Research article

Analysis of the selected antioxidant compounds in ice cream supplemented with Spirulina (*Arthrospira platensis*) extract

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Abstract: *The research is aimed at evidencing that ice-cream formulations incorporating algae can have health-benefiting effects on human body. The main task of the project is to design ice-cream product line that distinguish itself from regular ice-cream by increased anti-oxidant activity resulted from inclusion of the algae extract. The currently known research evidences that ice-cream can be effective as carriers of health-promoting probiotic bacteria, which in turn encourages also application of other microorganisms in particular algae of specific strains (e.g. Spirulina platensis) as a supplement to ice-cream. In submitted research, the level of polyphenols and antioxidant activity expressed as degree of inhibiting generation of cationo-free radical from DPPH•$^{+}$ solution were analyzed. Preliminary results based on antioxidative activity tests measured with potential to quench free radicals have shown that ice-cream formulations enriched with algae extract exhibit significantly higher potential achieving inhibition level of 39.7% in the mint ice cream samples as compared to 32.8% inhibition for the control sample without algae. Furthermore, each of the examined samples (dairy, pistachio, mint) ice creams versions supplemented with Spirulina were characterized by enhanced antioxidant activities expressed as potential to quench free radicals and the carotenoids content.*

Keywords: *ice creams, Spirulina, antioxidant.s.*

Introduction

Diet is an important determinant of health, physical well-being and well-being, so in the last decade more and more attention is paid to functional food production. Among such foods are milk and milk food products containing cultures of probiotic bacteria or enriched with ingredients that significantly increase the nutritional properties of the final product [1, 2, 3]. Ice cream is a milk product that can be a carrier of probiotic bacteria from the genus *Lactobacillus* and *Bifidobacterium* [4, 5]. It is important to maintain the rheological and physicochemical parameters of the ice cream. Another attempts to produce functional ice cream rely on zinc fortification [6] or substitution of sugars with low-caloric compounds like, orange fibre peeling [7, 8] or addition of honey [9]. Interesting were research concerning ice cream prepared on the basis of goat's milk, a raw material with excellent nutritional properties particularly fatty acid profile and mineral content are promising [10]. Addition of Amla (Indian gooseberry) [11], fermented acerola (*Malpighia emarginata DC*.) [12] or encapsulated carotenoids [13] were tested to increase antioxidant components such as vitamin C, provitamin A and polyphenols.

Algae can be used to improve the nutritional value of animal feed and food due to their chemical composition [14]. They play a key role in fish aquaculture as an ingredient in feeds and can be used as a supplement in the production of cosmetics. *Arthrospira platensis* – commercial name *"*Spirulina*"* – is a phototrophic, filamentous cyanobacteria, considered as an potential food additive, among others, due to its high protein (65-70% of dry matter), vitamins and minerals content [15, 16, 17]. It is because of high protein levels, Spirulina cells also contain significant amounts of phycocyanin, an antioxidant that is used as an ingredient in a variety of products developed in the cosmetic and pharmaceutical industries [18]. Until recently *Spirulina platensis* had been considered mostly only as an effective mean for alleviating malnutrition in children, whereas currently the research indicated that bio-fortification with algae can result in numerous health-benefiting effects like modification of the immune system [14].

Ice creams consumption in Poland continues to be characterized by seasonality, and in this situation buyers of dairy products pay particular attention to the nutritional and health value of ice cream [19]. As in the case of probiotic ice cream, for which the consumer would be willing to pay a slightly higher price, product innovation in the form of ice creams with microalgae could significantly increase the attractiveness of the small producers of the ice cream.
The purpose of this work was therefore to analyze selected parameters of ice cream with particular emphasis on their antioxidative potential. It is important to note that an crucial factor is the optimization of the level of *Spirulina* extract addition, which has a significant impact on the visual and sensory acceptance of the final product [20].

Experimental

Materials

Ice cream were prepared at the Vito Ice Factory (Biecka 17, 38-300 Gorlice) using traditional technology with the addition of the pistachio and mint paste without chemical stabilizers on the machines from Carpigiani (Anzola dell'Emilia, Italy). Below is a description of the fresh algae paste (*Spirulina* *platensis*) produced by a closed bioreactor system (EnerGaia Company Ltd, Bangkok, Thailand) which was introduced to the ice mass. Dosing of paste – 5% by weight in relation to the milk ingredient. After production process the samples were placed into 5 l plastic cuvettes and covered using the lids before being frozen at − 40 °C for hardening and storage up to 2 months.

Basic composition of the fresh algae paste (*Spirulina platensis*) according to the manufacturer data (EnerGaia Company Ltd):
Protein 18,18 g/100 g
Sugar 7.79 g/100 g
Lipids 1.46 g/100 g
Minerals (ash) 2.28 g/100 g
Moisture 70,29 g/100 g.

Table 1. Basic chemical parameters of the ice cream

Ice cream	Dairy	Mint	Pistachio
Fat (%)	7.0	7.5	9.6
Sugar (%)	19.4 (18.9*)	22.4 (21.3*)	21 (19.5*)
Protein (%)	3,9	3.5	4.26
Caloric value (kcal/100 g)	176.2	192.3	210.4

The fat content in the samples of ice cream were determined by the Gerber method and protein content using the Kjeldahl method. Sugar were analyzed by the colorimetric method based on reaction of dinitrosalicylic acid on reducing sugars and HPLC* technique.

Methods

Determination of the total phenolic content and antiradical activity of samples

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method with modification [21]. 10 g of samples were extracted with 90 mL 80% methanol for 3 hour on a stirrer in 20°C and left for 12h in dark for effective extraction. Next day samples were centrifuged for 10 min at 14000 rpm and supernatants just before analysis were filtered through 0.45 µm nylon syringe filter. 0.2 mL of such extract was added to the 10 mL tube containing 1.8 mL of distilled water. After added 1 mL Folin-Ciocalteu's reagent (Sigma-Aldrich, USA) and 2 mL 25% Na_2CO_3 tubes were mixed and incubated at 20 °C in dark for 20 minutes. After then the absorbance at 760 nm (Spekol® 1500, AnalytikJena, Germany) was measured. All samples were analyzed three times. Gallic acid (0-100 mg/L) was used as the reference standard, and the results were expressed as mg gallic acid equivalent per g sample (mg GAE/g).

The antioxidant activity of the samples was measured by a method with the synthetic free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) [22]. For this purpose, 0.1 mL of sample extract was added to 3.9 ml of freshly prepared 0.1 mM DPPH radical solution in 80% methanol and stirred. Samples were placed in dark place at 20°C for 30 minutes, after which the absorbance at 517 nm was measured using a Spekol® 1500 spectrophotometer (AnalytikJena, Germany). All samples were analyzed three times. The antiradical activity was calculated as follows: % inhibition = ($Abs_{control} - Abs_{sample} / Abs_{control}$) x 100.

Analysis of the carotenoids

Carotenoids were measured after extraction with acetone/ethanol (1:1, v/v, with 200 mg/L of butylated hydroxytoluene) by means of HPLC performed according to the method of Singgih et al. [23], with slight modifications. Carotenoid identification was carried out on a Gemini C8 phenyl column (250×4.6 mm, 3 µm) (Phenomenex, Torrance, CA, U.S.A.) with acetonitrile/methanol/2-propanol (85:10:5) as the mobile phase for isocratic elution. The detection was performed with a UV/visible detector at 450 nm and fluorimetric detector with excitation wavelength 445 nm and emission wavelength 530 nm. Identification of the carotenoid compound was compared with beta-carotene standard.

Statistical analysis

One-way analysis of variance followed by LSD test were performed using Statistica software (ver. 12.5). P-values ≤ 0.05 were regarded significant. Pearson's correlation coefficients were calculated based on multiply regression.

Results and Discussion

Bioactive parameters

Parameters that are determined in ice creams were the sum of polyphenols and the antioxidant activity expressed by the degree of inhibition for the formation of the cation free radical from DPPH solution were presented on the figures 1-2. These tests are commonly used to assess the antioxidant properties of a wide range of food products.

In tests for antioxidant activity measured by free radical scavenging potential, in each of the analyzed flavours of ice cream, a variant enriched with microalgae extract was found to have a significantly greater antioxidant potential. The lowest parameters were determined for dairy ice cream, although the addition of *Spirulina* increased the ability for quenching free radicals nearly by 8% (Fig. 2).

The same observations were made with pistachio ice cream and their supplemented version, where the level of attenuation increased by 9% as compared to the control sample (11.6% inhibition). The highest potential for the removal of free radicals was denoted for mint ice cream, which was significantly higher in both the conventional version and the one enriched with microalgae extract: 32.8% and 39.7% inhibition respectively, where the last one – was the most effective ice cream between all those tested in the range of antioxidant potential. At the same time, in the context of future consumer testing, the green colour of ice cream shows only minor differences in comparison to the starting raw material. The relatively low antioxidant potential of dairy and pistachio ice cream in relation to mint ice cream may be the effect of the initial concentration of the antioxidant compounds in these ingredients.

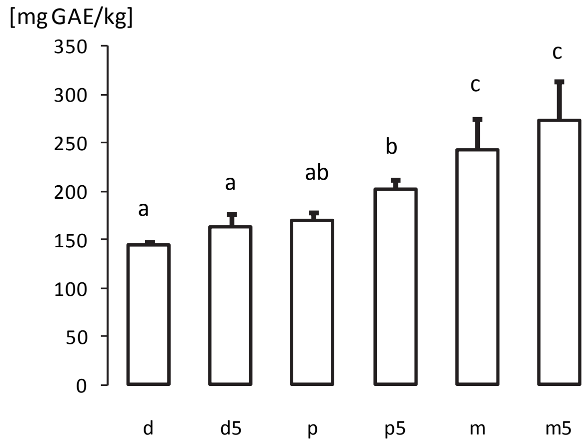

Figure 1. Antioxidative potential measured as total soluble phenolic content expressed in mg of gallic acid [GAE] equivalents per kg. Abbreviations: *d* – dairy ice-creams*; d5* – dairy ice creams supplemented with *Spirulina* extract*; p* – pistachio ice creams*; p5* – pistachio ice creams supplemented with *Spirulina* extract*; m* – mint ice cream*; m5* – mint ice cream supplemented with *Spirulina* extract. All data are presented as mean ± SD, n = 3. Bars with different letters are significantly different at P < 0.05

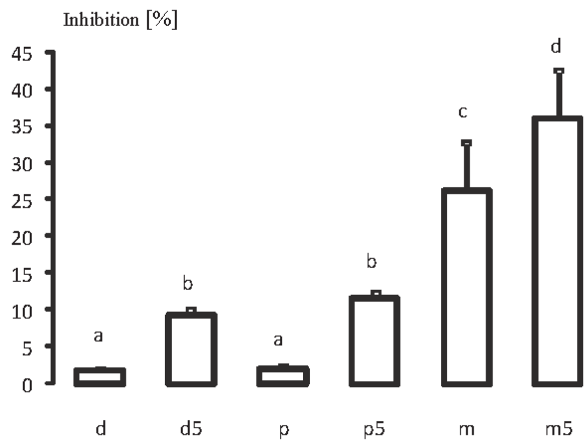

Figure 2. Antioxidative potential measured as inhibition level of free radicals released from cationradical of DPPH solution. Abbreviations: *d* – dairy ice creams*; d5* – dairy ice creams supplemented with *Spirulina* extract*; p* – pistachio ice creams*; p5*– pistachio ice creams supplemented with *Spirulina* extract*; m* – mint ice cream*; m5* – mint ice cream supplemented with *Spirulina* extract. All data are presented as mean ± SD, n = 3. Bars with different letters are significantly different at P < 0.05

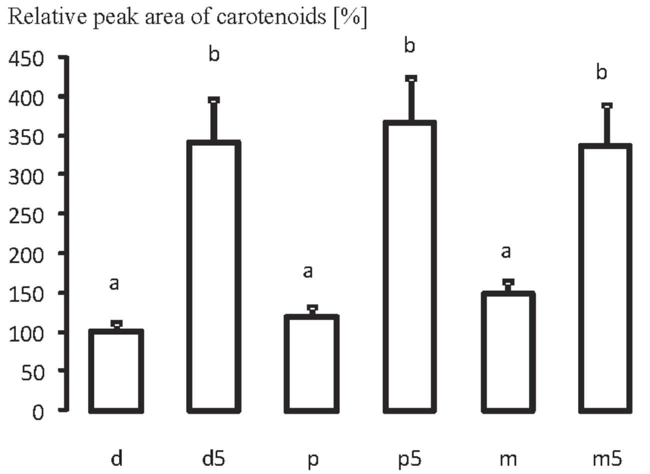

Figure 3. Sum of the carotenoids (as peak area in relation to dairy ice cream (100%)), alfa = 0,05 LSD test. Abbreviations: *d* – dairy ice-creams*; d5* – dairy ice creams supplemented with *Spirulina* extract*; p* – pistachio ice creams*; p5* – pistachio ice creams supplemented with *Spirulina* extract*; m* – mint ice cream*; m5* – mint ice cream supplemented with *Spirulina* extract. All data are presented as mean ± SD, n = 3. Bars with different letters are significantly different at $P < 0.05$

The high antioxidant potential of ice cream with the addition of *Spirulina* extract may be among others the result of the content of phycocyanin in microalgae biomass [15][14], which, with a relatively high degree of *Spirulina* supplementation (5%), directly influenced the analyzed parameters.

The sum of carotenoids for each of the analysed flavour versions of ice cream is a 3-3.5 higher magnitude for the samples enriched with *Spirulina* extract comparing to non-supplemented flavour versions (Fig. 3). Algae are known for their high contents of these nutraceuticals [24], so the results of this study confirm that the applied level of supplementation allows for exerting its bioactive potential.

Algae extracts are believed to yield positive effects in cell protection from oxidative stress through their nutrient composition parameters and antioxidants compounds. Furthermore, according to the recent data, *Spirulina* cells in combination with other products could be even more effective as antioxidants [14], such a tendency was documented in this brief study. However health benefiting impacts exerted from algae through food (e.g. ice-creams) that may incorporate them, still remain hardly explored.

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