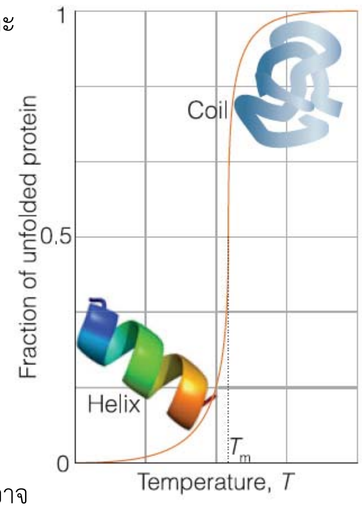
02738452 เคมีเชิงฟิสิกส์ทางวิทยาศาสตร์ชีวภาพ 1 ก.ย. 61 (09.00-12.00 น.)

สมดุลระหว่างเฟส (Phase Equilibria)

+++ การเปลี่ยนเฟสของสารชีวโมเลกุล +++

- โปรตีนและเมมเบรนทางชีวภาพ สามารถคงสภาพโครงสร้างได้ โดยอาศัยการกระทำระหว่างโมเลกุลในรูปแบบต่าง ๆ เช่น พันธะไฮโดรเจนและ hydrophobic interaction
- melting temperature T_m มีค่าสูงหรือต่ำ ขึ้นอยู่กับความแข็งแรงและจำนวนของตำแหน่งที่เกิดแรงกระทำทั้งในโมเลกุลเองและระหว่างโมเลกุล
- กรณีของ DNA นั้น ถ้ามีจำนวนคู่เบส C-G มาก ก็จะแข็งแรงมากขึ้น เพราะ C จับคู่กับ G ด้วยพันธะไฮโดรเจน 3 พันธะ

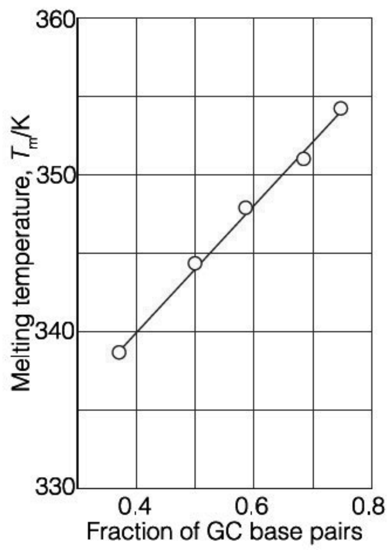


<u>ตัวอย่าง</u> หา T_m ของ DNA โดยใช้ differential scanning calorimetry ได้ข้อมูลดังข้างล่างนี้ โดยใช้สารละลาย 0.010 M Na_3PO_4(aq) กับชุดโมเลกุล DNA ที่มีองค์ประกอบคู่เบสแตกต่างกัน โดยมีสัดส่วนคู่เบส C-G ดังค่า f

f	0.375	0.509	0.589	0.688	0.750
T_m/K	339	344	348	351	354

จงคำนวณหา T_m ของโมเลกุล DNA ที่มีคู่เบส C-G อยู่ 40%

แนวคิด: ต้องหาความสัมพันธ์ระหว่าง T_m กับองค์ประกอบของ DNA โดยอาจจะพล็อตกราฟแล้วดูรูปร่างของเส้นโค้ง ถ้าเป็นความสัมพันธ์แบบเส้นตรง ก็หาได้จากสมการเส้นตรงที่สอดคล้องกับข้อมูล



วิธีทำ: พล็อตกราฟได้ดังภาพขวามือ จะได้สมการเส้นตรงคือ

T_m/K = 325 + 39.7f

และได้ T_m = 341 K สำหรับสัดส่วนคู่เบส C-G = 40.0%

แบบฝึกหัด จากข้อมูลต่อไปนี้ ใช้ 0.15 M NaCl(aq) กับชุดโมเลกุล DNA เดียวกับตัวอย่างข้างบน จงคำนวณหาค่า T_m ของโมเลกุล DNA ที่มีสัดส่วนคู่เบส C-G เป็น 40% ภายใต้สภาวะข้างล่างนี้

(ตอบ) 360 K

f	0.375	0.509	0.589	0.688	0.750
T_m/K	359	364	368	371	374

+++ การเปลี่ยนเฟสของเมมเบรนทางชีวภาพ +++

Lipid bilayer สามารถเปลี่ยนแปลงสถานะการเคลื่อนที่ (mobility) ได้ ขึ้นอยู่กับอุณหภูมิ

<u>คำถาม</u> สิ่งมีชีวิตสามารถสังเคราะห์ lipid ที่มีองค์ประกอบแตกต่างกัน ทำให้ได้เซลล์เมมเบรนที่มี T_m ใกล้เคียงกับอุณหภูมิของสิ่งแวดล้อม เหตุใดเซลล์แบคทีเรียและเซลล์พืชที่เจริญในอุณหภูมิต่ำจึงสังเคราะห์ phospholipids ที่มี unsaturated chain มากกว่าเซลล์ที่เจริญในอุณหภูมิสูง

<u>ตอบ</u> การมี unsaturated chain ทำให้ T_m ของเมมเบรนลดต่ำลง ใกล้เคียงกับอุณหภูมิของสภาพแวดล้อม

+++ กฎของ Henry และการละลายของก๊าซในสารละลาย +++

ที่อุณหภูมิคงที่ จำนวนก๊าซที่ถูกดูดซึมอยู่ในปริมาตรของของเหลว เป็นสัดส่วนต่อความดันของก๊าซในบรรยากาศ ที่ก๊าซได้ซึมเข้ามา โดยหาได้จากสูตร

$$[J] = K_H p_J$$

Table 3.2 Henry's law constants for gases dissolved in water at 25°C

	K_H/(mol m^{-3} kPa^{-1})
Carbon dioxide, CO_2	3.39×10^{-1}
Hydrogen, H_2	7.78×10^{-3}
Methane, CH_4	1.48×10^{-2}
Nitrogen, N_2	6.48×10^{-3}
Oxygen, O_2	1.30×10^{-2}

<u>ตัวอย่าง</u> การคำนวณหาว่าแหล่งน้ำธรรมชาติสามารถรองรับการดำรงชีวิตของสิ่งมีชีวิตในน้ำได้หรือไม่

ความเข้มข้นของ O_2 ในน้ำที่จำเป็นสำหรับการดำรงชีวิตของสิ่งมีชีวิตในน้ำ คือ 4.0 mg dm^{-3} จงหาความดันย่อย (partial pressure) ของออกซิเจนในบรรยากาศที่น้อยที่สุดที่ยังทำให้ได้ความเข้มข้นเท่านี้

แนวคิด: หาความดันย่อยของออกซิเจนตามกฎของ Henry

วิธีทำ: จากสมการข้างบน ได้ว่า $p_{O_2} = [O_2] / K_H$

ความเข้มข้นเป็นโมลาร์ของ O_2 หาได้จาก

$[O_2]$ = 4.0 x 10^{-3} g dm^{-3} / 32 g mol^{-1} = 4.0 x 10^{-3} mol / 32 dm^{3}

= 4.0 x 10^{-3} mol / 32 x 10^{-3} m^{3} = 4.0/32 mol m^{-3}

จากตารางขวามือ ค่า K_H สำหรับออกซิเจนในน้ำคือ 1.30 x 10^{-2} mol m^{-3} kPa^{-1} ดังนั้น ความดันย่อยที่ต้องใช้เพื่อให้ได้ความเข้มข้นดังกล่าวคือ

p_{O_2} = (4.0/32) mol m^{-3} / 1.30 x 10^{-2} mol m^{-3} kPa^{-1} = 9.6 kPa

หมายเหตุ ความดันย่อยของออกซิเจนในอากาศที่ระดับน้ำทะเลคือ 21 kPa (158 Torr) ซึ่งมีค่ามากกว่า 9.6 kPa (72 Torr) ดังนั้นในสภาวะปกติก็สามารถรักษาความเข้มข้นตามที่ต้องการได้

+++ Colligative properties +++

Table 3.4 Cryoscopic and ebullioscopic constants

Solvent	K_f/(K kg mol^{-1})	K_b/(K kg mol^{-1})
Acetic acid	3.90	3.07
Benzene	5.12	2.53
Camphor	40	
Carbon disulfide	3.8	2.37
Naphthalene	6.94	5.8
Phenol	7.27	3.04
Tetrachloromethane	30	4.95
Water	1.86	0.51

การเพิ่มขึ้นของจุดเดือด หาได้จาก $\Delta T_b = K_b b_B$ เมื่อ b_B = molality = mol solute / kg solvent

และการลดลงของจุดเยือกแข็ง $\Delta T_f = K_f b_B$

<u>แบบฝึกหัด</u> จงประมาณค่าการลดลงของจุดเยือกแข็งของสารละลายที่ได้จากการละลาย 3.0 กรัม (ประมาณ 1 ก้อน) ของน้ำตาลซูโครส ($C_{12}H_{22}O_{11}$) ในน้ำ 100 กรัม

ตอบ: 0.16 K

บทปฏิบัติการที่ 2 Impact of stabilizers on the freezing process, and physicochemical and organoleptic properties of coconut milk-based ice cream
ผลของสารเพิ่มความคงตัวต่อกระบวนการเยือกแข็ง สมบัติทางเคมีกายภาพและประสาทสัมผัสของไอศกรีมกะทิ

1. วัตถุประสงค์ของการทดลอง

2. inulin คือ ....

3. locus bean gum คือ ....

....

4. ในการทดลองนี้ ใช้ inulin ผสมด้วยความเข้มข้นสูงที่สุดคือ ร้อยละ .... โดยน้ำหนัก
และใช้ locus bean gum ผสมด้วยความเข้มข้นสูงที่สุดคือ ร้อยละ .... โดยน้ำหนัก

5. ให้ความหมายของคำต่อไปนี้
chemical composition ....
overrun ....
melting time ....
melting resistance ....
hardness ....

6. เมื่อเพิ่มความเข้มข้นของ inulin และ locus bean gum ส่งผลอย่างไรต่อ cryoscopic temperature และ melting time ของไอศกรีม

7. เมื่อเพิ่มความเข้มข้นของ inulin แต่ลดความเข้มข้นของ locus bean gum ส่งผลอย่างไรต่อไอศกรีม

8. สัดส่วนของสารเพิ่มความคงตัวเท่าใดที่ให้รสชาติเนื้อสัมผัสของไอศกรีมดีที่สุด

9. ไอศกรีม คือ

10. ข้อเสียของไอศกรีมที่ผลิตจากนมโค คือ

11. ข้อดีของไอศกรีมกะทิ คือ

12. ของผสมไอศกรีมก่อนเยือกแข็ง 1 ลิตร มีน้ำหนัก 1.08 กิโลกรัม หลังจากทำไอศกรีมแล้ว ไอศกรีม 1 ลิตร มีน้ำหนัก 0.97 กิโลกรัม จงคำนวณหา % overrun

13. สมบัติด้านประสาทสัมผัสของไอศกรีม วัดด้านใดบ้าง

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Impact of stabilizers on the freezing process, and physicochemical and organoleptic properties of coconut milk-based ice cream

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Locust bean gum (LBG)

ABSTRACT

The objective of the study was to characterize the effects of selected stabilizers on the freezing process and the physicochemical properties of coconut milk ice cream. Two stabilizers were used: inulin (0.8, 1.6, 2.4, 3.2 and 4 g/100 g of the mixture) and locust bean gum (LBG) (0.2, 0.4, 0.6 and 0.8 g/100 g of the mixture). Freezing process was performed in two stages. After the temperature of the ice cream mixtures reached −6 °C, they were hardened at −30 °C for 24 h. Then the samples were examined for their chemical composition, overrun, melting time and melting resistance, hardness and colour. The results showed that increasing concentration of inulin and LBG resulted in a decrease in the cryoscopic temperature and melting time of ice cream. On the other hand, ice cream produced from the mixtures containing higher inulin concentration and lower LBG concentration were characterized by higher overrun. Addition of inulin and LBG cause statistically significant changes in hardness of ice cream. The differences in colour of tested ice creams were shown. The highest scores in the sensory evaluation were obtained for ice cream with LBG and 0.8 g/100 g and 4 g/100 g of inulin.

1. Introduction

Consumer ice cream is one of the most popular desserts. The global consumption of this product is about 2 L per person per year. It is projected that the development of the industry and the increase in wealth of people will result in higher demand for ice cream (Agrawal, 2016). According to the definition of the European Ice Cream Association (Euroglaces, 2017), ice cream is a food product containing all food ingredients (amongst them all additives) authorized by the current provisions, which has structure and texture generated by freezing, and that is stored, transported, sold and consumed in the frozen state. Ice creams are physico-chemical complexes containing air bubbles which are dispersed in the continuous phase. This phase consists of the partially crystalline fat globules and casein micelles as discrete particles in a solution of sugars, insoluble mineral salts, dispersed whey protein, stabilizers, etc. The freezing process is a decisive factor affecting the properties of ice cream. There are two basic industrial methods of making ice cream: quiescent freezing, in which the liquid product is not stirred when freezing and active freezing which involves stirring (Clark, Jung, & Lamsal, 2014). The second method is more complicated but most often used. In this method freezing is carried out in two stages. The initial freezing of the mixture to −6 °C, until it achieves plasticity, is carried out in the freezers. The purpose of this stage of production is partial water crystallization. Approximately 55% of the freezable water is frozen. Such obtained type of ice cream is called soft serve. At this stage, the mixture (at about 4 °C) passes from the ageing tanks to overrun compartments. Freezers are most often built from a cylindrical drum surrounded by a cooling jacket through which the cooling agent flows. The resulting soft serve ice cream is subjected to a second freezing (hardening) step by reducing the temperature of the mixture from about −6 °C to −25 °C corresponding to 80–90% of the frozen water (Clark, 2012).

The main ingredient of commonly consumed ice cream is cow milk. Although it contains a number of substances beneficial to human body, such as proteins, milk fat, lactose, vitamins and mineral compounds, it is not recommended for some consumer groups e.g. people who are lactose intolerant (Lopata, 2010; Mattar, de Campos Mazo, & Carrilho, 2012; Misselwitz et al., 2013; Usai-Satta, Scarpa, Oppia, & Cabras,

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2012). Also, milk lipids after ingestion may increase blood cholesterol levels (Aboulfazli, Shori, & Baba, 2016). In addition, milk is known to be one of eight major human allergens and there is currently no effective way to completely reduce the allergy to cow's milk (Boye, Rajamohamed, & Britten, 2011; Lifschitz & Szajewska, 2015; Verhoeckx et al., 2015).

Substitutes of milk and its products make an alternative. The United Nations Food and Agriculture Organization (FAO) recommends substitutes for dairy products containing at least 3.5 g/100 g fat, 3.5 g/100 g protein and 5 g/100 g carbohydrates, which are all vegetable milk (Protein Advisory Group, 1972). Coconut milk is regarded a good choice as it is easily digested and a rich source of nutrients. Besides, it is abundant in calcium, phosphorus, potassium as well as C, E and B_6 vitamins. Notably, coconut milk contains proteins with large quantities of essential amino acids, lipids and antioxidants (Yuliana, Rangga, & Rakhmiati, 2010; Aboulfazli, Baba, & Misran, 2015; Fuangpaiboon & Kijroongrojana, 2015; Sridhar, Roopa, Varadaraj, & Vijayendra, 2015).

It is currently technologically possible to produce ice creams that are free of allergenic animal products and contain functional additives in their composition. Frozen desserts can serve as carriers for nutrients (Lin, 2012; Soukoulis, Fisk, & Bohn, 2014; Wichamanee, Acharaphan, & Santad, 2016) such as antioxidants and prebiotics (Aboulfazli et al., 2016; Soukoulis et al., 2014; Soukoulis, Lebesi, & Tzia, 2009).

The consumption of ice cream containing elderberry (*Sambucus nigra*) contributes to the deactivation of free radicals, vitamins and inhibits the lipid peroxidation processes. Elderberry is used in the prevention of cancer, vascular diseases and diabetes. It is characterized by antiviral and antimicrobial properties supporting the treatment of influenza and colds (Kong, 2009; Mikulic-Petkovsek, Ivancic, Schmitzer, Veberic, & Stampar, 2016; Sidor & Gramza-Michałowska, 2015). Other important ice cream ingredients treated as functional may be inulin and locust bean gum. Inulin is a polymer consisting of fructose molecules that naturally occurs in plants and certain microorganisms. It is often used as a fat substitute in food products. Thanks to its ability to bind water molecules, inulin plays an important role in stabilizing the texture of the product. It is also a nutritionally valuable prebiotic (Akbari, Eskandari, Niakosari, & Bedeltavana, 2016). Locust bean gum is a natural polysaccharide produced from carob tree seed. Like other polysaccharide hydrocolloids, it is a commonly used as stabilizing additive for ice creams due to its ability to thicken and gelate the product (Barak & Mudgil, 2014; Dłużewska, Gazda, & Leszczyński, 2003).

The objective of the work was to characterize the effects of selected stabilizers on the freezing process and the physicochemical properties of coconut milk ice cream.

2. Material and methods

2.1. Ingredients for ice cream production

The following ingredients were used for ice cream production: sterilized coconut milk (LIDL Stiftung, Co. KG. Germany), cane sugar (Sante, Poland), elderberry syrup (sugar, water, concentrated juice with elderberry fruits 19%) (Agros Nova Sp. Z o. o., Poland) and sunflower lecithin (Agnex, Poland). Composition of the mixture was modified by addition of different amounts of stabilizers (relative to the mass of the mixture): inulin (0.8; 1.6; 2.4; 3.2; 4 g/100 g) (Agnex, Poland) and LBG (0.2; 0.4; 0.6; 0.8 g/100 g) (Biozoon GmbH, Germany).

2.2. Preparation of ice cream

The ice cream mixture was prepared traditionally by weighing the components (81.3 g/100 g coconut milk, 8.1 g/100 g cane sugar, 10.2 g/100 g elderberry syrup, 0.4 g/100 g sunflower lecithin, inulin and LBG in above proportions), thorough blending and aeration. Simultaneously, a control sample (K) of ice cream without stabilizers was prepared.

2.3. Freezing process

Freezing was performed in an ice cream maker (Ariete Galatiera Compact, Italy) until the ice cream temperature was −6 °C. Temperature was recorded at every minute interval (twice) using a temperature mini data logger (Kimo KT 20, Germany). On the basis of the recorded data the freezing curves were plotted and the cryoscopic temperature was determined using Microsoft Office Excel 2007 (Rahman et al., 2002). Then the samples were placed in plastic containers and hardened at −30 °C for 24 h (chest deep freezer, Whirlpool, Poland).

2.4. Physiochemical analysis

Ice cream samples were stored at −30 °C for 7 d before physicochemical analyses. Dry matter of ice cream was estimated by drying the samples at 130 ± 1 °C for 3 h (AOAC, 2000). Fat content was determined according to AOAC (2000) using Soxhlet extractor, (Tecator Soxtec System HT 1043 extraction unit, Gemini, Apeldoorn) and protein content was assayed according to Kjeldahl method (AOAC, 2000). Ash content was analyzed according to PN-67/A-86430/Az2 (2002). Carbohydrates were calculated as the difference between 100% and the sum of the percentages of water, protein, total lipid (fat) and ash (US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory, 2016). All measurements were carried out in triplicate.

2.5. Determination of melting time, melting resistance and melting rate

In order to determine the melting time, a cooled metal ring in a form of a cone (stored at −30 °C for 24 h; 35 mm in height, 35 mm in upper diameter, 25 mm in lower diameter, volume – 30 mL) was driven into a sample. In the following step, the ice cream samples were removed from a ring, transferred on a funnel with a mesh and then placed in a controlled temperature chamber at 25 °C. The time required for the dripping of the first drop of melted ice cream was recorded (Jasińska, Trzciński, Dmytrów, & Mituniewicz-Małek, 2012).

Melting resistance was determined according to the methodology employed by Silva Junior and Lannes (2011) modifying the sample size and melting time. It was determined by measuring the dripped volume for 45 min every 5 min since the samples were placed in a controlled temperature chamber. The data recorded were used to determine the melting rate (mL/min).

2.6. Ice cream overrun

The overrun of the final product was determined according to the following formula (1) (Ismail, Al-Saleh, & Metwalli, 2013):

$$Overrun = \frac{W_1 - W_2}{W_2} \cdot 100\% \qquad (1)$$

where: W_1 – mass of unit volume of mixture (g), W_2 – mass of unit volume of ice cream (g).

2.7. Texture measurements

The evaluation of textural properties of ice cream: hardness and adhesiveness was performed in LFRA texture analyzer (Brookfield Engineering Laboratories, Inc., Middleboro, USA) using a penetration test and a chamber for backward extrusion. The chamber was filled with ice cream at temperature of −6 °C. To ensure a constant temperature during measurement, a specially designed insulated container filled with ice was used. It was adapted to a Brookfield texture analyzer, which provided stabilization of thermal conditions. Temperature control during texture measurement was carried out using a 16-channel MPI-L digital temperature scanner (Metronic, Poland).

Hardness was defined as a maximal force (N) required to push a probe into the sample on a half of its height. Adhesiveness was determined on the basis of a force recorded during withdrawal of a probe from the sample. The following parameters were assumed for analysis: probe diameter – 12 mm, chamber diameter – 30 mm, probe rate during penetration – 0.2 mm/s, and pressure force – 0.2 N (Tiwari, Sharma, Kumar, & Kaur, 2015).

2.8. Colour determination

Colour of ice cream was determined using a colorimeter LOVIBOND CAM-SYSTEM 500 (The Tintometer Ltd., Amesbury, UK) and expressed in CIE – L^*a^*b system where L^* – lightness of ice cream (1–100), a^* – redness (+100) and greenness (−100), b^* – yellowness (+100) and blueness (−100). The measurements were performed in triplicate. The total colour difference (ΔE) was calculated using the equation below (2):

$$\Delta E = \sqrt{(L^*_{sample} - L^*_{control})^2 + (a^*_{sample} - a^*_{control})^2 + (b^*_{sample} - b^*_{control})^2} \quad (2)$$

The values for background colours were: $L^* = 91.8$; $a^* = 0.4$; $b^* = -0.4$ (Tiwari et al., 2015).

2.9. Organoleptic evaluation

The organoleptic analysis of ice cream was carried out in laboratory conditions. The test participants were selected after previous pre-selection. Initially 30 people were subjected to the triangle test. The top 15 people were selected for the subsequent organoleptic test. The evaluation was carried out using the 5-point scale test. Distinctions of quality (colour, taste, smell, consistency) are presented in Table 1.

The number of points obtained in the sensory evaluation was analyzed and the level of ice cream quality was expressed in the following way: 5 – very good quality, 4 – good quality, 3- sufficient quality, 2 – insufficient quality, 1 – bad quality.

2.10. Statistical analysis

All measured values were treated with Dean-Dixon test for identification and rejection of outliers. Values indicating gross error were rejected from further analyzes (Rorabacher, 1991). The test of Shapiro-Wilk was applied. In order to determine the effect of the addition of the stabilizer and its percentage concentration, the two-factor ANOVA test was used in a complete randomisation system. Further tests to detect the significance of differences between particular groups were found using the Student *t*-test applied to compare independent samples in pairs, and variance analysis (ANOVA) was used for more than two groups. Detailed analysis was based on Tukey's confidence intervals. The Pearson's correlation coefficients were used to evaluate linear relationships between variables. All statistical tests were carried out at significance level of $\alpha = 0.05$. Statistical processing of results was performed using R program version 3.1.2 and Statgraphics 5.0.

Table 2
Cryoscopic temperature of coconut milk-based ice cream with inulin and locust bean gum (LBG) addition.

Sample	Cryoscopic temperature T_{cr} (°C)
Control (K)	−2.5
Inulin 0.8 g/100 g	−2.5
Inulin 1.6 g/100 g	−2.7
Inulin 2.4 g/100 g	−2.9
Inulin 3.2 g/100 g	−2.9
Inulin 4.0 g/100 g	−3.0
LBG 0.2 g/100 g	−2.7
LBG 0.4 g/100 g	−2.9
LBG 0.6 g/100 g	−2.9
LBG 0.8 g/100 g	−3.0

3. Results and discussion

3.1. Physiochemical characteristics of ice cream

One of the most important physical properties of food is the cryoscopic temperature. Because of its multidimensional importance it is extremely important to determine its value properly (Bogh-Sorensen, 2006; Kozłowicz & Kluza, 2012). The addition of both stabilizers to ice cream caused a variation in the cryoscopic temperature (Table 2). The values of T_{cr} determined based on analysis of the freezing curves were: −2.5 °C for the control (K) and −2.5 °C to −3.0 °C, and −2.7 °C to −3.0 °C, respectively, for ice cream with inulin and LBG. In this study a correlation between the amount of added stabilizer and the cryoscopic temperature of the ice cream was observed. The relationship between the amount of added stabilizer and the cryoscopic temperature was noted and then confirmed by Pearson's correlation coefficient. Its values for relationships between inulin content and T_{cr} and between LBG content and T_{cr} were, respectively, −0.948 and −0.923, indicating a strong negative correlation. Increasing contribution of the stabilizer corresponded to a drop in the cryoscopic temperature of ice cream. Muse and Hartel (2004), who studied ice creams produced with three types of sweetener and three different levels of emulsifier, noted cryoscopic temperatures ranging from −1.8 °C to −4.6 °C, whereas Alvarez, Wolters, Vodovotz, and Ji (2005) reported that T_{cr} ranged from −4.68 °C to −5.42 °C. According to the above authors, the resulting differences in the cryoscopic temperature did not affect the sensory evaluation of ice cream. The values of T_{cr} reported by Soukoulis et al. (2009) were −3.11 °C for ice cream containing 2 g/100 g of inulin and −1.78 °C for the samples with addition of 4 g/100 g of stabilizer. On the other hand, Aboulfazli et al. (2015) noted the cryoscopic temperature of −4.9 °C for coconut milk ice cream. The T_{cr} values recorded in this

Table 1
Quality indicators and discriminants for sensory evaluation of ice cream.

Point scale	Quality discriminants with quality indicators			
	Consistency (0.3)	Taste (0.15)	Colour (0.15)	Smell (0.25)
5	Uniform, dense, creamy, ice crystals undetectable	Characteristic for the type of milk and additives used, intensive	Characteristic for the type of milk and additives used, intensive	Clear, aromatic, characteristic for ice cream of the chosen taste
4	Uniform, ice crystals barely detectable	Characteristic for the type of milk and additives used, less intensive	Characteristic for the type of milk and additives used, less intensive	Clear, aromatic, less adequate for ice cream of the chosen taste
3	Uniform, ice crystals detectable	Indistinct	Colour slightly changed	Indistinct, barely adequate for ice cream of the chosen taste
2	Uniform, large ice crystals visible and detectable	Hard to recognise	Colour clearly changed	Clearly changed
1	Uniform, large ice crystals visible and detectable	Unrecognizable	Unusual colour	Unusual, foreign

Table 3
Chemical composition (mean ± standard deviation, n = 3) of ice cream with inulin and locust bean gum (LBG) in the 100 g of final product.

Sample	Fat [g/100 g]	Protein [g/100 g]	Ash [g/100 g]	Dry matter [g/100 g]	Carbohydrates [g/100 g]
	$\bar{x}$ ± SD				
Control (K)	20.78 ± 0.74[a]	2.20 ± 0.002[ab]	0.72 ± 0.005[e]	42.60 ± 0.03[a]	18.90 ± 0.72[a]
Inulin 0.8 g/100 g	20.83 ± 0.79[a]	2.23 ± 0.02[ab]	0.64 ± 0.002[bc]	43.72 ± 0.11[cd]	20.02 ± 0.78[ab]
Inulin 1.6 g/100 g	19.93 ± 0.58[a]	2.27 ± 0.002[ab]	0.63 ± 0.003[ab]	44.01 ± 0.01[d]	21.17 ± 0.58[ad]
Inulin 2.4 g/100 g	19.44 ± 0.54[a]	2.24 ± 0.002[ab]	0.71 ± 0.008[e]	45.27 ± 0.15[f]	22.88 ± 0.56[bd]
Inulin 3.2 g/100 g	20.03 ± 1.09[a]	2.19 ± 0.005[ab]	0.63 ± 0.003[ab]	46.29 ± 0.10[g]	23.44 ± 1.11[cd]
Inulin 4.0 g/100 g	19.91 ± 0.67[a]	2.23 ± 0.002[ab]	0.77 ± 0.006[f]	46.89 ± 0.16[h]	23.99 ± 0.73[d]
LBG 0.2 g/100 g	20.05 ± 0.14[a]	2.19 ± 0.002[ab]	0.64 ± 0.007[d]	43.02 ± 0.08[b]	20.14 ± 0.08[ab]
LBG 0.4 g/100 g	20.36 ± 0.32[a]	2.42 ± 0.02[b]	0.67 ± 0.002[bc]	43.57 ± 0.08[c]	20.11 ± 0.30[ab]
LBG 0.6 g/100 g	20.31 ± 0.24[a]	2.39 ± 0.22[b]	0.62 ± 0.002[a]	44.00 ± 0.06[d]	20.68 ± 0.45[abc]
LBG 0.8 g/100 g	21.93 ± 1.74[a]	2.10 ± 0.003[a]	0.65 ± 0.004[c]	44.58 ± 0.08[e]	19.90 ± 1.66[a]

[a,b,c,d,e,f,g,h] Means in the same column denoted by different letters were significantly different (P value < 0.05).

study did not differ significantly from those reported by other authors including values obtained for ice cream produced from traditional milk (Alvarez et al., 2005; Muse & Hartel, 2004; Soukoulis et al., 2009). Although Aboulfazli et al. (2015) obtained T_{cr} lower by 2.4 °C as compared to the control sample in this study.

The composition of ice cream is presented in Table 3. As expected, the addition of inulin and LBG increased the dry matter content. It ranged from 42.6 g/100 g to 46.89 g/100 g. It was found that the addition of inulin (0.8 g/100 g) influenced this parameter to a lesser extent than the addition of the same amount of LBG. The highest content of dry matter was found in ice cream containing 4.0 g of inulin/100 g. The high proportion of fat in ice cream was due to the specificity of ingredients that were used to make ice cream e.g. coconut milk contained 21 g of fat in 100 mL of the product. However, significant changes in the content of carbohydrates in ice cream were associated with the increasing content of dry matter.

Based on the results of the ANOVA test, it was found that the type of stabilizer and its percentage share significantly differentiate the individual chemical parameters of the ice cream. The type of stabilizer used had the greatest influence on the ash content (F = 235 at Pr = 9.06 × 10–9) and the dry matter (F = 319 at Pr = 1.78 × 10–9). Only in the case of protein, the type of stabilizer had no effect on its content in ice cream (F = 0.14 at Pr = 0.72).

Melting is an important factor in assessing quality of ice cream, correctness of selected technology and freezing parameters. Melting time is determined by a number of factors such as: total solids, size of the ice crystals, size of the fat particles and their amount. Its value depends to a large extent on the composition of the mixture (Muse & Hartel, 2004). The shortest melting time −16 min, was recorded for the sample with 4 g/100 g inulin, whereas the longest – 25 min for the control sample without stabilizers (Table 4). A strong negative correlation between inulin concentration and melting time (Pearson's correlation coefficient was −0.89) was observed. The increased inulin content from 0 g/100 g–4 g/100 g was accompanied by reduced melting time. The highest melting resistance (22.5 mL), after 45 min, was observed for the sample with 4 g/100 g addition of inulin. However, there were no significant differences in this parameter between the control sample and samples with 1.6 g/100 g and 3.2 g/100 g addition of inulin. Similar results were reported by Isik, Boyacioglu, Capanoglu, and Erdil (2011). Appearance of the first drop in the control sample (without the addition of stabilizers) was observed after 13.5 min, whereas in the samples with 8 g/100 g, 6.5 g/100 g and 5 g/100 g of inulin, respectively, after 11.5 min, 10.8 min, and 8.5 min. The increase of inulin content in ice cream reduced the time of first drop, but increased melting resistance (Akalın & Erişir, 2008). Ice cream with LBG did not melt. This may be due to the high content of this stabilizer in the mixture in combination with coconut milk which is rich in polysaccharides. LBG forms a gel-like net inside the ice cream structure. It is recommended to add from 0.1 g/100 g–0.5 g/100 g of LBG to traditional ice creams containing animal protein (Cropper, Kocaoglu-Vurma, Tharp, & Harper, 2013).

The ice cream overrun ranged from 8.76% to 15.31% (Table 5). The type of stabilizer (F 9548.0, Pr < 2.2 × 10–16) and its percentage share (F = 8658.3, Pr < 2.2 × 10–16) significantly differentiated the ice cream overrun. It was found that value of this parameter increased with increasing inulin content and decreased with increasing content of LBG. However, statistically significant differences were not found in the ice cream with inulin in the range of concentration from 1.6 g/100 g–4.0 g/100 g and LBG from 0.2 g/100 g–0.6 g/100 g. The lowest overrun was noted for the control (K) with no stabilizer added (8.76%). Akalın and Erişir (2008) also observed that the aeration level of ice cream with addition of 4 g/100 g of inulin was higher than for the control sample. The difference between results obtained for these samples was 53%, whereas in this study it was 43%. Tiwari et al. (2015)

Table 4
Melting time, rate and resistance (mean ± standard deviation, n = 3) of ice cream with inulin and locust bean gum (LBG).

Sample	Melting time	Melting rate (ml/min)					Melting resistance (ml)
	First drop (min)	After 25 min	After 30 min	After 35 min	After 40 min	After 45 min	After 45 min
	$\bar{x}$ ± SD						
Control (K)	25.00 ± 0.05[d]	0.04 ± 0.01[a]	0.17 ± 0.05[a]	0.31 ± 0.05[b]	0.40 ± 0.05[a]	0.48 ± 0.05[b]	21.0 ± 0.05[b]
Inulin 0.8 g/100 g	20.00 ± 0.05[c]	0.04 ± 0.01[a]	0.22 ± 0.05[c]	0.29 ± 0.05[a]	0.40 ± 0.05[a]	0.44 ± 0.05[a]	20.0 ± 0.05[a]
Inulin 1.6 g/100 g	20.00 ± 0.05[c]	0.06 ± 0.01[b]	0.17 ± 0.05[a]	0.36 ± 0.05[d]	0.41 ± 0.05[b]	0.48 ± 0.05[b]	21.0 ± 0.05[b]
Inulin 2.4 g/100 g	19.00 ± 0.05[b]	0.12 ± 0.01[c]	0.23 ± 0.05[d]	0.34 ± 0.05[c]	0.43 ± 0.05[c]	0.48 ± 0.05[b]	21.5 ± 0.05[c]
Inulin 3.2 g/100 g	19.00 ± 0.05[b]	0.12 ± 0.01[c]	0.20 ± 0.05[b]	0.36 ± 0.05[d]	0.41 ± 0.05[b]	0.44 ± 0.05[a]	21.0 ± 0.05[b]
Inulin 4.0 g/100 g	16.00 ± 0.05[a]	0.16 ± 0.01[d]	0.27 ± 0.05[e]	0.37 ± 0.05[e]	0.45 ± 0.05[d]	0.50 ± 0.05[c]	22.5 ± 0.05[d]

[a,b,c,d] Means in the same column denoted by different letters were significantly different (P value < 0.05).

Table 5
Hardness, adhesiveness and overrun (mean ± standard deviation, n = 3) of ice cream with inulin and locust bean gum (LBG).

Sample	Hardness (N)	Adhesiveness (Ns)	Overrun (%)
	$\bar{x}$ ± SD		
Control (K)	2.41 ± 0.22[a]	−1.69 ± 0.17[bc]	8.76 ± 0.32[a]
Inulin 0.8 g/100 g	2.60 ± 0.37[a]	−2.22 ± 0.23[ab]	10.92 ± 1.35[b]
Inulin 1.6 g/100 g	2.65 ± 0.65[a]	−1.60 ± 0.20[c]	13.66 ± 0.16[de]
Inulin 2.4 g/100 g	2.52 ± 0.69[a]	−1.36 ± 0.13[c]	13.88 ± 0.15[de]
Inulin 3.2 g/100 g	2.44 ± 0.43[a]	−1.80 ± 0.16[bc]	14.45 ± 0.30[de]
Inulin 4.0 g/100 g	2.92 ± 0.57[ab]	−2.18 ± 0.22[ab]	15.31 ± 0.56[e]
LBG 0.2 g/100 g	4.21 ± 0.72[bc]	−1.52 ± 0.10[c]	13.51 ± 0.42[ce]
LBG 0.4 g/100 g	4.58 ± 0.11[c]	−1.50 ± 0.20[c]	13.07 ± 0.17[cd]
LBG 0.6 g/100 g	4.15 ± 0.69[bc]	−2.44 ± 0.49[a]	11.64 ± 0.69[bc]
LBG 0.8 g/100 g	4.71 ± 1.14[c]	−1.43 ± 0.27[c]	9.96 ± 0.2[ab]

[a,b,c,d,e] Means in the same column denoted by different letters were significantly different (P value < 0.05).

Table 6
Colour values (mean ± standard deviation, n = 10) of ice cream with inulin and locust bean gum (LBG) (in fixed concentrations) after 48 h storage at −30 °C.

Sample	L^*	a^*	b^*	ΔE
	$\bar{x}$ ± SD			
Control (K)	69.40 ± 0.48[b]	−3.39 ± 0.28[a]	13.59 ± 0.67[a]	–
Inulin 0.8 g/100 g	68.63 ± 0.52[a]	−3.63 ± 0.30[a]	13.70 ± 0.46[ac]	0.81[c]
Inulin 1.6 g/100 g	68.67 ± 0.58[a]	−3.50 ± 0.56[a]	13.57 ± 0.55[ae]	0.74[b]
Inulin 2.4 g/100 g	68.40 ± 0.33[a]	−3.50 ± 0.36[a]	13.70 ± 0.46[a]	1.01[d]
Inulin 3.2 g/100 g	68.40 ± 0.31[a]	−3.63 ± 0.30[a]	14.23 ± 0.38[bf]	1.22[e]
Inulin 4.0 g/100 g	68.13 ± 0.27[a]	−3.63 ± 0.30[a]	14.77 ± 0.38[bdg]	1.75[g]
LBG 0.2 g/100 g	67.77 ± 0.24[c]	−4.43 ± 0.30[b]	14.78 ± 0.38[bc]	2.27[h]
LBG 0.4 g/100 g	69.87 ± 0.27[bd]	−3.50 ± 0.36[ac]	12.68 ± 0.55[adf]	1.02[d]
LBG 0.6 g/100 g	68.93 ± 0.27[bde]	−4.03 ± 0.38[b]	15.03 ± 0.38[bgh]	1.65[f]
LBG 0.8 g/100 g	69.40 ± 0.80[bdf]	−4.03 ± 0.38[b]	13.58 ± 0.83[aei]	0.65 [a]

L^* - lightness, a^* - redness and greenness, b^* - yellowness and blueness.
[a,b,c,d,e,f,g,h] Means in the same column denoted by different letters were significantly different (P value < 0.05).

did not observe any change in the level of ice cream aeration in relation to the inulin content, while Mahdian and Karazhian (2013) reported its decrease by 33% (2 g/100 g inulin) and 16% (4 g/100 g inulin) in comparison to the control. Dłużewska et al. (2003) observed that the overrun of ice cream was inversely proportional to the LBG content in the mixture. In this study we obtained much lower values of this parameter than those presented in the available literature. Ice cream overrun depends to a great extent on the protein content in the mixture (Mahdian & Karazhian, 2013), so the low aeration of the mixture observed in this study was probably due to a total absence of animal protein and low protein content in coconut milk (2.2 g).

Hardness of ice cream is affected by many factors, including the overrun, ice crystal size, ice phase volume, and extent of fat destabilization. A product that contains more large ice crystals is characterized by higher hardness. Ice creams with low hardness (so-called creamy ice cream) contain small ice crystals (Muse & Hartel, 2004). The lowest hardness of 2.41 N was determined for the control ice cream. Addition of both stabilizers to the mixtures did significantly affect hardness of ice cream (two-factor ANOVA test – F = 6024.24; Pr < 2.2×10^{-16}). Ice cream with LBG had higher hardness (4.15 N - 4.71 N) than the samples

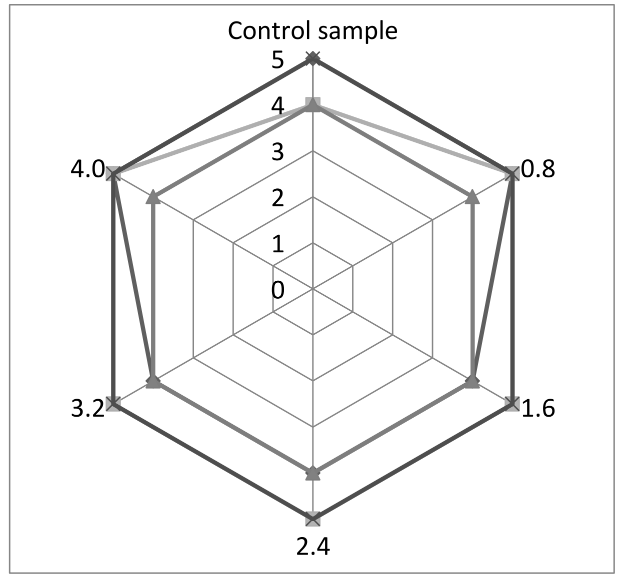

Fig. 1. Results of sensory evaluation of ice cream with inulin: (—) consistency, (—) taste, (—) colour, (—) smell.

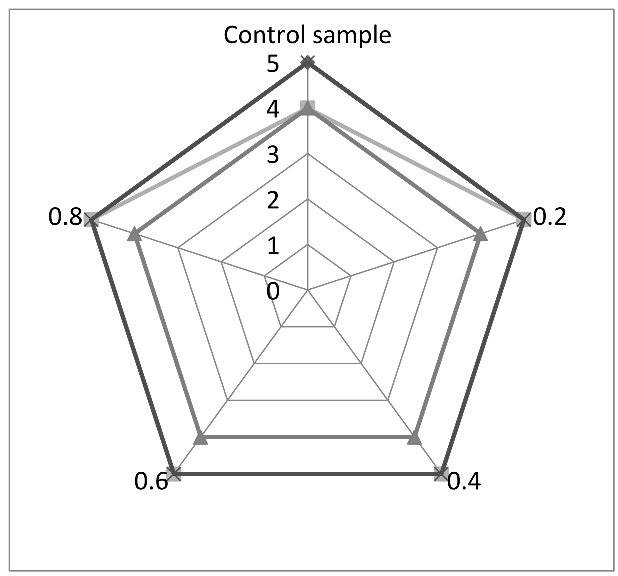

Fig. 2. Results of sensory evaluation of ice cream with LBG: (—) consistency, (—) taste, (—) colour, (—) smell.

with inulin (2.44 N- 2.92 N) (Table 5). There were slight differences in sample adhesion depending on the type of stabilizer used (in the two-factor ANOVA test - F = 16.88, Pr = 0.002) (Table 5). The lowest value of this parameter was found for ice cream with addition of 0.6 g/100 g LBG and 0.8 g/100 g inulin. The remaining samples were characterized by no statistically significant differences. Also, Akbari et al. (2016) did not observe statistically significant differences in hardness and adhesion of ice cream with 2, 3 and 4 g/100 g addition of inulin.

Colour of ice cream is one the most important characteristics that affect customer choice. Its values and total difference (ΔE) in the examined ice cream are summarized in Table 6.

The stabilizer concentration had an effect on the L * parameter defining brightness (F = 5.30, Pr = 0.0007). The values of this parameter in ice cream with inulin (ranged from 68.13 to 68.67) were significantly lower than in the control sample (69.40). This means that the addition of inulin led to darkening of ice cream. The colour parameter a^* ranged from −3.50 to −3.63, whereas the b^* value was in the range from 13.57 to 14.77, and the lowest value was obtained for the sample with 2.4 g/100 g of inulin, and the highest – for the one with 4 g/100 g inulin. The total colour difference ranged from 0.7 to 1.75. In the case of ice cream with LBG, the L^* value was the lowest for the sample with 0.6 g/100 g LBG and the highest – for the one with 0.2 g/100 g LBG. The control sample had the lowest a^* value, whereas the b^* value was in the range from 12.78 (0.4 g/100 g LBG) to 14.98 (0.6 g/100 g LBG). The statistically significant differences in ΔE were observed for ice cream containing both stabilizers (two factor ANOVA test – $F = 2980.3$; $Pr < 2.2 \times 10^{-16}$). According to the criteria adopted by the International Lighting Commission CIE (Heidelberg, 1999) absolute colour differences (ΔE) in the range from 0 to 2 are unrecognizable by the person experienced in distinguishing the colour. However, the observed differences in ice cream colour were lower than those found in the literature. Tiwari et al. (2015) obtained colour differences ranging from 3.6 to 7 in ice cream with variable inulin additions.

3.2. Organoleptic evaluation

Organoleptic assessment of food consists in determining its quality using the senses of smell, sight, taste and touch. This type of analysis is used primarily to assess non-measurable features and is conditioned by factors influencing consumers' perception and beliefs. Subjective organoleptic assessment depends primarily on the sensitivity of the evaluator and the conditions under which it is carried out (King & Arents, 1997). In the organoleptic assessment of the ice cream, such quality factors as consistency, taste, colour and smell were used. The control sample of ice cream was characterized by not too sweet taste and a uniform, not very soft consistency. In the testers' opinion, the addition of inulin and locust bean flour (in the whole range of shares used) caused that the ice cream had a better taste and a more creamy consistency, and they were rated higher by the consumers (Fig. 1, Fig. 2). Fuangpaiboon and Kijroongrojana (2015) reported that the addition of inulin to coconut ice cream did not alter the taste of ice cream compared to the control sample. They noticed, however, that the stabilizer addition had an effect on the sweetness of the ice cream and caused a noticeable change in their texture.

4. Conclusion

The ice cream obtained according to the recipe based on coconut milk with healthy additives and the chosen freezing technology, were characterized by physical characteristics similar to those of traditional products. Addition of the stabilizer influenced the cryoscopic temperature of ice cream. Both supplementation of the mixture with inulin and LBG resulted in a drop in the cryoscopic temperature of 0.5 °C.

Analysis of the chemical composition of the ice cream showed that the addition of inulin had a smaller effect on the increase in dry matter than the addition of the same amount of LBG. Ice-cream with coconut milk had high fat content, which resulted from the use of high-fat ingredients to make the mixture. The addition of stabilizers resulted in slight changes in the content of dry matter, ash, water and carbohydrates. The next parameters of the ice cream that are important for their quality are the melting time and the time of the first drop. The increased inulin content in the mixture reduced the time of the first drop during melting, but increased melting resistance of ice cream after 45 min. Ice cream with LBG did not melt, which was most probably due to too high concentration of stabilizer combined with polysaccharide-rich coconut milk.

Hardness, aeration, adhesiveness and colour are physical parameters that can be assessed instrumentally and which can also significantly affect the quality of ice cream. The addition of stabilizers influenced the hardness and aeration of ice cream and resulted in slight differences in their adhesiveness. The low overrun of the samples (from 8.76% to 15.31%) has been demonstrated, most probably due to the lack of animal products. Ice cream with LBG had higher hardness than the samples with inulin. Differences in the colour of individual ice cream samples were statistically significant. The effect of the stabilizer type on the brightness of the ice was found. The addition of inulin led to darkening of the ice cream.

The statistically significant differences in ΔE were observed for ice cream containing both stabilizers. The observed differences in ice cream colour were lower than those found in the literature.

Non-measurable ice cream features were evaluated using organoleptic evaluation. The addition of stabilizers, regardless of their participation, caused the higher rating of ice cream by the testers. According to the testers they had a better taste and a more creamy consistency. This confirms the legitimacy of using stabilizers in the production of coconut milk ice cream. To summarize, it has been found that it is possible to obtain ice creams that do not contain animal products in their composition using a freezing technology similar to that used for traditional ice cream.

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