LWT - Food Science and Technology 161 (2022) 113327

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Design and characterization of a plant-based ice cream obtained from a cereal/legume yogurt-like

Erica Pontonio [a], Marco Montemurro [a], Cinzia Dingeo [a,b], Michele Rotolo [c], Domenico Centrone [b], Vito Emanuele Carofiglio [b], Carlo Giuseppe Rizzello [d,*]

[a] Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Via Giovanni Amendola 165/A, 70126, Bari, Italy
[b] Celery SRL, Via Domenico Benedetti 9, 70044, Bari, Italy
[c] Città del Gelato Srl, Via T. Fiore 3, 70015, Noci, Italy
[d] Department of Environmental Biology, "Sapienza" University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy

ARTICLE INFO

Keywords:
Yogurt-like
Plant-based ice cream
Lactic acid fermentation
Lactose-free
Gluten-free

ABSTRACT

A gluten and lactose-free plant-based yogurt-like was used as the main ingredient to produce a novel plant-based ice cream. The integrated approach used to investigate the main properties of the novel ice cream demonstrated similar nutritional label between the plant- and milk-based products with the former showing higher protein digestibility and absence of lactose. The lactic acid bacteria fermentation occurring during the yogurt-like production ensured the decrease of the antinutritional compounds of the legume ingredients and the obtainment of suitable technological and sensory characteristics. Indeed, similar sensory profile with only slight differences in appearance, structure, taste, odor and texture parameters were found for both the plant- and milk-based products. However, structural and technological characteristics of the ice creams were significantly different; indeed, the overrun was higher in the plant-based ice cream, such as hardness, gumminess, and adhesiveness. A high cell density ($>10^7$ cfu/mL) of viable lactic acid bacteria during the 120-days storage at −20 °C confirmed the potential of the plant-based ice cream to be used as carrier of probiotics.

1. Introduction

A significant growth in the plant-based protein market, particularly for new alternatives to traditional meat and dairy, has been recently reported (Bouvard et al., 2015; Mintel, 2020; The Business Times, 2020, Willett et al., 2019). The adoption of a plant-based diet is growing trend across Western countries and those who follow a plant-based diet might choose to substitute animal products for vegetable options, without permanent restriction of animal foods (Alcorta, Porta, Tárrega, Alvarez, & Vaquero, 2021). Moreover, vegans and vegetarians have actively promoted the advantages of plant-based alternative foods over natural dairy milk-derived products, which include better health for preventing lactose intolerance and cow's milk allergy due to the benefits of lactose-free, cholesterol-free and low-calorie foods (Park, 2021).

Ice cream is a popular frozen dairy product with good taste and mouthfeel. However, its relatively high fat and cholesterol contents are often considered to be unfavorable for human health (Salem, Fathi, & Awad, 2005). The presence of lactose in the product may also cause lactose intolerance among lactase deficient consumers (Aboulfazli, Baba, & Misran, 2015). Hence consumer have moved towards ice cream without any dairy ingredients (Pinto et al., 2012). Nevertheless, producing plant-based ice cream is a technological challenge due to the unique flavor and structure given by milk and dairy ingredients (Pinto et al., 2012).

Recently, a biotechnological protocol to produce a novel gluten-free, lactose-free, and plant-based yogurt-like (YL) was optimized through the combination of rice, lentil and chickpea flours, a mild physical treatment, and fermentation by using the selected lactic acid bacteria. This innovative product was characterized by promising nutritional and functional features, technological properties, and overall sensory appeal (Pontonio et al., 2020).

In this framework, taking advantage of the competences acquired during the above-mentioned research project, this study aimed at optimizing a biotechnological protocol to produce a gluten-free, lactose-free, and plant-based ice cream, in which the YL was the main ingredient.

An integrated approach was used to investigate the main technological, nutritional, functional, and sensory properties of the novel ice

* Corresponding author.
E-mail address: carlogiuseppe.rizzello@uniroma1.it (C.G. Rizzello).

https://doi.org/10.1016/j.lwt.2022.113327
Received 13 January 2022; Received in revised form 20 February 2022; Accepted 4 March 2022
Available online 10 March 2022
0023-6438/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

cream.

Moreover, due to the rising interest towards healthy functional foods, e.g., food containing biomolecules, probiotics, and prebiotics (Sarwar et al., 2021), the survival of the lactic acid bacteria strains used as starters for the fermentation was monitored for 120 days of storage at −20 °C, to evaluate the potential of the plant-based ice cream to be used as a carrier of viable useful microorganisms.

2. Materials and methods

2.1. Raw ingredients and microorganisms

Commercial rice flour (Bioalimenta S.r.l., Fara San Martino, Italy) and chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris*) flours (Molino Favero s.r.l, Padova, Italy) were used in this study. Protein (total nitrogen × 6.25), lipids, moisture, total dietary fiber, and ash were determined according to Approved Methods 46-11A, 30–10.01, 44-15A, 32–05.01 and 08–01.01, respectively (American Association of Cereal Chemists, AACC, 2010). Carbohydrates were calculated as the difference [100 - (proteins + lipids + ash + total dietary fiber)]. Proteins, lipids, carbohydrates, total dietary fiber, and ash were expressed as % of dry matter (d.m.).

Lactiplantibacillus plantarum DSM33326 and *Levilactobacillus brevis* DSM33325 (Rizzello, Raho, Dingeo, Carofiglio, & Centrone, 2020 patent; Pontonio et al., 2020), belonging to the culture collection of the Celery Srl (Polignano a Mare, Italy) and deposited in the DSMZ (Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) culture collection, were used as starter for the fermentation. Strains were routinely propagated in De Man, Rogosa and Sharpe (MRS, Oxoid, Basingstoke, Hampshire, UK) at 30 °C for 24 h. When used as starters for the fermentation, lactic acid bacteria (LAB) were cultivated until the late exponential phase of growth was reached (16 h), harvested by centrifugation at 9000×*g* at 4 °C for 10 min, washed twice in 50 mmol/L phosphate buffer (4 °C, pH 7.0), resuspended in the tap water, and used for making the YL (final cell density 7 log10 cfu/g). Skimmed milk (Granarolo S.p.a., Bologna, Italy, brown sugar, Cocoa powder 22/24 (22–24% cocoa butter), Cocoa powder 10/12 (10–12% cocoa butter), Cocoa beans (powder), Modica chocolate (powder), tara pods (*Caesalpinia spinosa*) flour, Guar gum, and emulsifier E471 (mono- and di-glycerides mixture) were provided by Città del Gelato Srl, (Noci, Italy) and used in ice cream recipes.

2.2. Production of the yogurt-like

2.2.1. Processing

The biotechnological protocol to make the YL was adapted from that previously set-up by Pontonio et al. (2020) and is represented in Fig. 1.

In details, rice, lentil, and chickpea flours (ratio 4:2:1) were mixed with water (1:10) and homogenized with an Oster 6805 (Jarden Consumer Solutions Ltd., Cheadle, United Kingdom) mixer. Aiming at starch gelatinization, the mixture was treated at 80 °C for 15 min under stirring conditions (70 rpm). Then, the mixture was cooled down to 4 °C in 2 min and then wormed up to 30 °C prior the inoculum of *L. plantarum* DSM33326 and *L. brevis* DSM33325 (ratio 1:1). The initial cell density of each strain was 7.2 log10 cfu/mL. The fermentation was carried out at 30 °C for 16 h. At the end of fermentation, the mixture was cooled down to 4 °C in 5 min, packaged in a glass jar and analyzed within 2 h after the fermentation. Three independent productions were carried out and monitored.

2.3. Yogurt-like characterization

2.3.1. Microbiological analysis

The cell density of LAB was determined on 10 g of the YL, prior and after the fermentation, suspended in 90 mL of sterile sodium chloride (0.9%, w/vol) solution and homogenized in a Bag Mixer 400 P (Interscience, St Nom, France) at room temperature. Serial 10-fold dilutions were then plated into modified MRS (mMRS, maltose and fresh yeast extract were added to MRS at 1 and 5%, respectively, and the final pH was 5.6) supplemented with cycloheximide (0.1 g/L) and incubated at 30 °C for 48 h.

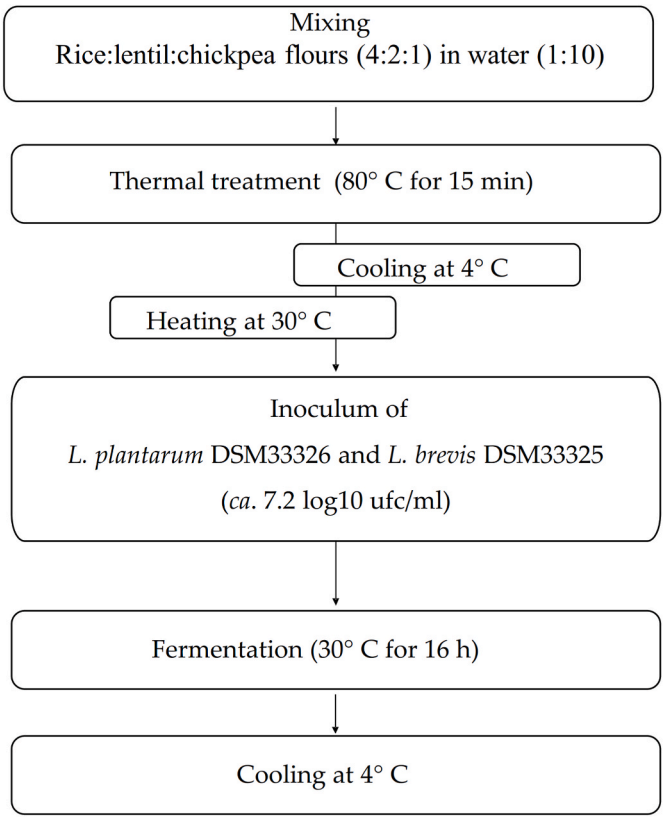

Fig. 1. Flow-chart of the biotechnological protocol to make the yogurt-like.

2.3.2. Biochemical and nutritional characteristics

The pH of YL was determined by a M.507 pH-meter (Crison, Milan, Italy) equipped with a food penetration probe while the total titratable acidity (TTA) was determined on 10 g of product homogenized with 90 mL of distilled water and expressed as the amount (mL) of 0.1 M NaOH to reach pH of 8.3. The apparent viscosity of YL samples, maintained at 25 °C for 30 min prior the analysis, was measured on 50 mL aliquots using the MYR VR3000 Model L viscometer (Viscotech Hispania, SL, Spain). Water/salt-soluble extracts (WSE) were prepared according to the method originally described by Osborne (1907) and modified by Weiss, Vogelmeier, and Görg (1993), and used to analyze organic acid and free amino acids (FAA). The sum of D- and L-lactic and acetic acids concentrations were determined by Megazyme kits (Megazyme International Ireland Limited, Bray, Ireland) K-DLATE and K-ACET, respectively, following the manufacturer's instructions. The fermentation quotient (FQ) was determined as the molar ratio between lactic and acetic acids. FAA were analyzed, after precipitation of proteins using 5-sulfosalicylic acid dihydrate (0.5% w/vol) (Sigma-Aldrich, Darmstadt, Germany), by a Biochrom 30+ series Amino Acid Analyzer (Biochrom Ltd., Cambridge Science Park, England) with a Li-cation-exchange column (20 by 0.46 cm inner diameter). Nutritional characterization evaluated macro- and micro-nutrient composition of YL. In details, ash, moisture, and protein contents were determined according to ISO 2171:2007, ISO 712:2009, and ISO/TS 16634–2:2019, respectively. Total fat was determined according to the method described in D. M. n. 4 of July 23, 1994 reported in G.U. n.186 of August 10, 1994 while saturated fatty acids after determination of methyl-esters according to

Reg. CEE 2568/1991. Carbohydrates were determined as a difference of nutrients according to D. lgs n. 77 (1993) while total fiber according to AOAC Official Method 985.29. Sugars (fructose, glucose, galactose, lactose, maltose, and sucrose) were evaluated by using K-FRUGL, K-LACGAL and K-MASUG kits (Megazyme), respectively. Calcium, iron, phosphorus, magnesium, potassium, sodium, and zinc contents were determined according to AOAC Official Method 984.27. Vitamins were evaluated by Food Safety Lab (Corato, Italy) according to internal validated methods. In details, vitamins characterization included vitamins A, B1, B2, B6, B9, B12, C, D2, D, 23 July 1994, and K1.

2.3.3. Antinutritional compounds

The presence of raffinose, phytic acid, saponins, and condensed tannins was determined. Raffinose and phytic acid concentrations were determined by using the K-RAFGA and K-PHYT kits (Megazyme), respectively, following the manufacturer's instructions. Total saponins were determined as reported by Lai, Hsieh, Huang, and Chou (2013). Briefly, the freeze-dried YL (0.5 g) was mixed with 10 mL of petroleum ether by shaking for 4 h. The residues (20 mg) were then extracted with 5 mL of 80% (vol/vol) aqueous methanol with shaking for 4 h. The extracts were kept at 4 °C in the dark until they were subjected to analysis. Total saponin content (TSC) was determined using vanillin solution and read by spectrophotometric method at 544 nm (Lai et al., 2013). Condensed tannins were determined using the acid butanol assay as described by Hagerman (2002) with some modification. Samples were homogenized with HCl:methanol (1:100 vol/vol) for 2.5 h at room temperature and centrifuged at 4000 rpm for 20 min. Extracts were covered from light and analyzed promptly. The total condensed tannins concentration was determined using acid butanol and iron reagents and read by spectrophotometric method at 550 nm (Hagerman, 2002).

2.3.4. Protein and starch digestibility

The *in vitro* protein digestibility (IVPD) of the YL was determined by the method proposed by Akeson and Stahmann (1964). Samples were subjected to a sequential enzyme treatment mimicking the *in vivo* digestion in the gastrointestinal tract. The IVPD was calculated as the percentage of digested (supernatant) on the total protein content (supernatant + precipitate) after their quantification in both fractions by the Bradford method (Bradford, 1976). The analysis of starch hydrolysis was carried out by the *in vitro* analysis, consisting in salivary amylase, pepsin, and α-amylase enzymatic treatments, mimicking the *in vivo* digestion of starch (De Angelis et al., 2009). The glucose content was measured with K-GLUC assay kit (Megazyme), and the starch digestion degree was expressed as the percentage of potentially available starch hydrolyzed after 3 h by α-amylase compared to total hydrolyzed starch after 16 h. The results were expressed as a hydrolysis index (HI) considering wheat bread as the reference product (HI = 100). The predicted pGI was calculated using the equation: pGI = 0.549 * HI + 39.71 (Capriles & Arêas, 2013).

2.4. Ice cream making

Ice creams were produced at pilot-plant level at Città del Gelato Srl (Noci, Italy). Formulation for obtaining the plant-based ice cream (pb-IC) and a conventional milk-based ice cream to be used as control (ct-IC) are reported in Table 1. Ingredients were mixed in a Smarty 7 ice cream machine (Valma, Volčja Draga, Slovenia); first, solid ingredients and powders were mixed; then, liquid ingredients, including cocoa powders previously resuspended in boiling water, were added. Three batches of 4 kg were obtained for each type of ice cream. Ice cream was extracted at −10 °C, packed in in 100 mL cup, and transferred into a Hiber chiller (Sedico, BL, Italy) at −18 °C.

Table 1
Recipes for making plant-based (pb-IC) and milk-based (ct-IC) ice-cream.

Ingredients (% w/w)	pb-IC	ct-IC
YL base	40.0	–
Skimmed milk	–	40.0
Brown sugar	22.0	22.0
Water	21.0	21.0
Cocoa powder 22/24	9.7	9.7
Cocoa powder 10/12	3.1	3.1
Modica chocolate (powder)	2.8	2.8
Cocoa beans (powder)	0.7	0.7
Emulsifier E471	0.3	0.3
Guar gum	0.2	0.2
Tara pods	0.1	0.1

2.5. Ice cream characterization

2.5.1. Biochemical analysis and proximal composition

The pH and TTA of ice cream were evaluated as described above. The proximal composition of ice cream was determined according to macronutrient composition (moisture, protein, fat, carbohydrates, fiber, and ash) as previously described. The energy value was calculated using the conversion factors listed in Annex XIV of Reg. CE 1169/2011.

2.5.2. Technological characterization

The dry matter in the ice cream was determined by drying the samples at 130 ± 1 °C for 3 h according to AOAC International Method 925.10.

Overrun, corresponding to the percentage of the incorporated air throughout the whipping–freezing process was calculated gravimetrically (Goff & Hartel, 2013) using a standard volume of aged mix and frozen end-product (50 mL) as follows:

Overrun (%) = 100 × [(weight of ice cream mix (g) − weight of ice cream (g)) ÷ weight of ice cream (g)]

Apparent viscosity of ice cream mixes (Kokini viscosity) was measured at 4 °C on 50 mL-aliquots of ice cream samples, using the MYR VR3000 Model L viscometer (Viscotech Hispania, SL, Spain) coupled with a circulating cooling bath at a shear rate of 50 s^{-1}, which represents the sensing shear rate in the mouth of low viscosity foods (Akhtar, Murray, & Dickinson, 2006; Stanley & Taylor, 1993). Before analysis, samples were conditioned at 4 °C for 3 h.

The chromaticity coordinates of the ice creams after hardening (obtained by a Minolta CR-10 camera) were reported in the form of a color difference, ΔE, as follows:

$$\Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2}$$

where Δa, Δb and ΔL are the differences for L, a, and b values between sample and reference (a white ceramic plate having L* = 93.4, a* = −1.8, and b* = 4.4).

The Instrumental Texture Profile Analysis (TPA) of the hardened ice cream samples (height 7.5 cm, diameter 5.5 cm) was performed with a FRTS-100N Texture Analyzer (Imada, Japan) equipped with a 20 mm diameter cylinder probe (FR-HA-20J, Imada, Japan). Before analysis, ice cream samples were reconditioned at - 15 °C for 30 min and attached to a 10 kg load cell. The penetration depth at the geometrical center of the samples was 10 mm, and the penetration speed was 2 mm/s (Tsevdou et al., 2019). The recorded parameters were hardness (N), adhesiveness (N × s), cohesiveness, springiness, and gumminess (N).

2.5.3. Sensory analysis

Sensory analysis of ice cream was performed by a trained panel group composed of twelve assessors (6 male and 6 female, mean age: 29 years, range: 25–47 years) with previous experiences in sensory evaluation. The sensory attributes, scored with a scale from 0 to 10 (with 10 the highest score), were discussed with the assessors during the introductory 2 h-training sessions. Attributes were chosen according to

descriptors previously used for ice cream sensory analysis (Cadena, Cruz, Faria, & Bolini, 2012; Roland, Phillips, & Boor, 1999; Thompson, Chambers, & Chambers IV, 2009; Warren & Hartel, 2014) and descriptors that were considered most suitable for the evaluation of commercial chocolate ice cream and chocolate ice cream obtained from plant-based YL. All the attributes are listed in Supplementary Table S1. Sensory evaluations were carried out in the library of the Department of Soil, Plant and Food Science of the University of Bari, Italy. Ice cream samples were frozen at −20 °C and were evaluated in portion of 20 g after a conditioning at room temperature of 5 min. Three independent sessions were carried out. A glass of water was used to clean the panelists mouth between samples.

2.5.4. Lactic acid bacteria survival

The LAB cell density on the ice cream was monitored for 120 days at 15-days intervals. The enumeration was carried out as described before.

2.6. Statistical analysis

Samples were produced in triplicate. All the chemical, microbiological, physical, and sensory analyses were carried out in triplicate for each batch of samples. Data were subjected to one-way ANOVA; paired comparison of treatment means was achieved by Tukey's procedure at $p < 0.05$, using the statistical software Statistica 12.5 (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Biochemical and nutritional characteristics of the yogurt like

Before fermentation, the pH of the mixture including water and raw ingredients was 6.50 ± 0.15, while TTA resulted 1.0 ± 0.2 mL NaOH 0.1M. After fermentation pH decreased to 4.25 ± 0.10 (TTA increased to 5.0 ± 0.3) as the consequence of the LAB fermentation. LAB cell density in YL base after fermentation was 9.7 ± 0.2 log10 cfu/mL. In detail, lactic and acetic acids were at concentration of 12.5 ± 0.6 and 4.0 ± 0.5 mmol/L, respectively. YL base viscosity was 4.30 ± 0.20 Pa × s.

As expected, a relevant concentration of TFAA was found (1150 ± 25 mg/kg). Compared to the unfermented matrix, an increase of the 35% was observed during fermentation due to the intense proteolytic activity of the selected starters used (Pontonio et al., 2020).

The proximal composition of the YL base was the follow: water 92.5 ± 1.1% (w/w); proteins 1,25 ± 0,24% (w/w), fat 0.20 ± 0.32% (w/w), total carbohydrates 5.20 ± 0.10% (w/w), ash 1.38 ± 0.06% (w/w). Total dietary fibers content was 1.22 ± 0.20, while salt was 0.012 ± 0.002% (w/w). Energy value corresponded to 127 ± 3 kJ/100g.

Among all the sugars analyzed, only glucose and sucrose were found at detectable concentration (0.03 ± 0.01 and 0.02 ± 0.01%, respectively). Among the vitamins analyzed, only folate was detected; its concentration was 0.02 ± 0.01 mg/kg.

Minerals were found at the following levels: Ca, 73 ± 3 mg/kg; Fe, 3 ± 1 mg/kg; P, 12 mg/kg; Mg, 52 ± 5 mg/kg; K, 388 ± 21 mg/kg; Na, 5 ± mg/kg; Zn, 12 ± 2 mg/kg.

Anti-nutritionl factors (ANF) that characterize the cereal/legume raw ingredients used for YL making were at the following concentrations: phytic acid, 16.4 ± 0.5 mg/100 mL; raffinose, 12.9 ± 0.3 mg/100 mL; saponins, 25.4 ± 0.8 mg/100 mL; condensed tannins 0.1 ± 0.1 mg/100 mL.

Digestibility indexes were also calculated by mimicking the digestion process through the gastro-intestinal tract. IVPD corresponded to 80 ± 2%, while HI was 12.4 ± 0.6. pGI of the YL, calculated on the basis of HI, resulted 46.5 ± 0.2.

3.2. Biochemical, nutritional characterization and LAB survival of the ice cream

The biochemical, nutritional and technological properties of the ice cream are summarized in Table 2. Overall, with the only exception of the TTA, proteins and ash contents which were significantly different between samples, ice creams showed similar pH and nutritional properties (Table 2). The use of plant-based ingredient led to higher TTA, due to the organic acids (lactic and acetic acids) produced by LAB during fermentation of the YL. The higher ash and lower protein contents are typical of the plant-derived ingredients compared to the milk. The cell density of the LAB of the pb-IC was monitored through the storage at −20 °C. Although it decreased from 8.2 Log10 cfu/mL to 7.4 Log10 cfu/mL after 120 days, the value was higher than 7.7 Log10 cfu/mL for the first 60 days of storage. (Fig. 2).

3.3. Technological properties and sensory analysis

According to the technological properties, the pb-IC showed significantly higher dry matter, overrun and apparent viscosity than ct-IC. Overrun resulted 2.5 times higher in pb-IC compared to ct-IC. Although the lightness and the other colorimetric coordinates did not significantly ($P > 0.05$) differ between the two samples, the ct-IC ΔE value was significantly ($P < 0.05$) higher in ct-IC compared to pb-IC (Table 3).

The use of plant-based ingredient had a significant impact on the texture of ice cream. Indeed, the hardness and gumminess of pb-IC were almost 3- and 2-times, respectively, higher than ct-IC. Adhesiveness was markedly (20-times) higher than ct-IC, while springiness of pb-IC was only slightly, but significantly, higher. On the contrary, cohesiveness was significantly ($P < 0.05$) lower when the plant-based ingredient was used (Table 3).

Fig. 3 shows the sensory profiles of the ice creams. Overall, with only few exceptions, they were similar. Indeed, according to the assessors, the use of the plant-based ingredient led to slightly higher score for size ice particulate and artificial taste, while the smell of chocolate, the bitterness, and the sweetness seemed to be more intense for the ct-IC. In details, the highest difference was found for chocolate perception which was 39% higher in ct-IC than pb-IC. Moreover, ct-IC was evaluated more bitter (+33%) and persistent (+12%) than the experimental plant-based counterpart. In both ice creams, scores of vegetable smell, size of particles, and acidic taste attributes were below 1.

4. Discussion

Today, the global food industry needs to answer the modern consumer requests for products that, in addition to the proper nutritional

Table 2
Biochemical and nutritional characteristics of the ice creams (pb-IC, plant-based ice cream; ct-IC, milk-based ice cream).

	pb-IC	ct-IC
Biochemical		
pH	6.5 ± 0.2[a]	7.3 0.5[a]
TTA	2.25 ± 0.3[a]	1.3 0.1[b]
Nutritional properties		
Moisture (%)	60.91 ± 1.18[a]	60.11 ± 1.01[a]
Proteins (%)	3.52 ± 0.15[b]	4.46 ± 0.17[a]
Fat (%)	4.65 ± 0.18[a]	4.65 ± 0.17[a]
Carbohydrates (%)	25.69 ± 0.37[a]	25.73 ± 0.31[a]
Ash (%)	0.67 ± 0.07[a]	0.27 ± 0.07[b]
Dietary fibers (%)	5.20 ± 0.04[a]	4.71 ± 0.05[b]
Energy value (kcal)	667± 4[a]	676± 5[a]

The data are the means of three independent experiments ± standard deviations (n = 3).
[a-b] Values in the same row with different superscript letters differ significantly ($p < 0.05$).

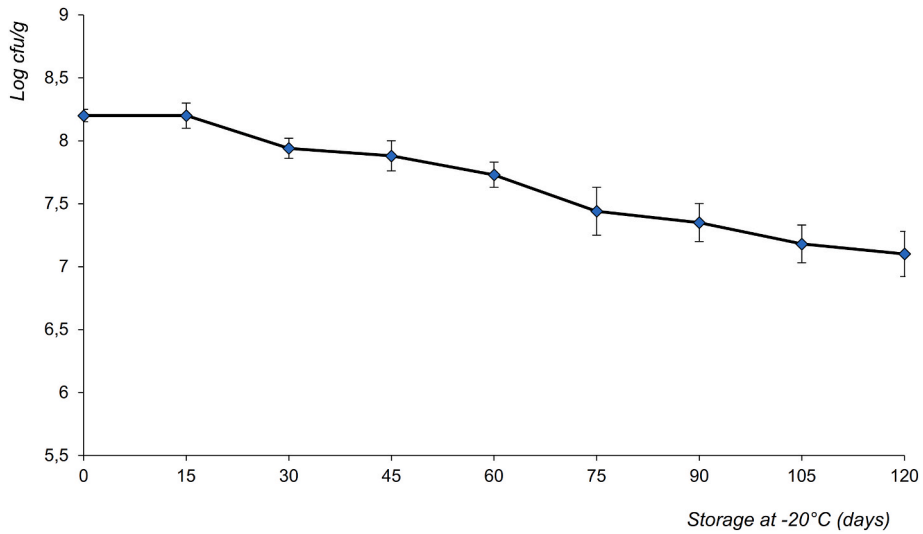

Fig. 2. Viable lactic acid bacteria cell density in the plant-based ice-cream during 120 days of storage at −20 °C. Values, determined at 15-days intervals, are the mean of three different measurements. Bars represent standard deviation.

Table 3
Technological properties of the ice creams (pb-IC, plant-based ice cream; ct-IC, milk-based ice cream).

	pb-IC	ct-IC
Dry matter	42.3 ± 0.3[a]	40.8 ± 0.3[b]
Overrun	19.3 ± 1.1[a]	7.7 ± 1.8[b]
Apparent Viscosity (Pa x s)	0.90 ± 0.01[a]	0.47 ± 0.01[b]
Colorimetric coordinates		
L*	25.55 ± 1.6[a]	25.92 ± 2.10[a]
a*	4.42 ± 0.30[a]	4.70 ± 0.81[a]
b*	6.81 ± 0.52[a]	7.11 ± 0.32[a]
ΔE*ab	2.29 ± 0.33[b]	3.05 ± 0.24[a]
Textural profile parameters		
Hardness (N)	19.99 ± 0.22[a]	7.08 ± 0.01[b]
Adhesiveness (N x s)	−19.18 ± 0.11[b]	- 0.80 ± 0.093[a]
Cohesiveness	0.19 ± 0.01[b]	0.25 ± 0.01[a]
Springiness	0.48 ± 0.01[a]	0.45 ± 0.01[b]
Gumminess (N)	3.74 ± 0.01[a]	1.78 ± 0.01[b]

The data are the means of three independent experiments ± standard deviations (n = 3).
a-b Values in the same row with different superscript letters differ significantly ($p < 0.05$).

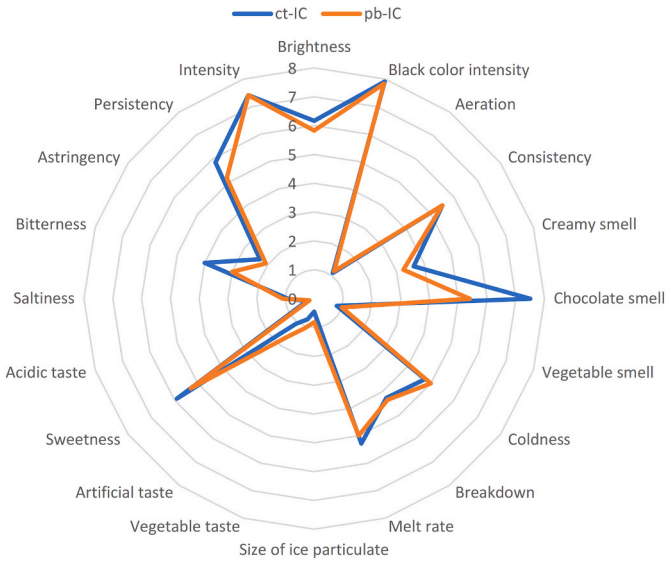

Fig. 3. Sensory profiles of the ice creams: pb-IC, plant-based ice cream; ct-IC, milk-based ice cream.

balance, meet new criteria that include long-term environmental sustainability, ethical requirements, absence of compounds capable of causing potential adverse reactions, such as gluten, lactose, and various allergens (Gobbetti, De Angelis, Di Cagno, Polo, & Rizzello, 2020)

In this context, scientific and industrial research community has recently evaluated the possibility of using plant-derived ingredients such as flours obtained from legumes and pseudo-cereals, overall characterized by nutritional characteristics of great interest such as high protein and dietary fibres content, abundance of bioactive compounds and polyphenols with antioxidant activity (De Pasquale, Pontonio, Gobbetti, & Rizzello, 2020; Gänzle, 2020; Gobbetti et al., 2020). When used in mixture with cereals, these ingredients allow the production of fortified foods, characterized by a nutritional and functional profile largely improved compared to the corresponding conventional products (Gobbetti et al., 2020; Montemurro, Coda, & Rizzello, 2019). Nevertheless, the use of these alternative ingredients poses several technological and organoleptic challenges. Furthermore, it is necessary to consider the presence both in legumes and in pseudo-cereals, of some anti-nutritional compounds that limit digestibility and in general their use on a large scale.

Overall, the fermentation of these matrices with selected microorganisms allows the overcoming of many of these limitations; the use of LAB, in particular, allows to improve the sensory profile and the technological properties, increase the protein digestibility, decrease the glycaemic index (De Pasquale et al., 2020; Gobbetti et al., 2020). Moreover, LAB selected for specific metabolic traits have the capability to decrease the level of anti-nutritional compounds such as phytic acid, condensed tannins, saponins, and raffinose and could enrich the fermented matrix in bioactive compounds such as peptides, GABA and exopolysaccharides (Gobbetti et al., 2020).

Among the categories of foods mainly interested by the recent innovation trend, that of the YL beverages is gaining attention by industry and consumer (Montemurro, Pontonio, Coda, & Rizzello, 2021; Pontonio & Rizzello, 2021; Ziarno & Cichońska, 2021). Such products are similar to conventional milk-derived yogurt in structure and viscosity; moreover, they share with the traditional counterpart the presence, at high cell density, of viable lactic bacteria, whose survival is guaranteed by storage in refrigerated conditions (Montemurro et al., 2021). Such products are made with vegetable substrates instead of

milk, such as cereals and legumes and requires a specific technology and the use of *ad hoc* selected LAB strains as starters for fermentation (Montemurro et al., 2021). The replacement of milk is challenging due to the optimal structuring properties of its proteins (Montemurro et al., 2021), and the use of gluten-containing ingredients could potentially provide effective solutions to the technological issues. Nevertheless, this choice does not fit to the design of formulations that are intended as alternative to dairy products, yogurt and ice-creams, that are naturally gluten-free. A biotechnological protocol to produce a YL lactose-free and gluten-free beverage obtained exclusively from gluten-free cereals and legumes (lentil and chickpea) has recently been developed and patented (Rizzello et al., 2020 patent).

In the present article, such biotechnological protocol was applied at pilot scale and the product obtained characterized to verify the adherence of its nutritional and functional features to the profile previously described in the patent. The YL had a creamy-viscous structure, similar to that of a milk-derived yogurt (viscosity 4.30 ± 0.20 Pa × s), thanks to the starch gelatinization promoted trough a thermal treatment in the first production step. The fermentation of the matrix was carried out at 30 °C with the use of *L. plantarum* DSM33326 and *Lev. brevis* DSM33325 (Pontonio et al., 2020). Both the strains were selected on the basis of the technological properties (e.g., fast acidification and high proteolytic activity) but also on the basis of the ability to degrade the anti-nutritional compounds typical of the legume component (Pontonio et al., 2020). Fermentation ensured the lowering of the pH of the matrix in a range that does not involve health and hygiene risks (pH 4.25 after 16 h of fermentation). The cell density in the product at the end of fermentation, according to the previous research (Pontonio et al., 2020) reached 10^9 cfu/mL. The YL contained relevant concentration of dietary fibers (5.20%) and proteins (3.52%), the latter previously characterized by a high biological value, thanks to the contribution of both rice and legumes (lentil and chickpea) protein fractions, having complementary amino acid profiles (Pontonio et al., 2020). Anti-nutritional compounds were all at very low levels (<25 mg/100 mL) thanks to the degradative activities that selected lactic bacteria strains carry out during the fermentation process (Pontonio et al., 2020). It should be noted that condensed tannins could act as bioactive compounds, thanks to capability to reduce starch digestibility and glycemic index (Gänzle, 2020), nevertheless, due to their capability to reduce protein digestibility (and micronutrients bioavailability) they are considered antinutritional in foods and beverages with limited protein content or containing legume-derived ingredients (Gobbetti et al., 2020; De Pasquale et al., 2020).

To evaluate diversified applications, a recipe for obtaining an entirely vegetable lactose-free and gluten-free ice cream, with the YL matrix as main ingredient, was developed. The experimental ice cream was then characterized for the main nutritional, structural and sensory feature and compared to a control ice cream obtained on a milk basis.

The comparison of the nutritional label of the two products showed almost similar characteristics. No differences in the concentration of fats and carbohydrates were observed, since mainly dependent by the same ingredients added respectively to the milk and to the YL. Significant differences were found in proteins, whose concentration was slightly lower in the plant-based ice cream (−27%), and in fibres, whose concentration was significantly higher (+10%) in the plant-based ice cream than in the milk-based control.

However, the YL used in the experimental product to replace milk has a higher protein digestibility compared to the milk (IVPD of 80% *vs* 26%) (Martínez-Padilla et al., 2020), and is characterized by the absence of lactose.

The use of the fermented vegetable matrix led to relevant difference in the structural and technological characteristics of the ice cream compared to that milk-based. Overrun, which corresponds to the air that the matrix incorporates during the production of the ice cream, mainly during the freezing of the mixture of ingredients, was significantly higher for the plant-based ice cream compared to the milk-based product. It can be hypothesized that such result depends on emulsifying and foaming properties characterizing legume proteins (Schwenke, 2001). The instrumental analysis of the texture also showed that the hardness, gumminess and adhesiveness were higher than the conventional ice cream. The increase of hardness, adhesiveness, springiness, cohesiveness, and gumminess values was previously observed in plant-based ice creams, in proportion to pea protein isolate supplementation (Guler-Akin, Avkan, & Akin, 2021). The sensory analysis was carried out by trained panelists; it showed very similar profiles for the two products, and negligible differences in the parameters related to appearance, structure, taste, odor and texture. No perception of the attributes commonly associated to plant-derived (vegetable smell and taste, bitterness, astringency) and fermented (such as the acidic taste) ingredients was reported. Also, the structural differences revealed by the instrumental (TPA) analysis did not correspond to perceived differences in textural properties of the two samples.

The plant-based ice cream was also characterized for the presence of viable LAB at high cell density. In particular, the viability was monitored for 120 days in the product stored at −20 °C. The analyses showed that the cell density was higher than 10^7 ufc/mL for the entire monitored storage period. The probiotic long-term (90–120 days at −20 °C) survival in conventional in ice cream formulations was previously observed by several authors (Kozłowicz, Góral, Góral, Pankiewicz, & Bronowicka-Mielniczuk, 2019; Sarwar et al., 2021).

Overall, the present study demonstrated the potentiality of the novel YL to be used as ingredient to produce plant-based ice-cream with peculiar nutritional, structural, and sensory properties which would be appreciated by the consumers. Moreover, based on the results the plant-based ice cream can therefore be considered as a potential carrier of viable LAB and/or probiotics in the diet.

Conflict of interest and authorship conformation form

- ○ All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- ○ This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
- ○ The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript:

CRediT authorship contribution statement

Erica Pontonio: Validation, Writing – review & editing. **Marco Montemurro:** Investigation, Data curation, Validation. **Cinzia Dingeo:** Investigation, Data curation. **Michele Rotolo:** Methodology, Investigation. **Domenico Centrone:** Conceptualization. **Vito Emanuele Carofiglio:** Conceptualization, Supervision. **Carlo Giuseppe Rizzello:** Methodology, Validation, Supervision, Writing – original draft, Reviewing and Editing.

Acknowledgements

Francesca Fiume (Città del Gelato Srl, Noci, Italy) is thanked for the technical support during the project. Daniele Santangelo is gratefully thanked for formal analyses carried out within his master's thesis project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113327.

References

Aboulfazli, F., Baba, A. S., & Misran, M. (2015). The rheology and physical properties of fermented probiotic ice creams made with dairy alternatives. *International Journal of Food Engineering, 11*(4), 493–504. https://doi.org/10.1515/ijfe-2014-0343

Akeson, W. R., & Stahmann, M. A. (1964). A pepsin pancreatin digest index of protein quality evaluation. *Journal of Nutrition, 83*, 257–261. https://doi.org/10.1093/jn/83.3.257

Akhtar, M., Murray, B. S., & Dickinson, E. (2006). Perception of creaminess of model oil-in-water dairy emulsions: Influence of the shear-thinning nature of a viscosity-controlling hydrocolloid. *Food Hydrocolloids, 20*, 839–847. https://doi.org/10.1016/j.foodhyd.2005.08.006

Alcorta, A., Porta, A., Tárrega, A., Alvarez, M. D., & Vaquero, M. P. (2021). Foods for plant-based diets: Challenges and innovations. *Foods, 10*(2), 293. https://doi.org/10.3390/foods10020293

Bouvard, V., Loomis, D., Guyton, K. Z., Grosse, Y., Ghissassi, F. E., Benbrahim-Tallaa, L., et al. (2015). Carcinogenicity of consumption of red and processed meat. *The Lancet Oncology, 16*, 1599–1600. https://doi.org/10.1016/S1470-2045(15)00444-1

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cadena, R. S., Cruz, A. G., Faria, J. A. F., & Bolini, H. M. A. (2012). Reduced fat and sugar vanilla ice creams: Sensory profiling and external preference mapping. *Journal of Dairy Science, 95*(9), 4842–4850. https://doi.org/10.3168/jds.2012-5526

Capriles, V. D., & Arêas, J. A. G. (2013). Effects of prebiotic inulin-type fructans on structure, quality, sensory acceptance and glycemic response of gluten-free breads. *Food & Function, 4*, 104–110. https://doi.org/10.1039/C2FO10283H

D, M. (23 July 1994). *Approvazione dei "Metodi ufficiali di analisi dei cereali e derivati - Supplemento n. 4". (GU Serie Generale n.186 del 10-08-1994 - Suppl. Ordinario n. 114)*. Retrieved from https://www.gazzettaufficiale.it/eli/id/1994/08/10/094A4960/sg. (Accessed 8 January 2022).

D. lgs n. 77 Attuazione della direttiva 90/496/CEE del Consiglio del 24 settembre 1990 relativa all'etichettatura nutrizionale dei prodotti alimentari. *(GU Serie Generale n.69*, (16 Febraury 1993). del 24-03-1993) Retrieved from https://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=1993-03-24&atto.codiceRedazionale=093G0116&elenco30giorni=false. (Accessed 8 January 2022).

De Angelis, M., Damiano, N., Rizzello, C. G., Cassone, A., Di Cagno, R., & Gobbetti, M. (2009). Sourdough fermentation as a tool for the manufacture of low-glycemic index white wheat bread enriched in dietary fibre. *European Food Research and Technology, 229*, 593–601. https://doi.org/10.1007/s00217-009-1085-1

De Pasquale, I., Pontonio, E., Gobbetti, M., & Rizzello, C. G. (2020). Nutritional and functional effects of the lactic acid bacteria fermentation on gelatinized legume flours. *International Journal of Food Microbiology, 316*, 108426. https://doi.org/10.1016/j.ijfoodmicro.2019.108426

Gänzle, M. G. (2020). Food fermentations for improved digestibility of plant foods–an essential ex situ digestion step in agricultural societies? *Current Opinion in Food Science, 32*, 124–132. https://doi.org/10.1016/j.cofs.2020.04.002

Gobbetti, M., De Angelis, M., Di Cagno, R., Polo, A., & Rizzello, C. G. (2020). The sourdough fermentation is the powerful process to exploit the potential of legumes, pseudo-cereals and milling by-products in baking industry. *Critical Reviews in Food Science and Nutrition, 60*(13), 2158–2173. https://doi.org/10.1080/10408398.2019.1631753

Goff, H. D., & Hartel, R. W. (2013). *Ice cream* (7th ed.). New York: Springer. https://doi.org/10.1007/978-1-4614-6096-1_11

Guler-Akin, M. B., Avkan, F., & Akin, M. S. (2021). A novel functional reduced fat ice cream produced with pea protein isolate instead of milk powder. *Journal of Food Processing and Preservation, 45*(11), Article e15901. https://doi.org/10.1111/jfpp.15901

Hagerman, A. E. (2002). Acid buthanol assay for proanthocyanidins. In A. E. Hagerman (Ed.), *The tannin handbook*. Oxford, UK: Miami University.

Kozłowicz, K., Góral, M., Góral, D., Pankiewicz, U., & Bronowicka-Mielniczuk, U. (2019). Effect of ice cream storage on the physicochemical properties and survival of probiotic bacteria supplemented with zinc ions. *Lebensmittel-Wissenschaft & Technologie, 116*, 108562. https://doi.org/10.1016/j.lwt.2019.108562

Lai, L. R., Hsieh, S. C., Huang, H. Y., & Chou, C. C. (2013). Effect of lactic fermentation on the total phenolic, saponin and phytic acid contents as well as anti-colon cancer cell proliferation activity of soymilk. *Journal of Bioscience and Bioengineering, 115*(5), 552–556. https://doi.org/10.1016/j.jbiosc.2012.11.022

Martínez-Padilla, E., Li, K., Blok Frandsen, H., Skejovic Joehnke, M., Vargas-Bello-Pérez, E., & Lykke Petersen, I. (2020). *In vitro* protein digestibility and fatty acid profile of commercial plant-based milk alternatives. *Foods, 9*(12), 1784. https://doi.org/10.3390/foods9121784

Mintel. (2020). *Plant-based push: UK sales of meat-free foods shoot up 40% between 2014-2019*. Retrieved from https://www.mintel.com/press-centre/food-and-drink/plant-based-push-uk-sales-of-meat-free-foods-shoot-up-40-between-2014-19. (Accessed 8 January 2022).

Montemurro, M., Coda, R., & Rizzello, C. G. (2019). Recent advances in the use of sourdough biotechnology in pasta making. *Foods, 8*(4), 129. https://doi.org/10.3390/foods8040129

Montemurro, M., Pontonio, E., Coda, R., & Rizzello, C. G. (2021). Plant-based alternatives to yogurt: State-of-the-art and perspectives of new biotechnological challenges. *Foods, 10*(2), 316. https://doi.org/10.3390/foods10020316

Park, Y. W. (2021). The impact of plant-based non-dairy alternative milk on the dairy industry. *Food Science of Animal Resources, 41*(1), 8. https://doi.org/10.5851/kosfa.2020.e82

Pinto, S. S., Fritzen-Freire, C. B., Muñoz, I. B., Barreto, P. L., Prudêncio, E. S., & Amboni, R. D. (2012). Effects of the addition of microencapsulated *Bifidobacterium* BB-12 on the properties of frozen yogurt. *Journal of Food Engineering, 111*(4), 563–569. https://doi.org/10.1016/j.jfoodeng.2012.03.016

Pontonio, E., Raho, S., Dingeo, C., Centrone, D., Carofiglio, V. E., & Rizzello, C. G. (2020). Nutritional, functional, and technological characterization of a novel gluten- and lactose-free yogurt-style snack produced with selected lactic acid bacteria and *Leguminosae* flours. *Frontiers in Microbiology, 11*, 1664. https://doi.org/10.3389/fmicb.2020.01664

Pontonio, E., & Rizzello, C. G. (2021). Milk Alternatives and non-dairy fermented products: Trends and challenges. *Foods, 10*(2), 222. https://doi.org/10.3390/foods10020222

Rizzello, C. G., Raho, S., Dingeo, C., Carofiglio, V. E., & Centrone, D. (2020). Nuovi ceppi di batteri lattici, composizione alimentare che li comprende, preparazione di tale composizione. *Patent*.

Roland, A. M., Phillips, L. G., & Boor, K. J. (1999). Effects of fat content on the sensory properties, melting, color, and hardness of ice cream. *Journal of Dairy Science, 82*(1), 32–38. https://doi.org/10.3168/jds.S0022-0302(99)75205-7

Salem, M. M., Fathi, F. A., & Awad, R. A. (2005). Production of probiotic ice cream. *Polish Journal of Food and Nutrition Sciences, 14*(3), 267.

Sarwar, A., Aziz, T., Al-Dalali, S., Zhang, J., Din, J., Chen, C., et al. (2021). Characterization of synbiotic ice cream made with probiotic yeast *Saccharomyces boulardii* CNCM I-745 in combination with inulin. *Lebensmittel-Wissenschaft & Technologie, 141*, 110910. https://doi.org/10.1016/j.lwt.2021.110910

Schwenke, K. D. (2001). Reflections about the functional potential of legume proteins A Review. *Food/Nahrung, 45*(6), 377–381. https://doi.org/10.1002/1521-3803(20011001)45:6<377::AID-FOOD377>3.0.CO;2. -G

Stanley, N. I., & Taylor, L. J. (1993). Rheological basis of oral characteristics of fluid and semi-solid foods: A review. *Acta Psychologica, 84*, 79–92. https://doi.org/10.1016/0001-6918(93)90074-2

The Business Times. (2020). *Impossible foods launches plant-based sausage product in Hong Kong*. Retrieved from https://www.businesstimes.com.sg/consumer/impossible-foods-launches-plant-based-sausage-product-in-hk. (Accessed 8 January 2022).

Thompson, K. R., Chambers, D. H., & Chambers IV, E. D. G. A. R. (2009). Sensory characteristics of ice cream produced in the USA and Italy. *Journal of Sensory Studies, 24*(3), 396–414. https://doi.org/10.1111/j.1745-459X.2009.00217.x

Tsevdou, M., Aprea, E., Betta, E., Khomenko, I., Molitor, D., Biasioli, F., et al. (2019). Rheological, textural, physicochemical and sensory profiling of a novel functional ice cream enriched with Muscat de Hamburg (*Vitis vinifera* L.) grape pulp and skins. *Food and Bioprocess Technology, 12*(4), 665–680. https://doi.org/10.1007/s11947-019-2237-3

Warren, M. M., & Hartel, R. W. (2014). Structural, compositional, and sensorial properties of United States commercial ice cream products. *Journal of Food Science, 79*(10), E2005–E2013. https://doi.org/10.1111/1750-3841.12592

Weiss, W., Vogelmeier, C., & Görg, A. (1993). Electrophoretic characterization of wheat grain allergens from different cultivars involved in bakers' asthma. *Electrophoresis, 14*(1), 805–816. https://doi.org/10.1002/elps.11501401126

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019). Food in the Anthropocene: The EAT-Lancet Commission on healthy diets from sustainable food systems. *The Lancet, 393*, 447–492. https://doi.org/10.1016/S0140-6736(18)31788-4

Ziarno, M., & Cichońska, P. (2021). Lactic acid bacteria-fermentable cereal-and pseudocereal-based beverages. *Microorganisms, 9*(12), 2532. https://doi.org/10.3390/microorganisms9122532