Food Chemistry 245 (2018) 439–445

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of (−)-epigallocatechin-3-gallate incorporation on the physicochemical and oxidative stability of myofibrillar protein–soybean oil emulsions

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ARTICLE INFO

Chemical compounds studied in the article:
(−)-Epigallocatechin-3-gallate (PubChem CID: 65064)
5,5′-Dithio-bis(2-nitrobenzoic acid) (PubChem CID: 6254)
8-Anilino-1-naphthalenesulfonic acid (PubChem CID: 1369)
β-Mercaptoethanol (PubChem CID: 1567)
2-Thiobarbituric acid (PubChem CID: 2723628)

Keywords:
Meat emulsions
Myofibrillar protein
Phenolic antioxidants
(−)-Epigallocatechin-3-gallate
Rheology
Fluorescence

ABSTRACT

The dose-dependent effects of (−)-epigallocatechin-3-gallate (EGCG; at 0, 50, 100, 200, 500, and 1000 mg/L) on the physical, chemical, and oxidative stability of porcine myofibrillar protein (MP)–soybean oil emulsion systems were investigated. The results showed EGCG at all levels effectively suppressed lipid oxidation in MP emulsion composite gels during the entire chill storage (at 4 °C for 0, 3, or 7 days). The incorporation of EGCG at higher concentrations (> 100 mg/L) promoted the loss of sulfhydryls, reduction of surface hydrophobicity, and aggregation and cross-linking of MP. As a result, high concentrations of EGCG (500 and 1000 mg/L) hampered emulsification and gel formation of MP. However, EGCG at lower concentrations (50–200 mg/L) improved the oxidative stability of meat emulsions without jeopardizing the textural stability.

1. Introduction

Meat products are an important source of high biological value protein, vitamins, minerals, and other nutritional substances (Williams, 2007). However, due to the abundance of saturated fatty acids (SFAs) and cholesterol, traditional meat products, such as emulsion-type sausages with the fat content up to 30%, are widely considered unhealthy. This is because the high intake of fatty and cholesterol-rich foods has been associated with increased risk of cardiovascular disease, diabetes, and other obesity-inflicted diseases (Houston et al., 2011).

One of the approaches to alleviating the health risk of processed meats is replacements of animal fat with selective healthier oils of plant sources whose characteristics are in line with health recommendations. Hence, vegetable oils having relatively high proportions of monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs), and desirable n-6/n-3 PUFA and PUFA/SFA ratios have been considered as good fat substitutes in processed meats (Asuming-Bediako et al., 2014). Numerous studies have shown that this strategy is effective for obtaining healthier meat products with acceptable technological and sensorial properties (Choi et al., 2010; Jiang & Xiong, 2016). However, due to the preponderance of unsaturated fatty acids (UFAs), such products become more susceptible to lipid oxidation (Josquin, Linssen, & Houben, 2012; Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Carballo, & Jiménez-Colmenero, 2015). The oxidation of UFAs always resulted in significant generation of dietary advanced lipid oxidation endproducts (ALEs, genotoxic and cytotoxic compounds), thus long-term consumption of oxidized fat/oil threatening human health has been reported widely (Kanner, 2007).

Plant extracts rich in phenolic acids and flavonoids are commonly added to emulsion-type meat products for flavor modification and oxidative stability (Jiang & Xiong, 2016; Kumar, Yadav, Ahmad, & Narsaiah, 2015). Moreover, polyphenols are recognized for

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http://dx.doi.org/10.1016/j.foodchem.2017.10.111
Received 24 August 2017; Received in revised form 17 October 2017; Accepted 22 October 2017
Available online 23 October 2017

their health benefits, for example, anti-artherogenic, anti-thrombotic, and antimicrobial properties (Maqsood, Benjakul, & Shahidi, 2013). Recent studies have also shown that many phenolic acids and flavonoids are capable of mitigation of genotoxicity in cooked and processed meats (Gibis & Weiss, 2012; Zhu, Zhang, Wang, Chen, & Zheng, 2016). Moreover, phenolics can interact with functional groups of proteins in meat emulsion (batter) through both covalent and noncovalent forces, resulting in altered techno-functional properties, such as gelation (Cao & Xiong, 2015; Jia, Wang, Shao, Liu, & Kong, 2017; Ozdal, Capanoglu, & Altay, 2013).

In a typical meat emulsion or "batter" product, protein-coated fat globules or oil droplets are imbedded within a continuous, three-dimensional myofibrillar protein (MP) gel matrix forming a heterogeneous composite structure (Wu, Xiong, & Chen, 2011). It is hypothesized that the masking of functional groups and aggregation of MP induced by binding with phenolic compounds and their oxidative quinone derivatives will affect the emulsifying properties and gel-forming ability of meat proteins. As reported recently, blocking of sulfhydryl groups in MP by N-ethylmaleimide or high concentrations of green tea extracts led to a reduced water-holding capacity and textural stability (Jongberg, Terkelsen, Miklos, & Lund, 2015; Wu et al., 2011), while treatment with polyphenols at appropriate concentration levels improved the gelling properties of meat proteins (Balange & Benjakul, 2009; Cao, True, Chen, & Xiong, 2016). However, knowledge of emulsifying properties of phenolic-treated MP and cross-linking of the emulsion droplets into a gel network upon heating is rather limited.

The objective of this study was to examine how (−)-Epigallocatechin-3-gallate (EGCG) would modify the structural and surface properties of MP, and how such modifications would affect the rheological characteristics of protein emulsions upon cooking. The EGCG was chosen because it is a kind of water-soluble phenolic compound abundantly present in green tea extracts that are broadly added to emulsion-type meat products as natural antioxidants (Senanayake, 2013).

2. Materials and methods

2.1. Materials

Longissimus muscle was collected from five pork carcasses (24 h postmortem) harvested at the University of Kentucky Meat Laboratory, a USDA-approved facility. Individual muscle samples (~100 g) were vacuum-packaged and stored in a −30 °C freezer until use. Soybean oil was purchased from Kroger Supermarket (Lexington, KY, USA) and used directly. (−)-Epigallocatechin-3-gallate (EGCG, ≥95%, product number E4143), 8-anilino-1-naphthalenesulfonic acid (ANS, 97%, product number A1028), piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES, ≥99%, product number P6575), and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, ≥98%, product number D8130) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were at least of analytical grade.

2.2. MP extraction and sample preparation

2.2.1. Extraction of MP

MP isolate was extracted from longissimus muscle according to the method described by Park, Xiong, and Alderton (2006). The whole preparation process was carried out in a walk-in cooler (2–4 °C). The final protein pellet (suspended in 0.1 M NaCl, pH 6.25) was stored in a tightly capped bottle, kept on ice, and utilized within 48 h. The protein concentration was measured by the Biuret method (Gornall, Bardawill, & David, 1949).

2.2.2. MP suspensions with EGCG

Stock MP (30 mg/mL) and EGCG (5 mg/mL) solutions were separately prepared with 15 mM PIPES buffer (containing 0.6 M NaCl, pH 6.25) and used immediately. A set of MP–EGCG complex suspensions (20 mg/mL protein and 50, 100, 200, 500, or 1000 mg/L EGCG, final concentrations) were prepared by pipetting various volume fractions of EGCG solutions into the MP suspension while gently vortexing. An MP suspension (20 mg/mL) without EGCG was used as the control.

2.3. Measurement of structural changes in MP

2.3.1. Intrinsic tryptophan fluorescence

Tryptophan fluorescence was measured using a FluoroMax-3 spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA) at room temperature (~22 °C). A series of dilute MP–EGCG complex suspensions (0.25 mg/mL protein in 15 mM PIPES buffer containing 0.6 M NaCl, pH 6.25) were excited at 295 nm, and the fluorescence emission spectra were recorded from 300 to 400 nm. Both the excitation and the emission slit widths were set to 5 nm and data was collected at a 500 nm/min rate. Background spectra under the same conditions were recorded and subtracted from the respective spectra of the EGCG treated MP samples.

2.3.2. Surface hydrophobicity (S_o)

MP suspensions with or without EGCG were diluted to 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL protein in 15 mM PIPES buffer containing 0.6 M NaCl, pH 6.25. To 5.0 mL of the diluted protein solution, 25 μL of 8.0 mM ANS were added and mixed thoroughly. After 15 min of incubation at room temperature, the fluorescence intensity (FI) was measured using a FluoroMax-3 spectrofluorometer where the excitation and emission wavelengths were set at 390 and 470 nm, respectively (slit width 5 nm). Sample blank (protein solution without added ANS) and reagent blank (PIPES buffer without protein, but ANS added) fluorescence was measured and subtracted from the ANS–protein conjugates samples (protein solution with ANS added). The initial slope of the FI versus protein concentration plot was calculated by linear regression analysis and used as S_o.

2.4. Emulsion preparation and property measurement

2.4.1. Emulsion preparation

Emulsions were prepared by mixing 2 g of soybean oil with 18 g of MP suspensions (20 mg/mL with or without EGCG) and homogenized with a Polytron blender (Brinkmann Instruments, Inc., Westbury, NY, USA) equipped with a low-foaming probe (PTA-20TS) at a speed setting of "6" (approximately 17,500 rpm) for 1 min. Prepared emulsions were immediately subjected to the following tests.

2.4.2. Total sulfhydryl (SH) content

The total SH content of MP emulsion samples with various amounts of EGCG were determined using the DTNB method (Ellman, 1959). Emulsion samples were diluted to 2 mg/mL protein with 15 mM PIPES buffer containing 0.6 M NaCl (pH 6.25). After reaction with DTNB for 15 min in the dark, 2 mL of chloroform were added, vortexed, and centrifuged at 1000*g* for 10 min to remove the oil, the absorbance of the aqueous phase was recorded at 412 nm against reagent and sample blanks. A molar extinction coefficient of 13600 M^{-1} cm^{-1} was used for the SH concentration calculation.

2.4.3. Protein cross-linking

The influence of EGCG addition on protein polymerization during the emulsifying process was observed using sodium dodecyl sulfate–-polyacrylamide gel electrophoresis (SDS–PAGE) according to Laemmli (1970) with a 4% polyacrylamide stacking gel and a 12% resolving gel. Diluted emulsion samples (2 mg/mL protein) were mixed with an equal volume of SDS–PAGE sample buffer (4% SDS, 20% glycerol, 0.125 M Tris, pH 6.8) with or without 10% β-mercaptoethanol (β-ME) and then boiled for 3 min. To each well, 35 μL of samples were loaded.