BIOTECHNOLOGICAL POTENTIAL OF APILARNIL AND ROYAL JELLY USED IN OBTAINING SOME FUNCTIONAL FOODS

Claudia Paşca[1*], D.S. Dezmirean[1], Otilia Bobiş[2], L.Al. Mărghitaș[1], Victorița Bonta[2]

[1]Faculty of Animal Science and Biotechnologies,
University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[2]Life Science Institute "King Michael I of Romania",
University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Abstract

Bees, through their activity, provide us with bee products containing a variety of biologically active compounds, which, in different proportions, have a positive impact on general human health, on the physical performance or mental status but also from a nutritional point of view. Through this research paper, it was sought the obtaining of two functional foods based on apilarnil, royal jelly and multifloral pollen with biotechnological potential. The products prototypes produced were characterized from a physico-chemical and organoleptic point of view. As a result to the analyzes, numerous polyphenolic compounds have been identified in this jellies, namely: rutin, chlorogenic acid, isoquercetin, naringenin, and which give antidepressant, immunomodulatory, antitumoral, antiinflammatory, hypolipidemic, antioxidant, DNA protection, proliferation of peroxisomes activities, compared to commercial jelly, where the amount and concentration of polyphenolic compounds is greatly diminished.

Key words: innovation, apilarnil, biotechnological potential, functional food, royal jelly

INTRODUCTION

In recent decades, the concept of functional foods has offered a new and practical approach to achieving optimal health by promoting the use of natural products with physiological benefits thus reducing the risk of various chronic diseases [1-3]. According to the definition, functional food is a part of human diet and is demonstrated to provide health benefits and to decrease the risk of chronic diseases beyond those provided by adequate nutrition [4-6]. Functional foods include: usual foods with naturally occurring bioactive substances (e.g. dietary fibre), foods supplemented with bioactive substances (e.g. probiotics, antioxidants), and derived food ingredients introduced to conventional foods (e.g. prebiotics) [5]. The health benefits such as decrease of cancer risk, improvement of heart health, enhancement of immune system [5, 7], reducing of menopause symptoms, enhancement of gastrointestinal health, preservation of urinary tract health, anti-inflammatory influences, diminution of blood pressure, protection of vision, antibacterial and antiviral activities, decline of osteoporosis and anti-obesity influences [6-7). Among foods that possess the characteristic of functionality, we may include all those originating in the beehive: bee pollen, royal jelly and apilarnil.

Bee pollen is a good source of healthy compounds due to the potential biotechnological such as phenolics, terpenes and flavonoids [8, 9], that are relevant for clinical applications against inflammatory diseases [10-12] source of proteins, essential amino acids, essential fatty acids, vitamin complexes, lipids, trace elements [10].

Royal Jelly is one of the most challenging bee products for biotechnologies [13], it is an important functional food item that possess

*Corresponding author:
claudia.pasca@usamvcluj.ro
The manuscript was received: 24.09.2019
Accepted for publication: 15.04.2021

several health promoting properties [9]. Biological activities of RJ are mainly attributed to the bioactive fatty acids, proteins and phenolic compounds as quercetine, kaempherol, galangine, pinocembrine, narynin, apigenin, hysperdine, acacetin, crisina, luteoline [9]. RJ has been demonstrated to possess numerous functional properties such as antibacterial activity, anti-inflammatory activity, vasodilative and hypotensive activities, disinfectant action, antioxidant activity, antihypercholesterolemic activity and antitumor activity [13, 14].

On the other hand, apilarnil is a natural bee product obtained by trituring larvae of drones harvested under special conditions, in whole, on the seventh day of hatching [9, 15]. Apilarnil has a higher nutitional value and biological active compounds as essential amino acids (treonin, leucine, isoleucine, methionine), vitamins (vitamin A, betacaroten, B1, B6, PP and choline), minerals (calcium, phosphorus, sodium, zinc, manganese, iron, copper and potassium) and it is also rich in sex hormones like testosterone, prolactin, progesterone and estradiol [16, 17].

In this research study, it was desirable to optimise some recipes of jellybeans based on bee products (royal jelly, apilarnil, multifloral pollen) that would bring added benefits due to the content in biologically active compounds with antioxidant, antiinfectious, vitalizant, biostimulatory and energizing properties, balancing of the endocrine and nervous system and falling within the category of functional foods.

MATERIAL AND METHODS

Chemicals and Bee Products Samples

All chemicals and reagent were analytical grade or chromategraphic grade purity and ultrapure water, purchased from Sigma, Cromatec and Merck. Fresh royal jelly and apilarnil were obtained from beekeepers; multifloral pollen from UASVM apiary. Other materials used were: *Sambucus L.* syrup, *Salvia officinalis* extract and gelling agent.

Bee Products Sample Preparation

Royal jelly and apilarnil were lyophilized using the method described by Nascimento *et al*. [18], for obtaining a powder and bee pollen was subjected to heat treatment at 40 Celsius degrees.

Jelly Technology

This process assumed the following steps: raw materials preparation, ingredients incorporation, raw materials mixing, boil mixture, cooling, apilarnil or RJ (0.15 gr/jelly) adding, bee pollen adding, mold transfer and refrigeration. The jelly weight varies between 10-15g.

Chemical Characterization of Experimental and Commercial Jelly

The two varieties of jellybeans based on royal jelly and apilarnil were analyzed by high-performance liquid chromatography with refractive index detection for sugar spectrum [19]. The instrument used was a Shimadzu apparatus equipped with a column: modified Alltima Amino 100Å, 5 µm, 250 x 4.6 mm; mobile phase flow: 1.3 ml/min; mobile phase: acetonitrile/water (75/25; v/v); column temperature: 30^{o}C; injection volume: 20 µl; separation time: 60 min. Sugar standard solutions are prepared similar to the analyzed sample. Results are expressed in g/100 g sample.

Biological active compounds

Identification of polyphenolic compounds from jellybeans based on royal jelly and apilarnil and the commercial jelly was made after Campos and Markham method [20], modified in our laboratory, using a liquid chromatograph Shimadzu 2010 EV (Kyoto, Japonia) coupled with Photodiode Array Detector (PDA). Compounds separation was achieved on a MEDITERRANEA SEA C18 reverse phase column. A binary gradient of two mobile phases was used: water at pH 2.5 (phase A) and acetonitrile (phase B). Elution was carried out with a flow rate of 1ml/min at 24˚C, with an injection volume of 10 µl. Spectral data for all peaks were registered in the range of 220-600 nm.

Determination of mineral content

To determine the levels of micro and macroelements: Na, Mg, K, Ca from obtained jellybeans, the atomic absorption spectrometry method was used. The mineralization of the samples was performed in a microwave furnace, Berghof digestion system MWS-2. Approximately 0.3 grams of the homogenized samples were placed in special Teflon tubes, 2 ml of 65% HNO_3 was added and let to react for 15 minutes, after which 3 ml of H_2O_2 was added before the container was sealed [21, 22]. At the end of the initiated program, the solution is transferred into plastic containers and the sample is diluted with ultrapure water to a volume of 50 ml. An Analyst 800 Atomic Absorption Spectrometer from Perkin-Elmer was used, equipped with a cross-linked graphite furnace. In the graphite furnace the sample is inserted into a small tube of electrically heated graphite. By increasing the tube temperature, the sample passes through the drying, pyrolysis and atomization phases [23].

Lipid and Protein Content

The total lipids and proteins for obtained jellybeans were determined by Soxhelt method and Kjeldahl method described by Bobiş *et al.*, in 2018 [23]. The moisture content was also determined by gravimetric method for calculating nutritional value of obtained jellybeans.

Sensory analysis

A number of 30 people, aged 18-40 years, by different sexes and occupations were questioned following the consumption of jellybeans with bee products.

RESULTS AND DISCUSSIONS

Two prototypes of products were achieved. One prototype contain: 160ml/200ml *Sambucus L.* syrup, 3 ml/200ml *Salvia officinalis* extract, 10g/200ml gelling agent, 25g/200ml multifloral pollen and 2g/200ml royal jelly and the other prototype contain: 160ml/200ml *Sambucus L.* syrup, 3ml/200ml *Salvia officinalis* extract, 10g/200ml gelling agent, 25g/200ml multifloral pollen and 2g/200ml apilarnil.

Those two functional foods (jellybeans with apilarnil and royal jelly) have a smaller nutritional value 179.6 kcal/100g for royal jelly and multifloral pollen jelly, and 141.9 kcal/100g for apilarnil and multifloral pollen jelly, compared to a commercial jelly product (according to the label), whose nutritional value is 340 kcal/100g. Chemical composition of experimental jellybeans with bee products are presented in Figure 1.

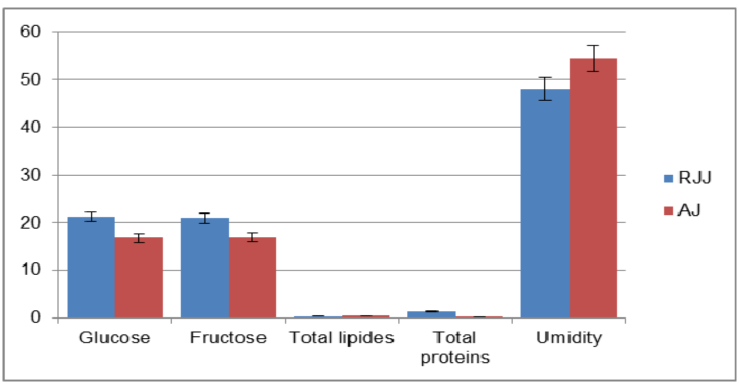

Fig. 1 Sugur content, total lipids, total proteins and umidity (%) of jellybeans with bee products.
*RJJ = royal jelly and multifloral pollen jelly; AJ = apilarnil and multifloral pollen jelly

The royal jelly and multifloral pollen jelly contain umidity in small amounts, but a higher contents in total lipids (0.234±0.02%), total proteins (1.25±0.01%) and total sugars (42.03±0.06%) compared to apilarnil and multifloral pollen jelly.

The content of some minerals from jellybeans with bee products are represented in Figure 2.

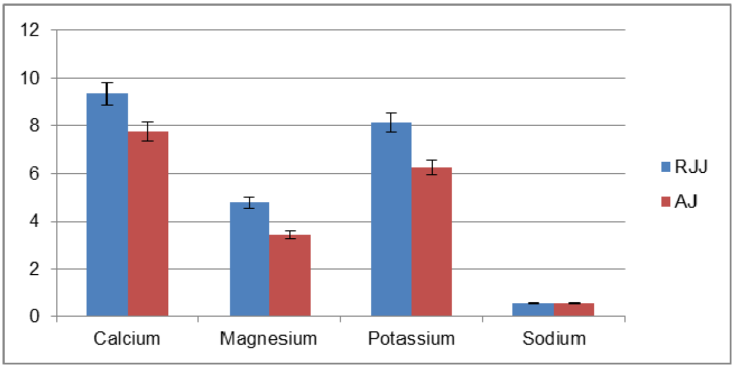

Fig. 2 Mineral content (μg/g) of jellybeans with bee products
*RJJ = royal jelly and multifloral pollen jelly; AJ = apilarnil and multifloral pollen jelly

The distribution of the elements among the two functional food were in favour of royal jelly and multifloral pollen jelly, much higher amonts being determined in royal jelly and multifloral pollen jelly compared to apilarnil and multifloral pollen jelly.

The biologically active potential of the two jelly samples obtained has been highlighted by identifying polyphenolic compounds (Table 1) and comparing the results achieved with those of an already existing on the market (Table 1). So, the royal jelly and multifloral pollen jelly have higher concentration in rutin, chlorogenic acid and isoquercetine while the cafeic acid and myricetin were found in small amounts. Rutin is an flavonoid predominantly found in plants, bee pollen [25] and presents antioxidant, cytoprotective, vasoprotective, anticarcinogenous, neuroprotective and cardioprotective activity [26]. On the other hand, chlorogenic acid present antibacterial, antioxidant and anticarcinogenous activity, specially hypoglycemic and hypolypidemics [27]. Also, isoquercetin have neuroprotective, cardioprotective, antioxidant, antiinflamtory, chemopreventive and antiallergic effects [27].

Analyses performed to apilarnil and multifloral pollen jelly confirmed higher amounts in rutin, chlorogenic acid and isoquercetine as well as naringenine, possessing both antidepressants, immunomodulatory, antitumor, anti-inflammatory, hypolypidemic and antioxidant activity, DNA protector and improves memory [28].

Table 1 Polyphenolic compounds of experimental and commercial jelly, containing plant extracts and bee products

Retention time (min)	Specification	Experimental jelly (apilarnil and multifloral pollen) [μg/g]	Experimental jelly (royal jelly and multifloral pollen) [μg/g]	Comercial jelly [μg/g]
11.11	protocatecuic acid	0.52	0.33	0.14
17.5	p-OH Benzoic acid	1.17	0.59	0.04
21.78	chlorogenic acid	6.79	7.01	0.14
23.48	caffeic acid	0.03	0.10	0.01
26.79	vanilin	-	0.40	1.04
29.37	p-coumaric acid	-	0.50	0.25
30.74	rutin	22.59	21.16	0.64
30.86	ferulic acid	-	-	0.17
31.2	isoquercetine	2.69	2.86	0.10
33.57	myricetin	0.17	0.25	-
41.26	naringenine	5.56	1.50	-
42.1	kaempferol	2.12	0.27	-

Comparing the bioactive compounds results for both functional foods, much higher concentrations were found in apilarnil and multifloral pollen jelly except for protocatecuic acid and isoquercetine.

In order to highlight the biologically active potential of jellybeans studied, comparisons were made with the results of polyphenolic compounds analysis for a commercial jelly (Table 1). It can be observed that, with regard to all the compounds recovered, concentrations are much lower range between 0.1-1.1 (μg/g) for commercial jelly, while the concentrations for RJJ and AJ are found between 0.03-23 (μg/g).

The main quality characteristics of jellybeans from consumer's perspective consist of being equilibrate in terms of aroma (21/30 responses), containing natural ingredients, without preservatives (20/30 replies) and not contain sugar (19/30 responses). Regarding organoleptic analysis of the two products, the replies received were mostly in the category "Excellent taste" (66.7% for RJJ and 60% for AJ), 30-40% expressed "Pleasant taste". The attention was equally given to both jellybeans of 73.3%; With regard to the changes they would like on these products, 56.7% were in favour of their shape, 23.3% on consistency and only 10% of respondents would bring improvements to taste.

CONCLUSIONS

Bee pollen, royal jelly and apilarnil are food products obtained from bees. All of them are important not only for their nutritional properties but also for their functional and biological properties. For their beneficial effects, the presented bee products can be used as potential ingredients for some functional foods, in our case jellybeans. Biotechnological potential of the functional foods obtained following this study are mainly attributed to the phenolic compounds such as flavonoids and phenolic acids and this potential of food can promote health, improve general well-being and reduce the risk of developing certain illnesses.

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