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REVIEW



Improvements of plant protein functionalities by Maillard conjugation and Maillard reaction products

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ABSTRACT

Plant-derived protein research has gained attention in recent years due to the rise of health concerns, allergenicity, trends toward vegan diet, food safety, and sustainability; but the lower techno-functional attributes of plant proteins compared to those of animals still remain a challenge for their utilization. Maillard conjugation is a protein side-chain modification reaction which is spontaneous, and do not require additional chemical additive to initiate the reaction. The glyco-conjugates formed during the reaction significantly improves the thermal stability and pH sensitivity of proteins. The modification of plant-derived protein using Maillard conjugation requires a comprehensive understanding of the influence of process conditions on the conjugation process. These factors can be used to establish a correlation with different functional and bioactive characteristics, to potentially adapt this approach for selective functionality enhancement and nutraceutical development. This review covers recent advances in plant-derived protein modification using Maillard conjugation, including different pretreatments to modify the functionality and bioactivity of plant proteins and their potential uses in practice. An overview of different properties of conjugates and MRPs, including food safety aspects, is given.

KEYWORDS

Maillard conjugation; plant protein; Maillard reaction products; protein modification

1. Introduction

A shift in the consumer choices toward vegan diet based on health or ethical preferences and sustainability has been observed in recent years, paving the way to research on replacing animal-based protein with plant-based ones (de Boer and Aiking 2011; Lam et al. 2018; Sack et al. 2015). Both animal and plant origin proteins are prone to structural deformation and instability due to the sensitivity to pH, temperature, and external stresses, therefore they can be readily hydrolyzed by peptic enzymes (de Oliveira et al. 2016). Moreover, the functional properties of proteins can also be enhanced by modifying its structure or glycation the protein (Kato 2002). Different approaches have been opted to enhance the solubility, emulsifying capacity, pH and temperature stability by modifying the structure of proteins or by coupling it with a carbohydrate, most of which are based on the application of thermal treatments, emulsification, fermentation, hydrolysis, dehydration, ultrafiltration and different enzymatic and non-enzymatic reactions (Sun-Waterhouse, Zhao, and Waterhouse 2014). Among the above approaches, non-enzymatic glycation of protein using Maillard conjugation is considered a promising approach to broaden the use of plant proteins in food processing as this reaction is cost effective, spontaneous and does not require any additives for its initiation (Arihara, Zhou, and Ohata 2017; Chen et al. 2019; Kato 2002; Liu et al. 2012).

Maillard reaction is a non-enzymatic reaction that has a crucial role in the formation of flavor and aroma in food and is catalyzed at high temperatures. While undergoing a complex set of reaction, the reducing sugars react with free amino groups of proteins in food to form different glyco-conjugates, while further reaction can form Maillard reaction products (MRPs). Apart from its contribution to flavor and color development, the MRPs can be used as natural, endogenous, free radical scavenging and antibacterial agents (Arihara, Zhou, and Ohata 2017; Chevalier et al. 2001; Habinshuti et al. 2019; Patrignani et al. 2019). Compared to non-covalent reaction approaches, such binding can irreversibly alter protein and carbohydrate structures. Hence, these interactions are more attractive as a novel approach in protein modification since the reactions are spontaneous and do not require any chemical additives. Furthermore, the neoglycoconjugates can potentially add to the functionality of the food (Keppler, Schwarz, and van der Goot 2020; Li et al. 2019). The terms, Maillard conjugation or glycation have been used interchangeably in literature, and they both refer to the same structural modification of protein by coupling it with carbohydrate during the initial stages of Maillard reaction. The interaction of carbohydrates and proteins during this reaction is mainly influenced by intrinsic factors including the complexity of the structure, food composition and extrinsic factors like temperature, humidity, and pH (Wu et al. 2010). Previous studies have mostly been based on the interaction of proteins on the single sugar

model systems (Guan, Wang et al. 2010; Guan et al. 2011; He et al. 2011; Ko et al. 2018; Li, Wu, and Yu 2018; Vhangani and Van Wyk 2013; Wang et al. 2011; Yu et al. 2016, 2017, 2019). This is mainly due to the complexity of food systems which makes it difficult to investigate individual reactions and predict their final impact on functionality (Keppler, Schwarz, and van der Goot 2020). Maillard reaction can also form advanced glycation end products (AGE), including 5-hydroxymethylfurfural (HMF), acrylamide and other MRPs in an uncontrolled state which can affect health, but AGEs formation can be avoided if this reaction is controlled (Ames 1990; Capuano and Fogliano 2011; Wei, Liu, and Sun 2018). Different mitigation strategies to control AGE formation have been studied over the years, including the utilization of competing amino acids, addition of bivalent cations and addition of enzymes. Apart from these methods, the use of phytochemicals was recently reported to stop the initial progression toward Amadori rearrangement by reacting with the amines to form adducts via Michael addition (Rannou et al. 2016; Rebollo-Hernanz et al. 2019).

To the best of our knowledge, there has not been a comprehensive review focusing on Maillard conjugates from plant proteins. There is a need to simplify the concept of Maillard conjugation to resolve misconceptions on the utilization of this method with regard to potential impacts on shelf stability of food products. Hence, this review will focus on the recent works in the production of conjugates and MRPs from plant-origin proteins. The review starts with a brief overview of reaction mechanism imperative in understanding the Maillard conjugation and the formation of desired reaction products, followed by recent advances in conjugate and MRP formation techniques. The effect of MRPs and the utilization of different carbohydrates during this process on the functional and bioactive properties of proteins, as well as future prospects are addressed.

2. A brief overview of maillard conjugation

Maillard reaction, first discovered by Louis C. Maillard in 1912, is a non-enzymatic reaction in which amino groups (proteins, peptides or amino acids) react with carbonyl groups (reducing sugars) undergoing a complex reaction network and parallel chemical transformations that lead to the formation of conjugates and different Maillard reaction products. The complexity of Maillard reaction is very extensive that even after 100 years, the reaction is not fully understood (Hemmler and Schmitt-Kopplin 2021). Hodge (1953) introduced the Hodge scheme to simplify the array of reactions occurring during Maillard reaction which remains precise even today. Hodge subdivided the reaction into seven major reactions extending over three stages (early, intermediate and advanced) with recently discovered free radical breakdown added to the list (Nursten 2005) (Figure 1). The early stage of Maillard reaction can be used for conjugation process with mitigation to enable its utilization in protein modification. The later stages of Maillard reaction cascade, mainly the intermediate stage can result in the formation of bioactive Maillard reaction products. The advanced stages of

reaction leading to the formation of AGEs are considered unfavorable. Hence, understanding these stages is crucial to exploit the reaction accordingly.

2.1. Factors affecting the conjugate and MRP formation

Maillard reaction, being a combination of diverse reactions is influenced by different conditions wherein each factor can influence the individual reaction differently. The most important factors among them are time, temperature, pH, water activity, the presence of sulfites, and the number of amino compounds and carbonyl groups available during the reaction (Ames 1990; Liu, Xia et al. 2020; Zeng et al. 2017).

Non-enzymatic modification of proteins via Maillard conjugation involves protein sidechain reactions that can manipulate the electrostatic charge and thereby change its hydrophobicity. According to de Oliveira et al. (2016), based on the reaction stage in which they are formed, Maillard reaction products can be both advantageous and detrimental to different food characteristics. Under controlled conditions of pH, temperature, humidity, and substrate concentration, certain beneficial products can be obtained while avoiding the formation of harmful MRPs. Liu, Ru, and Ding (2012) mentioned that under controlled reaction conditions, Maillard glycation could lead to distinct functionalities such as improved emulsification, solubility, and foaming properties, while the generated MRPs can be used as a natural additive to impart that functionality to different foods. Glycoconjugates do not belong to a single type of compound but are made up of different intermediates and unstable products that are combined into stable glycoforms. These glycated products are mainly affected by changes in temperature, pH, humidity and mass ratio of the reactants (carbonyl: amino group) (Van Boekel 2001). Earlier studies on glycation of proteins were based on the higher temperature and longer duration of time that resulted in the irreversible loss of functionality of the conjugates. The effect of mild reaction conditions (<60 °C, 80% RH, 24–48 h, molar ratio: 1:2) on the functional properties and glycation degree was studied by Martinez-Alvarenga et al. (2014) where it was found that more stable Amadori products can be obtained at lower temperatures with retained functionality. The water activity ranging from 0.5–0.7 was described to be optimum for this reaction (Van Boekel and Brands 1998). Still there is no established optimum range of temperature, time, and humidity conditions required for conjugation as the reaction is highly complex and dependent on the substrate used, with most studies quoting various range of conditions for this phenomenon (Aoki et al. 2001; Chen et al. 2018; Pirestani et al. 2018; Xu and Zhao 2019; Zhang et al. 2020).

2.2. Maillard conjugation methods

2.2.1. Dry state method

At the water activity lower than 0.5, proteins solutions with carbonyls exists as agglomerated solids in dry state (i.e., without free water). Such dry reactions can be initiated by freeze drying the protein-carbohydrate solutions and later by

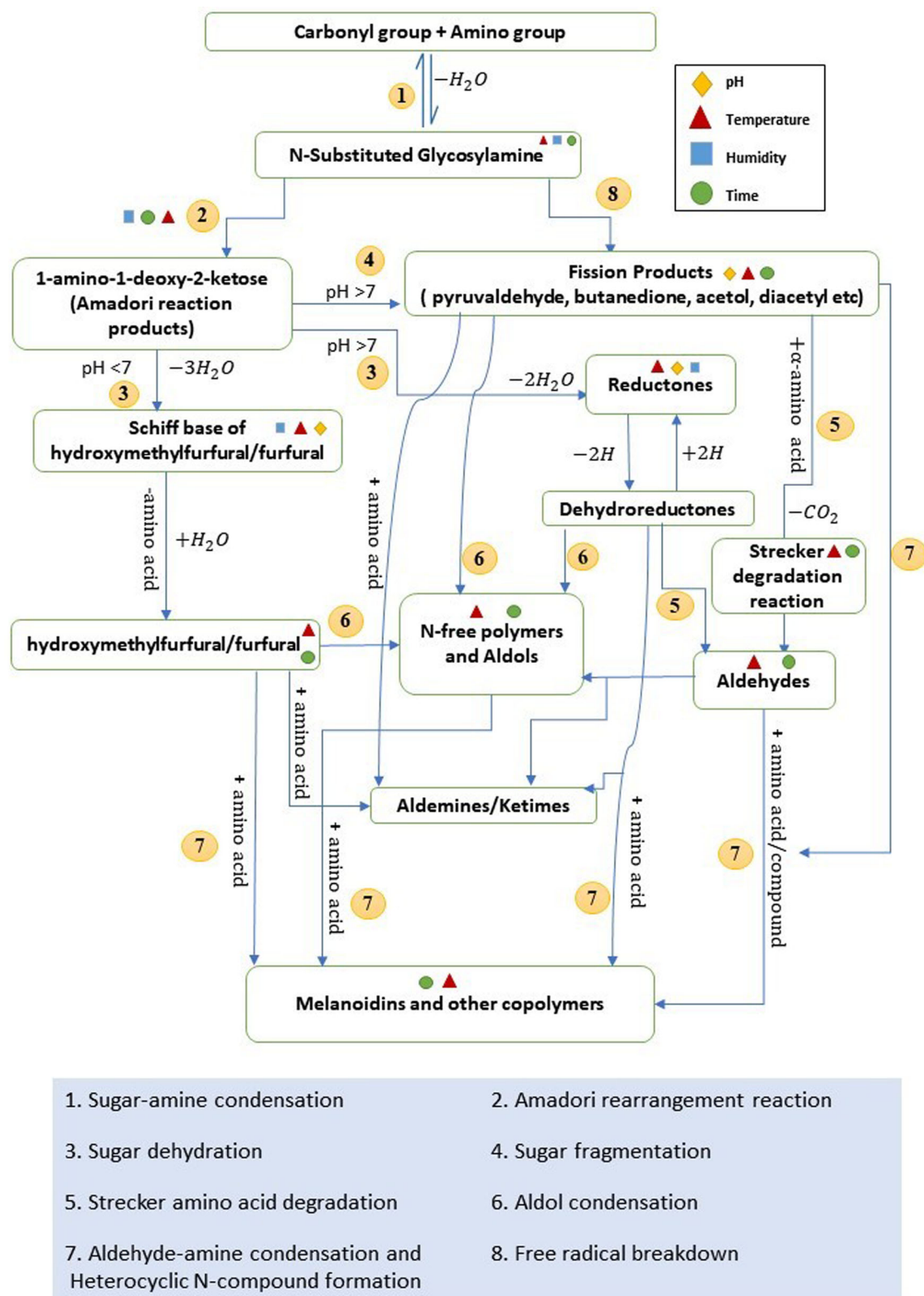


Figure 1. Maillard reaction schematic (adapted from Nursten (2005) with permission from The Royal Society of Chemistry).

heating the mixture in controlled conditions (temperature, time and humidity). In the dry state reaction, the aqueous solution of proteins and carbohydrates are mixed according to the required molar ratio, this solution is further lyophilized and then stored at a controlled temperature and humidity to complete the glycation process. This process can take from hours to weeks for conjugation, depending

upon the structure of proteins and the carbonyl groups present. Conventionally, the humidity is controlled by saturated salt solutions (KI/KBr) to provide favorable environment for conjugation (de Oliveira et al. 2016). A major drawback of this method is that some conjugation reactions involving globular protein might require over two weeks timespan (Nakamura and Kato 2000; Oliver, Melton, and Stanley

2006; Song et al. 2002; Usui et al. 2004). Moreover, most of the dry state Maillard conjugation uses freeze drying (A'Yun et al. 2020; Sedaghat Doost et al. 2020; Sheng et al. 2020; Zhu, Damodaran, and Lucey 2008) that provides lower reaction yield and is not feasible for large scale industrial application. This method also lacks control over AGEs formation, and thus more emphasis on replacing or modifying the method is crucial (Kutzli, Griener, Gibis, Schmid et al. 2020).

2.2.2. Wet state method

This method is based on macromolecular crowding effect. According to Gnutt and Ebbinghaus (2016), proteins show more stability in crowded macromolecular solutions than in dilute aqueous solutions. Due to their steric hindrance, macromolecules exclude other molecules from its vicinity, which restricts the addition of newer molecules. This restriction results in decreased random particle distribution and entropy which increases the free energy of the solute (Kuznetsova, Turoverov, and Uversky 2014). When Maillard conjugation is carried out under high concentration of macromolecules, a shift in the reaction is observed in the direction of species with smaller excluded volume that in turn stabilizes the protein structure (Zhuo et al. 2013). In this method, protein and carbohydrates are dissolved in the buffer. This mixed solution is pH adjusted and then heated for a timespan of few hours to initiate the glycation. The MRPs are then recovered by removal of excess solvent from the solution which adds to the overall steps and time required compared to the dry state method. Both concentrated and diluted mixtures can be used, with a lower timespan for glycation using concentrated mixtures. The absence of lyophilization step without humidity control requirement renders this method more energy-efficient, but the MRP separation and recovery steps add to the complexity.

2.3. Food safety related aspects

In terms of Maillard conjugation, only the initial reactions consisting of condensation and rearrangement are involved. These reactions modify the protein sidechains and are considered to be safe for protein modification (Ames 1992; Perrechil et al. 2014). Maillard reaction can produce different intermediates having many advantageous properties, but uncontrolled conditions can lead to the formation of AGEs is one of the significant points of concern in food safety and shelf stability. At present, five of the MRPs are shown to have some detrimental effect on health including HMF, acrylamide, furosine, heterocyclic amines and 3-Deoxyglucosone based on the amino acid-reducing sugars model studies (Ames 1990; Wei, Ni, Thakur, Liao, Hu et al. 2019). The nature of the generated compounds depends on the type of foods, i.e. cereal and starch-based products, coffee and its derivatives during thermal processing are prone to the formation of acrylamide, furans and furfurals while heterocyclic amines are mainly formed in meat and meat products (Rannou et al. 2016).

Among the compounds, the most potent mutagenic compounds were found to be 5-hydroxymethylfurfural, furan and acrylamide. Acrylamide is formed by deamination and decarboxylation of asparagine, whereas HMF is one of the intermediates formed due to direct dehydration of sugars in acidic pH (Capuano and Fogliano 2011). International agency for research on cancer (IARC) even declared furans, heterocyclic aromatic amines and acrylamide to be carcinogenic components (IARC 1993, 1994, 1995). Although possessing high risks, such compounds are mainly the advanced stage products and are not involved during the initial conjugation reactions or intermediate MRP formation.

Apart from the above-mentioned compounds, other neoglycoconjugates were found to be safe and did not show any mutagenicity in rat toxicity tests conducted by Jing and Nakamura (2005). Multiple measures can be opted to avoid the progression of reaction, such as avoiding acidic pH and low thermal processing. Liu, Li et al. (2020) and Rannou et al. (2016) provided a comprehensive collection of different techniques dividing them into preventive, protective, and removal methods that could control the reaction. Some of these methods include the addition of polyphenols, vitamins, salts, targeting the reactive sites of reaction, modification of reactants (i.e., sugars, amines), and/or using enzymes that could target the precursor amino acid. Recently, plant phytochemicals have also been investigated for their potential in mitigating the different stages of reactions by scavenging of hydroxyl and superoxide radicals, reduction of the generation of reactive carbonyl or dicarbonyl groups, and the chelation of metal ions promoting oxidative reactions (Rebollo-Hernanz et al. 2019). Hence such strategies can be potentially explored in selective formation of MRPs.

3. Maillard conjugation of plant origin proteins

Apart from providing high nutritional value, low allergenicity, ease of availability, and lower costs, plant origin proteins have shown to impart different functionalities when modified and hence are of keen interest in the food processing industries (Omura et al. 2021; Zha et al. 2019a). As per recent studies (Table 1), soy protein remains one of the most sought-after plant origin protein for conjugation, mainly due to their availability and lower costs. Composed of glycinin and β -conglycinin representing more than 70% of total proteins (Malaki Nik et al. 2009), soybean protein has been conjugated with mono- or disaccharides (glucose (Li et al. 2019), maltose + glucose (Cui et al. 2020), fructose (Wang et al. 2018)) and polysaccharides (fenugreek gum (Kasran, Cui, and Goff 2013), dextran (Zhuo et al. 2013), lentinan (Wen, Zhang, Qin et al. 2020), maltodextrin (Xue et al. 2013; Zhang et al. 2014), gum Acacia (Xue et al. 2013), carrageenan (Mao et al. 2018) and soy hull hemicellulose (Wang, Wu, and Liu 2017)) using conventional and modified glycation techniques. From these studies, it can be inferred that the Maillard conjugation has a significant effect on the functional properties like emulsion stability, solubility, thermal stability and surface hydrophobicity. These properties have been attributed to the improved steric

Table 1. Effect of Maillard conjugation on the different properties of plant origin proteins.

Protein	Carbohydrate	Method	Functional and bioactive properties							Other effects/findings	Reference
			EA/ES	S	TS	V	SH	AO	AM		
Soybean protein isolate	Fenugreek gum	Dry state	▲	–	–	–	▲	–	–	Unhydrolyzed gum provides better emulsification than hydrolyzed gum after Maillard conjugation.	Kasran, Cui, and Goff (2013)
	Dextran	Wet state	▲	▲	▲	–	–	–	–	Increased unordered coiling of protein structure.	Zhuo et al. (2013)
	Lentinan	Ultrasonic assisted wet state	▲	▲	▲	▲	–	–	–	Slit divergent ultrasound treatment enhanced the glycation efficiency.	Wen, Zhang, Qin et al. (2020)
	Maltodextrin gum Acacia	Dry state	▲	▲	–	–	▼	–	–	Enhanced foaming ability, foam stability	Xue et al. (2013)
		Dry state	▲	▲	–	▲	▼	–	–	Lower levels of free amino groups and higher degree of graft than gum Acacia.	
Maltodextrin		HTST assisted dry state	▲	–	–	–	–	–	–	Decreased the levels of α -helix, β -sheet and β -turn.	Zhang et al. (2014)
										Increased unordered coils level.	
Soy-hull hemicelluloses		Dry state	▲	–	▲	–	–	–	–	HTST enhanced the steric stabilization of the emulsion droplets.	Wang, Wu, and Liu (2017)
Glucose		Wet state	▲	–	–	–	▼	–	–	Improved physical stability of O/W emulsions for an extended storage period.	
										Flexibility was significantly and positively correlated with emulsifying properties of the conjugate.	Li et al. (2019)
										Changes in surface hydrophobicity, free sulfhydryl content and turbidity.	
Glucose + Maltose		Ultrasonic assisted dry state	▲	▲	▲	–	–	–	–	Increased degree of glycation.	Zhao et al. (2016)
										Reduction in browning intensity.	
										Reduction of weakening effect of glycation due to cavitation and microstreaming resulting in increased gelling ability.	Cui et al. (2020)
Glucose		Dry state	▲	▲	▲	–	–	–	–	Reduction in particle size of protein	
										Increased flexibility of protein structure	Wang et al. (2018)
Xylose/ fructose		Wet state	▼	▲	–	–	–	–	–	shortened reaction time, and charge density can be adjusted accordingly.	
I-Carrageenan		Dry state + Spray drying	▲	–	▲	–	–	–	–	The degree of graft of MRPs increased rapidly during spray drying.	Mao et al. (2018)
										Enhanced viability during in vitro digestion.	
										Probiotics-MRP complex sustained pasteurization.	

(continued)

Table 1. Continued.

EA/ES: Emulsion ability/stability, S: Solubility, TS: Thermal stability, V: Viscosity, SH: Surface hydrophobicity, AO: antioxidant activity, AM: Antimicrobial activity. ▽: Decreased ▲: Enhanced -: Not reported										
Protein	Carbohydrate	Method	Functional and bioactive properties							Reference
			EA/ES	S	TS	V	SH	AO	AM	
β-conglycinin	Dextran	Dry state	▲	-	-	-	-	-	-	Zhang, Wu et al. (2012)
	Maltodextrin	Ultrasonication assisted wet state	▲	▲	-	▲	-	-	-	Zhang, Chi, and Li (2014)
	gum Arabic	Dry state	▲	▲	-	-	-	-	-	Zha et al. (2019b)
	gum Arabic	Dry state	▲	-	-	-	▲	▲	-	Zha et al. (2019a)
Peanut protein isolate	Maltodextrin	Electrospun fiber assisted dry state	▲	▲	-	-	-	-	-	Kutzli, Griener, Gibis, Schmid et al. (2020)
	Pectin	Dry state	▲	-	▲	-	-	-	-	Tamnack et al. (2016)
	gum Arabic	Dry state	▲	-	-	-	-	▲	-	Zha et al. (2019)
	Maltodextrin	Ultrasound assisted wet state	▲	-	-	-	-	-	-	Chen et al. (2016)
Rice protein isolate	Dextran/gum Arabic	Ultrasound assisted dry state	▲	▲	-	-	-	-	-	Li, Xue et al. (2014)
	Glucomannan	Ultrasound assisted dry state	▲	▲	-	-	▲	-	-	Li, Huang et al. (2014)
Rice proteins	Dextran	Dry state	▲	▲	-	-	-	-	-	Cheng et al. (2018)
	Glucose/lactose/maltodextrin/dextran	Dry state	▲	▲	-	-	-	-	-	Li et al. (2013)
Rice protein hydrolysates	Dextran	Microwave assisted dry state	▲	▲	-	-	-	-	-	Cheng et al. (2021)
	K-carrageenan	Dry state	▲	▲	-	-	-	-	-	Du et al. (2013)

Rice dreg protein	Sodium alginate	Microwave assisted wet state	▲	▲	—	—	—	Increased emulsion stability at neutral and acidic pH. Smaller mean droplet diameter of emulsion. Improved immunomodulatory effect.	Meng et al. (2019)
Black rice glutelin	Arabinose/Lactose/Maltodextrin/sodium alginate	Dry state	▲	▲	—	▲	—	Improved grafting. Most effective conjugation obtained from arabinose as compared to other carbohydrates.	Liu, Li et al. (2020)
Wheat protein	Dextran	Dry state	▲	—	—	—	—	Enhanced steric stabilization of the emulsions at acidic pH.	Wong, Day, and Augustin (2011)
Wheat gluten	Maltose	Dry state	▲	▲	—	—	—	Decrease in β -fold and β -turn and the increase in α -helix at a certain degree. Conjugation has little effect on the irregular coiled structure. loss of β -sheet structures. Change in average particle size. Higher stability under acidic and ionic conditions. Dextran had higher solubility enhancement.	Song, Yang, and Li (2020)
Deamidated wheat gluten	Maltodextrin/citrus pectin	Dry state	▲	—	—	—	—	Extension of secondary structure induced by Maillard reaction. Improved stability to environmental stresses.	Wang et al. (2019)
Wheat germ protein	Xylose/glucose/lactose/ dextran/ maltodextrin	Wet state	▲	▲	—	—	—	Formation of uniform and smaller oil droplets with reduced coalescence.	Niu et al. (2011)
Oat protein isolate	β -glucan	Dry state	▲	▲	—	—	—	Enhanced swelling and fat binding capacity.	Zhong et al. (2019)
	Dextran	Dry state	▲	—	—	—	—	Higher reducing and DPPH scavenging activity compared to soy conjugate. Improved mouthfeel and taste continuity enhancing sensorial properties.	Zhang et al. (2015)
L-glutamine	Oat β -glucan	Dry state	▲	▲	—	▲	—	Enhanced sensory characteristics.	Sun et al. (2019)
Sunflower meal hydrolysate	Xylose	Dry state	▲	—	—	—	—	Enhanced sensory characteristics.	Habinshuti et al. (2019)
	Xylose	Wet state	—	—	—	—	—	Decreased flocculation during extended storage.	(Karangwa et al. 2013)
Corn meal hydrolysate	Xylose	Dry state	▲	—	—	—	—	Improved sensory characteristics.	Habinshuti et al. (2019)
Chickpea protein isolate	Endogenous reducing sugars/ oligosaccharide	Wet state	▲	—	—	—	—	Enhanced surfactant capacity. Ultrasonication increased efficacy of functional properties.	Mohammed (2018)
Flax seed protein hydrolysate	D-xylose	Dry state	▲	▲	—	—	—	Ultrasonication speeds up conjugation.	Wei, Thakur et al. (2018)
Buckwheat protein isolate	Dextran	Ultrasonic assisted dry state	▲	▲	—	—	—	Increased solubility, emulsification stability and surface hydrophobicity as compared to	Xue et al. (2017)
Mung bean protein isolate	Glucose	Ultrasonic assisted wet state	▲	▲	—	—	—		Wang et al. (2016)

(continued)

Table 1. Continued.

EA/ES: Emulsion ability/stability, S: Solubility, TS: Thermal stability, V: Viscosity, SH: Surface hydrophobicity, AO: antioxidant activity, AM: Antimicrobial activity. ▼: Decreased ▲: Enhanced -: Not reported									
Protein	Carbohydrate	Method	Functional and bioactive properties						Reference
			EA/ES	S	TS	V	SH	AO	AM
Canola protein isolate Black bean protein isolate	Gum Arabic	Wet state	▲	▲	-	▲	-	-	-
	Glucose	Ultrasound assisted dry state	▲	▲	-	-	▲	-	-
Plum seed protein isolate	Gum acacia	Dry state	▲	▲	▲	-	▲	-	-
Rapeseed protein isolate	Dextran	Ultrasound assisted wet state	▲	▲	▲	-	-	-	-

Other effects/findings

conventional wet state method. Conjugates formed had lower content of α -helix and unordered coil, and higher content of β -sheet and β -turn structure than isolate alone.

Higher viscosity and a better physical structure.

higher surface hydrophobicity, solubility, emulsification activity, emulsification stability and antioxidant activity as compared to untreated glycates.

Improved mechanical stability to external physical forces.

Improved solubility (at pH 5–6), emulsifying activity (at pH 4–10), emulsion stability (at pH 4–5 and 9–10), and thermal stability (at temperature 90–100 °C). Decreased digestibility of conjugates.

Pirestani et al. (2017)

Jin et al. (2019)

Li et al. (2020)

Qu et al. (2018)

stabilization of emulsion and the structural changes that results in higher molecular flexibility in both carbohydrates and proteins. Moreover, unhydrolyzed polysaccharides exhibit better emulsification ability after conjugation as they produce smaller emulsion droplet (smaller median droplet diameter) with monomodal distribution for a longer period of time in an emulsion compared to the hydrolyzed ones (Kasran, Cui, and Goff 2013). Modified Maillard conjugation techniques over soybean proteins using ultrasonication (Cui et al. 2020; Higuera-Barraza et al. 2016; Mu et al. 2010; Wen, Zhang, Qin et al. 2020; Zhang et al. 2018; Zhao et al. 2016), microwave (Guan et al. 2006; Žilić et al. 2014), high temperature-short time dry heating (Zhang et al. 2014) and PEF (Liu et al. 2014) have been studied over the years to improve the functional properties. As the solubility remains a key issue, ultrasonic treatment has been shown to affect structural stretching and molecular flexibility mainly due to ultrasonic cavitation and other resultant effects including microjet formation and interparticle shearing (Figure 4) that improves the solubility of the conjugate while simultaneously enhancing its functionality and glycation rate (Zhang, Wu et al. 2012; Zhang, Chi, and Li 2014; Zhuo et al. 2013).

Pea proteins, composed of legumin, vicilin and convicilins, have attracted interest mainly due to its low allergenicity and cost. Although it lacks lysine but the overall pea proteins exhibit good nutritional value. The overall functional properties of pea proteins are based upon the legumin: vicilin ratio, and the processing and environmental conditions during cultivation (Lam et al. 2018). Due to its low emulsification capability, low surface charge, and lower solubility compared to other plant origin proteins (Karaca, Low, and Nickerson 2011), structural modification of pea proteins by Maillard reaction using gum Arabic (Zha et al. 2019, 2019a, 2019b) and pectin (Tamnak et al. 2016) have been employed. It has been found that controlled MR can enhance the functionality (solubility, emulsification, thermal stability) of the low water-soluble plant origin protein, increase the antioxidant potential and reduce the typical leguminous beany flavor that could improve their utilization and acceptability. This enhancement has been linked to the improved electrostatic repulsions and/or steric hindrance effects. Apart from functionality enhancement, a neoglycoconjugate was also reported to be formed during Strecker degradation which added to the bioactivity and contributed in beany flavor mitigation. Further improvements in functionality were obtained using modified techniques like electrospraying which will be reviewed in section 5.3.

Peanuts are one of the important oilseeds. After extraction, the peanut meal left behind is used to obtain peanut protein flour, concentrates, and isolates composed of arachin and conarachin proteins. Although they show better functional properties compared to other leguminous proteins (except soybean), they are underutilized mainly due to low solubility. Different modifications using Maillard glycation have been used to improve its functional properties using dextran (Liu et al. 2012). There was a substantial increase in the thermal stability, emulsification, foaming and solubility which was linked to the modification of tertiary structure after conjugation with

dextran. It has also been noted that arachin is harder to glycosylate, which affects the glycation degree of the peanut protein. This problem could be overcome using ultrasonication-assisted Maillard glycation (Chen et al. 2016; Li, Xue et al. 2014; Li, Huang et al. 2014), in which the ultrasonic waves led to the increased utilization of conarachin instead of arachin for glycation, increased β -structures, reduced α -helix and increased the exposure of hydrophilic amino acid residues that led to improved solubility. Solubility of peanut protein after conjugation was found to be affected by the carbohydrate used and the pH during the reaction. Maillard conjugation has been shown to improve the sensory properties (taste, aroma and color) of the peanut meal as investigated by Qinzhu et al. (2018). The authors optimized the process for obtaining peanut meal MRP using xylose for conjugation. The resultant MRP also exhibited improved antioxidant activity which was attributed to the increase in phenolic compounds from 70% to 92% after conjugation reaction.

Rice proteins are mainly composed of prolamins (20–25%) and glutelins (60–65%) and small amounts of albumin and globulins that tend to polymerize to form hydrophobic high molecular weight insoluble complexes (Amagliani et al. 2017) that affect their functionality. Maillard reaction has been used to enhance those properties by glycation of the protein with different mono- and disaccharides (glucose (Li et al. 2013), arabinose (Liu, Li et al. 2020), lactose (Li et al. 2013; Liu, Li et al. 2020)) and polysaccharides (dextran (Cheng et al. 2018, 2021; Li et al. 2009, 2013), carrageenan (Du et al. 2013), sodium alginate (Meng et al. 2019), maltodextrin (Liu, Li et al. 2020)) that resulted in improved solubility, thermal stability, emulsion activity and stability index after conjugation. Further studies using ultrasonication pretreatment showed that the rice protein isolate performed poorly in terms of functionality optimization at the similar frequencies that were used for other plant origin protein modifications. This was due to the higher attenuation to the ultrasonic cavitation at these frequencies. Other methods like microwave-assisted Maillard glycation (Meng et al. 2019; Cheng et al. 2021) were proven to be useful in improving the unfolding and conformational flexibility of the rice protein that improved the emulsification and solubility of resultant conjugates.

Wheat consists of gliadins and glutenins (gluten proteins) which make up to 80–85% of total wheat proteins (Bos et al. 2005). Gluten exists in the form of aggregates due to the presence of extensive amino acid residues (leucine, glutamine and proline) in its structure that limits its functionality (Song, Yang, and Li 2020). Different studies of Maillard conjugation to improve the functionality have been undertaken that includes the formation of MRP by glycation of wheat gluten with maltose, xylose, lactose, glucose, dextran, maltodextrin, and citrus peel pectin (Niu et al. 2011; Song, Yang, and Li 2020; Wang et al. 2019; Wong, Day, and Augustin 2011). It was reported that Maillard conjugation of gluten or wheat proteins with saccharides improves their solubility, foaming ability/stability and other functional properties. These conjugates displayed better protection against physical and chemical degradation. They were able to stabilize oil-in-water emulsions when compared to non-conjugated ones, which was attributed to the number of saccharides conjugated with

proteins forming an interfacial steric layer and maintaining emulsion stability (Wang et al. 2019; Wong, Day, and Augustin 2011).

Oat is a cereal that consists of 70–80% of globulins, 4–14% of prolamins, and small amounts of albumins and glutelins. As this cereal lack gluten that is linked to celiac diseases, its utilization and modification have been particularly emphasized (Mäkinen et al. 2017). Oat protein has been glycosylated with dextran and β -glucan (Sun et al. 2018, 2019; Zhang et al. 2015; Zhong et al. 2019) that improved the emulsification ability and solubility due to alteration in secondary structure and increased random coiling of the protein conjugate. It was observed that the conjugate exhibited high viscosity ascribed to the functional properties of oat β -glucan and its concentration. Rheological studies also show that β -glucan-amino acid and peptide conjugates had higher storage modulus than the loss modulus indicating that conjugates showed solid-like behavior. There was a frequency-dependent increase of loss modulus in a high concentration system due to strong intermolecular interaction between molecules which stabilized it (Sun et al. 2018).

Similarly, studies of different nontraditional protein modification using Maillard conjugation have been conducted with black bean protein isolate-glucose (Jin et al. 2019), canola protein isolate-gum Arabic (Pirestani et al. 2018), flaxseed protein hydrolysate-xylose (Wei et al. 2018; Wei, Ni, Thakur, Liao, Huang et al. 2019), buckwheat protein isolate-dextran (Xue et al. 2017), mung bean protein isolate-glucose (Wang et al. 2016), sunflower/corn meal hydrolysate-xylose (Habinshuti et al. 2019), chickpea protein isolate-oligosaccharide (Mohammed 2018), rapeseed protein isolate-dextran (Qu et al. 2018) and plum seed protein isolate-gum acacia (Li et al. 2020) conjugates. In general, each protein mentioned above resulted in improved emulsification ability and stability, increased solubility, decreased flocculation, with some MRPs showing antimicrobial activity (Habinshuti et al. 2019), improved thermal stability, and radical scavenging activity that is dependent on the structure, amino acid composition, and its sequences, while the improved sensory attributes (overall taste continuity and mouthfeel) can be linked to the formation of low molecular weight Maillard peptides ranging from 1–5 kDa that mask the off-flavours of MRPs, and the reduction of bitter amino acids (Histidine, Methionine, Valine, Isoleucine, Phenyl-alanine, Arginine, Serine, Leucine) after Maillard reaction (Habinshuti et al. 2019; Karangwa et al. 2013; Lan et al. 2010). Most of these studies have been conducted by glycosylating single saccharide and amino acid (Maillard model system), hence these modifications might not exhibit similar effect when observed in complex foods. Moreover, nontraditional proteins are yet to be studied extensively in terms of other functional and bioactive improvements that can be enhanced after Maillard glycation.

4. Properties of plant protein derived Maillard conjugates and MRPs

4.1. Functional properties of conjugates

Functional properties define how the components behave during processing and their effects on different sensory and

nutritional attributes of the finished products. Based on the interaction of proteins with food systems, Sikorski (2001) compiled different functional properties of proteins which includes wettability, swelling, water retention, gelation, film-forming, emulsifying, stabilizing, binding, and solubilization ability. Protein functionality is primarily related to the structural transitions, Foegeding and Davis (2011) defined the general model of such changes during denaturation, where they described that the native structure is converted into an intermediate state reversibly, during which major changes occur in the tertiary structure while the most of the secondary structure remains unaffected to further unfold irreversibly into their denatured form. Based on such model, the structures associated with folding can be used to represent the denaturation phenomenon and hence its functionality. Most plant origin proteins due to their complex protein structure, require longer processing time for structural modifications, hence this also affects the functionality enhancement of such proteins.

Functional properties in terms of proteins are studied on the basis of how the proteins perform in solution, with the prediction of functionality often based on model food systems, rather than on real complex foods (Foegeding and Davis 2011). In terms of plant origin protein conjugates, the most relevant properties that is studied are emulsification and emulsion stabilization, solubility, viscosity and the resistance to high temperatures.

4.1.1. Emulsification and stabilization

Due to their unique structure, proteins can act as emulsifiers by interacting with both hydrophilic and hydrophobic phases, but such emulsions can be easily destabilized. This behavior was explained by Capek (2004), who described that the emulsion instability and degradation were due to the tendency of emulsions to minimize its interfacial area between dispersed phase and dispersing medium. This minimization is mainly accomplished by coagulation or coalescence (Tamnak et al. 2016; Wang, Wu, and Liu 2017; Wang et al. 2018; Zhang et al. 2014, 2015, 2018).

Conjugation of polysaccharide with protein and the resultant emulsions can have both positive and negative impacts on the stability. This change is based on different attributes of the carbohydrates that include the confirmation, mixing ratio, presence of reactive sites and its concentration (Lam and Nickerson 2013). Emulsions can also destabilize during storage due to gravity-based separation or flocculation, with one of the factors being the average droplet size. This parameter was studied by Wang, Wu, and Liu (2017) by determining droplet size of the emulsions prepared using Maillard conjugate (soy protein isolate-soy hull hemicelluloses). It was observed that the particle size distribution of the conjugate stabilized emulsions remained in mono-modal form for an extended period of time when compared with conventional ones. They explained that this phenomenon was due to the steric stabilization effect of carbohydrate combined with the emulsifying capacity of protein that prevented the re-coalescence of the oil droplets. Similarly, Kasran, Cui, and Goff (2013) utilized Maillard

reaction to conjugate soy-whey protein with hydrolyzed/unhydrolyzed fenugreek gum to study the particle size distribution and average particle size. It was reported that the conjugates produced O/W emulsion of relatively small droplet size much faster when compared to non-conjugated ones. The hydrolysis of gum had a significant effect on lowering the particle size, and it was observed that unhydrolyzed gums exhibits lower particle size of the droplet compared to hydrolyzed ones. Du et al. (2013) showed that emulsion stability of polysaccharide (K-carrageenan) rice dreg protein conjugate was improved due to the adsorption of hydrophobic protein fraction of the conjugate at the O/W interface that resulted in oil droplet stabilization, whereas the hydrophilic polysaccharide fraction extended over to the aqueous phase to improve its overall stability. Modification of pea protein isolate using Maillard conjugation with pectin and its effect on the O/W emulsion were investigated (Tamnak et al. 2016), which revealed that the reaction resulted in the formation of hybrid conjugated biopolymer with lower solubility but higher emulsification ability than the protein or carbohydrate alone. The emulsion formed using this conjugated polymer had better stability even after one month of storage due to its higher negative zeta potential and smaller droplet size compared to unconjugated emulsion.

Most of the investigations (Table 1) conducted on Maillard conjugates followed a similar trend that glycated conjugates increased the emulsification ability and emulsion stability. These studies infer that the glycation of proteins has a positive effect on the structure stabilization based on the ability of carbohydrates to provide steric hinderance between oil droplets while the proteins accumulate on the surface of droplets. The emulsification ability is improved as conjugation enhances protein solubility, mobility and flexible structural transition that results in faster oil-water interface migration and adsorption (Nooshkam, Varidi, and Verma 2020).

4.1.2. Solubility

A significant concern regarding plant origin proteins is their low solubility near its isoelectric point. Solubility and other functional properties are influenced by the protein type, molecular weight and extraction procedure (Ebert et al. 2020). Apart from its crucial role in emulsion formation, solubility is important in the formation of Maillard conjugates. A mixture of two biopolymers needs to remain in a blended state even after the solution has undergone the conjugation process, and while synthesizing such complexes using plant origin proteins, the lack of solubility can lead to reduced bond formation efficiency between the biopolymers (Ding et al. 2020). Most studies focusing on using polysaccharides for Maillard conjugation have demonstrated that the enhanced solubility had a direct effect on enhanced emulsification ability of the conjugates (Chen et al. 2018; Ding et al. 2020; Kasran, Cui, and Goff 2013; Kato 2002; Li et al. 2013, 2017; Sedaghat Doost et al. 2020; Wang et al. 2020; Wong, Day, and Augustin 2011; Zha et al. 2019a, 2019b; Zhuo et al. 2013).

Li et al. (2013) studied the functional properties of Maillard reaction products synthesized using different carbohydrates (glucose, lactose, maltodextrin (DE-20) and dextran) and rice protein hydrolysates. The authors reported that the solubility of rice proteins was increased by factor of 3.5 compared to the native rice proteins and was directly proportional to the reaction time in case of all the saccharides. Their study confirmed that the solubility enhancement induced by Maillard conjugation could also improve the functional properties of highly hydrophobic plant proteins. Wen, Zhang, Qin et al. (2020) used a polysaccharide obtained from the fruiting body of shiitake mushroom to conjugate with soy protein isolate, showing that the covalent attachment of hydrophilic sugars with proteins significantly enhanced the protein-water molecule interaction. Liu, Li et al. (2020) further focused on investigating the functional properties of black rice glutelin by conjugating it with arabinose, sodium alginate, lactose and maltodextrin. Compared to other carbohydrates studied, Maillard conjugates created using arabinose exhibited the most improvement in solubility from 20% to 80%. Similarly, monosaccharides (xylose, fructose) performed better than polysaccharides in solubility enhancement of soy-protein in the studies reported by Wang et al. (2018). Based on the above investigations, the functionality of proteins conjugated with mono-, oligo- or polysaccharides varies in their efficacy which can be attributed to the complexity of the structure of protein and the saccharides. The peanut protein isolate conjugated with gum Arabic performed better at alkaline pH but poorer in acidic pH (3–6) than the peanut protein isolate-dextran conjugate in terms of emulsification and solubility after ultrasonication. This effect has been ascribed to the action of Gum Arabic which causes the amino groups to disappear from the surface of the protein, affecting its solubility (Schmitt, Bovay, and Frossard 2005).

There have been reports of decreased solubility due to advanced protein crosslinking, increased protein aggregation due to increased surface hydrophobicity, and reaction of advanced glycation end products with amino groups (Le et al. 2013; Nooshkam, Varidi, and Verma 2020). Oliver, Melton, and Stanley (2006) mentioned that this contradictory behavior might be due to complexity and reactivity of proteins and carbohydrates, as the solubility is enhanced due to the limited degree of glycation, whereas peptide crosslinking and disulfide bridge formation can reduce protein solubility. Hence a controlled approach is required with the formation of Maillard reaction products to avoid the reaction to continue to advanced stages. Modification of protein prior to Maillard reaction has also been studied by Ettelaie, Zengin, and Lee (2014). It was reported that the hydrolysis of protein before conjugation with carbohydrate can enable the unfolding of protein and produce smaller peptides that show better solubility and surface activity. Ding et al. (2020) further used this approach to study the solubility of soybean protein by hydrolyzing the protein with trypsin and alcalase enzymes and the dry state Maillard reaction was initiated to form conjugates with maltodextrin. The author highlighted that the degree of hydrolysis must

be selected carefully to increase the solubility, as too lower hydrolysis causes no change in the solubility of proteins while too higher hydrolysis degree leads to generation of many small fragments that does not show any surface activity. In terms of soy proteins, 8% degree of hydrolysis was found to be optimum. Similar studies were carried out by Li et al. (2013) in which limited hydrolysis accompanied by Maillard glycation of rice protein was reported to be an efficient method for protein modification and functionality enhancement.

4.1.3. Viscosity

Rheological studies of Maillard conjugates by Sun et al. (2018) has shown that glycation has major effect on viscosity. It was found that the apparent viscosity of oat β -glucan conjugated with amino acids (L-glutamic acid, L-phenylalanine) and peptides (collagen, soybean) significantly changed after Maillard reaction. The conjugates presented shear thinning non-Newtonian pseudoplastic behavior over the entire shear rates due to disruption of random coil polymers and parallel alignment with flow stream during shearing. In comparison with native oat β -glucan, the amino acid conjugate was reported to have a higher apparent viscosity. On the contrary, a sharp decline in the apparent viscosity was noted in terms of peptide conjugates.

Mao et al. (2018) reported that the macroscopic viscosity index of the Maillard conjugates was influenced by the concentration of polysaccharide present and exhibited direct proportionality attributed to thickening properties of polysaccharides. A rapid increase in viscosity was observed 6 hours after spray drying due to increased degree of glycation, but further incubation resulted in viscosity decrease due to breakdown of polymer structure and formation of small molecular weight compounds. Overall, the higher glycation degree contributed to the higher viscosity and elasticity of the solution. Zhang, Qi et al. (2012) studied the apparent viscosity changes in β -conglycinin-dextran conjugates and found that the viscosity of the mixture increased from 0.01 Pa.s to 0.038 Pa.s within 6 hours of heating. The authors also observed that increased concentration of carbohydrate in the reaction leads to higher viscosity that can cause accelerated protein aggregation due to macromolecular crowding, whereas if the concentration of carbohydrate in the reaction keeps increasing, after a certain point the glycation rate decreases.

Similar to emulsification properties, advanced stages of Maillard reactions could lead to the formation of many insoluble complexes that can lead to the decline in system viscosity. However, this study was based on animal proteins, hence the effect of such conjugation using plant origin protein might show a different effect (Al-Hakkak and Al-Hakkak 2010).

4.1.4. Resistance to thermal processing

During thermal processing, most of the heat-labile bioactive components from food are lost and protein structure can be damaged, affecting the functionality and bioactivity of the

final products (de Oliveira et al. 2016; Jiménez-Castaño et al. 2005; Shen, Tang, and Li 2021). Pirestani et al. (2018) used a differential scanning calorimetry to determine Maillard reactions impact on the protein's stability by scanning canola protein isolate and canola protein-gum Arabic Maillard conjugates to obtain the thermograms. It was reported that the denaturation temperature used to describe thermal stability in canola protein isolate was much lower than that of the conjugate due to the effect of excluded volume and differential interaction confirming that glycation improved the thermal stability of the conjugate. Peptide degradation and crosslinking were observed during thermal degradation studies of xylose-soybean peptide system (Lan et al. 2010) and it was reported that peptides undergoing Maillard reaction glycated with sugars to form conjugate instead of degrading to free amino acids. Moreover, the content of free amino acids also decreased with increasing temperatures, due to crosslinking interactions among the sugars or its degraded products and amino acids. The notable selectivity of peptide to conjugate can help in reducing peptide degradation for enhanced thermal stability.

There has been inconsistency in the results for plant-derived protein conjugates as observed in the studies conducted by Li et al. (2019) on the surface functional properties of soybean protein isolate and glucose. It was found that the conjugation resulted in increased hydrophobicity that caused protein aggregation, thereby reducing the thermal resistance of the conjugates. Further studies in controlling the reaction to obtain conjugates that could withstand thermal processing would expand the applications of MRPs in food industries (Nooshkam, Varidi, and Verma 2020).

4.2. Bioactive properties of MRPs

As defined by Guaadaoui et al. (2014) bioactive properties are those that can interact with one or more components of the living tissues and exhibit wide ranges of health-enhancing effects. Major bioactive properties of Maillard reaction products includes antioxidant property, antimicrobial and antimutagenic properties that are described in this section and the reactions involved is illustrated in Figure 2.

4.2.1. Antioxidant property

During different metabolic processes in a biological system, oxidative stress is developed that can result in different pathologies, cardiovascular disorders, and initiation of tumor (Santos-Sánchez et al. 2019). Maillard reaction products have been reported to pose antioxidant activity that can reduce this oxidative damage due to free radicals using different chemical mechanisms (Santos-Sánchez et al. 2019; Patrignani et al. 2019). This property is mainly influenced by the type of protein and carbohydrates taking part in the reaction, their concentration, mode of processing and reaction conditions (pH, temperature-time, water activity) (Nooshkam, Varidi, and Bashash 2019). Recent investigations have noted that enzymatic hydrolysis can help in utilization of plant origin proteins with enhanced antioxidant

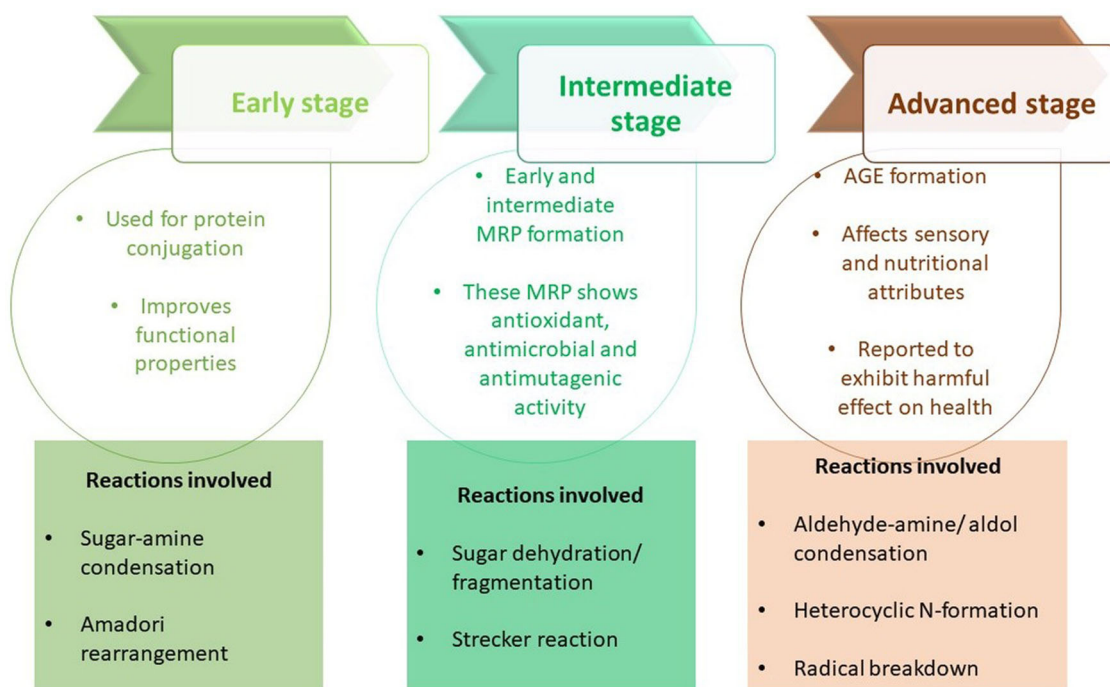


Figure 2. Different stages of Maillard reaction and their role.

activity to create Maillard conjugates (Wen, Zhang, Zhang et al. 2020).

Habinshuti et al. (2019) evaluated the antioxidant property of sunflower, soybean and corn hydrolysates conjugated with xylose. The authors compared the reducing power of the conjugates to butylated hydroxytoluene (BHT) and reported that sunflower MRP showed highest reducing power (0.832) followed by soybean (0.264), BHT (0.218) and corn (0.149). It was further explained that the antioxidant property was primarily due to the presence of reductones that help in breaking down the free radical chain reaction by donating electrons and transforming themselves into stable products. It was emphasized that similar results can be obtained due to polyphenolic components present in the samples. Similar result was reported by Karangwa et al. (2013). Nooshkam and Madadlou (2016a) reported that other mechanisms like metal ion chelation, hydrogen peroxide breakdown and reactive oxygen species (ROS) scavenging were also responsible for the antioxidant effect of the MRP. Lin et al. (2019) investigated the DPPH scavenging rate of the short linear glucan produced from corn and lysine Maillard conjugate. It was observed that scavenging rate of conjugates increased with increased weight ratio and exhibited 87.48% scavenging rate at 3:2 proportion compared to pure protein which had 3.76% scavenging rate. Further, the EC_{50} value (effective concentration of the conjugate to scavenge 50% of radical activity) was studied, with the ratio of 3:2 sample, the EC_{50} was 128.8 $\mu\text{g/mL}$ corresponding to the higher ability of the sample to scavenge free radicals. Vhangani and Van Wyk (2013) conducted the studies of DPPH, peroxy, hydroxy radical scavenging and reducing power of MRP based on the model systems of fructose-lysine and ribose-lysine. It was observed that in both the model systems, the

temperature increase (60 to 80 °C) resulted in increased radical scavenging activity whereas at higher temperature (121 °C) a significant decrease in the scavenging activity was noted. This decrease in reactivity was linked to the formation of advanced Maillard products that effectively decreased the radical scavenging. In terms of the peroxy radical reduction, reducing power and hydroxy radical scavenging, the conjugates exhibited higher radical chain breaking reaction activity although the antioxidant activity of the conjugates varied and was influenced by temperature, pH, time, reactant type, and concentration (Jaeger, Janositz, and Knorr 2010).

4.2.2. Antimicrobial property

Model system studies of antimicrobial activity of MRP have been investigated over the years and it was found that the MRPs displayed antimicrobial activity against different bacterial strains (*E. coli*, *S. aureus*, *L. monocytogens*) and yeasts as well (Hauser et al. 2014). The mechanism was investigated by Rufián-Henares and de la Cueva (2009) and it was found that the melanoidins produced during Maillard reaction tend to permeate through the outer and inner cell membranes along with magnesium ion (Mg^{2+}) chelation effect, leading to unavailability of the ion in the membrane which disrupts the cell membrane of bacteria. Apart from melanoidins, during the advanced stages, hydrogen peroxide is also synthesized which acts as an excellent antimicrobial component (Muscat et al. 2007). A recent study of minimum inhibitory concentration and inhibition zone formation using MRP generated from plant protein meals revealed that sunflower protein conjugate showed significant inhibitory concentration against *E. coli* (86 mg/mL) and *S. aureus* (70 mg/mL). This antimicrobial effect was reported to be due to the high amount of alcoholic and carboxylic groups

in the MRPs (Habinshuti et al. 2019; Kubo, Muroi, and Kubo 1995). Similarly, the antimicrobial effect of MRP generated from date palm sap sirups was investigated by Makhoul-Gafsi et al. (2018), showing a broad antimicrobial spectrum when tested against gram-positive bacteria due to presence of melanoidins and endogenous phenolic compounds. Thus, MRPs could act as a potential candidate to replace synthetic bacterio-static/-cidal agent in foods.

In contrast, Chevalier et al. (2001) studied the antimicrobial activity using disk diffusion method of MRP generated using β -lactoglobulin and different sugars (arabinose, galactose, glucose, lactose, rhamnose) and found that none of the samples exhibited antimicrobial effect. This result was explained to be due to the large spectrum of resistance mechanisms of the bacteria; hence more extensive studies are required to explore MRPs role as antimicrobial agents.

4.2.3. Antimutagenic property

The earliest study of antimutagenic properties was conducted by Chan et al. (1982) and later by Yen, Tsai, and Lii (1992) in which the MRPs were prepared using model systems comprising fructose, glucose and xylose with amino acids (lysine, arginine, glycine and tryptophan) and Ames assay was used to test the antimutagenic property of the MRPs. The authors reported that the mutagenic activity of 2-amino-3-methylimidazo(4,5-f) quinoline (IQ) and 2-amino-6-methyldipyrrodo [1,2-a:3',2'-d] imidazole was reduced by more than 50% in all the MRPs except fructose-arginine and fructose-glycine MRP that showed <50% inhibitory activity whereas tryptophan and xylose containing MRPs exhibited the highest inhibitory effect (>90%). The study indicated that the MRPs had a selective inhibitory effect on the mutagens under investigation and that the antimutagenic effect mainly occurs due to free radical scavenging.

To investigate the mechanism of antimutagenic activity Yen and Hsieh (2005) prepared MRP from xylose and lysine (1:2) and tested their antimutagenic activity against IQ. The results demonstrated that xylose-lysine MRPs reduced the IQ mutagenic activity by interacting with the metabolites of IQ and not by directly hindering the activation of hepatic microsome. Hence the study concluded that the antimutagenic activity of MRPs is due to the formation of inactive compounds by the interaction of MRPs with proximate IQ metabolites. Recent investigation was undertaken using a glucose-glycine model system by Ko et al. (2018) reporting that the antimutagenic activity increased with increase in MRP concentration in the model systems. The authors also pointed out that the antimutagenic activity in the alcoholic model system was higher as compared to the aqueous system. It must be noted that the antimutagenic effect of MRPs on food mutagens was demonstrated in vitro, and does not imply that the same effect will be demonstrated in vivo as the rate of absorption, excretion and distribution of the mutagens and antimutagens vary intensely (Yen, Tsai, and Lii 1992).

5. Technological advances in Maillard conjugation

In terms of plant-derived proteins, the Maillard conjugation rate is slow due to the compact protein structures that hinder the reaction of reactive amino groups with the carbonyl groups of sugars (Wen, Zhang, Qin et al. 2020), thus different techniques like ultrasonication, pulsed electric field, electrospraying, high pressure processing, and microwave techniques have been considered to increase the rate of conjugation by opening up the compact protein structures. A brief comparison of different methods is shown in Table 2.

5.1. Ultrasonication assisted Maillard conjugation

Ultrasound consists of soundwaves exceeding 20KHz frequency, generating compressions and rarefactions that leads to the creation of cavitation bubble, which implodes to produce high energy. This process, due to this acoustic cavitation effect has found major applications for modifying the properties such as emulsification, textural and other functional attributes of proteins (Bhargava et al. 2021). As explained by Wen et al. (2019), acoustic cavitation includes the following effects; the collapse of cavitation bubble that results in high temperature (4700 °C), pressure (100 MPa) and turbulence which intensifies the mass transfer, the formation of high shear stress and interparticle collisions that accelerate the rate of reaction and creation of reactive free radicals and superoxide that can affect the protein structure by crosslinking with it. While forming MRP and conjugates, the glycation rate is extremely slow due to the compact quaternary and tertiary structures of the proteins which hinder the reaction of carbonyls with the amino acids during Maillard reaction (Li, Xue et al. 2014). The above effects due to acoustic cavitation can help in speeding up the conjugation as illustrated in Figure 3.

Qu et al. (2018) described the possible mechanism of improvement in functional properties using ultrasonic-assisted Maillard reaction. The authors related this enhancement to the improved grafting efficiency leading to a better degree of glycation and random coil structure, tertiary structure extension, and loosened microstructure due to ultrasonication pretreatment. The authors observed that ultrasonication speed up the breaking of peptide bonds which increased the interaction of carbonyl with amino groups, resulting in the reduction of the time required for glycation. Initial studies were carried out by Yu et al. (2018) where the authors studied the effect of ultrasonication on different Maillard reaction model systems (Yu et al. 2018; Guan et al. 2011) and reported that ultrasonication pretreatment increases the depletion rate of reactant while enhancing the intermediate MRP formation.

Zhao et al. (2016) conducted an investigation on the effect of ultrasonic pretreatment on the physicochemical characteristics and rheological attributes of soy protein-glucose/maltose MRPs and reported that the MRPs prepared using ultrasonic pretreatment resulted in increased degree of glycation, lower browning intensity, particle size and increased unfolding of the tertiary structure compared to untreated samples. It was also noted that the cavitation and

Table 2. Comparison of different Maillard conjugation techniques.

Methods		Effects				
		Time required	Degree of glycation	Glycation rate	Structural changes	Neoglycoconjugate formation
Conventional method (Dry state/ Wet state) Pretreatment assisted methods	Ultrasonication: Acoustic cavitation leading to release of energy resulting in physical/chemical modifications in biopolymer (O'Sullivan et al. 2016).	✔	✔	✔	✔	✔
	High pressure processing: Pressure based unfolding and subsequent refolding of proteins after pressure is released resulting in conformational transitions, changes in volume and rearrangement of amorphous and crystalline regions of polysaccharides along with water intrusion (Bolumar et al. 2016).	✔	✔	✔	✔	✔
	Microwave: Microwave energy is absorbed by the polar groups of biopolymers which results in generation of free radicals resulting in chemical/physical modifications (Liu and Ma 2016).	✔✔	✔	✔	✔	✔
	Pulsed electric field: Increased electric field intensity resulting in changes in surface hydrophobicity due to polarization of amino acids and structural changes of biopolymer (Zhang et al. 2021).	✔	✔	✔	✔	✔
	Electrospinning: High electric voltage induced charged strands of biopolymer solution results in restructuring and molecular alignment of the biomolecules via Taylor cone (Rostamabadi et al. 2020).	✔	✔	✔	✔	✔

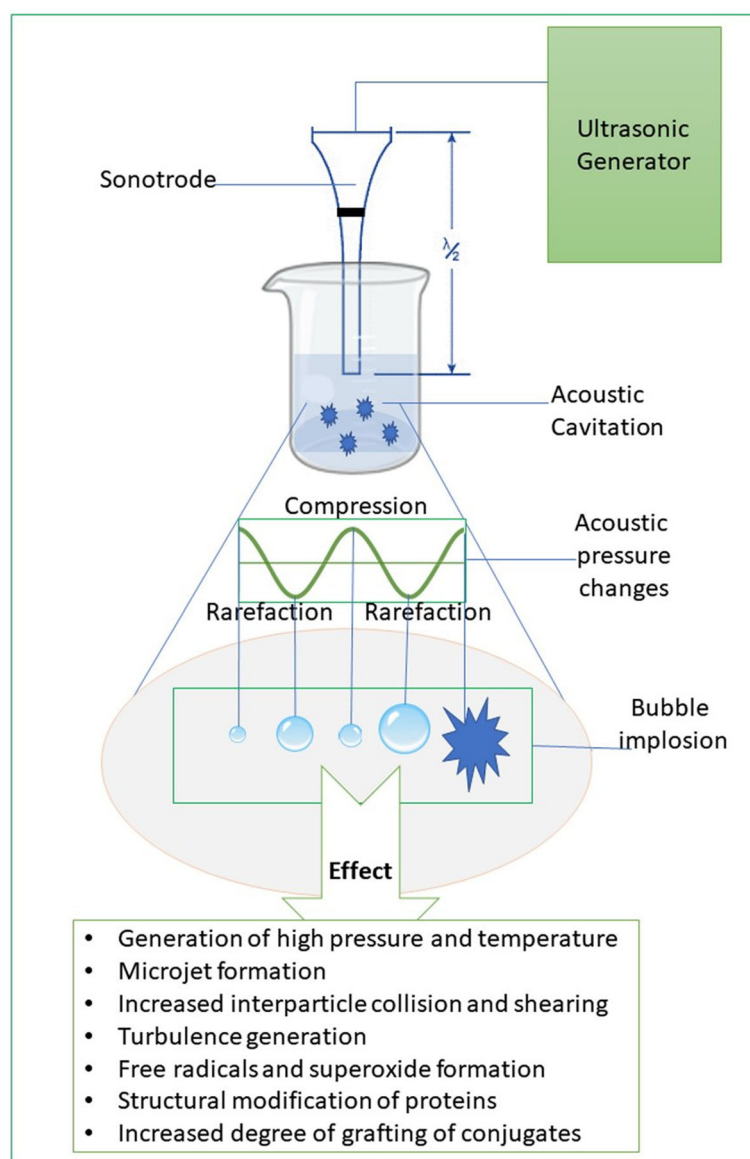


Figure 3. Effect of ultrasonication on Maillard conjugation.

microstreaming effect of the ultrasonication resulted in reduction of weakening effect of glycation on the gel network of the MRP that significantly improved gelling properties of the glycated products. Similar protein was further studied by Cui et al. (2020) to investigate the effect of conjugation (soy protein isolate-glucose) on the molecular flexibility and emulsification properties. It was observed that the rate of reaction increased significantly with the increase in ultrasonic intensity. There was a marked increase in the flexibility of the conjugates formed due to the reduction in particle size and structure loosening after the pretreatment and was correlated to the enhanced emulsification ability of the conjugates. Mu et al. (2010) grafted soy protein isolate with gum acacia using ultrasonication assisted MR and found that the time required for the grafting (34 degree of graft) using ultrasonication pretreatment was much lower (60 minutes) than that required using thermal MR (48 hours). While studying the conjugate further, it was reported that the emulsification stability and hydrophobicity

were enhanced with a marked decrease in the α -helix and β -sheet and increase in the unordered coiling of the secondary structure of the protein due to the pretreatment. The decrease in the lysine and arginine was observed due to covalent links formed during conjugation with the protein and polysaccharide. The effect of different protein concentrations on their functional properties during ultrasonication was investigated by Omura et al. (2021) showing that the functional properties were enhanced except for the rice protein. The physicochemical properties of aqueous protein dispersions (soy protein isolate, rice protein isolate, pea protein concentrate) were influenced by protein concentration showing that the higher concentration of proteins can hinder acoustic cavitation by affecting the frequency of bubble implosion. Although, very limited studies have been reported, excessive treatment has also resulted in the formation of HMF, furans and other AGEs that could hinder the potential use of ultrasonication. Hence, extensive studies must be undertaken to balance the pretreatment such that

the formation of advanced glycation products is avoided while enhancing the conjugation rate (Yu et al. 2020).

5.2. High-pressure assisted Maillard conjugation

In high pressure processing, the product to be processed is submerged inside pressure vessel system filled with liquid (water, silicon oil, Na-benzoate, ethanol, glycol, castor oil) that acts as a pressure transmitting medium (Hogan, Kelly, and Sun 2005). Studies on the influence of high-pressure processing treatment on emulsifying activity and stability of soy protein isolate, 7S and 11S globulins were undertaken. It was reported that the proteins showed improved surface hydrophobicity, emulsion stability and activity at different pressures (11S: 200 MPa, 7S: 400 MPa, soy protein isolate: 400 MPa) due to the partial or total denaturation of monomers of proteins that enhanced surface activity (Molina, Papadopoulou, and Ledward 2001). The earliest fundamental studies of the effect of high pressure on the Maillard reaction was carried by Tamaoka, Itoh, and Hayashi (1991) where they studied the reaction velocities of condensation and browning reactions between xylose, glyceraldehyde and glycolaldehyde with alanine amide, histidine, glycine, alanine and lysine at the applied pressure of 50–500 MPa and at 50 °C. It was reported that the high-pressure treatment suppressed the browning process but there was no effect on the initial condensation process. Model system studies based on glucose-lysine Maillard reaction with pressure of 400 MPa over pH range of 5–10 were undertaken by Moreno et al. (2003), showing that the Amadori rearrangement products formation was not affected by pressure treatment, but a notable suppression of MRP formation from intermediate and advanced stages was observed. This phenomenon was due to pressure induced dissociation of acids that resulted in pH drop at pH < 8, whereas at pH of 10.2, the high pressure accelerated the intermediate and advanced stages that led to formation of AGEs.

Pentosidine is one of the protein-bound fluorescent markers formed during advanced Maillard reaction, and hence is considered as an indicator to determine the stage and intensity of the Maillard reaction. The effect of high hydrostatic pressure over the formation of pentosidine during Maillard reaction was studied by Schwarzenbolz, Klostermeyer, and Henle (2000) where they tested the solution consisting of N-acetyl arginine, N-acetyl lysine and ribose over the pressure up to 600 MPa. They reported that a pressure dependent increase in the pentosidine content was contradictory to the model system studies where the degradation of Amadori products was reduced due to high pressure processing. The reports infer that different reactions during Maillard reaction tend to differ in their pressure dependence. Most studies have focused on model systems which shows that high pressure treatment could be beneficial in controlling the MRP formation and hindering the late-stage glycation, but more extensive studies are needed to explore its effect on complex foods.

5.3. Pulsed electric field (PEF) assisted Maillard conjugation

Pulsed electric field treatment is the process of exposing foods to variable electrical fields placed between the electrodes in a treatment chamber (Figure 4) (Alirezalu et al. 2020). It has been reported that the PEF treatment results in substantial changes in the functional properties mainly solubility and emulsion stability of Maillard conjugates along with increased free amino acid content and improves the conjugation to inhibit the heat-induced aggregation of proteins (Sun et al. 2011; Guan, Lin et al. 2010). PEF treatment can have a significant effect on the polysaccharide and protein structure which can positively influence the digestibility, structural and thermal properties (Zeng et al. 2016; Xiang et al. 2011; Liu et al. 2014).

The effects of PEF (10–40 kV/cm) on the functional and bioactive properties of Maillard conjugates has been studied over the years in asparagine-glucose (Guan, Wang et al. 2010), asparagine-fructose (He et al. 2011) and glycine-glucose (Wang et al. 2011, 2013; Xu et al. 2011) based Maillard model system. There was a marked increase in the rate of reaction and MRP formation over the intensity of 28 kV/cm, whereas no effect was observed below 24 kV/cm field strength. Significant increase in the DPPH radical scavenging activity with increased treatment time was reported which indicates the improved antioxidant capacity after the treatment. At the PEF from 20–40 kV/cm, there was no formation of 5-HMF and acrylamide. Moreover, there was a significant increase in the antioxidant and functional properties of the conjugates. PEF can reduce the extent of MR to advanced stages to reduce the HMF and AGEs formation, although in some cases it has been found that the HMF production was promoted after PEF due to the difference in PEF conditions and food matrices (Khaneghah et al. 2020).

5.4. Microwave assisted Maillard conjugation

When compared to the conventional Maillard conjugation methods, microwave-assisted Maillard conjugation is characterized by much milder reaction conditions and also has a consistent conjugation efficiency (Sun et al. 2020). Nooshkam and Madadlou (2016b) utilized microwave for isomerizing disaccharide and further using microwave-assisted Maillard conjugation to form the conjugate of isomerized sugar with protein isolates. It was observed that as Maillard conjugation progressed, the antioxidant activity increased along with improvement in the functional properties of the conjugate. Similar improvement in the functionality was reported in later studies (Nasrollahzadeh et al. 2017). This improvement in the functional properties can be attributed to the mechanism mentioned by Han et al. (2018) that in term of proteins, microwaves are absorbed by the polar groups leading to the generation of free radicals to modify the secondary and tertiary structures of proteins to influence the emulsification and solubility of proteins.

Guan et al. (2006) investigated the soy protein isolate-saccharides (maltose/lactose/dextran/soluble starch) graft reactions using microwave heating and reported that there was a

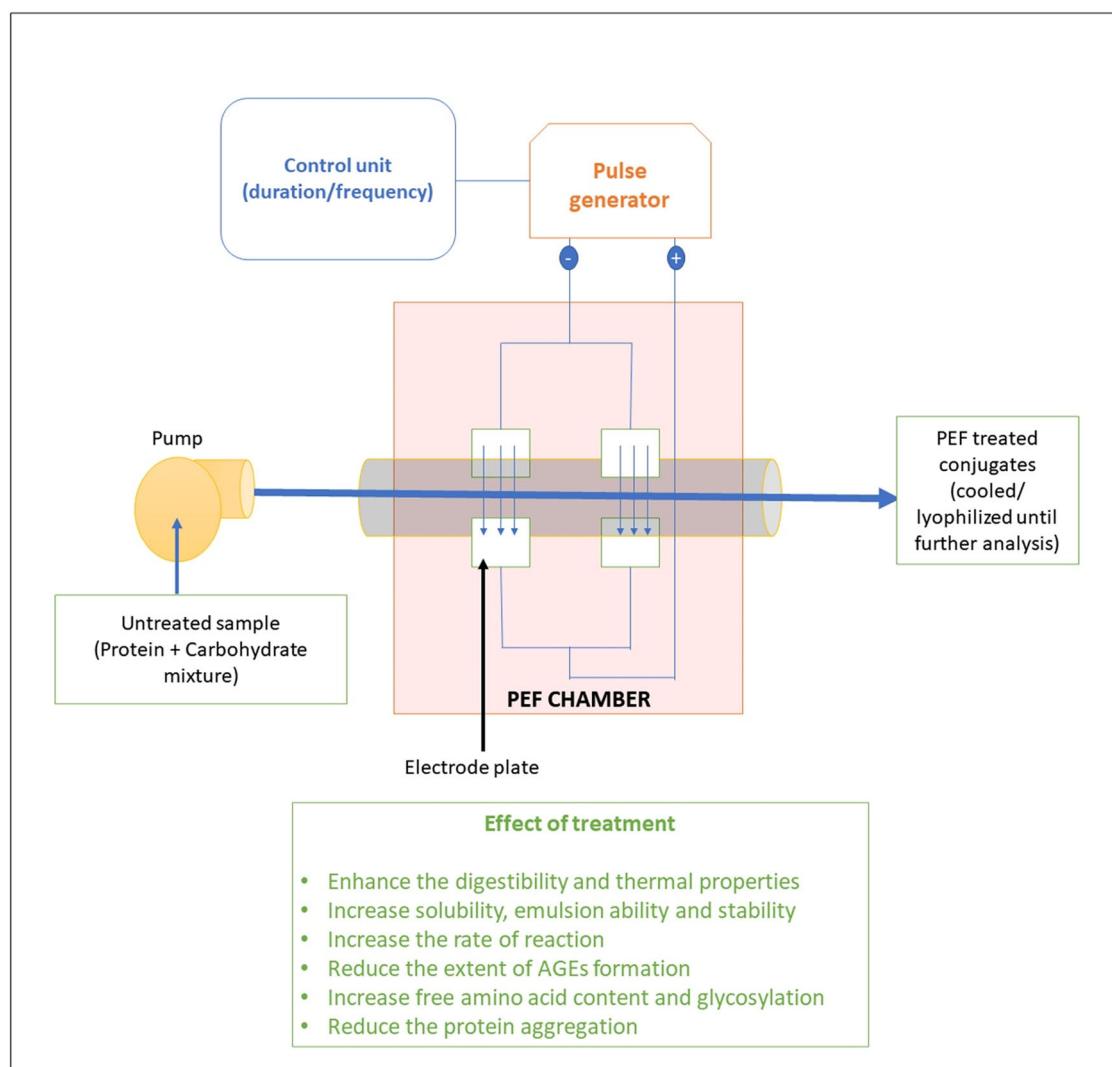


Figure 4. Schematic of PEF treatment and its effect on Maillard conjugates.

similar covalent binding of proteins ϵ -amino groups to the carbonyl groups of the saccharides. The finding implies that microwave heating shows similar covalent binding compared to conventional heating but requires much lower activation energy, and hence could avoid the formation of AGEs more efficiently than conventional heating. A detailed study on the effect of microwave-assisted Maillard conjugation in glucose-ammonium model system was conducted by Li, Wu, and Yu (2018). They reported that the microwave heating resulted in more effective glycation, higher absorbance, and lower chroma, indicating better conjugation. Microwave heating was found to have no effect on the pyrazine formation pathway but promoted the formation of flavor compounds like limonene. These compounds have been extensively explored to have bioactive properties like antioxidant, antidiabetic and anti-inflammatory potential (Vieira et al. 2018). Thus microwave-assisted Maillard reaction can be opted to enhance the flavor intensity of the food components along with enhancing bioactivity.

Plant origin protein studies were carried by Cheng et al. (2021) where they investigated the effect of microwave

processing on the Maillard conjugation between rice protein and dextran and reported that the microwave pretreatment enhanced the rate of glycation. It was also observed that there was a marked increase in solubility (64% within 5 min of microwave heating) whereas it was 24% for conventional heating in 5 minutes. The increase in solubility could be due to the expansion of tertiary structure of rice protein. The authors also reported that the activation energy of Maillard reaction using microwave treatment was much lower (46.7 kJ/mol) than conventional heating implying that the reaction would proceed faster. Recent report (Meng et al. 2019) on rice dreg protein-sodium alginate conjugates formed using microwave treatment also point out toward the enhancement of solubility and emulsion capacity. It was reported that the such modified protein conjugates show improved immunomodulatory effect when tested using cyclophosphamide-induced immunodeficiency animal model. The studies infer that the functional and bioactive properties were improved compared to conventional heating, demonstrating the advantages of microwave assisted conjugation.

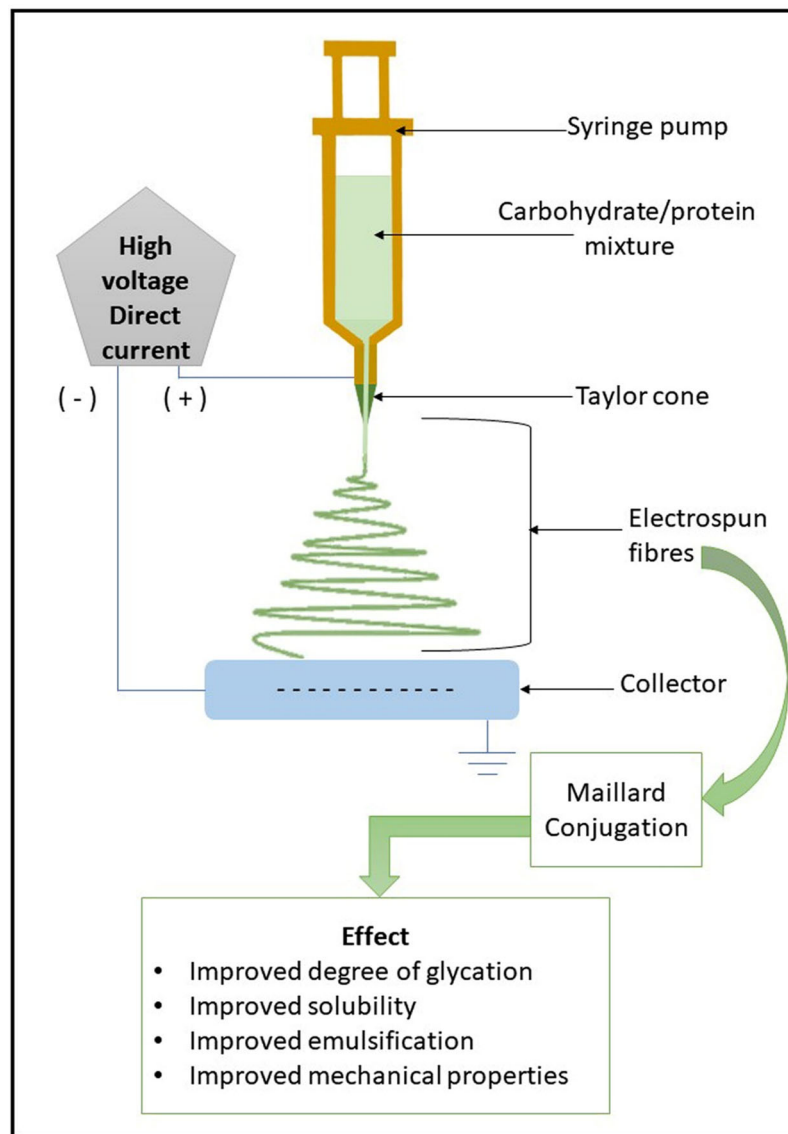


Figure 5. Schematic of electrospinning and its effect on Maillard conjugates.

5.5. Electrospinning assisted Maillard conjugation

Electrospinning is the electrostatic fiber fabrication technique (Figure 5) that can be used to structure the biomolecules into fibers (Bhardwaj and Kundu 2010). Electrospinning, due to its stretching and bending motions leads to molecular alignment of the biomolecular structures that become intact due to polymer chain entanglement and is mainly affected by the viscosity of the solutions, concentrations of polysaccharide, fiber size, mixing ratio of protein: polysaccharide, temperature and humidity (Baier et al. 2016).

Recent studies on production of pea protein isolate-maltodextrin based glycoconjugates using electrospun fibers was investigated, showing that different functional properties of the conjugates improved compared to conventional dry or wet heating methods. It was suggested that the glycation degree can be adjusted accordingly by changing the amount of carbonyl groups in the electrospun polysaccharide fiber. The solubility of the conjugate increased significantly (pH 2–4: 34–44%) indicating improvement of the degree of

glycation using the electrospun fibers. The increased solubility was related to the attachment of hydrophobic protein to hydrophilic carbohydrate that increases the hydrogen bonding capacity of the conjugate formed. Furthermore, emulsification property was also enhanced and exhibited monomodal behavior of the droplet size (36–55 μm at pH 2–7) due to the improved steric repulsion because of the attached carbohydrate (Kutzli, Griener, Gibis, Schmid et al. 2020; Kutzli, Griener, Gibis, Grossmann et al. 2020; Kutzli, Beljo et al. 2020). Electrospinning assisted Maillard conjugation has been used in film formation, and the effects on the wettability, mechanical properties, and biocompatibility of electrospun gelatin-glucose (Siimon, Siimon, and Järvekülg 2015) and zein/gelatin fibers-glucose (Deng et al. 2019) Maillard conjugate have been investigated. The results indicated that crosslinking resulted in improved elastic modulus, biocompatibility, with the resultant film showing tunable wettability and non-cytotoxicity. Globular proteins like soy cannot be electrospun individually and need a carrier to entrap into the electrospun networks. Fabra, López-Rubio, and Lagaron (2016) and Mendes, Stephansen, and

Chronakis (2017) provided a comprehensive review of improvement in different mechanical attributes and functional properties of food proteins and polysaccharides (soy, zein, gliadin, hordein, wheat, amaranth, starch, pullulan) after electrospinning and inferred that the protein aggregation, conformation, chain entanglement and shear thinning properties can be controlled and manipulated accordingly using this technique. The above studies exhibit the potential of electrospinning technique in the modification of proteins and as a pretreatment to facilitate the production of Maillard conjugates with improved functionality particularly in terms of plant proteins where solubility is a main concern (Kutzli, Griener, Gibis, Schmid et al. 2020).

The advanced processing methods for Maillard conjugation have their own advantages and limitations, but they can significantly improve the functional properties of the proteins while reducing the formation of advanced glycation end products. These methods can influence initial stages of Maillard reaction to improve the rate of conjugation, but more studies are essential to understand their effects on the advanced stages of the reaction and on the structural, functional, and bioactive properties of the conjugates. Some of the methods (PEF, electrospinning, HPP) require specialized equipment that can cost and energy intensive, while ultrasonication and microwave are easily available, cost effective, and do not require specialized facilities.

6. Future prospects

Extensive studies on various plant origin proteins like soybean, peas, peanuts, wheat, oat and others have been conducted, but to date, plant proteins have limited techno-functional properties to fully replace animal origin proteins. Nontraditional plant proteins from buckwheat, quinoa, amaranth have recently gained attention due to their better nutritional profile and lower allergenicity compared to traditional plant proteins, but are yet to be explored extensively to enhance their functional attributes. Previous attempts to conjugate the plant origin proteins have not been efficient due to lower yields, lower rate of glycation and undesirable reaction conditions (humidity, pH, temperature) affecting the process. Conventional methods such as dry or wet state methods require longer processing time due to additional steps, and the formation of advanced MRPs is hard to control (Kutzli, Griener, Gibis, Grossmann et al. 2020). The investigation on utilization of different pretreatment assisted Maillard conjugation process is thus needed. Several pretreatments have shown positive results in improving the shortcomings of conventional methods but most studies have been mainly conducted using single sugar or protein model systems which cannot be correlated to complex foods. Moreover, these pretreatments have significant effects on the structural and functional aspects of the reactants which are yet to be fully understood.

Further research on the effect of pretreatment assisted Maillard conjugation on different functional and bioactive properties of nontraditional plant origin proteins is required. In addition, simulated digestion studies of such Maillard

conjugates would be helpful to better utilize and develop plant origin functional foods. The changes in the rheological aspects of the foods by addition of MRPs or after conjugation still remain unexplored.

7. Conclusion

Maillard glycation shows the potential for modification of plant origin protein but requires in-depth investigations of different functional and bioactive attributes to ease the application of this process in selective functionality enhancement and nutraceutical developments. The type of carbohydrate used for glycation can have a significant effect on the conjugation process and the MRPs that are formed in the later stages. Apart from being a component of a complex flavor generation reaction, these early stage MRPs can potentially enhance bioactivity while the initial sidechain reactions can enhance the functionalities of the biomolecules. Earlier investigations have pointed out different methods of exploring the Maillard reaction for conjugation and for the generation of particular MRPs, with more studies required to elucidate the specific mechanism to intervene the initiation of advanced stages of this reaction while enabling the formation of the initial products. Furthermore, these studies are particularly focused on only a few of the traditional plant proteins like soybean, peas, rice or wheat while most of the nontraditional plant proteins have not been fully studied in terms of Maillard conjugation. Maillard conjugation of plant proteins shows a huge potential for application in the food industries and further investigations can be used to correlate the structural changes with their functional properties to allow for better utilization in functional food development.

Abbreviations

AGEs	Advanced glycation end products
ARP	Amadori reaction products
BHT	Butylated hydroxytoluene
HMF	Hydroxy methyl furfural
HPP	High pressure processing
IARC	International agency for research on cancer
MR	Maillard reaction
MRP	Maillard reaction products
PEF	Pulsed electric field
US	Ultrasonication

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Author's contributions

First author wrote the manuscript, all the authors discussed and provided critical feedback toward the manuscript.

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