SHORT COMMUNICATION

Use of *Spirulina platensis* and pomegranate peel extracts to increase the nutritional value of hard candies

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Summary
Recipes for enriched hard candies with extracts from *Spirulina platensis* and pomegranate peel powder were developed. The extracts were added at 4 %, 8 % and 12 % of the total content of sugar and molasses. *Escherichia coli* and *Bacillus subtilis* were used to study antimicrobial activity. The presence of volatile substances in hard candies was investigated by gas chromatography-mass spectrometry. It was found that hard candies with 12 % of pomegranate peel powder extract had satisfactory antimicrobial action and those with 12 % of spirulina extract contained a complex of valuable biologically active substances (palmitic, linolenic acid and others), which increased the biological value of the hard candies.

Keywords
hard candy; extract; *Spirulina platensis*; pomegranate peel; biological value

Hard candies in general, and in particular lollipops, are popular sweets available to different segments of the population [1]. They are delicious and nutritious because of containing saccharose, maltose and/or glucose. Hard candies can be also considered an affordable source of energy. Glucose ensures normal functioning of the brain, consuming approximately 80 % of this carbohydrate. Maintaining a stable glucose level is necessary for all metabolic processes in the body [2].

Sweets, such as modern lollipops, were known to the ancient Chinese, Egyptians and Arabs. People also noticed that lollipops were useful, for example they could help with cough. For this reason, the use of lollipops for medicinal purposes began almost 500 years ago. Today, caramel lollipops are used in pharmacology as carriers of active substances. The range of hard candies changed over time. The world's manufacturers take caramel production very seriously, constantly offering innovations both in taste and appearance. Customers choose confectionery products according to their taste preferences and price. When buying lollipops for children, parents choose those that contain natural dyes and flavours as well as vitamins. Elderly people also prefer lollipops with vitamins and other nutrients such as eucalyptus or honey. Currently, manufacturers tend to abandon the production of hard candies with artificial additives, using natural flavours and dyes, often with sugar substitutes such as isomalt or stevia [3].

A promising area is the development of new recipes with the inclusion of natural biologically active additives in the form of plant extracts. Sror et al. [4] revealed that anthocyanins of Egyptian red cabbage have good characteristics as a natural source of food dyes that can be used instead of synthetic additives. Therefore, the extract of Egyptian red cabbage is suitable as a dye in the production of hard candies. Al Khalaf et al. [5] demonstrated that the natural orange pigment of calendula can be used as a food colouring in hard candies. Yenrina et al. [6] revealed that to make hard candies in the form of a functional food, the kekombrang flower extract can be added, which enriches the product with health-promoting compounds. The results showed that the addition of kecombrang flower extract had a significant effect on antioxidant activity of the finished product. In general, the use of plant extracts in the production of hard candies allows to take full advantage of antioxidant, antitumour, immunotropic and other positive properties of the plants [7]. However, the classic recipe includes only simple carbohydrates.

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The disadvantage of hard candies, including lollipops, is the lack of valuable nutrients, such as vitamins, lipids as well as minor substances, for example dietary fibre and flavour enhancers. At the same time, the production of foods with functional properties has stable growth [8]. Therefore, a relevant and promising direction is the further development of the recipes and the technology for the production of hard candies enriched with natural additives.

Spirulina is a mass of cyanobacteria that can be used as a food supplement with many beneficial effects on humans including lipid-lowering action [9]. *Spirulina platensis* contains β-carotene, vitamin B12, γ-linolenic acid as well as a number of trace elements and amino acids [10, 11]. Pomegranate peel is also characterized by useful properties [12, 13]. It contains vitamin C, vitamin PP, B group vitamins, carotene, trace elements (iron, potassium, calcium, iodine, phosphorus) and, in general, contains much more nutrients than pulp or seeds of pomegranate. It also contains alkaloids, antioxidants, tannins and polyphenols. Pomegranate peel has anti-inflammatory, antiseptic and antimicrobial activities as well as a strong anthelmintic effect [14]. Therefore, it was tempting to use spirulina and pomegranate peel extracts to enrich hard candies and develop a new product with interesting nutritional properties.

MATERIALS AND METHODS

Raw materials

The spirulina and pomegranate peel extracts, sugar, maltose molasses and water were used for making hard candies in the form of lollipops. The spirulina extract was prepared from spirulina capsules made by NOW Foods (Bloomingdale, Illinois, USA). The pomegranate peel powder manufactured by HNCO Organics Private (Ahmedabad, India) was used to prepare the pomegranate peel extract. Other ingredients (maltose molasses, sugar, water) were obtained from the local market of the city of Chernihiv (Ukraine).

Tab. 1. Material formulations for making the hard candies.

Materials	Sample			
	A	B	C	D
Saccharose [g]	70	70	70	70
Maltose molasses [g]	30	30	30	30
Water [g]	30	26	22	18
Extract [g]	0	4	8	12
Total material [g]	130	130	130	130

Preparation of the extract

Spirulina and pomegranate peel extracts were prepared as follows: 1 part of the powder of spirulina or pomegranate peel was mixed with 9 parts of 70% aqueous ethanol and was kept at 15–20 °C with periodic stirring for 4 days. Then, the extract was drained, the remainder of raw materials was squeezed by a press, washed with a small amount of pure 70% ethanol solution and squeezed again. The extracts were pooled. The extracts were introduced to the recipe of the hard candies at a concentration of 4 %, 8 % or 12 %. The spirulina extract had a dark green colour, the pomegranate peel extract had burgundy colour.

Technology of hard candies

Ingredient formulae for making the hard candies are shown in Tab. 1. The materials were weighed, sugar, molasses and water mixed. The mixture was heated up to 65–70 °C, then the spirulina or the pomegranate peel extracts were added. After that, the mixture was heated up to 150 °C to remove ethanol (its boiling temperature is 78 °C). The resulting mass was poured into moulds and cooled. The finished hard candies were removed from the moulds and stored at 20 °C for maximum 6 months.

Methods

Physico-chemical parameters of the hard candies, namely, humidity, mass fraction of reducing sugars, titratable acidity, pH and mass fraction of ash were determined by standard methods [15, 16]. Sensitivity of bacteria to the active components of the *S. platensis* and the pomegranate peel extracts was studied by the disk diffusion method [16, 17]. *Escherichia coli* and *Bacillus subtilis* were used to study the antimicrobial activity of the extracts and the hard candies. Cultures of microorganisms for this study were provided by the Institute of Agricultural Microbiology and Agro-Industrial Manufacture of National Academy of Agrarian Sciences (Chernihiv, Ukraine). The extract of the hard candy sample with *S. platensis* (ingredient formula D) was subjected to analysis by gas chromatography-mass spectrometry (GC-MS). For this, 10 g of hard candies were soaked in methanol. The mixture was vigorously shaken and allowed to settle to form a suspension. The organic solvent with the extract was collected by filtering into a quartz beaker. The process was repeatedly carried out for two times. The collected aliquot was combined and purified by passing through an anhydrous sodium sulfate.

Gas chromatograph equipped with a mass selective detector DSQ (Thermo Electron, Waltham, Massachusetts, USA) was used. The electron ionization was 70 eV, the ion source temperature was 250 °C. Highly pure helium (99.9% purity) was used as a carrier gas, while HP-5ms column (30 mm × 0.25 mm × 0.32 μm; Agilent Technologies, Santa Clara, California, USA) was used. The initial thermostat temperature was 50 °C and it was held for 0.5 min. After that, temperature was increased at 25 °C·min^{-1} to 125 °C, then at 10 °C·min^{-1} to 255 °C and finally at 25 °C·min^{-1} to 300 °C. The holding time at 300 °C was 10 min. The components were identified by matching the retention times of chromatogram peaks and full mass spectra of individual components to suitable results for pure compounds contained in the NIST-5 mass spectrum library (National Institute of Standards and Technology, Gaithersburg, Maryland, USA) and using linear retention indices. The relative quantitative content of the components was calculated by the method of internal normalization of peak areas without adjustment for sensitivity factors.

Statistical processing throughout the study was performed for the 0.95 probability level (number of measurements 5).

RESULTS AND DISCUSSION

Physico-chemical and organoleptic parameters

The taste and colour of the obtained samples were characteristic for hard candies. The caramel had a homogeneous and vitreous structure. The intensity of colouring of the hard candies was the same, without stains, and the surface was dry and not sticky. The tint of the extract determined the colour of the hard candies. Hence, the hard candies with spirulina extract were dark green and the hard candies with the pomegranate peel extract had an attractive dark red colour. Physical and chemical indicators of the prepared candies are shown in Tab. 2. Humidity of the hard candies prepared with the addition of the extracts according to the ingredient formulae B, C, D was in the range of 2.2–2.3 % (Tab. 1). The lowest humidity had candies prepared according to the ingredient formula D, where the content of alcohol extract was 12 % (Tab. 2). This parameter is important because a lower humidity level provides a longer expiration of the finished product. Experts recommend the humidity of hard candies to be no more than 3.0–3.5 % [6].

Ash content is one of quality indices of candies. The lower ash content is obtained in candies, the clearer they will be. The addition of the *S. platensis* or the pomegranate peel extracts did not affect the ash content of the prepared hard candies (Tab. 2). Acidity is an important quality parameter of a caramel. A decrease in pH of hard candies is accompanied by an increase in the mass fraction of reducing sugars. At low pH, saccharose is converted into invert sugar or equimolar mixture of glucose and fructose increasing the hygroscopic properties of candies, which for this reason quickly become sticky during storage.

The pH value of the hard candies was in the range of pH 6.0–6.1 when the spirulina extract was used, and in the range of pH 5.6–6.1 in the case of the pomegranate peel extract addition (Tab. 2). Brand et al. [18] stated that pH 5.5 is critical for the tooth enamel because it is damaged at lower pH. Consequently, the lollipops with the *S. platensis* and the pomegranate peel extracts should not affect the tooth enamel. It contrasts to the results obtained for lollipops with fruit flavours [18], pH of which ranged from 2.3 to 2.4, and to the lollipops with cola and lemon flavours, pH of which was pH 2.2 and pH 2.4, respectively. Excessive

Tab. 2. Physical and chemical indicators of the prepared hard candies.

Indexes	A (control)	Extract added					
		Spirulina platensis			Pomegranate peel		
		B	C	D	B	C	D
Humidity [%]	2.4 ± 0.0	2.3 ± 0.1	2.2 ± 0.2	2.2 ± 0.0	2.3 ± 0.4	2.2 ± 0.0	2.2 ± 0.0
Reducing sugars [%]	10.1 ± 0.1	10.7 ± 0.0	10.5 ± 0.1	10.6 ± 0.1	10.8 ± 0.1	11.5 ± 0.1	11.8 ± 0.0
Titratable acidity [ml·kg^{-1}]	38.0 ± 0.1	39.0 ± 0.0	38.0 ± 0.1	38.0 ± 0.1	39.0 ± 0.1	40.0 ± 0.0	42.0 ± 0.1
pH	6.25 ± 0.08	6.05 ± 0.08	6.10 ± 0.06	6.08 ± 0.10	6.07 ± 0.06	5.78 ± 0.08	5.60 ± 0.08
Ash [%]	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

Reducing sugars and ash are expressed as mass fraction. Titratable acidity is expressed as millilitres of 0.1 mol·l^{-1} NaOH used to neutralize a kilogram of hard candies.
A, B, C, D – formulations are specified in Tab. 1.

consumption of such lollipops could lead to dental diseases, namely, tooth erosion, especially in people with low salivation and low buffering capacity of saliva. The presented results suggest that the ingredient formula D allowed to obtain hard candies with acceptable acidity and the best physico-chemical parameters.

Antimicrobial properties

The pomegranate peel extract showed antimicrobial activity against the gram-positive bacterium *B. subtilis* and against the gram-negative bacterium *E. coli* (Tab. 3). In the case of *B. subtilis*, application of discs soaked in the pomegranate peel extract resulted in the growth retardation zone that was 2.3-fold larger than that obtained by use of discs soaked in the spirulina extract. In the case of *E. coli*, application of discs soaked in the pomegranate peel extract resulted in the growth retardation zone of 18.2 ± 0.2 mm, which was three times larger than that obtained by use of discs soaked in the spirulina extract. The size of the growth retardation zone of the bacterial cultures when using the pomegranate peel extract indicated the pronounced antimicrobial effect of this extract. For this reason, the antimicrobial properties of the hard candies prepared with the addition of the pomegranate peel extract according to the ingredient formulae B, C and D were studied. When the ingredient formulae B and C were used, the growth retardation zones were small, not significant. In the case of the ingredient formula D, the growth retardation zone measured 14.0 ± 0.1 mm for *B. subtilis* and 11.4 ± 0.1 mm for *E. coli,* which indicated an antimicrobial effect.

Tab. 3. Antimicrobial activity of the extracts after 48 h.

Pure culture	Extract	
	Spirulina platensis	Pomegranate peel
Bacillus subtilis	8.7 ± 0.3	20.2 ± 0.3
Escherichia coli	6.1 ± 0.1	18.2 ± 0.2

Values represent the growth inhibition zone diameter (in millimetres). Number of samples was 5.

Tab. 4. Composition of the volatile fraction of the hard candies enriched with *Spirulina platensis* extract.

Compound	Mass fraction [%]
Tetradecane	34.6
α-Ionene	0.5
Pentadecane	3.2
2-Hexadecene	1.8
Hexadecane	2.2
Palmitonitrile	1.9
6(*Z*),9(*E*)-Heptadecadiene	1.2
Heptadecene	1.1
Heptadecane	39.7
Neophytadiene	2.1
Pentadecanitrile	2.2
Linolenic acid	1.0
Palmitic acid	0.8
Isophytol	1.1
Phytol	3.6

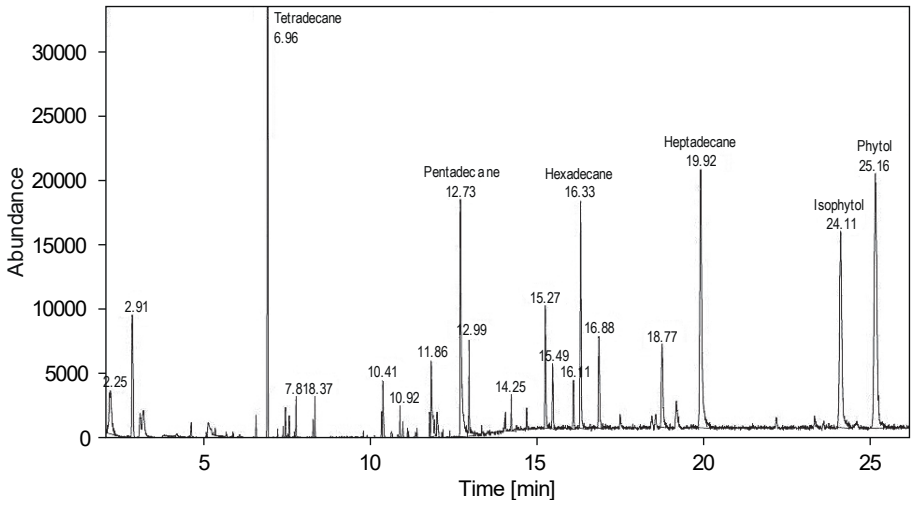

Fig. 1. GC-MS chromatogram of the methanol extract of the hard candies prepared by the ingredient formula D with the *Spirulina platensis* extract.

Thus, hard candies that inhibit bacteria can be obtained by adding of 12 % pomegranate peel extract to the recipe.

Volatile substances

The hard candies with the *S. platensis* extract prepared according to the ingredient formula D were subjected to GC-MS analysis because of their interesting physico-chemical parameters. It was established that the hard candies with the *S. platensis* extract prepared according to the ingredient formula D contained tetradecane, pentadecane, hexadecane and heptadecane. These hydrocarbons dominated among the volatile substances (Tab. 4, Fig. 1). It should be noted that these hydrocarbons are components of the essential oils of aerial organs of many medicinal herbs. The presence of heptadecane in algae and plants indicates powerful antioxidant, antitumour and antimicrobial activities. The mixture of volatile substances also contained phytol, isophytol and polyunsaturated fatty acids, such as palmitic or linolenic acids (Tab. 4, Fig. 1). It is known that phytol and isophytol are contained in medicinal plants, such as in medicinal jasmine. These substances help to overcome nervous exhaustion, depression and headache. Palmitic acid is a major component of breast milk. It is necessary for the child's proper development as well as for calcium absorption and the regulation of the digestive processes. Linolenic acid has a positive effect on the immune system and it is a potential inhibitor of cancer. It should be noted that linolenic acid is difficult to obtain it from food. In the childhood, this acid enters the child's body through breast milk.

CONCLUSION

Hard candies with higher biological value could be prepared when the *S. platensis* or the peel pomegranate extracts were used in the recipe. The use of 12 % *S. platensis* extract led to the enrichment of hard candies with valuable substances such as tetradecane, heptadecane or phytol. The hard candies prepared according to the ingredient formula D with 12 % of the extracts had the best organoleptic and physico-chemical parameters that are typical for this food product.

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