LWT - Food Science and Technology 85 (2017) 212–217

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Prebiotic green tea beverage added inclusion complexes of catechin and β-cyclodextrin: Physicochemical characteristics during storage

Rafaela Carvalho de Souza [a], Osvaldo Valarini Júnior [c], Keren Hapuque Pinheiro [a, b], Suellen Jensen Klososki [a], Tatiana Colombo Pimentel [a], Lucio Cardozo Filho [c], Carlos Eduardo Barão [a, *]

[a] Instituto Federal do Paraná (IFPR) – Campus Paranavaí, Rua José Felipe Tequinha, 1400, Jardim das Nações, Paranavaí, Paraná, 87703-536, Brazil
[b] Postgraduate Program in Food Sciences, State University of Maringá (UEM), Maringá, Brazil
[c] Departamento de Engenharia Química, Universidade Estadual de Maringá (UEM), Maringá, PR, Brazil

ARTICLE INFO

Article history:
Received 5 April 2017
Received in revised form
11 July 2017
Accepted 13 July 2017
Available online 14 July 2017

Keywords:
Catechin
Camellia sinensis
Cyclodextrin
Molecular inclusion
Oligofructose

ABSTRACT

The objective of this study was to evaluate the effect of the addition of β-cyclodextrin, catechin or inclusion complexes (catechin: β-CD) (physical mixture or with supercritical carbon dioxide) on prebiotic green tea beverage characteristics during storage at ambient temperature (90 days/25 °C). The prebiotic (oligofructose) stability was also evaluated. The addition of cyclodextrin and/orcatechin resulted in products with higher acidity. The physical process of complexation, which is simple and cheap, gave the best results in relation to the stability of the phenolic compounds content in the product. The supercritical carbon dioxide did not improve the protection of the phenolic compounds. A portion of 200 mL of the green tea had sufficient oligofructose content to be considered a prebiotic product during 90 days of storage. It was possible to formulate green tea beverages with high total phenolic compounds (2525–3307 mg CE/L) and oligofructose content (3.1 g/200 mL) sufficient to consider them prebiotic products, being recommended the physical process of complexation (catechin: β-CD). The green teas would maintain the beneficial effects for 90 days at ambient temperature (25 °C).

1. Introduction

Functional foods are foods that have beneficial effects on target functions in the body beyond nutritional effects, in a way that is relevant to health and well-being and/or the reduction of disease (Kaur & Singh, 2017). There are many components that could be added in functional foods, such as probiotics, prebiotics, antioxidants and others.

Prebiotics, such as oligofructoses, are non-viable food components, conferring host health benefits associated with the modulation of their microbiota (Fao/Agns, 2007). These components are related to the increase in the absorption of minerals from the diet, constipation relief, reduction of blood lipids; maintenance of blood sugars, inhibition of pathogens, and reducing the risk of colon cancer, among others (Al-Sheraji et al., 2013). However, in acidic environments, oligofructose can be hydrolyzed, resulting in the loss of nutritional, physicochemical and functional properties (Courtin, Swennen, Verjans, & Delcour, 2009; Pimentel, Madrona, Garcia, & Prudencio, 2015), being crucial the evaluation of its stability in food products.

Green tea is a beverage obtained from the plant *Camellia sinensis*, presenting high number of flavonoids and catechins. Catechins (CAT) are considered natural antioxidants (Krishnaswamy, Orsat, & Thangavel, 2012), and their consumption is associated with a reduction in the risk of cancer and cardiovascular diseases (Roychoudhury, Agarwal, Virk, & Cho, 2017; Shi et al., 2017). However, catechins undergo epimerization, degradation and oxidation reactions during food processing and storage, which limits the use of green tea catechins in functional food development (Bhushani, Kurrey, & Anandharamakrishnan, 2017). Moreover, catechins possess a very astringent and bitter flavor (Liu et al., 2016).

To circumvent these drawbacks, encapsulation has been proven to be a promising approach. Cyclodextrins (CDs) are macrocyclic carbohydrates consisting of six, seven and eight D-glucose units for the respective α-, β- and γ-CDs. They are well known for their

* Corresponding author.
E-mail address: carlos.barao@ifpr.edu.br (C.E. Barão).

http://dx.doi.org/10.1016/j.lwt.2017.07.022

ability to form complexes with various guest molecules and widely applied in pharmaceutical industry, foods, and agriculture, etc (Aree & Jongrungruangchok, 2016). The molecular encapsulation of food ingredients with CDs improves the stability of flavors, vitamins, colorants and unsaturated fats, etc., both in a physical and chemical sense, leading to extended product shelf life (Folch-Cano, Jullian, Speisky, & Olea-Azar, 2010).

There are several methods for the molecular inclusion in CDs. The physical mixture (PM) is a simple methodology and consists of macerating a dry mixture of the guest molecule and CD, whereas the supercritical method ($SSCO_2$) is an innovative method, in which the particles are added in a bed and then a supercritical gas (mainly carbon dioxide) is added (Valarini-Junior et al., 2017).

The inclusion complexation of catechin with cyclodextrins had been studied by other authors (Valarini-Junior et al., 2017; Folch-Cano et al., 2010), but none of them applied it in food products to verify their functionality. Therefore, the objective of this study was to verify the behavior of complexes formed between catechin and cyclodextrins (physical or supercritical methods) when applied in green tea beverage supplemented with oligofructose during storage at 25 °C for 90 days without light incidence. The formulations were compared to the products added free forms (catechin and/or pure cyclodextrin) and the oligofructose stability was also evaluated.

2. Material and methods

The materials and reagents used were: Green teas leaves available in local market; β-CD and +CAT (Sigma-Aldrich®, purity ≥90.0, Saint Louis, MO, USA), oligofructose (P95, Orafti®), CO_2 technical grade from White Martins (Rio de Janeiro, RJ, Brazil), ferric chloride hexahydrate ($FeCl_3.6H_2O$; Merck, Germany), hydrochloric acid and potassium ferrocyanide ($K_3[Fe(CN)_6]$; Merck, Germany). All reagents were of analytical grade.

2.1. Preparation of green tea beverages

Six formulations of green tea were prepared: (1) pure green tea, (2) green tea + pure β-CD (0.1% w/v), (3) green tea + pure CAT (0.1% w/v), (4) green tea +β-CD (0.1%) + CAT (0.1%) added separately, (5) green tea + molecular inclusion of β-CD:CAT (0.1% w/v; ≈0.02% of catechin) by supercritical carbon dioxide and (6) green tea + molecular inclusion by physical mixture of β-CD:CAT (0.1% w/v; ≈0.02% of catechin). All formulations were also supplemented with 2% (w/v) of oligofructose, as a prebiotic component.

The ingredients (β-CD, CAT and oligofructose) were placed in beakers, according to the respective formulation. Then, water (80 °C) and the green tea leaves (2 g/100 mL) were added and the mixture stirred for 10 minin a magnetic stirrer to ensure a better extraction of the phenolic compounds and to prevent the release of tannins that occurs with higher infusion time and imparts an astringent flavor to the tea (Nishiyama et al., 2010). At infusion time, tea was filtered, leaves discarded and pH reduced with citric acid (pH 4.5) to eliminate the possibility of microorganism growth during tea storage. Then, the tea formulations were placed in glass flasks of 80 mL of capacity (Farma®) and submitted to a pasteurization process (80 °C/35 min) in a water bath. Analyzes were performed on days 1, 15, 30, 60 and 90of storage at ambient temperature (25 °C) in the absence of light. The storage period was defined based on the shelf life of the Brazilian commercial teas.

2.2. Molecular inclusions of catechin by physical mixture (PM) and supercritical carbon dioxide (SSCO2) methods

The method of physical mixture was based on available studies with β-CD as the host molecule (Hu et al., 2012; Menezes et al., 2012, 2014). In summary, it consisted of macerating a mixture of dry + CAT and β-CD in a crucible at 1: 1 M ratio for 15 min, that is, the time taken to have a macroscopically homogeneous mixture. The supercritical carbon dioxide methodology was performed according to literature suggestions (Banchero, Ronchetti, & Manna, 2013; Banchero & Manna, 2012; Berna, Montón, & Subirats, 2001). SCCO2 at 90 bar and 40 °C was pumped through a packed bed formed by a 1: 1 M mixture of the considered guest and host compounds up to pressure and temperature stabilization. All the valves were closed and stagnant CO_2 was left in contact with the solid mixture for 60 min before its removal by system depressurisation (Valarini-Junior et al., 2017).

2.3. Chemical analysis of green tea beverages

The pH was determined using a digital potentiometer (MS Technopon®, Piracicaba, Brazil). The titratable acidity was measured according to AOAC (2004). A 10 mL aliquot of tea was taken and titrated with 0.1 moL/L NaOH and the end point was determined by pH at 7.0. The titratable acidity was expressed as the volume consumed in millilitres of 0.1 moL/L NaOH per 100 mL sample.

The juice color was measured in a Konica Minolta CR 400 (Japan) colorimeter, which provided the parameters L* (brightness), a* (red-green) and b* (yellow-blue). The oligofructose concentration was determined using a Fructan HK enzymatic kit (Megazyme International Ireland®).

Total phenolic content was determined with the Prussian blue method proposed by Margraf, Karnopp, Rosso, and Granato (2015). Briefly, an aliquot of 1000 μL of a 0.50 mmoL/L ferric chloride hexahydrate (FeCl3.6H2O) diluted in HCl0.01 moL/L was added to 1000 μL of properly diluted sample and left to react for 2 min. Then, 1000 μL of a 0.50 mmoL/L K3[Fe(CN)6] solution were added and shaken for 20 s. The absorbance was recorded at $\lambda = 725$ nm after 15 min reaction in the dark at 25 °C. The total phenolic content was expressed as mg catechin equivalents per liter of beverage (mg CE/L).

2.4. Statistical analysis

The physicochemical analyzes were conducted following a split plot design, with the green tea formulations being the main treatment and the storage time the secondary treatment. The experiment was repeated two times. In each replicate, analyzes were performed in duplicate. Data were submitted to analysis of variance (ANOVA) and Tukey comparison of the mean test ($p < 0.05$). Statistical analyzes were performed using the Statistical Analysis System program version 9.1.3.

3. Results and discussion

The physicochemical characteristics of the green tea formulations are shown in Table 1 and the total phenolic content is presented in Fig. 1. The green tea formulations had pH of 4.14–4.51, titratable acidity of 16.7–30.33 mL NaOH/100 mL solution and total phenolic compounds of 1363–3307 mg catechin equivalents per liter of beverage.

The pH of the green tea formulations was initially adjusted to 4.5 with citric acid to avoid bacterial contamination and also to maintain the stability of the catechins in the teas. In aqueous solutions, CATs are more stable at low pHs and become unstable at pH > 6. Therefore, in order to obtain the maximum amount of catechin during the tea infusion process, it is recommended to use boiling water and add pH-reducing ingredients, such as citric acid