



Review

Functionalization of sweet potato leaf polyphenols by nanostructured composite β -lactoglobulin particles from molecular level complexations: A review

Shadrack Isaboke Makori^{a,b}, Tai-Hua Mu^{a,*}, Hong-Nan Sun^{a,*}

^a Laboratory of Food Chemistry and Nutrition Science, Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Key Laboratory of Agro-Products Processing, Ministry of Agriculture and Rural Affairs, No. 2 Yuan Ming Yuan West Road, Haidian District, P.O. Box 5109, Beijing 100193, China

^b Food Technology Division, Kenya Industrial Research and Development Institute (KIRDI), P.O. Box 30650, GPO, Nairobi, Kenya

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 4,5-di-O-caffeoylquinic acid (PubChem CID: 6474309)
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ABSTRACT

Sweet potato leaf polyphenols (SPLPs) have shown potential health benefits in the food and pharmaceutical industries. Nowadays, consumption of SPLPs from animal feeds to foodstuff is becoming a trend worldwide. However, the application of SPLPs is limited by their low bioavailability and stability. β -lactoglobulin (β lg), a highly regarded whey protein, can interact with SPLPs at the molecular level to form reversible or irreversible nanocomplexes (NCs). Consequently, the functional properties and final quality of SPLPs are directly modified. In this review, the composition and structure of SPLPs and β lg, as well as methods of molecular complexation and mechanisms of formation of SPLPs β lgNCs, are revisited. The modified functionalities of SPLPs β lgNCs, especially protein conformational structures, antioxidant activity, solubility, thermal stability, emulsifying, and gelling properties including allergenic potential, digestibility, and practical applications are discussed for SPLPs future development.

1. Introduction

Polyphenols are secondary plant metabolites widely distributed in fruits and vegetables and are important determinants of the sensory and nutritional quality of plant-derived foods (Jiang et al., 2018). Polyphenols are separated into two general classes: flavonoids and phenolic acids. Flavonoids which can be classified by colour are divided as follows: flavones, flavanones, flavonols, flavanols, and isoflavones (Dias et al., 2021). On the other hand, phenolic acids occur as hydroxybenzoic

or hydroxycinnamic acids (Abbas et al., 2017). Polyphenols have several reported health benefits due to their high antioxidant capacity. For example, polyphenols possess significant antimicrobial, antidiabetic, antiobesity, anticarcinogenic, antimutagenic, and antihypertensive effects (Al-Shabib et al., 2018). The health-promoting effects of polyphenols have led to an unprecedented increase in research interest recently, due to their safety and therapeutic potentials (Makori et al., 2021). Moreover, a rise in lifestyle-related diseases and a progressively aging population in developed and some developing countries have

* Corresponding authors.

E-mail addresses: honey0329@163.com (T.-H. Mu), mutaihua@126.com (H.-N. Sun).

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necessitated the desire for healthier food choices (Kurata et al., 2019). Synthetic antioxidants are widely used in the food industry to prevent oxidation of fats and oils, and also prevent toxicities or mutagenicities in processed foods. However, numerous research publications have indicated the association between consumption of synthetic antioxidants and increased risk of cancer and liver damage (Sun et al., 2018). Consequently, exploration of effective natural antioxidants with minimal side effects to replace synthetic antioxidants would be rewarding for the food and pharmaceutical industries.

Sweet potato leaves, the aboveground parts of sweet potato (*Ipomoea batatas* L.), are high-yielding vegetables that can be harvested time and again during each growing season (Suárez et al., 2020). Attempts have been made to valorize sweet potato leaves i.e. freeze dry to produce sweet potato powder, sweet potato juice, and in some cases consumed as a fresh vegetable (Luo et al., 2020). However, due to the large quantities of sweet potato leaves being harvested each growing season, a greater portion is discarded or used as feed resulting in environmental pollution and wastage of this valuable resource (Jang & Koh, 2019). A previous study done by our research group showed that sweet potato leaf polyphenols (SPLPs) possessed significantly higher antioxidant activity than ascorbic acid, tea polyphenols, and grape seed polyphenols (Sun et al., 2018). Similarly, Frond et al. (2019) reported high amounts of total phenolics, total flavonoids and antioxidant activity in purple sweet potato than that of black carrot, eggplant and red onion. Moreover, the amount of polyphenols in sweet potato leaves was found to be much higher than that in the whole root, flesh tissue, and peel of sweet potato (Content et al., 2020; Sun et al., 2017); as well as other most commonly consumed commercial vegetables such as spinach, kale, amaranth, eggplant, cabbage, cauliflower, green peas and lettuce (Alam, 2021; Kurata et al., 2019). Considering these aspects, sweet potato leaves have a great potential to be used as sources of natural antioxidants in food and pharmaceutical applications (Alam, 2021). Nevertheless, the applications of SPLPs as functional ingredients are limited due to the following reasons: SPLPs are very unstable during food processing operations i.e. oxygen, light, and temperature; susceptible to degradation by digestive enzymes; poor solubility; alter the quality characteristics of food products such as a change in color, texture, flavor, and appearance (Milinčić et al., 2019).

To overcome the aforementioned limitations, the construction of nanoformulations using various carriers has been employed recently, to broaden the therapeutic effects of polyphenols (Yang et al., 2020). The complexation of β -lactoglobulin with dietary polyphenols has received immense research attention from both theoretical and practical points of view (Al-Shabib et al., 2018; Quan et al., 2019; Wu, Hui, Gong, et al., 2021). For instance, Su, Guo, Chen, Mao, et al. (2020) have developed functional composite β -lactoglobulin nanoparticles by conjugation of (–)-Epigallocatechin-3- gallate (EGCG), and innovatively incorporated it as Pickering emulsifier in the stabilization of a model lutein emulsion. Makori et al. (2021) constructed composite β -lactoglobulin or β -lactoglobulin-3,5-di-O-caffeoylquinic acid (3,5-di-O-CQA) non-covalent complexes, with possible application as functional ingredients in the food and pharmaceutical industry. Further, the incorporation of natural fruit pulps with high polyphenol content i.e. in yoghurts, and cheeses (pigmented by anthocyanins) have attracted a large consumer demand for healthier products, rich in natural antioxidants (Ferreira et al., 2016).

A literature search showed a progressive increase in the number of publications with keywords “SPLPs” and “nanostructured composite β -lactoglobulin particles” in the last two decades (2000–2020) (www.sciencedirect.com) (Fig. 1A). Consequently, the incorporation of SPLPs in human diets has evolved into an intense focus of research interest internationally due to their epidemiologically-proved health benefits (Krochmal-Marczak et al., 2020). Interaction between polyphenols and proteins resulting in “polyphenol-protein composites” is known to play a crucial role in the functional, sensorial, and nutraceutical quality of food products (Quan et al., 2019). For instance, Xu et al. (2019) investigated

the allergenicity potential and functional properties of whey protein isolate after covalent complexation with chlorogenic acid. The findings of their study demonstrated that whey protein isolate-chlorogenic acid conjugates significantly improved the functional properties and reduced IgE binding capacity *in vitro* of whey protein isolate, compared with non-conjugate whey protein isolate. Further, polyphenol-protein conjugation has been reported to reduce the surface hydrophobicity of proteins by donating a large number of hydroxyl groups, as well, improve the antioxidant properties of the modified proteins (Dai et al., 2019). Thus, polyphenols-protein conjugates could be considered as effective bioactive compounds in preventing oxidation in emulsion-based foods, and as a possible encapsulation delivery system in food and non-food applications (Zhang et al., 2020).

As far as the literature is concerned, there is no review to date focusing on the functionalization of SPLPs by nanostructured composite β -lactoglobulin particles from the molecular level complexation. Therefore, in this review, the composition and structure of SPLPs and β -lactoglobulin are first summarized. Next, the complexation methods on the functionalization of SPLPs- β -lactoglobulin nanoparticles and the mechanisms of formation are systematically reviewed. Lastly, the antioxidant and functional properties of SPLPs- β -lactoglobulin conjugates, their allergenic potential, and digestibility properties, including practical applications are also outlined. A complete understanding of the functionalization mechanisms of SPLPs- β -lactoglobulin nanocomplexes as well as their characteristics is essential to construct safe novel conjugates with superior antioxidant and functional properties.

2. Sweet potato leaf polyphenols

2.1. Chemical composition and structure of sweet potato leaf polyphenols

Polyphenols in sweet potato mainly constitute a mixture of caffeic acid (CA) and caffeoylquinic acids (CQAs) derivatives (Fig. 1B, C; Table 1) and are abundantly present in the leaves of sweet potato (Fu et al., 2016; Musilová et al., 2017). The content of CA and CQA in the stalks, stems, peel, and flesh is small (Kurata et al., 2019). CQAs are synthesized by the condensation of different acids such as quinic acid and transcinamic acids, and residues of certain transcinamic acids i.e. CA, p-coumaric acid, and ferulic acids (Abbas et al., 2017; Carrera et al., 2021; Usuki et al., 2017). The composition and molecular structure of CQAs derivatives will depend on the differences in positions, variety, and quantities of the esterification between quinic and transcinamic acids (Kremr et al., 2016; Krochmal-Marczak et al., 2020). It has become a consensus that mono-, di- and tri-CQAs are the most common in fruits and vegetables (Krochmal-Marczak et al., 2020; Kurata et al., 2019; Content et al., 2020; Suárez et al., 2020; Sun et al., 2018). Out of the many forms, 3-O-CQA (molecular formula $C_{16}H_{18}O_9$, and molar mass of 354.31 g/mol) is the most predominant form of CQAs in plants (Xue et al., 2016; Zhang et al., 2019). The antioxidant activity of CQAs has been linked to its double bond conjugated structure of catechol present in the phenyl ring (Tang et al., 2016). Recent research publications on cell lines and animal studies have shown the therapeutic properties of CQAs that include anti-inflammatory, anti-parasitic, anti-diabetic, anti-obese, antioxidant, neuroprotective, anti-cancer, radioprotective, anti-bacterial, and anti-viral effects (Nguyen et al., 2021). Composition of phenolic compounds in terms of the number of –OH groups in their molecular structure greatly determines their activities. For example, a study by Liang et al. (2019) indicated that di-CQAs i.e. 4,5-di-O-CQA, 3,4-di-O-CQA, and 3,5-di-O-CQA isomers exhibited greater affinity to ameliorate oxidative stress compared to mono-CQAs i.e. 3-O-CQA, 4-O-CQA, and 5-O-CQA isomers.

CQAs have been utilized as dietary supplements in food and non-food formulations due to their high antioxidant activity in addition to being natural antioxidants (Tang et al., 2016). However, the application of CQAs as functional ingredients are limited due to their low bioavailability, and poor stability to environmental conditions i.e. pH, ionic

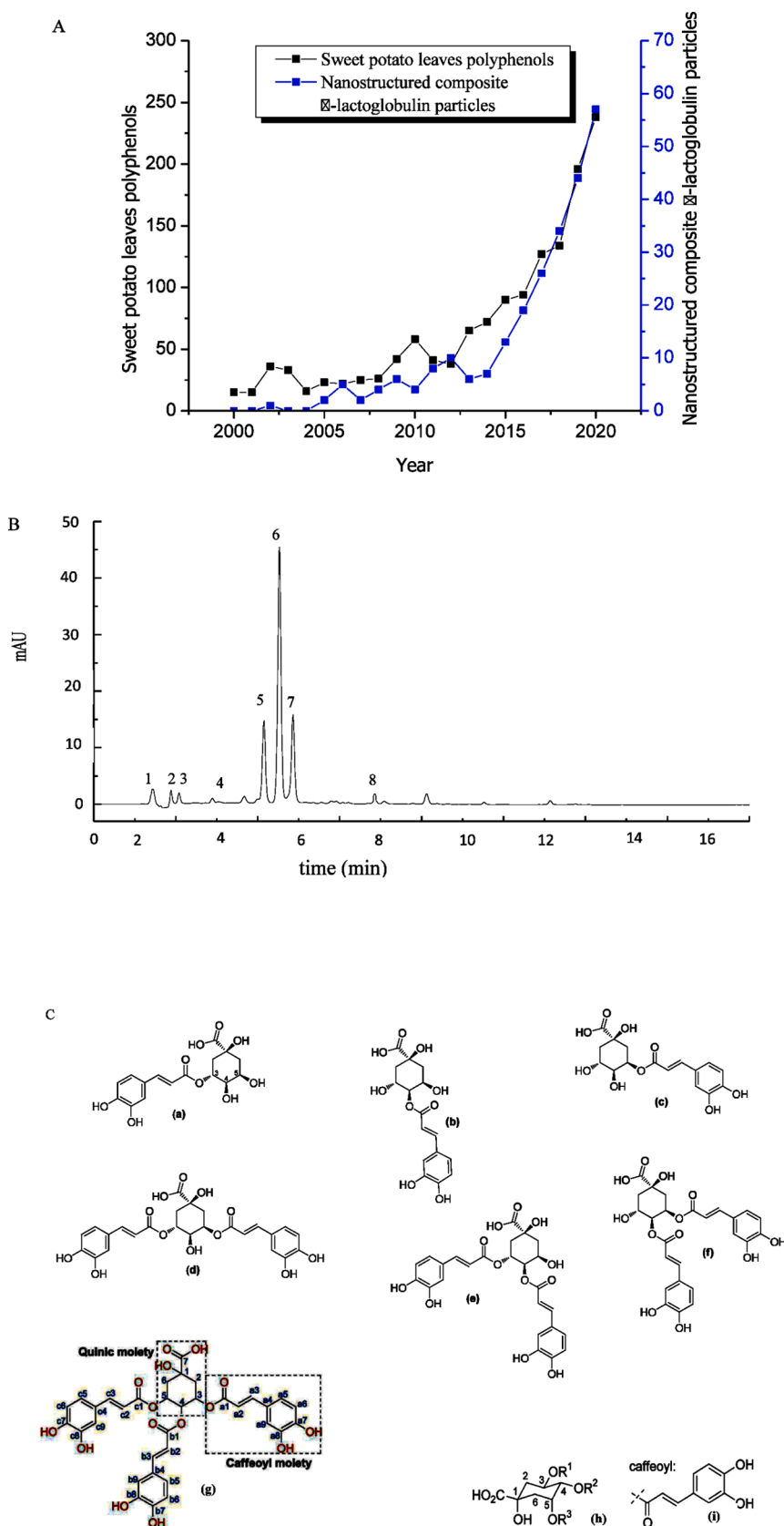


Fig. 1. Publication per year related to the term “sweet potato leaf polyphenols” and “nanostructured composite β-lactoglobulin particles” with the year from 2000 to 2020 (A). The HPLC chromatography of cultivar Yuzi No. 7 sweet potato leaf polyphenols (B). Peaks 1 to 8 denote 5-O-CQA, 3-O-CQA, 4-O-CQA, Caffeic acid, 4,5-di-O-CQA, 3,5-di-O-CQA, 3,4-di-O-CQA, 3,4,5-tri-O-CQA, respectively. Chemical structures of CQAs in sweet potato leaves (C). (a) 3-O-CQA, (b) 4-O-CQA, (c) 5-O-CQA, (d) 3,5-di-O-CQA, (e) 3,4-di-O-CQA, (f) 4,5-di-O-CQA, (g) 3,4,5-tri-O-CQA, (h) quinic acid, (i) caffeoyl. Key: R1, R2, and R3 denote different sites in the molecular structure of quinic acid. H stands for hydrogen, CQA denote caffeoylquinic acid. Note: Naming of the chemical compounds followed the IUPAC numbering system (Kremr et al., 2016). Figures adapted from Carrera et al. (2021), Kremr et al. (2016), Kurata et al. (2019), Tang et al. (2016), Usuki et al. (2017) and www.sciencedirect.com.

Table 1

Molecular structure of caffeoylquinic acids in sweet potato leaves (Kurata et al., 2019; Musilová et al., 2017; Sun et al., 2018). The codes R1, R2, R3, and H are similar to that depicted in the caption of Fig. 1. R1, R2, and R3 occurs at position 3, 4, and 5 in the molecular structure of quinic acid, respectively (Fig. 1C). Naming of the structures followed the IUPAC numbering system (Kremr et al., 2016).

Compound name	Abbreviation	Common name	R1	R2	R3	PubChem CID
3-O-caffeoylquinic acid	3-CQA	Chlorogenic acid	Caffeoyl	H	H	1,794,427
4-O-caffeoylquinic acid	4-CQA	Cryptochlorogenic acid	H	Caffeoyl	H	9,798,666
5-O-caffeoylquinic acid	5-CQA	Neochlorogenic acid	H	H	Caffeoyl	5,280,633
3,5-di-O-caffeoylquinic acid	3,5-diCQA	Isochlorogenic acid A	Caffeoyl	H	Caffeoyl	6,474,310
3,4-di-O-caffeoylquinic acid	3,4-diCQA	Isochlorogenic acid B	Caffeoyl	Caffeoyl	H	5,281,780
4,5-di-O-caffeoylquinic acid	4,5-diCQA	Isochlorogenic acid C	H	Caffeoyl	Caffeoyl	6,474,309
3,4,5-tri-O-caffeoylquinic acid	3,4,5-triCQA	–	Caffeoyl	Caffeoyl	Caffeoyl	6,440,783

strength, and temperature (Fan et al., 2017). Farah & Lima (2019) reported that only one-third of the consumed chlorogenic acid from coffee is absorbed in the human gastrointestinal tract. Besides, acrylamide formation was observed after thermal treatment of foods containing high levels of chlorogenic acid (Schouten et al., 2021). Accordingly, protein-based nanoemulsion or nanoparticle systems, exhibiting good chemical stability and lower cell toxicity than their synthetic counterparts, have been developed as efficient and promising delivery systems in labile bioactive compounds (Su, Guo, Chen, Mao, et al., 2020).

Sun et al. (2018) identified and quantified CQAs in sweet potato leaves. In their study, they found that sweet potato leaves consisted mainly of seven CQAs and a small amount of caffeic acid (Fig. 1B, C; Table 1). Further, the quantification results expressed in dry weight basis demonstrated that 3,5-di-O-CQA was highest ($36.30 \pm 0.19\%$) followed by 3,4-di-O-CQA ($25.01 \pm 0.42\%$), 4,5-di-O-CQA ($21.38 \pm 0.21\%$), 3,4,5-tri-O-CQA ($2.57 \pm 0.08\%$), 5-O-CQA ($2.42 \pm 0.07\%$), 3-O-CQA ($0.98 \pm 0.02\%$), 4-O-CQA ($0.95 \pm 0.01\%$) and caffeic acid ($0.09 \pm 0.01\%$). Other polyphenols that are present in minor quantities in sweet potato leaves include flavonoids (myricitrin, isoquercitrin, astragal, quercitrin, tiliroside, quercetin, kaempferol, rhamnetin) (Liu et al., 2019) and anthocyanins (cyanidin, pelargonidin, peonidin) (Thi Lan Khanh et al., 2018).

2.2. Challenges in food applications

The application of dietary polyphenols as functional ingredients faces immense challenges (Wu, Hui, Gong, et al., 2021). For instance, a scientific opinion from the European Food Safety Authority (EFSA) regarding the safety of green tea catechins has been published (Younes et al., 2018). It was noted that overexposure to green tea catechins at doses ≥ 800 mg EGCG/day could induce hepatotoxicity. Similarly, nutritional consequences as a result of protein–polyphenol complexation have been disclosed (José, 2020). It is worth mentioning here that formulation of functional food supplements via protein–polyphenol complexation requires proper optimization of ingredients and fabrication conditions therein (pH, and temperature) to produce the most desirable effect (José, 2020).

Likewise, there exist few challenges concerning practical applications of β -lactoglobulin nanoparticles. β -lactoglobulin is classified as one of the allergic proteins in bovine milk (Sun et al., 2018). Aside from the allergenic potential of β -lactoglobulin, complexation with polyphenols may result in haze formation in wines, juices, and also generate colloids in honey (Cosme et al., 2020); as well as disrupt the structure and lower the shelf life of other food ingredients (Lila et al., 2017). In addition, the formation of protein haze in white wine has been shown to result in instability unless removed before bottling (Jongberg et al., 2020). Depending on the mode of protein–polyphenol conjugation or methods of fabrication applied, the formation of undesirable side products may be produced such as melanoidins during Maillard reaction (Liu et al., 2017). Nonetheless, the aforementioned challenges should guide future researches in the construction of protein–polyphenol conjugates with health-enhancing effects.

3. β -lactoglobulin

β -lactoglobulin, the most abundant whey protein in cow milk, has been found to contain 162 amino acids with a molecular weight of 18.4 kDa (Fig. 2A, B). β -lactoglobulin belongs to the lipocalins family of proteins and is comprised of a free thiol (Cys121) and two disulfide bonds (Cys106-Cys119 and Cys66-Cys160) (Sengupta et al., 2018). Moreover, β -lactoglobulin possesses two tryptophan residues namely tryptophan 19 and tryptophan 61, located in the hydrophobic pocket and at the protein surface, respectively. The main binding site of β -lactoglobulin is made up of eight stranded antiparallel β -barrel. As a globular protein, β -lactoglobulin can form aggregates through hydrophobic and covalent interactions when heated above their denaturation temperatures (Bonarek et al., 2020; Broersen, 2020; Yue, 2019; Makori et al., 2021; Su, Guo, Chen, Mao, et al., 2020). β -lactoglobulin and dietary SPLPs may exist as isolated components or a mixture of different components with complex chemical structures. Their interactions at the molecular level occur between the hydroxyl (–OH) group of polyphenols and the amino group (–NH₂) of proteins. Moreover, previous studies have revealed complexation between globular proteins and polyphenols to occur through non-covalent forces i.e hydrogen bonds, hydrophobic interactions, and van der Waals attractions. By contrast, their covalent interactions take place through the enzymatic, thermal, or oxidative formation of o-quinones which in turn reacts with proteins (–NH₂ and –SH) (Keppler & Jan, 2020; Quan et al., 2019; Yildirim-Elikoglu & Erdem, 2018). Besides, molecular-level interactions between proteins and polyphenols depend on several factors namely: structural differences between the protein and dietary polyphenol, different binding mechanisms, environmental factors (pH, temperature), and disparate analysis fitting programs (Wu, Hui, Gong, et al., 2021).

3.1. Functionalities of β -lactoglobulin nanoparticles

A nanoparticle refers to a particle of microscopic size with dimensions and tolerances in the nanometer range of 1 to 100 nm. Nanoparticles are made up of a dense polymeric network with an active ingredient dispersed throughout the matrix (Dai et al., 2020). Manipulation of individual atoms and molecules at this length scale can result in the development of materials with novel properties and applications. More specifically, particle sizes below 1000 nm have been used in the pharmaceutical sciences because of their unique physicochemical properties when compared to their macroscale counterparts (Ha et al., 2019). In recent years, nanoparticles have been increasingly used in the food and pharmaceutical sciences, due to the high surface to volume ratio, ease of manipulation of particle size and surface characteristics, novel physicochemical properties, and their ability to control and sustain the release of active ingredients in the gastrointestinal environment (Makori et al., 2021; Su, Guo, Chen, Mao, et al., 2020). Moreover, these unique properties of the nanoparticles have made it possible to manipulate therapeutic effects of the drug in the body i.e distribution and clearance to minimize side effects (Fan et al., 2017).

β -lactoglobulin, the most abundant whey protein, has a high affinity for various hydrophobic and amphiphilic compounds and exists as a

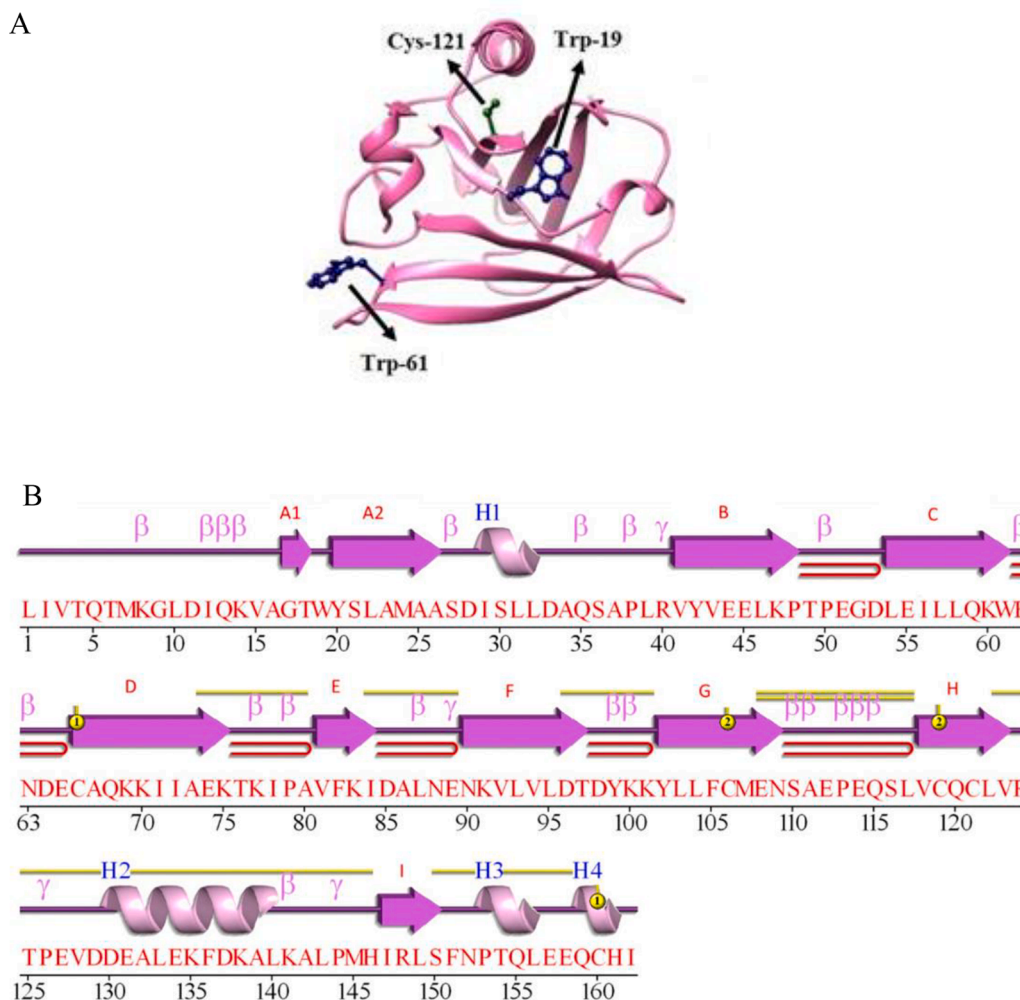


Fig. 2. The 3D structure of bovine β -lactoglobulin showing the position of two tryptophan (Trp) residues i.e. Trp 19 and Trp 61, and one free thiol group (Cys 121) (A). Primary structure and amino acid sequence of β -lactoglobulin (B). Key: H1–H4, helical segments; A1–I, beta sheets; β , beta turn; γ , gamma turn; 1 = 2, disulfide bonds. Figures adapted from [Broersen \(2020\)](#) and [Sengupta et al. \(2018\)](#).

mixture of monomers and dimers under neutral pH conditions (Makori et al., 2021; Su, Guo, Chen, Mao, et al., 2020). The unique nutritional and functional properties of β -lactoglobulin i.e ability to protect bioactive compounds such as caffeoylquinic acids, retinol, and β -carotene from heat, oxidation, or irradiation makes it a better candidate to be used as a nanoparticle carrier in food emulsion systems, and in various pharmaceutical applications (Wu, Hui, Gong, et al., 2021). Further, it has been shown that β -lactoglobulin is biocompatible, biodegradable, and GRAS (generally recognized as safe) raw material which can be used as hydrogel in certain pH conditions, and does not require chemical crosslinking agents in their construction (Wu, Hui, Gong, et al., 2021; Yildirim-Elikoglu & Erdem, 2018). These are the major requirements for any organic nanoparticle not only in pharmaceuticals but also in food and bioprocessing applications (Al-Shabib et al., 2018). Fan et al. (2017) showed that β -lactoglobulin can undergo thermal treatments (75–85 °C) to produce soluble or insoluble aggregates, increase the thiol group, and the level of exposure of hydrophobic amino acid residues due to protein unfolding. However, the physicochemical and functional properties of the formed nanoparticles will depend on several factors namely; temperature, time, pH, protein concentration, type, and level of added salts among other factors (Su, Guo, Chen, Mao, et al., 2020; Zhang et al., 2020). Additional illustrations on various functionalities of β -lactoglobulin nanoparticles as delivery materials are presented in Fig. 3.

3.2. Factors affecting the formation of β -lactoglobulin-sweet potato leaf polyphenols nanoparticles

Factors that affect the interaction between proteins and polyphenols can be divided into intrinsic (molecular structure and type of polyphenols and proteins) or extrinsic (pH, temperature) factors (Zhang et al., 2020). These factors are important in determining the mode of fabrication of protein–polyphenol complexes i.e covalent or non-covalent bonds, and also the conformational changes that occur in the protein structure after complexation (Quan et al., 2019). Nevertheless, the factors that affect protein–phenolic interactions have not been discussed here in detail, since this was not the focus of this present review, and also several profound and detailed reviews are available on this subject (Keppler & Jan, 2020; Quan et al., 2019; Wu, Hui, Gong, et al., 2021; Yildirim-Elikoglu & Erdem, 2018; Zhang et al., 2020).

3.3. The molecular level complexations of β -lactoglobulin-based sweet potato leaf polyphenols nanoparticles

The molecular level fabrications of β -lactoglobulin-based SPLPs nanoparticles with different molecular and physicochemical characteristics can be achieved by controlling the ingredients and preparation conditions (Makori et al., 2021). Recently, many studies have focused on the fabrication of β -lactoglobulin-based nanoparticles as potential nano-

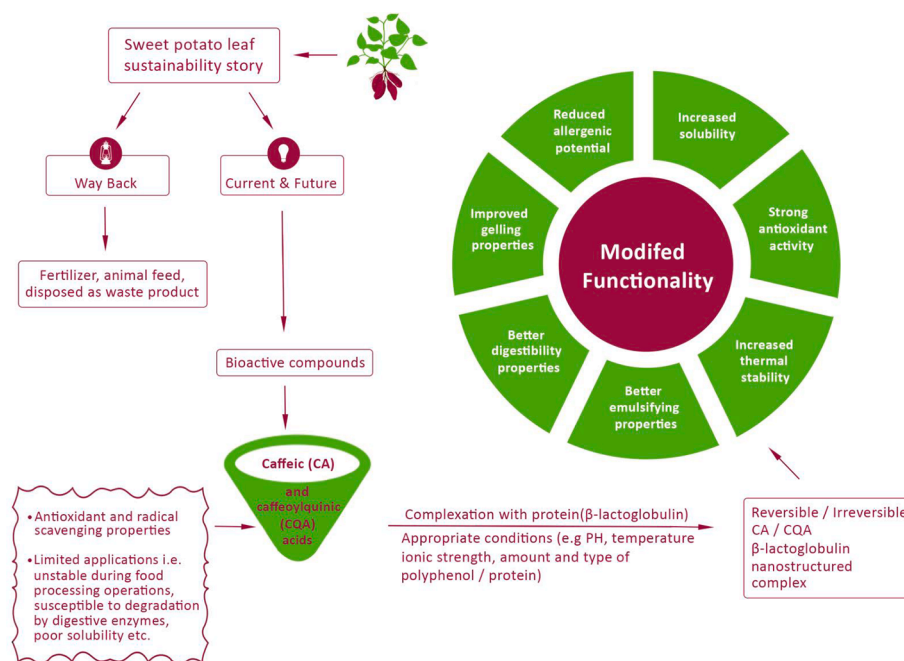


Fig. 3. Schematic representation of sweet potato leaf functionalization.

carriers with many applications associated with functional foods and nutraceutical delivery systems (Ali & Arafa, 2019; Ali, 2019; El-Maksoud et al., 2019). β -lactoglobulin-based nanoparticles encapsulating SPLPs are normally constructed using various methods. Formation of β -lactoglobulin-based nanoparticles can occur through self-assembly or self-organization of components depending on the medium conditions i.e solution pH, temperature, ionic strength, presence or absence of salt ions, β -lactoglobulin concentration, and type of coating molecules applied (Ali & Arafa, 2019; Jiang et al., 2018; Quan et al., 2019). Hence, there exist various protocols for the fabrication of β -lactoglobulin nanoparticles and it is not clear which combination of factors (mentioned above) is most suitable for a specific application (José, 2020). Optimization of ingredients and fabrication conditions is critical to obtain the most desirable effects (Makori et al., 2021).

Su, Guo, Chen, Mao, et al. (2020) reported the fabrication of self-assembled composite nanoparticles composed of β -lactoglobulin and EGCG for the delivery of Lutein. Least degradation of lutein was reported (87.2% of lutein retained after 30 days of storage) in emulsions stabilized by β -lactoglobulin-EGCG composite nanoparticles. In our previous study, we constructed self-assembled composite β -lactoglobulin or β -lactoglobulin nanoparticles-3,5-di-O-CQA with superior physico-chemical and antioxidant activity, with average diameters of 144.48–211.10 nm for β -lactoglobulin-3,5-di-O-CQA composite particles and 153.37–236.14 nm for β -lactoglobulin-3,5-di-O-CQA composite nanoparticles (Makori et al., 2021). Chemical modification is also a common fabrication technique of β -lactoglobulin nanoparticles. For example, Tao et al. (2019) prepared EGCG-modified β -lactoglobulin as a biomacromolecule with enhanced antioxidant activity and protective effect against low-density lipoprotein peroxidation. Liang et al. (2016) created chitosan/ β -lactoglobulin nanoparticles with average diameters of 100–500 nm via ionic complexation and the degradation of EGCG in the gastrointestinal tract was effectively slowed by the formation of chitosan/ β -lactoglobulin conjugate nanoparticles delivery system. Additional studies on the molecular level fabrications of β -lactoglobulin nanoparticles are depicted in Table 2.

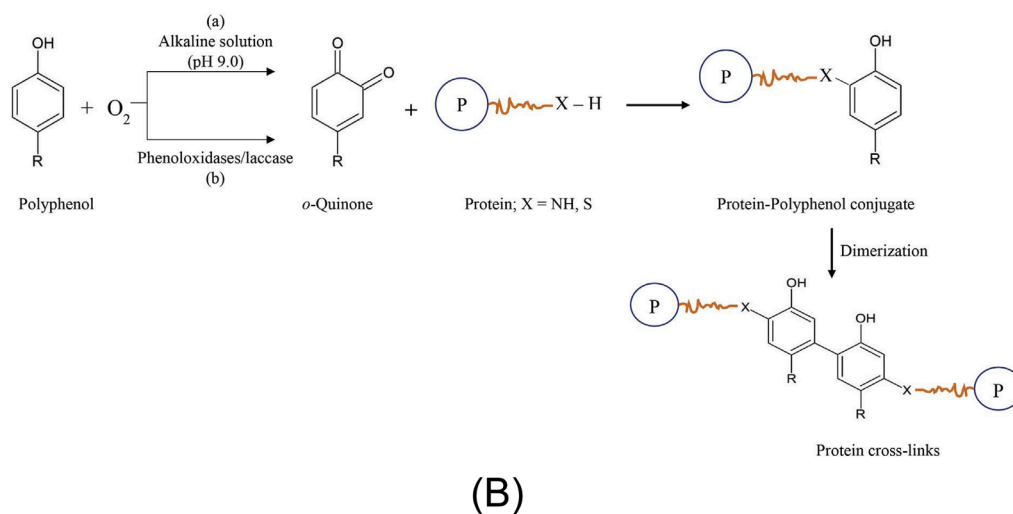
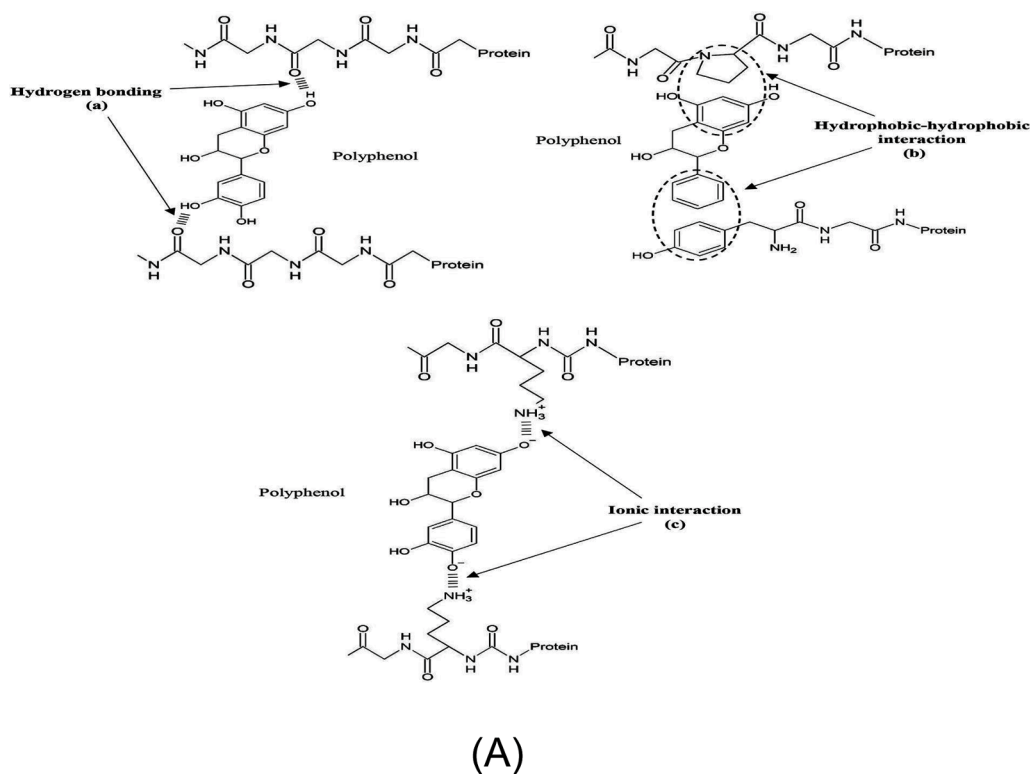
3.4. Mechanism for the formation of β -lactoglobulin-sweet potato leaf polyphenols conjugates nanoparticles

The mechanisms of interactions between proteins and polyphenols have attracted a lot of research attention due to the effect of these interactions in foods i.e structural organization in terms of the physico-chemical, sensory, and nutritional properties of foods (Liu et al., 2017). Besides, protein–polyphenol interactions have been reported to result in the development of specialized functional ingredients in the food and pharmaceutical industry (Jia et al., 2017). A number of studies reveal that the mechanisms of interactions between proteins and polyphenols occur via covalent, also known as irreversible (enzymatic or non-enzymatic methods) or non-covalent bonds, also known as reversible binding (hydrogen, hydrophobic, and ionic) (Wu, Hui, Gong, et al., 2021). Covalent interactions result in irreversible protein–polyphenol binding as opposed to non-covalent interactions which form reversible protein–polyphenol complexes (Yildirim-Elikoglu & Erdem, 2018).

3.4.1. Reversible interaction between proteins and polyphenols

Non-covalent complexes formed between proteins and polyphenols occur between the hydroxyl (–OH) group of polyphenols and the amino (–NH₂) group of proteins (Fig. 4A, a-c) (Jiang et al., 2018). Non-covalent bonds are usually weaker than covalent binding, and always are reversible. Non-covalent protein–polyphenol complexes occur through hydrophobic bridging, van der Waals attractions, hydrogen bonding, and ionic interactions (Quan et al., 2019; Yildirim-Elikoglu & Erdem, 2018). Polyphenol compounds are known to be excellent hydrogen donors able to form hydrogen bonds with carboxylic groups of proteins resulting in the formation of soluble or insoluble aggregates (Fig. 4A, a) (Zhang et al., 2020). Su, Guo, Chen, Mao, et al. (2020) revealed that the phenolic hydroxyl group of EGCG can interact with the carbon, oxygen, and amino group of the β -lactoglobulin polypeptide chain to form hydrogen bonding and hydrophobic bridging. The binding sites of rutin- β -lactoglobulin conjugates were Glu62, Asn88, Asn90, Asn109, Glu114, and Ser116 at site 1, and Leu1, Lys91, Lys91, Glu108, Ser110, Ala111, and Glu112 at site 2, to form hydrogen bonds (Al-Shabib et al., 2018). Liu et al. (2017) documented that curcumin was able to interact with β -lactoglobulin via Pro38 residues by hydrogen bonding.

Studies have demonstrated that hydrophobic interactions mostly



occur between the hydrophobic amino acid residues of proteins and the nonpolar aromatic rings of polyphenols (Fig. 4A, b) (Quan et al., 2019). Besides, proteins and polyphenols can interact through hydrophobic stacking of the aromatic groups of the protein and polyphenols (Quan et al., 2019; Su, Guo, Chen, Mao, et al., 2020; Yildirim-Elikoglu & Erdem, 2018). The binding sites of β -lactoglobulin with curcumin conjugates were Phe105, Val41, Lys60, Leu46, Leu54, Ile56, Val92 to form hydrophobic interactions (Liu et al., 2017).

Other protein–polyphenol interactions such as ionic bonds (Fig. 4A, c) contribute to a lesser extent in the complexation process (Keppler & Jan, 2020). The formation of ionic bonds may occur between the protein (i.e. ϵ -amino groups of lysine) and negatively charged groups of polyphenols (Zhang et al., 2020). Despite the unstable nature of non-covalent interactions (Keppler & Jan, 2020), studies have

Fig. 4. Mechanisms of formation of non-covalent and covalent complexes (enzyme and non-enzyme catalyzed methods) between proteins and polyphenols. Non-covalent mode of interaction of proteins with polyphenols and protein cross-linking via hydrogen bonding (Fig. 4A, a), hydrophobic-hydrophobic interaction (Fig. 4A, b), and ionic interaction (Fig. 4A, c). Covalent conjugation of protein and polyphenol and protein cross-linking via alkaline (Fig. 4B, a) and enzymatic (Fig. 4B, b) reactions. . Adapted from Quan et al. (2019)

demonstrated the potential for application as functional ingredients in the foods and pharmaceutical industry (2018).

3.4.2. Irreversible interaction between proteins and polyphenols

Irreversible associations between polyphenols and proteins involve the formation of covalent bonds via oxidation of phenolic compounds resulting in the formation of *o*-quinones or *o*-semi-quinones, which may react with the nucleophilic groups on the protein molecule (Tao et al., 2019). Irreversible associations in the formation of protein–polyphenol conjugates may occur through non-enzymatic or enzymatic methods (Fig. 4B, a and b) (Czubinski & Dwiecki, 2017).

For the non-enzymatic catalyzed method, the phenolic compounds are oxidized using various mechanisms i.e. autooxidation mechanism where an active oxygen species results in the oxidation of phenolic

Table 2

Complexation methods and modified functionalities of representative protein–polyphenol complexes.

Complexation methods	Complexation conditions	Proteins	Polyphenols	Influence on functional properties/bioactivities of complexes	References
Non-covalent complex formation	pH 7.4, at 0.05 M phosphate buffer solution, through dissolution	β -lactoglobulin(β lg)	Chlorogenic acid	Reduced surface hydrophobicity	(Jia et al., 2017).
	Through thermal pretreatment and dissolution	β lg	3,5-di-O-caffeoylquinic acid (3,5diCQA)	Decreased particle size, increased negative surface potentials, enhanced antioxidant activity	(Makori et al., 2021).
	pH 3.5 or 7.0, through mixing	Whey protein isolate (WPI)	Caffeic acid (CA)	Improved antioxidant activity, enhanced stability against degradation in simulated gastrointestinal (GIT) environment	(de Morais et al., 2020).
	pH 7.0, at 25 °C, 100 mM phosphate buffer, through mixing.	WPI	Chlorogenic acid	Improved antioxidant activity, solubility, foaming properties, and reduced surface hydrophobicity.	(Jiang et al., 2018).
	pH 3.5 or 7.0, at 25 °C for 1 h (in the dark), through mixing	WPI	CA	Increased thermal stability, reduced surface hydrophobicity, and reduced IgE-binding capacity	(2018).
Covalent complex formation	pH 2.5, 6.0, and 8.5, through covalent coupling method (crosslinker chemistry)	β lg	CA	Improved antioxidant activity, water absorption index, water solubility index, oil absorption index, thermal stability, and inhibition of lipid oxidation in emulsions.	(Abd El-Maksoud et al., 2018).
	pH 9.0, through alkaline method	WPI	Chlorogenic acid	Decreased free amino and thiol groups, increased surface hydrophobicity, and increased isoelectric point	(Ali & Arafa, 2019).
	Through atom transfer radical polymerization. (Tetra ethylene glycol used as a linker between caffeic acid units and β lg).	β lg	CA	Improved antioxidant activity, enhanced inhibition of oxidative rancidity, increased electrical and thermal stability, decreased creaming and polydispersity index	(El-Maksoud et al., 2019).
	Free radical induced grafting method	β lg	Chlorogenic acid	Improved antioxidant activity, chemical stability, and <i>in vitro</i> controlled release of (–)-Epigallocatechin-3- gallate (EGCG) nanoparticle in GIT	(Fan et al., 2017).
	Alkali dissolution-acid precipitation method, maillard reaction	WPI	Chlorogenic acid	Improved antioxidant activity, enhanced oxidative, thermal, freeze–thaw, and storage stabilities	(Liu et al., 2020).
	pH 3.5, at 25 °C, 1 h, through mixing in the dark	Native β lg and transglutaminase cross-linked β lg	CA	Improved antioxidant activity, enhanced inhibitory effects on α-glucosidase, α-amylase, and pancreatic lipase enzymes	(Stănciuc et al., 2020).
	Free radical induced grafting method	β lg	Chlorogenic acid.	Improved antioxidant activity, increased thermal stability, reduced content of free amino groups, decreased allergenic potential	(Wu et al., 2018).
	Free radical induced grafting method.	WPI	Chlorogenic acid	Reduced IgE binding capacity, decreased content of free amino and free thiol groups, increased digestibility, solubility, emulsifying activity, foaming properties, and improved antioxidant activity.	(Xu et al., 2019).

compounds; redox pair of initiator system such as free-radical grafting, alkaline reaction, ascorbic acid, hydrogen peroxide systems among others (Keppler & Jan, 2020; Quan et al., 2019). Since a full discussion on the non-enzymatic catalyzed methods would be prohibitively long, this review has focused on the non-enzymatic catalyzed alkaline reaction method specifically related to mechanisms of formation (Fig. 4B, a). Oxidation of phenolic compounds may occur at a higher pH range in the presence of an active oxygen species to form *o*-quinone or *o*-semi-quinone, radicals which readily react with nucleophilic residues in the protein side chains (Guo et al., 2021). Thus, the covalent cross-link (C–N or C–S) can be formed between protein and polyphenol (Fig. 4B, a) (Liu et al., 2017; Quan et al., 2019). Ali and Arafa (2019) found that chlorogenic acid and rosmarinic acid were covalently bound to whey protein isolate as demonstrated by the decrease in free amino and thiol groups. Moreover, it has been reported that chlorogenic acid can form covalent bonds with whey protein isolate, and the whey protein isolate bound chlorogenic acid could contribute to lower IgE binding capacity, and enhanced functional and antioxidant properties of proteins (Xu et al., 2019).

For the enzyme-catalyzed method, oxidation occurs in the presence of oxygen, phenolic compound, and the enzyme polyphenol oxidase i.e catechol oxidases and laccases (Fig. 4B, b) (Jiang et al., 2017). In the presence of molecular oxygen, the enzyme polyphenol oxidase can

catalyze the oxidation of phenolic compounds to form quinones (Fig. 4B, b). Moreover, catechol oxidases can catalyze hydroxylation and oxidation of monophenols into *o*-diphenols and *o*-diphenols into *o*-quinones, respectively, in the presence of oxygen (Quan et al., 2019). Cresolase and catecholase are the specific enzymes involved in the catalytic oxidation of phenolic compounds (Biundo et al., 2020). Specifically, catecholase which involves laccases can oxidize *o*-diphenols and *p*-diphenols into quinones, which form cross linked proteins or polyphenols by interacting with the nucleophile amino acid residues in the protein chain (Fig. 4B, b). In addition, *o*-semi-quinone radical may be generated via reverse disproportionation in excess of *o*-diphenols and *o*-quinones (Liu et al., 2017; Quan et al., 2019; Yildirim-Elikoglu & Erdem, 2018; Zhang et al., 2020). Polyphenol-protein conjugates fabricated by enzymatic methods are considered to be more specific and environmental friendly. Moreover, enzymatically fabricated polyphenol-protein complexes exhibit strong radical scavenging activity against superoxide anion radicals, but the fabrication methods are costly and complex (Gu et al., 2017).

3.5. Conjugation with other proteins/peptides

Polyphenols are able to interact with bioactive peptides or with the precursor proteins resulting in reversible or irreversible interactions as

mentioned. Several changes in the molecular digestion, absorption, metabolism and function could be anticipated as a result of these interactions (Pérez-Gregorio et al., 2020). Bioactive peptides in the human body arise mainly from dietary proteins (plant or animal derived bioactive peptides), or from the action of the microbiome and cell metabolism (Sánchez & Vázquez, 2017). Proline-rich peptides have been shown to be the most active bioactive peptides with the ability to confer resistance to cleavage by digestive enzymes (Karami & Akbari-adergani, 2019). Moreover, polyphenols have a higher affinity to bind to the proline-rich peptides derived from biological proteins (i.e. salivary proline-rich peptides) and food proteins (i.e. gliadin) (Delimont et al., 2017). The high binding activity is mainly attributed to the occurrence of proline residues which result in a more open and extended conformation of the peptidic chain (Pérez-Gregorio et al., 2020).

A part from the bioactive peptides, proteins from different sources i.e. animal, botanical or recombinant sources of food proteins have been utilized in developing protein–polyphenol conjugates (Quan et al., 2019). Some of the most commonly used animal proteins include those derived from meat (i.e. collagen, gelatin), milk (i.e. casein, whey proteins), eggs (i.e. egg white and egg yolk), and silk (from the cocoons of the silkworm) (Fathi et al., 2018; Reddy & Rapisarda, 2021). Likewise, plant proteins containing high prolamin content have been employed to produce food prolamin based nanoparticles (Song et al., 2021). Prolamin based nanoparticles have been extensively explored, especially as these form conjugate nanoparticles due to their amphiphilic effects (Brotons-Canto et al., 2021; Cheng et al., 2020; Song et al., 2021). Gliadin, hordein, secalin, zein, kafirin and avenin are some of the commonly used prolamins (Hu et al., 2017). Prolamin based nanoparticles are excellent carrier materials which can be used as delivery vehicles for drugs, and as biomolecules for controlled release applications (Hu et al., 2017; Reddy & Rapisarda, 2021). Curcumin was loaded in zein nanoparticles through desolvation method, to improve its oral bioavailability and pharmacokinetic properties in Wistar rats (administered a single oral dose of 250 mg/kg of zein-curcumin nanoparticles) (Brotons-Canto et al., 2021). The formulation containing zein-curcumin nanoparticles was well tolerated and demonstrated a 9 fold dose advantage in oral bioavailability over native curcumin. Furthermore, zein-curcumin nanoparticles developed in this study demonstrated superior bioavailability profile when compared to other curcumin complexes currently available in the market (Brotons-Canto et al., 2021). Cheng et al. (2020) prepared gliadin nanoparticles pickering emulgels through antisolvent procedure combined with microfluidization, to improve the delivery of β -carotene. Pickering emulgels stabilized with gliadin nanoparticles at 1% and 1.5% concentration exhibited improved protection and bioaccessibility at different storage conditions as well as under simulated gastrointestinal environment conditions. Pu et al. (2020) also reported a hollow resveratrol-loaded kafirin nanoparticle formulation crosslinked by gallic acid, which displayed stability against pancreatin and controlled-release of resveratrol in simulated digestion.

4. Insights into modified functionality and nutritional profiles of protein–polyphenol nanocomplexes

4.1. Protein conformational structures

As earlier mentioned, mechanisms of interactions between proteins and polyphenols involve reversible (non-covalent) and non-reversible (covalent) interactions (Wu, Hui, Gong, et al., 2021). As a consequence of this interaction, modification of secondary and tertiary structures of protein may take place (El-Maksoud et al., 2019). Molecular fabrication of protein–polyphenol nanocomplexes may result in alterations of protein secondary structures, for instance, the hydrophobicity or polarity of the aromatic amino acid residues (Jiang et al., 2017; Zhang et al., 2020). The non-covalent complexation between tannic acid and sodium caseinate led to an increase in the proportions of β -sheet on the expense of α -helix, β -turn, and random coil (Zhan et al., 2018). In

comparison, the covalent conjugation of EGCG and chlorogenic acid via the free radical method led to a slight decrease in the contents of α -helix and β -sheet structures with a corresponding increase in the unfolded structures, indicative of more protein unfolding after conjugation (Wu et al., 2018). In a different study, Zhang et al. (2020) observed that the binding interactions of selected phenolic compounds i.e. gallotannin 1,2,3,4,6-penta-*O*-galloyl- β -D-glucopyranose, ellagic acid and gallic acid at high concentrations with bovine serum albumin caused changes in the secondary structure of bovine serum albumin. The gradual addition of phenolic compounds to bovine serum albumin led to a decrease in the content of α -helix with a corresponding increase in the contents of unordered structures (Zhang et al., 2020). In this respect, the degree of modification of proteins after complexation with phenolic compounds will depend on several factors which include intrinsic (molecular nature and structure of proteins and phenolics) or extrinsic factors (pH, temperature) (Czubinski & Dwiecki, 2017). More studies are needed to make clear the precise interaction-protein structure association mechanisms for future industrial applications.

4.2. Antioxidant and functional properties of protein–polyphenol nanocomplexes

Proteins and polyphenols have unique functional properties. Therefore, the complexation of proteins and polyphenols may lead to the formation of conjugates with novel or multifunctional properties (Jia et al., 2017; Jiang et al., 2018). Previous studies have reported several functionalities of protein–polyphenol conjugates which include, among others, solubility, emulsifying, gelling, foaming, and enhanced antioxidant properties (Liu et al., 2017; Quan et al., 2019). Additional studies on the functionalities of β -lactoglobulin-SPLPs conjugates are summarized in Table 2.

4.2.1. Antioxidant activity

The antioxidant activity of proteins could be greatly enhanced due to the strong antioxidant activity of polyphenols and the ability of polyphenols to scavenge free radicals, prevent the generation of reactive oxygen species, and protect against oxidation of biological molecules (Liu et al., 2017, 2019). Several studies have shown that protein–polyphenol conjugates possess stronger antioxidant activity than original proteins. Moreover, various *in vitro* assays have been employed in the analysis of the antioxidant activity of protein–polyphenol conjugate i.e. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), ferric ion reducing antioxidant power (FRAP), oxidation inhibition assays, etc. (El-Maksoud et al., 2019; Liu et al., 2020; Stănciuc et al., 2020; Wang et al., 2020). Stănciuc et al. (2020) found that the antioxidant activity of CA- β -lactoglobulin and CA- β -lactoglobulin transglutaminase complexes possessed significantly higher antioxidant activity and inhibitory effects against metabolic syndrome enzymes.

Xu et al. (2019) prepared whey protein isolate-chlorogenic acid conjugate based on free radical grafting and evaluated their antioxidant properties by DPPH radical and ABTS radical scavenging activities assays. The antioxidant activities of the developed whey protein isolate-chlorogenic acid conjugates were significantly higher compared to the control (whey protein isolate), which was attributed to the introduction of a higher number of hydroxyl groups responsible for enhanced antioxidant activity of the developed complexes. Similarly, we found that the radical scavenging capacity was significantly increased in a dose-dependent manner when β -lactoglobulin was conjugated with 3,5-di-*O*-CQA (Makori et al., 2021). Abd El-Maksoud et al. (2018) produced multi-functional phenolic emulsifiers at different pH conditions by covalent coupling of β -lactoglobulin to CA using crosslinker chemistry and evaluated their antioxidant activities by DPPH radical and hydroxyl radical scavenging activities assays. It was noticed that DPPH radical scavenging activity increased in a dose-dependent manner when β -lactoglobulin was covalently coupled with CA.

El-Maksoud et al. (2019) also found that DPPH, ABTS, and hydroxyl radical scavenging activities assays of β -lactoglobulin with polymeric units containing CA presented higher antioxidant activity compared to native β -lactoglobulin. Moreover, a thiobarbituric acid test revealed that β -lactoglobulin with polymeric units containing CA inhibited oxidative rancidity up 4–5 fold compared to native β -lactoglobulin. In summary, the ability of protein–polyphenol complexes to enhance the antioxidant properties of the resulting conjugates makes them have potential uses in the food and non-food systems. For example, protein–polyphenol composites can be used as functional ingredients in food and non-food solutions, and to improve the oxidative stability of lipid-based foods. As well, protein–polyphenol composites can act as effective antioxidant emulsifiers and stabilizers with potential uses as delivery vehicles in fortified foods and pharmaceutical products.

4.2.2. Solubility and thermal stability

4.2.2.1. Solubility. Protein solubility or insolubility plays a crucial role in determining the functionality of proteins in food solutions since insoluble proteins have been shown to exhibit limited functionalities (Jiang et al., 2018). The good water solubility of food ingredients is a key factor in determining the application of these protein–polyphenol functional ingredients in different food systems. For example, the application of protein–polyphenol conjugates in food fortification systems including fortified waters, sports drinks, and nutritional beverages (Liu et al., 2017). Protein–polyphenol conjugation may increase or decrease the water solubility of the protein depending on some of the intrinsic (i.e. amino acid composition, amino acid sequence) and extrinsic (pH, temperature, ionic strength) factors (Zhang et al., 2020). Similarly, the mode of formation of the resulting conjugates i.e. through covalent or non-covalent bonding mechanism plays a role in protein solubility (Quan et al., 2019). For instance, the pH solubility profiles and electrical characteristics of proteins may be altered during covalent bonding with charged polyphenols (Liu et al., 2017). On the other hand, the complexation of proteins with nonpolar polyphenols may increase their surface hydrophobicity and surface activity, which in turn may lead to reduced water solubility (Zhang et al., 2020). Moreover, cross-linking of proteins may be promoted during protein–polyphenol conjugation, which may also alter their water solubility (Quan et al., 2019). Xu et al. (2019) investigated the functional properties of whey protein isolate-chlorogenic acid covalent conjugate prepared by free-radical grafting. The solubility of whey protein isolate was significantly increased by 21% after conjugation with chlorogenic acid. These findings were attributed to the phenolic hydroxyl groups produced by chlorogenic acid, which resulted in enhanced hydrophilicity of the protein surface.

Similarly, Jiang et al. (2018) revealed that the protein solubility of casein and whey protein isolate increased by 18.2% and 15.1%, respectively, after conjugation with 240 μ mol/g chlorogenic acids. The increase in water solubility was attributed to the partial protein unfolding with the addition of chlorogenic acid, reduced surface hydrophobicity, and enhanced hydrophilicity of the protein surface brought about by the phenolic hydroxyl and carboxyl groups of chlorogenic acid (Jiang et al., 2018). Abd El-Maksoud et al. (2018) also reported a significant increase in water solubility of β -lactoglobulin after covalent coupling to CA using crosslinker chemistry at different pH conditions. The highest water solubility index (84.4%) of β -lactoglobulin-CA complex was obtained at pH 8.5 (Abd El-Maksoud et al., 2018). In summary, protein–polyphenol interactions could affect the secondary and tertiary structures of the proteins which consequently may lead to a change in the solubility of the formed conjugates (Liu et al., 2017). The amount of water that can interact with proteins depends on many factors which include the number of exposed polar groups, type of polyphenol, type of protein, pH value, and chemical interactions (Abd El-Maksoud et al., 2018; Jiang et al., 2018).

4.2.2.2. Thermal stability. Changes in the thermal stability of the resulting composites may occur when phenolic compounds interact with proteins. Thermal processing of food may result in thermal denaturation and aggregation of globular proteins, which may affect food quality (Liu et al., 2017). However, an improvement in thermal stability of globular proteins can be realized by complexation with polyphenols (Wang et al., 2020). Besides, the complexation of proteins with polyphenols may result in changes in enthalpy (Zhang et al., 2020). Liu, Wang, et al. (2016) documented that thermal aggregation of lactoferrin at neutral pH was inhibited after heating and conjugation with polyphenols (chlorogenic acid and EGCG). This phenomenon was attributed to the occurrence of steric hindrance and electrostatic repulsion and enhanced thermal stability of the protein after conjugation (Liu, Wang, et al., 2016). Liu, Ma, et al. (2016) observed that the thermal stability of lactoferrin was increased after its conjugation with chlorogenic acid. Differential scanning calorimetry results showed higher transition temperature and lower enthalpy change for the ternary conjugates i.e. (chlorogenic acid-lactoferrin)-dextran conjugates, compared to lactoferrin alone (Liu, Ma, et al., 2016). Moreover, the denaturation temperature for covalently coupled β -lactoglobulin-CA conjugate was significantly higher than that of unmodified β -lactoglobulin (Abd El-Maksoud et al., 2018). Covalently linking CA to β -lactoglobulin at pH 6 increased the denaturation temperature to 128 °C for the β -lactoglobulin-CA conjugate, compared to 105 °C for the unmodified β -lactoglobulin (Abd El-Maksoud et al., 2018). Additionally, conjugation of β -lactoglobulin with polymeric units containing CA led to an increase in the endothermal peak i.e. 157.5 °C, compared to 108.9 °C for the unmodified β -lactoglobulin (El-Maksoud et al., 2019). Thus, alterations in thermal behaviours of protein–polyphenols conjugates are important in improving the thermal stability of proteins during thermal processing. Furthermore, thermal stable protein–polyphenol complexes would play a key role in the development of novel products with desired functionalities.

4.2.3. Emulsifying properties

In general, a food emulsion system is made up of an oil phase and an aqueous phase. For a food ingredient to be used as an emulsifier, it must be amphiphilic, able to reduce the interfacial tension at the oil/water interface, including inhibition of droplet aggregation, flocculation, and coalescence (Kim et al., 2020). Conjugation of proteins with polyphenols has been widely used for their high stability against thermal treatment, aggregation or flocculation, coalescence, phase separation, and the ability to exhibit high optical clarity and improved bioavailability when applied in food and non-food solutions i.e. in the pharmaceutical industry (Liu et al., 2017; Li, 2021). It has been demonstrated that the amphiphilic part of the protein can form a coating around oil droplets, at the oil–water interface, while the phenolic part acts as an antioxidant in polyunsaturated lipids against oxidation (Liu et al., 2019). The development of protein–polyphenol conjugates as novel emulsifiers has received a lot of research attention lately, due to their unique emulsification, GRAS, and biocompatible properties (Liu et al., 2017, 2019).

Chlorogenic acid-whey protein isolate-dextran ternary conjugates prepared using Maillard reaction were reported to have better emulsion stability under different environmental stresses i.e. different ionic strength, freeze–thaw conditions, and thermal treatments, than whey protein isolate alone (Wang et al., 2020). Puerarin-whey protein isolate composites formed through hydrogen bonding were found to improve the emulsifying properties of whey protein isolate in a dose dependent manner (Zhong et al., 2021). Further, a decrease in the interfacial tension of puerarin-whey protein isolate composite at the oil–water interface was observed, when compared to unmodified whey protein isolate (Zhong et al., 2021). El-Maksoud et al. (2019) also constructed β -lactoglobulin with polymeric units containing CA via conjugated polymerization. The resulting conjugates exhibited better emulsion stability and could afford up 4–5-fold oxidative stability (inhibit oxidative rancidity) of the emulsified fish oils. Xu et al. (2019)

synthesized whey protein isolate-chlorogenic acid conjugates via a free radical grafting procedure. The covalent conjugation of chlorogenic acid with whey protein isolate showed better emulsifying activity than whey protein isolates alone. The enhanced emulsifying properties of the resulting conjugates were attributed to changes in the surface hydrophobicity of the whey protein isolate and increased exposure of aromatic residues resulting in increased affinity of the whey protein isolate towards the oil/water interface (Xu et al., 2019).

Polyphenol moieties can covalently be attached to protein causing an increase in surface hydrophobicity, thereby lowering the interfacial tension (Czubinski & Dwiecki, 2017). Nonetheless, different types of polyphenols will have a difference in the degree of protein conformation resulting in a difference in emulsion activity or emulsion stability. For instance, in a recent study by Ali and Arafa (2019), rosmarinic acid was reported to carry out strong interaction with whey protein isolate, resulting in better emulsion activity and emulsion stability values than chlorogenic acid. Interestingly, conjugation of rosmarinic acid with whey protein isolate led to a decrease in isoelectric point compared with unmodified one, but a slightly higher isoelectric point when compared with whey protein isolate modified by chlorogenic acid. Further, large droplets of whey protein isolate-chlorogenic acid emulsion were observed due to the low solubility of whey protein isolate-chlorogenic acid conjugate, which resulted in an increase in the isoelectric point (Ali & Arafa, 2019). In conclusion, emulsifying properties of protein–polyphenol conjugates will depend on some factors which include polyphenol type, type of protein, as well as their associated structures, and the specified conditions of conjugation or complexation i.e pH, and temperature.

4.2.4. Gelling properties

A gel is made up of molecules linked by covalent or non-covalent forces. The covalent or non-covalent forces link together to form a continuous three-dimensional gel network (Liu et al., 2017). Proteins and polyphenols can be covalently modified to form gels with increased strength, rigidity, and thermal stability via cross-linking effects of the oxidized polyphenols (Keppler & Jan, 2020). Polyphenols could affect the interaction and cross-linking of protein molecules in the gelation process resulting in change in the physicochemical properties (Guo & Xiong, 2021). Guo and Xiong (2019) disclosed stronger myosin tail–tail interaction and formation of a more elastic gel network when myofibrillar protein was cross-linked with gallic acid.

Similarly, Erge and Eren (2021) disclosed that mechanically separated chicken meat residue gelatin gels cross-linked by caffeic acid at 2.5% caffeic acid concentration, 12.5 min oxidation period, and 50 °C cross-linking temperature caused an increase in gel strength, which was 63% higher than the uncross-linked chicken gelatin. It was deduced that caffeic acid could be used as an efficient natural cross-linking factor in enhancing the gel strength of mechanically separated chicken meat residue gelatin gels (Erge & Eren, 2021). Casas-Forero et al. (2021) also found that cryoconcentrate blueberry juice incorporated into gelatin gel at 20 g/100 g concentration led to an increase in gel strength i.e. an increase of 28% in relation to the initial gel strength. This phenomenon was further supported by Zhao and Sun (2018) who found that cross-linking gelatin with polyphenol resulted in high gel strength i.e 136.9 N/mm² at a concentration of 360 µg polyphenols/g gelatin compared to 98.4 N/mm² for genipin at 300 mg genipin/g dry gelatin concentration. In their concluding remarks, cross-linked gelatin-polyphenol complex resulted in superior gels with high denaturation temperature and better thermal stability than gels produced by cross-linking of gelatin with genipin (Zhao & Sun, 2018). As aforementioned, gels formed by covalent interactions are more compact and are characterized by high denaturation temperatures than those developed by non-covalent interactions (Guo & Xiong, 2021; Quan et al., 2019; Zhang et al., 2020). However, non-covalent interactions may also reinforce the formation of a gel structure at higher polyphenol concentrations (Keppler & Jan, 2020). Therefore, the effect of protein–polyphenol cross-linking to form

a gel will depend on many factors: type of protein and polyphenols, protein or polyphenol concentration (ingredients optimization), and mode of interaction employed.

4.2.5. Allergy and digestibility

4.2.5.1. Allergenicity properties of β -lactoglobulin-sweet potato leaf polyphenols nanocomplexes. Bovine milk allergy possesses a serious public health concern with limited application in the food and pharmaceutical industry (Wu, Hui, Gong, et al., 2021). β -lactoglobulin is a major whey protein considered an important nutrient of dairy products (Wu et al., 2018). However, the allergenic potential of β -lactoglobulin has been reported to limit its use and application in various food and non-food solutions. Sun et al. (2018) attributed the allergenic potential of β -lactoglobulin to its high stability and the fact that β -lactoglobulin is not found in human milk. Bovine milk allergy is mainly caused by immunoglobulin E (IgE) mediated reactions which can result in various clinical symptoms such as diarrhea, growth retardation, effect on the skin and respiratory system, or in severe cases may cause systemic anaphylactic reactions (2018). The complexation of allergic proteins to phenolic compounds may mask or alter IgE epitopes or block the IgE binding sites which provide a reliable way to develop hypoallergenic functional matrices with health imparting effects (Zhang et al., 2020).

Most studies have revealed hypoallergenic effects of phenolic-protein complexation *in vitro*, through covalent and non-covalent interactions. The IgE binding capacity of whey protein isolate (β -lactoglobulin and α -lactalbumin) was reduced by approximately 35% at 100 µg/ml in the digested whey protein isolate-chlorogenic acid complex compared to the unmodified whey protein isolate (Xu et al., 2019). The reduction in the IgE-binding capacity was attributed to changes in the conformational and linear epitopes, most likely brought about by covalent binding of chlorogenic acid with the nucleophilic amino acid side chains of whey protein isolate after conjugation. Similarly, covalent complexation of β -lactoglobulin with EGCG and chlorogenic acid resulted in reduced IgE-binding capacity which was linked to increased inaccessibility to epitopes or destruction of epitope structures (Wu et al., 2018). In their study, (2018) also recorded similar decreases in IgE-binding capacity for β -lactoglobulin and bovine serum albumin upon complexation with CA and EGCG at pH 3.5 or 7.0. However, only whey protein isolate complexed with EGCG showed a significant reduction, statistically, at both pH conditions. To further corroborate the reduced IgE-binding capacity of allergenic proteins upon complexation with phenolics, explorations on oral sensitization capacity and the allergenic properties *in vivo*, should be assessed (2018).

4.2.5.2. Digestion properties of β -lactoglobulin-sweet potato leaf polyphenols nanocomplexes. A better understanding of the factors that influence the digestibility or nutritional value of protein–polyphenol complexes is important to optimize the biological application of proteins for the food and nutraceutical industry (de Morais et al., 2020). Digestibility properties and nutritional characteristics of proteins after complexation with phenolic compounds should be assessed (Xu et al., 2019). Functionalized β -lactoglobulin-SPLPs nanocomplexes including ingredients or products containing added complexes have been shown to have very different bioaccessibilities (Hu et al., 2017). *In vitro* digestibility assay of menhaden oil emulsions were effectively hindered and delayed after conjugation with EGCG and genipin-mediated interfacial cross-linking (Fan et al., 2018). Moreover, EGCG conjugation and genipin-mediated interfacial cross-linking were found to reduce the rate and extent of lipolysis after 120 min as follows: 89.50% for whey protein isolate, 73.46% for whey protein isolate-genipin-mediated interfacial cross-linking conjugate, 81.23% for whey protein isolate-EGCG conjugate, and 57.61% for whey protein isolate-EGCG-genipin-mediated interfacial cross-linking conjugate (Fan et al., 2018). He et al. (2019) revealed that the digestibility of ovalbumin was increased after covalent

conjugation with EGCG. Further, complexing whey protein isolate-gum arabic conjugate with EGCG significantly improved the bioaccessibility of EGCG as evidenced by its slow and sustained release in intestinal fluid (Chen et al., 2019). In addition, the extent of lipolysis and curcumin bioaccessibility was remarkably improved after conjugation with ovotransferrin-gallic acid-carboxymethyl dextran conjugates (Wei et al., 2019).

On the contrary, studies on the *in vitro* bioavailability of β -carotene-loaded emulsions containing lipid droplets coated with egg white protein or with catechin-egg white protein conjugates showed no significant difference when they were passed through a simulated gastrointestinal tract (Gu et al., 2018). However, emulsions constructed with catechin-egg white protein complexes displayed a higher overall *in vitro* bioavailability, which was ascribed to improved stability of the carotenoids to chemical alteration (Gu et al., 2018).

On the other hand, the conjugation of proteins with polyphenols may result in changes in the nutritional value or content of the resulting conjugate in the gastrointestinal tract. For example, Malik and Saini (2017) reported a decrease in the contents of amino acids such as lysine in sunflower protein isolates containing polyphenols. The authors noted that the interaction between the nucleophilic ϵ -amino group of lysine and the oxidized form of phenolic compounds i.e. electrophilic quinones may have resulted in reduction of lysine content in the extracted protein isolates (Malik & Saini, 2017). In this regard, due attention should be given to further elucidate the metabolic consequences as regards to nanocomplexation of polyphenol compounds with proteins. Moreover, more mechanistic investigations are required to make clear the connection between type and structure of the conjugates, and bioaccessibility in the gastrointestinal tract. Besides, more *in vivo* models should be established to conduct safety tests of the developed nanocomplexes beneficial to human health.

5. Practical applications

Only a few publications have reported the practical functionality of protein-polyphenol complexes with applications in specific formula foods or as drug delivery tools (Wu, Hui, Gong, et al., 2021). The particular application of these functional protein-polyphenol capsules will depend on many factors which include particle size, food matrix, and physicochemical characteristics (Su, Guo, Chen, Dong, et al., 2020). It was revealed that the complexation of tannic acid with bovine serum albumin, egg ovalbumin, or β -lactoglobulin was enhanced at acidic pH, which was an important factor for gut-targeted drug delivery system (Xie et al., 2017). Abdel-Hamid et al. (2020) demonstrated the ability of producing a functional yoghurt enriched with polyphenol rich Chinese sweet tea extract to attenuate symptoms of chronic diseases such as cancer and cardiovascular disease. It was observed that at 1% Chinese sweet tea extract addition, the highest antioxidant activity, antihypertensive activity, and inhibition of the Caco-2 cell line were obtained i.e. 92.43%, 82.03% and 67.46%, respectively.

Several studies also registered a reduction in the IgE-binding capacity of allergenic proteins after complexation with phenolics (Pessato et al., 2018; Wu et al., 2018; Xu et al., 2019). Nevertheless, no data is available on the specific biological effects regarding the relevant dosage of polyphenols (José, 2020).

On the other hand, maltodextrin and whey protein were utilized as encapsulating agents for wheatgrass juice (Akbas et al., 2017). It was documented that encapsulated wheatgrass powders exhibited not only good thermal stability but also good stability in gastric juice. A recent study by Wu, Hui, Stipkovits, et al. (2021) incorporated functionalized whey protein-blackcurrant concentrate conjugate particles into cookies resulting in novel fortified cookies with better functional characteristics. In light of this, the incorporation of protein-polyphenol functional capsules into specific formula foods seems to be a promising strategy to improve the nutritional quality and functional properties of foods. Further, onion skin extract was encapsulated using carbohydrate or

protein-based coating materials to obtain thermal stable onion extracts with the possibility of application in thermally treated food products i.e. baked and fried foods (Akdeniz et al., 2018). Similarly, Wu, Hui, Mu, et al. (2021) constructed novel protein ingredients with high protein and anthocyanin content, indicating the possibility of producing functional ingredients with the ability to improve the protein value of starchy foods and also regulate postprandial blood sugar.

6. Concluding remarks and future trends

In summary, the application of SPLPs as functional ingredients is limited due to their low solubility, poor stability, and active efflux process in the gastrointestinal tract, which in turn result in poor absorption and bioavailability of these natural antioxidants. Proteins-polyphenol complexes can be formed through non-covalent interactions (hydrophobic bridging, hydrogen bonding, and ionic interactions) or covalent interactions brought about by enzymatic or non-enzymatic methods. The molecular level functionalization of β -lactoglobulin-based SPLPs nanoparticles can be optimized by controlling the ingredients (amount of added β -lactoglobulin or SPLPs) and medium conditions (solution pH and temperature). β -lactoglobulin nanoparticles may improve the functional properties of SPLPs and inhibit oxidation or degradation of SPLPs in the gastrointestinal tract via nanocomplexation. Moreover, β -lactoglobulin nanoparticles have shown the potential to improve the bioaccessibility of SPLPs by disrupting tight junctions and increasing transepithelial absorption via endocytosis. Meanwhile, nanocomplexation of β -lactoglobulin with SPLPs may mask IgE epitopes or blunt the IgE binding sites present in β -lactoglobulin which provides a reliable way to construct hypoallergenic supplements with epidemiologically-proved health effects. Thus, the synergistic mechanisms of nanocomplexation between β -lactoglobulin nanoparticles with SPLPs show the potential to exert health stimulating effects and augment the functional and biological properties of the resulting conjugates. Nonetheless, the complexation of proteins with polyphenols from different food sources should be greatly explored to construct novel supplements with superior functional and nutritional properties, and at the same time improve their bioaccessibility. In the future, more research attention should be directed towards the practical use of these functional complexes into specific food formulas as natural antioxidants, colourants, and to improve the protein value of starchy foods. Furthermore, toxicological risks and nutritional consequences of consuming these functionalized protein-polyphenol nanocomplexes including specific biological effects regarding the relevant dosage of polyphenols warrant more deliberate attention in further investigations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., Ishtiaq, A., Hussain, S., & Suleria, H. A. R. (2017). Natural polyphenols: An overview. *International Journal of Food Properties*, 20(8), 1689–1699. <https://doi.org/10.1080/10942912.2016.1220393>
- Abd El-Maksoud, A. A., Abd El-Ghany, I. H., El-Beltagi, H. S., Anankabil, S., Banerjee, C., Petersen, S. V., Pérez, B., & Guo, Z. (2018). Adding functionality to milk-based protein: Preparation, and physico-chemical characterization of β -lactoglobulin-phenolic conjugates. *Food Chemistry*, 241, 281–289. <https://doi.org/10.1016/j.foodchem.2017.08.101>

- Abdel-Hamid, M., Huang, Z., Suzuki, T., Enomoto, T., Hamed, A. M., Li, L., & Romeih, E. (2020). Development of a Multifunction Set Yogurt Using *Rubus suavissimus* S. Lee (Chinese Sweet Tea) Extract. *Foods*, 9(9), 1163. <https://doi.org/10.3390/foods9091163>
- Akbas, E., Kilercioglu, M., Onder, O. N., Koker, A., Soyler, B., & Oztot, M. H. (2017). Wheatgrass juice to wheat grass powder: Encapsulation, physical and chemical characterization. *Journal of Functional Foods*, 28, 19–27. <https://doi.org/10.1016/j.jff.2016.11.010>
- Akdeniz, B., Sumnu, G., & Sahin, S. (2018). Microencapsulation of phenolic compounds extracted from onion (*Allium cepa*) skin. *Journal of Food Processing and Preservation*, 42(7), e13648. <https://doi.org/10.1111/jfpp.v42.710.1111/jfpp.13648>
- Al-Shabib, N. A., Khan, J. M., Malik, A., Alsenaidy, M. A., Rehman, M. T., AlAjmi, M. F., Alsenaidy, A. M., Husain, F. M., & Khan, R. H. (2018). Molecular insight into binding behavior of polyphenol (rutin) with beta lactoglobulin: Spectroscopic, molecular docking and MD simulation studies. *Journal of Molecular Liquids*, 269, 511–520. <https://doi.org/10.1016/j.molliq.2018.07.122>
- Alam, M. K. (2021). A comprehensive review of sweet potato (*Ipomoea batatas* [L.] Lam): Revisiting the associated health benefits. *Trends in Food Science and Technology*, 115(April), 512–529. <https://doi.org/10.1016/j.tifs.2021.07.001>
- Ali, M., & Arafa, S. (2019). Characterization of Whey Protein Isolate Covalently Modified with Phenolic Compounds 2: Physicochemical and Emulsifying Properties. *Journal of Food and Dairy Sciences*, 10(1), 1–8. [10.21608/jfds.2019.36132](https://doi.org/10.21608/jfds.2019.36132)
- Ali, M. (2019). Chemical, structural and functional properties of whey proteins covalently modified with phytochemical compounds. *Journal of Food Measurement and Characterization*, 13(4), 2970–2979. <https://doi.org/10.1007/s11694-019-00217-1>
- Biundo, A., Braunschmid, V., Pretzler, M., Kampatsikas, I., Darnhofer, B., Birner-Gruenberger, R., Rompel, A., Ribitsch, D., & Guebitz, G. M. (2020). Polyphenol oxidases exhibit promiscuous proteolytic activity. *Communications Chemistry*, 3(1), 1–8. <https://doi.org/10.1038/s42004-020-0305-2>
- Bonarek, P., Loch, J. I., Tworzydło, M., Cooper, D. R., Milto, K., Wróbel, P., Kurpińska, K., & Lewiński, K. (2020). Structure-based design approach to rational site-directed mutagenesis of β -lactoglobulin. *Journal of Structural Biology*, 210(2), 107493. <https://doi.org/10.1016/j.jsb.2020.107493>
- Broersen, K. (2020). Milk processing affects structure, bioavailability and immunogenicity of β -lactoglobulin. *Foods*, 9(7), 874. <https://doi.org/10.3390/foods9070874>
- Brotons-Canto, A., González-Navarro, C. J., Gil, A. G., Asin-Prieto, E., Saiz, M. J., & Llabrés, J. M. (2021). Zein nanoparticles improve the oral bioavailability of curcumin in wistar rats. *Pharmaceutics*, 13(3), 1–14. <https://doi.org/10.3390/pharmaceutics13030361>
- Carrera, C., Zelaya-Medina, C. F., Chinchilla, N., Ferreira-González, M., Barbero, G. F., & Palma, M. (2021). How Different Cooking Methods Affect the Phenolic Composition of Sweet Potato for Human Consumption (*Ipomoea batata* (L.) Lam). *Agronomy*, 11(8), 1636. <https://doi.org/10.3390/agronomy11081636>
- Casas-Forero, N., Moreno-Osorio, L., Orellana-Palma, P., & Petzold, G. (2021). Effects of cryoconcentrate blueberry juice incorporation on gelatin gel: A rheological, textural and bioactive properties study. *LWT - Food Science and Technology*, 138, 110674. <https://doi.org/10.1016/j.lwt.2020.110674>
- Chen, W., Lv, R., Muhammad, A. I., Guo, M., Ding, T., Ye, X., & Liu, D. (2019). Fabrication of (–)-epigallocatechin-3-gallate carrier based on glycosylated whey protein isolate obtained by ultrasound Maillard reaction. *Ultrasonics Sonochemistry*, 58, 104678. <https://doi.org/10.1016/j.ultsonch.2019.104678>
- Cheng, C.e., Gao, Y.i., Wu, Z., Miao, J., Gao, H., Ma, L.i., Zou, L., Peng, S., Liu, C., & Liu, W. (2020). Gliadin nanoparticles Pickering emulsions for β -carotene delivery: Effect of particle concentration on the stability and bioaccessibility. *Molecules*, 25 (18), 4188. <https://doi.org/10.3390/molecules25184188>
- Cosme, F., Fernandes, C., Ribeiro, T., Filipe-Ribeiro, L., & Nunes, F. M. (2020). White wine protein instability: Mechanism, quality control and technological alternatives for wine stabilisation—an overview. *Beverages*, 6(1), 1–28. <https://doi.org/10.3390/beverages6010019>
- Czubinski, J., & Dwiecki, K. (2017). A review of methods used for investigation of protein-phenolic compound interactions. *International Journal of Food Science and Technology*, 52(3), 573–585. <https://doi.org/10.1111/ijfs.2017.52.issue-310.1111/ijfs.13339>
- Dai, T., Chen, J., McClements, D. J., Hu, P., Ye, X., Liu, C., & Li, T. (2019). Protein-polyphenol interactions enhance the antioxidant capacity of phenolics: Analysis of rice glutelin-procyanidin dimer interactions. *Food and Function*, 10(2), 765–774. <https://doi.org/10.1039/c8fo02246a>
- Dai, W., Ruan, C., Sun, Y., Gao, X., & Liang, J. (2020). Controlled release and antioxidant activity of chitosan and β -lactoglobulin complex nanoparticles loaded with epigallocatechin gallate. *Colloids and Surfaces B: Biointerfaces*, 188(110802).
- F.P.R. de Moraes T.B. Pessato E. Rodrigues L. Peixoto Mallmann L.R.B. Mariutti F.M. Netto 133 2020 109104 10.1016/j.foodres.2020.109104.
- Delimont, N. M., Rosenkranz, S. K., Haub, M. D., & Lindshield, B. L. (2017). Salivary proline-rich protein may reduce tannin-iron chelation: A systematic narrative review. *Nutrition and Metabolism*, 14(1), 1–16. <https://doi.org/10.1186/s12986-017-0197-z>
- Dias, R., Pereira, C. B., Pérez-Gregorio, R., Mateus, N., & Freitas, V. (2021). Recent advances on dietary polyphenol's potential roles in Celiac Disease. *Trends in Food Science and Technology*, 107, 213–225. <https://doi.org/10.1016/j.tifs.2020.10.033>
- A.A.A. El-Maksoud S. Anankanbil Y. Zhou I.H.A. El-Ghany H.S. El-Beltagi C. Banerjee S. V. Petersen Z. Guo Grafting phenolics onto milk protein via conjugated polymerization for delivery of multiple functionalities: Synthesis and characterization Food Chemistry 301 April 2019 125298 10.1016/j.foodchem.2019.125298.
- Erge, A., & Eren, Ö. (2021). Chicken gelatin modification by caffeic acid: A response surface methodology investigation. *Food Chemistry*, 351, 129269. <https://doi.org/10.1016/j.foodchem.2021.129269>
- Fan, Y., Liu, Y., Gao, L., Zhang, Y., & Yi, J. (2018). Oxidative stability and in vitro digestion of menhaden oil emulsions with whey protein: Effects of EGCG conjugation and interfacial cross-linking. *Food Chemistry*, 265(May), 200–207. <https://doi.org/10.1016/j.foodchem.2018.05.098>
- Fan, Y., Zhang, Y., Yokoyama, W., & Yi, J. (2017). β -Lactoglobulin-chlorogenic acid conjugate-based nanoparticles for delivery of (–)-epigallocatechin-3-gallate. *RSC Advances*, 7(35), 21366–21374. <https://doi.org/10.1039/c6ra28462k>
- A. Farah J. de Lima P. Consumption of chlorogenic acids through coffee and health implications Beverages 5 1 2019 10.3390/beverages5010011.
- Fathi, Milad, Donsi, Francesco, & McClements, David Julian (2018). Protein-Based Delivery Systems for the Nanoencapsulation of Food Ingredients. *Comprehensive Reviews in Food Science and Food Safety*, 17(4), 920–936. <https://doi.org/10.1111/crf3.2018.17.issue-410.1111/1541-4337.12360>
- Ferreira, I. C. F. R., Barros, L., Carrocho, M., Antonio, A. L., Caleja, C., & Oliveira, M. B. P. P. (2016). Fortification of yogurts with different antioxidant preservatives: A comparative study between natural and synthetic additives. *Food Chemistry*, 210, 262–268. <https://doi.org/10.1016/j.foodchem.2016.04.114>
- Fronđ, A. D., Iuhas, C. I., Stirbu, I., Leopold, L., Socaci, Sonia, Andreea, S., Ayvaz, H., Andreea, S., Mihai, S., Diaconescu, Z., & Carmen, S. (2019). Phytochemical Characterization of Five Edible. *Molecules*, 1–22.
- Fu, Z. F., Tu, Z. C., Zhang, L., Wang, H., Wen, Q. H., & Huang, T. (2016). Antioxidant activities and polyphenols of sweet potato (*Ipomoea batatas* L.) leaves extracted with solvents of various polarities. *Food Bioscience*, 15, 11–18. <https://doi.org/10.1016/j.fbio.2016.04.004>
- Jiao Ge Xueyang Yue Shuo Wang Jinpeng Chi Jin Liang Yue Sun Xueling Gao Pengxiang Yue 116 2019 336 345 10.1016/j.foodres.2018.08.045.
- Daniel Granato Andrei Mocan José S. Câmara 98 2020 162 166.
- Gu, L., Pan, C., Su, Y., Zhang, R., Xiao, H., McClements, D. J., & Yang, Y. (2018). In Vitro Bioavailability, Cellular Antioxidant Activity, and Cytotoxicity of β -Carotene-Loaded Emulsions Stabilized by Catechin-Egg White Protein Conjugates. *Journal of Agricultural and Food Chemistry*, 66(7), 1649–1657. <https://doi.org/10.1021/acs.jafc.7b05909>
- Gu, L., Peng, N., Chang, C., McClements, D. J., Su, Y., & Yang, Y. (2017). Fabrication of Surface-Active Antioxidant Food Biopolymers: Conjugation of Catechin Polymers to Egg White Proteins. *Food Biophysics*, 12(2), 198–210. <https://doi.org/10.1007/s11483-017-9476-5>
- Guo, A., & Xiong, Y. L. (2019). Glucose oxidase promotes gallic acid-myofibrillar protein interaction and thermal gelation. *Food Chemistry*, 293(May), 529–536. <https://doi.org/10.1016/j.foodchem.2019.05.018>
- Guo, Anqi, & Xiong, Youling L. (2021). Myoprotein-phytophenol interaction: Implications for muscle food structure-forming properties. *Comprehensive Reviews in Food Science and Food Safety*, 20(3), 2801–2824. <https://doi.org/10.1111/crf3.v20.310.1111/1541-4337.12733>
- Guo, J., Wang, L., Zhang, X., Yu, H., & Zhang, N. (2021). Hemp (*Cannabis sativa* L.) Seed Protein-EGCG Conjugates. *Covalent Bonding and Functional Research. Foods*, 10 (1618). [10.3390/foods10071618](https://doi.org/10.3390/foods10071618).
- Ha, H. K., Rankin, S. A., Lee, M. R., & Lee, W. J. (2019). Development and characterization of whey protein-based nano-delivery systems: A review. *Molecules*, 24(18), 1–17. <https://doi.org/10.3390/molecules24183254>
- He, W., Xu, H., Lu, Y., Zhang, T., Li, S., Lin, X., Xu, B., & Wu, X. (2019). Function, digestibility and allergenicity assessment of ovalbumin-EGCG conjugates. *Journal of Functional Foods*, 61(February), Article 103490. <https://doi.org/10.1016/j.jff.2019.103490>
- Hu, B., Liu, X., Zhang, C., & Zeng, X. (2017). Food macromolecule based nanodelivery systems for enhancing the bioavailability of polyphenols. *Journal of Food and Drug Analysis*, 25(1), 3–15. <https://doi.org/10.1016/j.jfda.2016.11.004>
- Jang, Y., & Koh, E. (2019). Antioxidant content and activity in leaves and petioles of six sweet potato (*Ipomoea batatas* L.) and antioxidant properties of blanched leaves. *Food Science and Biotechnology*, 28(2), 337–345. <https://doi.org/10.1007/s10068-018-0481-3>
- Jia, J., Gao, X., Hao, M., & Tang, L. (2017). Comparison of binding interaction between beta -lactoglobulin and three common polyphenols using multi-spectroscopy and modeling methods. *Food Chemistry*, 228, 143–151. <https://doi.org/10.1016/j.foodchem.2017.01.131>
- Jiang, J., Zhang, Z., Zhao, J., & Liu, Y. (2018). The effect of non-covalent interaction of chlorogenic acid with whey protein and casein on physicochemical and radical-scavenging activity of in vitro protein digests. *Food Chemistry*, 268(June), 334–341. <https://doi.org/10.1016/j.foodchem.2018.06.015>
- Jiang, Z., Yuan, X., Yao, K., Li, X., Zhang, X., Mu, Z., Jiang, L., & Hou, J. (2017). Laccase-aided modification: Effects on structure, gel properties and antioxidant activities of α -lactalbumin. *LWT - Food Science and Technology*, 80, 355–363. <https://doi.org/10.1016/j.lwt.2017.02.043>
- Jongberg, S., Andersen, M. L., & Lund, M. N. (2020). Covalent Protein-Polyphenol Bonding as Initial Steps of Haze Formation in Beer. *Journal of the American Society of Brewing Chemists*, 78(2), 153–164. <https://doi.org/10.1080/03610470.2019.1705045>
- Karami, Z., & Akbari-adergani, B. (2019). Bioactive food derived peptides: A review on correlation between structure of bioactive peptides and their functional properties. *Journal of Food Science and Technology*, 56(2), 535–547. <https://doi.org/10.1007/s13197-018-3549-4>
- Keppeler, J. K., Schwarz, K., & van der Goot, A. J. (2020). Covalent modification of food proteins by plant-based ingredients (polyphenols and organosulphur compounds): A commonplace reaction with novel utilization potential. *Trends in Food Science and*

- Technology, 101(October 2019), 38–49. <https://doi.org/10.1016/j.tifs.2020.04.023>.
- Kim, W., Wang, Y., & Selomulya, C. (2020). Dairy and plant proteins as natural food emulsifiers. *Trends in Food Science and Technology*, 105(June), 261–272. <https://doi.org/10.1016/j.tifs.2020.09.012>
- Kremr, D., Bajer, T., Bajerová, P., Surmová, S., & Ventura, K. (2016). Unremitting problems with chlorogenic acid Nomenclature: A review. *Quim. Nova*, 39(4), 530–533. [10.5935/0100-4042.20160063](https://doi.org/10.5935/0100-4042.20160063).
- Krochmal-Marczak, Barbara, Cebulak, Tomasz, Kapusta, Ireneusz, Oszmianski, Jan, Kaszuba, Joanna, & Żurek, Natalia (2020). The content of phenolic acids and flavonols in the leaves of nine varieties of sweet potatoes (*Ipomoea batatas* L.) depending on their development, grown in central Europe. *Molecules*, 25(15), 3473. <https://doi.org/10.3390/molecules25153473>
- Kurata, R., Sun, H.-N., Oki, T., Okuno, S., Ishiguro, K., & Sugawara, T. (2019). Sweet potato polyphenols. In *Sweet Potato* (pp. 177–222). Elsevier Inc.. <https://doi.org/10.1016/b978-0-12-813637-9.00007-7>
- Liang, J., Yan, H., Yang, H. J., Kim, H. W., Wan, X., Lee, J., & Ko, S. (2016). Synthesis and controlled-release properties of chitosan- β -lactoglobulin nanoparticles as carriers for oral administration of epigallocatechin gallate. *Food Science and Biotechnology*, 25 (6), 1583–1590. <https://doi.org/10.1007/s10068-016-0244-y>
- Liang, N., Dupuis, J. H., Yada, R. Y., & Kitts, D. D. (2019). Chlorogenic acid isomers directly interact with Keap 1-Nrf2 signaling in Caco-2 cells. *Molecular and Cellular Biochemistry*, 457(1), 105–118. <https://doi.org/10.1007/s11010-019-03516-9>
- Lila, M. A., Schneider, M., Devlin, A., Plundrich, N., Laster, S., & Foegeding, E. A. (2017). Polyphenol-enriched berry extracts naturally modulate reactive proteins in model foods. *Food and Function*, 8(12), 4760–4767. <https://doi.org/10.1039/C7FO00883J>
- Liu, C., Jin, H., Yu, Y., Sun, J., Zheng, H., Zhang, Y., Xu, J., & Zhu, X. (2020). The improvement of nanoemulsion stability and antioxidant via protein-chlorogenic acid-dextran conjugates as emulsifiers. *Nanomaterials*, 10(6), 1–20. <https://doi.org/10.3390/nano10061094>
- Liu, Jingwen, Jiang, L., Zhang, Y., Du, Z., Qiu, X., Kong, L., & Zhang, H. (2017). Binding behaviors and structural characteristics of ternary complexes of β -lactoglobulin, curcumin, and fatty acids. *RSC Advances*, 7(73), 45960–45967. <https://doi.org/10.1039/c7ra09012a>
- Liu, Jun, Yong, H., Yao, X., Hu, H., Yun, D., & Xiao, L. (2019). Recent advances in phenolic-protein conjugates: Synthesis, characterization, biological activities and potential applications. *RSC Advances*, 9(61), 35825–35840. <https://doi.org/10.1039/c9ra07808h>
- Luo, Dan, Mu, Tai-Hua, Sun, Hongnan, & Chen, Jingwang (2020). Optimization of the formula and processing of a sweet potato leaf powder-based beverage. *Food Science and Nutrition*, 8(6), 2680–2691. <https://doi.org/10.1002/fsn3.v8.6.10.1002/fsn3.1555>
- Shadrack Isaboke Makori Tai-Hua Mu Hong-Nan Sun Total Polyphenol Content, Antioxidant Activity, and Individual Phenolic Composition of Different Edible Parts of 4 Sweet Potato Cultivars Natural Product Communications 15 7 2020 1934578X2093693 10.1177/1934578X20936931.
- Makori, Shadrack Isaboke, Mu, Tai-Hua, & Sun, Hong-Nan (2021). Physicochemical properties, antioxidant activities, and binding behavior of 3,5-di-O-caffeoylquinic acid with β -lactoglobulin colloidal particles. *Food Chemistry*, 347, 129084. <https://doi.org/10.1016/j.foodchem.2021.129084>
- Malik, M. A., & Saini, C. S. (2017). Polyphenol removal from sunflower seed and kernel: Effect on functional and rheological properties of protein isolates. *Food Hydrocolloids*, 63, 705–715. <https://doi.org/10.1016/j.foodhyd.2016.10.026>
- Milincić, Danijel D., Popović, Dušanka A., Lević, Steva M., Kostić, Aleksandar Ž., Tešić, Živoslav Lj., Nedović, Viktor A., & Pešić, Mirjana B. (2019). Application of polyphenol-loaded nanoparticles in food industry. *Nanomaterials*, 9(11), 1629. <https://doi.org/10.3390/nano9111629>
- Musilová, J., Bystrická, J., Árvay, J., & Harangózo, Ľ. (2017). Polyphenols And Phenolic Acids In Sweet Potato (*Ipomoea Batatas* L.) Roots. *Potravinárstvo Slovak Journal of Food Sciences*, 11(1), 82–87.
- Nguyen, H. C., Chen, C., Lin, K., Chao, P., Lin, H., & Huang, M.-Y. (2021). Bioactive Compounds, Antioxidants, and Health Benefits of Sweet Potato Leaves. *Molecules*, 26 (1820), 1–13.
- Pérez-Gregorio, Rosa, Soares, Susana, Mateus, Nuno, & de Freitas, Victor (2020). Bioactive peptides and dietary polyphenols: Two sides of the same coin. *Molecules*, 25(15), 3443. <https://doi.org/10.3390/molecules25153443>
- T.B. Pessato F.P.R. de Moraes N.C. de Carvalho A.C.M. Figueira L.G.R. Fernandes R. de Zollner L., & Netto, F. M. Protein structure modification and allergenic properties of whey proteins upon interaction with tea and coffee phenolic compounds Journal of Functional Foods 51 October 2018 121 129 10.1016/j.jff.2018.10.019.
- Pu, C., Tang, W., Liu, M., Zhu, Y., & Sun, Q. (2020). Resveratrol-loaded hollow kafirin nanoparticles via gallic acid crosslinking: An evaluation compared with their solid and non-crosslinked counterparts. *Food Research International*, 135(April), Article 109308. <https://doi.org/10.1016/j.foodres.2020.109308>
- Quan, T. H., Benjakul, S., Sae-leaw, T., Balange, A. K., & Maqsood, S. (2019). Protein–polyphenol conjugates: Antioxidant property, functionalities and their applications. *Trends in Food Science & Technology*, 91, 507–517. <https://doi.org/10.1016/j.tifs.2019.07.049>
- Reddy, N., & Rapisarda, M. (2021). Properties and applications of nanoparticles from plant proteins. *Materials*, 14(13), 1–41. <https://doi.org/10.3390/ma14133607>
- Sánchez, A., & Vázquez, A. (2017). Bioactive peptides: A review. *Food Quality and Safety*, 1(1), 29–46. <https://doi.org/10.1093/fqs/fyx006>
- Schouten, Maria Alessia, Tappi, Silvia, Angeloni, Simone, Cortese, Manuela, Caprioli, Giovanni, Vittori, Sauro, & Romani, Santina (2021). Acrylamide formation and antioxidant activity in coffee during roasting – A systematic study. *Food Chemistry*, 343, 128514. <https://doi.org/10.1016/j.foodchem.2020.128514>
- Sengupta, B., Das, N., & Sen, P. (2018). Monomerization and aggregation of β -lactoglobulin under adverse condition: A fluorescence correlation spectroscopic investigation. *Biochimica et Biophysica Acta - Proteins and Proteomics*, 1866(2), 316–326. <https://doi.org/10.1016/j.bbapap.2017.11.007>
- Song, Jingru, Sun, Cuixia, Gul, Khalid, Mata, Analucia, & Fang, Yapeng (2021). Prolamin-based complexes: Structure design and food-related applications. *Comprehensive Reviews in Food Science and Food Safety*, 20(2), 1120–1149. <https://doi.org/10.1111/crf3.v20.2.10.1111/1541-4337.12713>
- Stănciuc, N., Răpeanu, G., Bahrim, G. E., & Aprodu, I. (2020). The interaction of bovine β -lactoglobulin with caffeic acid: From binding mechanisms to functional complexes. *Biomolecules*, 10(8), 1–14. <https://doi.org/10.3390/biom10081096>
- Suárez, S., Mu, T., Sun, H., & Anón, M. C. (2020). Antioxidant activity, nutritional, and phenolic composition of sweet potato leaves as affected by harvesting period. *International Journal of Food Properties*, 23(1), 178–188. <https://doi.org/10.1080/10942912.2020.1716796>
- Sun, Hong Nan, Mu, T. H., & Xi, L. S. (2017). Effect of pH, heat, and light treatments on the antioxidant activity of sweet potato leaf polyphenols. *International Journal of Food Properties*, 20(2), 318–332. <https://doi.org/10.1080/10942912.2016.1160410>
- Sun, Z., Wang, M., Han, S., Ma, S., Zou, Z., Ding, F., Li, X., Li, L., Tang, B., Wang, H., Li, N., Che, H., & Dai, Y. (2018). Production of hypoallergenic milk from DNA-free beta-lactoglobulin (BLG) gene knockout cow using zinc-finger nucleases mRNA. *Scientific Reports*, 8(1), 1–11. <https://doi.org/10.1038/s41598-018-32024-x>
- Tang, B., Huang, Y., Yang, H., Tang, P., & Li, H. (2016). Molecular mechanism of the binding of 3,4,5-tri-O-caffeoylquinic acid to human serum albumin: Saturation transfer difference NMR, multi-spectroscopy, and docking studies. *Journal of Photochemistry and Photobiology B: Biology*, 165, 24–33. <https://doi.org/10.1016/j.jphotobiol.2016.10.017>
- Tao, Fei, Xiao, Chaogeng, Chen, Wenwei, Zhang, Yongyong, Pan, Jiarong, & Jia, Zhenbao (2019). Covalent modification of β -lactoglobulin by (–)-epigallocatechin-3-gallate results in a novel antioxidant molecule. *International Journal of Biological Macromolecules*, 126, 1186–1191.
- Thi Lan Khanh, Phan, Chittrakorn, Sasivimon, Rutnakornpituk, Boonjira, Phan Tai, Huan, & Ruttarattanamongkol, Khanitta (2018). Processing effects on anthocyanins, phenolic acids, antioxidant activity, and physical characteristics of Vietnamese purple-fleshed sweet potato flours. *Journal of Food Processing and Preservation*, 42(9), e13722. <https://doi.org/10.1111/jfpp.v42.9.10.1111/jfpp.13722>
- Usuki, T., Onda, S., Yoshizawa-Fujita, M., & Rikukawa, M. (2017). Use of [C4mim]Cl for efficient extraction of caffeoylquinic acids from sweet potato leaves. *Scientific Reports*, 7(1), 1–7. <https://doi.org/10.1038/s41598-017-07291-9>
- Wang, Hao, Yan, Yong, Feng, Xiangru, Wu, Zijian, Guo, Yatu, Li, Heyu, & Zhu, Qiaomei (2020). Improved physicochemical stability of emulsions enriched in lutein by a combination of chlorogenic acid–whey protein isolate–dextran and vitamin E. *Journal of Food Science*, 85(10), 3323–3332. <https://doi.org/10.1111/jfds.v85.10.10.1111/1750-3841.15417>
- Wei, Z., Zhang, H., & Huang, Q. (2019). Curcumin-loaded Pickering emulsion stabilized by insoluble complexes involving ovotransferrin-gallic acid conjugates and carboxymethyl dextran. *Food and Function*, 10(8), 4911–4923. <https://doi.org/10.1039/c9fo01162e>
- Wu, X., Lu, Y., Xu, H., Lin, D., He, Z., Wu, H., Liu, L., & Wang, Z. (2018). Reducing the allergenic capacity of β -lactoglobulin by covalent conjugation with dietary polyphenols. *Food Chemistry*, 256, 427–434. <https://doi.org/10.1016/j.foodchem.2018.02.158>
- Xie, L., Wehling, R. L., Ciftci, O., & Zhang, Y. (2017). Formation of complexes between tannic acid with bovine serum albumin, egg ovalbumin and bovine beta-lactoglobulin. *Food Research International*, 102, 195–202. <https://doi.org/10.1016/j.foodres.2017.10.007>
- Xu, Haoxie, Zhang, Tingting, Lu, Yuqin, Lin, Xiao, Hu, Xiaopeng, Liu, Lizhong, He, Zhenan, & Wu, Xuli (2019). Effect of chlorogenic acid covalent conjugation on the allergenicity, digestibility and functional properties of whey protein. *Food Chemistry*, 298, 125024. <https://doi.org/10.1016/j.foodchem.2019.125024>
- Xue, M., Shi, H., Zhang, J., Liu, Q. Q., Guan, J., Zhang, J. Y., & Ma, Q. (2016). Stability and degradation of caffeoylquinic acids under different storage conditions studied by high-performance liquid chromatography with photo diode array detection and high-performance liquid chromatography with electrospray ionization collision-induc. *Molecules*, 21(7), 1–13. <https://doi.org/10.3390/molecules21070948>
- Shizhang Yan Fengying Xie Shuang Zhang Lianzhou Jiang Baokun Qi Yang Li 609 2021 125641 10.1016/j.colsurfa.2020.125641.
- Yang, Bingyan, Dong, Yixin, Wang, Fei, & Zhang, Yu (2020). Nanoformulations to enhance the bioavailability and physiological functions of polyphenols. *Molecules*, 25 (20), 4613. <https://doi.org/10.3390/molecules25204613>
- Yildirim-Elikoglu, S., & Erdem, Y. K. (2018). Interactions between milk proteins and polyphenols: Binding mechanisms, related changes, and the future trends in the dairy industry. *Food Reviews International*, 34(7), 665–697. <https://doi.org/10.1080/87559129.2017.1377225>
- Younes, Maged, Aggett, Peter, Aguilar, Fernando, Crebelli, Riccardo, Dusemund, Birgit, Filipić, Metka, ... Wright, Matthew (2018). Scientific opinion on the safety of green tea catechins. *EFSA Journal*, 16(4). <https://doi.org/10.2903/j.efsa.2018.5239>
- Zhan, F., Yang, J., Li, J., Wang, Y., & Li, B. (2018). Characteristics of the interaction mechanism between tannic acid and sodium caseinate using multispectroscopic and thermodynamics methods. *Food Hydrocolloids*, 75, 81–87. <https://doi.org/10.1016/j.foodhyd.2017.09.010>
- Zhang, C., Liu, D., Wu, L., Zhang, J., Li, X., & Wu, W. (2019). Chemical Characterization and Antioxidant Properties of Ethanolic Extract and Its Fractions from Sweet Potato (*Ipomoea batatas* L.) Leaves. *Foods*, 18, 1–14.
- Zhang, L., Liu, Y., Hu, X., Xu, M., & Wang, Y. (2020). Studies on interactions of pentagalloyl glucose, ellagic acid and gallic acid with bovine serum albumin: A

- spectroscopic analysis. *Food Chemistry*, 324, Article 126872. <https://doi.org/10.1016/j.foodchem.2020.126872>
- Zhao, Y., & Sun, Z. (2018). Effects of gelatin-polyphenol and gelatin-genipin cross-linking on the structure of gelatin hydrogels. *International Journal of Food Properties*, 20(00), S2822–S2832. <https://doi.org/10.1080/10942912.2017.1381111>
- Zhong, Yejun, Zhao, Jincheng, Dai, Taotao, Ye, Jiangping, Wu, Jianyong, Chen, Tingting, & Liu, Chengmei (2021). Fabrication of oil-in-water emulsions with whey protein isolate-puerarin composites: Environmental stability and interfacial behavior. *Foods*, 10(4), 705. <https://doi.org/10.3390/foods10040705>