Block freeze-concentration of coffee extract: effect of freezing and thawing stages on solute recovery and bioactive compounds

F.L. Moreno, M. Raventós, E. Hernández, Y. Ruiz

PII: S0260-8774(13)00400-7
DOI: http://dx.doi.org/10.1016/j.jfoodeng.2013.07.034
Reference: JFOE 7502

To appear in: *Journal of Food Engineering*

Received Date: 28 May 2013
Revised Date: 17 July 2013
Accepted Date: 21 July 2013

Please cite this article as: Moreno, F.L., Raventós, M., Hernández, E., Ruiz, Y., Block freeze-concentration of coffee extract: effect of freezing and thawing stages on solute recovery and bioactive compounds, *Journal of Food Engineering* (2013), doi: http://dx.doi.org/10.1016/j.jfoodeng.2013.07.034

Block freeze-concentration of coffee extract: effect of freezing and thawing stages on solute recovery and bioactive compounds

Moreno[1,2,3] F.L., Raventós[2] M., Hernández[2] E. and Ruiz[3]* Y.

[1]Biosciencies Doctoral Program, Universidad de La Sabana, Campus Universitario del Puente del Común, Km 7 Autopista Norte de Bogotá, Chía – Cundinamarca – Colombia.

[2]Agri-Food Engineering and Biotechnology Department, Universidad Politécnica de Cataluña (UPC) C/ Esteve Terradas, 8. 08860 Castelldefels –Barcelona- España

[3]Agroindustrial Process Engineering, Universidad de La Sabana, Campus Universitario del Puente del Común, Km 7 Autopista Norte de Bogotá, Chía – Cundinamarca – Colombia.

*Corresponding Author: tel: (+571) 8615555 Ext 25217; e-mail: ruth.ruiz@unisabana.edu.co

Abstract

Coffee extract was freeze-concentrated using the total block technique. The effects of four parameters were evaluated: the initial coffee mass fraction (5 and 15% w/w), the cooling temperature (-10 and -20 °C), the heating temperature (20 and 40 °C) and the freezing direction (parallel and counter-flow to the thawing direction). The solid concentration was measured during the thawing stage to quantify the solute recovery and the concentration index for one stage of freeze concentration. The coffee mass fraction, the freezing direction and the cooling temperature significantly influenced the solute recovery. A concentration index between 1 and 2.3 was obtained in one cycle. The effect of block freeze concentration on the bioactive compound concentration and the antioxidant activity was measured. The coffee bioactive compounds were distributed in proportion to the total solid content in the ice and liquid. Therefore, block freeze concentration is an effective technique to preserve functional properties of coffee extracts.

Keywords: Cryoconcentration; solute yield; coffee; chlorogenic acids; antioxidant activity

1. Introduction

Coffee is the most traded food in the world, and its production has great economic and social importance worldwide (Esquivel & Jiménez, 2012; Vignoli et al., 2011). For the consumer, the value of coffee is provided by its sensory and functional properties; for this reason, technologies that promote quality preservation are

highly valued in coffee processing. In the production of freeze-dried coffee, freeze concentration (FC) technology is used to remove water from coffee extracts to increase the solid content and reduce the time and cost of the freeze-drying process. At the same time, the sensory properties of the product are preserved using low temperatures (Boss et al., 2004; Joët et al., 2010; Sánchez et al., 2009).

Water removal in FC is achieved by cooling the solution until the ice crystals form and separate (Miyawaki et al., 2005). Three techniques are used according to the ice crystal growth: suspension FC, film FC (progressive or falling film FC) and block FC (total or partial) (Aider & de Halleux, 2009; Sánchez et al., 2009). Suspension FC is a unique technique implemented at the industrial level. Different techniques, such as falling film FC, (Chen et al., 1998; Sánchez et al., 2011), progressive FC (Miyawaki et al., 2005) and block FC (Aider & Ounis, 2012; Nakagawa et al., 2010a), are being developed to reduce operational costs.

In the block FC method, also known as freeze–thaw concentration, the solution to be concentrated is completely frozen and then partially thawed to recover a fraction of liquid with a higher concentration (Aider & de Halleux, 2009; Nakagawa et al., 2010b). Block FC consists of three stages: freezing, thawing and separation of the concentrated liquid fraction (Moreno et al., 2013). These stages define the separation efficiency (Nakagawa et al., 2009). Additionally, the process can be repeated in successive cycles to increase the concentration index (Aider & Ounis, 2012).

The technical viability of the block FC method has been proposed recently by several researchers (Gao et al., 2009; Nakagawa et al., 2010a; Aider & Ounis, 2012; Boaventura et al., 2012; Miyawaki et al., 2012; Petzold et al., 2013). During the freezing stage, heat and mass transfer phenomena can modify the solute occlusion, which should be as low as possible. Chen et al. (2001) reported that the solute elution in the freezing front in FC depends on the molecular size of the compounds. Certain authors have reported that the solute separation is controlled by the thawing stage (Nakagawa et al., 2010b). For coffee solutions, Moreno et al. (2013) studied the use of aids in the separation stage. These authors reported the influence of the FC protocol and solution type on solute recovery and the concentration index; for this reason, there is no agreement on the significance of the process variables. The effects of the process variables of block FC on the separation efficiency of coffee extracts have not been reported.

Coffee can be considered to be a functional beverage due to its radical scavenging capabilities (Cheong et al., 2013; Esquivel & Jiménez, 2012). Several studies have reported the health benefits of coffee consumption related to the components with antioxidant activity, such as the group of chlorogenic acids and caffeine.

Chlorogenic acid (3-caffeoylquinic acid), cryptochlorogenic acid (4-caffeoylquinic acid), neoclorogenic acid (5-caffeoylquinic acid) and caffeine are the major bioactive compounds present in coffee (Ferruzzi, 2010; Fujioka & Shibamoto, 2008; Sopelana et al., 2013; Vignoli et al., 2011). The block FC method has been shown to retain nutritional and functional properties of the product using low processing temperatures (Belén et al., 2013; Boaventura et al., 2012); however, this effect has not been tested for coffee extracts.

The aim of the present study was to evaluate the effect of the initial coffee mass fraction, the cooling temperature, the heating temperature and the freezing direction on the solute yield and concentration index of block freeze-concentrated coffee extracts. Additionally, the impact of the technique on bioactive compound concentration and the antioxidant activity of the coffee extract was tested.

2. Materials and Methods

2.1. Materials

Coffee solutions were prepared from freeze-dried soluble coffee supplied by the company Buencafé Liofilizado de Colombia (Colombian Coffee Growers Federation, Colombia) for the FC tests. The coffee was added to distilled water at 35 °C and mixed for 20 min. The samples were stored at 4 °C for 12 h. The solid concentration is expressed as the coffee mass fraction (X_S), which is defined as the mass of coffee solids per unit of coffee solution mass. The relationship between Brix degrees and X_S is represented by the equation X_S=0.0087 °Brix (R^2=0.991). This expression was obtained by preparing coffee solutions at 10, 20, 30, 40 and 50 °Brix and by measuring coffee mass fraction using the weight loss technique in the oven at 103 °C for four hours according to technical standard NTC4602 (Icontec, 2009). The measurements were performed in triplicate. The coffee mass fraction of the solutions was ascertained immediately before the FC tests by refractometry (Atago Pal 100, Japan). A liquid coffee extract was used for the measurement of bioactive compounds. This extract belonged to the same batch of soluble coffee and was also provided by Buencafé Liofilizado de Colombia.

2.2. Methods

2.2.1 Freeze concentration protocol

The effects of the initial coffee mass fraction (X_S), cooling temperature (T_C), heating temperature (T_H) and the freezing direction (F_D) were studied. A full factorial design with four factors and two levels was used for a total number of 16 tests (Table 1). The coffee solutions were subjected to one cycle of freezing,

thawing and separation to study the effect of process variables on solute yield after one cycle of FC.

The block FC device is shown in Fig. 1. In total, 160 g of the coffee sample was placed into a cylindrical container (1) measuring 52.5 mm in diameter and 85 mm in height. The container is a double jacket device for the flux of cooling and heating fluids. The internal jacket is 19 mm in diameter (2). The cooling/heating fluid was a mixture of ethylene glycol and water (53% w/w) coming from two circulated baths (4 and 5) (Polystat, Cole Parmer, USA). The baths were temperature controlled (6 and 7) at an interval from -35 °C to 150 °C +/- 0.01 °C. The baths pumped the heat exchange fluid to the jackets through a system of ducts and valves (7).

During the tests, the heat exchange fluid temperature was settled in one bath. After the fluid reached the temperature, it was circulated to the jackets to freeze the solution inside. The heat transfer was in the radial direction from the internal wall (for freezing parallel to thawing) or from the external wall (for freezing in counter-flow to thawing). Meanwhile, the heating temperature of the second bath was settled. When the sample was frozen and the temperature was approximately constant, the thawing stage was begun by pumping the heating fluid through the external jacket. The exit valve (9) was opened and the liquid fraction was separated in a collector vessel (10) on a scale (11) (Ohaus PA3102, USA) with a capacity of 3100 g and a precision of 0.01 g for weight measurement. During the thawing stage, the temperature of the internal jacket was maintained one Celsius degree below the freezing point of the coffee solution to avoid thawing the internal side and to preserve unidirectional thawing. Ten liquid fractions of the same mass were collected. Lastly, the coffee mass fraction (X_S) was measured by refractometry (Atago Pal 100, Japan).

2.2.2. Temperature profile

The FC device seen in Fig. 1 has four temperature sensors (12) inside of the container to measure the temperature profile during one test. These sensors were used during the temperature measuring tests but not during the FC tests. The sensors PT100-IP65 (Testo, Germany) had a 2 mm diameter and a measuring interval of -50 to 300 °C +/- 0.01 °C and were placed equidistant from the centre of the container (sensor 1) and the external wall (sensor 4). The sensors were connected to a datalogger 176 T2 (Testo, Germany) connected to a PC for data collection.

2.2.3 Data analysis

2.2.3.1 Thawing fraction (f)

A thawing fraction (f) was used to follow the development of the process. The f was measured as the ratio between the thawed mass and the mass of the original solution, defined by Eq. 1. (Miyawaki et al., 2012; Nakagawa et al., 2010a):

$f = m_{liq}/_{m0}$ (1)

where

f: thawing fraction

m_{liq}: collected liquid mass

m_0: initial mass.

2.2.3.2 Solute Yield (Y)

Solute yield was calculated for analysing the solute recovery. Y was defined as the relationship between the mass of solute present in the separated liquid and the mass of solute present initially in the original solution, as seen in Eq. 2. (Moreno et al., 2013; Nakagawa et al., 2010a):

$Y=m_{s\ liq}/m_{s\ 0}$ (2)

where

Y: solute yield

$m_{s\ liq}$: solute mass in the liquid fraction

$m_{s\ 0}$: initial solute mass.

2.2.3.3 Concentration Index

The concentration index (CI) was used to express the concentration of solutes reached after the FC process. CI was defined as the relationship between the solid concentration in the liquid fraction and the solid concentration in the initial solution. CI is also known as relative concentration (Nakagawa et al., 2009):

$CI = X_{s\ liq} / X_{s\ 0}$ (3)

where

CI: concentration index

$X_{s\ liq}$: coffee mass fraction in the freeze-concentrated liquid fraction

$X_{s\ 0}$: coffee mass fraction in the initial solution.

When the CI is calculated using the mixture of the thawed fractions at a given time, Eq. 3. can be expressed as the cumulative index (CI_{cum}). Cumulative CI is the relationship between X_S in the accumulated liquid fraction and X_S in the initial solution.

Eq. 4. was obtained by combining Eq. 2. and Eq. 3.:

$Y = CI_{cum} * f$ (4).

2.2.3.4 Area under curve Y vs. f

During the thawing stage of FC, the graph Y against f represents the percentage of coffee solids that was recovered from the initial solution for each thawed liquid fraction. The behaviour of a freeze concentration test can be represented by Fig. 2, as proposed by Nakagawa, et al. (2010a).

The diagonal line represents the case in which the thawed liquid fraction had the same concentration as the initial solution; therefore, there was no FC. A higher curve from the diagonal indicates the amount of recovered solute for a given f and the efficiency of the separation were greater. An ideal situation would be a curve very close to the y-axis in which all of the solute was recovered at the beginning of the thawing stage. Therefore, the area under the curve Y vs. f can be used as a single parameter to compare the efficiency of the separation process and to examine the effect of the studied factors.

The area under the curve represents the integral of the function Y vs. f. The area value is bounded between 0 when the solutes are not recovered and by 1 when all the solutes are recovered instantaneously and there is no solute occlusion. The diagonal line of no-concentration has an area of 0.5. An area value closer to one indicates a better result of the FC process. The area under the curve can be understood as the sum of the solute yield achieved in a thawing fraction during the FC. The area under curve Y vs. f was used as an identifying parameter of the effect of each studied variable.

Freezing front growth rate

The average freezing front growth rate was calculated by measuring the distance from the cooling surface to the front of the ice during the freezing stage with a calliper. The average of the ratios of distance to time was used as the rate. The rate was expressed in micrometres per second.

2.2.4 Bioactive compound measurement

The major bioactive compounds of coffee extract, chlorogenic acid (CGA), cryptochlorogenic acid (c-CGA) and caffeine, were measured for the initial solution, the freeze-concentrated liquid recovered at a f value of 50% and for the residual ice at the same f as a comparative parameter. The measurements were performed for tests 1 and 16 (Table 1), which correspond to the extreme values of X_S, T_C, T_H and total process time. The measurements were performed in triplicate.

The concentration of bioactive compounds was determined by reversed-phase high performance liquid chromatography (RP-HPLC) as described by Fujioka & Shibamoto (2008) and Owen et al. (2003) with modifications. The RP-HPLC apparatus, LaChrom (Merck-Hitachi, Germany-Japan), was equipped with a quaternary pump, degasification system and a diode array detector (UV/VIS). The separation was achieved in a Gemini column C-18 (Phenomenex, USA) measuring 250 mm * 4.6 mm and 5 µm at 25 °C. The mobile phase used was acetic acid 2% (A) and methanol (B). The gradient was adjusted as follows: 0-10 min, A/B 96/4; 65 min, 85/15; 75 min, 75/25; and 85 min, 25/75 at a flow rate of 1 mL·min^{-1}. CGA and c-GCA were detected at 325 nm, and caffeine was detected at 276 nm. The injection volume was 5 µL. The concentrations of bioactive compounds were calculated using a regression equation of their concentrations and the peak area obtained from pattern grade HPLC (Sigma-Aldrich, USA).

The loss of bioactive compounds in the residual ice due to FC was calculated using Eq. 5. (Ramos et al., 2005):

$$IL = C_{RI} / (C_{RI} + C_{FCL}) * 100 \quad (5)$$

where

IL: ice loss

C_{RI}= concentration of bioactive compounds in the residual ice

C_{FCL}= concentration of bioactive compounds in the freeze-concentrated liquid.

2.2.5. Antioxidant activity

The antioxidant activity of coffee samples was determined by the ABTS (2,20-azinobis (3-ethylbenzothiazoline-6-sulphonic acid assay) and DPPH (2,2-diphenyl-1-picrylhydrazyl assay) methods.

ABTS methodology

Antioxidant activity was estimated in terms of radical scavenging activity using the procedure described by Vignoli et al. (2011) with modifications. Briefly, ABTS radical cations ($ABTS^+$) were produced by reacting 3.5 mM ABTS stock solution

with 12.5 mM potassium persulphate prepared in a 10% phosphate buffer solution at a pH of 7.4 in distilled water. The solution was stored in the dark at room temperature for 12 h. Before the analysis, the solution was settled at 0.8 +/- 0.2 nm. Lastly, 50 µL of the coffee sample was added to 200 µL of ABTS+ solution and the absorbance was read after 30 min of incubation in complete darkness using an iMark Microplate Reader spectrophotometer (Bio-Rad, USA). The same procedure was conducted for calibration with ethanol solutions containing known concentrations of Trolox between 3 and 15 $\mu L \cdot mL^{-1}$. The results were expressed in g of Trolox per 100 g of coffee (dry matter). The experiments were performed in triplicate.

DPPH methodology

The DPPH technique was performed according to Vignoli et al. (2011). A DPPH solution was prepared at 0.6 mM of methanol. The absorbance was settled to 1.1 nm before the tests. For the analysis, 50 µL of DPPH solution was added to 75 µL of each coffee sample. The absorbance was measured at 515 nm after 30 min of incubation at room temperature in complete darkness. The calibration was performed with Trolox at concentrations between 3 and 15 $\mu L \cdot mL^{-1}$. The antioxidant activity was expressed as mg of Trolox/mL. The experiments were performed in triplicate using methanol as a blank.

2.3 Statistical analysis

All the tests were performed in triplicate. The area under the curve of Y vs. f was obtained by a spline regression procedure. A response surface regression procedure was used to determine the effect of each studied factor listed in Table 1 on the area under the curve with a confidence interval of 95%. One-way analysis of variance (ANOVA) was applied to the results of the area under the curve followed by a LSD test with a level of significance of 95%. For the bioactive compound measurement, the mean values were calculated and a correlation analysis was performed by comparing the Pearson coefficients. All statistical analysis were performed using the SAS 9.2 software package

3. Results and discussion

3.1 Temperature profiles

The temperature profiles during FC tests for tests 1 and 8 described in Table 1 are shown in Fig. 3. These tests corresponded to the lowest and highest overall process time; therefore, the other tests were within this time interval. Temperature sensor 1 was located beside the internal wall of the container and sensor 4 was located in the external wall. In test 1, the freezing was achieved from the centre

and the thawing from the external wall. For this reason, the temperature dropped first in sensor 1 and last in sensor 4, as can be seen in Fig. 3a; during the thawing stage, the order was reversed.

Point “a” shows the nucleation phenomenon that caused a temperature increase due to the latent heat of the phase change. The latent heat released from the portion of liquid closest to the centre (line 1) caused a temperature increasing of the external portion before it was frozen (line 4). For this reason, a temperature increasing in the interval 0°C to 5°C was observed. After that, the whole solution was frozen and the temperature tended to be constant. A similar behaviour was reported by Nakagawa et al. (2010b).

When the temperature was stable, the thawing phase began (point b). A change in the temperature was observed in the opposite order than it was in the freezing stage. For test 1, the thawing time was 180 min and the average freezing growth rate was 1.84 $\mu m \cdot s^{-1}$. Alternatively, in test 8 (Fig. 3b), the freezing and the thawing were both achieved from the external wall in parallel. The first portion that was frozen and thawed corresponded to sensor 4, which was located beside the external wall. A freezing time of 45 min and an average ice growth rate of 7.17 $\mu m \cdot s^{-1}$ were obtained.

3.2 Freeze-concentration tests

The results of the block FC tests are shown in Table 2 in descending order of the area under the curve of Y vs. f. The greatest area was obtained for test 1, which corresponded to the lowest coffee mass fraction, the greatest cooling temperature, the lowest heating temperature and freezing direction in counter-flow to the thawing. The treatments showed significant differences at $p<0.05$. The LSD test indicated differences among treatments for X_S 0.05. Differences in F_D and T_C can be observed. On the contrary, the tests with the highest X_S did not show significant difference because the solid concentration is predominant over the effect of the other factors.

Table 2. Freeze concentration tests in descending order of area under the curve

The values for Y and f when the CI was equal to one are shown in Table 2. At this value of f, solute yields between 67 and 83% were obtained. At this point, the cumulative CI values were between 1.10 and 1.67. A CI of 1.8 for one cycle of FC was reported by Aider & Ounis (2012). For falling film FC, a CI between 2 and 3 was reported by Sánchez et al. (2011) and Belén et al. (2012). Miyawaki et al. (2005) reported a CI between 2 and 3 for progressive FC. However, all of these authors reported that the results depended on the fluid being concentrated, the type and size of the equipment and the process time.

The maximum CI obtained was 2.38 for test 8. The maximum CI for each test was reached in the first thawing fractions and these values descended during the thawing stage. The concentrated fraction percolates through the frozen matrix (Petzold et al., 2013) and its concentration descended until values close to zero during the thawing stage. It therefore was possible to know the f value at which the CI reached a value of 1. This situation corresponded to the moment at which it becomes convenient to separate the thawing fraction to avoid a cumulative concentration decrease. The f values are shown in Table 2 (f at CI=1) and varied from 0.5 to 0.8. The f value at which the thawing stage has to be stopped depends on the process conditions.

Freezing front growth rates from 1.8 to 7.5 $\mu m \cdot s^{-1}$ were obtained. The values depended on the cooling temperature, the heat transfer area and the initial coffee mass fraction. The area under the curve tended to be higher for lower freezing rates. The result is more evident at low solid concentrations. For high solid concentrations the effect of freezing rate was not observed because of the effect of solid interactions. No concentration was obtained for a freezing rate of 7.5 $\mu m \cdot s^{-1}$. At this rate, the ice occluded solutes during the freezing stage. A critical rate value was also obtained by Nakagawa et al. (2010a). The authors reported that for velocities higher than 8 $\mu m \cdot s^{-1}$, the freezing was too fast to expect a considerable separation of the concentrated solution phase.

3.3 Effect of each operational factor on solute recovery

A regression analysis was performed to determine the statistical significance of the factors of the study on the area under the curve of Y vs. f. The result is shown in Table 3. The analysis showed a good fitting of the experimental data (R^2=0.9874 and RMSE=0.0123). The parameters with a Pr<0.05 significantly affected the area under the curve. The main effects X_S, T_C and F_D and the interaction terms $T_C \cdot X_S$ and $F_D \cdot X_S$ significantly affected the freeze concentration.

The coffee mass fraction had the greatest influence with a negative correlation; in other words, the grade of concentration achieved with the block FC decreased with the increase in X_S. The second main effect was the freezing direction followed by the cooling temperature. The interactions between X_S and the other two mean factors were also significant, indicating the influence of these variables. Alternatively, the T_H did not significantly affect the studied intervals. Nakagawa et al. (2010b) and Moreno et al. (2013) reported that the T_H influenced the solute yield when lower thawing temperatures were compared. This result depended on the FC protocol and the level of T_H.

3.3.1. Effect of initial coffee mass fraction (X_S)

The curves Y against f and CI against f for tests 5 and 13 are shown in Fig. 4. These test had different values of X_S, but the other factors were constant. A higher solute recovery was obtained for $X_S = 0.05$. This result was the same for all of the tests. Comparing the curve with the diagonal line of no-concentration, the area under the curve was higher for the lowest X_S.

This result can be explained by different factors. First, during the freezing stage, the ice grows by the diffusion of water molecules to the ice surface and the counter-diffusion of solutes to the liquid phase (Petzold & Aguilera, 2009). The diffusion rate of solutes decreases when the solid concentration increases due to the interactions between molecules; consequently, the achieved concentration decreases. The same result was reported by Chen et al. (2001) and Hindmarsh, Russell, & Chen (2005). Second, the coffee solution viscosity increases with X_S; this factor can cause the separation of the liquid phase during the thawing stage to be difficult (Raventós et al., 2007). Additionally, the ice tends to grow in dendritic form for high solid concentrations, occluding higher amounts of solutes (Yee et al., 2003). Lastly, the volume of water that can be frozen and separated is lower for higher concentrations (Aider & de Halleux 2008a). The combination of these four effects explains why X_S was the factor with the greatest effect on Y and CI.

In the CI vs. f curve, the value at which the CI crossed the horizontal line of CI=1 is shown in Fig. 4 to 7. This intersection corresponded to the moment at which the thawing stage has to be completed to avoid a sample dilution and to recover as much solute as possible. For test 1, at f=0.5, 83% of the coffee solids had been recovered and the cumulative CI was 1.67. These results indicate a good separation efficiency.

3.3.2. Effect of freezing direction (F_D)

Tests 1 and 2 are shown in Fig. 5a. Tests 9 and 10 are shown in Fig. 5b. Table 2 shows the differences in freezing direction. A better FC was obtained for the tests in which the freezing and thawing directions were opposite.

For the F_D during counter-flow, the CI began in maximum values and descended during the thawing. This result indicates that the solutes moved during the freezing stage to the farthest zone from the container centre, which was the last area frozen and the first area thawed. Additionally, the lower heat transfer area in the internal wall compared to the external wall facilitated the solute elution due to the slower ice front growth. Alternatively, the initial CI was lower than the CI for the tests when the F_D was in parallel. The solutes moved from the external region of the container, as evidenced by the smaller X_S compared to the initial X_S. This finding can be explained by the elution phenomenon, in which a movement of the solutes was

produced by counter-diffusion during ice crystals formation that expelled the solutes to the liquid fraction. This result was also observed by Nakagawa et al. (2009). Moreover, Chen et al. (2001) reported that elution depends on the molecular size of the solutes. The effect is smaller with increasing initial solute contents.

For the tests when the F_D was in parallel, the CI was lower than counter-flow direction at the beginning, and then it increased at f values from 0.2 to 0.4. This result could have occurred because the solutes diffused from the concentrated liquid fractions to the droplet of water that had melted during thawing. This phenomenon is known as sweating. This result was also reported by Nakagawa et al. (2009). Likewise, the concentrated portions were the first fractions to be thawed and separated due to the difference in densities, as reported by Yee et al. (2003). The results show the freezing direction as a variable of interest in the study of block FC to promote the intensification of solute recovery.

3.3.3. Effect of cooling temperature (T_C)

Fig. 6 shows the tests comparing the effect of T_C. Better outcomes were obtained at -10 °C. The heat transfer rate was slower at higher cooling temperatures. The ice crystals were able to grow in a more ordered pattern that occluded a smaller amount of solutes. For the elution to occur, the solutes mass transfer rate must be greater than the ice growth rate (Caretta et al., 2006; Petzold & Aguilera, 2009). In addition, the ice crystal size depends on the cooling rate, which can affect the level of occlusion (Pardo et al., 2002). Certain authors have reported that the cooling temperature is not a significant factor impacting block FC (Aider & de Halleux, 2008b; Gao et al., 2009). These reports suggest that the effect of T_C depends on the FC protocol during both the freezing and thawing stages and depends on the separation mode of the liquid phase.

3.3.4. Effect of heating temperature (T_H)

A slightly higher solute recovery was obtained at a T_H of 20 °C (Fig. 7). The average temperature in the empty region of the container after each liquid fraction separation during the thawing was 10 °C (Fig. 3). The treatments with the lowest T_H allowed for a slower thawing and avoided the dilution of the concentrated phase. However, the effect was not significant at the studied levels. The T_H may be significant at different levels, as reported by Moreno et al. (2013) where the tested heating temperatures were closer to the freezing point.

3.4 Bioactive compounds and the antioxidant activity of coffee

The concentrations of the major bioactive compounds in coffee solutions were determined for the initial solution (C_0), the liquid freeze-concentrated liquid (C_{FCL}) and the residual ice (C_{RI}) obtained for a thawing fraction of 50%. A typical chromatogram is shown in Fig. 8. Chlorogenic acids were the major component in the solutions. The bioactive compounds concentration and the ice loss percentage are shown in Table 4.

The ice loss percentage was approximately 16% for the lowest X_S and 41% for the highest X_S. This factor was related to the concentration index. When the ice loss (IL) was calculated on a coffee dry matter basis, the result was approximately 50%. This result indicates that the functional compounds were equally distributed in the ice and liquid fractions. There was a greater amount of bioactive compounds in the liquid phase because the concentrated liquid had a higher X_S. The results correspond to tests 1 and 16 (Table 1), which had extreme values of X_S, T_C, T_H and total process time. All of the other tests were inside the intervals of tests 1 and 16.

The CI of total coffee solids for tests 1 and 16 at f=0.5 were 1.60 and 1.15, respectively as seen in table 4. These values were statistically equal to the CI for the bioactive compounds, according to the LSD test. A higher significant correlation (1.00) was found ($p<0.01$) between X_S and the concentrations of CGA, 4-CQA and caffeine. The same correlation between CI and %LI was found. Consequently, the amount of bioactive compounds was maintained in proportion to the amount of total coffee solids. Therefore, the concentration of bioactive compounds was enhanced through freeze concentration and the bioactive compounds of the beverage were preserved by block FC.

Highly significant correlations ($p<0.01$) between antioxidant activity measured by DPPH and the concentrations of CGA, c-CGA and caffeine were demonstrated, as seen in table 5. The correlations of the ABTS measurements were significant ($p<0.05$). These results confirm that the antioxidant activity of coffee depends on the CGA and caffeine content, as reported by Fujioka & Shibamoto (2008).

A ratio between the antioxidant activity of the liquid fraction and the initial solution was calculated to represent the antioxidant activity relative index (C_{FCL}/C_0) (Table 4). There was no significant difference between the antioxidant activity relative index and the CI of the total coffee solids. The antioxidant activity was increased until 2.4 in one FC cycle. The increase of the antioxidant activity of mate extract was also reported by Boaventura et al. (2012) using block freeze concentration. This finding suggests block FC is an effective technique to preserve the functional properties of coffee extracts.

4. Conclusions

Coffee extract was freeze-concentrated by the total block technique. A significant effect of the initial coffee mass fraction, freezing direction and cooling temperature on solute recovery was found. The highest solute recovery was achieved at the lowest coffee mass fraction, when the freezing direction was in counter-flow to the thawing direction and at the highest cooling temperatures. The thawing fractions at which completion of the thawing stage was convenient were found between the values of 0.5 and 0.8. The initial coffee mass fraction was the factor with the highest influence on the solute yield and the concentration index. Using a freezing direction in counter-flow to the thawing direction represents an interesting alternative to increase solute recovery due to solute elution. Furthermore, the coffee bioactive compounds were distributed in the ice and liquid phase in proportion to the total solid content. Very significant correlations between the antioxidant activity and chlorogenic acid and caffeine contents in the freeze-concentrated extract were found. Consequently, the freeze concentration method increased the bioactive compound concentration and the antioxidant activity of the coffee extract. The block freeze concentration method is a potential technique to remove water and preserve the functional properties of coffee extracts.

5. Acknowledgements

The research was supported by Universidad de La Sabana and COLCIENCIAS with the project 1230521-28461 (2011). The authors thank Eng. Carlos Osorio of Buencafe Liofilizado de Colombia (Colombian Coffee Growers Federation) for providing the coffee and assisting with the research. Author Moreno F L thanks COLCIENCIAS for its condonable grant for doctoral studies (2013). Author Ruiz Y thanks COLCIENCIAS for its condonable grant for doctoral studies (2004).

Nomenclature

CI	Concentration index
CI_{cum}	Cumulative concentration index
C_{FCL}	Concentration of bioactive compounds in the freeze-concentrated liquid
C_{RI}	Concentration of bioactive compounds in the residual ice
f	Thawing fraction
F_D	Freezing direction
IL	Ice loss percentage
$m_{s\,0}$	Initial solute mass

$m_{s\ liq}$	Solute mass in the liquid fraction
m_0	Initial mass
m_{liq}	Collected liquid mass
T_C	Cooling temperature
T_H	Heating temperature
$X_{s\ 0}$	Coffee mass fraction in the initial solution
X_S	Coffee mass fraction
$X_{s\ liq}$	Coffee mass fraction in the freeze-concentrated liquid fraction
Y	Solute yield

6. References

Aider, M., & Ounis, W. B. (2012). Skim milk cryoconcentration as affected by the thawing mode: gravitational vs. microwave-assisted. *International Journal of Food Science & Technology*, 47(1), 195-202.

Aider, M., & de Halleux, D. (2008a). Production of concentrated cherry and apricot juices by cryoconcentration technology. *LWT - Food Science and Technology*, 41(10), 1768-1775.

Aider, M., & de Halleux, D. (2008b). Passive and microwave-assisted thawing in maple sap cryoconcentration technology. *Journal of Food Engineering*, 85(1), 65-72.

Aider, M., & de Halleux, D. (2009). Cryoconcentration technology in the bio-food industry: Principles and applications. *LWT - Food Science and Technology*, 42(3), 679-685.

Belén, F., Benedetti, S., Sánchez, J., Hernández, E., Auleda, J. M., Prudêncio, E. S., Petrus, J. C. C., et al. (2013). Behavior of functional compounds during freeze concentration of tofu whey. *Journal of Food Engineering*, 116(3), 681-688.

Belén, F., Sánchez, J., Hernández, E., Auleda, J. M., & Raventós, M. (2012). One option for the management of wastewater from tofu production: Freeze concentration in a falling-film system. *Journal of Food Engineering*, 110(3), 364-373.

Boaventura, B. C. B., Murakami, A. N. N., Prudêncio, E. S., Maraschin, M., Murakami, F. S., Amante, E. R., & Amboni, R. D. D. M. C. (2012).

Enhancement of bioactive compounds content and antioxidant activity of aqueous extract of mate (Ilex paraguariensis A. St. Hil.) through freeze concentration technology. *Food Research International*. doi:10.1016/j.foodres.2012.07.042

Boss, E. A., Filho, R. M., & de Toledo, E. C. V. (2004). Freeze drying process: real time model and optimization. *Chemical Engineering and Processing: Process Intensification*, 43(12), 1475-1485.

Caretta, O., Courtot, F., & Davies, T. (2006). Measurement of salt entrapment during the directional solidification of brine under forced mass convection. *Journal of Crystal Growth*, 294(2), 151-155.

Chen, P., Chen, X. D., & Free, K. W. (1998). Solute Inclusion in Ice Formed From Sucrose Solutions on a Sub-cooled Surface-an Experimental Study. *Process Engineering*, 38(1), 1-13.

Chen, Y. H., Cao, E., & Cui, Z. F. (2001). An experimental study of freeze concentration in biological media. *Trans IChemE*, 79, 35-40.

Cheong, M. W., Tong, K. H., Ong, J. J. M., Liu, S. Q., Curran, P., & Yu, B. (2013). Volatile composition and antioxidant capacity of Arabica coffee. *Food Research International*, 51(1), 388-396.

Esquivel, P., & Jiménez, V. M. (2012). Functional properties of coffee and coffee by-products. *Food Research International*, 46(2), 488-495.

Ferruzzi, M. G. (2010). The influence of beverage composition on delivery of phenolic compounds from coffee and tea. *Physiology & behavior*, 100(1), 33-41.

Fujioka, K., & Shibamoto, T. (2008). Chlorogenic acid and caffeine contents in various commercial brewed coffees. *Food Chemistry*, 106(1), 217-221.

Gao, W., Habib, M., & Smith, D. W. (2009). Removal of organic contaminants and toxiciy from industrial effluents using freezing processes. *Desalination*, 245(1-3), 108-119.

Hindmarsh, J. P., Russell, a. B., & Chen, X. D. (2005). Measuring dendritic growth in undercooled sucrose solution droplets. *Journal of Crystal Growth*, 285(1-2), 236-248.

Icontec. (2009). NTC 4602-1, Determination of extraction yield and soluble solids in coffee beverage. Colombia.

Joët, T., Laffargue, A., Descroix, F., Doulbeau, S., Bertrand, B., Kochko, A. D., & Dussert, S. (2010). Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. *Food Chemistry*, 118(3), 693-701.

Miyawaki, O., Liu L., Shirai, Y., Sakashita, S., Kagitani, K. (2005). Tubular ice system for scale-up of progressive freeze-concentration. *Journal of Food Engineering*, 69, 107-113.

Miyawaki, O., Kato, S., & Watabe, K. (2012). Yield improvement in progressive freeze-concentration by partial melting of ice. *Journal of Food Engineering*, 108(3), 377-382.

Moreno, F. L., Robles, C. M., Sarmiento, Z., Ruiz, Y., & Pardo, J. M. (2013). Effect of separation and thawing mode on block freeze-concentration of coffee brews. *Food and Bioproducts Processing*, doi: 10.1016/j.fbp.2013.02.007

Nakagawa, K., Maebashi, S., & Maeda, K. (2009). Concentration of aqueous dye solution by freezing and thawing. *The Canadian Journal of Chemical Engineering*, 87(5), 779-787.

Nakagawa, K., Maebashi, S., & Maeda, K. (2010a). Freeze-thawing as a path to concentrate aqueous solution. *Separation and Purification Technology*, 73(3), 403-408.

Nakagawa, K., Nagahama, H., Maebashi, S., & Maeda, K. (2010b). Usefulness of solute elution from frozen matrix for freeze-concentration technique. *Chemical Engineering Research and Design*, 88(5-6), 718-724.

Owen, R. ., Haubner, R., Mier, W., Giacosa, a, Hull, W. ., Spiegelhalder, B., & Bartsch, H. (2003). Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food and Chemical Toxicology*, 41(5), 703-717.

Pardo, J M, Suess, F., & Niranjan, K. (2002). An investigation into the relationship between freezing rate and mean ice crystal size of coffee extarcts. *Trans IChemE*, 80, 176-182.

Petzold, G., & Aguilera, J. M. (2009). Ice Morphology: Fundamentals and Technological Applications in Foods. *Food Biophysics*, 4(4), 378-396.

Petzold, G., Niranjan, K., & Aguilera, J. M. (2013). Vacuum-assisted freeze concentration of sucrose solutions. Journal of Food Engineering, 115(3), 357-361.

Ramos, F. a., Delgado, J. L., Bautista, E., Morales, a. L., & Duque, C. (2005). Changes in volatiles with the application of progressive freeze-concentration to Andes berry (Rubus glaucus Benth). *Journal of Food Engineering*, 69(3), 291-297.

Raventós, M., Hernández, E., Auleda, J., & Ibarz, A. (2007). Concentration of aqueous sugar solutions in a multi-plate cryoconcentrator. *Journal of Food Engineering*, 79(2), 577-585.

Sopelana, P., Pérez-Martínez, M., López-Galilea, I., de Peña, M. P., & Cid, C. (2013). Effect of ultra high temperature (UHT) treatment on coffee brew stability. *Food Research International*. 50(2), 682-690.

Sánchez, J., Hernández, E., Auleda, J. M., & Raventós, M. (2011). Freeze concentration of whey in a falling-film based pilot plant: Process and characterization. *Journal of Food Engineering*, 103(2), 147-155.

Sánchez, J., Ruiz, Y., Auleda, J. M., Hernández, E., & Raventós, M. (2009). Review. Freeze Concentration in the Fruit Juices Industry. *Food Science and Technology International*, 15(4), 303-315.

Sánchez, J., Ruiz, Y., Auleda, J. M., Hernández, E., & Raventós, M. (2009). Review. Freeze Concentration in the Fruit Juices Industry. *Food Science and Technology International*, 15(4), 303-315.

Vignoli, J. A., Bassoli, D. G., & Benassi, M. T. (2011). Antioxidant activity , polyphenols , caffeine and melanoidins in soluble coffee : The influence of processing conditions and raw material. *Food Chemistry*, 124(3), 863-868.

Yee, P. L., Wakisaka, M., Shirai, Y., & Hassan, M. A. (2003). Effects of single food components on freeze concentration by freezing and thawing technique. *Japanese Journal of food engineering*, 4, 77-82.

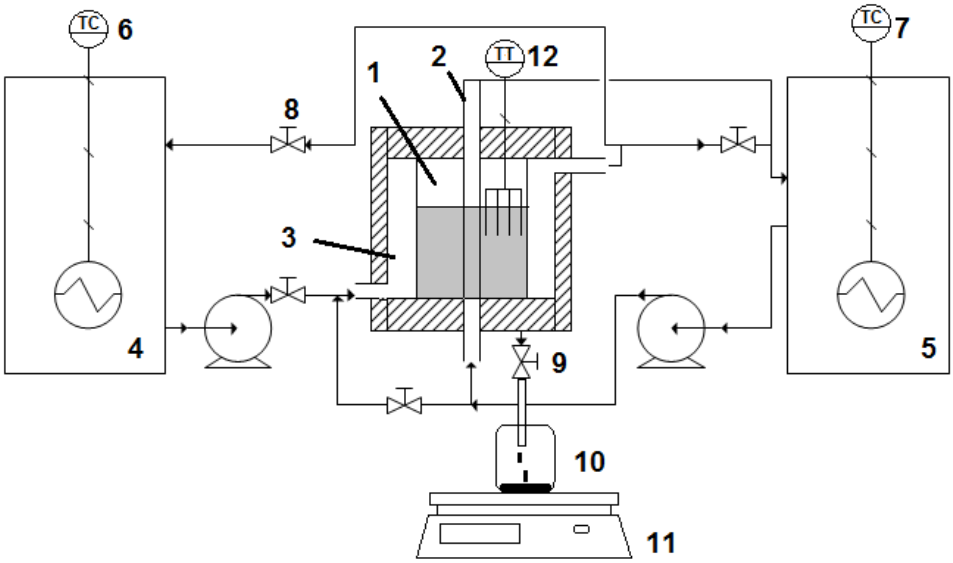


Fig. 1. Experimental set up for block freeze concentration

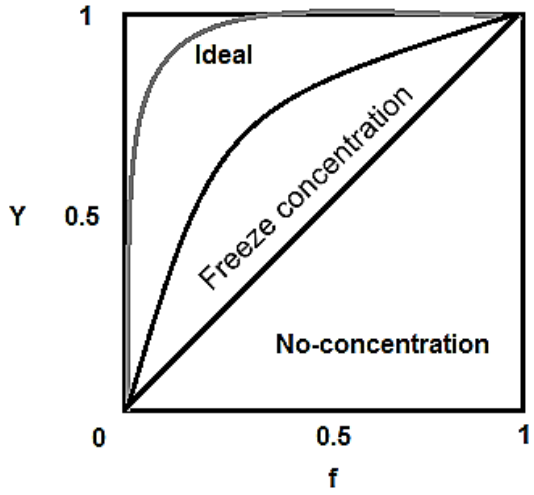


Fig. 2. Solute yield during freeze concentration tests. Adapted from (Nakagawa et al., 2009)

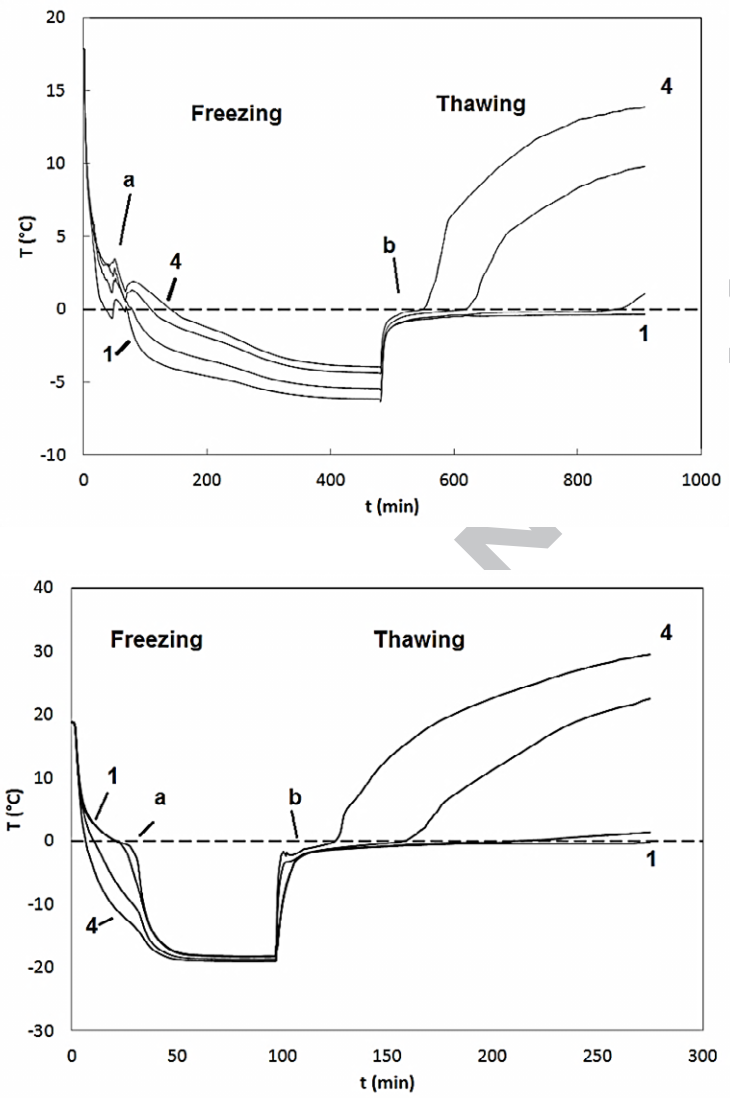
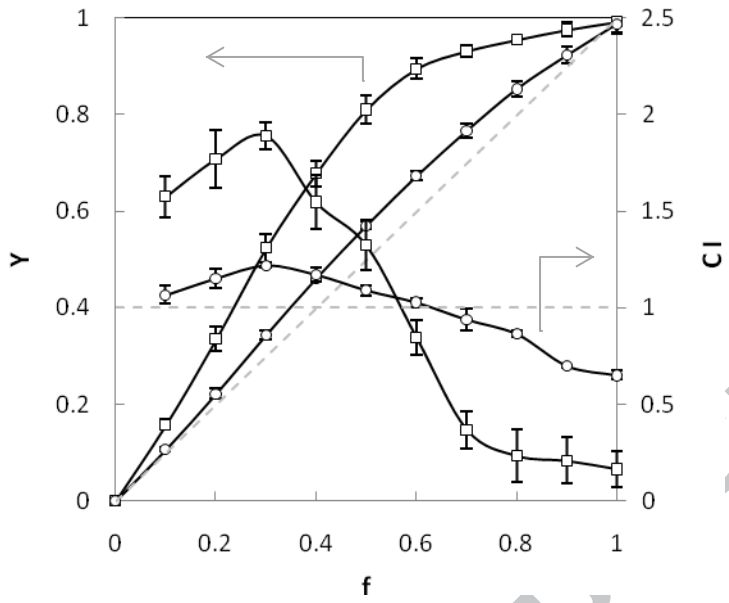
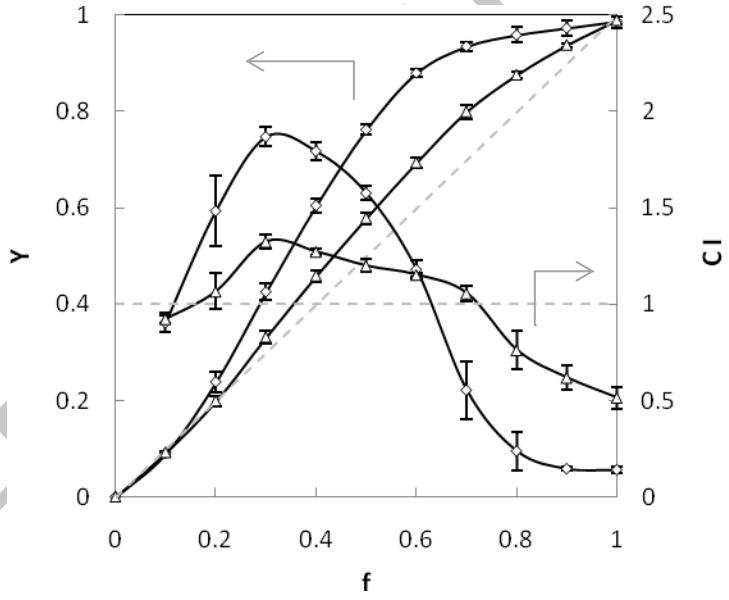


Fig. 3. Temperature profile during freezing and thawing stages. a) Test 1. T_C = -10 °C, T_H = 20 °C; b) Test 8 T_C = -20 °C, T_H = 40 °C. 1: Temperature sensor 1 (interior) and 4: temperature sensor 4 (exterior)

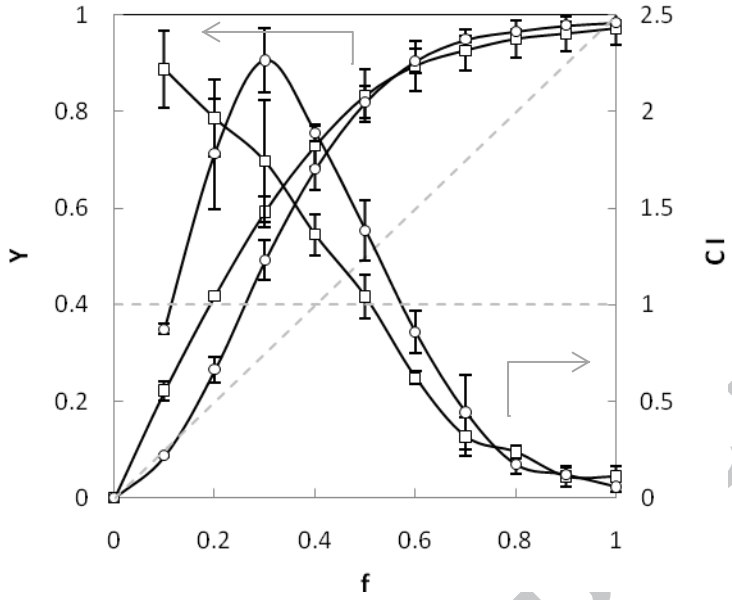


(a)

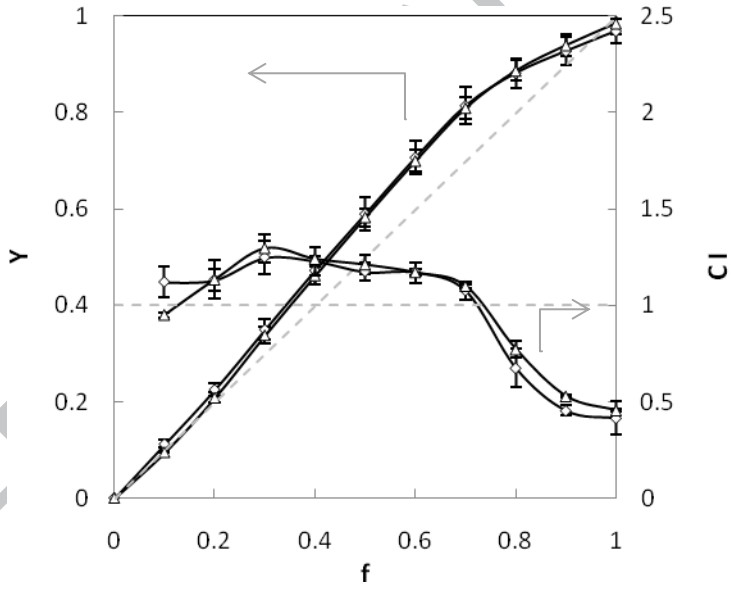


(b)

Fig. 4. Effect of coffee mass fraction on solute yield and the concentration index. a) Test 5, X_S = 5% (□); Test 13, X_S = 15% (○); b) Test 6, X_S = 5% (◊); Test 14, X_S = 15% (Δ)

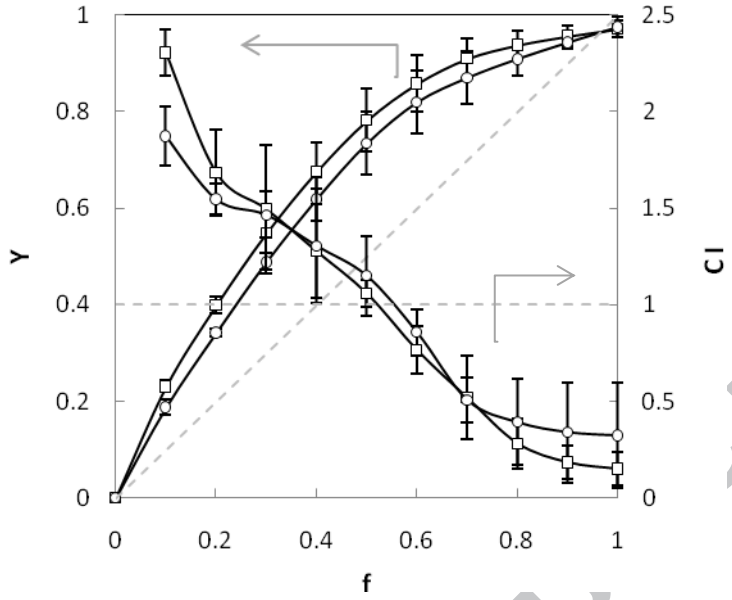


(a)

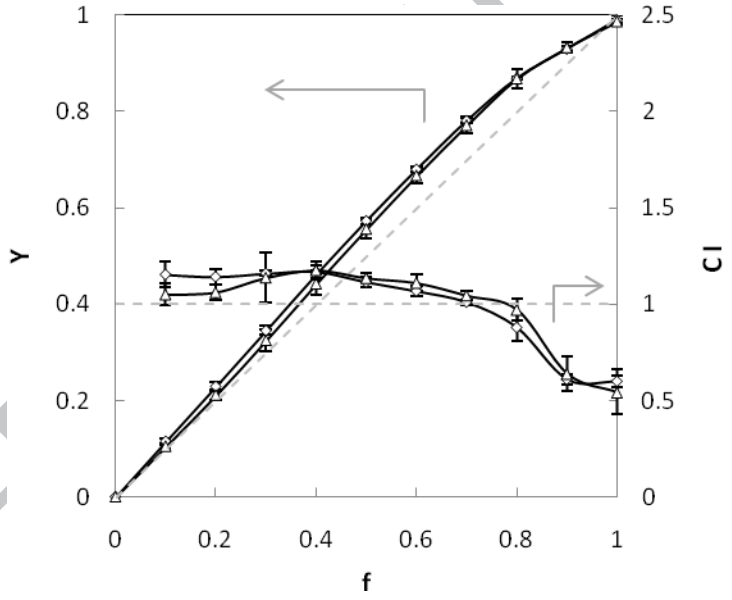


(b)

Fig. 5. Effect of freezing direction on solute yield and the concentration index. a) Test 1, F_D = +1 (□); Test 2, F_D = -1 (○); b) Test 9, F_D = +1 (◊); Test 10, F_D = -1 (Δ)

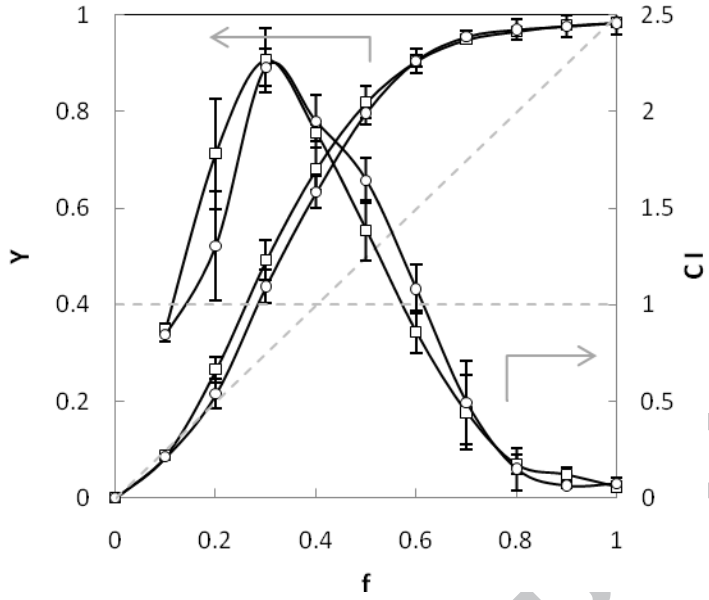


(a)

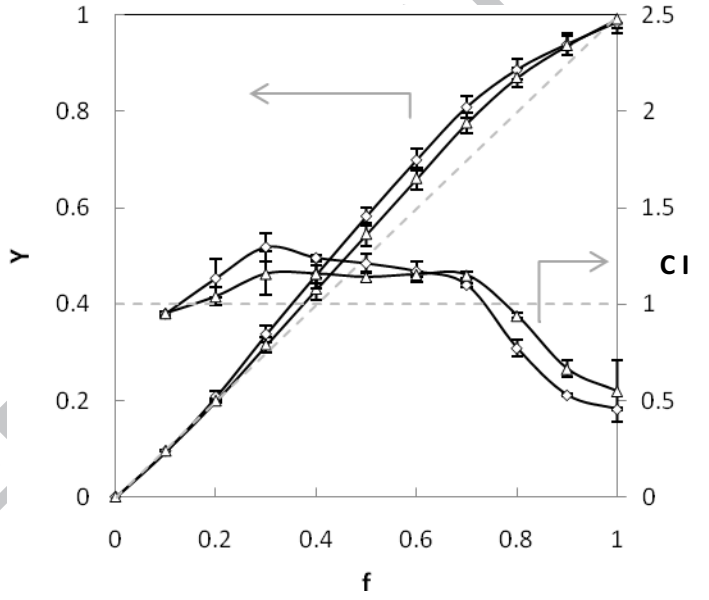


(b)

Fig. 6. Effect of cooling temperature on solute yield and the concentration index. a) Test 3, T_C = -10 °C (□); Test 7, T_C = -20 °C (○); b) Test 11, T_C = -10 °C (◊); Test 15, T_C = -20 °C (Δ)



(a)



(b)

Fig. 7. Effect of heating temperature on solute yield and the concentration index. a) Test 2, T_H = 20 °C (□); Test 4, T_H = 40 °C (○); b) Test 10, T_H = 20 °C (◊); Test 12, T_H = 40 °C (Δ)

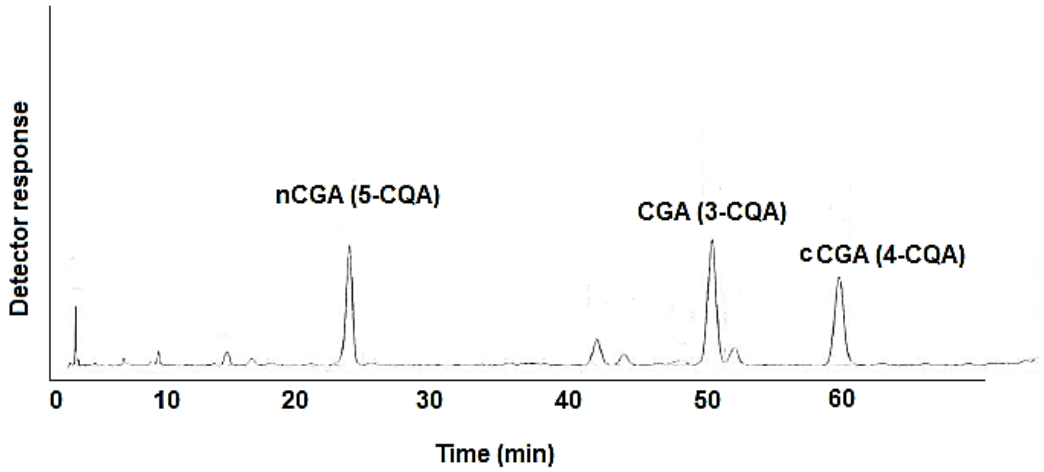


Fig. 8. Typical chromatogram of a coffee solution sample: Test 1.

Table 1. Experimental design

Test	X_S	T_C	T_H	F_D
1	0.05	-10	20	1
2	0.05	-10	20	-1
3	0.05	-10	40	1
4	0.05	-10	40	-1
5	0.05	-20	20	1
6	0.05	-20	20	-1
7	0.05	-20	40	1
8	0.05	-20	40	-1
9	0.15	-10	20	1
10	0.15	-10	20	-1
11	0.15	-10	40	1
12	0.15	-10	40	-1
13	0.15	-20	20	1
14	0.15	-20	20	-1
15	0.15	-20	40	1
16	0.15	-20	40	-1

F_D +1: counter-flow to thawing; F_D -1: parallel to thawing

Table 2. Freeze concentration tests in descending order of area under the curve

TEST	X_S	T_C	T_H	F_D	Area under curve	f at CI=1	Y at CI=1	Cumulative CI at CI=1	CI max	Freezing front growth (μs^{-1})
1	0.05	-10	20	1	0.802 [a]	0.5	0.83	1.67	2.22	1.84
3	0.05	-10	40	1	0.778 [a,b]	0.5	0.78	1.56	1.49	1.83
5	0.05	-20	20	1	0.777 [b,c]	0.5	0.81	1.62	1.89	2.87
2	0.05	-10	20	-1	0.762 [b,c,d]	0.5	0.82	1.64	2.26	3.71
4	0.05	-10	40	-1	0.746 [e,c,d]	0.6	0.91	1.51	2.23	3.71
7	0.05	-20	40	1	0.741 [e,d]	0.5	0.73	1.47	1.87	2.87
6	0.05	-20	20	-1	0.736 [e,d]	0.6	0.88	1.47	1.79	7.17
8	0.05	-20	40	-1	0.735 [e]	0.6	0.90	1.50	2.38	7.17
9	0.15	-10	20	1	0.657 [f]	0.7	0.81	1.16	1.25	1.19
10	0.15	-10	20	-1	0.653 [f]	0.7	0.81	1.16	1.29	5.10
11	0.15	-10	40	1	0.652 [f]	0.7	0.78	1.12	1.17	1.19
14	0.15	-20	20	-1	0.647 [f]	0.7	0.80	1.14	1.32	7.53
16	0.15	-20	40	-1	0.646 [f]	0.8	0.89	1.11	1.23	7.53
13	0.15	-20	20	1	0.644 [f]	0.6	0.67	1.12	1.22	2.58
15	0.15	-20	40	1	0.640 [f]	0.7	0.77	1.10	1.18	2.58
12	0.15	-10	40	-1	0.635 [f]	0.7	0.78	1.11	1.16	5.10

Different letters indicate statistically significant differences ($p<0.05$)

Table 3. Significance analysis of surface response for freeze concentration factors

Parameter	Estimator	Pr > |t|	
Intercept	0.917	<0.001	
X_S	- 1.617	0.0002	*
T_C	0.004	0.039	*
T_H	- 0.001	0.077	
F_D	0.037	0.010	*
$T_C \cdot X_S$	- 0.020	0.049	*
$T_H \cdot X_S$	- 0.006	0.184	
$T_H \cdot T_C$	- 0.001	0.545	
$F_D \cdot X_S$	- 0.133	0.019	*
$F_D \cdot T_C$	0.001	0.141	
$F_D \cdot T_H$	0.001	0.358	

* statistically significant at $\alpha<0.05$

Table 4. Changes in bioactive compounds and the antioxidant activity of coffee during freeze concentration

Test	Compound	C_0 (mg/mL)	C_{FCL} (mg/mL)	C_{RI} (mg/mL)	% Ice loss	% Ice loss (dry basis)	C_{FCL}/C_0
1	CGA	0.29 ± 0.01	0.48 ± 0.04	0.09 ± 0.02	16.03 ± 4.05	48.68 ± 0.37	1.66 ± 0.14 [a]
	c-CGA	0.20 ± 0.00	0.33 ± 0.03	0.06 ± 0.01	16.28 ± 3.89	49.21 ± 0.79	1.66 ± 0.12 [a]
	Caffeine	1.21 ± 0.01	2.02 ± 0.17	0.39 ± 0.08	16.07 ± 3.83	48.79 ± 0.77	1.67 ± 0.13 [a]
	Total solids	0.05 ± 0.00	0.08 ± 0.01	0.01 ± 0.00	16.87 ± 4.30	50.0 ± 0.00	1.60 ± 0.14 [a]
	DPPH*	2.61 ±0.39	4.01 ± 2.42	1.17 ±0.25	27.10 ± 13.5	61.50 ± 19.82	1.58 ± 1.00
	ABTS*	24.02 ±2.64	58.22 ± 22.70	12.63 ±1.97	18.84 ±5.97	53.10 ± 10.40	2.47 ± 1.08
16	CGA	0.93 ± 0.01	1.07 ± 0.02	0.76 ± 0.01	41.50 ± 0.74	49.62 ± 0.77	1.16 ± 0.03 [b]
	c-CGA	0.63 ± 0.01	0.73 ± 0.01	0.52 ± 0.01	41.65 ± 0.70	49.79 ± 0.70	1.17 ± 0.12 [b]
	Caffeine	3.93 ± 0.03	4.56 ± 0.08	3.28 ± 0.03	41.81 ± 0.68	49.96 ± 0.58	1.16 ± 0.03 [b]
	Total solids	0.15 ± 0.00	0.17 ± 0.01	0.12 ± 0.00	41.86 ± 0.43	50.0 ± 0.00	1.15 ± 0.01 [b]
	DPPH*	53.15 ±3.99	72.90 ±9.01	33.5 ±2.97	30.77 ±0.51	37.92 ± 0.32	1.41 ± 0.31
	ABTS*	136.90 ±9.01	171.31 ±1.50	114.8 ±8.20	39.52 ±2.25	47.32 ± 2.08	1.27 ± 0.13

Different letters indicate statistically significant differences ($p<0.05$)

* Expressed as mg Trolox/mL

Table 5. Correlations between antioxidant activity and bioactive compounds concentration

	CGA	cCGA	CAFFEINE	ABTS	DPPH
CGA	1	1.00**	1.00**	0.557*	0.913**
cCGA	1.00**	1	1.00**	0.561*	0.915**
CAFFEINE	1.00**	1,000**	1	0.561*	0.914**
ABTS	0.557*	0.561*	0.561*	1	0.744**
DPPH	0.913**	0.915**	0.914**	0.744**	1

** The correlation is significant p<0.01 (bilateral).

* The correlation is significant p<0.05 (bilateral).

Highlights

Coffee extract was freeze-concentrated by the block technique.

The initial coffee mass fraction influenced the solute recovery.

The control of freezing direction improved the concentration.

The bioactive compounds were distributed in proportion to the total solid content.

The technique preserved the functional properties of the coffee extract.