resulting film was reported to have high rigidity because of the compatibility between the bacterial cellulose and buckwheat protein matrix (Wang et al. 2017). There has been an increased emphasis on the development of environmentally friendly materials from agricultural resources or byproducts of food processing. Considering its film-forming and antimicrobial properties, buckwheat proteins have the potential for use in developing antimicrobial films and bio-adhesives for food and biomedical applications.

Conclusion

Buckwheat contains considerable amounts of underutilized proteins that have potential as raw materials for product development in the food industry. As discussed in this review, buckwheat proteins have desirable features, for instance their functional properties, which support their potential use in structuring food products. Moreover, several studies have demonstrated the use of buckwheat proteins in preparing bioactive hydrolysates and peptides. The most notable bioactivity reported is cholesterol lowering activity, which is facilitated by the low digestibility of some buckwheat proteins. The protein digestibility depends greatly on the cell structure and presence of phenolic compounds and protease inhibitors in buckwheat seeds. Consequently, there is a need to develop suitable processing methods for enhancing the quality and facilitating the comprehensive utilization of buckwheat proteins. Available research on buckwheat can also facilitate value-added utilization of its processing fractions. For example, the byproduct of polyphenol extraction from buckwheat in the production of "Ku Qiao Tea" (buckwheat tea) can be further processed to recover proteins for new product development. As buckwheat protein is gluten-free and contains a high amount of lysine, the limiting amino acid of cereals, there is a tremendous opportunity in its use as an alternative plant-based protein for a wide variety of food, health and biomaterial applications.

Disclosure statement

All of the authors declare that there are no conflicts of interest.

Funding

J.J. was supported by the Sichuan Science and Technology Support Program, Key Laboratory of Coarse Cereals Processing; Ministry of Agriculture, Chengdu University (2018CC14); China Scholarship Council; and Longshan Talents Program of Southwest University of Science and Technology (17LZX549). C.C.U. received funding from the Natural Sciences and Engineering Research Council of Canada (RGPIN-2018-06839), and the University Research Chair Program of the University of Ottawa.

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References

Ahmed, A., N. Khalid, A. Ahmad, N. Abbasi, M. Latif, and M. Randhawa. 2014. Phytochemicals and biofunctional properties of buckwheat: A review. *The Journal of Agricultural Science* 152 (3): 349–69. doi: 10.1017/S0021859613000166.

Arts, M. J., G. R. Haenen, L. C. Wilms, S. A. Beetstra, C. G. Heijnen, H.-P. Voss, and A. Bast. 2002. Interactions between flavonoids and proteins: Effect on the total antioxidant capacity. *Journal of Agricultural and Food Chemistry* 50 (5):1184–7. doi: 10.1021/jf010855a.

Bai, J. C., M. Fried, G. R. Corazza, D. Schuppan, M. Farthing, C. Catassi, L. Greco, H. Cohen, C. Ciacci, R. Eliakim, World Gastroenterology Organization, et al. 2013. World Gastroenterology Organisation global guidelines on celiac disease. *Journal of Clinical Gastroenterology* 47 (2):121–6., doi: 10.1097/MCG.0b013e31827a6f83.

Bejosano, F. P., and H. Corke. 1999. Properties of protein concentrates and hydrolysates from Amaranthus and Buckwheat. *Industrial Crops and Products* 10 (3):175–83. doi: 10.1016/S0926-6690(99)00021-7.

Berryman, C. E., H. R. Lieberman, V. L. Fulgoni, III, and S. M. Pasiakos. 2018. Protein intake trends and conformity with the Dietary Reference Intakes in the United States: Analysis of the National Health and Nutrition Examination Survey, 2001–2014. *The American Journal of Clinical Nutrition* 108 (2):405–13.

Boachie, R., S. Yao, and C. C. Udenigwe. 2018. Molecular mechanisms of cholesterol-lowering peptides derived from food proteins. *Current Opinion in Food Science* 20:58–63. doi: 10.1016/j.cofs.2018.03.006.

Boye, J., R. Wijesinha-Bettoni, and B. Burlingame. 2012. Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *British Journal of Nutrition* 108 (S2):S183–S211. doi: 10.1017/S0007114512002309.

Cadée, J. A., C.-Y. Chang, C.-W. Chen, C.-N. Huang, S.-L. Chen, and C.-K. Wang. 2007. Bovine casein hydrolysate (C12 peptide) reduces blood pressure in prehypertensive subjects. *American Journal of Hypertension* 20:1–5.

Chen, P., Y. Guo, Q. Yan, and Y. Li. 2011. Molecular cloning and characterization of Fag t 2: A 16-kDa major allergen from Tartary buckwheat seeds . *Allergy* 66 (10):1393–5. doi: 10.1111/j.1398-9995.2011.02657.x.

Chen, X. W., Y. J. Chen, J. Y. Li, Y. H. Wang, and X. Q. Yang. 2019. Enzyme-assisted development of biofunctional polyphenol-enriched buckwheat protein: physicochemical properties, in vitro digestibility, and antioxidant activity. *Journal of the Science of Food and Agriculture* 99 (6):3176–85. doi: 10.1002/jsfa.9534.

Chen, X.-W., D.-Y. Luo, Y.-J. Chen, J.-M. Wang, J. Guo, and X.-Q. Yang. 2019. Dry fractionation of surface abrasion for polyphenol-enriched buckwheat protein combined with hydrothermal treatment. *Food Chemistry* 285:414–22. doi: 10.1016/j.foodchem.2019.01.182.

Choi, S. M., and C. Y. Ma. 2006. Extraction, purification and characterization of globulin from common buckwheat (Fagopyrum esculentum Moench) seeds. *Food Research International* 39 (9):974–81. doi: 10.1016/j.foodres.2006.06.004.

Cirkovic Velickovic, T. D., and D. J. Stanic-Vucinic. 2018. The role of dietary phenolic compounds in protein digestion and processing technologies to improve their antinutritive properties. *Comprehensive Reviews in Food Science and Food Safety* 17 (1): 82–103. doi: 10.1111/1541-4337.12320.

Clemente, A. 2000. Enzymatic protein hydrolysates in human nutrition. *Trends in Food Science & Technology* 11:254–62.

Deng, Y., O. Padilla-Zakour, Y. Zhao, and S. Tao. 2015. Influences of high hydrostatic pressure, microwave heating, and boiling on chemical compositions, antinutritional factors, fatty acids, *in vitro* protein digestibility, and microstructure of buckwheat. *Food and Bioprocess Technology* 8 (11):2235–45.

Duodu, K., A. Nunes, I. Delgadillo, M. Parker, E. Mills, P. Belton, and J. Taylor. 2002. Effect of grain structure and cooking on sorghum and maize *in vitro* protein digestibility. *Journal of Cereal Science* 35 (2):161–74. doi: 10.1006/jcrs.2001.0411.

Fagbemi, T., A. Oshodi, and K. Ipinmoroti. 2005. Processing effects on some antinutritional factors and *in vitro* multienzyme protein digestibility (IVPD) of three tropical seeds: Breadnut (*Artocarpus altilis*), cashewnut (*Anacardium occidentale*) and fluted pumpkin (*Telfairia occidentalis*). *Pakistan Journal of Nutrition* 4:250–6.

FAO. 2011. Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. *Food Nutrition Paper* 92: 1–66. http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf.

FAO, IFAD, UNICEF, WFP and WHO. 2019. The state of food security and nutrition in the world 2019. Safeguarding against economic slowdowns and downturns. Rome: FAO. https://www.wfp.org/publications/2019-state-food-security-and-nutrition-world-sofi-safeguarding-against-economic.

FAOSTAT (Statistics Division of Food and Agriculture Organization of the United Nations). 2018. http://www.fao.org/faostat/en/#data/QC

Fujimura, M., Y. Minami, K. Watanabe, and K. Tadera. 2003. Purification, characterization, and sequencing of a novel type of antimicrobial peptides, Fa-AMP1 and Fa-AMP2, from seeds of buckwheat (Fagopyrum esculentum Moench.). *Bioscience, Biotechnology, and Biochemistry* 67 (8):1636–42. (doi: 10.1271/bbb.67.1636.

Fuller, S. J. 1989. Effect of tannins on the *in vitro* digestibility of jojoba meal proteins. MSc thesis., San Jose State University. http://scholarworks.sjsu.edu/cgi/viewcontent.cgi?article=4064&context=etd_theses.

Guo, Y. Z., Q. F. Chen, L. Y. Yang, and Y. H. Huang. 2007. Analyses of the seed protein contents on the cultivated and wild buckwheat *Fagopyrum esculentum* resources. *Genetic Resources and Crop Evolution* 54 (7):1465–72. doi: 10.1007/s10722-006-9135-z.

Guo, X., and H. Yao. 2006. Fractionation and characterization of tartary buckwheat flour proteins. *Food Chemistry* 98 (1):90–4. doi: 10.1016/j.foodchem.2005.05.055.

Guo, X., K. Zhu, H. Zhang, and H. Yao. 2007. Purification and characterization of the antitumor protein from Chinese tartary buckwheat (*Fagopyrum tataricum* Gaertn.) water-soluble extracts. *Journal of Agricultural and Food Chemistry* 55 (17):6958–61. doi: 10.1021/jf071032+.

Guo, X., K. Zhu, H. Zhang, and H. Yao. 2010. Anti-tumor activity of a novel protein obtained from tartary buckwheat. *International Journal of Molecular Sciences* 11 (12):5201–11. doi: 10.3390/ijms11125201.

Guo, X., and Y. L. Xiong. 2013. Characteristics and functional properties of buckwheat protein–sugar Schiff base complexes. *Lwt - Food Science and Technology* 51 (2):397–404. doi: 10.1016/j.lwt.2012.12.003.

Han, S.-W., K.-M. Chee, and S.-J. Cho. 2015. Nutritional quality of rice bran protein in comparison to animal and vegetable protein. *Food Chemistry* 172:766–9. doi: 10.1016/j.foodchem.2014.09.127.

Hassan, H., A. Afify, A. Basyiony, A. Ahmed, and T. Ghada. 2010. Nutritional and functional properties of defatted wheat protein isolates. *Australian Journal of Basic and Applied Sciences* 4:348–58.

Hübner, F., and E. K. Arendt. 2010. Studies on the influence of germination conditions on protein breakdown in buckwheat and oats. *Journal of the Institute of Brewing* 116 (1):3–13. doi: 10.1002/j.2050-0416.2010.tb00392.x.

Ikeda, K., and M. Kishida. 1993. Digestibility of proteins in buckwheat seed. *Fagopyrum* 13:21–4.

Ikeda, K., K. Arioka, S. Fujii, T. Kusano, and M. Oku. 1984. Effect on buckwheat protein quality of seed germination and changes in trypsin inhibitor content [*Fagopyrum esculentum*]. *Cereal Chemistry* 61: 236–8.

Ivanciuc, O., C. H. Schein, T. Garcia, N. Oezguen, S. S. Negi, and W. Braun. 2009. Structural analysis of linear and conformational epitopes of allergens. *Regulatory Toxicology and Pharmacology: RTP* 54 (3 Suppl):S11–S19. doi: 10.1016/j.yrtph.2008.11.007.

Janssen, F., A. Pauly, I. Rombouts, K. J. Jansens, L. J. Deleu, and J. A. Delcour. 2017. Proteins of amaranth (*Amaranthus* spp.), buckwheat (*Fagopyrum* spp.), and quinoa (*Chenopodium* spp.): A food science and technology perspective. *Comprehensive Reviews in Food Science and Food Safety* 16 (1):39–58. doi: 10.1111/1541-4337.12240.

Jin, J., O. D. Okagu, A. E. A. Yagoub, and C. C. Udenigwe. 2021. Effects of sonication on the in vitro digestibility and structural

properties of buckwheat protein isolates. *Ultrasonics Sonochemistry* 70:105348 doi: 10.1016/j.ultsonch.2020.105348.

Jin, J., H. Ma, C. Zhou, M. Luo, W. Liu, W. Qu, R. He, L. Luo, and A. E. G. A. Yagoub. 2015. Effect of degree of hydrolysis on the bioavailability of corn gluten meal hydrolysates. *Journal of the Science of Food and Agriculture* 95 (12):2501–9. doi: 10.1002/jsfa.6982.

Joye, I. 2019. Protein digestibility of cereal products. *Foods* 8 (6):199.

Jurkovic, N., and I. Colic. 1993. Effect of thermal processing on the nutritive value of wheat germ protein. *Die Nahrung* 37 (6):538–43. doi: 10.1002/food.19930370604.

Kayashita, J., H. Nagai, and N. Kato. 1996. Buckwheat protein extract suppression of the growth depression in rats induced by feeding amaranth (Food Red No. 2). *Bioscience, Biotechnology, and Biochemistry* 60 (9):1530–1. doi: 10.1271/bbb.60.1530.

Kayashita, J., I. Shimaoka, and M. Nakajyoh. 1995. Hypocholesterolemic effect of buckwheat protein extract in rats fed cholesterol enriched diets. *Nutrition Research* 15 (5):691–8. doi: 10.1016/0271-5317(95)00036-I.

Kayashita, J., I. Shimaoka, M. Nakajoh, and N. Kato. 1996. Feeding of buckwheat protein extract reduces hepatic triglyceride concentration, adipose tissue weight, and hepatic lipogenesis in rats. *The Journal of Nutritional Biochemistry* 7 (10):555–9. doi: 10.1016/S0955-2863(96)00110-6.

Kayashita, J., I. Shimaoka, M. Nakajoh, N. Kishida, and N. Kato. 1999. Consumption of a buckwheat protein extract retards 7,12-dimethylbenz[alpha]anthracene-induced mammary carcinogenesis in rats. *Bioscience, Biotechnology, and Biochemistry* 63 (10):1837–9. doi: 10.1271/bbb.63.1837.

Kayashita, J., I. Shimaoka, M. Nakajoh, M. Kondoh, K. Hayashi, and N. Kato. 1999. Muscle hypertrophy in rats fed on a buckwheat protein extract. *Bioscience, Biotechnology, and Biochemistry* 63 (7):1242–5. doi: 10.1271/bbb.63.1242.

Kayashita, J., I. Shimaoka, M. Nakajoh, M. Yamazaki, and N. Kato. 1997. Consumption of buckwheat protein lowers plasma cholesterol and raises fecal neutral sterols in cholesterol-fed rats because of its low digestibility. *The Journal of Nutrition* 127 (7):1395–400.

Kayashita, J., I. Shimaoka, M. Yamazaki, and N. Kato. 1995. Buckwheat protein extract ameliorates atropine-induced constipation in rats. *Cellulose* 5:941–6.

Koyama, M., S. Hattori, Y. Amano, M. Watanabe, and K. Nakamura. 2014. Blood pressure-lowering peptides from neo-fermented buckwheat sprouts: A new approach to estimating ACE-inhibitory activity. *PLOS One* 9 (9):e105802 doi: 10.1371/journal.pone.0105802.

Koyama, M., K. Naramoto, T. Nakajima, T. Aoyama, M. Watanabe, and K. Nakamura. 2013. Purification and identification of antihypertensive peptides from fermented buckwheat sprouts. *Journal of Agricultural and Food Chemistry* 61 (12):3013–21. doi: 10.1021/jf305157y.

Lee, C., S. In, Y. Han, and S. Oh. 2016. Reactivity change of IgE to buckwheat protein treated with high-pressure and enzymatic hydrolysis. *Journal of the Science of Food and Agriculture* 96 (6):2073–9. doi: 10.1002/jsfa.7321.

Leung, E. H., and T. Ng. 2007. A relatively stable antifungal peptide from buckwheat seeds with antiproliferative activity toward cancer cells. *Journal of Peptide Science: An Official Publication of the European Peptide Society* 13 (11):762–7. doi: 10.1002/psc.891.

Li, C. H., T. Matsui, K. Matsumoto, R. Yamasaki, and T. Kawasaki. 2002. Latent production of angiotensin I-converting enzyme inhibitors from buckwheat protein . *Journal of Peptide Science: An Official Publication of the European Peptide Society* 8 (6):267–74. doi: 10.1002/psc.387.

Ličen, M., and I. Kreft. 2005. Buckwheat (Fagopyrum esculentum Moench) low molecular weight seed proteins are restricted to the embryo and are not detectable in the endosperm. *Plant Physiology and Biochemistry* 43 (9):862–5. doi: 10.1016/j.plaphy.2005.08.002.

Liu, K., J. Zheng, and F. Chen. 2019. Effect of domestic cooking on rice protein digestibility. *Food Science & Nutrition* 7 (2):608–16. doi: 10.1002/fsn3.884.

Lohi, S., K. Mustalahti, K. Kaukinen, K. Laurila, P. Collin, H. Rissanen, O. Lohi, E. Bravi, M. Gasparin, A. Reunanen, et al. 2007. Increasing prevalence of coeliac disease over time. *Alimentary Pharmacology & Therapeutics* 26 (9):1217–25., doi: 10.1111/j.1365-2036.2007.03502.x.

Ma, M.-S., I. Y. Bae, H. G. Lee, and C.-B. Yang. 2006. Purification and identification of angiotensin I-converting enzyme inhibitory peptide from buckwheat (*Fagopyrum esculentum* Moench). *Food Chemistry* 96 (1):36–42. doi: 10.1016/j.foodchem.2005.01.052.

Ma, Y., and Y. L. Xiong. 2009. Antioxidant and bile acid binding activity of buckwheat protein *in vitro* digests. *Journal of Agricultural and Food Chemistry* 57 (10):4372–80. doi: 10.1021/jf803670u.

Ma, Y., Y. L. Xiong, J. Zhai, H. Zhu, and T. Dziubla. 2010. Fractionation and evaluation of radical scavenging peptides from *in vitro* digests of buckwheat protein. *Food Chemistry* 118 (3):582–8. doi: 10.1016/j.foodchem.2009.05.024.

Maat-Bleeker, F. D., and S. Stapel. 1998. Cross-reactivity between buckwheat and latex. *Allergy* 53:538–9.

Manninen, A. H. 2009. Protein hydrolysates in sports nutrition. *Nutrition & Metabolism* 6:38. doi: 10.1186/1743-7075-6-38.

Markus, G. 1965. Protein substrate conformation and proteolysis. *Proceedings of the National Academy of Sciences of the United States of America* 54 (1):253–8. doi: 10.1073/pnas.54.1.253.

Martín-Cabrejas, M. A., Y. Aguilera, M. M. Pedrosa, C. Cuadrado, T. Hernández, S. Díaz, and R. M. Esteban. 2009. The impact of dehydration process on antinutrients and protein digestibility of some legume flours. *Food Chemistry* 114 (3):1063–8. doi: 10.1016/j.foodchem.2008.10.070.

Matsumoto, R., K. Fujino, Y. Nagata, S. Hashiguchi, Y. Ito, Y. Aihara, Y. Takahashi, K. Maeda, and K. Sugimura. 2004. Molecular characterization of a 10-kDa buckwheat molecule reactive to allergic patients' IgE . *Allergy* 59 (5):533–8. doi: 10.1046/j.1398-9995.2003.00412.x.

McCarthy, A., Y. O'Callaghan, and N. O'Brien. 2013. Protein hydrolysates from agricultural crops—bioactivity and potential for functional food development. *Agriculture* 3 (1):112–30. doi: 10.3390/agriculture3010112.

Metzger, B. T., D. M. Barnes, and J. D. Reed. 2007. Insoluble fraction of buckwheat (*Fagopyrum esculentum* Moench) protein possessing cholesterol-binding properties that reduce micelle cholesterol solubility and uptake by Caco-2 cells. *Journal of Agricultural and Food Chemistry* 55 (15):6032–8. doi: 10.1021/jf0709496.

Nagaoka, H., and H. Kayahara. 2000. Resolution and synthesis of optically active alcohols with immobilized water-soluble proteins from green pea, soybean and buckwheat as new bio-catalysts. *Bioscience. Bioscience, Biotechnology, and Biochemistry* 64 (4):781–4. doi: 10.1271/bbb.64.781.

Noma, T., I. Yoshizawa, N. Ogawa, M. Ito, K. Aoki, and Y. Kawano. 2001. Fatal buckwheat dependent exercisedinduced anaphylaxis. *Asian Pacific Journal of Allergy and Immunology* 19:283–6.

Nosworthy, M. G., A. Franczyk, A. Zimoch-Korzycka, P. Appah, A. Utioh, J. Neufeld, and J. D. House. 2017. Impact of processing on the protein quality of pinto bean (*Phaseolus vulgaris*) and buckwheat (*Fagopyrum esculentum* Moench) flours and blends, as determined by *in vitro* and *in vivo* methodologies. *Journal of Agricultural and Food Chemistry* 65 (19):3919–25. doi: 10.1021/acs.jafc.7b00697.

Nwachukwu, I. D., and R. E. Aluko. 2019. Structural and functional properties of food protein-derived antioxidant peptides . *Journal of Food Biochemistry* 43 (1):e12761 doi: 10.1111/jfbc.12761.

Ogodo, A. C., O. C. Ugbogu, R. A. Onyeagba, and H. C. Okereke. 2019. Microbiological quality, proximate composition and *in vitro* starch/protein digestibility of *Sorghum bicolor* flour fermented with lactic acid bacteria consortia. *Chemical and Biological Technologies in Agriculture* 6 (1):7. doi: 10.1186/s40538-019-0145-4.

Ohanenye, I. C., A. Tsopmo, C. E. Ejike, and C. C. Udenigwe. 2020. Germination as a bioprocess for enhancing the quality and nutritional prospects of Legume proteins. *Trends in Food Science & Technology* 101:213–22.

Okolie, C., A. Aryee, and C. Udenigwe. 2018. Detection and deactivation of allergens in food. In *Proteins in food processing*, 367–87. Duxford: Elsevier/Woodhead Publishing.

Oparin, P. B., K. S. Mineev, Y. E. Dunaevsky, A. S. Arseniev, M. A. Belozersky, E. V. Grishin, T. A. Egorov, and A. A. Vassilevski. 2012.

Buckwheat trypsin inhibitor with helical hairpin structure belongs to a new family of plant defence peptides. Biochem J 446 (1):69–77. doi: 10.1042/BJ20120548.

Park, J., D. Kang, C. Kim, S. Ko, H. Yum, K. Kim, C. S. Hong, and K. Lee. 2000. Identification and characterization of the major allergens of buckwheat. *Allergy* 55 (11):1035–41. doi: 10.1034/j.1398-9995.2000.00763.x.

Park, S.-S., K. Abe, M. Kimura, A. Urisu, and N. Yamasaki. 1997. Primary structure and allergenic activity of trypsin inhibitors from the seeds of buckwheat (*Fagopyrum esculentum* Moench). *FEBS Letters* 400 (1):103–7. doi: 10.1016/s0014-5793(96)01367-1.

Phiarais, B. N., B. D. Schehl, and E. K. Arendt. 2008. Protein changes during malting of buckwheat. *Journal of the American Society of Brewing Chemists* 66 (2):127–35. doi: 10.1094/ASBCJ-2008-0102-01.

Radović, S. R., V. R. Maksimović, and E. I. Varkonji-Gašić. 1996. Characterization of buckwheat seed storage proteins. *Journal of Agricultural and Food Chemistry* 44 (4):972–4. doi: 10.1021/jf950655x.

Rajendran, S. R. C. K., C. E. C. C. Ejike, M. Gong, W. Hannah, and C. C. Udenigwe. 2017. Preclinical evidence on the anticancer properties of food peptides. *Protein and Peptide Letters* 24 (2):126–36. doi: 10.2174/0929866523666160816152755.

Riedl, K. M., and A. E. Hagerman. 2001. Tannin-protein complexes as radical scavengers and radical sinks. *Journal of Agricultural and Food Chemistry* 49 (10):4917–23. doi: 10.1021/jf010683h.

Rizzello, C. G., L. Nionelli, R. Coda, M. De Angelis, and M. Gobbetti. 2010. Effect of sourdough fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ. *Food Chemistry* 119 (3):1079–89. doi: 10.1016/j.foodchem.2009.08.016.

Roberts, P. R., J. Burney, K. W. Black, and G. P. Zaloga. 1999. Effect of chain length on absorption of biologically active peptides from the gastrointestinal tract. *Digestion* 60 (4):332–7. doi: 10.1159/000007679.

Sadhu, S., R. Thirumdas, R. Deshmukh, and U. Annapure. 2017. Influence of cold plasma on the enzymatic activity in germinating mung beans (*Vigna radiate*). *LWT- Food Science and Technology* 78: 97–104. doi: 10.1016/j.lwt.2016.12.026.

Schiffner, R., B. Przybilla, T. Burgdorff, M. Landthaler, and W. Stolz. 2001. Anaphylaxis to buckwheat. *Allergy* 56 (10):1020–2021. doi: 10.1034/j.1398-9995.2001.00386.x.

Seppo, L., T. Jauhiainen, T. Poussa, and R. Korpela. 2003. A fermented milk high in bioactive peptides has a blood pressure–lowering effect in hypertensive subjects. *The American Journal of Clinical Nutrition* 77 (2):326–30. doi: 10.1093/ajcn/77.2.326.

Sharrett, A. R., C. Ballantyne, S. Coady, G. Heiss, P. Sorlie, D. Catellier, and W. Patsch. 2001. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein (a), apolipoproteins AI and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 104 (10):1108–13. doi: 10.1161/hc3501.095214.

Silk, D., G. Grimble, and R. Rees. 1985. Protein digestion and amino acid and peptide absorption. Proceedings of the Nutrition Society, *44*, 63–72.

Sivam, A., D. Sun-Waterhouse, C. Perera, and G. Waterhouse. 2013. Application of FT-IR and Raman spectroscopy for the study of biopolymers in breads fortified with fibre and polyphenols. *Food Research International* 50 (2):574–85. doi: 10.1016/j.foodres.2011.03.039.

Škrabanja, V., and I. Kreft. 2016. Nutritional value of buckwheat proteins and starch. In *Molecular breeding and nutritional aspects of buckwheat*, 169–76. Elsevier/Academic Press, London, United Kingdom.

Skrabanja, V., H. N. Laerke, and I. Kreft. 2000. Protein-polyphenol interactions and *in vivo* digestibility of buckwheat groat proteins. *Pflugers Archiv : European Journal of Physiology* 440 (5 Suppl): R129–R131.

Starzyńska-Janiszewska, A., B. Stodolak, R. Duliński, M. Bączkowicz, B. Mickowska, A. Wikiera, and Ł. Byczyński. 2016. Effect of solid-state fermentation tempe type on antioxidant and nutritional parameters of buckwheat groats as compared with hydrothermal processing. *Journal of Food Processing and Preservation* 40:298–305.

Stember, R. H. 2006.Buckwheat allergy. *Allergy and Asthma Proceedings* 27 (4):393–5. doi:10.2500/aap.2006.27.2879.

Tang, C. H. 2007. Functional properties and in vitro digestibility of buckwheat protein products: Influence of processing. *Journal of Food Engineering* 82 (4):568–76.

Tang, C.-H., J. Peng, D.-W. Zhen, and Z. Chen. 2009. Physicochemical and antioxidant properties of buckwheat (*Fagopyrum esculentum* Moench) protein hydrolysates. *Food Chemistry* 115 (2):672–8. doi: 10.1016/j.foodchem.2008.12.068.

Tang, C. H., X. Y. Wang, F. Liu, and C. S. Wang. 2009. Physicochemical and conformational properties of buckwheat protein isolates: Influence of polyphenol removal with cold organic solvents from buckwheat seed flours. *Journal of Agricultural and Food Chemistry* 57 (22):10740–8. doi: 10.1021/jf901928h.

Tavares, T., M. Á. Sevilla, M. J. Montero, R. Carrón, and F. X. Malcata. 2012. Acute effect of whey peptides upon blood pressure of hypertensive rats, and relationship with their angiotensin-converting enzyme inhibitory activity. *Molecular Nutrition & Food Research* 56 (2):316–24. doi: 10.1002/mnfr.201100381.

Tomotake, H., I. Shimaoka, J. Kayashita, M. Nakajoh, and N. Kato. 2002. Physicochemical and functional properties of buckwheat protein product. *Journal of Agricultural and Food Chemistry* 50 (7): 2125–9. doi: 10.1021/jf011248q.

Tomotake, H., I. Shimaoka, J. Kayashita, F. Yokoyama, M. Nakajoh, and N. Kato. 2000. A buckwheat protein product suppresses gallstone formation and plasma cholesterol more strongly than soy protein isolate in hamsters. *The Journal of Nutrition* 130 (7):1670–4.

Tomotake, H., N. Yamamoto, H. Kitabayashi, A. Kawakami, J. Kayashita, H. Ohinata, H. Karasawa, and N. Kato. 2007. Preparation of tartary buckwheat protein product and its improving effect on cholesterol metabolism in rats and mice fed cholesterol-enriched diet. *Journal of Food Science* 72 (7):S528–S533.

Tomotake, H., N. Yamamoto, N. Yanaka, H. Ohinata, R. Yamazaki, J. Kayashita, and N. Kato. 2006. High protein buckwheat flour suppresses hypercholesterolemia in rats and gallstone formation in mice by hypercholesterolemic diet and body fat in rats because of its low protein digestibility. *Nutrition* 22 (2):166–73.

Udenigwe, C. C., and R. E. Aluko. 2011. Chemometric analysis of the amino acid requirements of antioxidant food protein hydrolysates. *International Journal of Molecular Sciences* 12 (5):3148–61. doi: 10.3390/ijms12053148.

Udenigwe, C. C., and R. E. Aluko. 2012. Food protein-derived bioactive peptides: production, processing, and potential health benefits . *Journal of Food Science* 77 (1):R11–R24. doi: 10.1111/j.1750-3841.2011.02455.x.

Udenigwe, C., and K. Rouvinen-Watt. 2015. The role of food peptides in lipid metabolism during dyslipidemia and associated health conditions. *International Journal of Molecular Sciences* 16 (5):9303–13. doi: 10.3390/ijms16059303.

Varga, E.-M., D. Kollmann, M. Zach, and B. Bohle. 2011. Anaphylaxis to buckwheat in an atopic child: A risk factor for severe allergy to nuts and seeds? *International Archives of. International Archives of Allergy and Immunology* 156 (1):112–6. doi: 10.1159/000321916.

Wang, C., S. Yuan, W. Zhang, T. Ng, and X. Ye. 2019. Buckwheat antifungal protein with biocontrol potential to inhibit fungal (*Botrytis cinerea*) infection of cherry tomato. *Journal of Agricultural and Food Chemistry* 67 (24):6748–56. doi: 10.1021/acs.jafc.9b01144.

Wang, T., S. Shyur, D. Wen, Y. Kao, and L. Huang. 2006. Buckwheat anaphylaxis: An unusual allergen in Taiwan. *Asian Pacific Journal of Allergy and Immunology* 24 (2–3):167–70.

Wang, X., N. Ullah, X. Sun, Y. Guo, L. Chen, Z. Li, and X. Feng. 2017. Development and characterization of bacterial cellulose reinforced biocomposite films based on protein from buckwheat distiller's dried grains. *International Journal of Biological Macromolecules* 96:353–60. doi: 10.1016/j.ijbiomac.2016.11.106.

Wang, J., J. Xiao, X. Liu, F. Geng, Q. Huang, J. Zhao, D. Xiang, and G. Zhao. 2019. Analysis of tartary buckwheat (*Fagopyrum tataricum*) seed proteome using offline two-dimensional liquid chromatography

and tandem mass spectrometry . *Journal of Food Biochemistry* 43 (7):e12863 doi: 10.1111/jfbc.12863.

Wieslander, G., and D. Norbäck. 2001. Buckwheat allergy. *Allergy* 56 (8):703–4. doi: 10.1034/j.1398-9995.2001.056008703.x.

Wijngaard, H., and E. K. Arendt. 2006. Buckwheat. *Cereal Chemistry Journal* 83 (4):391–401. doi: 10.1094/CC-83-0391.

Wüthrich, B., and A. Trojan. 1995. Wheatburger anaphylaxis due to hidden buckwheat. *Clinical & Experimental Allergy* 25 (12):1263 doi: 10.1111/j.1365-2222.1995.tb03052.x.

Xia, N., J.-M. Wang, Q. Gong, X.-Q. Yang, S.-W. Yin, and J.-R. Qi. 2012. Characterization and *in vitro* digestibility of rice protein prepared by enzyme-assisted microfluidization: Comparison to alkaline extraction. *Journal of Cereal Science* 56 (2):482–489. doi: 10.1016/j.jcs.2012.06.008.

Xue, F., Z. Wu, J. Tong, J. Zheng, and C. Li. 2017. Effect of combination of high-intensity ultrasound treatment and dextran glycosylation on structural and interfacial properties of buckwheat protein isolates. *Bioscience, Biotechnology, and Biochemistry* 81 (10): 1891–1898. doi: 10.1080/09168451.2017.1361805.

Yang, Z. H., C. Li, Y. Y. Li, and Z. H. Wang. 2013. Effects of Maillard reaction on allergenicity of buckwheat allergen Fag t 3 during thermal processing. *Journal of the Science of Food and Agriculture* 93 (6):1510–1515. doi: 10.1002/jsfa.5928.

Yano, M., R. Nakamura, S. Hayakawa, and S. Torii. 1989. Purification and properties of allergenic proteins in buckwheat seeds. *Agricultural and Biological Chemistry* 53:2387–2392.

Yiming, Z., W. Hong, C. Linlin, Z. Xiaoli, T. Wen, and S. Xinli. 2015. Evolution of nutrient ingredients in tartary buckwheat seeds during germination. *Food Chemistry* 186:244–248. doi: 10.1016/j.foodchem.2015.03.115.

Yousif, N. E., and A. H. El Tinay. 2000. Effect of fermentation on protein fractions and *in vitro* protein digestibility of maize. *Food Chemistry* 70 (2):181–184. doi: 10.1016/S0308-8146(00)00069-8.

Yousif, N. E., and A. H. El Tinay. 2001. Effect of fermentation on sorghum protein fractions and *in vitro* protein digestibility. *Plant Foods for Human Nutrition* 56 (2):175–182. doi: 10.1023/A:1011140602122.

Zhang, C., R. Zhang, Y. M. Li, N. Liang, Y. Zhao, H. Zhu, Z. He, J. Liu, W. Hao, R. Jiao, et al. 2017. Cholesterol-lowering activity of Tartary buckwheat protein. *Journal of Agricultural and Food Chemistry* 65 (9):1900–1906., doi: 10.1021/acs.jafc.7b00066.

Zhang, X., J. M. Yuan, X. D. Cui, and Z. H. Wang. 2008. Molecular cloning, recombinant expression, and immunological characterization of a novel allergen from tartary buckwheat. *Journal of Agricultural and Food Chemistry* 56 (22):10947–10953. doi: 10.1021/jf801855a.

Zhang, Z., X. Zhang, W. Chen, and P. Zhou. 2018. Conformation stability, *in vitro* digestibility and allergenicity of tropomyosin from shrimp (*Exopalaemon modestus*) as affected by high intensity ultrasound. *Food Chemistry* 245:997–1009. doi: 10.1016/j.foodchem.2017.11.072.

Zhou, X., S. Cheng, Y. Yang, Y. Zhou, W. Tang, X. Zhang, Q. Wang, and Z. Li. 2011. Toward a novel understanding of buckwheat self-defensive strategies during seed germination and preliminary investigation on the potential pharmacological application of its malting products. *Journal of Medicinal Plants Research* 5:6946–6954.

Zhou, X., L. Huang, W. Tang, Y. Zhou, Q. Wang, and Z. Li. 2013. A novel buckwheat protein with a beneficial effect in atherosclerosis was purified from *Fagopyrum tataricum* (L.) Gaertn. *Archives of Biological Sciences* 65 (2):767–772. doi: 10.2298/ABS1302767Z.

Zhou, X., L. Wen, Z. Li, Y. Zhou, Y. Chen, and Y. Lu. 2015. Advance on the benefits of bioactive peptides from buckwheat. *Phytochemistry Reviews* 14 (3):381–388. doi: 10.1007/s11101-014-9390-0.

Zhou, X.-L., B.-B. Yan, Y. Xiao, Y.-M. Zhou, and T.-Y. Liu. 2018. Tartary buckwheat protein prevented dyslipidemia in high-fat diet-fed mice associated with gut microbiota changes. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association* 119:296–301. doi: 10.1016/j.fct.2018.02.052.

Zhu, F. 2016. Chemical composition and health effects of Tartary buckwheat. *Food Chemistry* 203:231–245. doi: 10.1016/j.foodchem.2016.02.050.