

Effects of thermally modified green tea catechins on the oxidative and hydrolytic stability of butter

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ABSTRACT

Green tea catechins are classified as (-)-epi-forms (2R, 3R) or (-)-forms (2S, 3R) with respect to stereoisomerism. The (-)-forms (2S, 3R) in catechin preparations obtained from green tea amounts to approximately 10% of total catechins, whereas the other 90% are (-)-epi-forms (2R, 3R). High temperature induces the conversion of (-)-epi-forms (2R, 3R) to (-)-forms (2S, 3R). This study investigated the effect of catechin preparations containing 10, 20 and 30% (-)-forms (2S, 3R) on the oxidative and hydrolytic stability of butter. For comparison, butter with δ -tocopherol and BHT and butter without stabilizer were analysed. Butter stability was examined under conditions of refrigeration (8 °C) and freezing (-22 °C) and at temperature of 50 °C and 100 °C. Catechin preparations were more efficient butter stabilizers than BHT and δ -tocopherol. Thermal modification of catechins that led to the generation of 20% of (-) forms (2S, 3R) improved their antioxidative efficacy, but longer treatment leading to the formation of 30% of (-) forms (2S, 3R) decreased their antioxidative activity. The hydrolytic stability of butter, however, increased as the amount of (-) forms (2S, 3R) increased.

Keywords: Oxidative; Hydrolytic Stability; Antioxidants; Catechins

1. INTRODUCTION

In recent years a number of studies have been carried out that have shown a positive effect of green tea on humans, mainly due to polyphenols. Green tea polyphenols have been reported to have antiatherosclerotic [1], anticarcinogenic [2,3] and anti-inflammatory [4,5] effects. Flavan-3-ols (catechins) form the most numerous group of non-fermented green tea polyphenols. The flavan-3-ol

molecule comprises two stereogenic centres situated on the C-ring and constituted by the C2 and C3 carbon atoms. Tea catechins always have an R configuration at the C3 carbon atom. Based on configuration at the C2 carbon atom, catechins can be classified into two groups: (-) epi-forms (2R, 3R) and (-) forms (2S, 3R) [6]. In catechin preparations obtained from tea the main components are (-) epi-forms (2R, 3R): (-) epigallocatechin gallate (EGCG), (-) epicatechin gallate (ECG), and (-) epicatechin (EC). At high temperatures and in anaerobic conditions the epimerization reaction results in more (-) epi-forms (2R, 3R) being transformed to (-) forms (2S, 3R) [7,8]. Catechins that belong to the (-) forms (2S, 3R) include: (-) galocatechin gallate (GCG), (-) catechin gallate (CG), and (-) catechin (C). These compounds efficiently limit the absorption of cholesterol [9,10] and lipid hydrolysis products in the digestive tract [11-14]. Ikeda reported that catechin gallates, being (-) forms (2S, 3R), were most effective in eliminating cholesterol from bile acid micelles [10]. The antioxidative and antiseptic properties of catechins suggest that these compounds could be used as food stabilizers. Moreover, catechin preparations that are rich in (-) forms (2S, 3R) seem to be ideal stabilizers of high-fat foods that are also high in cholesterol. The aim of this study was to determine the influence of catechin preparations that differed in flavan-3-ols content on the oxidative and hydrolytic stability of butter stored at 8 °C and -20 °C or exposed to the temperature of 50 °C and 100 °C.

2. MATERIALS AND METHODS

2.1. Thermal Modification of Catechin Preparations

Catechin solutions (50 mg/ml of redistilled water) were prepared from Sigma Polyphenon 60 and subjected to thermal modification at 140 °C for 40 minutes (preparation HMC1) or 80 minutes (preparation HMC2). Modifications were carried out in Scott tubes under gaseous nitrogen.

2.2. Determination of Chemical Composition of Catechin Preparations

The composition of flavan-3-ols in thermally modified and non-modified (NMC) catechin preparations was determined by the HPLC method as described by Lin [15]. Standards of (-) ECG, (-) EGCG, (-) GCG, (-) CG, (-) C, (-) EC and catechin solutions (diluted 200-fold) were filtered (0.45 μm) and injected (50 μl) into a LUNA 5U C18(2) (250 x 4.6 mm) chromatographic column. The Phase A elution solution comprised methanol, formic acid and redistilled water (20:0.3:79.7 v/v/v) and phase B was composed of methanol and formic acid (99.7:0.3 v/v). Samples were eluted from the column using the following programme: (0—10 min: 100% of phase A; 10—25 min: 100% \rightarrow 90% of phase A, 0% \rightarrow 10% of phase B, linear gradient; 25—60 min: 90% \rightarrow 70% of phase A, 10% \rightarrow 30% of phase B, linear gradient). Liquids were filtered and degassed. The flow rate was 1 ml per minute and the detection wavelength was 280 nm.

2.3. Preparation of Butter Samples

Catechin preparations (NMC, HMC1 or HMC2) were introduced into home-made butter at concentrations of 50, 100, 200 and 400 mg per 100 g of butter. For comparison, samples of butter stabilized with δ -tocopherol and 3,5-di-*tert*-4-butylhydroxytoluene (BHT) in 50 mg per 100 g of butter were used. Butter samples stabilized with catechin preparations of various amounts of different (-) forms (2S, 3R), BHT, δ -tocopherol and a negative control without stabilizer (K₋) were stored at 8 °C for two weeks or at -20 °C for four weeks and then were incubated at 50 °C or 100 °C for one hour. In addition, samples prepared from a stabilizer and fresh butter with no additions served as positive controls (K₊).

2.4. Elucidation of Oxidative and Hydrolytic Stability of Butter

In order to determine butter stability, compounds that reacted with thiobarbituric acid (TBARS) and total acid number (TAN) were analysed. TBARS were detected using the method described by Buege and Aust [16] and were expressed as μmol of malondialdehyde (MDA) released from 100 g of butter. TAN was determined by titration of butter samples with 0.1 M KOH in ethanol and was expressed as mmol of free fatty acids (FFA) per 100 g of butter.

The concentration of FFA was determined by the HPLC method as described by Fliszar [17] and expressed as mmol of FFA per 100 g of butter. Standards of lauric acid, myristic acid, oleic acid, palmitic acid, stearic acid and butter extract were filtered (0.45 μm) and injected (20 μl) into a LUNA 5 μm C18(2) (250 x 4.6 mm) chromatographic column. The column temperature was maintained at 40 °C, the flow rate was 1 ml per minute and

detection was performed at 220 nm. Liquids were filtered and degassed. A gradient of acetonitrile (ACN) and o-phosphoric acid (0.1%) was employed to achieve suitable separation. The linear gradient used was as follows: 30% \rightarrow 75% of ACN from 0 to 10 min; 75% \rightarrow 80% of ACN from 10 to 20 min; 88% \rightarrow 99% of ACN from 20 to 22 min; then held for 23 min. An equilibration time of 5 min was employed between injections.

2.5. Statistical Analysis

Data were analysed using Statgraphics Plus for Windows. Results were compared with multifactor ANOVA and significant differences were determined using the LSD test at $p < 0.05$.

3. RESULTS AND DISCUSSIONS

3.1. Thermal Modification of Catechin Preparations

The differences in total catechin content among the HMC1, HMC2 and NMC preparations were not significant (**Table 1**). In the NMC preparation (-) form catechins (2S, 3R) constituted approximately 10% of the total catechins. As a consequence of thermal modification of the preparation the amount of (-) forms (2S, 3R) in HMC1 and HMC2 increased to 20 and 30% of total catechins, respectively. It is known that high temperatures cause γ -pyran ring opening, which enables rotation of the group situated at position C2 of the ring and a change in its location in relation to the plane of the whole molecule. The (-) forms (2S, 3R) in which the groups at the asymmetric carbon atoms are in trans orientation to each other are more stable than their (-) epimers (2R, 3R) [7]. The rate of epimerization depends not only on the temperature and on the time of exposure but also on the presence of metal ions and the pH of the solution [8,18].

3.2. Oxidative Stability of Butter Enriched with Catechins

During storage under refrigeration (8 °C) all catechin preparations inhibited butter oxidation to an extent that did not differ from the positive control (**Table 2**). δ -Tocopherol and BHT, however, were less effective stabilizers, although the amounts of MDA assayed in butter samples stabilized with these agents were 39.2% and 88% lower, respectively, in comparison to samples without stabilizer stored at 8 °C for 14 days (negative control, K₋).

The amount of MDA assayed in butter samples stored at -20 °C for four weeks corresponded with the amount of MDA in the positive control for samples stabilized with the NMC and HMC1 preparations. On the other hand, the amount of MDA in samples stabilized with the HMC2 preparation, δ -tocopherol and BHT did not differ from the

Table 1. The percentage of catechins ((-) EGCG, (-) ECG, (-) EC, (-) GCG, (-) CG, (-) C) and GA gallic acid) in a dry mass of thermally modified (HMC1, HMC2) and non-modified (NMC) catechin preparations. Different superscripts within a column indicate significant differences between catechin preparations (at $p < 0.05$). All analyses were carried out in quadruplicate.

Catechin preparations	(-) epiform (2R, 3R) [% d.m.]				(-) form (2S, 3R) [% d.m.]			Total catechins
	GA	(-) EGCG	(-) ECG	(-) EC	(-) GCG	(-) CG	(-) C	
NMC	0.24 ^a	49.88 ^c	11.30 ^b	7.02 ^b	4.20 ^a	1.63 ^a	1.64 ^a	75.92 ^a
HMC1	0.35 ^b	45.10 ^b	9.78 ^a	5.93 ^a	8.53 ^b	3.03 ^b	3.10 ^b	75.91 ^a
HMC2	0.47 ^c	36.04 ^a	9.62 ^a	5.42 ^a	16.04 ^c	3.95 ^c	4.48 ^c	76.03 ^a

Table 2. Concentrations of MDA assayed in 100 g of butter. All stabilizers were added at doses of 50 mg per 100 g of butter. Different superscripts within a row indicate significant differences between samples (at $p < 0.05$). All analyses were carried out in quadruplicate. HMC1, HMC2 = thermally modified catechin preparations. NMC = non-modified catechin preparation. K₊ denotes the amount of MDA in fresh butter.

Thermal treatment	MDA $\mu\text{mol}/100\text{ g butter}$						
	Butter stabilizers				Butter without stabilizer		
	NMC	HMC1	HMC2	δ -Tocopherol	BHT	K ₊	K ₋
Refrigeration 8 °C, 2 weeks	0.84 ^a	0.85 ^a	0.86 ^a	3.66 ^c	1.34 ^b	0.77 ^a	5.52 ^d
Freezing -20 °C, 4 weeks	0.82 ^a	0.77 ^a	1.12 ^b	1.19 ^b	1.21 ^b	0.77 ^a	1.20 ^b
Heating 50 °C, 1 h	1.05 ^{cd}	0.85 ^a	0.96 ^b	1.11 ^d	1.02 ^{bc}	0.77 ^a	1.30 ^c
Heating 100 °C, 1 h	1.08 ^b	1.18 ^b	1.20 ^b	1.62 ^c	1.25 ^b	0.77 ^a	1.67 ^c

Table 3. Concentrations of FFA assayed in 100 g of butter. All stabilizers were added at doses of 50 mg per 100 g of butter. Different superscripts within a row indicate significant differences between samples (at $p < 0.05$). Mean values from four independently performed experiments. HMC1, HMC2 = thermally modified catechin preparations. NMC = non-modified catechin preparation. K₊ denotes the amount of MDA in fresh butter.

Thermal treatment	FFA $\text{mmol}/100\text{ g butter}$						
	Butter stabilizers				Butter without stabilizer		
	NMC	HMC1	HMC2	δ -Tocopherol	BHT	K ₊	K ₋
Refrigeration 8 °C, 2 weeks	14.17 ^{cd}	13.72 ^{bc}	13.58 ^b	14.85 ^c	15.00 ^c	4.19 ^a	14.67 ^{dc}
Freezing -20 °C, 4 weeks	4.15 ^a	4.51 ^a	4.42 ^a	4.46 ^a	4.17 ^a	4.19 ^a	4.15 ^a
Heating 50 °C, 1 h	4.24 ^a	4.30 ^a	4.42 ^a	4.28 ^a	4.21 ^a	4.19 ^a	4.38 ^a
Heating 100 °C, 1 h	4.33 ^a	4.35 ^a	4.28 ^a	4.15 ^a	4.24 ^a	4.19 ^a	4.42 ^a

negative control.

Butter samples stabilized with the HMC1 preparation incubated at 50 °C did not show any changes in the amounts of TBARS compared to the fresh butter (K₊). In the conditions of the assay only the HMC1 preparation effectively stopped the oxidation process. On the other hand, δ -tocopherol was the least efficient butter stabilizer (35.9% less MDA generated during incubation than in the negative control K₋). Analysis of the results obtained for

samples incubated at 100 °C did not demonstrate any differences between the extent of oxidation of butter stabilized with catechin preparations of various amounts of (-) forms (2S, 3R) and BHT. These stabilizers limited the amounts of oxidation products emerging during heating at 100 °C by 46.7 up to 65.6% compared to the negative control. As in the case of incubation at 50 °C, δ -tocopherol was the least efficient stabilizer because no differences were observed between butter stabilized with

δ -tocopherol and the negative control. Neither of the preparations used at a rate of 50 mg per 100 g of butter stopped the oxidation of butter heated at 100 °C. In order to establish whether any of the catechin preparations were capable of stopping the butter oxidation process at the level corresponding to the positive control at 100 °C, higher doses of preparations were used (**Figure 1**).

At doses of up to 100 mg of catechins per 100 g of butter, the efficacies of all preparations tested were similar. For doses higher than 100 mg of catechins per 100 g of butter, the HMC1 preparation containing 20% of (-) forms (2S, 3R) proved to be the most effective stabilizer. HMC1 stopped the oxidation process at the level corresponding to the positive control at a dose of 400 mg per 100 g of butter. The least effective was the HMC2 preparation with the highest proportion of (-) forms (2S, 3R). Increasing the concentration from 200 to 400 mg per 100 g of butter did not decrease the rate of oxidation during a one-hour incubation of butter at 100 °C.

Experiments determining the efficacy of non-modified green tea catechins as stabilizers in poultry meat [19], beef [20,21], pork [22], fish [21] and vegetable oils [23] have been reported. O'Sullivan and co-workers [19] showed that catechins increase the durability of meat under conditions of refrigeration through inhibition of the oxidation process, but not as efficiently as BHT. However, Chen [23] showed that catechins stopped oxidation more efficiently than BHT in oil heated to 95 °C. These results suggest that the (-) forms (2S, 3R) that emerge following heating demonstrate higher antioxidative activity than the stereoisomers classified as (-) epiforms (2S, 2R). Xu and co-workers [24] demonstrated, however, that the highest difference, in favour of epiforms (2S, 2R), occurs between (-) EGC and (-) GC whilst the other pairs of stereoisomers (-) EGCG \rightarrow (-) GCG; (-) EC \rightarrow (-) C; (-)ECG \rightarrow (-)CG had a similar antioxidative activity. In our study the highest antioxidative activity in the HMC1 preparation, in which the proportion of stereoisomers classified as (-) forms (2S, 3R) amounted to 20%, was probably due to the synergistic effect of both catechin stereoisomers. A decrease in the antioxidative activity of the HMC2 preparation (containing 30% of (-) forms (2S, 3R)) in samples exposed to 50 and 100 °C was most probably due to further conversions of the (-) forms (2S, 3R) to polymeric structures typical for fermented teas.

3.3. Hydrolytic Stability of Butter Enriched with Catechins

Incubation of butter samples for one hour at 50 °C and 100 °C and further storage at -20 °C for four weeks did not increase the amount of FFA (**Table 3**).

Alterations in the amounts of FFA were observed only for butter samples stored at 8 °C for 14 days. When stabilizers were used at a level of 50 mg per 100 g of butter, a decrease in the amounts of free fatty acids released as

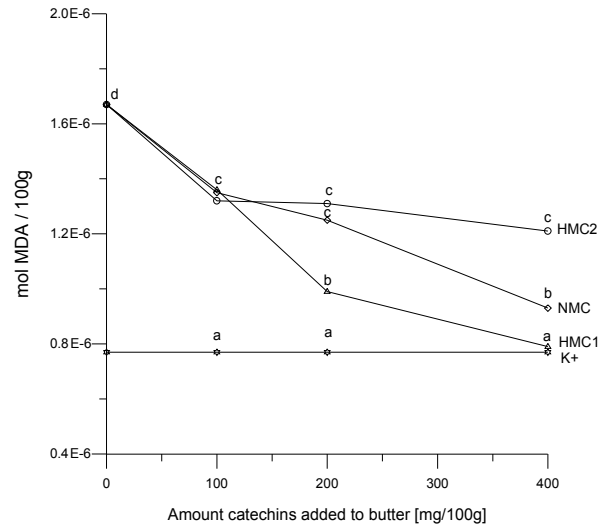


Figure 1. The influence of the concentration of catechin preparations (NMC, HMC1, HMC2) added to 100 g of butter on the amount of dimalonic aldehyde released during a one-hour incubation at 100 °C. Individual letters denote statistically significant differences (at $p < 0.05$). All analyses were carried out in quadruplicate. K₊ denotes the amount of MDA in fresh butter.

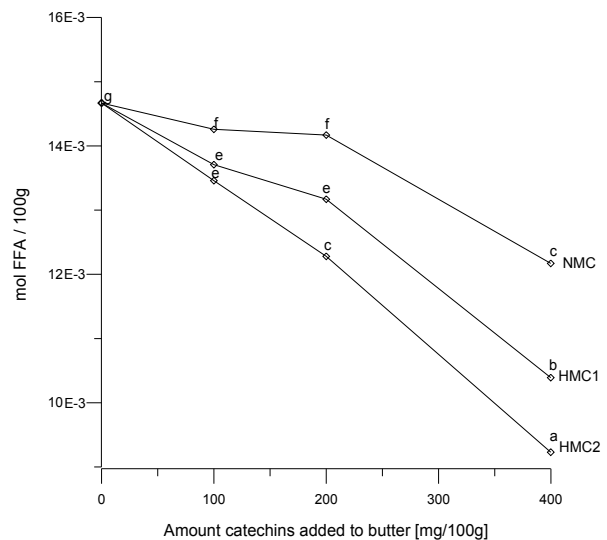


Figure 2. The influence of the concentration of catechin preparations (NMC, HMC1, HMC2) added to 100 g of butter on the amount of free fatty acids (FFA) released during two weeks storage at 8 °C. Individual letters denote statistically significant differences (at $p < 0.05$). All analyses were carried out in quadruplicate.

compared to the negative control was observed only with thermally modified catechins. The other compounds did not influence the hydrolytic stability of butter. However, the amount of fatty acids was lower by only 13.3% for the HMC2 catechin preparation and by 10.9% for the HMC1 preparation in comparison to the negative control. In order to improve the hydrolytic stability of butter under refrigeration conditions (8 °C) the amounts of cate-

chins added were increased (**Figure 2**).

With increasing dose and an increased proportion of catechins classified as (-) forms (2S, 3R), the amount of free fatty acids decreased during storage at 8 °C. For the HMC2 and HMC1 preparations at the highest dose the amounts of FFA were lower by 52.1% and 40.6% respectively, compared to the negative control. Inhibition of the hydrolysis of butter lipids is most probably a secondary effect caused by suppression of microbial growth. A number of data in the literature confirm the antiseptic activity of polyphenols, including catechins [25,26].

In conclusion, catechin preparations were more efficient butter stabilizers than BHT and δ -tocopherol. Thermal modification of the catechin preparation that led to the formation of 20% (-) forms (2S, 3R) improved its antioxidative efficacy; however, further increases in (-) form catechins (2S, 3R) to 30% of these molecules led to a decrease in the antioxidative stability of butter. The hydrolytic stability of butter, on the other hand, increased as the concentration of catechins classified as (-) forms (2S, 3R) increased.

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