

Green Tea Polyphenols-proteins Nanocomplexes Foaming Behavior, Nanoemulsions and Their Applications

Marie Alice TUYISHIME*

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, 214122 Jiangsu, P.R China

Emmanuel MATABARO

Key Laboratory of Carbohydrate Chemistry and Biotechnology, School of Biotechnology, Jiangnan University, 1800 Lihu Road, Wuxi, 214122 Jiangsu, P.R China

Ildephonse HABINSHUTI

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, 214122 Jiangsu, P.R China

Aloys HITABATUMA

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, 214122 Jiangsu, P.R China

Sameh A. Korma

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, 214122 Jiangsu, P.R China

Abdelmoneim H. Ali

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, 214122 Jiangsu, P.R China

Department of Food Science, Faculty of Agriculture, Zagazig University, 44511 Zagazig, Egypt

Department of Food Science, Faculty of Agriculture, Zagazig University, 44511 Zagazig, Egypt

*Corresponding author (Tel: +8613003362502, email : tmarialice@yahoo.com, sameh9251@yahoo.com)

Abstract – The formulation of food products with health promoting compounds has recently gained considerable attention due to increasing incidence of diseases associated with oxidative stress. Green tea polyphenols exhibit invaluable properties such as antioxidant activity, antitumor and anti-mutagenic and antibacterial activities. Green tea polyphenols-proteins nanocomplexes are among the most promising delivery systems for bioactive compounds, food ingredients and nutraceuticals. However, the formation of green tea polyphenols-proteins nanocomplexes may affect both protein technological properties and polyphenols biological activity. Little is known about the current knowledge of green tea polyphenols-proteins nanocomplexes and their properties and applications in food industry and health care. Therefore there is a need of enough knowledge to understand their properties and how these systems can be used to create novel products. Therefore, in the present paper, an attempt is made to review green tea polyphenols-proteins nanocomplexes, their foaming behavior, nanoemulsions, rheological characterization and oxidative stability as well as their applications.

Keywords – Green Tea, Polyphenol, Protein, Nanoemulsion.

I. INTRODUCTION

Green tea is rich in polyphenols including mainly phenolic acids and flavonoids [1] and contain several other bioactive compounds and antioxidants for human health [2]. Green tea polyphenols possess many physiological effects combined with antimutagenic, anticarcinogenic [3-5], hypolipidaemic [6], antitumorigenic [7], anti-inflammatory [8], anti-hypercholesterolemic, antibiotic and antiviral effects [9]. Polyphenols decrease levels of markers for cardiovascular diseases [10,11] and have anti-obesity and antidiabetic properties [12]. However, the poor water solubility and poor bioavailability of polyphenols [13] and their instability under food/pharmaceutical products processing (temperature, light, pH) or during storage (light, oxygen) limit the beneficial properties and potential health benefits of these compounds in functional food or pharmaceutical products [14,15]. Maintain stability of oxidation-sensitive bioactive molecules and drugs for increasing their

bioavailability and bio-functions is needed [16]. Proteins have been studied as the hydrophilic emulsifiers in external aqueous phase of W/O/W double emulsions [17]. Gelatin has been used for encapsulation of polyphenols due to its biodegradability and electrical properties [18] for controlled release of active food ingredients [19, 20]. In addition, the use of whey proteins as functional ingredients in food industry is increasing because of their role in human nutrition, health and β -lactoglobulin (β -lg) and caseinomacropptide (CMP) as good foaming agents [21]. Therefore, effective oral delivery of green tea catechins via encapsulation in nanometric size carriers, such as oil in water (O/W) nanoemulsions to improve their bioavailability and their efficacy [22]. Those bio-derived particles may also improve the transdermal transport of emulsions for applications in topical drug delivery as well as stabilization of foam and emulsion systems and prevention of lipid oxidation in food grade emulsions [23]. Moreover, phenolic compounds protect some important biomolecules (such as DNA, lipids and proteins) against oxidative damage induced by reactive oxygen species (ROS) and their addition to food alters its nutritional and rheological properties [24-26]. The formation of protein-green tea polyphenols complexes may affect both protein technological properties and polyphenols biological activity [26] but their complexes and nanoemulsions have nutritional benefits [24]. Therefore, complex formation between tea polyphenols and proteins improves the stability of these molecules as the research on binding of green tea catechins with casein micelles reported milk proteins as an ideal delivery system of these bioactive molecules [27]. They are needed in formulation of foods, beverages, drugs and their delivery systems and they have many other applications. This review focuses on green tea polyphenols-protein nanocomplexes, their foaming behavior, green tea nanoemulsions and their rheological characterization. Their oxidation stability in various systems and applications are overviewed. This review provides an outlook for future work to improve our understanding of their properties and how these systems can be used to create novel products.

II. GREEN TEA POLYPHENOLS-PROTEIN NANOCOMPLEXES

The formation of protein–polyphenol complexes is deeply influenced by the nature of the protein, the nature of the polyphenol (i.e. number of aromatic rings), temperature and presence of other components (i.e. carbohydrates) that can affect the interaction [26]. Green tea polyphenols contain mainly phenolic acids and flavonoids (**Figure 1**), flavonoids themselves contain flavan-3-ols and galloylated catechins that are powerful antioxidants and free iron scavengers [4]. Catechins are the major constituent of tea flavonoids which consist of epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), and their epimerization isomers gallocatechin gallate (GCG),

gallocatechin (GC), catechin gallate (CG), and catechin (C) [28]. Flavan-3-ols have a generic backbone while the presence of a galloyl moiety or an additional hydroxy substitution on the B-ring determines the exact compound [29]. The ability of polyphenols to associate with proteins involves hydrophobic interactions and hydrogen bridging [27] which adversely affect the antioxidant activities of polyphenols [25]. Circular dichroism spectropolarimeter (CD) spectral studies showed alterations in the secondary structure of collagenase on treatment with higher concentration of catechin and EGCG [30]. Formation of non-covalent protein–polyphenol interactions can lead to the formation of aggregates that precipitate from the reaction mixture and polyphenols might bind to proteins [31] forming complexes (**Table 1**).

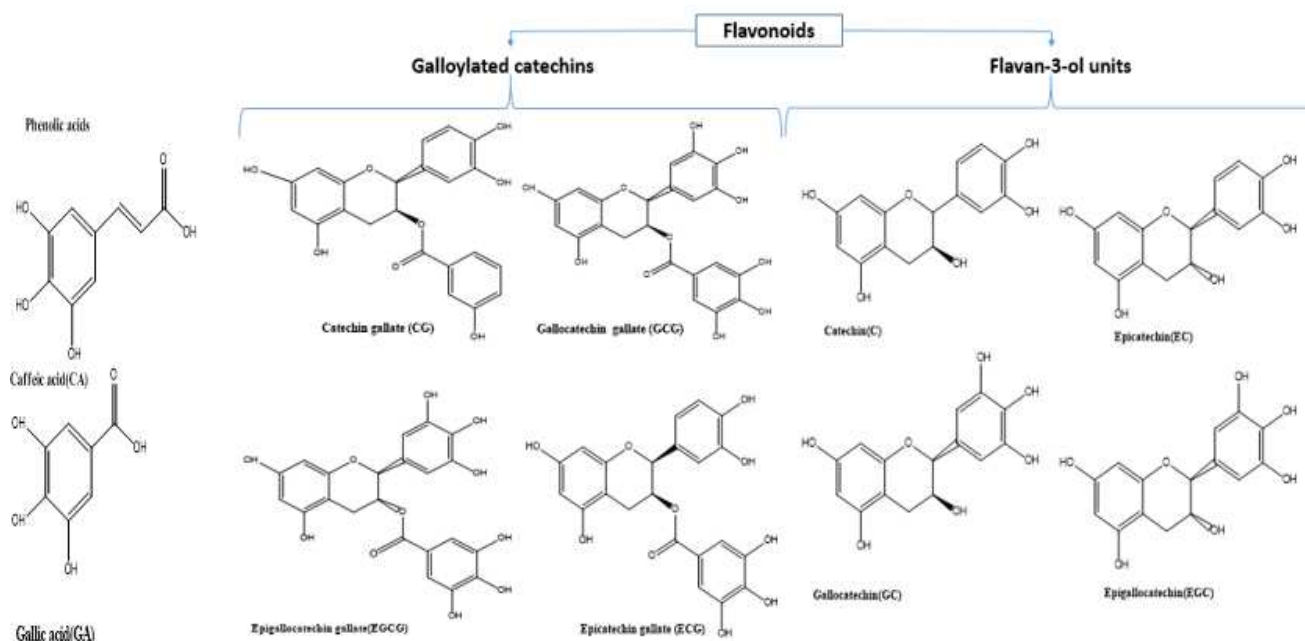


Fig. 1. Classifications and chemical structures of green tea polyphenols

Table 1. Some properties of proteins used in green tea polyphenols –proteins nanocomplexes

Protein	Function and properties	References
β -lactoglobulin (β -lg)	Emulsifier	[32]
	Carrier of polyphenols, heat denatured, rapidly aggregate to form gels	[33]
Caseinomacropetide (CMP)	Great foaming capacity superior to β -lg, but minor foam stability	[34]
	pH-dependent	[35, 36]
α -lactalbumin (α -La)	aggregation (maximum at about pH 4.6 and slow for pH 5.5)	[37]
Human serum albumin (HSA)	Heat-stable protein High binding capacity for polyphenols like EGCG	[38]

Polyphenols localization and concentration in different regions of a multiphase system will depend on their polarity, solubility and affinity for structural

constituents [32]. The changes in pH, temperature, age and ionic strength may affect activity and solubility of these materials in water [23]. Particle size and charge of protein–

polyphenol complexes depend on protein nature and pH, the bigger complexes were observed at pH 4.5 for β -lg and at pH 3 for casein protein CMP[26]. EGCG bind well to proteins with a high number of charges (positive and negative). The positive net charge of lactoferrin promotes better its binding to protein due to due to stronger ionic interactions [38]. However, plant proteins are normally considered inferior to animal proteins (e.g. gelatin, egg white and whey protein) in terms of gelling properties [39]. Polymerized polyphenols which form a coat around a protein by a surface adsorption mechanism lead to the protein insolubility, this loss in protein solubility and decrease in protein pattern are associated the formation of covalent-crosslinking between phenol and protein molecules [25,38]. The fabrication of protein nanoparticles and micro particles rely on the ability of proteins to form gels [40, 41]. The formation of protein-polyphenol complexes promotes the gelation of both β -lg and CMP as indicated by shorter Tgel and lower Tgel values, denaturation of β -lg and proteins aggregation change the viscoelastic characteristics of β -lg gels [26]. Many amino acid residues contribute in polyphenol-protein complexation with extended H-bonding network [42]. An excess of either proteins or polyphenols will reduce the size of the complex and avoid precipitation[29]. Protein-flavan-3-ol interaction has ability to modulate biological activity [31], polyphenol- beta lacto globulin (BLG) systems were stable at pH values of the gastrointestinal tract , bio accessibility and bioavailability of flavan-3-ols have been revealed [43]. The structures of catechins affect the affinities of tea catechins for casein micelles in the green tea polyphenols-milk system[28]. Moreover, the studies reported that the binding of green tea catechins to milk proteins increased as the number of OH group increased with this order of increasing affinity C ~ EC < EGC < EGCG in the case of α - and β -casein [44], whereas for β -lactoglobulin in the order of C ~ EC < EGC < EGCG [31, 42]. Previous researches reported that polyphenol-protein interactions involve mainly hydrophobic bonding between galloyl rings of polyphenol molecules and proline-rich peptides, dominated by cover of the polyphenolic rings against hydrophobic surfaces which are reinforced by hydrogen bonding [26, 43, 44]. Larger polyphenols with a higher number of aromatic rings and hydroxyl groups are more efficient to form complexes with the proline-rich peptides [31, 42, 44]. The initial binding of polyphenols to whey proteins is a non-selective hydrophobically driven interaction, surface charge effects decide the insolubility of the complex formed , control the degree of aggregation and the size of particles formed[26]. It has been shown that serum albumins bind best to the first group of polyphenols (Phlorentin, Hesperetin and EGCG), caseins to the second group (procyanidin B2) and lactoferrin to the fourth group which are all other polyphenols [38].

III. FOAMING BEHAVIOR OF GREEN TEA POLYPHENOL-PROTEIN NANOCOMPLEXES

A foam is a gas-in-liquid dispersion, the dispersed and continuous phases in foams and emulsions are immiscible [23]. Foam formation is controlled by the adsorption of the foaming agent at the air-water interface and its ability to rapidly decrease surface tension , many bio macromolecules exert surface activity at air-liquid and liquid-liquid interfaces [23]. The interfacial interactions between proteins and polyphenols may inhibit foam column falling [21]. Hydroxyl groups in phenolic compounds are able to interact with proteins, thus modifying the structure and function of proteins [25]. They also alter the colloidal stability of the system and gelation properties [26]. Caseinomacropeptide (CMP) possess a great foaming capacity, superior to β -lg, but minor foam stability[21]. The formation of β -lg-polyphenols nano-complexes would not participate in production of a viscoelastic film with good rheological properties due to polyphenols stacked to the hydrophobic side chains of the aminoacids [32]. Recently, most consumers prefer food foamed products with the creamy and soft mouth sensations triggered by the small gas bubbles [21]. The addition of 3% of polyphenol concentrated extract and freeze dried extract decreased the hardness of milk chocolate [45]. It was reported that 0.25-0.4% w/w of instant green tea has succeeded to improve foam expansion and stability of egg albumen proteins [46]. Recently, many studies have focused on the use of partially hydrophobic particles for the stabilization of foams and emulsions due to their ability to adsorb strongly at the air-liquid and liquid-liquid interfaces to limit coarsening, as well as to slow down drainage and syneresis by structuring in the continuous phase [23]. However, foam stabilization requires different surface properties such as the formation of a cohesive viscoelastic film via intermolecular interactions [21]. The amphiphilic molecules prevent phase separation and reduce the energy of the air-liquid or liquid-liquid interfaces in these systems [23]. The intra and intermolecular hydrophobic interactions between proteins needed to form an elastic film which would be restricted by the binding of polyphenols [32]. Furthermore, the binding of catechins to caseins reduces their sensation of astringency in the mouth [27].

IV. GREEN TEA POLYPHENOLS NANOEMULSIONS PARTICLES AND RHEOLOGICAL CHARACTERIZATION

An emulsion is a liquid-in-liquid dispersion, the dispersed and continuous phases in emulsions are immiscible [23]. A nanosized emulsion is a stable emulsion composed of surfactant and oil suspended in water with a particle diameter typically less than 500 nm [22]. Nanoemulsions increased bioavailability upon ingestion due to increased solubility at lesser particle size

[7]. Storage temperature, pH and ionic strength greatly influence the physical and chemical stability of nanoemulsions [19]. It has been found that the presence of polyphenols accelerate the gelation of both β -lactoglobulin (β -lg) and caseinomacropptide (CMP) and mainly affect the viscoelasticity of β -lg gels more than caseinomacropptide gels [26]. Nanoemulsions preserve the properties of bioactive compounds in near natural form when subjected to different storage conditions [7]. Nanoemulsions can be formulated with proteins and polysaccharides showing minimal impact on organoleptic properties of food [7]. Green tea polyphenols are applied in nano-emulsions in the food industry due to increased antioxidant properties [47]. Total polyphenol content, flavonoid content and antioxidant activity of catechins nanoemulsions were compared with unencapsulated extracts to confirm the presence of catechins in their active forms [7]. The concentration of catechin in green tea extract (GTE) and nano green tea extract (NGTE) groups indicated that the nanoemulsion successfully captured the major active compounds during the emulsification process whereas phenolic compounds and total flavonoid content were lower in NGTE than the levels in GTE [22]. The rheological properties of nanoemulsion showed the Newtonian behavior of formulations similar to milk, polyphenol content and flavonoid content are confirmed as active species encapsulated into the sunflower oil (SFO) and peanut oil (PO) based emulsions [7]. The Brownian motion of the particles produces a three-dimensional isotropic and random distribution and as the shear rate increases, the particles become more ordered along the stream lines and form layers that offer less resistance to the fluid flow and therefore produce a decrease in viscosity [48]. Hydroxyl groups in phenolic compounds are capable to interact with proteins, thus modifying the structure and function of proteins [25] and alter the colloidal stability of the system as well as other functional properties like gelation [26]. Nanoscale emulsification may change the composition of green tea extract, which could influence its antioxidant activity and related lipid metabolism [22].

V. OXIDATION STABILITY

Green tea polyphenols have been used as food additives to improve the antioxidant properties and shelf-life of foods [13]. Selection of a stable W/O primary emulsion can increase the stability of W/O/W multiple emulsions [47]. It was reported that at high temperature (37 °C) storage, there was a change in terms of pH, conductivity and refractive index that could lead to a decrease in stability of the emulsions [10]. In addition, ROS and lipid oxidation of oils not only deteriorate food safety but also causing unpleasant odors, off flavors and discoloration, but also decreases the nutritional quality and adversely affect human health [49]. It has been found that fatty acid composition, pH and ionic composition, type and concentration of antioxidants and prooxidants, emulsion droplet and interfacial properties, lipid droplet

characteristics, concentration and physical state influence the physical and oxidative stability of emulsions [50]. Green tea polyphenols have enhanced the oxidative stability of liver fish oil [32]. Due to their chemical structure, polyphenols have powerful antioxidant activities being able to scavenge a wide range of ROS such as hydroxyl radicals and superoxide radicals [51]. Moreover, encapsulated extracts possess a significant amount of antioxidant activities for maintaining the lag phase in oxidation of nanoemulsions [7, 13]. The smallest and most stable nanocomplexes between β -lg and polyphenols was found at pH of 6.0 [26]. The extract catechins from green tea leaves with improved EGCG yield were used in preparation of water-in-oil (W/O) green tea nanoemulsions with soy, peanut, sunflower, and corn oils, where the green tea/peanut oil emulsion displayed the highest oxidative stability [48]. The complexation of tea polyphenols with milk proteins can change the antioxidant activity of tea compounds and the protein secondary structure [42]. The EGCG-loaded GA-g-CS-caseinophosphopeptide (CPP) nanoparticles (84–90% for encapsulation efficiency) showed improved delivery property, controlling release of EGCG under simulated gastrointestinal environments, preventing its degradation under neutral and alkaline environments, and amplifying its anticancer activity against Caco-2 cells which indicated that the nanoparticles with antioxidant activity could stabilize EGCG under alkaline environments [16]. Moreover, the specific surface area of the nanoemulsions was very high and with an average value of about 40 m²/mL [48]. The most common method to increase the oxidative stability of emulsion systems is probably the use of antioxidants [50]. The EGCG release profiles showed a delayed release of the encapsulated antioxidant in aqueous solutions while free EGCG in PBS lost a 30% of their antioxidant activity being completely degraded in 100 h. on the other hand, encapsulated EGCG retained its whole antioxidant activity within this time period [18]. Encapsulation efficiency of the EGCG-loaded capsules of 96% \pm 3% has been found, gelatin capsules were capable of stabilizing EGCG against degradation in aqueous solution (pH 7.4) and as its antioxidant activity was better preserved in this media when encapsulated than in its free form [18]. Antioxidant activities of protein and polyphenols influence radical scavenging activity of the complexes, greater polyphenol to protein ratios increased first order degradation rates, consequently decreasing formation of oxidation products [29]. In addition, the antioxidant activities of tea polyphenols-protein conjugates enhanced with increasing of the protein content [49]. Another research showed that the β -lg conformation was altered in the presence of polyphenols with an increase in β -sheet and α -helix suggesting protein structural stabilization. This explains the mechanism by which the antioxidant activity of tea compounds is affected by the addition of milk [42]. Many studies have shown that if the quantity of polyphenol binding sites on proteins is roughly equivalent to the concentration of polyphenols, large complexes may form, resulting in precipitation and loss of stability [29].

Phenolic compounds have been documented to possess complexing properties towards protein in both unoxidized and oxidized forms [14,52]. Polymer nanoparticles assembled from gallic acid (GA) grafted chitosan (CS, GA-g-CS for GA grafted CS) and caseinophosphopeptides (CPP) showed strong antioxidant activity and cytotoxicity against Caco-2 colon cancer cells [16]. The proteins were never saturated with polyphenols, thus the loss of EGCG stability was caused by oxidation and not by precipitation, the presence of galloyl and hydroxy moieties was linked with higher stability of monomeric flavan-3-ols with increasing protein [29]. Phenolic compounds participated to olive oil stability, even more than other compounds [50]. The oxidation of nanoemulsions was successfully inhibited by the catechins [1].

VI. APPLICATION OF GREEN TEA POLYPHENOLS – PROTEIN NANOCOMPLEXES

A. Health Benefits of Green Tea Polyphenols – Protein Nanocomplexes

Green tea has attracted many researchers in food industry because of its invaluable active components which have shown to improve human health. Green tea polyphenols, mainly epigallocatechin gallate (EGCG) contribute to many health benefits such as prevention of coronary heart diseases, tumor growth, LDL cholesterol, anti-angiogenic and antioxidant effects [53]. Green tea also exhibited anticancer and antidiabetic activities [54-56]. Milk proteins have been shown to improve intestinal transport of green tea catechins [31]. Casein glycomacropptides process responses of immune system prevent adhesion of virus and bacteria, encourage the growth of bifidobacteria, control blood circulation and prevent secretions of stomach [35]. Interaction of polyphenols and proteins is important in chemo preventive modality [57]. Gallic acid (GA) possess the potent ability for treatment and prevention of gastric cancer metastasis [58]. The EGCG-loaded GA-g-CS-caseinophosphopeptide (CPP) nanoparticles (84–90% for encapsulation efficiency) showed improved delivery property, controlling release of EGCG under simulated gastrointestinal environments, preventing its degradation under neutral, alkaline environments and amplifying its anticancer activity against Caco-2 cells [16]. The catechins of green tea can improve or prevent the absorption of nutrients from food which directly depend on the catechin concentration, oxidation and processing as well as their interactions with proteins [59]. Reduction of the allergenicity of peanut proteins due to formation of insoluble complexes with various phenolic acids, has recently been shown [59]. Natural lipophilic functional foods have excellent nutritional value, anti-inflammatory and wound healing [60]. Additionally, inhibition enzymatic activities of collagenases and elastases by green tea polyphenols plant polyphenols might be promising ingredients in anti-aging skin care formulations as EGCG showed a dose-dependent inhibition of collagenase activity (IC₅₀ = 0.9

mM) [61]. The results showed that a wide range of natural polyphenols have a strong potential in creation of less allergenic food products, minimizing the risks of food-allergy reactions in sensitized individuals or treatments of obesity and related hypersensitivity disorders [59].

B. Green Tea Polyphenols–protein Nanocomplexes in Food Industry

It has been reported that green tea polyphenols can be used as antioxidants and as antimicrobial in whey protein based systems [26] and their activity depends on the type of system into which they are incorporated (aqueous, oil or emulsified systems) [32]. Whey protein has excellent emulsifying properties and rich in cysteine which has potent intracellular antioxidant capacity [17]. Adding green tea extract to biscuit can not only prevent rancidity, but also make it as functional food which provides healthy properties to biscuits in the consumer's mind (Sharma and Zhou 2011). Difference in antioxidant activity may be due to various antioxidant compounds formed during baking is associated to antioxidant capacity in polyphenol enriched breads [62]. Similarly, addition of green tea extract (GTE) in the gelatin-based film possessed the highest antioxidant activity, reducing power and retardation of lipid oxidation due to polyphenol compounds [63]. More recently, tea extracts have been used in various industrial products such as cosmetics, foods, and beverages, meat, oil/fat, dairy, and other foods as antioxidant additives [64]. Additionally, most consumers prefer food foamed products with soft and creamy mouth sensations triggered by the small gas bubbles [21]. The cross-linking effect of green tea flavonoids on milk proteins can be used for manufacturing of novel milk products with desired textural properties because of potential effects on textural properties of milk products, e.g., yogurt and cheese [44]. Moreover, it was also reported that green tea extracts improve the quality and shelf life of various food products [65].

C. Application of Green Tea Polyphenols Nanoemulsions

The best stable formulation that delivers nanoemulsion consisting of major catechins from the decaffeinated green tea which are useful in the field of nutraceuticals [7]. The oil-in-water nanoemulsions are used for delivery of hydrophobic bioactives in food systems and can be used in beverages due to their clarity and translucent appearance [66]. Delivery of food bioactive molecules is very important in production of functional foods and the nutraceuticals [41]. Unfortunately, lipophilic compounds have been limited in their application to food system due to their extremely poor aqueous solubility, low oral bioavailability, easy to oxidize, and immiscible feature with other major hydrophilic compounds [60]. Though polyphenols present many health advantages in nutrition, their bio-accessibility is affected by the degradation, epimerization, hydrolysis and oxidation under gastrointestinal conditions [67]. Many studies indicated that nanosized nutritional supplements improve delivery of lipids, vitamins and nutraceuticals [68]. Therefore, nanoemulsions as delivery systems may be used to overcome the problem of their bioavailability and improve

their efficacy [22]. The application of both polyphenols and emulsions using unsaturated fatty acids as the oil phase of polyphenol-encapsulated emulsion can achieve the delivery of multiple nutrients (unsaturated fatty acids and polyphenols) [69]. The average size of the nanoemulsions (approximately 300nm diameter) was tested within the range of nanoparticles that may increase bioavailability through enhanced intestinal uptake *in vivo*[22]. The utilization of encapsulated tea polyphenol mixtures can significantly improve their storage stability, mask the taste as well as increase water solubility [68] while maintaining their antioxidant activity [69]. Besides, consumer's rejection of chemical additives, use of natural additives with antimicrobial properties seems to be a promising alternative for food industry [70]. Therefore research focused on the delivery of natural antimicrobial substances to food products has gained attention in strong food packaging industry [65]. When green tea extract incorporated into edible films improves shelf life and quality of food as a deliver polyphenols which may have potential beneficial effects [71]. Moreover, enriching films with green tea antioxidants enhances nutritional and aesthetic quality of food products [65]. Nanoemulsification increased hypocholesterolemic effects of green tea extract *in vivo* due to increased bioavailability [22].

VII. CONCLUSION AND FUTURE PROSPECTS

Green tea polyphenols (phenolic acids and flavonoids) possess antimicrobial, antioxidant and so many health-promoting properties. Recently, the development of functional milk products and other products containing proteins using polyphenols has increased. However, formulation of green tea polyphenols-proteins nanocomplexes affect both rheological properties of proteins and biological activity of green tea polyphenols. Protein interactions can be applied in stabilizing flavan-3-ols through thermal processing. Green tea polyphenols-proteins nanocomplexes and nanoemulsions are used in nutraceuticals and functional foods formulation as delivery systems of food bioactive molecules. In addition, combination of polyphenols and whey proteins is a promising chemo-preventive modality. Green tea polyphenols-proteins nanocomplexes are used in pharmaceutical products formulation, prevent some diseases linked to oxidative stress as well as formulation of anti-aging skin care due to antioxidant, antimicrobial, anti-obesity and anticarcinogenic properties of polyphenols. They are used in food packaging, food industries for formulations of foamed foods as well as food products and beverages with various functional and nutritional properties. The larger part of the work on green tea polyphenols – proteins nanocomplexes has been using whey protein as a protein source. Recently, there is an increasing interest in using alternative protein sources such as plant-derived protein sources. However, few researches on these other protein sources with green tea polyphenols are available. In future, more researches are

needed to investigate different properties of nanocomplexes of green tea polyphenols with these alternative protein sources, their designs as well as their applications to facilitate production of novel products.

REFERENCES

- [1] Gadkari, P.V. and M. Balaraman, Catechins: Sources, extraction and encapsulation: A review. *Food and Bioproducts Processing*, 2015. **93**: p. 122-138.
- [2] Vodnar, D.C. and C. Socaciu, Selenium enriched green tea increase stability of *Lactobacillus casei* and *Lactobacillus plantarum* in chitosan coated alginate microcapsules during exposure to simulated gastrointestinal and refrigerated conditions. *LWT - Food Science and Technology*, 2014. **57**(1): p. 406-411.
- [3] Manea, A.-M., B.S. Vasile, and A. Meghea, Antioxidant and antimicrobial activities of green tea extract loaded into nanostructured lipid carriers. *Comptes Rendus Chimie*, 2014. **17**(4): p. 331-341.
- [4] Du, G.J., et al., Epigallocatechin Gallate (EGCG) Is the Most Effective Cancer Chemopreventive Polyphenol in Green Tea. *Nutrients*, 2012. **4**(11): p. 1679-1691.
- [5] Yang, C.S., et al., Cancer prevention by tocopherols and tea polyphenols. *Cancer Letters*, 2013. **334**(1): p. 79-85.
- [6] Lv, H.-P., et al., Bioactive compounds from Pu-erh tea with therapy for hyperlipidaemia. *Journal of Functional Foods*, 2015. **19, Part A**: p. 194-203.
- [7] Gadkari, P.V. and M. Balaraman, Extraction of catechins from decaffeinated green tea for development of nanoemulsion using palm oil and sunflower oil based lipid carrier systems. *Journal of Food Engineering*, 2015. **147**: p. 14-23.
- [8] Kemperman, R.A., et al., Impact of polyphenols from black tea and red wine/grape juice on a gut model microbiome. *Food Research International*, 2013. **53**(2): p. 659-669.
- [9] Villanueva Bermejo, D., et al., High catechins/low caffeine powder from green tea leaves by pressurized liquid extraction and supercritical antisolvent precipitation. *Separation and Purification Technology*, 2015. **148**: p. 49-56.
- [10] van Schothorst, E.M., et al., Direct comparison of health effects by dietary polyphenols at equimolar doses in wildtype moderate high-fat fed C57BL/6JOLA^{Hsd} mice. *Food Research International*, 2014. **65, Part A**: p. 95-102.
- [11] Quiñones, M., M. Miguel, and A. Aleixandre, Beneficial effects of polyphenols on cardiovascular disease. *Pharmacological Research*, 2013. **68**(1): p. 125-131.
- [12] Haidong, L., et al., Study on preparation of β -cyclodextrin encapsulation tea extract. *International Journal of Biological Macromolecules*, 2011. **49**(4): p. 561-566.
- [13] Rashidinejad, A., et al., Delivery of green tea catechin and epigallocatechin gallate in liposomes incorporated into low-fat hard cheese. *Food Chemistry*, 2014. **156**: p. 176-183.
- [14] Ghitescu, R.-E., et al., Encapsulation of polyphenols into pHEMA e-spun fibers and determination of their antioxidant activities. *International Journal of Pharmaceutics*, 2015. **494**(1): p. 278-287.
- [15] Pasrija, D., et al., Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. *LWT - Food Science and Technology*, 2015. **64**(1): p. 289-296.
- [16] Hu, B., et al., Polymer nanoparticles composed with gallic acid grafted chitosan and bioactive peptides combined antioxidant, anticancer activities and improved delivery property for labile polyphenols. *Journal of Functional Foods*, 2015. **15**: p. 593-603.
- [17] Mohammadi, A., et al., Application of nano-encapsulated olive leaf extract in controlling the oxidative stability of soybean oil. *Food Chemistry*, 2016. **190**: p. 513-519.
- [18] Gómez-Mascaraque, L.G., J.M. Lagarón, and A. López-Rubio, Electrospayed gelatin submicroparticles as edible carriers for the encapsulation of polyphenols of interest in functional foods. *Food Hydrocolloids*, 2015. **49**: p. 42-52.

- [19] Sari, T.P., et al., Preparation and characterization of nanoemulsion encapsulating curcumin. *Food Hydrocolloids*, 2015. **43**: p. 540-546.
- [20] Özvural, E.B., Q. Huang, and M.L. Chikindas, The comparison of quality and microbiological characteristic of hamburger patties enriched with green tea extract using three techniques: Direct addition, edible coating and encapsulation. *LWT - Food Science and Technology*, 2016. **68**: p. 385-390.
- [21] Rodríguez, S.D., M. von Staszewski, and A.M.R. Pilosof, Green tea polyphenols-whey proteins nanoparticles: Bulk, interfacial and foaming behavior. *Food Hydrocolloids*, 2015. **50**: p. 108-115.
- [22] Kim, Y.J., et al., Nanoemulsified green tea extract shows improved hypocholesterolemic effects in C57BL/6 mice. *The Journal of Nutritional Biochemistry*, 2012. **23**(2): p. 186-191.
- [23] Lam, S., K.P. Velikov, and O.D. Velev, Pickering stabilization of foams and emulsions with particles of biological origin. *Current Opinion in Colloid & Interface Science*, 2014. **19**(5): p. 490-500.
- [24] Świeca, M., et al., Bread enriched with quinoa leaves – The influence of protein–phenolics interactions on the nutritional and antioxidant quality. *Food Chemistry*, 2014. **162**: p. 54-62.
- [25] Nie, X., et al., Preparation and characterization of edible myofibrillar protein-based film incorporated with grape seed procyanidins and green tea polyphenol. *LWT - Food Science and Technology*, 2015. **64**(2): p. 1042-1046.
- [26] von Staszewski, M., et al., Nanocomplex formation between β -lactoglobulin or caseinomacropeptide and green tea polyphenols: Impact on protein gelation and polyphenols antiproliferative activity. *Journal of Functional Foods*, 2012. **4**(4): p. 800-809.
- [27] Sabouri, S., J. Geng, and M. Corredig, Tea polyphenols association to caseinate-stabilized oil–water interfaces. *Food Hydrocolloids*, 2015. **51**: p. 95-100.
- [28] Ye, J., et al., Interactions of black and green tea polyphenols with whole milk. *Food Research International*, 2013. **53**(1): p. 449-455.
- [29] Song, B.J., C. Manganais, and M.G. Ferruzzi, Thermal degradation of green tea flavan-3-ols and formation of hetero- and homocatechin dimers in model dairy beverages. *Food Chemistry*, 2015. **173**: p. 305-312.
- [30] Madhan, B., et al., Role of green tea polyphenols in the inhibition of collagenolytic activity by collagenase. *International Journal of Biological Macromolecules*, 2007. **41**(1): p. 16-22.
- [31] Xie, Y., et al., Milk enhances intestinal absorption of green tea catechins in in vitro digestion/Caco-2 cells model. *Food Research International*, 2013. **53**(2): p. 793-800.
- [32] von Staszewski, M., V.M. Pizones Ruiz-Henestrosa, and A.M.R. Pilosof, Green tea polyphenols- β -lactoglobulin nanocomplexes: Interfacial behavior, emulsification and oxidation stability of fish oil. *Food Hydrocolloids*, 2014. **35**: p. 505-511.
- [33] Mehalebi, S., T. Nicolai, and D. Durand, Light scattering study of heat-denatured globular protein aggregates. *International Journal of Biological Macromolecules*, 2008. **43**(2): p. 129-135.
- [34] Martínez, M.J., et al., Interactions between β -lactoglobulin and casein glycomacropeptide on foaming. *Colloids and Surfaces B: Biointerfaces*, 2012. **89**: p. 234-241.
- [35] Thomä-Worringer, C., J. Sørensen, and R. López-Fandiño, Health effects and technological features of caseinomacropeptide. *International Dairy Journal*, 2006. **16**(11): p. 1324-1333.
- [36] Majhi, P.R., et al., Electrostatically Driven Protein Aggregation: β -Lactoglobulin at Low Ionic Strength. *Langmuir*, 2006. **22**(22): p. 9150-9159.
- [37] McGuffey, M.K., et al., Solubility and aggregation of commercial α -lactalbumin at neutral pH. *International Dairy Journal*, 2007. **17**(10): p. 1168-1178.
- [38] Nagy, K., et al., Non-covalent binding of proteins to polyphenols correlates with their amino acid sequence. *Food Chemistry*, 2012. **132**(3): p. 1333-1339.
- [39] Nieto-Nieto, T.V., et al., Inulin at low concentrations significantly improves the gelling properties of oat protein – A molecular mechanism study. *Food Hydrocolloids*, 2015. **50**: p. 116-127.
- [40] Davidov-Pardo, G., I.J. Joye, and D.J. McClements, Chapter Nine - Food-Grade Protein-Based Nanoparticles and Microparticles for Bioactive Delivery: Fabrication, Characterization, and Utilization, in *Advances in Protein Chemistry and Structural Biology*, D. Rossen, Editor. 2015, Academic Press. p. 293-325.
- [41] wang, Y., et al., 14 - Hydrogel particles and other novel protein-based methods for food ingredient and nutraceutical delivery systems, in *Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals*, N. Garti and D.J. McClements, Editors. 2012, Woodhead Publishing. p. 412-450.
- [42] Kanakis, C.D., et al., Milk β -lactoglobulin complexes with tea polyphenols. *Food Chemistry*, 2011. **127**(3): p. 1046-1055.
- [43] Moser, S., et al., The effect of milk proteins on the bioaccessibility of green tea flavan-3-ols. *Food Research International*, 2014. **66**: p. 297-305.
- [44] Hasni, I., et al., Interaction of milk α - and β -caseins with tea polyphenols. *Food Chemistry*, 2011. **126**(2): p. 630-639.
- [45] Belščak-Cvitanović, A., et al., Innovative formulations of chocolates enriched with plant polyphenols from *Rubus idaeus* L. leaves and characterization of their physical, bioactive and sensory properties. *Food Research International*, 2012. **48**(2): p. 820-830.
- [46] Wu, W., M. Clifford, and N.K. Howell, The effect of instant green tea on the foaming and rheological properties of egg albumen proteins. *Journal of the Science of Food and Agriculture*, 2007. **87**(10): p. 1810-1819.
- [47] Mohammadi, A., et al., Nano-encapsulation of olive leaf phenolic compounds through WPC–pectin complexes and evaluating their release rate. *International Journal of Biological Macromolecules*, 2016. **82**: p. 816-822.
- [48] Lante, A. and D. Friso, Oxidative stability and rheological properties of nanoemulsions with ultrasonic extracted green tea infusion. *Food Research International*, 2013. **54**(1): p. 269-276.
- [49] Wang, Y., et al., Tea polysaccharides as food antioxidants: An old woman's tale? *Food Chemistry*, 2013. **138**(2–3): p. 1923-1927.
- [50] Poyato, C., et al., Oxidative stability of O/W and W/O/W emulsions: Effect of lipid composition and antioxidant polarity. *Food Research International*, 2013. **51**(1): p. 132-140.
- [51] Wittenauer, J., et al., Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity. *Fitoterapia*, 2015. **101**: p. 179-187.
- [52] Belščak-Cvitanović, A., et al., Protein-reinforced and chitosan-pectin coated alginate microparticles for delivery of flavan-3-ol antioxidants and caffeine from green tea extract. *Food Hydrocolloids*, 2015. **51**: p. 361-374.
- [53] Öztürk, B., et al., Change of enzyme activity and quality during the processing of Turkish green tea. *LWT - Food Science and Technology*, 2016. **65**: p. 318-324.
- [54] Zhong, R.-z. and D.-w. Zhou, Oxidative Stress and Role of Natural Plant Derived Antioxidants in Animal Reproduction. *Journal of Integrative Agriculture*, 2013. **12**(10): p. 1826-1838.
- [55] Zielinski, A.A.F., et al., A comparative study of the phenolic compounds and the in vitro antioxidant activity of different Brazilian teas using multivariate statistical techniques. *Food Research International*, 2014. **60**: p. 246-254.
- [56] Kunyanga, C.N., et al., Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients. *LWT - Food Science and Technology*, 2012. **45**(2): p. 269-276.
- [57] Weng, C.-J. and G.-C. Yen, Chemopreventive effects of dietary phytochemicals against cancer invasion and metastasis: Phenolic acids, monophenol, polyphenol, and their derivatives. *Cancer Treatment Reviews*, 2012. **38**(1): p. 76-87.
- [58] Ho, H.-H., et al., Anti-metastasis effects of gallic acid on gastric cancer cells involves inhibition of NF- κ B activity and downregulation of PI3K/AKT/small GTPase signals. *Food and Chemical Toxicology*, 2010. **48**(8–9): p. 2508-2516.
- [59] Tantoush, Z., et al., Green tea catechins of food supplements facilitate pepsin digestion of major food allergens, but hampers their digestion if oxidized by phenol oxidase. *Journal of Functional Foods*, 2012. **4**(3): p. 650-660.

- [60] Shin, G.H., J.T. Kim, and H.J. Park, Recent developments in nanoformulations of lipophilic functional foods. *Trends in Food Science & Technology*, 2015. **46**(1): p. 144-157.
- [61] Wittenauer, J., et al., Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity. *Fitoterapia*, 2015. **101**: p. 179-187.
- [62] Culetu, A., et al., Effect of theanine and polyphenols enriched fractions from decaffeinated tea dust on the formation of Maillard reaction products and sensory attributes of breads. *Food Chemistry*, 2016. **197, Part A**: p. 14-23.
- [63] Li, J.H., et al., Preparation and characterization of active gelatin-based films incorporated with natural antioxidants. *Food Hydrocolloids*, 2014. **37**: p. 166-173.
- [64] Karaosmanoglu, H. and P.A. Kilmartin, 9 - Tea extracts as antioxidants for food preservation, in *Handbook of Antioxidants for Food Preservation*, F. Shahidi, Editor. 2015, Woodhead Publishing. p. 219-233.
- [65] Li, J.-H., et al., Preparation and characterization of active gelatin-based films incorporated with natural antioxidants. *Food Hydrocolloids*, 2014. **37**: p. 166-173.
- [66] Augustin, M.A. and C.M. Oliver, 1 - An overview of the development and applications of nanoscale materials in the food industry A2 - Huang, Qingrong, in *Nanotechnology in the Food, Beverage and Nutraceutical Industries*. 2012, Woodhead Publishing. p. 3-39.
- [67] López-Gutiérrez, N., et al., Identification and quantification of phytochemicals in nutraceutical products from green tea by UHPLC–Orbitrap-MS. *Food Chemistry*, 2015. **173**: p. 607-618.
- [68] Riviere, J.E., 2 - Potential health risks of nanoparticles in foods, beverages and nutraceuticals A2 - Huang, Qingrong, in *Nanotechnology in the Food, Beverage and Nutraceutical Industries*. 2012, Woodhead Publishing. p. 40-52.
- [69] Lu, W., A.L. Kelly, and S. Miao, Emulsion-based encapsulation and delivery systems for polyphenols. *Trends in Food Science & Technology*, 2016. **47**: p. 1-9.
- [70] Kurek, M., et al., Antimicrobial efficiency of carvacrol vapour related to mass partition coefficient when incorporated in chitosan based films aimed for active packaging. *Food Control*, 2013. **32**(1): p. 168-175.
- [71] López de Lacey, A.M., et al., Bioaccessibility of green tea polyphenols incorporated into an edible agar film during simulated human digestion. *Food Research International*, 2012. **48**(2): p. 462-469.

AUTHOR'S PROFILE



TUYISHIME Marie Alice was born at Rwanda, Muhanga district on 3rd October 1989. She obtained a Bachelor's Degree with honor in crop sciences in 2013 from the former National University of Rwanda located at Huye district in Rwanda. Now she is a student in nutrition and food hygiene in Master's degree program, school of food science and technology at Jiangnan

University, Wuxi city, Jiangsu province in People's Republic of China. She was TEACHER in 2009, ENUMERATOR of Harvest Plus in 2013, MARKETING AGENT of Harvest Plus in 2014. ENUMERTOR of Rural Sector Support Project in 2015. JUDGE in National High School Academic Debate and Speech Tournament in Jiangsu province in People's Republic of China.