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# Integrating bacterial cellulose in artisanal ice cream: a farm-to-fork sustainable approach



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This study explored the use of bacterial cellulose (BC) in milk-based cocoa ice cream as possible dietary fibers. BC fibers were added in 1%, 3%, and 5% wt. amounts, resulting in notable changes in texture, density, melting behavior. The ice cream transitioned from a Bingham to a Herschel-Bulkley fluid, while density rose with higher fiber content. Although hardness and gumminess slightly increased, the 5% wt. BC sample formed a more cohesive network structure when melted. Ice nucleation was delayed, with the first melt drop appearing later as BC content rose up to 22 minutes for the 5% wt. sample. Caloric content dropped by up to 21% corresponding to the highest amount of BC, making it a healthier dessert option. However, sensory testing showed that 1% and 3% wt. BC formulations were the most appealing to consumers.

Ice cream is an extremely popular dessert. Generally speaking, “ice cream” refers to a wide range of frozen dessert varieties. All types of ice cream have a similar base composition: they are sweetened, flavored, and contain ice. Unlike other frozen food, ice cream varieties are typically consumed in their original state, by people of all ages and conditions, due to the energy intake and the cooling effect<sup>1–3</sup>.

In terms of physico-chemical composition, ice cream is a multiphase system made up of air cells, fat globules, ice crystals, protein-hydrocolloid structures, and a serum phase that consists in dissolved carbohydrates, proteins, and salts dispersed/solubilized in unfrozen water<sup>4</sup>. Air bubbles have an important role in establishing the stability, sensory properties and physical attributes of ice cream<sup>4</sup>. Also, fat globules are present in ice cream in two different forms: as clusters of globules formed after freezing, or as separate, distinct globules formed during homogenization<sup>5</sup>. A larger degree of fat instability during freezing is indicated by a higher level of these partially coalescent globule clusters. The physical characteristics of ice cream, particularly melting, are significantly impacted by the existence of these globule clusters<sup>6,7</sup>.

In the past decade, consumer preferences have increasingly shifted towards more natural foods, often referred to as “bio-foods.” These are characterized by having a controlled origin, derived from sources as natural

as possible, and are free from artificial additives<sup>8,9</sup>. This shift has led to a new approach in food processing, emphasizing a “farm-to-fork” concept<sup>10,11</sup>.

The increased understanding of the food-health connection has led consumers to demand an ice cream that matches their physical and mental health needs<sup>1,8,12</sup>.

Recent studies have turned ice cream into a functional food that contains different and new ingredients of animal or vegetable nature<sup>13–17</sup>, namely, dietary fiber<sup>18–22</sup>, probiotic bacteria<sup>12,23</sup>, fruit pulp or syrup<sup>24</sup>, food colorings and natural sweeteners<sup>25–27</sup>.

To offer enhanced stability against salt, pH, heat treatment, freeze-thaw, and other variables that affect the behavior of various kinds of ice cream, a single polysaccharide or protein is insufficient. These days, there is a lot of interest in the investigation of boosting fiber consumption by adding more biopolymers derived from natural resources to ice cream and other food products. Protein-polysaccharide composites have been widely used in the stabilization of the food formulation systems due to their enhanced performance, which stems from the interactions between the protein and polysaccharide components<sup>28,29</sup>.

Bacterial cellulose (BC) produced extracellularly in a culture medium with carbon and nitrogen supplies by non-pathogenic aerobic bacteria, holds promise as a low-calorie substitute for traditional dietary fibers. While

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BC and plant cellulose share the same chemical composition, BC is a highly pure material lacking lignin, pectin, hemicellulose, or other associated components<sup>30–32</sup>.

By improving the cultivation conditions (temperature, pH), expanding the variety of substrates used (including food waste), and changing the operating methods (static, continuous, with or without stirring), the production of BC has significantly improved recently<sup>33–35</sup>. As a result, BC is now available in a variety of forms, such as sheets, films, granules, etc.<sup>29,36,37</sup>.

Bacterial cellulose was identified as safe by the Food and Drug Administration (FDA) of the United States and classified as an insoluble dietary fiber in the human gut. Natural hydrocolloids, dietary fibers have a variety of uses in food items, such as holding water, forming gels, and having characteristics similar to fat<sup>37,38</sup>. They can also be used to texturize, stabilize, and prevent sticking. Furthermore, the potential of dietary fibers to improve the consistency of small ice crystals, regulate the formation of ice, and promote the growth of small crystals during the freezing-storage of products could be responsible for their ability to store water. Food scientists have reported using BC in food applications such as thickening, stabilizing, and binding water agent, and as packaging in the form of intact functional membranes, dissociated nanofibrils, and crystals<sup>38,39</sup>.

Potential uses of BC in the food industry have been investigated due to its suspension stability, water retention, thickening, and emulsification properties<sup>29,40,41</sup>. Furthermore, BC fibers are not affected by changes in pH, temperature or ionic strength and exhibit a strong association with the protein matrix<sup>40</sup>. Other studies have shown that adding fiber, particularly BC, to ice cream, in the form of cream, improves melting characteristics, smooths the texture, and lowers the overall calorie content of the dessert<sup>42</sup> while maintaining the shape of the ice cream for at least 60 minutes at room temperature after it has been removed from the freezer.

Despite the limitations of large-scale BC production, including the long duration of the production process and the low yield relative to the amount of raw materials, BC has significant potential in the food industry. However, the utilization of BC in the dairy industry is still in its infancy<sup>28</sup>.

This study builds upon the research conducted over the past decade at the Mass Transfer Laboratory within the Faculty of Chemical Engineering and Biotechnologies at the National University of Science and Technology Politehnica Bucharest, Romania, focusing on BC synthesis from fruits wastes and the collaboration with commercial partners dedicated to the development of functional food products. This work has contributed significantly to the development of innovative BC-based food products.

Notably, in 2019, one of the authors, Chef Alexandru Ștefan (representative of the National Junior Chef Team in pastry domain), was awarded the Innovation Prize for the creation of low-calorie BIOS Cake (in GastroPan contest) in which a strawberry BC-based jelly was incorporated into a traditional cake formulation.

Beginning with the idea of “farm to fork,” which stands for the new challenge in the fight for sustainable food<sup>10,11</sup>, this study aims to introduce a novel concept: artisanal ice cream made from locally sourced, natural ingredients enriched with dietary fiber to transform its nutritional profile. Thus, this is a preliminary study in which different content of BC fibers was introduced in a classical milk-based ice cream formulation.

In contrast to the study developed by Mehta et al.<sup>43</sup>, which incorporated guar gum as an additive in the ice cream formulation and utilized a BC content of 2.8% dry matter on a wet basis, the present study adopts a non-additive formulation approach and explores a broader range of BC concentrations, specifically, 1%, 3%, and 5% dry matter on a wet basis.

By integrating BC fibers into a traditionally energy-dense dessert, one of the main purposes of this research is to create a lighter, functionally superior food product. The core objective is to assess how BC fibers influence on one hand, the product's textural characteristics, thermal stability, rheology behavior, and emulsification, as well as its visual appeal, melting behavior, and consumer sensory perception on the other. Consequently, this preliminary study has a dual focus on sustainable sourcing and nutritional innovation positioning the study at the intersection of human health improvement and environmental responsibility.

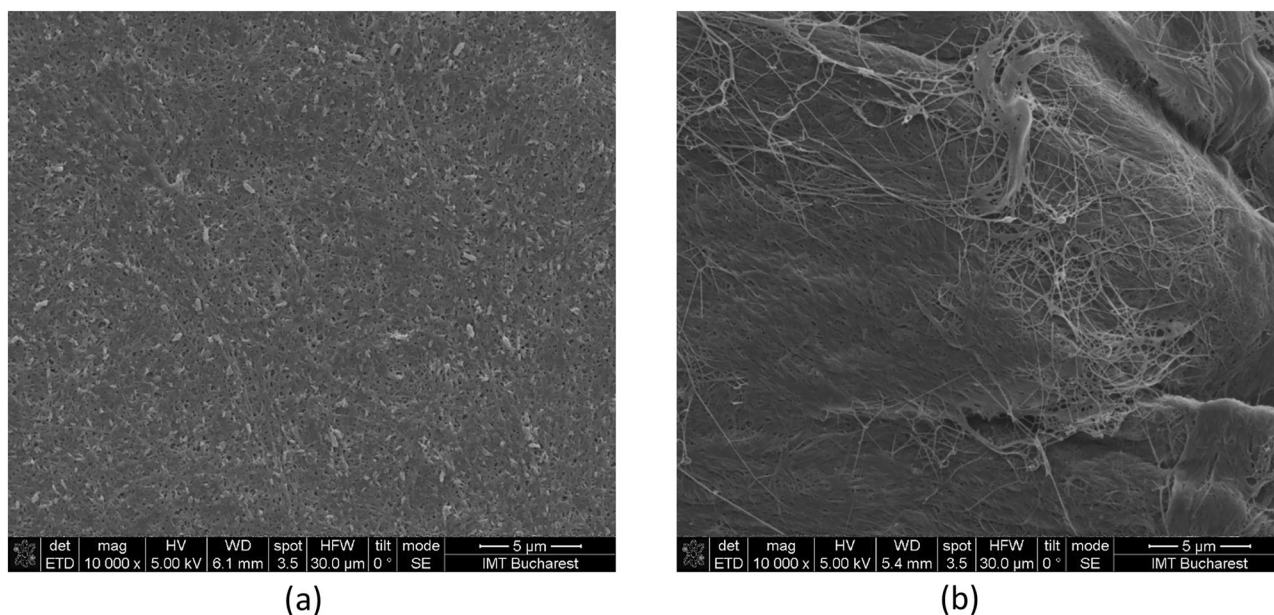
## Results

### Scanning electronic microscopy (SEM) analysis of the BC fiber

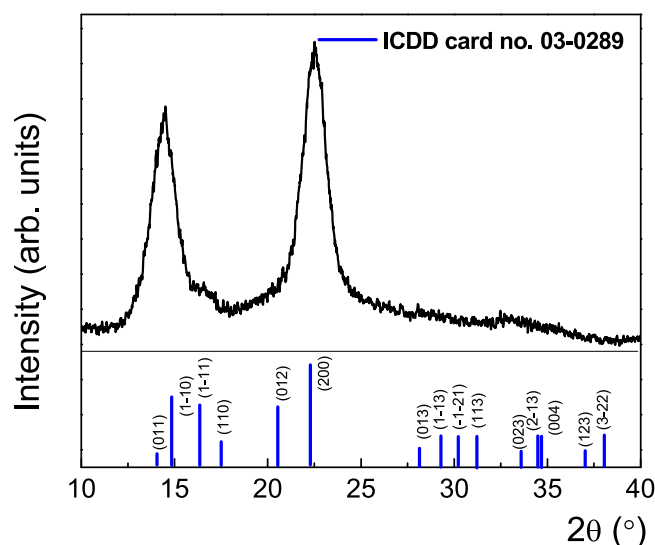
Following the purification procedure described in *Method section* and subsequent grinding, the fibers were dried and subjected to SEM analysis (Fig. 1). The morphology of BC was investigated before purification (Fig. 1a), and after purification and grinding (Fig. 1b). The aspect of grinded BC could influence several other characteristics of the final food product which will be discussed further in the manuscript.

### X-ray diffraction (XRD) analysis of the BC fiber

The experimental XRD pattern (Fig. 2) displays two sharp diffraction peaks at  $2\theta = 14.47^\circ$  and  $22.59^\circ$ , as well as two diffraction features at  $16.63^\circ$  and  $32.69^\circ$ . The observed diffraction peaks were attributed to  $I_\beta$  cellulose (see blue lines), with characteristic diffraction peaks at  $14.09^\circ$ ,  $14.90^\circ$ ,  $16.49^\circ$ ,



**Fig. 1 | The morphology of BC fiber before purification. a and after purification and grinding (b) determined by SEM analysis.**



**Fig. 2 | XRD pattern of the investigated samples.** The blue lines indicate the theoretical position of  $I_{\beta}$  cellulose (ICDD card no. 03-0289).

**Table 1 | Density values for ice cream formulations**

| Sample codes | Density, g·cm <sup>-3</sup> |
|--------------|-----------------------------|
| IC           | 0.771 ± 0.017               |
| ICBC1        | 0.888 ± 0.022               |
| ICBC3        | 0.932 ± 0.028               |
| ICBC5        | 0.965 ± 0.34                |

17.34°, 20.64°, 22.84°, 28.22°, 29.14°, 29.06°, 30.63°, 31.80°, 33.84°, 34.13°, 34.54°, 34.87°, 36.62°, 37.24° and 38.18°, according to ICDD card no. 03-0289. Also, the diffraction peaks could be assigned unambiguously to different reflections of Nishiyama cellulose with  $a = 0.835$  nm,  $b = 1.028$  nm, and  $c = 0.796$  nm with monoclinic space group<sup>44, 45</sup>.

A key structural aspect for cellulose is related to the size of the crystalline domains, since the different crystallite sizes affect the thermal degradation temperature of natural fibers<sup>46</sup>. One of the most common ways to evaluate the crystal quality is based on the Scherrer's equation. The Scherrer equation is based on an assumption that broadening is related to crystallite size alone. It gives the correlation between the size of the crystalline domains and the Full Width at Half Maximum (FWHM) in the following way<sup>47–49</sup>:

$$\tau = k\lambda/\beta \cos \theta \quad (1)$$

where  $k$  is a shape factor usually taken as 0.93,  $\lambda$  is the wavelength of the incident X-ray beam (0.15418 nm),  $\beta$  is the Full Width at Half Maximum (FWHM) of the diffraction peak in radians and  $\theta$  is the angular position. Based on the pseudo-Voigt fit of the (200) cellulose reflection, the FWHM is 1.5°, which implies a mean crystallite size of ~ 6 nm.

#### Density determination of the non-frozen BC-based formulations

The densities varied in the range of 0.771 up to 0.965 g/cm<sup>3</sup> and increased with the content of BC fibers added in the classical ice cream formulation as presented in Table 1. The values obtained are in good agreement with the literature data<sup>18,50–52</sup>, and in our case, these are attributed to the rehydration of the BC fibers that absorb the water content of the liquid phase of the ice cream components. Additionally, the rehydration behavior of BC fibers limits the amount of air that can be incorporated into the mixtures, as their swelling capacity reduces space for air entrapment.

#### Rheology of the non-frozen BC-based formulations

Viscosity indicates a fluid's resistance to flow in a quantitative way. Figure 3 shows the flow curves resulted from rheological measurements with the rotational viscometer giving the shear stress (Pa) as a function of deformation rate or shear rate (1/s) when the shear rate was increased from zero to a maximum level (up-curve – black lines), respectively decreased (down-curves – red lines) in the same period of time. Thus, our focus in these formulations was also directed to the behavior of the ice cream formulations before and after shearing.

#### Proximate composition

Table 2 shows the proximate composition and nutritional characteristics of the analyzed ice cream samples.

#### Mouth-feel related texture analysis

In this study, the samples were analyzed in melted state to correlate the rheological properties with the mouth-feel related texture characteristics of the samples after melting as shown in Table 3. Moreover, the texture analysis performed in melted state is more suitable for the products that incorporate new fibers or the addition of functional ingredients.

#### Dynamic scanning calorimetry (DSC) of the BC-based formulations

For a clearer and more precise analysis of melting/freezing stages the BC pristine sample and BC-based ice cream samples developed in this study were analyzed by DSC coupled with optical microscopy (OM).

DSC analyses coupled with OM were carried out for the BC sample, under nitrogen atmosphere, up to 325 °C (Fig. 4), respectively (Fig. 5).

For the BC-based ice cream samples the DSC analysis highlighted the thermal transitions (melting and freezing) of the food products between -60 and 60 °C as can be observed in Fig. 6, while the thermophysical properties of all samples were registered in Table 4. This temperature interval was selected considering that this food product will not be exposed to lower/higher temperature ranges during the deposition or consumption.

Optical microscopy images of the ice creams during heating from -60 °C to 60 °C show the textural changes associated with ice and fat melting (Fig. 7).

#### Melting analysis

The chemical composition of ice cream formulations and the physical structure of the ice cream itself affect the melting dynamics. The melting curves for all tested ice cream samples are presented in Fig. 8.

#### Color analysis

The color of the ice cream is one of the most important characteristics that determine the choice of the product by the consumer. The values of the color parameters (" $a$ ", and " $b$ ") and brightness (" $L^*$ ") in the examined ice cream samples are summarized in Table 5 and described in Figs. 9, and 10).

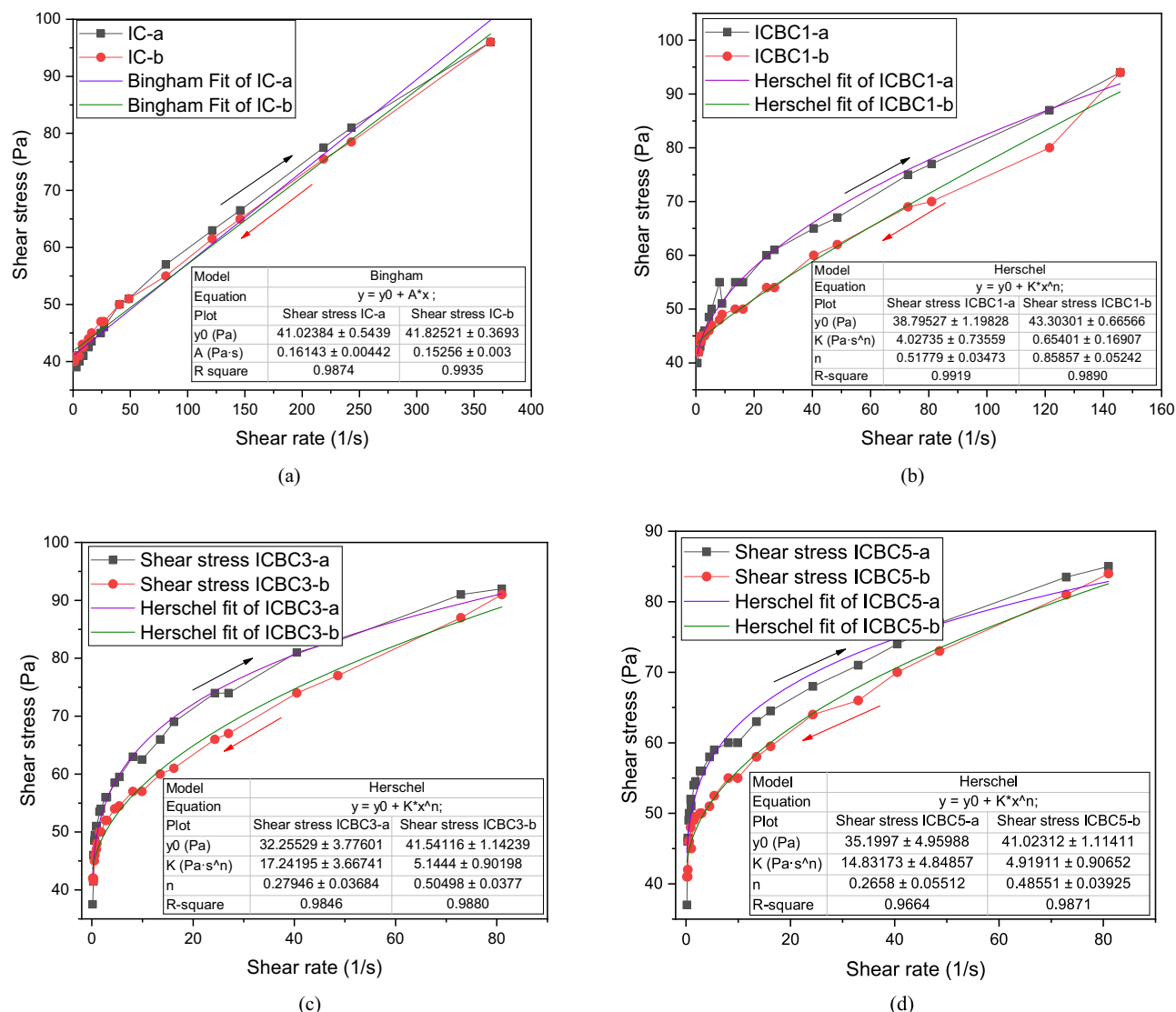
#### Sensorial and hedonic analysis of the final BC-based ice cream products

For the descriptive sensorial analysis, a questionnaire has been prepared as described in the Methods section with scoring scale for each parameter. The results obtained in the descriptive test are summarized in Table 6, while in Fig. 11 the spider diagram indicates the results of the descriptive test performed for all samples.

#### Discussion

To determine the morphology and to prove the purity of the BC fiber, the first analysis involved the use of SEM and XRD techniques (Figs. 1 and 2).

As shown in Fig. 1 the morphology of the BC fibers prior to purification appeared slightly compact with characteristic porous regions, typical of BC membranes formed via the acetic fermentation process (Fig. 1a). The morphology underwent a notable transformation, with the ground fibers



**Fig. 3 | Rheology of the non-frozen BC-based ice cream formulations.** Shear stress (Pa) as a function of shear rate (1/s) for classical ice cream IC (a), and the modified formulations (ICBC1) (b), (ICBC3) (c), (ICBC5) (d); shear rate up and down curves

are presented as black/square marker (a) and red/dot marker (b) with both fitting curves (purple line - a, and green line -b).

**Table 2 | Proximate composition of all ice cream formulations (reported as g/100 g sample)**

| Indicator        | U.M.        | IC                  | ICBC 1              | ICBC 3              | ICBC 5              |
|------------------|-------------|---------------------|---------------------|---------------------|---------------------|
| Energetic value  | Kcal /100 g | 254                 | 248                 | 227                 | 200                 |
| Energetic value  | Kj /100 g   | 1059                | 1035                | 948                 | 835                 |
| Protein          | %           | $3.77 \pm 0.113^a$  | $3.81 \pm 0.156^a$  | $3.85 \pm 0.184^a$  | $3.66 \pm 0.163^a$  |
| Carbohydrates    | %           | $22.49 \pm 0^d$     | $22.96 \pm 0^c$     | $23.05 \pm 0^a$     | $22.99 \pm 0^b$     |
| from which sugar | %           | $16.58 \pm 0.368^b$ | $17.46 \pm 0.311^b$ | $19.72 \pm 0.368^a$ | $17.24 \pm 0.311^b$ |
| Fats*            | %           | $16.56 \pm 0.283^a$ | $15.67 \pm 0.269^b$ | $13.26 \pm 0.24^c$  | $10.33 \pm 0.226^d$ |
| Moisture         | %           | $56.05 \pm 0.071^d$ | $56.54 \pm 0.141^c$ | $58.78 \pm 0.113^b$ | $61.95 \pm 0.071^a$ |
| Dry weight       | %           | $43.95 \pm 0.002^a$ | $43.46 \pm 0.001^b$ | $41.22 \pm 0.006^c$ | $38.05 \pm 0.003^d$ |
| Ash              | %           | $1.13 \pm 0.002^a$  | $1.02 \pm 0.001^c$  | $1.06 \pm 0.005^b$  | $1.07 \pm 0.003^b$  |

\*Calculated by difference. The results are mean  $\pm$  standard deviation ( $n = 2$ ). Values followed by different superscript letters in the same row are significantly different ( $p < 0.05$ ).

forming a much more compact structure, though some regions still exhibited entangled BC fibers as evidenced in Fig. 1b.

In terms of food products, the purity of raw materials is essential. In our case, demonstrating that the cellulose resulted from the fermentation process is free from lignin, hemicellulose, and pectin, XRD analysis was

performed on the BC ground purified fibers (Fig. 2). Moreover, XRD analysis can confirm the crystallinity and purity of the BC by comparison with the cellulose standard which is vital for the final behavior of the food product in terms of water retention, protein and fats interaction, and overall texture.



In contrast to our previously published work<sup>46</sup> on cellulose derived from *Gleditsia triacanthos* pods functionalized with *Phytolacca americana* fruit extract, where additional diffraction peaks corresponding to phenolic compounds were observed, the present study revealed no such additional peaks. This absence confirms the high purity of the ground and purified BC sample from this study.

According to literature data dedicated to different fluids or food products<sup>53,54</sup> a material/fluid is considered non-thixotropic when the black and red curves fully overlap, while its fluid thixotropy increases with the height of the hysteresis loop.

In our rheological experiments, the shear rate was increased or decreased while measuring the shear stress, and it was noticed that for the classical formulation of ice cream without BC, the mixture has a Newtonian behavior before freezing only if a certain value of the yield stress is exceeded. Thus, for IC (blank sample without BC), the trendline of the curves represented as blue, respectively green lines were linear when the yield stress,

$y_0$ , exceeded 41.02, respectively 41.82 Pa, indicating a Bingham rheological behavior<sup>55</sup> (see values from the table inserted in Fig. 3a). It is worth mentioning that the almost identical overlap of the two curves (red and black – Fig. 3-a) indicates that the formulation has no thixotropy, confirmed also by the close values of parameters A, and yield stress,  $y_0$ . Thus, by exceeding the value of the yield stress,  $y_0$ , the ice cream IC formulation will behave as a Newtonian fluid<sup>56</sup> in which the viscosity is directly proportional to the deformation. It is worth mentioning that below these values registered for the shear stress,  $y_0$ , the mixture will not flow.

By increasing the concentration of BC to 1%, 3%, respectively 5% wt., the rheological behavior of the fluid formulations changed from Bingham to Herschel-Bulkley (Fig. 3b, c, and d)<sup>57</sup>. The curves fitted very well the Herschel-Bulkley model since the values of  $R^2$  were close to unity for all BC-based ice cream formulations<sup>21</sup>.

Three parameters characterize the non-Newtonian Herschel-Bulkley rheological model following the expression of Eq. 2, in which  $y$  is the shear stress (Pa),  $y_0$  is the yield stress (Pa),  $K$  is the consistency index ( $\text{Pa}\cdot\text{s}^n$ ),  $\dot{\gamma}$  is the deformation or shear rate ( $\text{s}^{-1}$ ), and  $n$  is the dimensionless flow index<sup>21</sup>:

$$y = y_0 + K\dot{\gamma}^n \quad (2)$$

For ICBC1, ICBC3, and ICBC5, the formulations will behave as non-deformable solids if the shear rate  $\dot{\gamma}$  is lower than  $y_0$ , and as fluids if  $\dot{\gamma}$  is higher than  $y_0$ . To put these into other words, the formulations will flow once the shear stress exceeds the values of  $y_0$ , namely 38.79 Pa for ICBC1, 32.25 Pa for ICBC3, respectively and 35.19 Pa for ICBC5. It is important to note that no clear trendline could be found between the  $y_0$  values of the yield shear stress for any of the samples that had been modified with BC due to the challenges in evenly distribute the BC fibers throughout the milk-based formulations.

Parameter  $n$  indicates the degree of which the fluid is closer to a shear-thinning or a shear-thickening behavior. In both cases  $n$  has values below 0.3, respectively below 0.5 for the hysteresis indicating a clear pseudoplastic shear-thinning formulation<sup>18,47</sup> before the freezing process that involves the transfer of the fluid into molds by pouring or pumping procedures.

In industrial applications the shear-thinning behavior of fluids, regardless of their use, is preferred considering that the immediate consequence implies that apparent viscosity will decrease while the shear rate increases. Thus, the flow resistance of the fluid will decrease at higher shear rates during mixing or pouring process.

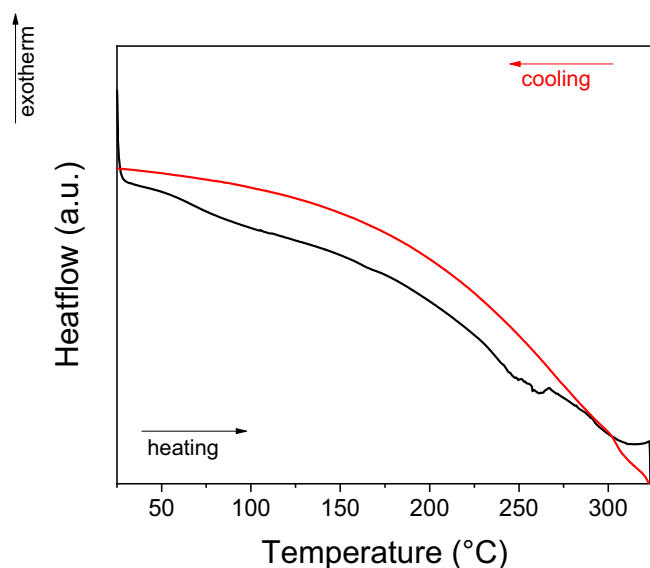
It is worth mentioning that the hysteresis in a flow curve represents the energy difference between the up and down shear sweeps, often associated with thixotropic behavior, where the material's structure breaks down and rebuilds over time under shear<sup>48</sup>.

To explain the thixotropic behavior of BC-based samples, the hysteresis loop area was determined using the method of Wang et al.<sup>58</sup>. Classical IC formulation did not register a thixotropic behavior, while by adding 1% wt. to the classical ice cream formulation an increased value of the loop area of  $789.25 \text{ Pa}\cdot\text{s}^{-1}$  was recorded for ICBC1. By further increasing the BC concentration to 3% and 5% wt., a notable decrease in the hysteresis loop area was observed, with ICBC3 registering  $459.17 \text{ Pa}\cdot\text{s}^{-1}$ , and ICBC5 showing the lowest value at  $320.65 \text{ Pa}\cdot\text{s}^{-1}$ .

**Table 3 | Texture parameters for the BC-based formulations in the melted state**

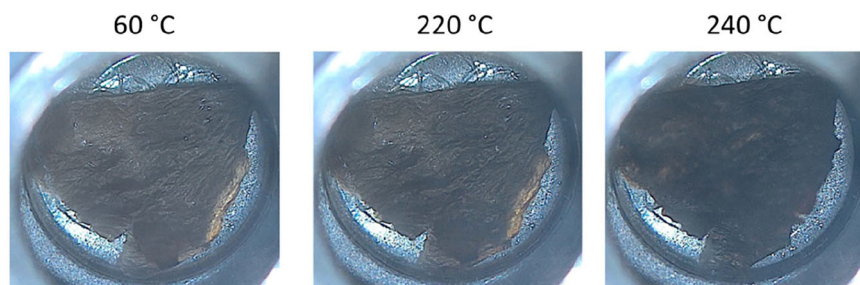
| Sample code | Hardness (Force 40%) (N) | Cohesiveness       | Elasticity         | Gumminess (N)          |
|-------------|--------------------------|--------------------|--------------------|------------------------|
| IC          | $0.02 \pm 0^b$           | $0.94 \pm 0.036^a$ | $0.97 \pm 0.024^a$ | $0.02 \pm 0.006^b$     |
| ICBC1       | $0.02 \pm 0^b$           | $0.79 \pm 0.025^b$ | $0.99 \pm 0.015^a$ | $0.02 \pm 0^{a,b}$     |
| ICBC3       | $0.04 \pm 0.005^a$       | $0.59 \pm 0.042^c$ | $0.98 \pm 0.005^a$ | $0.03 \pm 0.005^a$     |
| ICBC5       | $0.04 \pm 0.005^a$       | $0.61 \pm 0.071^c$ | $1 \pm 0.026^a$    | $0.03 \pm 0.006^{a,b}$ |

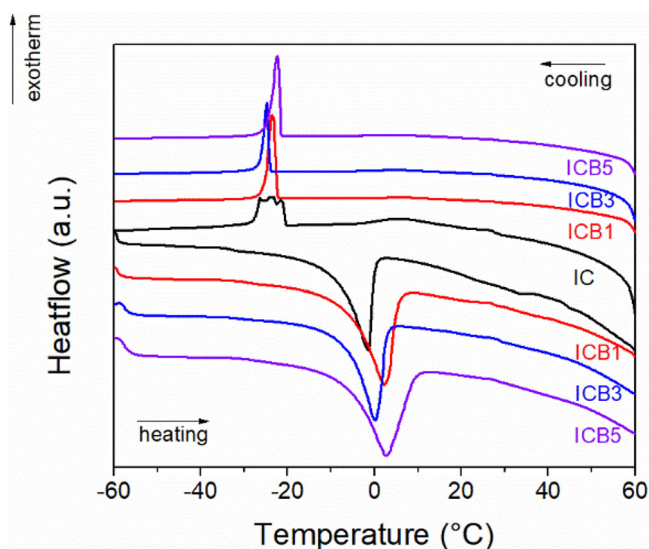
- values followed by different superscript letters in the same row are significantly different ( $p < 0.05$ ).



**Fig. 4 | DSC analysis of BC pristine sample.**

**Fig. 5 | Optical microscopy of BC-based samples at different temperatures.** Optical microscopy images acquired on the BC sample at 60, 220, and 240 °C.





**Fig. 6 | DSC analysis of the BC-based ice cream samples.** DSC analyses of the BC-based ice cream samples between  $-60^{\circ}\text{C}$  and  $+60^{\circ}\text{C}$ .

**Table 4 | Thermophysical properties and freezable water content of ice creams prepared with bacterial cellulose**

| Sample | T <sub>g</sub> (°C) | m.p. (°C) | $\Delta H$ (Jg <sup>-1</sup> ) | f.p. (°C) | FW* (%) |
|--------|---------------------|-----------|--------------------------------|-----------|---------|
| IC     | -34.3               | -8.1      | 51.0                           | -17.6     | 15.3    |
| ICB1   | -31.5               | -5.5      | 87.9                           | -22.0     | 26.3    |
| ICB3   | -33.5               | -5.8      | 90.0                           | -23.9     | 26.9    |
| ICB5   | -32.1               | -5.3      | 92.3                           | -21.2     | 27.6    |

\* FW = freezable water, computed as  $FW = 100 \frac{\Delta H_{\text{sample}}}{\Delta H_{\text{water}}}$ , where  $\Delta H_{\text{water}} = 334 \text{ Jg}^{-1}$

Thus, ICBC1 has a higher degree of thixotropy, the formulation exhibiting more pronounced structural breakdown and recovery under shear<sup>48,59</sup> while ICBC3, and ICBC5 indicate reduced thixotropic behavior that can be attributed to the aggregation of the BC fiber at higher concentrations as evidenced by OM images (Fig. 7) that resists deformation and does not readily recover as ICBC1. In the case of higher BC content, a denser/rigid network is probably formed but with less uniform characteristics in terms of homogenization of the ingredients due to the difficulty of incorporating higher fiber amounts, the ICBC3 and ICBC5 being less susceptible to structural changes induced by the deformation rate.

Thus, the intermolecular structure of the formulations takes longer to recover to its initial state when the BC content is increased, which is consistent with published findings on the rheological behavior of milk-based ice cream formulations<sup>60</sup>. Nevertheless, the decrease in thixotropic behavior at higher BC concentrations is likely due to the formation of polysaccharide fiber-protein-fat aggregates<sup>61</sup> resulting in a more stable but less shear-responsive network within the ice cream matrix.

In terms of proximate composition, based on the experimental data, a clear inverse relationship is observed between energy intake and the concentration of added BC fibers (see Table 2). Specifically, energy intake decreases by 2.36% at 1 wt.% BC fiber content, 10.63% at 3 wt.%, and 21.26% at 5 wt.%. These findings support the primary objective of this study. Comparing the IC formulation with the BC-based samples, there were no significant changes from one sample to another in terms of proteins and carbohydrates. However, the major changes were registered for moisture content that increased from 56.05% for IC to 61.95% for ICBC5. This increase effectively reduces the relative concentration of other components on a wet basis, particularly for fats that were calculated by difference as mentioned in the Results part (see Table 2). However, it is worth mentioning, that although a standard practice, all measurement errors from

moisture, protein, carbohydrate, and ash are accumulated in the fat value when using this calculation. Higher values for carbohydrates and sugar content, were recorded by ICBC3. In certain instances, variations in component proportions may suggest limitations in the purification process of BC fibers, potentially resulting in trace impurities such as residual nutrients or microbial metabolites. Nevertheless, in this study, the purity of the cellulosic fibers was confirmed through XRD analysis<sup>46</sup> which revealed no structural impurities within the cellulose. However, given that the highest sugar concentration was observed in the ICBC3 sample (19.72%), it is possible that if such impurities are present, their levels fall below the detection threshold of the XRD technique. Nevertheless, some variations in composition among different specimens can also be attributed to the non-uniform distribution of BC fibers, which became increasingly difficult to mix uniformly as their content increased. This challenge is likely due to the pressing process, during which most of the water content was removed to obtain 1, 3, and 5 g of fibers. These results are close to some studies in the literature<sup>7,24,51,61</sup> which introduce dietary fiber into dairy-based food products, and indicate the composition of the basic formulation.

In this study, samples were evaluated in their melted state to establish a correlation between rheological properties and mouthfeel-related textural attributes, as presented in Table 3. Analyzing texture in the melted state is particularly appropriate for products containing novel fibers or added functional ingredients, as it more accurately reflects their impact on sensory characteristics.

Texture is a crucial factor for sensory approval. Texture analysis of ice cream is generally performed in its frozen state and is directly connected with hardness and chewiness and can be utilized to forecast how a semisolid food will behave in the mouth.

Given the importance of taste for food products texture analysis is highly recommended as a preliminary result in terms of routine quality control in food processing<sup>54,62,63</sup> taking into account hardness, cohesivity, elasticity and gumminess as main parameters. Apart from its direct impact on consumer acceptability, texture plays a crucial role in regulating taste release and perception<sup>40,64</sup>.

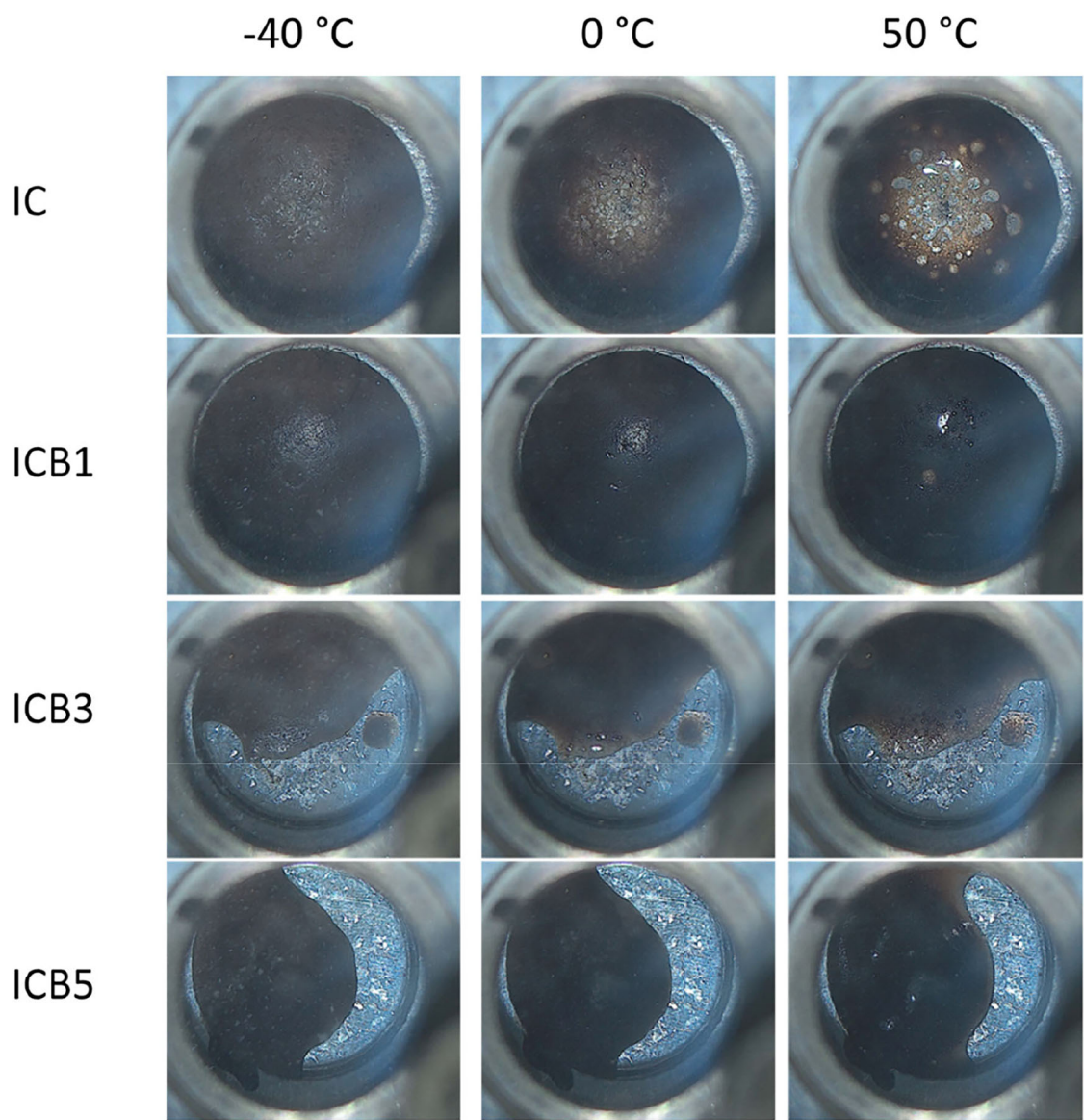
The hardness is defined as the maximum force on a product that displays resistance to deformation and is calculated from the peak force of the first compression of the melted products in this study. The hardness did not vary for the samples IC and ICBC1 and it was similar for samples ICBC3 and ICBC5. A slight increase was observed for ICBC3.

The cohesiveness was calculated as the area of work during the second compression divided by the area of work during the first compression (Area 2/Area 1) and represents how well the melted formulations withstand a second deformation relative to the first one. All samples exhibited a maximum cohesiveness situated in the range of 0.61–0.94. The cohesiveness decreased with the increase of BC addition. The samples ICBC3 and ICBC5 did not present different cohesiveness. The cohesiveness can be indirectly defined as the strength of internal bonds constituting the texture of the melted product. These results reached a consensus with Vo et al.<sup>65</sup> who found that the cohesiveness decreased with a higher level of bacterial cellulose powder contained in the dietary fiber-enriched castella cake.

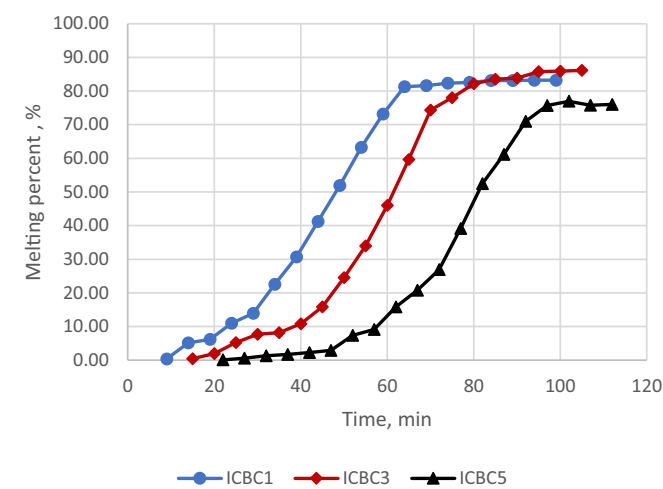
The first two samples (without BC and with reduced BC content) had a slightly higher cohesiveness, which confirms the corresponding behavior of samples with a lower liquid fraction, compared to the other two (ICBC3 and ICBC5), which have a higher fraction of moisture, thus increasing the degree of inhomogeneity.

The elasticity did not vary significantly between the samples. Elasticity refers to the ability of ice cream to deform under stress and return to its original shape when the stress is released. However, in this case, the elasticity is performed in a melted state, the elasticity being correlated with the fiber content of BC added in the formulations.

Gumminess characterizes semi-solid foods and is calculated from Hardness \* Cohesiveness. Gumminess was defined as the characteristic of the ice cream to be held together, resemble taffy, and form curls during scooping<sup>66</sup>. In our case, since the tests were performed on the melted product, the gumminess was related to the capability of the ingredients to be



**Fig. 7 | Optical microscopy of the BC-based ice cream samples.** Optical microscopy images of the BC-based ice creams samples upon heating at different temperatures (−40, 0, and 50 °C).



**Fig. 8 | Melting rate of the BC-based ice-cream samples.** Graph representing the meting percentage in time for the BC-based formulations (blue line/dot marker – ICBC1; red line/square marker– ICBC 3; black line/triangle marker ICBC5).

**Table 5 | Color parameters of ice cream samples**

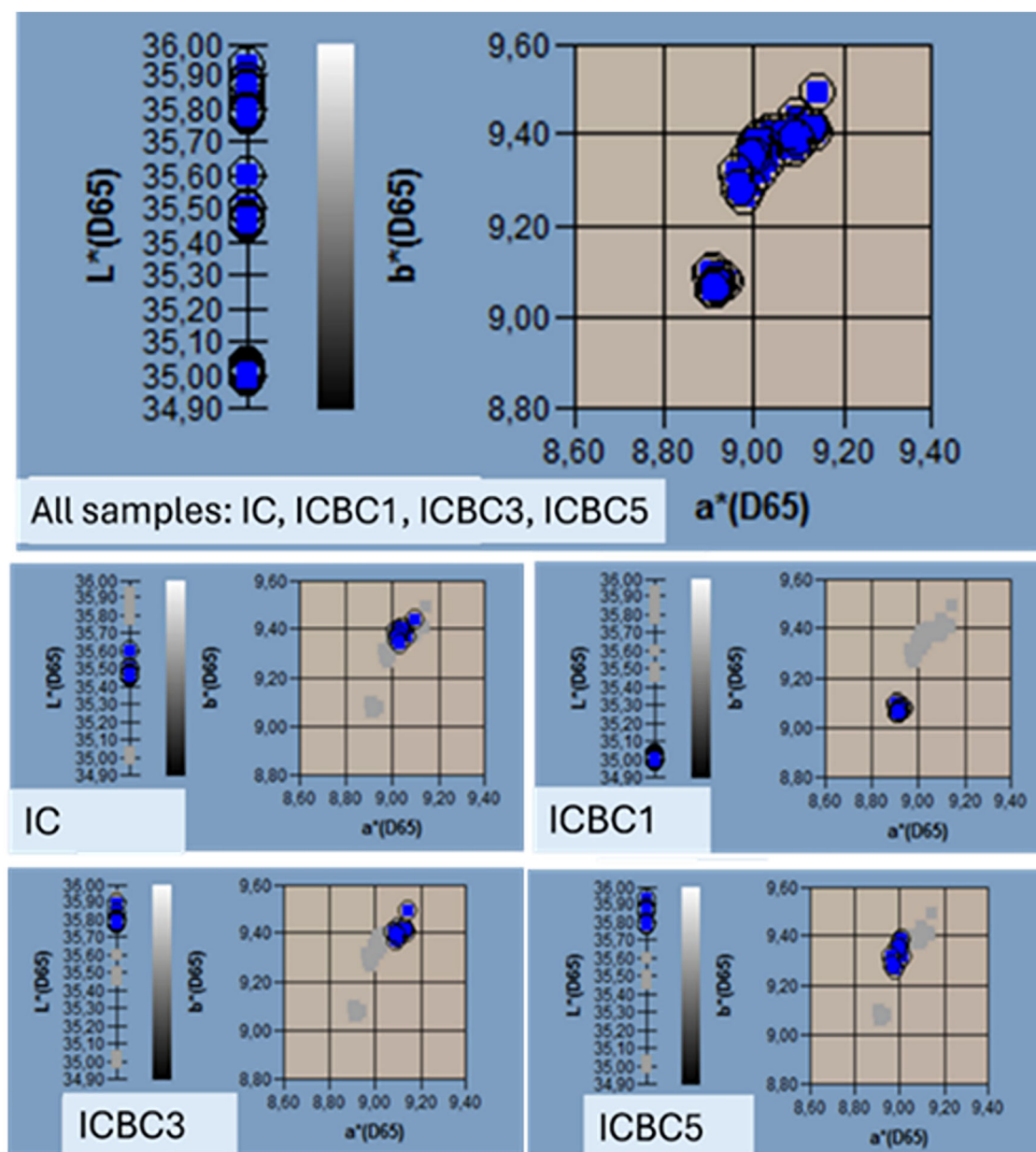
| Sample | <i>L</i> <sup>*</sup> (D65) | <i>a</i> (D65)           | <i>b</i> (D65)           | Δ <i>E</i> |
|--------|-----------------------------|--------------------------|--------------------------|------------|
| IC     | 35.49 ± 0.04 <sup>b</sup>   | 9.04 ± 0.03 <sup>b</sup> | 9.38 ± 0.03 <sup>a</sup> | -          |
| ICBC 1 | 35.01 ± 0.01 <sup>c</sup>   | 8.92 ± 0.01 <sup>d</sup> | 9.07 ± 0.01 <sup>c</sup> | 0.58       |
| ICBC 3 | 35.81 ± 0.03 <sup>a</sup>   | 9.11 ± 0.02 <sup>a</sup> | 9.41 ± 0.03 <sup>a</sup> | 0.33       |
| ICBC 5 | 35.83 ± 0.05 <sup>a</sup>   | 8.99 ± 0.02 <sup>c</sup> | 9.32 ± 0.04 <sup>b</sup> | 0.35       |

Results are mean standard deviation (*n* = 10 determinations). Values followed by different superscript letters (a, b, c, and d) in the same column are significantly different (*p* < 0.05).

hold together and avoid separation. Thus, by the presence of BC led to a slight increase of gumminess, this being related with the capacity of BC to bond water, proteins and fats.

The DSC analysis shows the melting of the crystalline domains inside the BC sample as a broad endothermic event between 240 and 260 °C occurred (Fig. 4)<sup>67</sup>. Optical microscopy images acquired in situ show that the cellulose melting occurs with visible decomposition around 220 °C (Fig. 5) and that the transformation is irreversible as evidenced by the image captured at 240 °C. A total heat of fusion of 9.0 Jg<sup>−1</sup> was obtained.





**Fig. 9 | Color analysis of BC-based ice-cream samples.** Color analysis of ice cream samples and registration of brightness (“L\*” parameter), reddish color (“a\*” parameter), and yellowish color (“b\*” parameter) for all BC-based ice creams; the image was extracted from Color Data Software CM-S100w, SpectraMagic™ NX.

Thus, in the case of BC, it was observed that this sample was thermally stable on a large temperature range.

DSC analysis performed for all samples evidenced similar thermal behavior on heating, consisting of the water glass transition between  $-40$  and  $-25$  °C, followed by ice melting between  $-10$  and  $+5$  °C<sup>68</sup>. The melting of milk fat takes place between  $20$  and  $40$  °C<sup>69</sup>. The fat melting is reversible, with two freezing events being noticed on the cooling runs (Fig. 6). Water freezes with significant supercooling, between  $-15$  and  $-35$  °C.

The addition of bacterial cellulose (BC) leads to an increase in both melting points and heat of fusion with respect to the ice cream without BC (Table 4). The decrease of water melting point is proportional to the quantity of dissolved substances, so a larger freezable water content implies a higher melting point. The ice creams freezing points are lower for the samples containing BC, indicating that ice nucleation is suppressed by the cellulose addition which is in good agreement with literature data<sup>70,71</sup>.

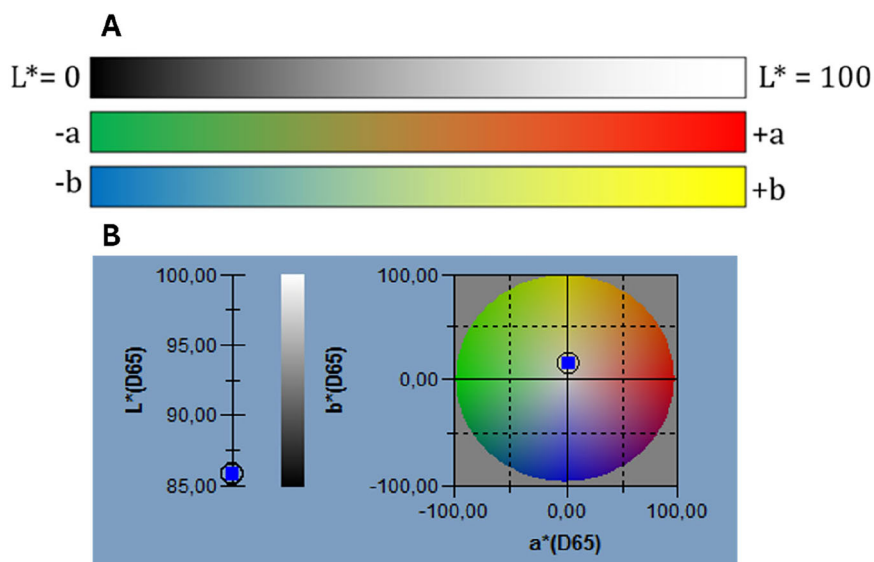
No significant changes in the ice glass transition could be noticed (Table 4)<sup>69</sup>

From OM analysis of the blank IC formulation, it was observed that by increasing the temperature from  $-40$  to  $0$  °C a slight separation of the fats occurs, which was considerably enhanced when the temperature reached  $50$  °C (Fig. 7). By the addition of only 1%wt. BC fibers in sample ICBC1, the fats separation was no longer observable probably to a hindering effect of the fat diffusion in the sample structure, which is in good agreement with the cohesivity results obtained in the melted state and mentioned previously in the texture analysis section, and correlated with the rheological data. Additionally, this effect can also be assigned to the cellulose fibers capability to ensure emulsification between the ingredients of the BC-based ice cream<sup>70,71</sup>.

By increasing the BC content, samples ICBC3, and ICBC5 had similar behavior in the whole temperature range. For both samples, the ice crystals were observable between  $-40$  and  $0$  °C as the fibers absorbed and separated the water from the formulation during mixing and before the freezing process. As a consequence, at  $50$  °C we have a clear distinct area represented by the melted ice for ICBC3, and ICBC5. This could be attributed to the



**Fig. 10 | Color attribution for brightness, reddish and yellowish color parameters.** Color attribution for  $L^*$ ,  $a^*$ , and  $b^*$  parameters (A), and overall color determination (B) extracted from Color Data Software CM-S100w, SpectraMagic™ NX.



**Table 6 | Sensorial characteristics of ice cream samples**

| Sensorial characteristics         | Sample IC                 | Sample ICBC1               | Sample ICBC3               | Sample ICBC5             |
|-----------------------------------|---------------------------|----------------------------|----------------------------|--------------------------|
| External appearance - color       | 4,29 ± 0,99 <sup>a</sup>  | 4,29 ± 0,91 <sup>a</sup>   | 4,43 ± 0,65 <sup>a</sup>   | 4,29 ± 0,83 <sup>a</sup> |
| External appearance - homogeneity | 4,29 ± 0,91 <sup>a</sup>  | 4,29 ± 0,61 <sup>a</sup>   | 4,07 ± 0,92 <sup>a</sup>   | 3,71 ± 1,33 <sup>a</sup> |
| Taste - sweet                     | 3,93 ± 0,92 <sup>a</sup>  | 3,43 ± 1,02 <sup>a</sup>   | 3,36 ± 0,93 <sup>a</sup>   | 2,71 ± 0,99 <sup>a</sup> |
| Taste - sour                      | 1 ± 0 <sup>a</sup>        | 1 ± 0 <sup>a</sup>         | 1,07 ± 0,27 <sup>a</sup>   | 1 ± 0 <sup>a</sup>       |
| Taste - bitters                   | 2,14 ± 1,03 <sup>a</sup>  | 2 ± 1,04 <sup>a</sup>      | 2,14 ± 1,29 <sup>a</sup>   | 1,71 ± 0,73 <sup>a</sup> |
| Taste - of cocoa                  | 4,43 ± 0,65 <sup>a</sup>  | 4,43 ± 0,65 <sup>a</sup>   | 4 ± 1,04 <sup>a</sup>      | 3,64 ± 1,08 <sup>a</sup> |
| Taste - chemical                  | 1,36 ± 0,75 <sup>a</sup>  | 1,21 ± 0,58 <sup>a</sup>   | 1,5 ± 1,09 <sup>a</sup>    | 1,36 ± 0,63 <sup>a</sup> |
| Flavor - pleasant cocoa           | 4,5 ± 0,65 <sup>a</sup>   | 4,43 ± 0,51 <sup>a,b</sup> | 3,86 ± 0,95 <sup>a,b</sup> | 3,43 ± 1,16 <sup>b</sup> |
| Consistency - creamy              | 3,43 ± 1,16 <sup>a</sup>  | 3,43 ± 1,22 <sup>a</sup>   | 3,36 ± 1,65 <sup>a</sup>   | 3,93 ± 1,07 <sup>a</sup> |
| Consistency - unctuous            | 3,21 ± 1,05 <sup>a</sup>  | 2,93 ± 1,07 <sup>a</sup>   | 3,57 ± 1,4 <sup>a</sup>    | 3,5 ± 1,23 <sup>a</sup>  |
| Consistency - with ice crystals   | 2,21 ± 1,19 <sup>a</sup>  | 2,86 ± 1,61 <sup>a</sup>   | 3,21 ± 1,42 <sup>a</sup>   | 2,43 ± 1,45 <sup>a</sup> |
| Texture - firm                    | 2,93 ± 1,21 <sup>a</sup>  | 3 ± 1,11 <sup>a</sup>      | 4 ± 0,96 <sup>a</sup>      | 3,5 ± 1,23 <sup>a</sup>  |
| After taste - pleasant            | 4,21 ± 0,8 <sup>a,b</sup> | 4,43 ± 0,51 <sup>a</sup>   | 3,93 ± 1 <sup>a,b</sup>    | 3,21 ± 1,05 <sup>b</sup> |
| After taste - unpleasant          | 1 ± 0 <sup>a</sup>        | 1 ± 0 <sup>a</sup>         | 1,64 ± 1,15 <sup>a</sup>   | 2,07 ± 1,54 <sup>a</sup> |

Results are mean standard deviation ( $n = 14$ ). Values followed by different superscript letters in the same column are significantly different ( $p < 0.05$ ).

The results of the hedonic analysis are shown in Table 7. Samples ICBC 1 and IC were scored with the expression above mentioned in Characterization section.

tendency for fiber entanglement as BC content increases, leading to the formation of aggregates that can interact with proteins and fats<sup>40</sup>. These interactions may create a more rigid network structure as previously confirmed by thixotropic behavior of the samples with increased BC content, reducing the matrix's ability to retain water uniformly.

The melting dynamics of ice cream depends on its chemical composition and the physical structure determined by the amount of ice and air incorporated and BC fibers. The melting analysis aimed to monitor the time of appearance of the first drop, and respectively the melting percentage of the analyzed ice cream formulations (Fig. 8). The experimental data indicate linear melting rates of 1.522%/min for ICBC1, 1.99%/min for ICBC3, and 1.46%/min for ICBC5. The appearance of the first melt drop was recorded after 9 minutes for ICBC1, 15 minutes for ICBC3, and 22 minutes for ICBC5, respectively. These results suggest a progressive delay in the onset of melting with increasing concentrations of bacterial cellulose (BC) fibrils in the ice cream formulation. This trend aligns with the known suppressive effect of BC fibers on melting, as supported by differential scanning calorimetry (DSC) analysis coupled with optical microscopy (OM).

Complete melting of ICBC1 was observed, with only traces of foam remaining on the support. In contrast, ICBC3 exhibited a slower initial melting rate, although the final stage of melting occurred more rapidly than in ICBC1, leaving behind both foam and BC fibrils. The melting behavior of ICBC5 was markedly slower and more irregular, likely due to the leakage and accumulation of fibrils between the supporting threads. After melting, both foam and fibrillar agglomerates remained on the support.

These findings confirm that the incorporation of BC fibers reduces the overall melting rate, consistent with previously reported data<sup>43</sup>. Notably, ICBC3 displayed the highest melting rate among the samples, which may be attributed to structural discontinuities in the BC fibril network and disruptions in the lipid matrix that would otherwise form in the absence of BC. In the ICBC1 formulation, the dispersed fibrils may enhance the lipid matrix by swelling, as suggested by OM observations at various temperatures, thus contributing to the reduced melting rate. In the case of ICBC5, the high concentration of BC fibers appears to form a reinforcing matrix within the ice cream, resulting in irregular melting and the detachment of intact sections agglomerated around fibrils, as also evidenced at the microscopic level through DSC and OM analysis.

**Fig. 11 | Spider diagram describing sensorial characteristics of the BC-based ice cream samples.** Spider diagram regarding the descriptive test of the ice cream samples based on sensorial characteristics: (1) external appearance – color; (2) external appearance – homogeneity; (3) taste – sweet; (4) taste – sour; (5) taste – bitter; (6) Taste – of cocoa; (7) Taste – chemical; (8) flavor – pleasant cocoa; (9) consistency – creamy; (10) consistency – unctuous; (11) consistency – with ice crystals; (12) texture – firm; (13) aftertaste – pleasant; (14) aftertaste – unpleasant.

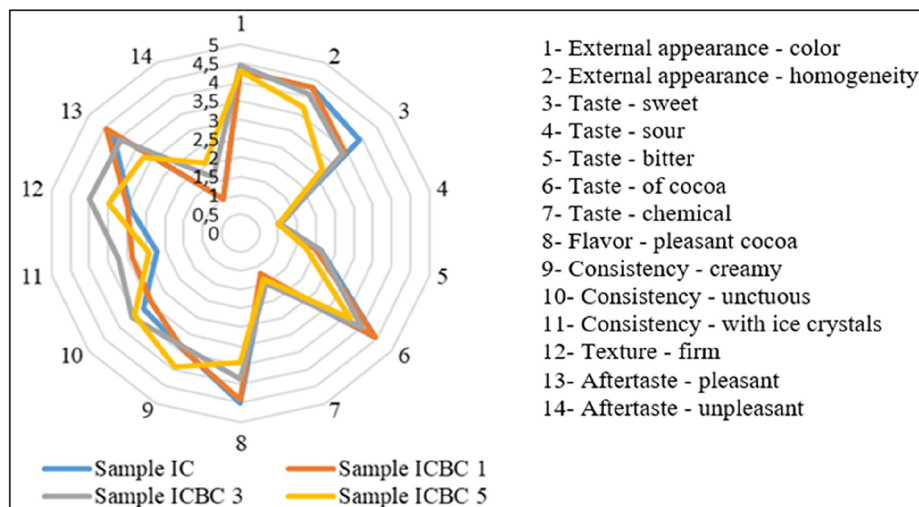


Table 5 summarizes the color parameters of ice cream samples. The concentration of BC fiber addition had effectively an impact on the “*L\**” parameter that defines the brightness of the samples (Figs. 9, and 10). The values of this parameter increased for ICBC3 and ICBC5 (values of  $35.81 \pm 0.03^a$  and  $35.83 \pm 0.05^a$ ) than in the control sample IC ( $35.49 \pm 0.04^b$ ). This means that the addition of BC fibers leads to a lightening of the color of the ice cream. The darkest color was registered by the ICBC1 sample ( $35.01 \pm 0.01^c$ ) with a “*L\**” value lower than the control sample.

Regarding the first color parameter “*a*”, the ICBC 3 sample recorded the highest value ( $9.11 \pm 0.02^a$ ), which represents a more reddish color almost like the classical formulation – control sample IC ( $9.04 \pm 0.03^b$ ). Less reddish sample is ICBC 1 ( $8.92 \pm 0.01^d$ ). Second color parameter, “*b*”, responsible for yellow color, the IC and ICBC 3 samples showed a slightly pronounced yellowish color. ICBC 3 recorded again the maximum value of “*b*” parameter ( $9.41 \pm 0.03^a$ ).

According to the criteria adopted by the International Illumination Commission CIE<sup>72</sup> absolute color differences ( $\Delta E$ ) in the range from 0 to 2 are unrecognizable by the person experienced in color discrimination.

Therefore, the addition of BC fibers in cocoa ice cream would not represent a negative factor in the perception of color and the decision to purchase the product.

According to the statistical analysis, the only notable differences in the 4 ice cream samples refer to the following two characteristics: pleasant cocoa aroma and pleasant aftertaste (Table 6, and Fig. 11). The highest score for “pleasant cocoa flavor” was registered by sample IC, while the lowest was attributed to sample ICBC 5. The highest score for “pleasant aftertaste” was for sample ICBC 1, while the lowest was registered by ICBC 5, probably due to the high concentration of BC fibers, which gives a strong cardboard taste. All other sensory characteristics of the 4 ice cream samples do not differ significantly.

None of the formulas addressed achieved score 5, despite the fact that it is the maximum for a positive outcome (Table 6). Naturally, for some parameters, ICBC3 and ICBC5 formulations reported values greater than 4. As a result, the ice cream’s mouthfeel and general acceptability were negatively impacted by the addition of 5% wt. BC fiber, indicating that the ice cream was coarser and rougher overall. However, based on the results registered in Table 6, a proportion between 1% and 3% wt. of BC can be suggested to lower the energy intake of ice cream and raise the intake of protein and dietary fiber, according to the corroboration of the results of the sensory assessment, color, and texture.

For hedonic analysis, in proportion of 35% the respondents chose the following order of evidence in terms of total acceptability (Table 7). Starting from the most accepted to the least accepted: I-

**Table 7 | Scoring percentage on the hedonic scale**

| Scoring scale            | Sample IC | Sample ICBC 1 | Sample ICBC 3 | Sample ICBC 5 |
|--------------------------|-----------|---------------|---------------|---------------|
| 9 I like it extremely    | 14%       | 14%           | 14%           | 14%           |
| 8 I like it very much    | 50%       | 57%           | 21%           | –             |
| 7 I like it moderately   | 21%       | 21%           | 36%           | 29%           |
| 6 I like it easy         | 14%       | 7%            | 7%            | –             |
| 5 Impassive              | –         | –             | 14%           | –             |
| 4 I dislike easily       | –         | –             | –             | 29%           |
| 3 I moderately dislike   | –         | –             | 7%            | 14%           |
| 2 I really dislike it    | –         | –             | –             | 14%           |
| 1 I dislike it extremely | –         | –             | –             | –             |

Sample ICBC 1; II- Sample IC – classic formulation; III- Sample ICBC 3; IV- Sample ICBC 5.

Compared to the study performed by Mehta et al.<sup>43</sup>, which incorporated 2.8% bacterial cellulose (BC) dry matter on a wet basis, and the research by Xavier and Ramana<sup>39</sup>, which utilized higher BC concentrations of 17% and 30% (wet weight), our preliminary investigation explored a different range, specifically between 1% and 5% wt. obtained after pressing the BC fibers. Notably, Xavier and Ramana reported that higher BC content adversely affected the acceptability of the food product, a finding that aligns with our observations, where increased BC concentrations led to diminished sensory quality. Thus, in this study, the preferences for the acceptability of the BC fiber incorporated in the ice cream did not exceed 1%, respectively 3% wt.

Since ICBC 1 sample ranked highest among the classifications, the results of the sensory and hedonic analysis are encouraging, and the usage of BC fibers in ice cream compositions going forward might be taken into consideration.

In conclusion, this work highlights the benefits of including BC fibers into traditional cocoa ice cream formulation to design an innovative milk-based dessert with an enhanced fiber content and nutrition attributes. The added BC fibers in different concentrations (1%, 3%, and 5% wt. to the classical formulation) influenced the rheological properties of the non-frozen formulations. In contrast with the initial formulation, the increasing content of BC fiber led to a more shear-sensitive system governed by

reorganization of intermolecular interactions determined by hydrogen bonding and hydrophobic association between protein and cellulose chains. The densities varied in the range of 0.771 up to 0.965 g/cm<sup>3</sup> and increased with the content of BC fibers added in the classical ice cream formulation. One of the major purposes of this study was achieved given by the energetic value of the final product that was reduced to 2.26% in the case of ICBC1, 10.48% for ICBC3, and 21.15% for ICBC5. DSC analysis copupled with optical microscopy and performed on pristine BC and ice cream formulations confirmed the functional role of BC in improving thermal stability and modifying phase transition dynamics while suppressing ice nucleation. These findings were correlated with the melting rate values that indicated a delay in melting resistance up to 22 minutes.

Compared with blank sample, incorporating 1% and 3% BC into the ice cream formulation enhances texture (performed in melted state) and sensory attributes, whereas higher concentration brings more formulation challenges like diminished cohesiveness and decreased sensory appeal. Thus, incorporating 1% to 3% BC (obtained after pressing) offers a promising strategy to enhance the nutritional profile of traditional ice cream without compromising quality. Since ICBC 1 sample ranked highest among the classifications, the results of the sensory and hedonic analysis are encouraging, and the usage of BC fibers in ice cream compositions going forward might be taken into consideration. Thus, the results of this study open new avenues not only for the use of BC in other fiber-enriched dairy frozen desserts, but also brings new opportunities for the development of sustainable, “farm-to-fork” products, further reinforcing/confirming the role of BC as a versatile and functional food ingredient.

## Methods

### Materials

The bacterial cellulose (BC) utilized in this article was produced in 2024 at the National University of Science and Technology Politehnica Bucharest's Bioreactors laboratory using an unconventional substrate - food waste coming from pulp-free juice production. The other ingredients used to make the ice cream samples were purchased from the local market, namely: whipping cream (35% wt. fat, Olympus, SC. Fabrica de Lapte, Braşov, Romania), sugar (Coroniţa, Agrana Romania), milk (3.5% wt. fat, Napolact, Friesland Campina, Romania), low-fat cocoa (11% wt. fat, Dr. Oetker RO S.R.L., Oetker-Group), salt (SALROM S.A., Romania). The reagents used for the analysis methods are of analytical purity and purchased from Sigma-Aldrich in 2023.

### Synthesis and BC purification processes

BC was obtained by employing a modified method of Hestrin-Schramm [MHS] culture medium with 2% wt. carbon source using a substrate of berries waste from pulp-free berries juice production with the addition of peptone and mineral salts in a batch static culture at 28 °C for 7 days<sup>31,73</sup>. Afterwards, the BC membranes were microbiologically inactivated in an aqueous solution of NaOH 0.5 N at 90 °C for 1 hour and then rinsed repeatedly with deionized water until neutral pH. For the ice cream formulation BC was wet grinded and sterilized in wet form at 121 °C for 15 min. The water content for BC fibers was 98% wt after sterilization.

### Preparation of the BC-based ice cream formulations

The mixture was pre-heated up to 40 °C to ensure complete solubilization of sugar, salt and cocoa. A 1:1 volumetric ratio of milk to whipping cream was maintained for the liquid phase, to which 250 g/L sugar, 200 g/L cocoa, and 6 g/L salt were added. This mixture, designated as the classical formulation (IC), was homogenized and pasteurized using a 2 L capacity Vorwerk Thermomix TM6 at 60 °C for 60 minutes. For the ice-cream formulations the sterilized BC fibers were pressed and weight until we obtained 1 g, 3 g, and 5 g, respectively of total BC fibers corresponding to the ICBC1, ICBC3, and ICBC5 specimens. Thus, to clarify, 1 g, 3 g, and 5 g of BC fibers were added to individual ice cream formulations, each with a total weight of 100 g (referred in the manuscript as 1%, 3%, and 5% wt.). The classical formulation (IC) and BC-based ice creams samples were aged at 4 °C for

24 hours followed by a partially freezing procedure performed at −5 °C for 20 min to ensure the slow formation of small ice crystals. Partially frozen ice cream was moved into molds and frozen completely in the freezer for at least 4 hours before performing analyses.

### Scanning electron microscopy (SEM) micrographs of BC fibers

The structural characterization of all BC fibers was obtained before and after purification and grinding with a Nova NanoSEM 630 Scanning Electron Microscope (FEI Company, Hillsboro, OR, USA) at an acceleration voltage of 10 kV. Prior to SEM analysis all samples were sputtered with a thin layer of Au of 5 nm to increase the resolution of the micrographs.

### X-ray diffraction analysis of BC fibers

X-ray diffraction (XRD) patterns of the BC fibers were recorded using a 9 kW Rigaku SmartLab diffractometer (Osaka, Japan), equipped with a CuKα1 source that provides a monochromatic wavelength,  $\lambda = 0.15406$  nm. During the measurements of the film-like sample, the scan was initiated in 2θ mode (grazing incident mode), the scan step being 0.01°/min, while the speed was set as 5°/min. The data obtained from XRD analysis was plotted and baseline-corrected using Origin 2018 software.

### Density determination of the non-frozen BC-based formulations

In order to estimate the overrun, the weight of 80 mL of molten ice cream was determined at 25 °C under atmospheric pressure. The values were collected as an average of 4 determinations for each sample and standard deviation was also included.

### Rheology of the non-frozen BC-based formulations

Using Rheotest 2, a coaxial cylinder viscometer, outfitted with rotating cylinders (assigned as “N”, “S”, and “H”) designed for fluids with low, medium and high viscosities range, the rheological behavior of the molten ice creams was determined at a temperature of 25 °C. The cylinders were selected according to the ice creams viscosities as follows S1 for IC (low viscosity), S2 for ICBC1 (medium viscosity), and S3 (medium-high viscosity) for ICBC3, and ICBC5, respectively. The Ostwald-de-Waele model was applied to determine the rheological parameters, namely the flow index, *n* (dimensionless), the shear stress (Pa), and the flow consistency index, *m* (Pa·s<sup>*n*</sup>). The readings fell in a deviation of 1%. The results were obtained by plotting the shear stress values as a function of shear deformation using Origin 2018 software.

### Determination of proximate composition of samples

Proximate composition consists in the determination of fat, protein, sugar and dry matter content following the procedure of the approved European standards for the specific analyses of food products.

The composition of the samples was determinate as follows: in the determination of moisture content using the oven drying method (AACC. Method 44–15.02)<sup>74</sup>, protein content by the Kjeldahl method, Nx6.25, fat content by Soxhlet method using the extraction with petroleum ether under reflux conditions and ash through gravimetric method by burning the sample at 550 °C in a furnace, sugar content by Scroll and dry matter content following the procedure of the approved European standards for the specific analyzes of food products. The preparation of dairy samples was done according to STAS 6343–81<sup>75</sup>.

Determination of protein substances in dairy products using the Kjeldahl method. After mineralization, the samples were allowed to cool to room temperature. Distillation and titration were performed using a specialized system. The solutions required for titration included 40% sodium hydroxide, 4% boric acid, and 0.1 N hydrochloric acid. The results can be expressed in terms of nitrogen content and protein content (g/100 g) by applying the transformation factor *F* = 6.25. Determination of protein content was done according to ISO 8968-1:2014. The analysis was performed in duplicate for each sample, and the average value was registered.

Fat extraction by acid hydrolysis: for a 10 g sample, add 100 mL of hydrochloric acid while stirring continuously. Once homogenized, the



sample is refluxed at its boiling point for 15 minutes under atmospheric pressure. Refrigerant residues are collected at the bottom and passed through a porous filter until the litmus paper shows no further color change. The resulting precipitate is dried in an oven for one hour at  $103 \pm 2^\circ\text{C}$ . All remaining material is extracted and placed into the Soxhlet apparatus cartridge. Extraction is performed using petroleum ether at a rate of 10–12 siphons per hour. The solvent is then evaporated in a water bath, and the flask is dried at  $103 \pm 2^\circ\text{C}$  for one hour, cooled in a desiccator, and weighed. The analysis was performed in duplicate for each sample, and the average value was registered.

Determination of sugar content by Scroll. Quantitatively transfer 5 g of the sample into a 250 ml volumetric flask and fill up to three quarters of the of the flask's volume with distilled water. After homogenization, add 5 ml each of potassium ferrocyanide 10% and zinc sulfate 15% and then add distilled water to the required volume. After filtration, the sample (50 ml of filtrate) is hydrolyzed with acid for five minutes at  $67\text{--}70^\circ\text{C}$  in a water bath. The samples are brought to room temperature and neutralized using 30% sodium hydroxide and phenolphthalein to a pale pink color. Each sample was analyzed in duplicate, and the mean value was reported.

Determination of dry matter using the oven drying method. Samples were heated up to  $105^\circ\text{C}$  for 4 hours using an aluminum ampoule used for humidity, with 15 g of sea sand and a small glass rod and 5–10 g of sample. After drying, the sample was cooled in a desiccator and then weighed. The dry matter content is calculated as the inverse of the moisture content, and expressed as a percentage and relies on 2 measurements for each sample (STAS 6344-88)<sup>76</sup>.

### Mouth-feel related texture analysis

The mouth-feel texture properties of the ice cream samples were measured with the Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA), using a compression plunger with a diameter of approximately 12 mm. The samples were analyzed in the melted state at room temperature. Methods of analysis included a cycle of two compressions up to 10 mm, lift pause of 1 second, and again compression and lift. The test parameters were: compression speed: 1.6 mm/min; load cell: 50 N. The texture analysis was performed to measure the rheological parameters that can have a correspondence with sensorial attributes/mouth-feel-related texture characteristics. Using the Bluehill 3.13 software, 4 texture parameters (hardness, cohesiveness, elasticity, and gumminess) were calculated as the average of 4 measurements for each sample.

### Dynamic scanning calorimetry (DSC) analysis of the BC-based formulations

Differential scanning calorimetry (DSC) measurements were performed using a Mettler Toledo DSC 3 calorimeter, at a scanning rate of  $10^\circ\text{C min}^{-1}$ , under  $80\text{ mL min}^{-1}$  nitrogen flow. DSC analyses were performed between  $-60$  and  $60^\circ\text{C}$  and were represented using Origin 2018 software. Melting points (m.p.) and freezing points ( $^\circ\text{C}$ ) were computed as onset temperatures, while glass transition temperatures ( $T_g$ ) were determined as midpoint temperature. In situ optical microscopy (OM) was acquired using an Olympus SC50 digital microscope, at a frame rate of  $1\text{ image min}^{-1}$ .

### Melting point analysis

Melting tests for the BC-based ice cream formulations were conducted following methodologies previously reported in the literature<sup>39,43,57,77–79</sup> with modifications adapted to the present experimental conditions. Ice cream samples stored at  $-19.0^\circ\text{C}$  for three days were used for testing. Each sample, in the shape of a parallelepiped (dimensions:  $20 \times 30 \times 30\text{ mm}$ ; approximate mass: 90 g), was placed on a perforated plastic wire support suspended above a graduated glass vessel. This setup was placed in a thermostatic chamber maintained at  $22.0 \pm 1^\circ\text{C}$ . Melted ice cream was collected in the vessel and weighed at 5 minutes intervals over a period of approximately 1 hour. The onset of melting was recorded as the time at which the first drop was observed. The extent of melting was expressed as the percentage of

melted ice cream, calculated using the equation:

$$\text{percentage of melted ice cream}(\%) = \left( \frac{m_t}{m_p} \right) 100\% \quad (3)$$

where  $m_t$  is the mass of the melted ice cream collected in time (g), and  $m_p$  is the initial mass of the ice cream sample (g). The percentage of melted ice cream was plotted against melting time (minutes) and was used to determine the melting rate of the ice cream. All measurements were performed in triplicate.

### Color analysis

Color is one of the important characteristics for sensory evaluation of food. The determination was made with the Colorimeter Konica Minolta (Spectrophotometer CM-5) using the software Color Data Software CM-S100w, SpectraMagicTM NX. The color of the sample was measured using reflectance and the determined color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) (Figs. 9, and 10) in which:

“ $L^*$ ” – brightness scale evaluated from 0 to 100 units,  $L^* = 0$  attributed to black and  $L^* = 100$  is attributed to white;

“ $a^*$ ” – color of the sample from green to red ( $-a^*$  = green and  $+a^*$  = red);

“ $b^*$ ” – color of the sample from blue to yellow, ( $-b^*$  = blue and  $+b^*$  = yellow).

Before starting the analysis of the samples, the device is calibrated. The preparation of the samples consisted of placing the samples (at room temperature) in the cylinder with a diameter of 45 mm and a height of 17 mm, with great care to avoid the formation of air voids on the bottom of the cylinder.

### Sensorial and hedonic analysis of the final BC-based ice cream products

The analysis was carried out in the sensory analysis laboratory with a panel of trained examiners. The samples, coded beforehand, were placed on the plates shortly before to avoid melting. Each sample was analyzed individually, with room-temperature water used as a neutralizing agent for mouth rinsing between tastings. Both descriptive and hedonic analyses were performed using an evaluation questionnaire. The panel consisted of 14 participants — 8 women and 6 men — with an average age of 29 years.

In the panel sensory analysis, we used both descriptive and hedonic analysis. Therefore, we drew up an evaluation questionnaire in which we monitored the following attributes:

External appearance (Color [very light ... very dark]; Homogeneity [very inhomogeneous ... very homogeneous]);

Interior appearance (Color [very light ... very dark], Homogeneity [very inhomogeneous ... very homogeneous]);

Taste (Sweet [very faint ... very intense], Sour [very faint ... very intense], Bitter [very weak ... very intense], Cocoa [very weak ... very intense], Chemical [very weak ... very intense]);

Flavor (Cocoa Cake [slightly enjoyable ... very enjoyable]);

Consistency (Creamy [slightly creamy ... very creamy], Unctuous [slightly unctuous ... very unctuous], With ice crystals [slightly noticeable ... very noticeable]);

Texture (Firm [slightly firm ... very firm]).

Descriptive sensory evaluation, for ice cream samples, used a 1-5 scoring scale, where: imperceptible (1); easily noticeable (2); neutral (3); slightly intense (4); very intense (5) (Table 6).

Hedonic analysis includes the following scale of values: 9 - I like it extremely; 8 - I like it very much; 7 - I like moderately; 6 - I like it easy; 5 - Impassive; 4 - I dislike easily; 3 - I moderately dislike; 2 - I really dislike it; 1 - I dislike it extremely.

The questionnaire ended after the following 3 requirements:

- Sort the samples from the most accepted to the least accepted
- Please justify the choice made previously
- Other observations

All samples were analyzed at least in duplicate and results were expressed as mean  $\pm$  standard deviation (SD). Results were statistically analyzed by using Minitab statistical software version 20. One-way analysis of variance (one-way ANOVA) followed by Tukey's test was used to evaluate the statistical significance between samples. The chosen level of significance was set at  $p < 0.05$ .

### Data availability

The data are available upon request from the authors.

### Code availability

The code used in this work is not publicly available due to third-party licensing restrictions. However, relevant methods are fully described in the Methods section.

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### Author contributions

G.O.I., R.G.S., and A.M. contributed towards experimental design, investigation, data analysis, data interpretation, supervision and writing the original draft. A.M. contributed towards rheology analysis and data interpretation. D.E.G. and G.D.C.S. contributed towards proximate composition analysis and interpretation. R.A.M. contributed towards DSC analysis and data interpretation. A.S. contributed towards experimental design and data interpretation. C.R. contributed to XRD analysis and data interpretation. O.A.B. contributed to SEM analysis and data interpretation. All authors contributed towards reviewing and editing.

### Competing interests

The authors declare no competing interests.

### Additional information

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