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Block freeze concentration by centrifugation and vacuum increases the content of lactose-free milk macronutrients

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Title: Block freeze concentration by centrifugation and vacuum increases the content of lactose-free milk macronutrients

Associate Editor: Mauer, Lisa

Dear Editor

Firstly, we would like to thank you for the opportunity to reconsider our manuscript for publication in **Journal of Food Science**. We also would like to point out that all the comments made by Reviewers have been taken into account. We believed that all corrections were essential for the improvement of our manuscript. As suggested by Reviewers, changes were made in the manuscript, and the comments have been discussed and incorporated in this enhanced version, which is highlighted in red.

Reviewers' comments:

Reviewer #1:

1. Lump citations such as (Canella et al., 2019a; D. Arend et al., 2022; Demoliner 116 et al., 2020; Sequera et al., 2019) is not recommended. Please specify each liquid food application for each citation/reference.

Author's comments: We agree with the Reviewer. Therefore, we have rewritten these sentences (See Page 7, Lines 166–170).

2. Please state a good reason for the combination of PFC and BFC. State any past research on PFC and BFC and their results, then find the research gap/limitation that leads to the PFC and BFC combination.

Author's comments: Thank you for the observations. Although PFC is not the object of study of this work, we have rewritten the Introduction addressing your comment. Therefore, we have added information related to a good reason for the combination of PFC and BFC, studies on PFC and BFC combination, and gaps related to PFC and BFC combination. To see this information, and also to have a better understanding of our purpose, please read page 7 line 166 until page 9, line 212.

3. Please proofread the paper as there are some words that I think are not suitable to be used. Example: Protein analysis was "realized" according to the Kjeldahl method (line 159). The word "realized" can be replaced with another word.

Author's comments: We have agreed with the reviewer and replaced the word "realized" (See Page 10, Line 231). Thank you for your constructive comment.

4. Define lactose-free milk.

Authors' comments: We agree with the Reviewer and this comment was addressed in the Introduction section (See Page 4, Lines 93–125). We have discussed some details involved in the production and definition of lactose-free milk.

5. Concentrate yield for the effect of centrifugal time is not even higher than 50%. Please add any past researches that did the same method and their results. Please also add a statement on either centrifugal time is an important parameter or not for this study.

Authors' comments: Thank you for the recommendation. We did a broad study on the topic and improved the discussion (See Page 16, Lines 361–399).

6. How this method is applicable to milk manufacturing? Please mention it in the conclusion.

Author's comments: We appreciate your comment. Reviewer 2 also asked us to make changes to the *Conclusion* section. So we have rewritten it.

7. What is the difference amount of concentrate 1 and 2, in mass?

Authors' comments:

	1st cycle
Initial sample mass (milk)	~734g
Concentrate mass	~33g

continuation below

	2nd cycle
Initial sample mass (ice)	~692g
Concentrate mass	~137g

8. Please mention the analytical equipment used to analyze the products as the authors claim this method can maintain the sensorial characteristic of the milk.

Author's comments: Descriptive analysis presents great applicability for the monitoring and adjustment of sensory characteristics of milk products. This method does not require training, has a low financial impact, and optimizes time and resources in dairy industries. In addition, it gives information highly correlated with traditional methods, providing a total assessment of products and taking all sensory traits into account. Therefore, for a better understanding, the following comment was removed from the manuscript: "Then, it is expected that lactose-free milk submitted to these FC processes may have distinct sensorial characteristics."

9. Do the results from this study compared with the current method used in the industry? Please mention it.

Author's comments: We agree with the Reviewer and this comment was addressed. Therefore, the following information was added in the final version of the manuscript:

"According to Beldie and Moraru (2021), the concentration of milk in the dairy industry is typically achieved by thermal evaporation or membrane technology such as reverse osmosis. The efficiency of these processes is very similar to those obtained by the freeze concentration process used in the present study. In thermal evaporation, the maximum concentration level achievable is 55% for skim milk and 50% for whole milk, while for the membrane process, the maximum concentration varies between 25% and 30% solids. Thermal evaporation is also known to reduce the quality of the final concentrated product due to prolonged exposure to heat, which negatively affects the color, taste, and nutritional value of milk. Furthermore, evaporators are prone to biofilm formation by spore-forming mesophilic or thermophilic bacteria. A major drawback of membrane separation is that the process is significantly affected by membrane fouling, which leads to flux decline and limits the final achievable concentration of the product (Beldie & Moraru, 2021). Given the above, we can verify that block freeze concentration by centrifugation and vacuum can be used by the industry aiming for the macronutrient concentration of lactose-free milk." (See Page 21, Lines 484–498)

Reviewer #2:

This research article presents an interesting evaluation of block freeze concentration by centrifugation and vacuum to increase the content of lactose-free milk macronutrients. However, some aspects must be considered in order to improve the manuscript.

Author's comments: We understand the Reviewer's point of view and highlight the relevance of the comments.

Line 27: It is alright to use pronouns, but try to limit their usage

Author's comments: We agree with the Reviewer and this comment was addressed. (See Page 2, Line 36).

Line 28: Don't have to mention the experimental design in the abstract; it's alright to give a 2-3 brief intro and the relevance of the current research

Author's comments: We agree with the reviewer regarding the brief presentation in the Abstract, and therefore, we have made changes in this section (See Page 2, Lines 30–36).

Concerning the mention of the experimental design, our research group doesn't see problems in this sense, since the same approach was used in the abstract of Santana et al. (2020), who performed assays of blueberry juice freeze concentration. Even so, we have suppressed the specific design (2^3) (see page 2, lines 36 and 37). We didn't remove more information than this one because the sentences that follow each other would be meaningless or confused.

Reference:

Santana, T., Moreno, J., Petzold, G., Santana, R., & Sáez-Trautmann, G. (2020). Evaluation of the temperature and time in centrifugation-assisted freeze concentration. *Applied Sciences*, *10*, Article 9130. https://doi.org/10.3390/app10249130

Line 30: “were” analyzed

Author’s comments: We agree with the reviewer, and therefore, we have rewritten the Abstract section (See Page 2, Lines 35–39).

Line 31: Too exaggerated: do not use “greater effect”. What does a greater effect translate to?

Author’s comments: We agree with the reviewer, and therefore, we have rewritten the Abstract section (See Page 2, Lines 39 and 40). The phrase “The centrifugation temperature had a greater effect on the concentrate yield and concentration index” was replaced by “Concentrate yield and concentration index were mainly affected by the centrifugation temperature”.

Line 32: It seems that there is an article usage problem here.

Line 32: The noun phrase significant effect seems to be missing a determiner before it. Consider adding an article.

Author’s comments: We agree with the reviewer, and therefore, we have rewritten the Abstract section (See Page 2, Lines 40–42). The phrase “Regarding **the** efficiency, individual factors did not have **significant effect** on the response, but their interactions did.” was replaced by “On the other hand, individual factors did not have **a significant effect** on the efficiency, only their interactions.”

Line 49: you can also include the sustainability aspect of it.

Author’s comments: We agree with the reviewer and this comment was addressed. (See Page 3, Lines 58–65)

Line 51: The singular verb is does not appear to agree with the plural subject contents. Consider changing the verb form for subject-verb agreement.

Author’s comments: We agree with the reviewer, and therefore, we have rewritten this sentence (See Page 3, Lines 58–60). The phrase “In turn, milk with higher solids contents is gaining in popularity in much research of dairy products” was replaced by “In turn, dairy products that provide a high amount of solids (especially protein) are gaining in popularity among consumers, with consequent interest from researchers”.

Line 54-55: Rewrite

Author’s comments: We appreciate your valuable comment and have chosen to remove this sentence, not least because there is a word limit established by the journal for this section.

Line 78: It may be unclear who or what “This” refers to. Consider rewriting the sentence to remove the unclear reference.

Author’s comments: We agree with the reviewer, and therefore, we have rewritten the Introduction section (See Page 4, Lines 77–84).

Line 74-97: These two paragraphs can be combined into one rather than being vague in certain lines

Author’s comments: Based on your comment, as well as the suggestions of reviewer 1, we have made changes in the Introduction section. As per your suggestion, all content of milk and intolerance was kept in one paragraph. As recommended by reviewer 1, more information about lactose-free milk was added.
(See Page 4, Lines 77–125).

Line 87-90: This appears to be a sentence fragment. Consider rewriting it as a complete sentence.

Author’s comments: We agree with the reviewer, and therefore, we have rewritten all these sentences (See Page 4, Lines 81–93).

Line 94-97: Not clear what’s precisely being conveyed here

Author’s comments: We understand the reviewer's point of view, but we need to emphasize that after all the context presented, the sentence itself is clear and understandable.

In the previous sentences, it was discussed what lactose malabsorption is, and all the negative consequences that this situation can lead to human health. In lines 97–105, we reported that despite all these problems, lactose malabsorbers don’t need to restrict milk or dairy from their diet, thanks to existing technologies for the production of lactose-free milk and dairy products. Still, our society is constantly changing in terms of the way we feed and nourish ourselves, and as a consequence, the industry tries to keep up with this movement. The production of concentrated lactose-free milk could support both the industry and consumers. That's because, the consumer can do direct use of this product, depending on its nutritional requirements (related to sports practices, lifestyle, and age), socio-cultural factors, and intrinsic product characteristics. Likewise, from concentrated milk, the industry can innovate in obtaining lactose-free derivatives, as we know that concentration is often the first step in their manufacture. Among the many products that utilize milk or milk components, we can mention supplements for athletes, infant formulas, supplements for the elderly, as well as more common products such as cheeses, concentrated yogurts, ice cream, and dairy beverages.

Line 111: Please elaborate on the previous studies on milk/whey and establish

Author's comments: We agree with the reviewer. The following sentences were added:

"More specifically, Camelo-Silva et al. (2022) freeze concentrated milk until the third stage and used the sample from the first stage for ice cream production (Ice cream 1). This ice cream was contrasted to the ice cream from regular milk (Ice cream 2), and the authors concluded that Ice cream 1 presented good chemical, physical, rheological, and microstructural properties. Likewise, in the approach of Barros et al. (2022), FC was employed as an indirect technology for ice cream manufacture. However, instead of milk, cheese whey was used as a raw material for the FC process, and the concentrate from the second stage was evaluated as a milk substitute in ice cream. After testing 4 replacement levels, the authors concluded that the incorporation of concentrated whey at a 50% substitution level was better in terms of color, flavor, and texture attributes. In addition to the structural characteristics of the final product, the other advantage of this study was the added value to the whey, which is often seen only as a by-product of the cheese industry. De Liz et al. (2020) also utilized freeze concentrated whey in their experiments, but with another proposal: goat's whey freeze concentrate was used as an encapsulant agent for probiotic encapsulation (*Bifidobacterium animalis* ssp. *lactis* BB-12) by spray drying. The powdered probiotic samples from the encapsulation process presented good stability after storage at 4 °C and 25 °C for 40 days (> 7 log CFU g^{-1}), showing that goat whey concentrates had an excellent behavior as a cell protective material. Also, it was noted that, as a wall material, goat whey concentrate exhibited favorable thermal properties before and after encapsulation. Equally, Machado Canella et al. (2020), worked with a based-goat raw material. However, their sample did not consist of goat whey, but semi-skimmed goat milk. After optimization of the FC process, it was obtained a concentrate yield of 77.97%. Moreover, the content of total solids changed from 9.94 to 32.87 (g 100 g^{-1}), with an emphasis on protein (from 3.53 to 9.43 g 100 g^{-1})."

To understand the full context, please read page 6, lines 126–165.

Line 126-132: It's not evidently established what differentiates your research from that of Dantas et al. (2021); rewrite and introduce your hypothesis here more effectively

Author's comments: We agree with Reviewer 2 and this comment was addressed. Note that the work realized by Dantas et al. (2021) used a different technique of freeze concentration. This work (Dantas et al., 2021) evaluated the behavior of lactose-free milk when submitted to the Progressive freeze concentration. Given the considerable amount of total solids retained in the solid fraction (ice), this ice was thawed, frozen again, and subjected to the vacuum-assisted BFC. In the present study, it was explored a different technique, i. e., the lactose-free milk was submitted to the centrifugation-assisted block freeze concentration process. In the sequence, as performed by Dantas et al. (2021), the ice fraction was subjected to the vacuum-assisted BFC aiming for the better utilization of the nutrients that were retained there. Finally, as recommended by Reviewer 2 and attending to other suggestions from Reviewer 1, this part was rewritten. (See Page 7, Lines 166–212).

Line 136: Please clearly present the hypothesis and contribution of your study in the Introduction section.

Author's comments: We agree with the reviewer. Therefore, we have rewritten the Introduction as discussed in the previous comment.

Line 128-131: Rewrite

Author's comments: We agree with the reviewer.

So, the phrases

"Recent work (Dantas et al., 2021) suggests the combination of progressive FC and BFC supported by vacuum, as a strategy for concentration of lactose-free milk. However, given the interesting results obtained by other authors, when who worked with centrifugation-assisted BFC, we have realized how valuable it would be if we could expand the study with lactose-free milk through use of this technique. Therefore, the approach of this research provides data of the use of BFC processes to concentrate carbohydrates and protein from lactose-free milk."

were replaced by:

"From previous studies on centrifugation-assisted BFC (Casas-Forero et al., 2021b; Guerra-Valle et al., 2022; Orellana-Palma et al., 2021), the present work considers the application of this technology at a first moment. Subsequently, as performed by Dantas et al. (2021), an ice fraction was subjected to the vacuum-assisted BFC aiming for better utilization of the nutrients retained in this portion. Therefore, the approach of this research provides data on the use of BFC processes (both centrifugation-assisted BFC and vacuum-assisted BFC) to concentrate carbohydrates and protein from lactose-free milk."

(See Page 9, Lines 201–208).

Line 133: Don't have to mention the design here. Could this be moved to a separate section under the sub-heading "experimental approach," and could you detail it for approaches used.

Author's comments: We agree with the reviewer, and therefore we have removed the following sentences:

"For this purpose, a factorial experimental design was employed to investigate the milk concentration by centrifugation-assisted BFC. Aiming to improve the process parameters, a second stage was studied using the vacuum-assisted BFC."

Line 146: Why was UHT milk considered versus a lactose-free skim milk

Author's comments: This comment is unclear. There were no 2 types of milk to be investigated. All the work was performed with only one type of milk: commercial skim lactose-free milk previously submitted to the ultra-high-temperature processing (UHT).

Taking into account the objective of our work, it was more advantageous to acquire commercial milk already standardized, than to use raw milk, for example, and standardize it. The difficulty in standardization would start with the removal or hydrolysis of lactose. Our laboratory is not specialized in enzyme kinetics or membrane separation. So, the raw material chosen is the one that best adapts to our conditions and, at the same time, best represents industrial sequencing.

Line 182: How would you justify this with other concentration technologies that are commercially available (from a cost/time manufacturer standpoint)

Author's comments: In the present study, the lactose-free milk was frozen at -20 °C and maintained in a static freezer for 12 hours. In this case, the objective was to form large ice crystals. This is because according to Samsuri et al. (2015), large ice crystals contain smaller amounts of impurities and solids than small ice crystals. Therefore, slow freezing is recommended to obtain a good separation process of the concentrate and ice. For this reason, more information was added to the manuscript. (Please see Page 11, Lines 251–256, and Page 21, Lines 484–498).

Reference cited:

Samsuri, S., Amran, N. A., & Jusoh, M. (2015). Spiral finned crystallizer for progressive freeze concentration process. *Chemical Engineering Research and Design, 104*, 280-286. https://doi.org/10.1016/j.cherd.2015.06.040

Line 185: global data respecting?? It's recommended that the manuscript be carefully revised for research writing language.

Author's comments: We have agreed with the reviewer. The word "global" was deleted and the word "respecting" was replaced (See Page 11, Line 259). Thank you for your constructive comment.

Line 191: based on what this was selected/optimized: temperature, centrifuge rotation speed, and time

Author's comments: We agree with the Reviewer and this comment was addressed. (See Page 12, 266–274).

Line 218: Concentrate 1 and Ice 1 (can be abbreviated)

Author's comments: The terms "Concentrate 1" and "Concentrate 2" have been simplified. Please see page 13, line 300, as well as the rest of the document.

Line 285: Needs further explanation

Author's comments: We are grateful for your comment. The explanation was improved accordingly (See Page 16, Lines 361–399).

Line 293-296: Consider rewriting the sentence

Author's comments: Author's comments: We agree with the reviewer. The sentences were rewritten (See Page 17, Lines 402–406).

The phrases

"All individual factors, as well as the interaction between all of them, had a significant negative effect on the *CI*. This reinforces what has been commented above, where, through Table 2, it can be seen that the centrifugation rotation speed also affected the Concentration Index. That is, the lowest *CI* values were found at the highest rotation speed (4500 rpm)."

were replaced by:

"All individual factors, as well as the interaction between all of them, had a significant negative effect on the *CI*. This is in accordance with what was discussed above regarding the time. From Figure 2a and Table 2, it can be seen that the centrifugation rotation speed also negatively affected the *CI*, since the highest values were found at the lowest rotation speed (3500 rpm)".

Line 313: The use of and/or is severely frowned upon in formal writing. Consider using only one conjunction or rewriting the sentence.

Author's comments: We agree with the reviewer, so we used only one conjunction (See Page 18, Line 423).

Line 323: It appears that you have an unnecessary comma before the dependent clause marker since. Consider removing the comma. Please review throughout the manuscript for similar issues with comma and preposition use

Author's comments: We would like to highlight that the word "nevertheless" in this context is not a preposition (in fact in any context, it would never be a preposition). Current grammarians classify it as a "conjunctive adverb".

Also, it is important to note that the phrase that follows "Nevertheless," is an **independent clause**, not a dependent clause. The use of commas after introductory words like *therefore*, *nevertheless*, *instead*, *otherwise*, *furthermore*, etc. is absolutely needed and provided for according to the grammatical rules of the English language.

References:

https://www.pristineword.com/comma-however-nevertheless-though/#:~:text=At%20the%20start%20of%20a,in%20front%20of%20%22though%22.

https://www.aje.com/arc/editing-tip-commas-conjunctive-adverbs/

https://www.ndsu.edu/pubweb/~dasulliv/style/conjadv.htm

https://onlinewritingtraining.com.au/however-therefore-furthermore/

https://www.britannica.com/dictionary/eb/qa/Nevertheless

Line 355: It seems that the verb outweighs does not agree with the subject. Consider changing the verb form.

Author's comments:

"…suggesting **the effect** of centrifugation-assisted BFC on the other milk constituents (minor components) outweighs the effect on the macronutrients studied."

The words "the effect", as well as the words that follow, were designed to be understood in the singular. That is, the subject of the clause is singular. Look:

the effect… = singular form; it can be replaced by **it**. ➡ suggesting **it outweighs** the effect on the macronutrients studied.

Line 392: More discussion is anticipated

Author's comments: Thank you for the recommendation. We have added more discussion to point *3.3 Validation of experimental results*.

Line 394: Rewrite to better reflect the scope of this current study. Also, include a comment indicating the potential use of the proposed method at the industrial level/scale-up challenges/costs, etc.

Author's comments: Thank you for the recommendation. The *Conclusion* was enhanced with additional information.

Figure 1: Redraw this: looks like two different fonts were used/looks skewed.

Author's comments: We agree with the reviewer and appreciate your feedback. The figure was redrawn. Please, see Figure 1.

Finally, we believed that all corrections were essential for the improvement of our manuscript. Thank you for your time and patience.

Best regards,

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Block freeze concentration by centrifugation and vacuum increases the content of lactose-free milk macronutrients

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Short version of title (running head): Cryoconcentration of lactose-free milk

Choice of journal/topic where article should appear in *Journal of Food Science:*

Food Engineering, Materials Science, and Nanotechnology

Abstract

Lactose-free milk is rising in popularity among consumers due to its claim to be a better digestible product compared to regular fluid milk. For that reason, concentrating this food is a good alternative for increasing its versatility and usability in different dairy industry segments. Block freeze concentration (BFC) is a simple technology used to concentrate liquid foods through ice crystal formation and subsequent removal of water. Thus, this work aimed to test two variants of the BFC technique on lactose-free milk concentration. In the first approach, it was investigated the centrifugation-assisted BFC of skim lactose-free milk by applying a factorial experimental design. Temperature, time, and rotation speed were the factors; and the response variables included the concentrate yield, concentration index, and efficiency of the process. Concentrate yield and concentration index were mainly affected by the centrifugation temperature. On the other hand, individual factors did not have a significant effect on the efficiency, only their interactions. In the case of centrifugation-assisted BFC in a single step, the condition at 40 °C, 70 min, and 4500 rpm was considered the best, given the highest values of efficiency and concentrate yield (80.87 and 67.02, respectively), and still an excellent value for concentration index (2.05). Conversely, the condition at 30 °C, 45 min, and 3500 rpm was chosen to integrate a freeze concentration process in two-stage. Then, the ice obtained from the first cycle was subjected to the vacuum-assisted BFC, which consisted in the second cycle. The concentrate obtained from the vacuum-assisted BFC presented contents of total solids, carbohydrates, and protein 2.95, 3.00, and 2.91 times more than the initial lactose-free milk, respectively. Therefore, we

believe that the concentrates obtained can be used for the development of innovative lactose-free dairy products.

Practical Application

Using concentration processes in the dairy industry can significantly contribute to enhancing the overall efficiency of milk processing since huge quantities of water from milk can be reduced, increasing the total solids content. In turn, dairy products that provide a high amount of solids (especially protein) are gaining in popularity among consumers, with consequent interest from researchers. In addition, milk concentration shows advantages in terms of processing, packaging, transportation, and handling. Since most changes occur in an aqueous environment, the removal of some part of water results in the preservation of milk. It is noteworthy that dairy industries are concerned principally with food preservation, green technologies, and the production of high-quality products. Thus, concentration processes could favor the development of milk products rich in proteins to meet certain demands on functional and nutritional properties, for example in beverages and formulated food.

1. Introduction

Dairy is an important source of essential nutrients including high-quality proteins, calcium, vitamins (vitamins B12, vitamin A, riboflavin, thiamin), and micronutrients (Mg and K), in many populations. However, milk consumption has reduced over the last few decades, notedly in developed countries, where unfavorable gastrointestinal symptoms are a frequent cause for avoidance. In turn, full dairy avoidance from the diet may enhance the risk of nutrient insufficiency and thus contribute to metabolic bone disease, low bone mineral density, or metabolic syndromes (Appleby et al., 2007; Pearlman & Akpotaire, 2019). The presence of the disaccharide lactose in milk is commonly assigned to these dairy restrictions due to a health status known as lactose malabsorption (Shrestha et al., 2021). There are some reasons for the manifestation of lactose malabsorption (such as celiac disease, microbial infections, or malnutrition that damages the intestinal villi), but the main one is the lactase non-persistence (LNP) (OMIM#223100), a phenotypic enzyme deficiency that affects different cultures (EFSA, 2010). The enzyme in question is lactase-phlorizin hydrolase, simply called lactase, which is responsible for hydrolyzing lactose to galactose and glucose, thus facilitating its digestion. In LNP clinical cases, there is a decrease in the lactase activity in the intestinal lumen after weaning (Kuchay, 2020). As a consequence, LNP individuals may experience distinct kinds of systemic and intestinal symptoms: vomiting, nausea, headache, abdominal pain, flatulence, gut distension, constipation, diarrhea, muscle pain, loss of concentration, allergies, mouth ulcers, heart arrhythmia, and increased micturition (Qibtia et al., 2021). Taking into account this entire scenario, it is required to produce innovative and alternative technologies to serve lactose malabsorbers who do not wish to limit dairy products from their diet, given the significance of specific nutrients contained in milk. In this context, fluid lactose-free milk is already a well-established way to help this population, as well as those who are tolerant but also want to avoid lactose for any other reasons (as discussed in the work by Castellini and Graffigna [2022]). Only in the United States, this kind of milk had 201 million gallons sold in 2020, experiencing 19% growth on both a dollar and volume basis compared to the previous year (Dairy Foods, 2021). There are two most common forms of industrial production of lactose-free or lactose-reduced milk. The first is lactose enzymatic hydrolysis, via using of β-galactosidase from *Kluyveromyces lactis*, *Kluyveromyces faragilis*, or *Aspergillus oryzae*, releasing galactose and glucose into milk as a result of the reaction (De Oliveira Neves & de Oliveira, 2021; Inanan, 2022). The other technology frequently approached are ultrafiltration and nanofiltration membranes, which mechanically separate out the lactose molecules (Winkless, 2021). Nevertheless, researchers are constantly searching for better filtration performance and separation capacity, for example, the work by Morelos-Gomez et al. (2021), who developed graphene oxide membranes for lactose separation. In any of the procedures, the final product must present a lactose concentration in accordance with the guidelines of the country or region. For instance, according to the European Food Safety Agency (EFSA), final products labeled as "lactose-free" must have a lactose concentration lower than 0.1 g 100 mL^{-1} (EFSA, 2010). This same limit is established by the Brazilian guidelines published by the Health Surveillance Agency (ANVISA) (BRASIL, 2017). In the same way, ANVISA establishes that dairy products classified as "lactose-reduced" or "low-lactose" are those that contain lactose in a range of 0.1–1.0 g 100 mL^{-1}. On the other hand, the Food and Drug Administration (FDA) does not define the terms "lactose-free" or "lactose-reduced", but suggests that these claims should be truthful and not misleading. These products or those with similar claims are expected to be free of milk allergen, or in this case, free of lactose (FDA, 2022).

Freeze concentration (FC) is an environmentally friendly and emerging technology employed to concentrate food solutions with heat-sensitive constituents such as coffee, milk, and fruit juices. Regarding the milk, thermal treatment in a temperature variation from 70 to 100°C may denature the whey protein (e.g., β-lactoglobulin and α-lactalbumin) and provoke the formation of aggregates (Qian et al., 2017). Thus, FC has been revealed to be very efficient in the maintenance of bioactive compounds, volatile compounds, and biological activity (cytotoxic activity and antioxidant) due to the low temperature (below 0 °C) used in the process (Gunathilake, 2020). Thereby, the beneficial effects of FC have been newly reported in blueberry juice (Casas-Forero et al., 2020a, b; Casas-Forero et al., 2021a), strawberry juice (Adorno et al., 2017), orange juice (Haas et al., 2022), pineapple juice (Orellana-Palma et al., 2020a), waste potato juice (Kowalczewski et al., 2019), apple juice (Ding et al., 2019; Qin et al., 2019), broccoli extract (Azhar et al., 2020), green tea (Meneses et al., 2021), milk (Camelo-Silva et al., 2022; Machado Canella et al., 2020), and whey (Barros et al., 2022; De Liz et al., 2020). More specifically, Camelo-Silva et al. (2022) freeze concentrated milk until the third stage and used the sample from the first stage for ice cream production (Ice cream 1). This ice cream was contrasted to the ice cream from regular milk (Ice cream 2), and the authors concluded that Ice cream 1 presented good chemical, physical, rheological, and microstructural properties. Likewise, in the approach of Barros et al. (2022), FC was employed as an indirect technology for ice cream manufacture. However, instead of milk, cheese whey was used as a raw material for the FC process, and the concentrate from the second stage was evaluated as a milk substitute in ice cream. After testing 4 replacement levels, the authors concluded that the incorporation of concentrated whey at a 50% substitution level was better in terms of color, flavor, and texture attributes. In addition to the structural characteristics of the final product, the other advantage of this study was the added value to the whey, which is often seen only as a by-product of the cheese industry. De Liz et al. (2020) also utilized freeze concentrated whey in their experiments, but with another proposal: goat's whey freeze concentrate was used as an encapsulant agent for probiotic encapsulation (*Bifidobacterium animalis* ssp. *lactis* BB-12) by spray drying. The powdered probiotic samples from the encapsulation process presented good stability after storage at 4 °C and 25 °C for 40 days (> 7 log CFU g^{-1}), showing that goat whey concentrates had excellent behavior as a cell protective material. Also, it was noted that, as a wall material, goat whey concentrate exhibited favorable thermal properties before and after encapsulation. Equally, Machado Canella et al. (2020) worked with a based-goat raw material. However, their sample did not consist of goat whey, but semi-skimmed goat milk. After optimization of the FC process, it was obtained a concentrate yield of 77.97%. Moreover, the content of total solids changed from 9.94 to 32.87 (g 100 g^{-1}), with an emphasis on protein (from 3.53 to 9.43 g 100 g^{-1}). As well, FC has ever been used to immobilize extracellular ice nucleators (Zhou et al., 2014).

Among FC processes, block freeze concentration (BFC) has been studied to concentrate various liquid foods. For example, coffee extract in the work by Sequera et al. (2019), beet (*Beta vulgaris L.*) by-products extract in the work by D. Arend et al. (2022), goat milk by Canella et al. (2019a), sapucaia nut cake milk in the study by Demoliner et al. (2020), and strawberry juice by Jaster et al. (2018). In this technique, the solution to be concentrated is totally frozen, followed by partial gravitational thawing. Thus, the ice block acts as a solid carcass through which the concentrated food traverses (Machado Canella et al., 2018). Aiming to improve separation efficiency, the BFC can be assisted by other techniques, such as ultrasound, centrifugation (Baykal & Dirim, 2019), and vacuum (Orellana-Palma et al., 2017a).

The associated use of different concentration methods has also been described in the literature; for example, progressive FC (PFC) of coconut water followed by controlled thawing of ice (Jayawardena et al., 2020), and suspension FC and centrifugal filtration of apple juice (Qin et al., 2021). Thereby, the combination of PFC and BFC has been seen as an interesting opportunity to increase the amount of concentrate extracted from the ice fraction. In addition, the process could reach a higher phase separation than an isolated FC method. Studies addressed by Miyawaki and Inakuma (2021) and Prestes et al. (2022) demonstrated that each method (individually) also presents acceptable extraction results. However, as a complete unit, both methods are enhanced, and in turn, it achieves a more effective extraction, leading to a higher quality in the final concentrate, with excellent process parameters results. In this context, a recent work proposed the combination of PFC and BFC supported by vacuum to desalinize salt solutions (Hernández et al., 2021). These saline solutions simulated seawater fluid, indicating that PFC and vacuum-assisted BFC can have practical applicability. Moreover, Dantas et al. (2021) suggested the combination of PFC and vacuum-assisted BFC as a strategy for the concentration of lactose-free milk, with a significant increase of total solids (from 8.8 to 18.5 g 100 g $^{-1}$). Firstly, the authors evaluated the behavior of the sample when submitted to the PFC. Then, given the considerable amount of total solids retained in the ice, this fraction was subjected to the vacuum-assisted BFC. In conclusion, despite the potential of PFC and BFC combination, there are still gaps in this separation method, since there are no studies on other liquid foods such as juices or extracts. Also, there is no investigation of effects on the final amount, physicochemical parameters, bioactive components, and antioxidant capacity. And finally, there is no consideration of the initial parameters of the sample (density, viscosity, solutes, among others), or even, the option to use BFC and later PFC.

From previous studies on centrifugation-assisted BFC (Casas-Forero et al., 2021b; Guerra-Valle et al., 2022; Orellana-Palma et al., 2021), the present work considers the application of this technology at a first moment. Subsequently, as performed by Dantas et al. (2021), an ice fraction was subjected to the vacuum-assisted BFC aiming for better utilization of the nutrients retained in this portion. Therefore, the approach of this research provides data on the use of BFC processes (both centrifugation-assisted BFC and vacuum-assisted BFC) to concentrate carbohydrates and protein from lactose-free milk. Our general objective is to evaluate the viability of these processes as non-thermal technologies to enhance the content of important nutrients in lactose-free milk. The development of concentrated liquid milk, and its transference to the food industry, is expected to increase the sustainability of the food system (processing), the health of consumer diet, and the food industry competitiveness.

2. Material and methods

2.1 Material

UHT lactose-free skim milk (CARREFOUR®, Madrid, Spain) from a local supermarket in Barcelona (Spain) was used in the freezing concentration experiments. It contained 8.80 g 100 g^{-1} of total solids, 3.2 g 100 g^{-1} of proteins, 4.8 g 100 g^{-1} of carbohydrates (lactose <0.01%), and lipid <0.5 g 100 g^{-1}.

2.2 Physicochemical analysis

The total solids content of initial lactose-free milk, concentrated milk fractions, and ice fractions was determined exactly as the protocol described by Dantas et al. (2021). Therefore, a standard curve of total solids content against °Brix readings was plotted employing different concentrations of lactose-free skim milk. Thus, at the end of each freeze concentration test, the °Brix result was converted and expressed as total solids content (g 100 g^{-1}) through a linear regression ($y = 0.8715x – 0.3553$, $R^2 = 0.999$).

Protein analysis was performed according to the Kjeldahl method (AOAC, 2005). In turn, galactose, glucose, and lactose were determined following Schuster-Wolff-Bühring et al. (2011), with some modifications. Therefore, the sample (1 mL) was initially diluted in distilled water (8 mL) and mixed. In this solution were added 0.5 mL of Carrez Reagent 1 and 2, which was vortexed for 1 min. Then, after 15 min of rest, a nylon syringe filter (0.45 µm of diameter pore) (Agilent, Santa Clara, California, United States) was used to filter the mixture. Each sample was injected in triplicate onto a carbohydrate column (ION 300) (Interaction Chromatography, San Jose, CA, USA) of an HPLC system (Hewlett Packard Series 1100, Agilent Technologies, Waldbronn, Germany). A refraction index (Detector Beckman 156, San Ramon, California, United States) was employed as detector. The column temperature was maintained at 28 °C, and the mobile phase used was a sulfuric acid solution (0.013 M), with a flow rate of 0.4 mL min^{-1}.

2.3 Freeze concentration systems of lactose-free skim milk

Two freeze concentration protocols (centrifugation-assisted BFC and vacuum-assisted BFC) were used for the lactose-free milk concentration.

2.3.1 Centrifugation-assisted BFC

The centrifugation-assisted BFC process was performed in accordance with the methodology proposed by Orellana-Palma et al. (2017b), but with some modifications. Lactose-free milk (45 mL) was placed in centrifugal plastic tubes (internal diameter equal to 27 mm) and frozen in a static freezer (12 h, − 20 °C), since according to Samsuri et al. (2015), this time helps in the formation of large ice crystals, contributing positively to the concentration process during the phases separation. The tubes were previously enveloped with polystyrene foam. After the freezing, the frozen samples were rapidly transferred to a centrifuge (Hettich model Rontanta 460R, Tuttlingen, Germany) with temperature control. The data from four tubes were considered as a single batch, that is, 4 tubes were put in the centrifuge and their data were collected together. This procedure was replicated three times for each predetermined condition. Since the samples were centrifuged, the ice fraction was separated from the concentrate fraction using a filter.

A completely randomized 2^3 factorial design was performed. The independent variables (centrifuge rotation speed, temperature of centrifugation, and assay time) and their respective levels are shown in Table 1. The choice of these parameters was based on preliminary tests, as well as on correlated works. For example, Santana et al. (2020) verified the best separation conditions at longer assay times. Baykal and Dirim (2019), who studied the centrifugal block freeze crystallization of milk with different fat contents, applied 4500 rpm for a time of 35 min in their experiments, which provided values of efficiency of concentration between 63−83%. The preliminary tests were essential for the establishment of centrifugation temperatures since the temperatures generally used in the works (i.e., 15, 20, and 25 °C) did not allow the efficient phase separation of lactose-free milk within the time settled (up to 70 min). The responses (dependent variable) were efficiency (η), concentration index (*CI*), and concentrate yield (*Y*).

The efficiency of concentration was defined as the increase in the solids concentration of the concentrate fraction relative to the solids content retained in the ice fraction. Thus, this parameter was determined using Equation (1):

$$\eta\ (\%) = \left(\frac{C_f - C_i}{C_f}\right) x\ 100 \qquad (1)$$

where C_f is the total solids content of the concentrate fraction (g 100 g^{-1}), and C_i is the total solids content (g 100 g^{-1}) of the ice fraction.

The concentration index was given as described by Moussaoui et al. (2018). So, a ratio between the solids content in the concentrated liquid and the initial milk was calculated using Equation 2.

$$CI = \frac{concentrate\ fraction\ total\ solids\ (g\ 100\ g^{-1})}{initial\ milk\ total\ solids\ (g\ 100\ g^{-1})} \quad (2)$$

The concentrate yield was calculated according to Machado Canella et al. (2020), using Equation (3).

$$Y(\%) = \frac{C_f\ m_f}{C_0\ m_0} x\ 100 \quad (3)$$

where C_f is the total solids amount of the concentrated sample (g 100 g^{-1}), C_0 is the initial total solids amount of milk (g 100 g^{-1}), m_f is the concentrated sample weight (g), and m_0 is the initial milk weight (g).

After all conditions were evaluated, one of them was considered the best option in the case of single-step freeze concentration. On the other hand, we also elected another condition that can be submitted to a new freeze concentration cycle (second cycle). For this case, the fractions obtained by the centrifugation-assisted BFC were termed Conc 1 (from concentrated liquid) and Ice 1 (from frozen solution).

2.3.2 Vacuum-assisted BFC

The Ice 1 was used as a feed solution for the second freeze concentration cycle (Figure 1). For this, the ice produced after centrifugation was immediately subjected to the vacuum-assisted BFC. The vacuum was applied on the condition of 10 kPa (absolute pressure) during 50 min. The suction was performed by linking the bottom of the frozen sample to a vacuum pump. The choice of pressure and time was based on preliminary tests and the work of Machado Canella et al. (2020). The fractions obtained from second cycle were denoted as Conc 2 and Ice 2. Equations 4–7 were used to calculate the *CI* of second cycle, in terms of total solids, protein, and carbohydrates:

$$CI = \frac{Conc\ 2\ total\ solids\ (g\ 100\ g^{-1})}{Ice\ 1\ total\ solids\ (g\ 100\ g^{-1})} \quad (4)$$

$$CI = \frac{Conc\ 2\ protein\ (g\ 100\ g^{-1})}{Ice\ 1\ protein\ (g\ 100\ g^{-1})} \quad (5)$$

$$CI = \frac{Conc\ 2\ glucose\ (g\ 100\ g^{-1})}{Ice\ 1\ glucose\ (g\ 100\ g^{-1})} \quad (6)$$

$$CI = \frac{Conc\ 2\ galactose\ (g\ 100\ g^{-1})}{Ice\ 1\ galactose\ (g\ 100\ g^{-1})} \quad (7)$$

Efficiency and Concentrate yield were also calculated for vacuum-assisted BFC from the adaptation of Equations 1 and 3, respectively.

2.4 Validation of results

As presented by Machado Canella et al. (2020) and Muñoz et al. (2018), a mass balance was made and therefore the experimental results were compared to the theoretical values (Equation 8).

$$w_{pred} = \frac{initial\ milk\ total\ solids\ (g\ 100\ g^{-1}) - concentrate\ fraction\ total\ solids\ (g\ 100\ g^{-1})}{ice\ fraction\ total\ solids\ (g\ 100\ g^{-1}) - concentrate\ fraction\ total\ solids\ (g\ 100\ g^{-1})} \quad (8)$$

where W_{pred} is the predicted ice fraction mass ratio (kg ice/kg lactose-free milk).

To determine the quality of the fit between experimental and theoretical data, the root mean square (RMS) was calculated using Equation 9, as follow:

$$RMS\ (\%) = 100\sqrt{\frac{\sum\left(\frac{w_{exp} - w_{pred}}{w_{exp}}\right)^2}{N}} \quad (9)$$

where W_{exp} is the ratio of experimental ice mass ratio ($mass_{ice}$ / $mass_{initial\ lactose\text{-}free\ milk}$), and N is the number of assay repetitions.

2.5 Statistical analyses

To evaluate significant differences ($P < 0.05$) between treatments, one-way analysis of variance (ANOVA) and the *t*-test were used. The software STATISTICA version 13.3 (TIBCO Software Inc., Palo Alto, CA, USA) was employed for these statistical analyses, and the results were expressed as mean ± standard deviation (means found in triplicate).

3. Results and discussion

3.1 Experimental design

Table 2 presents the results of the response variables investigated, including the efficiency, concentration index, and concentrate yield. As cited above, the centrifugation implementation as a supported technique to the BFC process was tested to improve its separation efficiency. Thus, the parameter efficiency (η, %) reached values of approximately 78%, results that were similar to those found by Casas-Forero et al. (2021a) and Orellana-Palma et al. (2020a) after the first centrifugation-assisted BFC cycle. In turn, Orellana-Palma et al. (2020a), who studied this technique applied to pineapple juice, highlighted that these high values are due to the porous form of the ice fraction since this structure has channels between the ice crystals and permits to extract solutes easer with the centrifugal force.

Regarding the Concentration index (*CI*) and Concentrate yield (*Y*), the centrifugation time produced an important effect in these responses, with significant differences ($P < 0.05$) for each condition. A gradual increase in *Y* values was observed as time progressed, since 70 min presented better results than 45 min, with 45% and 29%, respectively, in the condition at 30 °C and 4500 rpm. In contrast, at 45 min of centrifugation, the *CI* values were higher than those at 70 min, as the same example at 30 °C and 4500 rpm. Dantas et al. (2021) found similar results when applying PFC in lactose-free milk: in general, *CI* was maximum when *Y* was minimum, and this was associated with the amount of ice produced. In the present work, the condition at 40 °C, 3500 rpm, and 45 min (*Y* = 45.25%, *CI* = 3.04) resulted in an ice fraction of ~82%; meanwhile, at 40 °C, 3500 rpm, and 70 min (*Y* = 62.46 %, CI = 2.26), approximately 70% of ice fraction was produced. A smaller amount of ice formed consequently indicates a larger amount of unfrozen solution. Thereby, the longer the assay time, the diluent (water) present in the ice fraction tends to be incorporated into the concentrated fraction, decreasing its solids content. This situation is correlated with the final temperature of the process, since longer rotation times of the centrifuge generate an increase in the rotor temperature, causing that part of the ice to transform into water and be expelled to the outside together with the concentrate, harming the phase separation of the system. Conversely, an insufficient centrifugation time would not achieve an efficient separation, and thus, the process parameters would indicate low concentrate recovery percentages (Santana et al., 2020). Therefore, these results (higher *CI* values when time is shorter) could be justified due to the ice fraction staying relatively intact (without breaking and/or thawing) employing 45 min as centrifugation time, and thus, only freeze concentrate was extracted from the ice fraction. This pattern was also observed by Orellana-Palma et al. (2020b), who employed centrifugal BFC to fresh calafate juice. On the other hand, a larger amount of unfrozen solution leads to an increase in *Y*, since this parameter takes into account the final mass of the concentrated fraction (Equation 3). As a result, it was observed that the highest separation conditions presented better results than the lowest centrifugation temperature, time, and rotation speed conditions. Other studies indicated *Y* values higher than our data. For example, Guerra-Valle et al. (2021) obtained *Y* values between 70% to 90% in the freeze concentration of Endemic Patagonian berries juices under unidirectional BFC at −20 °C, 20 °C of centrifugal temperature, 20 min of centrifugal time, and 4000 rpm of rotation speed. In the same way, Orellana-Palma et al. (2017b) achieved a *Y* value close to 75% under unidirectional BFC at −20 °C, 20 °C for 15 min at 4000 rpm to concentrate blueberry juice. Therefore, apart from the centrifugal time, the different *Y* results can also be explained by the separation technique and the multiple conditions applied to the sample, i.e., the freezing conditions (axial or unidirectional), rotation speed, initial sample amount, in addition to the nature of the sample.

Figure 2 shows the effects of temperature, centrifuge rotation speed, and time on the three responses studied using Pareto diagrams. Figure 2a illustrates the effects of these three factors on *CI*. All individual factors, as well as the interaction between all of them, had a significant negative effect on the *CI*. This is in accordance with what was discussed above regarding the time. From Figure 2a and Table 2, it can be seen that the centrifugation rotation speed also negatively affected the *CI*, since the highest values were found at the lowest rotation speed (3500 rpm). In opposition, interactions between two factors were non-significant and had a positive effect on the response. Concerning the Efficiency (Figure 2b), the individual factors had no significant effect, only two interactions (time:temperature:rotation speed and time:rotation speed). Santana et al. (2020) also calculated the concentration efficiency for blueberry juice when subjected to the centrifugation-assisted freeze concentration, and likewise, individual factors did not affect this response. A different result was noted for *Y* (Figure 2c), where it was observed that all individual factors and two interactions had a significant effect on the response.

In view of all the above, the experimental condition at 40 ºC, 70 min, and 4500 rpm (Treatment 8) was considered ideal for the freeze concentration in a single step, given the highest values of efficiency and concentrate yield (80.87% and 67.02%, respectively), and still an excellent value for concentration index (2.05). Dantas et al. (2021), who used progressive FC combined with vacuum-assisted BFC to concentrate lactose-free milk, found an estimated *CI* value of 1.42. On the other hand, this present work proposes a one-step approach, with a highest value of *CI*. Therefore, these findings contribute to its industrial application and process agility. The variations in process parameter terms and in final solute concentration can be explicated by the ice front expanse for each FC technology. Centrifugation-assisted BFC exhibited lower solute retention and better final concentration in C_i than progressive FC due to a phenomenon denominated constitutional supercooling, i.e., the ice growth rate is determined by the heat transfer area and the cooling temperature, which is a relevant factor in the design of FC apparatus (Orellana-Palma et al., 2020a). The concentrated lactose-free milk from Treatment 8 presented the following values (g 100 g^{-1}) for protein, lactose, glucose, and galactose, respectively: 6.50 ± 0.26, 0.27 ± 0.01, 5.53 ± 0.07, and 5.27 ± 0.06. The initial values for each of these components are shown in Table 3. Thus, it is clear that the obtained amounts were at least 2-fold higher when compared to the initial milk sample.

Nevertheless, we also investigated the FC in two steps in order to further improve the results and decrease the total solids in the frozen matrix, since it was noted that Treatment 1 had the highest content of retained solids in the ice (7.28 g 100 g^{-1}). Thus, we are interested in the recovery of these solids. As already mentioned, the second cycle was performed using another methodology (vacuum-assisted BFC). In this case, the vacuum was applied to the ice immediately after the centrifugation step. The integrity of the ice in the condition at 30 °C, 45 min, and 3500 rpm was also decisive in the choice of this treatment for the application of vacuum.

3.2 Vacuum-assisted BFC

As enlightened by the maker, the lactose-free skim milk employed in this work was lactose-free due to prior enzymatic hydrolysis with β-galactosidase. Thus, the resulting milk presented large quantities of the monosaccharides galactose and glucose (about 2.4 g 100 g^{-1} of each). Therefore, the contents of glucose, galactose, lactose, and protein of two cycles were measured and are shown in Table 3. A high separation of solids content (CI = 3.57 for the concentrate) was obtained using vacuum-assisted BFC. Carbohydrates and protein contents were also maximized using a second cycle. In addition, the solids content verified in Ice 2 was reduced considerably. Machado Canella et al. (2020) stated that this behavior is expected because the natural division of gravitational thawing is improved due to external driving force (vacuum). Equally, η(%) and *Y*(%) values were improved when vacuum-assisted BFC was performed (91.20 ± 0.91 and 71.50 ± 1.31, respectively).

While investigating the values more deeply, the results suggest that centrifugation and vacuum have different effects on milk components. This is because the *CI* of Conc 1 for protein was equal to 3.66, while the *CI* of Conc 2 was equal to 3.97. Similar behavior occurred for carbohydrates (average of galactose and glucose values): *CI* of Conc 1 was equal to 3.49, while the *CI* of Conc 2 was equal to 3.76. That is, vacuum-assisted BFC seems to have a slightly better performance compared to centrifugation-assisted BFC in terms of these macronutrients. However, when analyzing the total solids (*CI* of Conc 1 = 4.06, *CI* of Conc 2 = 3.57), we noticed an inverse behavior, suggesting the effect of centrifugation-assisted BFC on the other milk constituents (minor components) outweighs the effect on the macronutrients studied. Kawasaki et al. (2006) developed a work that can help to understand these results. They studied PFC of multicomponent solutions, and found that the small molecular mass solutes concentrated and separated more satisfactorily than the higher molecular mass solutes. This coincided well with the magnitude of the diffusion coefficient of each solute. Likewise, Nakagawa et al. (2010) noted that phenol (M.M = 94 g mol^{-1}) was more concentrated in the liquid zone than dye (M.M = 993 g mol^{-1}). So, the dissimilarities found between total solid solids content vs. carbohydrates and proteins could be explained due to the difference in the mobility when the substances meet the freezing front. The molecular size and the concentration affect the mobility of the solutes. Therefore, as a suggestion for future research on lactose-free milk FC, we propose an extensive investigation of mineral and vitamin contents, in addition to specific determinations of the protein fraction (for instance, αs1, αs2-, β-, and κ-casein), given the increasing demand for kinds of milk that offer additional advantages over common milk in terms of digestive health. In this regard, Canella et al. (2019b), who freeze concentrated skim goat milk by BFC, found the content of important minerals (such as magnesium and calcium) can be enhanced by the increasing freeze concentration cycles. Moreover, it was discussed that a substantial increase in the flavor and taste of food products can be verified when unitary operations based on vacuum and low temperatures are used (Sun & Zheng, 2006).

According to Beldie and Moraru (2021), the concentration of milk in the dairy industry is typically achieved by thermal evaporation or membrane technology such as reverse osmosis. The efficiency of these processes is very similar to those obtained by the freeze concentration process used in the present study. In thermal evaporation, the maximum concentration level achievable is 55% for skim milk and 50% for whole milk, while for the membrane process, the maximum concentration varies between 25% and 30% solids. Thermal evaporation is also known to reduce the quality of the final concentrated product due to prolonged exposure to heat, which negatively affects the color, taste, and nutritional value of milk. Furthermore, evaporators are prone to biofilm formation by spore-forming mesophilic or thermophilic bacteria. A major drawback of membrane separation is that the process is significantly affected by membrane fouling, which leads to flux decline and limits the final achievable concentration of the product (Beldie & Moraru, 2021). Given the above, we can verify that block freeze concentration by centrifugation and vacuum can be used by the industry aiming for the macronutrient concentration of lactose-free milk.

3.3 Validation of experimental results

Table 4 shows the values of W_{exp}, W_{pred}, and RSM. The results were similar to those found by Orellana-Palma et al. (2017b) (0.6–0.9 for ice mass ration, W_{exp} or W_{pred}) in the cryoconcentration of blueberry juice. They noted a decrease in values as the freeze concentration cycles increased, and attributed this to the increase in the percentage of concentrate over consecutive stages. Our results showed analogous behavior, since, when analyzing the first and second cycle, the W_{pred} values were 0.95 and 0.79, respectively. Similarly, Orellana-Palma et al. (2017c) noticed a decrease in W values as the test time increased, and this was also attributed to the increase in the percentage of concentrate over time. Likewise, our results showed this trend, such as Treatments 2 and 4, where conditions were kept the same except for the increase in time. By comparing these treatments, we observed a significant decrease in W_{exp} and W_{pred} values, as well as Treatments 5 and 7. Additionally, the RMS values fluctuated between 1.0% and 3.7%, indicating an excellent fit, since a bad fit corresponds to RSM values over 25% (Lewicki, 2000). Therefore, the experimental conditions used to concentrate lactose-free milk macronutrients were ideal. Moreover, the RMS values were lower than the values obtained by Petzold et al. (2015) and Orellana-Palma et al. (2017b) (4.9%, and 8.7%, respectively).

4. Conclusions

The results of this study suggest that it is possible to obtain an interesting concentrated sample without damaging its initial properties, obtaining a final product post BFC process with a high content of macronutrients. While investigating the effect of process parameters on centrifugation-assisted BFC of lactose-free milk, we observed that all individual factors presented a significant effect on concentrate yield and concentration index. On the other hand, the same did not occur to efficiency, where it was observed that only two interactions had a significant effect on this response, which in turn presented values from 78% to 81%. To obtain concomitantly the maximum concentrate yield and efficiency values, the operating condition can be set at a time of 70 min, centrifugation temperature of 40 ºC, and rotation speed of 4500 rpm. The results of this approach were promising, considering the simplicity and ease of the procedure. Nonetheless, a second stage for the freeze concentration process was proposed (by vacuum-assisted BFC), which also demonstrated to be an efficient method to concentrate skim lactose-free milk. In this system, the protein was well concentrated in the liquid phase (~3.97-fold higher than Ice 1), as well as the carbohydrates (glucose and galactose) (~3.7-fold higher than Ice 1). These findings support the idea that the concentration of lactose-free milk by centrifugation-assisted BFC jointly with an additional separation by vacuum-assisted BFC could be considered an innovative technology to obtain a liquid sample rich in macronutrients. Either concentrate 1 or concentrate 2 could be used for the development of new lactose-free dairy products. In this sense, future studies must be carried out to evaluate the feasibility of scaling the BFC system from laboratory scale to pilot plant scale, aiming the production on a mass scale. Thus, this technique would be an attractive alternative for dairy industries. Furthermore, an interesting challenge is the application of the two stages-BFC in other liquid food matrices, such as juices and/or extracts, and to study its effect on different properties. For instance, some properties currently valued are the presence of bioactive components and the antioxidant capacity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1 Factors values at the low (− 1) and high (+ 1) levels studied.

	Factors		
	Centrifugation temperature (°C)	Rotation speed (rpm)	Centrifugation time (min)
− 1	30	3500	45
+ 1	40	4500	70

Table 2 Results of efficiency, concentration index, and concentrate yield.

Treatment	Independent variables			Responses		
	Centrifugation temperature (°C)	Rotation speed (rpm)	Time (min)	Efficiency (η) (%)	Concentration index	Concentrate yield (%)
1	30	3500	45	79.41 ± 0.62[ab]	4.06 ± 0.04[a]	18.09 ± 0.44[e]
2	30	4500	45	79.54 ± 1.30[a]	3.61 ± 0.29[a]	29.77 ± 2.87[d]
3	30	3500	70	78.76 ± 1.26[ab]	3.74 ± 0.32[a]	23.39 ± 2.68[de]
4	30	4500	70	78.51 ± 1.20[ab]	2.82 ± 0.01[b]	45.11 ± 3.62[c]
5	40	3500	45	79.56 ± 1.11[a]	3.04 ± 0.15[b]	45.25 ± 3.41[c]
6	40	4500	45	78.23 ± 0.82[ab]	2.36 ± 0.02[c]	56.42 ± 0.83[b]
7	40	3500	70	76.65 ± 0.08[b]	2.26 ± 0.04[c]	62.46 ± 0.25[ab]
8	40	4500	70	80.87 ± 0.93[a]	2.05 ± 0.06[c]	67.02 ± 2.11[a]

[a,b,c,d,e]Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) between the treatments.

Table 3 Carbohydrate and protein content (mean ± standard deviation) of concentrates and ice fractions from first and second freeze concentration cycles.

	Lactose-free milk	Conc 1	Ice 1	Conc 2	Ice 2
Total solids (g 100 g^{-1})	8.8	35.38 ± 0.31	7.28 ± 0.18	26.02 ± 0.25	2.29 ± 0.22
Protein (g 100 g^{-1})	3.2	11.71 ± 0.31	2.35 ± 0.35	9.34 ± 0.09	0.83 ± 0.08
Glucose (g 100 g^{-1})	~ 2.4*	8.64 ± 0.32	2.02 ± 0.02	7.62 ± 0.02	0.64 ± 0.01
Galactose (g 100 g^{-1})	~ 2.4*	8.14 ± 0.29	1.91 ± 0.02	7.18 ± 0.04	0.63 ± 0.01
Lactose (g 100 g^{-1})	< 0.01	0.523 ± 0.005	0.174 ± 0.001	0.448 ± 0.001	< 0.01

Conc 1 and Ice 1 were fractions obtained by centrifugation-assisted freeze concentration at 30 ºC, 45 min, and 3500 rpm. Conc 2 and Ice 2 are fractions obtained by vacuum-assisted block freeze concentration from Ice 1. *The manufacturer does not provide data for monosaccharides, but only for total carbohydrates (4.8 g 100 g^{-1}). Therefore, glucose and galactose contents were expressed as approximate values, as discussed in section *3.2 Vacuum-assisted BFC* of this work.

Table 4 Experimental results validation for three different factors.

Treatment	Centrifugation temperature (°C)	Rotation speed (rpm)	Time (min)	W_{pred}	W_{exp}	RSM (%)
1	30	3500	45	0.95 ± 0.01^{a}	0.94 ± 0.01^{a}	1.39
2	30	4500	45	0.91 ± 0.02^{a}	0.89 ± 0.02^{b}	1.77
3	30	3500	70	0.93 ± 0.02^{a}	0.92 ± 0.01^{ab}	1.48
4	30	4500	70	0.82 ± 0.01^{b}	0.82 ± 0.02^{c}	1.00
5	40	3500	45	0.84 ± 0.03^{b}	0.82 ± 0.02^{c}	2.32
6	40	4500	45	0.736 ± 0.003^{c}	0.743 ± 0.001^{d}	1.02
7	40	3500	70	0.73 ± 0.01^{c}	0.70 ± 0.01^{e}	3.66
8	40	4500	70	0.63 ± 0.02^{d}	0.65 ± 0.02^{f}	2.76

[a,b,c,d,e]Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) between the treatments.

Figure Legends

Figure 1: Freeze concentration schematic diagram of lactose-free milk in two steps.

Figure 2: Effect of temperature, rotation speed, and time on the (a) Concentration Index, (b) Efficiency, and (c) Concentrate Yield.

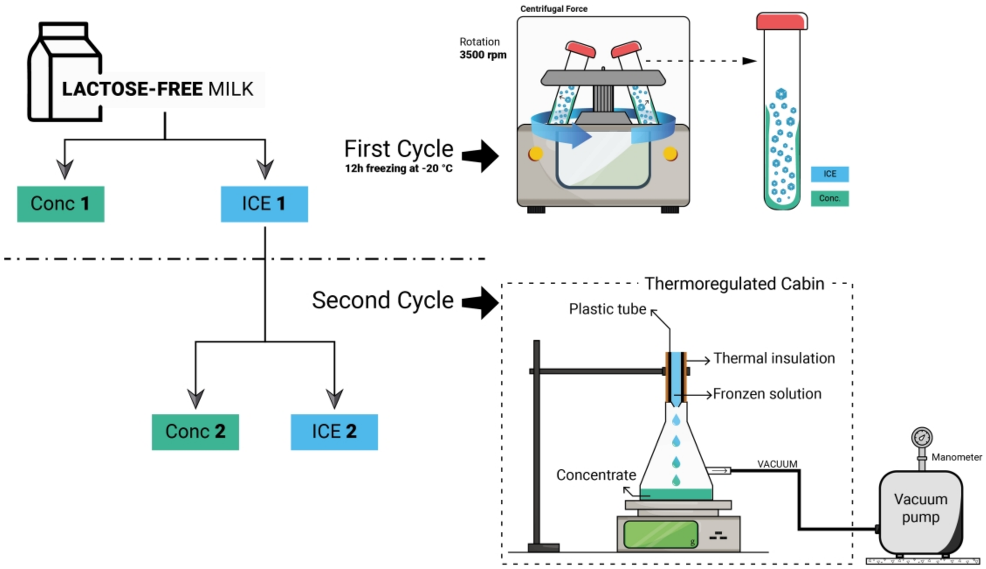
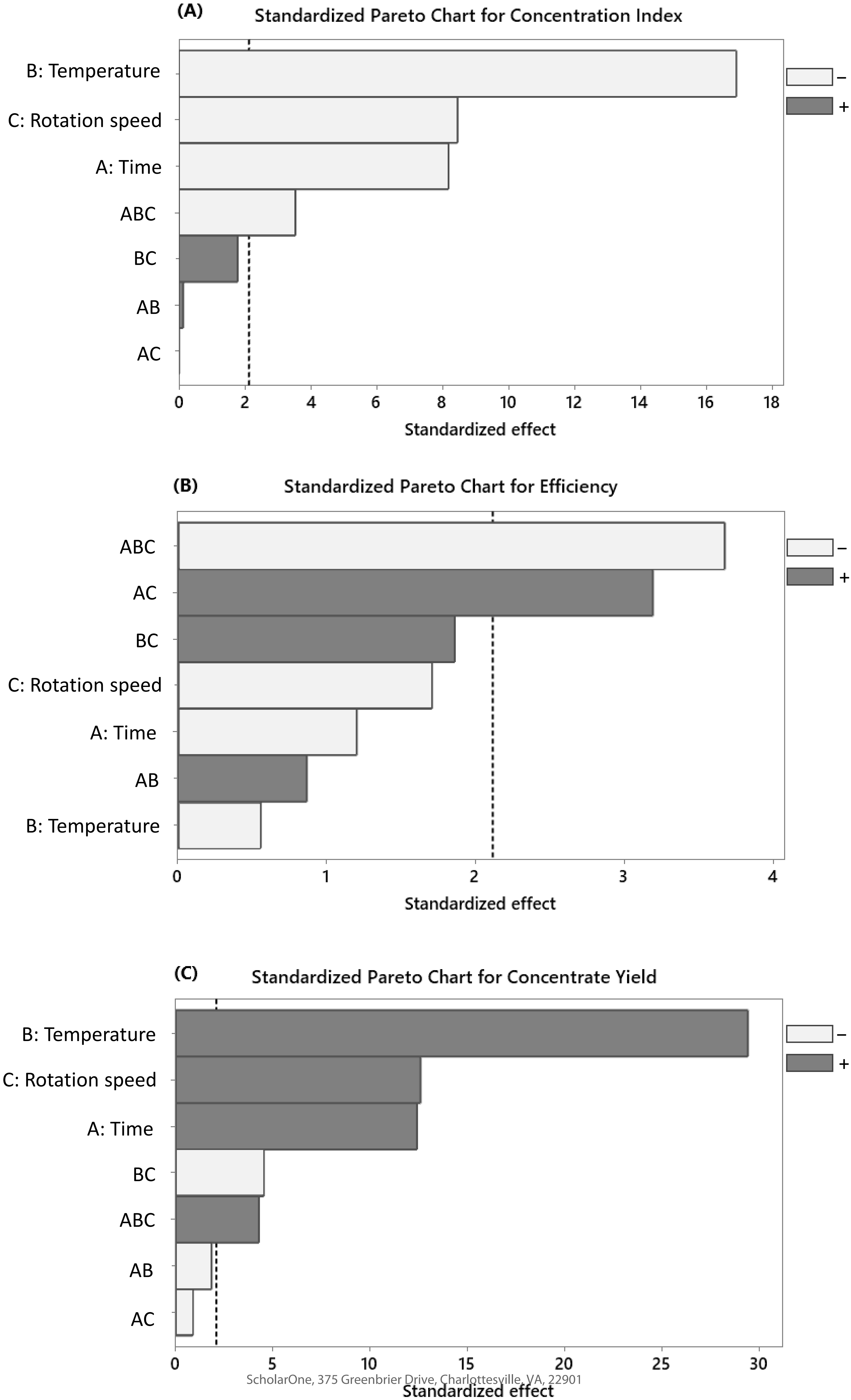


Figure 1: Freeze concentration schematic diagram of lactose-free milk in two steps.

828x506mm (118 x 118 DPI)


(A)
Standardized Pareto Chart for Concentration Index
B: Temperature
C: Rotation speed
A: Time
ABC
BC
AB
AC
–
+
0
2
4
6
8
10
12
14
16
18
Standardized effect
(B)
Standardized Pareto Chart for Efficiency
ABC
AC
BC
C: Rotation speed
A: Time
AB
B: Temperature
–
+
0
1
2
3
4
Standardized effect
(C)
Standardized Pareto Chart for Concentrate Yield
B: Temperature
C: Rotation speed
A: Time
BC
ABC
AB
AC
–
+
0
5
10
15
20
25
30
Standardized effect