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The effect of milk alpha-casein on the antioxidant activity of tea polyphenols

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ABSTRACT

In this study, we report how the antioxidant capacities of major tea polyphenols are affected by their interactions with milk alpha-casein (milk protein) using three complimentary oxidation methods: $ABTS^{\cdot+}$ radical cation scavenging, cyclic voltammetry and lipid peroxidation inhibition. We found that using the $ABTS^{\cdot+}$ assays, the antioxidant activity of all polyphenols was lowered by 11–27% in the presence of caseins. Using cyclic voltammetry, the overall current measured at the electrode was decreased by the presence of the protein, from 21% to 61%. The peak potentials were also shifted to higher values varying from 13 to 41 mV. However, using lipid peroxidation method, we noticed of the antioxidant activity of all the polyphenols changed (from 6% up to 75%) after the addition of alpha-casein. The results show using this method the larger gallate esters containing polyphenols epicatechingallate (ECG) and (epigallocatechingallate (EGCG) were less affected by the presence of casein than smaller polyphenols catechins (C), epicatechin (EC) and epicgallocatechine (EGC). Alpha-casein caused a small effect on the chain breaking antioxidant capacity of theaflavins as well. Therefore, casein has different effects on the overall antioxidant capacities of tea compounds depending on the methods used. We aim to understand those results with the types of protein–polyphenol interactions that take place in various settings and their effects on the antioxidant capacities of those compounds.

1. Introduction

Naturally occurring polyphenols are found in a wide variety of dietary sources such as tea, the second most consumed beverage in the world after water, and red wine. Tea and red wine show high levels of polyphenol content. For comparison, polyphenol concentrations found in green teas ranges from 800 to 2400 mg/L [1] and varies between 1000 and 4000 mg/L in red wines [2,3].

The main polyphenols found in teas are catechins and flavanoids with a hydroxyl group at position 3 (flavan-3-ols) [4] (Scheme 1). Flavan-3-ols are potent antioxidants found in plants and many of them show better reducing abilities than vitamin C on a molar basis [5,6] with major health benefit. The chemical structures of tea polyphenols are very similar. The skeleton consists of two phenyl rings linked by to a pyran cycle (C_6–C_3–C_6). The number and positions of hydroxyl groups on the aromatic rings determine the polyphenol's antioxidant capacity. Past studies on green tea show that epigallocatechin gallate (EGCG), the gallic acid ester of epicatechin, is the most abundant catechin [4], with the highest intrinsic antioxidant capacity as determined by cyclic voltammetry [7].

Free radicals are viewed as the major cause of several chronic and degenerative diseases (Alzheimer's, arteriosclerosis, diabetes, hypertension, Parkinson's) and other health disorders (coronary heart diseases, cancer, allergies) [8]. There is strong evidence that daily intake of fruits rich in polyphenols reduces the incidence frequency of diseases [9] due to their antioxidant, anti-inflammatory, antithrombogenic, and vasodilatating effects [10]. Thus, polyphenols are now viewed as essential in human health for lowering the detrimental effects of reactive free radicals at a cellular level [11].

However, the antioxidant activity of polyphenols may be affected when adding milk to your tea. For instance, previous studies showed that the addition of milk to tea lowers the free radical scavenging ability of its antioxidant components [7,12] and [12]. Furthermore, clinical studies showed that milk inhibits bioavailability of cocoa polyphenols, thus reducing the beneficial effects of those antioxidants [13,14]. However, similar experiments showed that milk did not have any significant effect on the bioavailability and antioxidant capacities of tea polyphenols [15]. The above inconsistencies concerning the effect of milk on the antioxidant activity of tea compounds prompted us to design the present investigation monitoring the effect of milk casein on the antioxidant capacity of tea polyphenols.

The interaction of catechins with milk was shown to depend on milk proteins rather than its lipid fraction [7]. Caseins, the most

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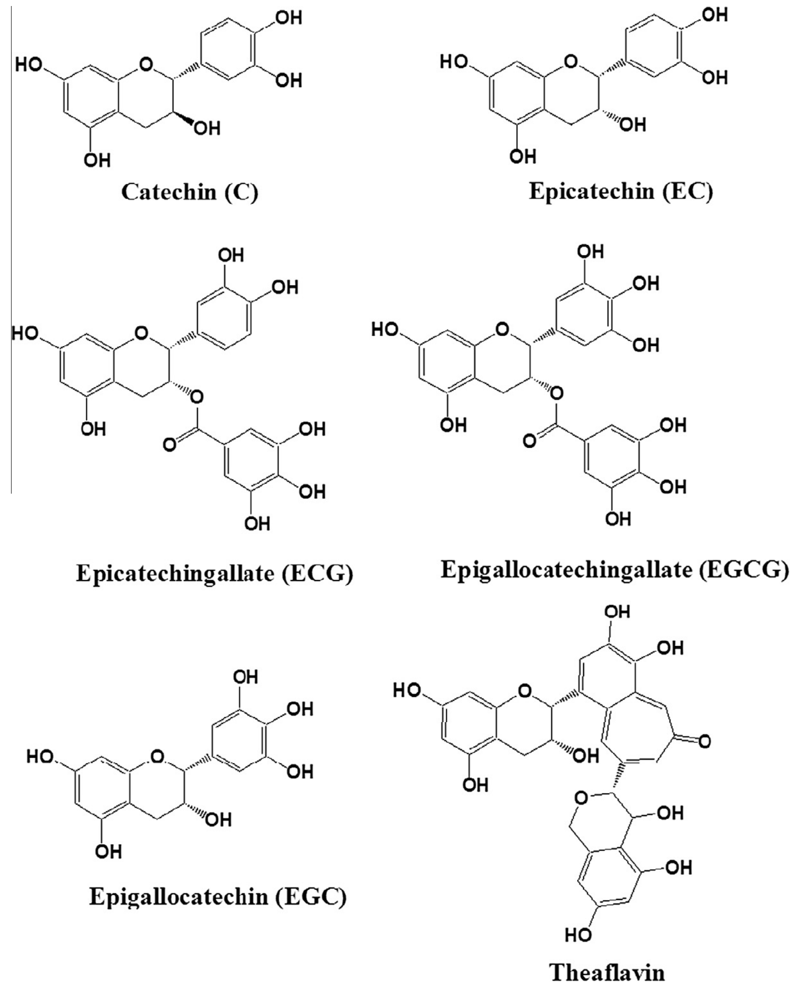

Scheme 1. Chemical structures of polyphenols.

abundant proteins in milk, form colloidal complexes that function as carriers for the delivery of proteins, calcium, potassium and phosphorus to newborns [16,17]. Those proteins are found in three different forms: alpha, beta, and κ. Alpha and beta caseins have similar molecular weight (24 kDa) [18,19] and contain high levels of proline residues. Such proteins are reported to have a greater binding affinity with polyphenols [20]. The binding affinity of polyphenols increases with the molecular weight and the number of hydrophilic hydroxyl groups [21]. Larger polyphenols like those found in black teas (theaflavins, a type of polymeric polyphenol produced during the enzymatic oxidation of tea leaves) are reported to be more likely to interact with caseins. This binding can affect the electron donation capacity of the catechins by reducing the number of hydroxyl groups available for oxidation in the media.

Recent spectroscopic studies from our laboratory showed strong polyphenol–casein interaction, which induced major protein secondary structural changes in the presence of polyphenols [22]. The changes observed on both protein conformation and polyphenol structure showed casein–polyphenol complexation leads to the alterations of protein structure and the antioxidant activity of polyphenols in aqueous solution [22].

Here, we report the antioxidant activity of tea catechins with and without alpha-casein, using three different oxidation methods: $ABTS^{\cdot+}$ radical cation scavenging, lipid peroxidation and cyclic voltammetry. The antioxidant capacities measured by these methods were compared and the mechanism by which the antioxidant capacity of tea polyphenols affected by milk alpha-casein is reported here.

2. Materials and methods

2.1. Materials

All polyphenols (catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate and theaflavin black tea extracts) (Scheme 1), alpha-casein (80% purity) was purchased from Sigma–Aldrich and used without further purification. ABTS, potassium persulfate, AAPH, linoleic acid and PBS were also from Sigma–Aldrich and used as supplied.

2.2. $ABTS^{+}$ scavenging radical capacity

An aqueous solution of stable $ABTS^{\cdot+}$ radical cation was prepared and left in the dark, overnight, for 16 h before use according to published method [23] with minor modifications. Briefly, (0.0132 ± 0.0001 g; 48.8 μmol) of potassium persulfate was added to 2,2′-azinobis(3-ethylbenzothiaaoline-6-sulfonate) diammonium salt (0.0768 ± 0.0001 g; 140 μmol) in 20 mL of distilled water to form a dark blue solution. For the test, 2 mL of the ABTS solution was diluted into 150 mL of distilled water to obtain stable initial absorbance of 0.700 ± 0.010 at 734 nm, yielding the same working concentration as reported [23].