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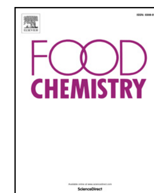

Dual effects of propyl gallate and its methylglyoxal adduct on carbonyl stress and oxidative stress

Hengqing Cui[a,1], Fei Tao[c,1], Yu Hou[a], Yongling Lu[a], Tiesong Zheng[a], Shengmin Sang[b], Lishuang Lv[a,*]

[a] Department of Food Science and Technology, Nanjing Normal University, 122# Ninghai Road, Nanjing 210097, PR China
[b] Laboratory for Functional Foods and Human Health, Center for Excellence in Post-Harvest Technologies, North Carolina Agricultural and Technical State University, North Carolina Research Campus, 500 Laureate Way, Kannapolis, NC 28081, United States
[c] College of Standardization, China Jiliang University, 258# Xueyuan Street, Hangzhou 310018, PR China

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ABSTRACT

In the present study, we investigated the trapping of methylglyoxal (MGO) by propyl gallate (PG), a known food grade antioxidant, and the anti-carbonyl and anti-oxidative properties of the mono-MGO adduct of PG (MM-PG). Our result indicated that more than 77.5% MGO was suppressed by PG after a 30 min incubation of PG with MGO, which was much more effective than gallic acid (15.2%). For the first time, MM-PG was purified, and its structure was elucidated based on the analysis of its ^{1}H, ^{13}C, and 2D-NMR data. We also demonstrated that MM-PG had strong anti-oxidative and anti-carbonyl activities. Furthermore, PG could trap the MGO generated during the preparation of roasted pork, and both mono- and di- MGO adducts of PG were detected in the roasted pork system using LC/MS technique. Thus, PG could be widely applied in the food system for inhibiting the formation of both carbonyl species and oxidative species.

1. Introduction

Reactive carbonyl species (RCS), especially α-dicarbonyl compounds (methylglyoxal (MGO) and glyoxal), are reactive intermediates, originating from both exogenous and endogenous pathways, such as protein glycation (Thornalley, Langborg, & Minhas, 1999), lipid peroxidation (Angelika & Spiteller, 1994), sugar autoxidation (Wolff, Bascal, & Hunt, 1989), enzyme fermentation (Bravo et al., 2008), and UV-photo damage (Mizutari, Ono, Ikeda, Kayashima, & Horiuchi, 1997). α-Dicarbonyl compounds from dietary sources, such as coffee, yogurt, cookie, oil and highly processed foods (Degen, Hellwig, & Henle, 2012), make important contributions to the endogenous levels of RCS (Poulsen et al., 2013; Sharma, Kaur, Thind, Singh, & Raina, 2015; Uribarri et al., 2005), and lead to the development of a series of chronic diseases, such as diabetes, ageing (Rabbani & Thornalley, 2015). It has been well studied that the increasing of reactive oxygen species (ROS) levels *in vivo* could also result in dramatic damage to the structures of proteins, DNA or RNA, induce inflammatory responses, and cause many different chronic diseases (Manoharan et al., 2016; Panth, Paudel, & Parajuli, 2016). Recent studies indicated that in many cases an increase in RCS concentrations was a consequence of oxidative stress, and an increase in ROS levels resulted from carbonyl stress (Ramlagan et al., 2017; Semchyshyn & Lushchak, 2012). Therefore, there is an urgent need to find dual inhibitors of RCS and ROS to improve food qualities.

Numerous studies reported that the flavonoids, such as quercetin (Li, Zheng, Sang, & Lv, 2014; Zhao, Wang, Chen, & Sang, 2016), genistein (Lv, Shao, Chen, Ho, & Sang, 2011; P. Wang, Chen, Sang, 2016), phloretin (Shao et al., 2008), rutin (Cervantes-Laurean et al., 2006), epicatechin (Totlani & Peterson, 2006), are dietary antioxidants and could effectively trap MGO *in vitro* and *in vivo*. However, flavonoids have not been widely used as food additives in food industries, which might due to either that some flavonoids were not authorized as a food additive or their addition would affect the color and flavor of food. Propyl gallate (PG, E310) (Fig. 1A) (Additives, 2014), a synthetic antioxidant, plays a significant role in preventing lipid oxidation and degradation of food products containing high levels of lipids or fats. It has been widely used as a preservative in the foods (C.R.C., 2016), cosmetics (Wang et al., 2013), and pharmaceutical industries (Listed, 2007). However, there is no study on the effects of PG trapping MGO. The purpose of this paper is to investigate the trapping of MGO by PG in

Abbreviations: DM-PG, di-MGO adduct of PG; GA, gallic acid; MGO, methylglyoxal; MM-PG, mono-MGO adduct of PG; PG, propyl gallate; RCS, reactive carbonyl species; ROS, reactive oxygen species; V_C, vitamin C

* Corresponding author.
E-mail addresses: ssang@ncat.edu (S. Sang), lulishuang@njnu.edu.cn (L. Lv).

[1] These authors contributed equally to this study.

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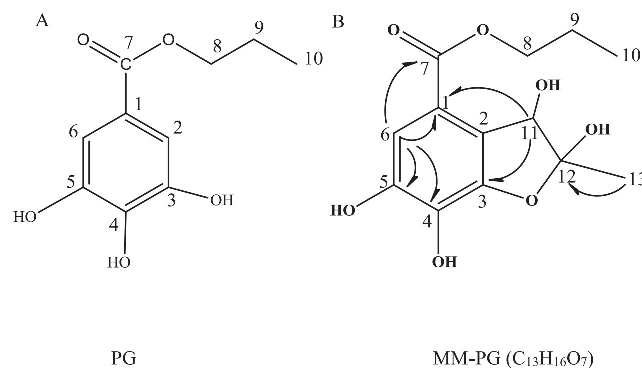

Fig. 1. Chemical structures of PG, MM-PG, and significant HMBC (H → C) correlations of MM-PG. (MM-PG, mono-MGO adduct of PG).

buffer and in roasted pork, and the anti-carbonyl and anti-oxidative effects of the mono-MGO adduct of PG (MM-PG).

2. Materials and methods

2.1. Materials

Methylglyoxal (MGO, 40% in water), 1,2-diaminobenzene (DB), 2,3-butanedione, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St Louis, MO, USA). Propyl gallate was purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Corn oil (without antioxidant) was provided by Bunge (Nanjing) Grains & Oils Co., Ltd. Pork was purchased from local supermarket (Nanjing, China). Sodium dihydrogen phosphate ($NaH_2PO_4{\cdot}2H_2O$), disodium hydrogen phosphate ($Na_2HPO_4{\cdot}12H_2O$) and other chemicals were of analytical grade.

2.2. Trapping of MGO by PG and gallic acid in phosphate buffer

MGO (0.5 mM) was incubated with PG or gallic acid (GA) (0.5 mM) in phosphate buffer (pH 7.0, 0.2 M) at 100 °C for 0, 5, 10, 15, and 30 min. At each time point, 1.0 mL of sample was collected and cooled in ice water and then stored at − 80 °C. MGO levels were analyzed according to our published method (Li et al., 2014). DB was used as the derivatization agent and 2,3-butanedione was used as the internal standard. Samples were mixed with DB and kept at 37 °C for 1 h, and acetaldehyde was added to react with the rest of the DB. The mixture was extracted with methylene chloride for GC analysis. All determinations were performed in triplicate.

2.3. Preparation of the major MGO adduct of PG

PG (0.325 M, 0.689 g) and MGO (3.25 M) were dissolved in 5.0 mL of phosphate buffer (0.2 M, pH 7.0) and kept at 80 °C for 3 h. The reaction mixture was loaded onto a reverse phase silica gel C_{18} column and eluted with 20% methanol about two times the volume of bed, and then with 40% methanol to obtain MM-PG (48.0 mg) (Fig. 1B).

2.4. Nuclear magnetic resonance (NMR) analysis

^{1}H, ^{13}C, and two dimensional (2D) ^{1}H-^{13}C HMQC (heteronuclear multiple quantum correlation) and HMBC (heteronuclear multiple band correlation) spectra were acquired on a Bucker AVANCE 400 MHz instrument (Bruker, Inc., Silberstreifen, Rheinstetten, Germany).

^{1}H and ^{13}C NMR data of PG and MM-PG are listed in Table 1a. Both compounds were analyzed in DMSO.

Table 1a
δ_H (400 MHz) and δ_C (100 MHz) NMR Spectroscopic Data of PG and MM-PG (DMSO, δ in ppm and J in Hz).

	PG		MM-PG	
	δ_H	δ_C	δ_H	δ_C
1		120.463 s		124.074 s
2	7.075 s	108.896 s		117.736 s
3		144.981 s		148.154 s
4		138.287 s		134.952 s
5		144.981 s		146.838 s
6	7.075 s	108.896 s	6.992 s	110.232 d
7		167.476 s		165.994 s
8	4.188 t J 6.4	66.177 s	4.152 t J 6.4	66.064 t
9	1.755 q	21.763 s	1.706 q	22.147 t
10	1.025 t J 7.2	9.522 s	0.976 t J 7.2	10.994 q
11			4.901 s	77.480 d
12				112.057 s
13			1.548 s	22.114 q

2.5. Trapping of MGO by MM-PG in phosphate buffer

Following the procedure described under “trapping of MGO by PG in phosphate buffer”, the effects of MM-PG to trap MGO was determined. For structure elucidation of the MGO adducts of MM-PG by LC-MS, MM-PG (0.325 M) was incubated with MGO (1:10) in phosphate buffer (0.2 M, pH 7.0) at 100 °C for 30 min.

2.6. LC-MS/MS analysis

LC-MS/MS analysis was carried out with an Agilent Masshunter System consisting of a 1290 G4220A BinPump, a 1290 G4226A Wellplate sampler, a G4212A diode array detector, and a 6460 QQQ mass detector Agilent 6520 LC-Q-TOF-MS. (Agilent, Santa Clara, CA, USA) incorporated with an electrospray ionization (ESI) interface. A 250 × 4.6 mm i.d., 5 µm, Kromasil 100–5 C_{18} column was used for separation at a flow rate of 0.6 mL/min. The mobile phases were composed of water (mobile phase A) and methanol (mobile phase B). The linear gradient elution had the following profile: 30% B for 3 min, 30–70% B from 3 to 20 min, 70% B from 20 to 23 min, and then 70–30% B from 23 to 25 min. The column was then re-equilibrated with 30% B for 5 min. The LC eluent was introduced into the ESI interface. The negative ion polarity mode was set for ESI ion source with the voltage on the ESI interface maintained at approximately 5 kV. Nitrogen gas was used as the sheath gas at a flow rate of 45 arbitrary units (au) and the auxiliary gas at 5 au, respectively. The structural information of PG and the major MGO-adducts was obtained by tandem mass spectrometry (MS/MS) through collision-induced dissociation (CID) with a relative collision energy setting of 15%. Data acquisition was performed with Qualitative Analysis of Masshunter (Agilent, Santa Clara, CA, USA).

2.7. Effect of MM-PG and PG on the formation of MGO in roasted pork system

Pork was cut into slices (5 × 5 × 1 cm), and immersed in the brine (ingredients: salt 25 g/kg, sugar 150 g/kg, monosodium glutamate 3 g/kg, soy sauce 10 g/kg, white spirit 25 g/kg) served in a sealed container, stored at 4 °C for 2 h (Chen, He, Qin, Chen, & Zeng, 2017). The sliced pork rubbed by 5.0 mL corn oil containing MM-PG or PG (0.05, 0.1, 0.2 g/kg) was roasted at 220 °C in oven for 10 min (5 min for each side). The roasted pork samples were lyophilized and finely ground and then stored at -80 °C for further analysis.

Ground pork samples (1.0 g) were extracted by 100 mL methanol for 2 h, and centrifuged at 8000 rpm for 10 min. The supernatant was

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