FROM BY-PRODUCTS TO VALUE: ENHANCING BEETROOT EXTRACTS WITH CRYOCONCENTRATION AND CENTRIFUGATION

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ABSTRACT – Cryoconcentration assisted by centrifugation was applied to produce extracts rich in bioactive compounds from beetroot coproducts. Process performance was assessed by comparing the physicochemical and biological properties (total phenolic and betalains content, antioxidant capacity, and *in vitro* gastrointestinal simulation) of the *in natura* and concentrated extract. Concentrated extract, in the fourth cryoconcentration cycle, presented a total solid content 10 times higher than the *in natura* extract and a concentration index of 98%. Thus, centrifugation-assisted cryoconcentration has proven to be a sustainable approach for producing concentrated natural extracts.

KEYWORDS: freeze concentration; beetroots coproducts; antioxidant activity.

RESUMO - A crioconcentração assistida por centrifugação foi aplicada para produzir extratos ricos em compostos bioativos a partir de coprodutos de beterraba. O desempenho do processo foi avaliado pela comparação das propriedades físico-químicas e biológicas (conteúdo total de fenólicos e betalaínas, capacidade antioxidante e simulação gastrointestinal in vitro) do extrato *in natura* e do extrato concentrado. O extrato concentrado, no quarto ciclo de crioconcentração, apresentou um conteúdo de sólidos totais 10 vezes superior ao do extrato *in natura* e um índice de concentração de 98%. Assim, a crioconcentração assistida por centrifugação mostrou ser uma abordagem sustentável para a produção de extratos naturais concentrados.

PALAVRAS-CHAVE: concentração por congelamento; coprodutos de beterraba; atividade antioxidante.

1. INTRODUCTION

Innovative and alternative concentration processes have recently been developed to improve the bioactivity of numerous natural compounds in liquid foods (Bredun et al., 2023). Nowadays, the most common unit operation used to concentrate products in the food industry is evaporation, a thermal-based process. Despite the high concentration performance, the high temperatures applied in this method cause undesirable functional properties changes, affecting the food products' technological (organoleptic) aspects, resulting in possible rejection by consumers (Zielinski et al., 2019). Furthermore, most of the biological compounds in liquid foods (phenolics, pigments, and others) are thermolabile, being degraded during the evaporation concentration (Dehghannya et al., 2023).

In this context, cryoconcentration is an environmentally friendly technology used to concentrate liquid foods. This technique allows the concentration of liquid samples by freezing the water and separating the ice fraction from the liquid concentrate (Almeida et al., 2023). Cryoconcentration assisted by centrifugation has emerged as a recent method to improve gravitational concentration. This technology applies a higher driving force through centrifugation to replace the gravitational force, allowing the accelerated recovery of the solutes and increasing the efficiency of block cryoconcentration (Orellana-Palma et al., 2019).

In another context, food waste and by-products have been recognized as a significant global problem, compromising the sustainability of the food supply chain. Beetroot (*Beta vulgaris L.*) is one of the most cultivated vegetables worldwide, playing an important role in the human diet (Bangar et al., 2022). Its industrial processing generates underused by-products, such as leaves and stalks, which reach, in some situations, losses of 75%. Furthermore, its by-products are a rich source of hydrophilic and non-toxic pigments, such as betacyanin and betaxanthin (betalains) (Calva-Estrada et al., 2022). Thus, the present work aimed to investigate the concentration of aqueous beetroot by-product extracts employing cryoconcentration assisted by centrifugation to increase the content of bioactive compounds.

2 MATERIAL AND METHODS

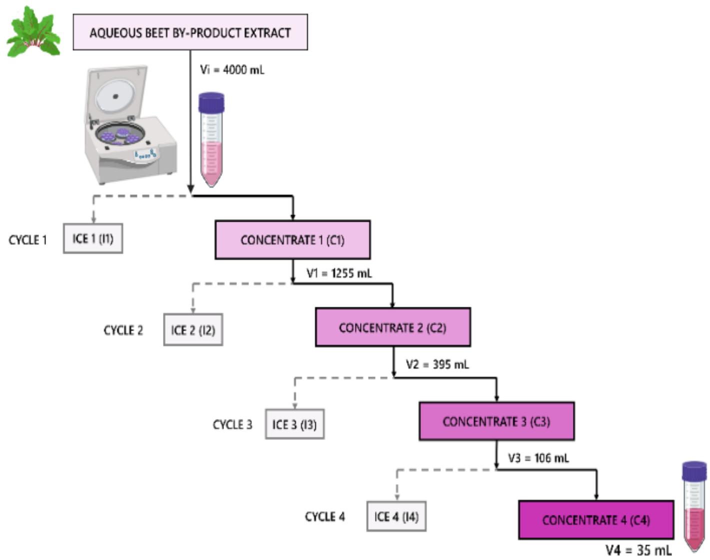
2.1 Beetroot by-products preparation and extraction procedure

The beetroots (*Beta vulgaris* L.) by-product (composed of stalks and leaves) was acquired commercially by a local supplier (Paulo Lopes, Santa Catarina state, Brazil). The sample preparation and extraction of the bioactive compounds was performed according to Arend et al. (2022).

2.2 Cryoconcentration process

The cryoconcentration procedure was performed according to the methodology proposed by Orellana-Palma et al. (2019), and the general experimental protocol is depicted in **Figure 1.** First, centrifuge tubes (internal diameter of 22 mm) were filled with 40 mL of extract. Then, the samples were frozen (Bosch, KND 42) by indirect cooling at −20 ± 2 °C for 12 h. Four consecutive cryoconcentration cycles were used to obtain the final concentrated extract. The centrifugation tubes with the frozen beetroot extract solutions were transferred to a centrifuge to obtain two fractions, a concentrated solution (C) and a frozen solution corresponding to ice (I). The concentrated volume of the first cycle (C1) was frozen again and used as a feed solution for the second cryoconcentration cycle. This procedure was repeated in the third and fourth cycles, resulting in four concentrated volumes (C1-first, C2-second, C3-thrid, and C4-fourth cycle) and four volumes of residual ice (I1-first, I2-second, I3-thrid, and I4-fourth cycle). The centrifugations were performed at 3500.g for 25 min. At each cycle concentrated sample was withdrawn and frozen at − 20 °C for further analysis.

Figure 1. General flowchart of cryoconcentration assisted by centrifugation of beetroot by-product (leaves and steams) extract.



2.3 Cryoconcentration performance and physical-chemical analysis

Total solids content was determined as described by AOAC (2012) and was determined with the assistance of a digital refractometer. The measurements were performed in duplicate at a room temperature of 20 ± 2 °C, and the results were expressed as ºBrix. The concentration index (CI) was determined according to the methodology proposed by Aider and Halleux (2008). CI was calculated for concentrate and ice fractions for each cryoconcentration cycle based on the ratio of the mass of the total solid content of each fraction (ice or concentrate) and the total solid content of the feed extract, according to Equation 1.

$$CI = \frac{TSC_i}{TSC_{fi}} \qquad (1)$$

where, TSC_i is the total solid content (mg L^{-1}) for each cryoconcentration cycle (i = 1, 2, 3 and 4th cycles) and TSC_{fi} is the total solid content (mg L^{-1}) of the feed extract of the respective cycle.

4th cycles) and TSC_{fi} is the total solid content (mg L^{-1}) of the feed extract of the respective cycle.

Color properties were determined for the *in natura*, concentrated and ice fractions using a colorimeter (Minolta CR400), calibrated in a white, for measurements according to the CIE-Lab system (L*: darkness–lightness, a*: green–red axis and b*: blue–yellow axis). Moreover, to observe the differences between the cryoconcentrate (C1-C4) and the *in natura* extracts, the total color difference (ΔE* - the Euclidean distance between two points in a 3D space) was calculated according to Equation (2).

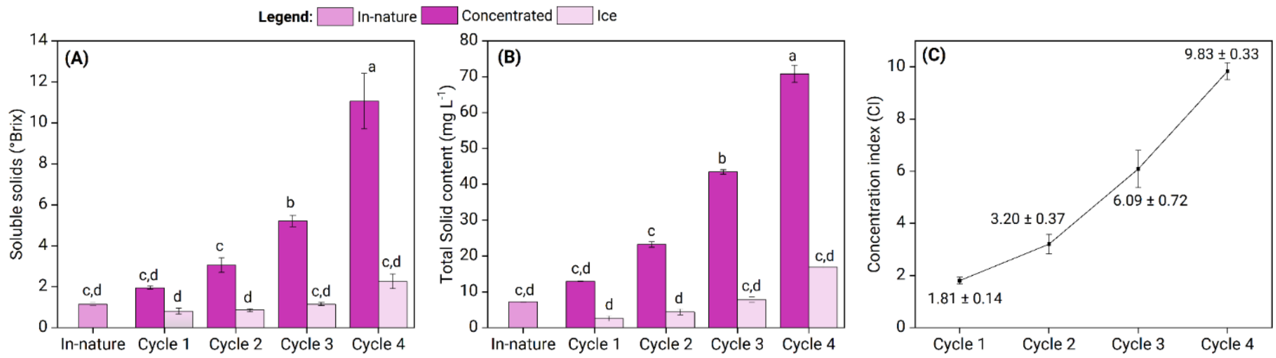
$$\Delta E = \sqrt{\Delta L^{\cdot 2} + \Delta a^{\cdot 2} + \Delta^{\cdot 2}} \quad (2)$$

3 RESULTS AND DISCUSSION

The improvement of total solid content (mg L^{-1}), soluble solids (°Brix), and concentration index (CI) observed in each cryoconcentration cycle are shown in Figure 2. A progressive increase in the total solid content was observed with the progress of freeze concentration cycles according to Figure 2(A). Furthermore, total solid content (dry matter) from the *in natura* (7.19 mg L^{-1}) extract was almost 10-fold lower than the extract from the fourth cryoconcentrate cycle (C4, 70.78 mg L^{-1}), showing the good performance of the cryoconcentration method. The total solid content observed in the present study (44.76 mg L^{-1}) was higher than those observed by Arend et al. (2022) on beetroot aqueous extracts (31.48 mg L^{-1}). In the cryoconcentration assisted by centrifugation process, the ice act as a porous solid through which the concentrated solution is forced by an external force to percolate through drainage channels between ice crystals, increasing the total solids content in the concentrate fraction (Orellana-Palma et al., 2019).

Following the same trends, soluble solids values significantly increased as the cycles advanced (Figure 2B), indicating an increase of 0.8, 1.9, 4.0, and 9.9 °Brix, concerning the *in natura* extract (1.15 °Brix), for C1, C2, C3, and C4, respectively. This upward tendency can be explained by the cryoconcentrated solution obtained in the first cycle being used as feed in the second cycle. Consequently, as the cycles progressed, a solute with high soluble solids concentration was collected as a new feed solution for the next cycle. In addition, a significant increase of 5.8 °Brix was observed in the fourth cycle, compared with the third, indicating a high solute recovery in the last process step.

Figure 2. (a) Soluble solids (°Brix); (b) total solid content (mg L^{-1}) in the *in natura*, concentrated, and ice fractions; and (c) concentration index for each cryoconcentration cycle of beetroot by-product extracts.



Different letters indicate a significant difference ($p < 0.05$) in each column by Tukey's test.

According to the results obtained for the color parameters, presented in Table 1, the L* value of the concentrated fractions decreased (C4: 20.43) compared with the *in natura* extract (33.28), as expected, the concentrated fractions were becoming darker than the *in natura* extract, with the cryoconcentration progress.

Table 1. Color parameters L*, a*, and ΔE obtained for the in-nature extract and concentrated and ice fractions of the cryoconcentration assisted by centrifugation of beetroot by-products extracts.

Sample	L*	a*	b*	ΔE	Color
In-nature	$33.28^{c} \pm 0.07$	$15.87^{d} \pm 0.06$	$4.16^{e} \pm 0.02$	-	
C1	$25.27^{f} \pm 0.15$	$14.36^{e} \pm 0.30$	$5.47^{c} \pm 0.08$	$8.26^{e} \pm 0.10$	
I1	$40.45^{a} \pm 0.01$	$8.78^{g} \pm 0.02$	$1.86^{j} \pm 0.01$	$10.34^{c} \pm 0.01$	
C2	$24.08^{g} \pm 0.01$	$16.29^{c} \pm 0.04$	$5.93^{b} \pm 0.02$	$9.38^{d} \pm 0.06$	
G2	$37.23^{b} \pm 0.01$	$12.10^{f} \pm 0.01$	$2.87^{g} \pm 0.01$	$5.61^{f} \pm 0.01$	
C3	$22.45^{h} \pm 0.01$	$11.79^{f} \pm 0.01$	$4.03^{f} \pm 0.01$	$11.57^{b} \pm 0.07$	
I3	$31.33^{d} \pm 0.01$	$17.45^{b} \pm 0.03$	$5.07^{d} \pm 0.01$	$2.67^{h} \pm 0.04$	
C4	$20.43^{i} \pm 0.01$	$6.29^{h} \pm 0.02$	$2.04^{i} \pm 0.01$	$16.17^{a} \pm 0.09$	
I4	$26.61^{e} \pm 0.01$	$18.16^{a} \pm 0.01$	$6.51^{a} \pm 0.01$	$7.44^{e} \pm 0.06$	

Legend: C-concentrated, I-ice and 1 to 4-corresponds to the cryoconcentration cycle; Different superscript lowercase letters indicate a significant difference ($p < 0.05$) in each column by Tukey's test.

This behavior could be associated with increased total solid and betalains content. The lightness parameter (L*) may also be related to the water content in the extracts; the higher the water content in the extract, the lighter the product and, consequently higher the L* value. This effect is clear when comparing the L* value of the initial extract (33.28) and the concentrated extract from cycle 4 (20.43). This phenomenon indicated cycles 3 and 4 enhanced the dark aspect of the extract compared with the initial extract.

4 CONCLUSIONS

Cryoconcentration assisted by centrifugation was applied as an effective concentration method to concentrate beetroot by-product extract. Four cycles were necessary to concentrate the total solid content 10 times compared with the in-nature extract. Finally, using cryoconcentrate beetroot by-products extracts could be a sustainable strategy to produce natural additives to supplement and enrich foods, improving their sensorial, nutritional, and antioxidant properties.

REFERENCES

AIDER, M., DE HALLEUX, D. Passive and microwave-assisted thawing in maple sap cryoconcentration technology**. Journal of Food Engineering**, v. 8, n. 1, p. 65–72, 2008

ALMEIDA, É. DA S., KNAPP, M. A., ROCHA, J. D. G. DA, HOTZA, D., OLIVEIRA, D. de. Multi-stage block freeze concentration via gravitational method applied to increase of the nutritional content of Morinda citrifolia L. tea. **Food Bioscience**, v. 51, 2021

AOAC. Official **Methods of Analysis of the AOAC International**. AOAC, 18 th ed., 2012

AREND, G. D., ALMEIDA, É. S., BYRUCHKO, R. T., PINTO, M. E. G., DA CRUZ, A. B., VERRUCK, S., DI LUCCIO, M., REZZADORI, K. Gravitational and microwave-assisted multi-stages block freeze concentration process to obtain enriched concentrated beet (Beta vulgaris L.) by-products extract: bioactive compounds and simulated gastrointestinal profile. **Food and Bioproducts Processing**, v. 133, p. 77–86, 2022

BREDUN, M. A., PRESTES, A. A., PANCERI, C. P., PRUDÊNCIO, E. S., BURIN, V. M.. Bioactive compounds recovery by freeze concentration process from winemaking by-product. **Food Research International,** v.173, p.113220, 2023

DEHGHANNYA, J., RASTGOU-OSKUEI, S., DADASHI, S. Influence of pulsed microwave on betacyanins, betaxanthins and physical properties during drying of red beetroot. **Applied Food Research**, v. 3, n. 1, 2023

DEHGHANNYA, J., RASTGOU-OSKUEI, S., DADASHI, S. Influence of pulsed microwave on betacyanins, betaxanthins and physical properties during drying of red beetroot. **Applied Food Research**, v.3, n.1, 2023.

ORELLANA-PALMA, P., GONZÁLEZ, Y., PETZOLD, G. Improvement of Centrifugal Cryoconcentration by Ice Recovery Applied to Orange Juice. **Chemical Engineering and Technology**, v. 42, n. 4, p. 925–931, 2023.

ZIELINSKI, A. A. F., ZARDO, D. M., ALBERTI, A., BORTOLINI, D. G., BENVENUTTI, L., DEMIATE, I. M., NOGUEIRA, A. Effect of cryoconcentration process on phenolic compounds and antioxidant activity in apple juice. **Journal of the Science of Food and Agriculture**, v. 99, n. 6, p. 2786–2792, 2019.