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# Oxidative stability and rheological properties of nanoemulsions with ultrasonic extracted green tea infusion

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ABSTRACT

Epigallocatechin-3-gallate (EGCG) is considered the most significant tea catechin because it has the highest free radical scavenging activity and because of its role in preventing carcinogenesis. Thus, adding green tea infusion to food products imparts a safe, natural antioxidant that prevents rancidity and promotes good health. Even if mild technologies could be developed to optimize the extraction of EGCG from green tea, questions remain regarding the best means of delivering EGCG through food and its disposition in the body after ingestion. Recent reports indicate that the bioavailability of EGCG is very poor due to its large molecular size and number of hydrogen bonds. The present study demonstrates the use of ultrasound to extract catechins from green tea leaves with improved EGCG yield, and subsequent preparation of water-in-oil (W/O) green tea nanoemulsions with soy, peanut, sunflower, and corn oils. The green tea/peanut oil emulsion displayed the highest oxidative stability. All W/O emulsions examined demonstrated a shear thinning behavior in good agreement with the Carreau model ($R^2 = 0.980 \pm 0.033$). Values for shear viscosity at a shear rate of 10 $s^{-1}$ were found to be compatible with the semi-empirical equation of Larson and McClements, with an effective volume fraction slightly higher than the actual volume fraction and still increasing with homogenization time. Moreover, the specific surface area of the nanoemulsions was very high and with an average value of about 40 $m^2$/mL.



## 1. Introduction

Green tea (GT) is generally recognized as an important source of bioactive compounds, prompting the claim that green tea promotes good health (Zaveri, 2006). From a technological aspect, GT extract represents a cheap, natural, food-grade additive that inhibits the growth of foodborne pathogens and lipid oxidation, thereby increasing the shelf life of foods. Consequently, due to its strong antioxidant properties and generally recognized as safe (GRAS) status, researchers have proposed including green tea as an ingredient in foods as diverse as biscuits, cereals, cakes, dairy products, meats, fish, and fried snacks (Perumalla & Hettiarachchy, 2011; Sharma & Zhou, 2011).

Among flavonols of the catechin type, the well-studied epigallocatechin-3-gallate (EGCG) is considered the most significant tea catechin because it has the highest free radical scavenging activity and because it plays a role in preventing carcinogenesis (Peng, Wargovich, & Dixon, 2006). Even though differences in processing technologies can impact extraction of EGCG, it is clear that the chemical composition of green tea leaves, and thus that of GT infusions, is affected by other factors, including tea variety and crop and environmental conditions.

Extraction by heating or boiling the leaves is the traditional first step in recovering water-soluble compounds such as EGCG from tea, but many researchers have reported that the increase in temperature during boiling leads to epimerization of tea catechins. During traditional tea brewing, EGCG can change to the corresponding isomers GCG or gallocatechin gallate (Ananingsih, Sharma, & Zhou, 2011). The use of procedures in which extraction can be carried out at lower temperatures could avoid these reactions and thus improve EGCG yield. A simple and efficient alternative method may be the use of ultrasound, as reported by Xia, Shi, and Wan (2006), who investigated the effect of ultrasonic-assisted extraction on the chemical and sensory quality of tea infusions. Using this technique, the authors reported increases in the concentration of polyphenols, probably due to disruption of plant cell walls by ultrasonically induced cavitation. Wijngaard, Hossain, Rai, and Brunton (2012) reported similar observations in a study involving ultrasound-assisted extraction to improve recovery of polyphenols from plant by-products. However, even though mild ultrasound-based technologies are useful for optimizing the extraction of bioactive compounds from tea, questions remain regarding the best means of delivering these compounds through food products and their disposition in the body following ingestion.

Several authors have recently reported that the bioavailability of epigallocatechin-3-gallate is very poor due to its large molecular size and large number of hydrogen bonds. To overcome problems

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with bioavailability, liposomes, oil-in-water (O/W) emulsions, and nanocapsules have been proposed as safe alternative delivery systems for improving the efficacy of green tea (Kim et al., 2012; Ru, Yu, & Huang, 2010). Nanoemulsions characterized by higher surface area-to-mass ratios may be a promising tool for delivering bioactive compounds such as those found in green tea in a basic functional margarine. Mason, Wilking, Meleson, Chang, and Graves (2006) suggested that water-in-oil (W/O) nanoemulsions are typically easier to create than O/W emulsions, even though the use of vegetable oils, which addresses the need to reduce dietary ingestion of saturated fats (Iqbal, Hameed, Baloch, & McClements, 2012; Nehir El & Simsek, 2012) might enhance the already high susceptibility of O/W emulsions to oxidation (Di Mattia, Sacchetti, Mastrocola, & Pittia, 2009; Poyato et al., 2012). Thus, the use of nanoemulsions containing GT infusions can be used to impart foods with natural antioxidants to preventing rancidity and to deliver functional ingredients with higher bioavailability. The present study demonstrates the possibility of extracting catechins from green tea leaves with improved EGCG yield through the use of ultrasound and the creation of W/O green tea nanoemulsions using soy, peanut, sunflower, and corn oils. The oxidative stability and rheological properties of these nanoemulsions were also determined.

## 2. Materials and methods

### 2.1. Preparation of green tea infusions

Based upon comparisons of the results of different trials, GT infusions were prepared by sonicating 1 g of dried tea leaves (New Foods, Verona, Italy) in 40 mL of distilled water at 60 °C for 15 min using a Bandelin Ultrasonic Sonopuls GM 200 sonicator (Germany, microtips MS 72). Infusions were centrifuged at 5000 rpm for 15 min and filtered using 0.45-μm filter membranes (Millipore, MA, USA). As control, a conventional extraction using boiling distilled water (95 °C) was carried out under the same conditions.

### 2.2. HPLC analysis of green tea infusions

Catechins extracted from tea leaves were identified and quantified using a Thermo Finnigan Spectra System UV6000LP HPLC (Thermo Finnigan, San Jose, CA, USA) equipped with a diode array detector and Supelcosil™ LC-18 column (Sigma-Aldrich, St. Louis, MO, USA). The column temperature was maintained at 40 °C and the detector was set to a wavelength range of 200–600 nm. The analysis time course was 100 min. The mobile phase, which consisted of a mixture of 100% methanol and double-distilled water acidified with sulphuric acid (pH 2.5), was programmed as described by Zocca, Lomolino, and Lante (2011). Chemicals used as standards were purchased from Sigma-Aldrich. All experiments were performed in triplicate.

### 2.3. Preparation of water-in-oil emulsions

To create emulsions like margarine, using edible oils and green tea infusion, water-in-oil emulsions were composed of GT infusion (19.2 wt.%, pH 5.4), commercial soy lecithin (0.04 wt.%) (Lecinova, Nutrition & Santè, S.p.a., Italia) previously dissolved in 3 g (3.9 wt.%) of distilled water using an ultrasonic bath, and 76.9 wt.% corn, peanut, soybean, or sunflower oil (purchased from a local supermarket). In brief, the aqueous (23.1 wt.%) and oil (76.9 wt.%) phases were combined and homogenized in an ice bath using an Ultra Turrax T-25 high-speed homogenizer (Janke and Kunkel, Germany) operated at 12,000 rpm for 180, 300, 600 or 900 s for preparation of corn, peanut, soybean, or sunflower oil emulsions, respectively. The final W/O emulsions were withdrawn from their respective containers to evaluate droplet size, oxidative stability, and rheological characteristics.

**Table 1**
Proportion of major catechins in ultrasound green tea (GT) infusion, as determined by HPLC analysis.

| Compound | Concentration (%) |
|---|---|
| −Epigallocatechin (EGC) | 8.41 |
| +Catechin (C) | 1.60 |
| −Epigallocatechin-3-gallate (EGCG) | 85.27 |
| −Epicatechin (EC) | 1.07 |
| −Epicatechin-3-gallate (ECG) | 3.65 |

### 2.4. Determination of droplet size

The size of emulsion droplets was determined by dynamic light scattering using a Malvern Zetasizer Nano S. particle size analyzer (Malvern, Worcestershire, UK) equipped with a 4 mW He–Ne laser and operated at 633 nm. Signals were acquired using a high sensitivity avalanche photodiode detector. All measurements were made at a fixed scattering angle of 173° and a temperature of 25 ± 0.1 °C. Droplet size was estimated based upon the average of three measurements and presented as the mean diameter of the number-based particle size distribution.

### 2.5. Oxidative stability

Oxidative stability was assessed using a Rancimat apparatus (Metrohm, model 743, Herisau, Switzerland), which conductometrically measures the formation of volatile acids produced by free radical chain reactions. Each determination was made at 110 °C with a 20 L $h^{-1}$ air flow, using 3 g of corn, peanut, soybean, or sunflower GT W/O emulsion prepared with 60 mL of distilled water. Water-in-oil emulsions without GT infusion were used as controls. Volatile decomposition products were detected using a conductivity cell. The induction time for each model system was determined in triplicate analyses. The antioxidant activity index was calculated from the measured induction times as described by Lante, Nardi, Zocca, Giacomini, and Corich (2011).

### 2.6. Rheological measurements

Rheological characteristics were assessed using a viscometer (HAAKE Viscotester VT550) equipped with a sensor system consisting of coaxial cylinders (MV3). The radius and height of the inner cylinder were 15.2 and 60.0 mm, respectively, while the outer cylinder radius was 21.0 mm. Measurement of shear stress vs. shear rate was performed at 25 °C by varying the shear rate over the range 1–100 $s^{-1}$. All experiments were performed in triplicate and data are reported as the mean and standard deviation.

**Table 2**
Oxidative stability of GT water-in-oil emulsions, as determined using the Rancimat® test.

| Sample | IT (h) | AAI |
|---|---|---|
| GT corn oil | 12.52 ± 0.28[b] | 1.42 |
| Corn oil | 8.58 ± 0.43 | |
| GT peanut oil | 14.46 ± 0.38[a] | 1.71 |
| Peanut oil | 8.52 ± 0.6 | |
| GT soybean oil | 9.59 ± 0.33[c] | 1.50 |
| Soybean oil | 6.38 ± 0.32 | |
| GT sunflower oil | 6.23 ± 0.18[d] | 1.30 |
| Sunflower oil | 4.74 ± 0.45 | |

a, b, c, d: $P < 0.05$.
IT = induction time, which refers to the time (h) at the break point of the two extrapolated straight portions of the curve obtained using the Rancimat® apparatus.
AAI = antioxidant activity index. Calculated as the IT of W/O emulsions with green tea infusions/IT of W/O emulsions without green tea infusions.
Time of homogenization of W/O emulsions was 300 s.