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QUANTITATIVE ANALYSIS OF BIOACTIVE COMPOUNDS IN FIVE BLACK TEA SORTIMENTS OF THE ROMANIAN MARKET

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Abstract

Five black tea types, Assam, Darjeeling, Earl Grey, English Breakfast and Oolong were tested for their chlorophyll, carotenoid, flavonoid, total phenolic content and essential oil content. English Breakfast had the highest chlorophyll content (834 mg/kg) and Earl Grey had the highest carotenoid content (360 mg/kg). Total phenolic content was the highest in Darjeeling (an average of 40,874 mg/kg), while flavonoids, specifically, reached the highest concentrations in Oolong (498 mg/kg). Total essential oils were maximum in Assam tea blend (4,600 mg/kg). Tea pH values ranged between 6.13-6.66.

Introduction

Tea is one of the most popular beverages sold worldwide, being the second most consumed beverage after bottled water. The total amount produced and consumed yearly surpasses 5 million tonnes (FAO 2015). Other applications include cold drinks, various types of foods and desserts.

The raw materials for producing proper teas are the dried leaves of the shrub *Camellia sinensis* (L.) Kuntze (Fam. Theaceae). Depending on the processing method used, and plant variety involved, there are several types of tea. The main way to distinguish teas is by their degree of oxidation. There are white, yellow, green (minimally oxidized), black (fully oxidized) and Oolong (intermediary) teas. Besides this, teas can be flavoured with a wide variety of aromatic plants or dry fruits. Black teas are extremely popular, due to their energizing properties and strong flavour. It is widely consumed especially in South Asia, the Middle East and Eastern Europe, but also in other parts of the world. For producing black tea, green *C. sinensis* leaves are withered, macerated, aerated, shaped and then dried and graded. Oxidation of leaf tissues is complete, until a dark color is obtained. In Oolong tea, the process is similar, but the oxidation is stopped by heating, at an interval longer that that needed for green tea prduction. As a result, the degree of oxidation of leaf tissues is intermediate (Kamunya *et al*. 2019). Tea is known for contianing large amounts of varous types of of bioactive compounds.

Chlorophylls (of which land plants contain clorophylls a and b) are the main photosynthetic pigments. For consumers, they have anti-inflammatory properties and help wound healing. They are also known to lower the alimentary uptake of some known carcinogenic substances, while also inhibiting the accumulation of calcium oxalate dihydrate (the precursor of kidney stones). Furthermore, clorophylls are among the most important antioxidants found in food, helping prevent oxidative stress-associated diseases, cardiovascular affections and even cancer (Inanç 2011).

Carotenoids (which include compounds such as carotenes, lutein, lycopene and zeaxanthin) are accessory photosynthetic pigments found in all plants (especially in leaves, flowers and fruits). For human consumers, they play an important role by being the raw material for the biosynthesis

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of retinol and melanin, thus essential to eye and skin health. They are also known to be have significant effective antioxidant and antiproliferative properties (Eldahshan and Singab 2013).

Phenolic compounds are a wide variety of chemicals compounds, including flavonoids, tannins, phenolic acids etc., which work as plant pigments or as antimicrobial and antifungal protective agents (Kivrak and Kivrak 2014). For human diet and health, they are important as the main class of antioxidant compounds, radical scavengers, lipid oxidation inhibitors and reducing agents (Zymonė *et al.* 2018).

Among phenolic compounds, flavonoids (low-mass polyphenolic compounds) are some of the most valuable, having strong antioxidant, antibacterial, antifungal, antiviral, antiinflammatory and antiproliferative properties (Kivrak and Kivrak 2014).

Volatile essential oils are aromatic mixtures of polyphenols (monoterpenes, sesquiterpenes, flavonoids), hydrocarbons and derivatives, alcohols, aliphatic aldehydes, esters, etc., of which more than 90% are volatile. They are responsible for giving the specific flavour to vegetable foods and beverages, while they also have antioxidant and antibacterial properties (Orphanides *et al.* 2011).

The objective of this paper was to comparatively assess the content of the above-mentioned compound classes in some common black tea sortiments available to Romanian consumers.

Materials and Methods

Five black tea sortiments were acquired from local vendors, in the city of Constanța, Romania.

Assam tea groups several varieties growing in the Assam State (India), a hot and humid highland region that favors the development of a malty flavor. The production process involves crushing, tearing and curling of tea leaves.

Darjeeling teas come from plants grown in the northern, colder, Himalayan areas of West Bengal State, India. Darjeeling tea has a more astringent taste and it is appreciated as a high-quality tea.

Earl Grey is a traditional British type of tea, usually produced from Sri Lankan cultivars and flavored with bergamot oil.

English Breakfast is one of the so-called breakfast blends, a mixture of various South Asian and East African cultivars, with a strong and bitter taste, usually consumed with milk and/or sugar.

Oolong (Wu Long) tea originates from the southern Chinese provinces of Guangdong and Fujian. The main characteristic of this tea type is the intermediate degree of oxidation (varying between 10-85%) (Lachman *et al.* 2003).

Chlorophyll a and b and total carotenoid contents were determined by extracting tea leaf tissue in 80% acetone (80%), filtering and reading spectrophotometric absorption (S106 WPA spectrophotometer) at 663, 647, 470 nm (Popoviciu *et al.* 2020). Compound content was calculated according to Lichtenthaler and Buschmann (2001).

Flavonoid contents were determined by extraction in water : methanol (4 : 8) mixture and reading spectrophotometric absorption at 340 nm wavelength (Szabo *et al.* 2012, Popoviciu *et al.* 2020).

The content of total phenolic compounds was determined by using a spectrophotometric version of the Folin-Ciocâlteu method. Leaf tissue was extracted with methanol and incubated for 30 minutes with Folin-Ciocâlteu reagent (10%) and sodium bicarbonate (7.5%). Spectrophotometric absorbance was read at 765 nm. A calibration curve was prepared, using standard gallic acid concentrations (Stanković 2011, Siddiqui *et al.* 2017, Popoviciu *et al.* 2020).

Essential oils were determined by gravimetric means. Ground tea tissue was extracted in petroleum ether (25 g solvent per 5 g tissue). Extracts were evaporated at 35°C and the residue was weighed (Orphanides *et al.* 2011).

Tea pH was determined by hot brewing of 1.5 g tea in 120 mL boiling water for 3 minutes (Shrestha *et al.* 2010). The pH values were determined by using a HI98103 pH tester (Hanna Instruments).

Triplicate samples were performed for each analysis.

Results and Discussions

Among the five sortiments, English Breakfast had the highest content of chlorophylls – 834 mg/kg – followed by Earl Grey. The lowest amounts were found in Darjeeling (228 mg/kg). Between Chlorophylls a and b, chlorophyll b was dominant in all teas, with a ratio of 1.45-1.01 : 1 (Fig. 1). Chlorophylls are considered to be among the main indicators of tea quality. However, they are known to undergo major decreases due to prolonged storage (after 2-5 months), depending on sortiment (Ošťádalová *et al.* 2014).

Carotenoids had their maximum concentrations in Earl Grey (360 mg/kg) and the minimum ones in Darjeeling (56 mg/kg) (Fig. 2). Carotenoids in teas are a less studied aspect – a research conducted on 31 tea cultivars in China found 324.80-528.80 mg/kg total carotenoids. However, it should be noted that this study involved fresh leaves and not processed tea (Wei *et al.* 2016).

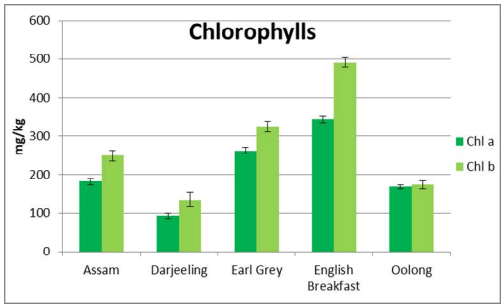

Fig. 1. Concentrations of chlorophylls a and b in selected black tea types (average values; mg/kg).

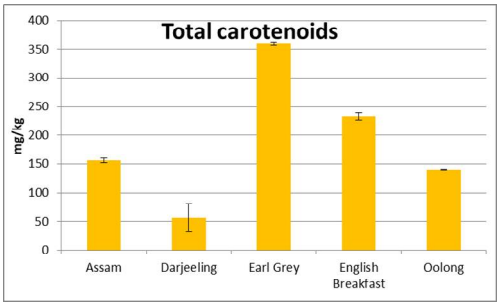

Fig. 2. Concentrations of total carotenoid pigments in selected black tea types (average values; mg/kg).

Total phenolic contents (TPC) were highly variable, ranging from 40,874 to 14,733 mg/kg GAE (Fig. 3). Tea is a well-known source of phenolic and polyphenolic compounds, which can amount up to 30% of the leaf weight (Blumberg *et al.* 2015). The exact amount is highly variable and depends on tea type, cultivar, processing and storage etc. The main factors influencing the resulting phenolic content are leaf age (with young leaves usually having significantly higher values) and extraction time, which also operates during normal tea brewing (Chan *et al.* 2007, Lachman *et al.* 2003). Usually, green teas tend to yield higher amounts of phenolics, as shown by scientific literature (Lachman *et al.* 2003, Unachukwu *et al.* 2010), including a survey on some common sortiments available in Romanian commerce, that found 31,390-56,205 mg/kg GAE (Popoviciu *et al.* 2020).

For comparison, various types of black teas around the world were found to contain from 20,000 to over 130,000 mg/kg. Of this theoretical amount, only a fraction can be extracted in normal brewing, up to 70,000 mg/kg in some Assam varieties and 55,000 mg/kg in Darjeeling (Lachman *et al.* 2003, Shrestha *et al.* 2010).

Of the total phenolic content, flavonoids constituted only a small fraction, between 20-498 mg/kg, with the highest amount in Oolong and the lowest in Darjeeling (Fig. 4) – much lower than the 2,627-5,370 mg/kg found by a previous research in the most common green teas available on the Romanian market (Popoviciu *et al.* 2020). Flavonoids are also known to vary among tea types. In fresh leaves, they make up 3-30% of total phenolic compounds (Blumberg *et al.* 2015).

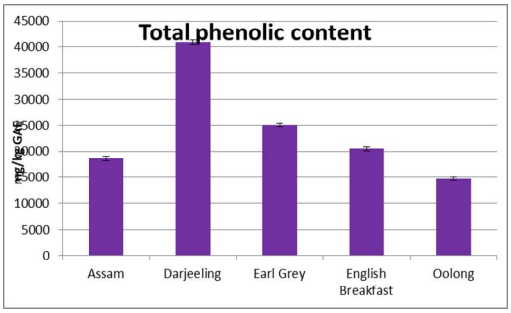

Fig. 3. Concentrations of total phenolic content in selected black tea types (average values; mg/kg).

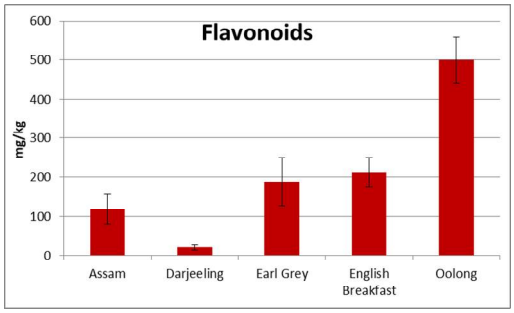

Fig. 4. Concentrations of flavonoids in selected black tea types (average values; mg/kg).

Essential oils had the highest levels in Assam (4,600 mg/kg), English Breakfast and Oolong, while only 2,600 mg/kg were found in Darjeeling (Fig. 5). Essential oils in tea leaf tissues are derived from the oxidation reactions of carotenoids, lipids, terpene derivatives and glycosides. Their content is highly variable, from one cultivar to another and also depending on oxidation degree and storage (Zheng *et al.* 2016). A study conducted on several black tea types, for instance, found 900-6,300 mg/kg total concentrations (Rehman *et al.* 2008). These values are higher than those found in common green tea sortiments in Romania (1,467-1,800 mg/kg) (Popoviciu *et al.* 2020).

pH values ranged between 6.13 (Earl Grey) and 6.66 (Darjeeling) (Fig. 6). Tea acidity is relevant, among other reasons, because on a long term, it can lead to corrosion of dental enamel. The critical value is 5.5. All analyzed teas surpasses this value, being similar to that found in most teas and herbal infusions (Akyuz and Yarat 2010).

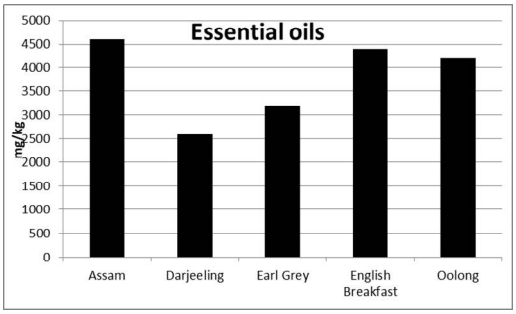

Fig. 5. Concentrations volatile essential oils in selected black tea types (average values; mg/kg).

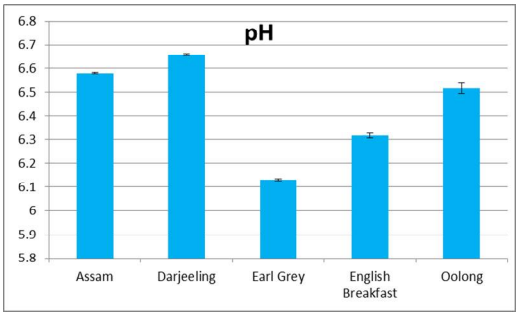

Fig. 6. pH of selected black tea types (average values; mg/kg).

Among the five black tea types, English Breakfast (834 mg/kg) and Earl Grey (587 mg/kg) had the highest average content of chlorophylls, while for carotenoids, the maximum values were found in Earl Grey (360 mg/kg) and English Breakfast (233 mg/kg).

Total phenolic compounds had their highest content in Darjeeling (40,874 mg/kg GAE), while Assam, Earl Grey and English Breakfast tea had almost similar levels of total phenolic compounds, ranged from 18,641 to 25,061 mg/kg. Oolong, on the other hand, had the highest amounts of flavonoids (498 mg/kg).

Total essential oil concentration was similar in Assam, English Breakfast and Oolong, with over 4,000 mg/kg.

In conclusion, while phenolic content is usually the main focus concerning tea nutritional benefits (in this case, maximum in Darjeeling tea), each of the analyzed teas has its benefits considering the concentration of different classes of bioactive, potentially antioxidant compounds.

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