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The effect of pectin and other constituents on the antioxidant activity of tea

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ABSTRACT

The effect of pectin on the antioxidant capacity and phenolic content of black, green, mountain tea and *Pelargonium purpureum* infusions was investigated. Antioxidant capacity was determined by the Ferric Reducing Antioxidant Power (FRAP) assay and total phenolics by the Folin-Ciocalteu method. Green tea was the richest in phenolics and had the highest antioxidant capacity, followed by black and the remaining two infusions. High methoxy pectin, added at various concentrations, did not affect the studied properties of all the infusions. As a further step, milk, lemon juice or sugars (sucrose, fructose) were added to black and green tea infusions, in the presence and absence of pectin. For green tea infusions, no significant differences were reported for the studied properties, for all the constituents, both in the presence and absence of pectin. For black tea infusions statistically different values were observed when pectin was combined with the constituents. These results suggest that pectin does not mask the antioxidant capacity of tea infusions and therefore, it may be added to tea infusions for stabilizing or thickening reasons and create new food formulations with improved health benefits.

1. Introduction

Pectin is a general term for a group of valuable natural polysaccharides extracted from edible plant material where they occur as structural materials. Its main sources are citrus peel and apple pomace. Pectin has quite a wide range of applications. For example, it is used as dietary fibre with positive effects on human health (Stasse-Wolthuis et al., 1980; Thibault & Ralet, 2001). Moreover, its pharmaceutical activities like antidiarrhea, detoxicant regulation and protection of gastrointestinal track are utilized in medical preparations (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Pectin also finds application in drug delivery and tissue engineering (e.g. Lin & Yeh, 2010). A recent work by Xin et al. (2013) reports the utilisation of pectin for the fabrication of scaffolds for bacterial inhibition which can be used for wound dressings and food packaging. Regarding food packaging, pectin is also exploited in the formation of environmental friendly, low cost, biodegradable edible films (Pérez Espitia, Du, de Jesús Avena-Bustillos, de Fátima Ferreira Soares, & McHugh, 2013). These films cam incorporate antioxidants, antimicrobial, colourants, flavours, fortified nutrients and spices (Tripathi, Mehrotra, & Dutta, 2010). For example, Kang et al. (2007) studied pectin edible coating enriched with green tea powder.

However, its major application is in the food industry, as gelling, stabilizing and thickening agent (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002). It is used as gelling agent in jams, jellies, preserves, bakery fillings and toppings. It is also used to stabilise and thicken mayonnaise, salad creams, tomato ketchup, protein foams, cloudy juices, beverages and ice creams (Voragen et al., 1995).

Antioxidants are another basic topic for the Food Industry. Antioxidants are substances used in order to overcome the effects of free radicals to human health and quality of foods. Their efficiency is due to their capability to bind free radicals thus, preventing their destructive oxidant activity (Blasa, Gennari, Angelino, & Ninfali, 2010). There are natural and synthetic antioxidants, with the natural ones being safer. Fruits, vegetables, grains, herbs are some sources of natural antioxidants. Tocopherols, flavonoids and phenolic acids are the most important groups of natural antioxidants and can be found in microorganisms and plants (mainly tea and herbs).

Tea is the second most commonly consumed beverage in the world (Paquay et al., 2000) and is the major source of natural antioxidants (Dubeau, Samson, & Tajmir-Riahi, 2010). It contains carbohydrates, vitamins and most importantly polyphenols that account for up to 30% of its dry weight (Balentine, Wiseman, &

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Bouwens, 1997). Catechins are the major polyphenols in tea leaves (Giménez, López de Lacey, Pérez-Santín, López-Caballero, & Montero, 2013) and the most powerful antioxidant among the known plant phenols (Bancirova, 2010). According to their manufacturing process, tea can be classified into green (non fermented), oolong (semi fermented) and black tea (fermented) (Bancirova, 2010). The antioxidant power of green tea is considerably higher than black (Benzie & Szeto, 1999).

Apart from tea, other herbal infusions have also been studied for their health benefits and their possible use as food additives, due to their high content of phenolics or essential oils. Among them, infusions from mountain tea species (*Sideritis sp.*) which may exert specific pharmacological actions in human organism and their constituents could have a variety of uses (Gonzalez-Burgos, Carretero, & Gomez-Serralinos, 2011). *Pelargonium purpureum* is another aromatic plant rich in flavonoids, which is used in cooking and may exert specific bioactivity in organ tissues (Koutelidakis et al., 2009).

Nowadays, and in order to satisfy the consumers' needs and requests for new food products with improved health benefits, new formulations are under investigation. Tea infusions are very popular products. In the aspect of creating new formulations, pectin could be added to new tea infusion products for stabilizing or thickening reasons. As already mentioned, tea infusions are rich in phenolics, and as a result, posses high antioxidant activity. The present work was focused on evaluating the effect of pectin on the antioxidant capacity and polyphenol content of these infusions, which seems worth exploring when studying new products.

To the best of our knowledge, this is the first study that investigates the effect of pectin on these highly interesting properties of teas and herbs. The out coming results are of great interest as they will answer the question of whether the addition of pectin masks the antioxidant properties of herbal infusions or not. This is a basic first question that has to be answered when food products that contain pectin and herbal infusions are to be developed.

As a first step, various concentrations of high methoxy pectin were added to tea (green and black) and herbal (mountain tea and *P. purpureum*) infusions. The colorimetric Folin-Ciocalteu method (Singleton & Rossi, 1965) was used in order to estimate the total phenolic compounds in our samples A modified Ferric Reducing Antioxidant Power (FRAP) assay (Benzie & Strain, 1996) was used for the determination of their antioxidant capacity. As a further step, milk, sugars and lemon were also added to black and green tea infusions containing pectin and the corresponding values of the same two properties were also determined.

2. Materials and methods

2.1. Materials

Two commercial teas (Lipton, Unilever, Greece), "Green" (green tea, Batch number: L0010AE056) and "Gold" (black tea, Batch number: L0064OH023), bought from local supermarket, were used. Both Greek mountain tea (*Sideritis sp.*) flower bundles and *P. purpureum* leaves were collected from four different plants in one day in the area of Lamia (Greece) and Crete (Greece), respectively. The mountain tea flower bundles were stored at room temperature, before being analyzed. The *P. purpureum* leaves were dried in the dark for 2 months and pulverized just before the experiment. Pectin (DE: 70-75%; High methoxy pectin) from apples was obtained from Fluka (Buchs, Germany). Lemon juice (100% lemon juice, "Realemon" Jotis, Greece, Batch number: L8350 A), sucrose (normal food grade, Hellenic sugar industry s.a, Greece, LOT 29908264) and milk (condensed, 7.5 wt% fat, "NOYNOY", Friesland Campina Hellas S.A., Greece) were purchased locally. Folin-Ciocalteu reagent was from Merck (Darmstadt, Germany). The remaining reagents were all purchased from Sigma-Aldrich (Steinheim, Germany). Distilled water was used throughout.

2.2. Preparation of tea infusions

All infusions were prepared by steeping of 1 g of either dried leaves (black and green tea, *P. Purpureum*) or flower bundles (mountain tea) in 100 g of boiling water. Boiling was continued for 10 min and followed by filtration. When present, pectin was dispersed in the infusion and dissolved at high temperature (~90 °C) using agitation. Three different concentrations of pectin were used (0.1, 0.5 and 1.0 wt%). Sugars (when present) (10 g) were then added and dissolved at high temperature. Milk (10 mL) and lemon juice (1 mL) were also present in some samples. In this case, they were the last constituents added to the infusions. In all samples, in the presence or absence of the constituents, the final weight of the infusion was kept constant at 100 g.

2.3. Determination of total polyphenol contents

The colorimetric Folin-Ciocalteu method (Singleton & Rossi, 1965) was used in order to estimate the total phenolic compounds in our samples. Prior to analysis, all infusions were diluted with distilled water (1:31v/v) and 0.5 mL of each diluted sample was mixed, in a test tube, with 2.25 mL of distilled H_2O and 0.25 mL of Folin-Ciocalteu reagent. The mixtures were agitated for 1 min and 8 min later, 2 mL of Na_2CO_3 (7.5% w/v) were added. The tubes were left in the dark, for 1 h, for colour development. At the end of the incubation time, absorbance was measured at 765 nm, with a double-beam UV-Vis spectrophotometer (Jasco V-530, Tokyo, Japan) against a blank solution. Each analysis was performed in triplicate. The determination of total phenol content was achieved by comparison to a calibration curve constructed by gallic acid. The results were expressed as mg equivalents of gallic acid per g of the samples.

2.4. Determination of antioxidant capacity

A modified Ferric Reducing Antioxidant Power (FRAP) assay (Benzie & Strain, 1996) was used for the determination of the antioxidant capacity of all tea infusions. According to this method, initially, 100 μL HCl 40 mM were added to 20 μL of diluted with distilled water sample (1:71 v/v), put in a well of a 96 well plate reader, and absorbance at 595 nm was read immediately. The same procedure was repeated in order to read the absorbance at 30 min. In this step, the HCl solution was replaced by 100 μL of FRAP reagent. FRAP reagent consists of 25 mL acetate buffer (0.3 M, pH 3.6), 2.5 mL of $FeCl_3.6H_2O$ 20 mM and 2.5 mL of TPTZ (2,4,6-Tri (2-Pirydil)-s-triazine) 10 mM. Absorbance was measured with a multiwell absorbance plate reader (ELx808TM, BioTek Instruments Inc., Vermont, USA) at a constant temperature of 37 °C. Each analysis was in triplicate for each sample. Quantitative determination was made with the help of a standard curve of $FeSO_4.7H_2O$ in HCl 0.01 N. FRAP values were expressed as μmol of $FeSO_4$ per g of the samples.

2.5. Pectin extraction

Pectin present in all teas and herbs used in the present work was determined according to the method described by Ele-Ekouna, Pau-Roblot, Courtois, and Coutois (2011), slightly modified. Initially, 3 g of either dried leaves (black and green tea, *P. Purpureum*) or flower bundles (mountain tea) were grinded to a powder (where necessary) and then suspended, at 4 °C for 5 min, in 100 mL of sodium