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Multi-stage block freeze-concentration of green tea (*Camellia sinensis*) extract

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Abstract

Tea is the third most consumed beverage in the world. It has bioactive compounds that provide health benefits; for that reason, concentrating this product is a good alternative for different industries. Block freeze-concentration is a technology with the potential to be used to concentrate food solutions, preserving functional compounds. The aim of this work was to study the effect of block freeze-concentration on the total solids content, catechin content, polyphenol content, antioxidant activity and sensorial profile of green tea. Green tea extract at a 4 % (w/w) solid mass fraction was freeze-concentrated in three successive stages at -25°C. The effects of some parameters were evaluated, such as the concentration index, solid recovery yield, and concentration efficiency. After three stages, the solution was concentrated from 4% (w/w) to 14.1 % (w/w) with a distribution coefficient of 0.43 and a total concentration efficiency of 81.6%. The efficiency of concentration decreased with the number of stages. The levels of catechins and polyphenols were increased by 4.5- and 3.4-fold in the final liquid fraction, respectively. The antioxidant activity was preserved after three stages of freeze concentration. The physicochemical parameters were maintained: of the 27 sensory attributes evaluated, 18 of them were retained. The results demonstrated that block freeze-concentration is a useful technique for preserving and enhancing the bioactive compound content and the functional properties of green tea extracts.

1. Introduction

Some liquid products need to be concentrated to reduce transportation costs, reduce the amount of water contained and increase its useful life as the number of microorganisms that can grow is lower (Bozkir and Baysal, 2017). Some liquid products have bioactive compounds with great benefits, but their concentrations are deficient within the matrix, as is the case with green tea (Reygaert, 2017).

Tea is one of the most consumed beverages worldwide (Unno and Osakabe, 2018; Viljoen et al., 2016). There are different types of tea depending on the production method, and green tea is the only tea that is not fermented (Ho et al., 2015). Recently, interest in the consumption of natural beverages has increased (Conger and Singg, 2019) due to their different benefits. In the case of green tea the benefits include the prevention of heart disease, insulin resistance, autoimmune disorders, and cancer prevention (Daglia et al., 2017). There are many studies about its antioxidant activity, where the capacity to capture free radicals is attributed to green tea (Ortiz-López et al., 2016). These benefits are attributed to compounds known as catechins, which represent 70% of the bioactive compounds in green tea extract. The catechin that is present in 80% of the total catechins, the highest quantity, is 9-epigallocatechin-3-gallate (EGCG) (Yu et al., 2014). However, catechin stability is affected when subjected to high temperatures, generating epimerization of the molecules (Bhushani et al., 2017); thus, if necessary, it is more advantageous to use non-thermal technology to concentrate green tea than traditional evaporation.

Non-thermal technologies are widely used in the food industry for their ability to preserve product quality by preventing the thermal degradation of temperature-sensitive compounds (Chemat et al., 2017). These technologies can help reduce microbial load, avoid food degradation, or increase food concentration (Pereira and Vicente, 2010). Freeze-concentration or cryoconcentration is a non-thermal technology that allows the concentration of different solutions (Aider and de Halleux, 2009); such technology increases the concentration by separating the water from the solution, obtaining a very diluted phase and a concentrated phase (Correa et al., 2017). There are three different techniques of freeze concentration: the best-known at an industrial level is suspension (Miyawaki et al., 2012). This technology is efficient in the separation process (Ding et al., 2019), but at the operational level it has removable parts that require higher cost (Sanchez et al., 2009); the other technologies are progressive freeze-concentration and block freeze-concentration.

Block freeze-concentration (BFC) allows the solution to be completely frozen and then partially thawed (Moreno et al., 2014a). The technique is also known as freeze-thaw concentration. The first recovered fractions at the thawing step have the highest level of solids. (Moreno et al. 2014b; Petzold et al., 2016). The process can be performed in successive stages to increase the concentration index (Aider and Ounis, 2012). This technology could have economic advantages. This technology may be more efficient than other techniques if the ammount of solids trapped in the diluted phase decreases (Aider and de Halleux, 2009). Some challenges remain in the industrial application of block freeze-concentration related to increasing the separation efficiency and establishing the effect of the technique on the bioactive compounds, bioactivity, and sensory attributes among other quality characteristics of the products. Moreover, a sensorial analysis identifies the parameters of smell and flavour, employing different notes, (Lu et al., 2009) to help identify the changes in green tea extract before and after freeze-concentration.

BFC has been studied on several fluids (Moreno et al., 2014a, 2013; Orellana-Palma et al., 2017; Petzold et al., 2016, 2015). However, to our best knowledge, no references have yet been found on the block freeze-concentration of green tea. Thus, this paper aimed to evaluate the effect of three stages of block freeze-concentration on the solids content, polyphenol content, catechin content, antioxidant activity and sensory quality of aqueous green tea extract.

2. Material and Methods

2.1. Materials

Green tea extract was obtained from commercial dried tea leaves supplied by Agrícola Himalaya (Bitaco) from Cali Colombia. First, 130 g of dried leaves were placed in 1 L of distilled water at 80 °C for 30 min to obtain a solution with an initial solid content of 4% (w/w) ; then, extraction was performed according to the protocol proposed by Vuong et al., (2011). The solution was stored at 4°C. The °Bx of all samples was measured by a refractometer (Atago Pal 100, Tokyo, Japan) and a correlation between °Bx and solid content was obtained. The solids content (Cs) was determined in an oven at 103 ± 2°C (NTC 4396,1998). The equation obtained was Cs= 0.789* °Bx (R^2 =0.99). Cs represent the total amount of solids present in the tea extract. All measurements were performed in triplicate.

104

105 **2.2. Freeze-concentration tests**

106 The block freeze-concentration tests were performed on the equipment located in the
107 laboratories of Universidad de La Sabana, Chia, Colombia, developed by Moreno et al
108 2014a (Figure 1). First, 300 mL of tea extract at 4 % (w/w) was placed in the vessel (1) to
109 be complete frozen and consequent thawed by using as the cooling and heating fluid,
110 ethylene glycol provided by the thermostatic baths (2) (Cole Parmer, Vernon Hills, USA)
111 (4,5) with a Cooling and heating power at 20 C of 800 W and 1 kW respectively. The bath
112 temperature was set to -25 °C, and the freezing time was 8 h. After the freezing stage, the
113 thawing stage was conducted by heating the external jacket of the container with the
114 heating fluid settled at 20 °C (3). Ten thawed fractions were collected in separated
115 containers (4) in the thawing stage by gravitational separation and weighed with a scale
116 (5) (Mettler Toledo, Greifensee, Switzerland). The thawing time varied between 4 and 6 h.
117 Finally, the concentration was measured by refractometry (Atago model PAL-100; Tokyo,
118 Japan) and converted to a content of solids with the correlated equation Cs= 0.789 * °Bx.
119 This equation was generated by this work as explained before.

120 The experimental design was performed in three stages as shown in Figure 2. Two
121 streams with the mixing of some collected fractions were obtained after freeze
122 concentration, one diluted and other concentrated. The definition of the number of
123 fractions to be mixed was performed according to the results shown in figure 2. The
124 fractions in which the concentration index (defined in equation 1) was greater than one
125 were mixed and identified as the concentrated stream (liquid fraction). The fractions with a
126 concentration index less than one were mixed and identified as the diluted stream (ice
127 phase). The diluted streams were discarded. The concentrated phase was used to the
128 next stage. The first stage was preformed four times and the second stage twice to
129 complete the volume necessary to the next stage. A total of three stages with seven tests
130 were performed as shown in Figure 2. The overall process was performed in triplicate.

131

132 **2.3. Evaporation test**

133 An evaporation test was performed to compare the catechin concentration results with a
134 method of concentrating at higher temperatures. The evaporation process was performed
135 using 100 g of green tea extract (4% w/w) obtained under the same conditions as the

136 extract used in the freeze-concentration tests. The sample was evaporated at 92°C, the
137 boiling point at Bogotá, using a beaker on a heating plate (Thermo Fischer Scientific,
138 Waltham, USA). The sample was evaporated at 92 °C for the necessary time to obtain the
139 same concentration that results in the three stages of BFC. The solids content was
140 measured by refractometry. The samples were stored at 4 °C until further analysis.

141 **2.4. Response variables**

142 The following response variables were calculated to evaluate the behaviour of the freeze
143 concentration tests:

144 **Concentration index (CI):** This factor was defined as the ratio between the solids content
145 in the liquid fraction and the solids content in the initial fraction (Moreno et al., 2014a).

146 $$CI = \frac{C_{sLIQ}}{C_{s0}} \quad (1)$$

147 Where, CI is the concentration index, C_{sLIQ} is the total solid content (%w/w) in the
148 individual liquid fraction and Cs_0 is solid content of the initial solution.

149 **Thawing fraction (f):** This was defined as the ratio between the mass thawed and the
150 initial mass, and this variable can be used to follow the development of the process
151 (Moreno et al., 2014a).

152 $$f = \frac{m_{LIQ}}{m_0} \quad (2)$$

153 where m_{LIQ} and m_0 are the liquid and initial mass, respectively.
154
155 **Solid Yield (Y):** This factor was defined to analyse the solids recovery; it was calculated
156 as the ratio between the solid mass in the liquid and the solid mass present in the initial
157 solution (Moreno et al., 2013):

158 $$Y = \frac{m_{sLIQ}}{m_{s0}} \quad (3)$$

159 where m_{sLIQ} is the solid mass in the recollected liquid, and m_{s0} is the solid mass in the initial
160 solution.
161
162 **Concentration efficiency (eff):** This was defined as the concentration of solids recovery
163 in the liquid solution with respect to the concentration of solids present in the ice phase.
164 The equation is as follows (Gulfo et al., 2014):
165

$$eff = \frac{C_{sLIQ} - C_{sICE}}{C_{sLIQ}} * 100 \quad (4)$$

Where, C_{sLIQ} and C_{sICE} are the total solid content (%w/w) in the liquid and ice fractions respectively.

The average distribution coefficient: This is the ratio between the solids in the ice phase and the solids in the liquid phase (Moreno et al., 2014b).

$$k = \frac{C_{sICE}}{C_{sLIQ}} \quad (5)$$

Productivity: This factor is defined as the rate of separation of water from the initial solution (Moreno et al., 2015).

$$Productivity = m_{ice} * \frac{(1 - C_{sICE})}{100 * (m_0 * t)} \quad (6)$$

Where, m_{ice} is the mass of the ice produced during freeze concentration, C_{sICE} is the total solid concentration in the ice, m_0 is mass of the initial solution, and t is the total processing time of the freeze concentration in hours. The productivity of the operation represent the rate of water separation from the solution achieved by the freeze concentration process.

2.5. Bioactive compounds and bioactivity of green tea extract

The following measurements were performed for each of the samples before and after the three stages of BFC and the evaporation concentration.

Catechin content

The catechins were measured by high-performance liquid chromatography (HPLC). The column used was a Zorbax SB-C8 with a UV detector at 280 nm, the mobile phases were methanol (25%) and trifluoroacetic acid (TFA) (57%), and the catechins were reported in mg/mL of green tea extract (Gadkari et al., 2014).

Total polyphenol content

The polyphenol content was measured by Folin-Ciocalteu according to ISO 14502–1 (2005). A sample of 1 mL of tea extract was mixed with 1 mL of gallic acid at different concentrations and 5 mL of Folin-Ciocalteau reagent; the reaction was left for 60 min at room temperature, and then 4 mL of 7.5% Na_2CO_3 was added. The absorbance was measured at 765 nm, using a spectrophotometer (prove 600 Merk, Darmstadt, Germany), and a blank sample was included. The results were expressed in mg equivalent gallic acid per gram of tea leaf (dry basis).

Antioxidant activity

The antioxidant activity was measured by three different methods: DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and ORAC (oxygen radical absorbance capacity). Different methods were used to show the reproducibility of the results.

The DPPH technique was performed using 2 mL of green tea extract and mixed with DDPH reactive and a 2 mL of solution ethanol; the absorbance was measured at 517 nm, and the results were expressed as grams of TEAC Trolox per 100 grams of tea leaves or mL of solution. (Yu et al., 2007). The ABTS technique was performed using 0.025 mL of green tea extract mixed with a previously prepared Trolox ethanolic solution. The mixture was left for approximately 2 h in a dark room; then, the samples were read in a spectrometer at 734 nm (Damiani et al., 2014). The results were expressed as mmol/L Trolox equivalents per 100 grams of tea leaves. Finally, the ORAC technique was performed using 20 µl of sample and mixed with Trolox reactive. The pH was adjusted to 7.7, and the samples were incubated in boxes with 96 plates for 10 min at 37°C; the measurements were performed at 538 nm. The results were expressed as µmol of Trolox equivalents per mL of tea extract (µmol TE/100 mL) (Liu et al., 2018).

2.6. Sensory analysis

A panel of seven trained judges between 25 and 60 years at the Laboratory of Sensory Analysis of Universidad de Antioquia (Medellin, Colombia) evaluated the sensory properties of the initial and freeze-concentrated green tea extract. The samples were re-dissolved and standardized at the same final solid content (1.5 % w/w) This content was settled to compare the sensory quality of a possible tea beverage produced from the freeze concentrated extract. Panellists evaluated the appearance, texture, taste, flavour, and odour according to the Colombian National Standard NTC 3501 and NTC 3932.

2.7. Statistical analysis

Data were analysed using SAS9.2 (SAS Institute Inc., Cary, USA) software package. All tests were performed in triplicate. Data were reported with the deviation, and the variance (ANOVA) was evaluated to identify the differences among the data with a level of significance of 95%. Pearson's correlation coefficients were used to compare the relationship between the bioactive compounds and antioxidant activity.

3. Results and discussion

3.1. Freeze-concentration tests

The first analysis corresponds to the solid content of the individual thawing fractions. The results of the concentration index (CI) for each stage of BFC at the ten fractions obtained following the thawing process are shown in Figure 3. The concentration index represents the number of times the initial concentration of the solution was increased for each collected fraction during the thawing stage. It is important to note that each thawing fraction was analysed separately, thus the CI in the figure 3 represents the individual concentration index. The behaviour was similar for the three stages. The graph shows that the solid content of the first fractions recovered in the thawing stage had the highest concentration, with CI values of approximately 2.6. This is because the drops of liquid that were being separated in the first thawed fractions were enriched with the solids eluted from the frozen matrix during the thawing stage (Nakagawa et al., 2010; Robles et al., 2016). Consequently, the solid content decreased at each thawing fraction until the concentration index values approached 0.

The second analysis corresponds to the result of the three stages of freeze concentration. The horizontal line of the figure 3 represents the case in which CI=1, which indicates that the fractions that are being separated have the same solid content than the initial solution. At this point, two streams can be separated. The first, with the highest solid content, composed by the mixture of the first fractions is the concentrated phase, and the last, with the lowest solid content, containing the last fractions is the diluted phase. The average f at CI=1 of each test was determined and showed in the table 1. and a mass balance was made at this f, to calculate the final solid content of each stream as shown in Figure 4. The values are expressed as a percent w/w. The solid content in the liquid phase increased from 4% (w/w) to 14.1% (w/w) in the three stages, whereas the solids content in the ice phase was 5.86% (w/w); the overall concentration efficiency of the process was 81.57%. The results are similar to those reported for the BFC of other products (Moreno el al., 2014b, Petzold et al., 2015).

The response variables of the block freeze-concentration at the average f value when CI=1 are shown in Table 1. These values can be calculated with the equations 1 to 6. The total solids content increased by 3.52-fold in the three stages. In each stage the concentration index was approximately 1.5, but in the second stage this value was higher. The Y_{stage} represents the solid yield, or the amount of solids that can be recovered from the initial

solution at the end of each BFC stage. In the first two stages it was similar; 78% of the solids were recovered. In the third stage the value decreased slightly due to the difficulty of recovering solids in more concentrated solutions. Thus, there was always a similar solids recovery. The highest value of the average distribution coefficient was obtained in the third stage. This is because the concentration was higher at the third stage, and the solids increased the viscosity and reduced the mobility of solids during the thawing stage, allowing more solids to be trapped in the ice (Dunaway et al., 2010; Moreno et al., 2014a; Nakagawa et al., 2010; Osorio et al., 2018). Finally, the productivity of the operation was 0.04 kg water removed per kg extract per hour. This value is lower than that reported by Moreno et al.2015 for coffee extract due to the lower temperature needed to completely freeze the green tea sample.

The results demonstrated that BFC is an effective technique for concentrating tea and increasing the solids content. Similar values were reported by Moreno et al. (2014) in coffee extract. (Moreno et al., 2014a); however, the processing time is relatively high, approximately 8 h for each BFC. Similar results were obtained in coffee extract (Moreno et al. 2015). The high processing time is maybe the greatest disadvantage of block freeze concentration. Several alternatives in the thawing and separation stages such as microwave thawing, vacuum, and centrifugal separation are being explored to increase the concentration efficiency or reduce the processing time (Orellana-Palma et al., 2017; Petzold et al., 2016).

Finally, a third analysis was done in this section. A multistage process of bock freeze concentration was proposed. Figure 5 shows a scheme of three BFC stages and the solids content of the concentrated and diluted phases. The arrows pointing downwards represent the ice phase (diluted), and the right arrows the concentrated phase which enter to the new stage. BCF3 effluent (5.83% (w/w)) can be recirculated to the stage 2 to recover the solids contained in that stage. In contrast, the ice obtained from stages 1 and 2, because they are very diluted, can be defined as final effluents. A mass balance was calculated with this process strategy and the overall concentration efficiency and concentration index were calculated. In addition, an experimental equation to calculate the concentration index as a function of the concentration of liquid at each sage was used. An overall process efficiency of 85.3% can be achieved with this process configuration, with a concentration index of 3.52, an f value of 0.16 and a solute yield of 0.56. In contrast, the process without recirculation achieved a concentration efficiency of 81.1%, an f value of 0.11 and a solute

yield of 0.41. The results showed an improve of the process. Moreover, the multistage process can be applied with a higher number of stages to reach a final concentration to obtain a more concentrated extract. The limit is determined by the solid-liquid equilibrium of the system. Further research on the topic is necessary. This kind of product can be commercialized as a concentrated liquid to be reconstituted after storage, or even as a product prior to freeze-drying to obtain freeze-dried green tea extract.

3.2. Biocompounds and bioactivity of the extract

Catechins, polyphenols, and antioxidant activity in the extract before and after block freeze-concentration and the comparison between the values obtained with evaporation are shown in Table 2. In general, the results indicate that both bioactive compounds and bioactivity were significantly increased at each stage of BFC. The table shows the value of the concentration index reached at the third stage (CI Stage 3). This value corresponds to how many times the concentration of the compound or the activity was increased with respect to the initial concentration or activity. The polyphenol content and catechin concentration were increased by a similar factor as the increase in total solids content. The catechins were slightly more concentrated than the total solids. This could be obtained by a possible selective separation due to differences in polarity or molecular size. A further experimentation is recommended to explain this result and to determine the useful of this selectivity. This result showed that the BFC preserved the bioactive compounds of the extract. In contrast, the evaporation technique used with the same solids content showed a decrease in most of the bioactive compounds and bioactivity (CI<1).

Similarly, the antioxidant activity measured by DPPH, ABTS and ORAC was increased after BFC but with a lower concentration index than the bioactive compound preservation. However, the CI obtained for the antioxidant activity of BFC was higher than when using the evaporation technique. The higher CI obtained by ORAC, ABTS and DPPH presented similar values of CI, between 1.48 and 2.02 for BFC; in contrast, evaporation obtained values ranging from 0.59 to 1.58. The DPPH values were higher with respect to the other two techniques (Kopjar et al., 2015); similar values were found in matrices such as yogurt (Jaster et al., 2018) and yerba mate(Dudonné et al., 2009) . The catechins increased by 4.45-fold, but the activities increased by 1.48 to 2.02; hence, the prolonged exposure of 12 h to cold could affect the ability to capture free radicals. On the other hand, the polyphenols were increased; thus, the technique of freeze-concentration is highly efficient

in preserving these bioactive compounds; similar results were found for matrices such as wine (Zhang et al., 2016). In contrast, evaporation decreased the concentration of bioactive compounds and the bioactivity of the green tea extract.

The results of the correlation analysis between bioactive compounds and antioxidant activity in BFC are shown in Table 3. Pearson's correlation coefficient is reported. This value represents the linear relationship between the variables, and it can be used to stablish a correlation between the compounds and the bioactivity. A good correlation between the bioactive compounds and the antioxidant activity was found. This result indicates that the enhancement of the activity after BFC can be explained by the increase in the catechin and polyphenol contents. In addition, a direct correlation between catechins and polyphenols of 0.983 was obtained; catechins have a correlation with antioxidant activities with values between 0.771-0.936, and polyphenols have a correlation with values between 0.733-0.953. BFC preserves the compounds and the functional quality of green tea extract. The evaporation test did not yield a good correlation.

3.3. Sensory analysis

A sensory evaluation of green tea extract can directly reflect the quality of the samples. The sensory profile of extract tea in a concentrated liquid after three stages of BFC and without treatment is shown in the spider web diagram in Figure 6. The panellists evaluated 27 parameters among flavour (F), odour (O), texture (T), appearance (A), and sensorial sensation (Ss), and they did not perceive any significant difference in 18 of the 27 evaluated parameters. Some parameters of the freeze-concentrated sample obtained a higher score than the initial extract at the same concentration and other parameters a lower value. However, the overall quality was statistically preserved in the tea extract before and after freeze concentration. This result shows that BFC is an effective technique for preserving the sensory quality of tea extract.

Despite the overall quality preservation, some attributes such as tobacco, bitterness, green colour, sweetness, herbal flavour and sweet odour were parameters that presented a significant difference with $p<0.05$. This means, the probability to reject the hypothesis of the statistical difference is less than 5%. The overall quality did not show statistical differences. The changes in the freeze-concentrated extract, compared with the extract without treatment, can be related to the volatile compounds found in the polyphenols and

other bioactive compounds (Ahmad et al., 2015; Wu et al., 2017) that were present in high quantity in the BFC extract, as the samples were diluted with water at the same concentration as the extract without treatment; at the same time, exposure to temperatures below zero can cause changes in odour (Zhang et al., 2016). Similar results were obtained for other matrices such as coffee (Moreno et al., 2015) and strawberry pulp (Jaster et al., 2018). These results show that the sensorial quality can be preserved using block freeze-concentration.

4. Conclusions

It was possible to concentrate the green tea extract (*Camellia sinensis*) from 4 % (w/w) solids to 14.1 % (w/w) after three stages of block freeze-concentration. The efficiency of concentration was 81.3% after three stages and the solute yield 42%. These values improve to 85.3% and 56% respectively when a recirculation strategy is applied. The concentration of the bioactive compounds, catechins, and polyphenols can be increased during freeze concentration. In contrast, these compounds were degraded during concentration by evaporation. The antioxidant activity was improved with BFC. A correlation between the compounds and bioactivity was found. The sensory profile of most attributes was preserved during freeze concentration. The overall sensory quality of the beverage was preserved by BFC. The multistage process can be applied to obtain higher concentrations depending on the number of stages. The results show that block freeze-concentration is an efficient technique for increasing the solids and bioactive compound contents, increasing the antioxidant activity and preserving the sensory quality of green tea extract.

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Table 1 Initial solids concentration, concentration index, solid yield and distribution coefficient at each stage of BFC for green tea extract, at CI=1

Stage	Cs_{LIQ} (% w/w)	Cs_{ICE} (% w/w)	Y	CI	k	f	Eff(%)	Productivity (kg water/(kg extract. h))
1	5.20 ± 0.00^{c}	1.25 ± 0.01^{c}	0.79 ± 0.062^{a}	1.53 ± 0.025^{b}	0.25 ± 0.075^{b}	0.65 ± 0.029^{a}	75.38 ± 4.21^{a}	0.043 ± 0.002^{b}
2	8.94 ± 0.01^{b}	2.08 ± 0.03^{b}	0.77 ± 0.075^{a}	1.73 ± 0.084^{a}	0.24 ± 0.064^{b}	0.45 ± 0.058^{b}	76.79 ± 3.52^{a}	0.044 ± 0.004^{b}
3	14.1 ± 0.11^{a}	5.76 ± 0.18^{a}	0.68 ± 0.007^{b}	1.55 ± 0.078^{b}	0.43 ± 0.013^{a}	0.40 ± 0.009^{b}	59.10 ± 2.87^{b}	0.046 ± 0.001^{a}

Average values in the same column with different superscript letters are significantly different ($p < 0.05$).

Table 2. Catechin content, total polyphenols content, and antioxidant activity at each stage of BFC of green tea extract compared with evaporation at 92°C.

Block freeze concentration					
Stage	Initial	Stage 1	Stage 2	Stage 3	CI stage 3
Solid concentration (% w/w)	4.0 ± 0.04^{d}	5.2 ± 0.06^{c}	8.9 ± 0.09^{b}	14.1 ± 0.11^{a}	3.53 ± 0.06
Catechins (mg/mL)	6.25 ± 0.58^{d}	11.30 ± 0.95^{c}	16.42 ± 1.38^{b}	27.80 ± 0.45^{a}	4.45 ± 0.50
Polyphenols (mg eq GAE/mL)	14.48 ± 0.69^{d}	18.71 ± 1.64^{c}	34.88 ± 3.75^{b}	50.33 ± 6.31^{a}	3.48 ± 0.60
DPPH (mg eq Trolox/mL)	95.90 ± 2.76^{b}	148.11 ± 4.67^{b}	157.78 ± 5.36^{a}	166.08 ± 4.69^{a}	1.73 ± 0.10
ABTS (mg eq Trolox/mL)	68.32 ± 1.98^{d}	75.61 ± 2.04^{c}	97.64 ± 3.22^{b}	101.41 ± 3.44^{a}	1.48 ± 0.09
ORAC (mg eq Trolox/mL)	50.74 ± 2.92^{d}	78.52 ± 6.01^{c}	85.54 ± 3.28^{b}	102.48 ± 3.29^{a}	2.02 ± 0.19
Evaporation					
Solid concentration (% w/w)	4.0 ± 0.02^{d}	5.2 ± 0.03^{c}	8.9 ± 0.12^{b}	14.1 ± 0.24^{a}	3.53 ± 0.06
Catechins (mg/mL)	4.65 ± 0.12^{a}	0.91 ± 0.12^{b}	0.81 ± 0.09^{c}	0.71 ± 0.07^{d}	0.15 ± 0.01
Polyphenols (mg eq GAE/mL)	12.01 ± 0.24^{b}	10.48 ± 1.40^{b}	16.64 ± 2.27^{a}	8.08 ± 1.23^{c}	0.67 ± 0.11
DPPH (mg eq Trolox/mL)	91.54 ± 6.87^{a}	79.25 ± 3.74^{b}	63.48 ± 1.27^{c}	54.35 ± 2.51^{d}	0.59 ± 0.06
ABTS (mg eq Trolox/mL)	63.40 ± 1.81^{d}	98.79 ± 2.16^{c}	101.45 ± 1.35^{b}	100.73 ± 3.51^{a}	1.58 ± 0.09
ORAC (mg eq Trolox/mL)	44.32 ± 2.75^{d}	58.22 ± 2.49^{b}	66.79 ± 1.55^{a}	54.31 ± 2.43^{c}	1.23 ± 0.12

Average values in the same row with different superscript letters are significantly different ($p < 0.05$).

Table 3. Correlations between bioactive compound concentration and antioxidant activity.

	Catechins	Polyphenols	DPPH	ABTS	ORAC
Catechins	1.000	0.983	0.771	0.905	0.936
Polyphenols	0.983	1.000	0.733	0.953	0.901
DPPH	0.771	0.733	1.000	0.810	0.944
ABTS	0.905	0.953	0.810	1.000	0.897
ORAC	0.936	0.901	0.944	0.944	1.000

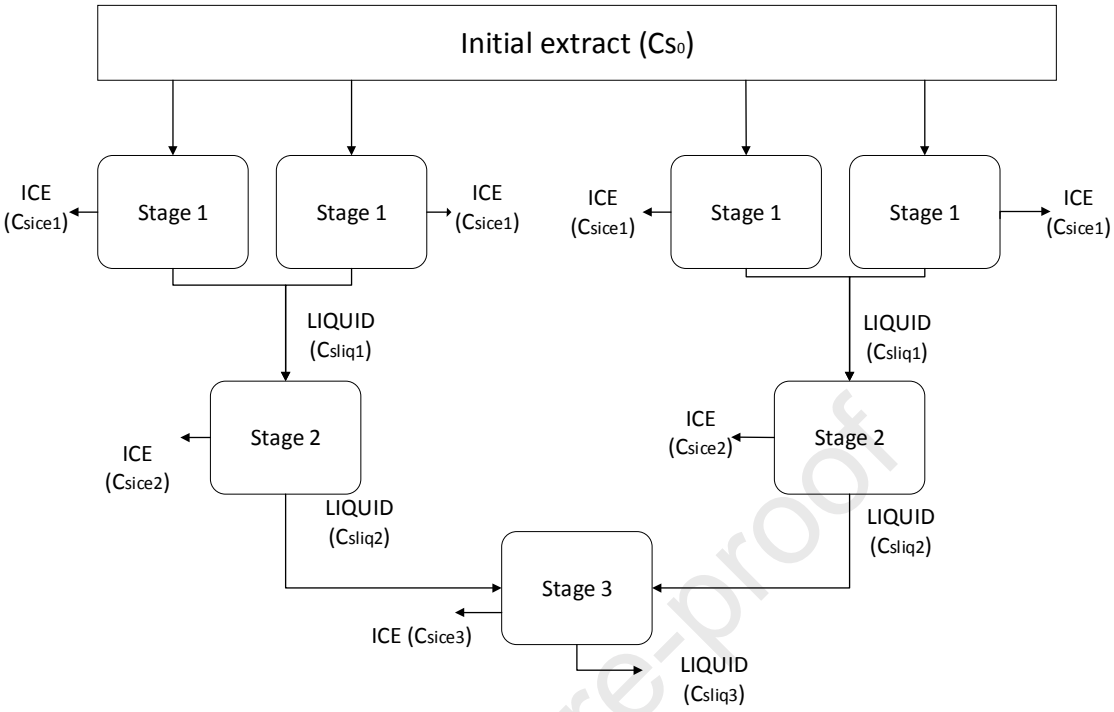

Figure 2. Multistage block freeze concentration process applied in the tests. C_{s0} (initial solids concentration), C_{sice} (solids concentration in the ice phase), and C_{sLiq} (solids concentration in the liquid phase). ICE streams were discarded.

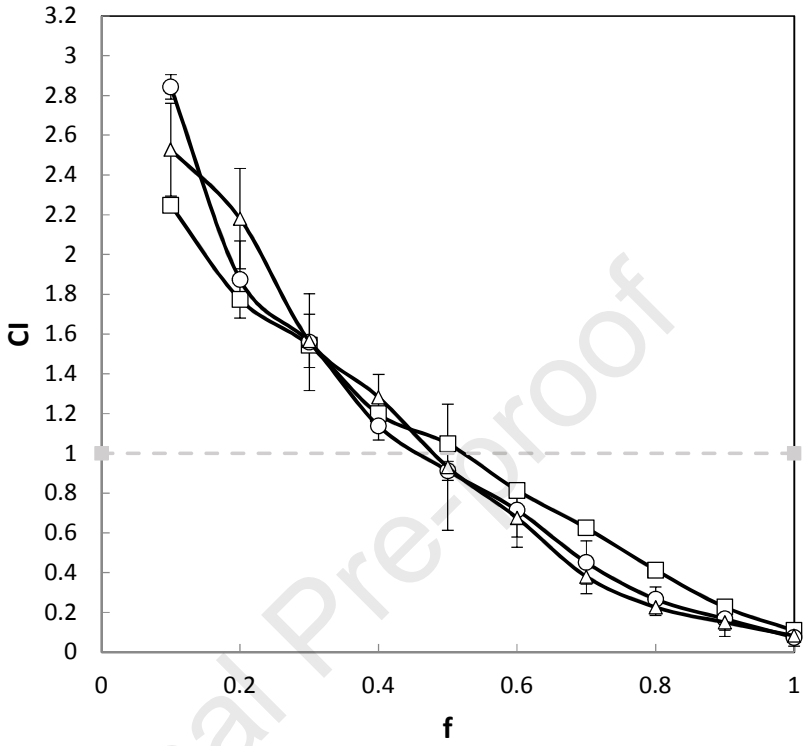

Figure 3. Concentration index as a function of thawing fraction; (□) First stage (○) second stage, and (Δ) third stage

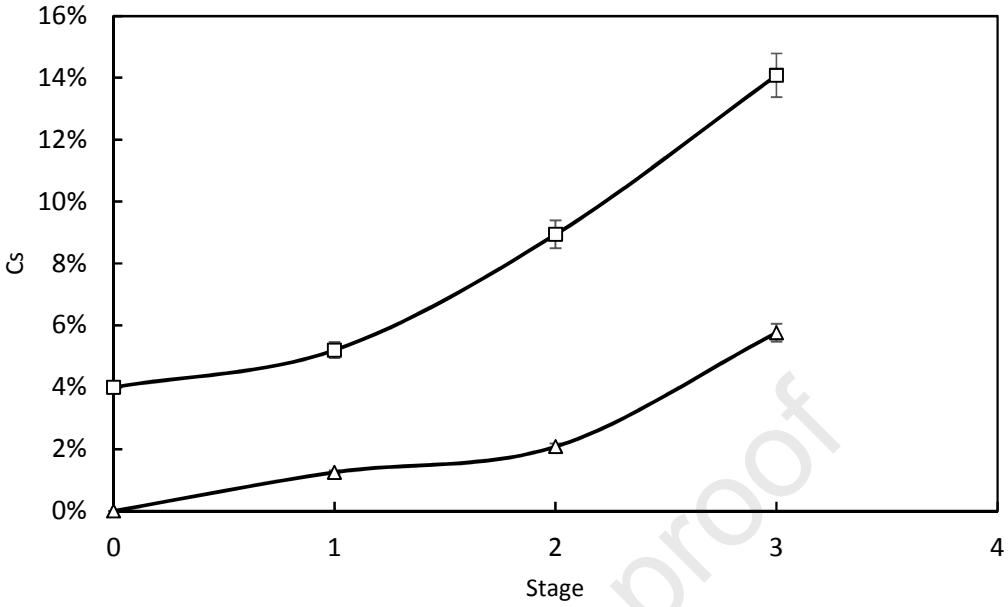

Figure 4. Solids concentration in liquid and ice at each BFC stage. (Δ) Ice phase and (□) Liquid phase.

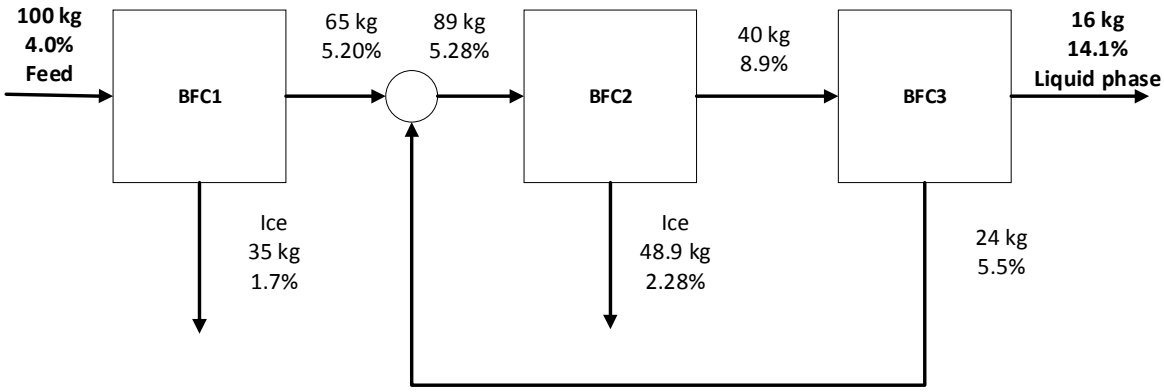

Figure 5. The proposed process of freeze-concentrating green tea extract using block freeze-concentration (BFC). Ice streams of stages 1 and 2 are final effluents. The liquid phase in the stage 3 is the final concentrated product. Solid concentration is expressed as % (w/w),

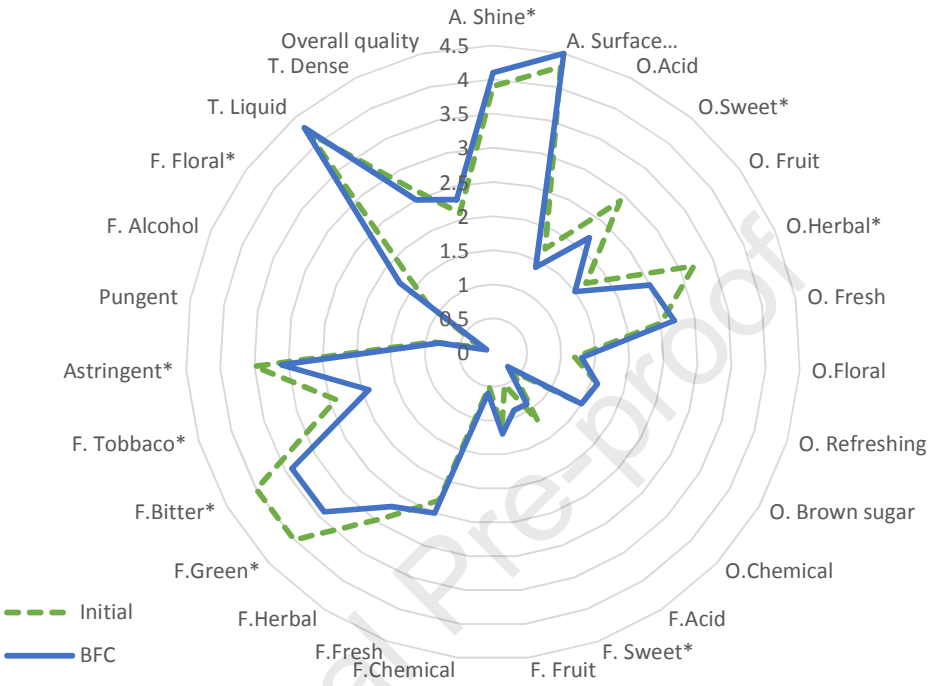

Figure 6. Sensory profile of the liquid after three stages of (—) BFC and (– –) the initial green tea extract without treatment at the same solid content. Parameters marked (*) showed a significant difference between BFC and the initial extract.

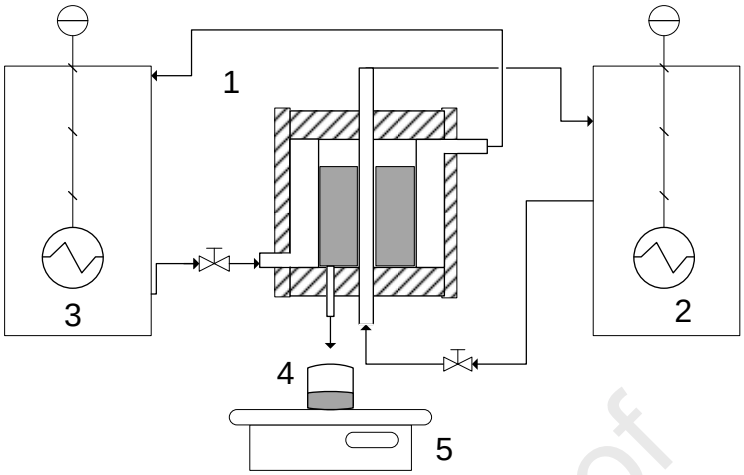

Figure 1. Experimental set-up for block freeze-concentration

Adapted from (Moreno et al., 2014a)

Highlights

Green tea extract was freeze-concentrated by the block technique.

The content of bioactive compounds was increased after the concentration.

The technique preserved the functional properties of the green tea extract.

The sensory attributes of the extract were preserved.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: