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BEE POLLEN AS ANTIOXIDANT INGREDIENT IN READY-TO-SERVE CITRUS JUICE

Laura STAN

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
3-5 Mănăştur Street, 400372, Cluj-Napoca, Romania

Corresponding author email: laurastan@usamvcluj.ro

Abstract

Bee pollen is a highly nutritious food supplement, which can be easily added to any juice type and enrich its nutritional and sensory quality. The aim of the study was to develop a citrus juice recipe enriched with bee pollen, highly nutritional and with good acceptability of consumers and to compare the polyphenols content and radical scavenging activity of regular citrus juice (RJ) versus enriched citrus juice with bee pollen (JBP). Both products were evaluated for vitamin C content, antioxidant activity (DPPH method), total polyphenols (Folin Ciocalteu method) and sensory evaluation by consumers (N=125) using nine scale hedonic test. The addition of bee pollen and honey to the citrus juice did modify significantly ($p<0.05$) the total polyphenols content and antioxidant activity in JBP compared to RJ. The hedonic score of JBP was 7.29 ± 1.61, slightly lower than RJ (8.04 ± 0.73). More than 70% respondents declared they would buy this drink due to the beneficial effects of bee pollen. Bee pollen is one convenient option of many available on market used to increase the nutritional value of beverages.

Key words*: bee pollen, antioxidant activity, polyphenols, sensory evaluation, hedonic test.*

INTRODUCTION

The number of studies about consumers of bee pollen is scarce. Even more, studies about using bee pollen as ingredient in fruit juices are completely lacking as far as the author knows. Most of the studies published so far on bee pollen are focused either on bee pollen quality and composition (Bogdanov, 2004; Stangaciu, 2015; Salazar-González et al., 2016; Bobiş et al., 2017), either on the biological effects of different classes of compounds (Carpes et al., 2007; Komosinska-Vassev et al., 2015, Urcan et al., 2017). Previous literature data prove that bee pollen has the real potential to be used in nutrition and therapy (Campos et al., 2010; Bogdanov, 2014; Komosinska-Vassev et al., 2015). However, most of the studies were carried out on bee pollen extracts and not on the whole product itself (Campos et al., 2010; Bogdanov, 2014). It is debated that the outer layer of the pollen grain (exine) is heavily digestible, therefore, practitioners of Ayurveda and Traditional Chinese Medicine, recommend to add bee pollen to a slightly acidic drink or food (e.g. yoghurt) prior to ingestion in order to increase its bioavailability (Campos et al., 2010; Bogdanov, 2014; Stangaciu, 2015; Urcan et al., 2017). Recently, proposals on standardization criteria provided valuable information especially for producers and quality control services (Campos et al., 2008; Campos et al., 2010). Even in EU Regulation 1924/2006 bee pollen is viewed as functional food with beneficial effects on health. “Long term ingestion of pollen and special pollen preparations (cracked pollen, pollen extracts) can improve the physical performance and fitness of sportsmen and elderly people”, as well as "pollen intake can improve gut, gastroenterological and liver health" (EU Regulation 1924/2006). This increasing interest of the scientists in bee pollen proves its marvellous market potential (FP7-SME-2008-2). Moreover, this overlaps with the market trend for healthy “greener” food and drinks (Kasriel-Alexander et al., 2016), therefore, the consumers’ attention moved from fast-food to more conscious choices (Byers et al., 2002; Story et al., 2008).

This study aims at evaluating the influence of bee pollen in fruit juices, more specifically citrus fresh juices. Consumers have different sensorial reasons to like citrus juice during any season of the year e.g. sweet-sour or and slightly bitter taste, wonderful colour, cooling

sensation during drinking, while other consumers are attracted to its nutritional qualities - rich source of vitamins and fibers (Drewnowski et al., 2000; Fernández-Vázquez et al., 2011). Still, for consumers who are looking for a more nutritious drink, regular citrus juice is far from perfect.

In this context, the first objective of this work was to develop a citrus juice enriched with bee pollen, highly nutritional and with good acceptability of consumers. Second objective was to evaluate some quality parameters (vitamin C, polyphenols, sensory quality) of the citrus juice enriched with bee pollen.

MATERIALS AND METHODS

Preparation of citrus juices

Citrus fruits (lemons, grapefruits and oranges) were obtained from local market and kept in the refrigerator (+4°C) until further use. The fruits were washed, cut in halves, and squeezing was carried out by using a rotary hand extractor in order to obtain the juice. Fresh bee pollen samples obtained from local beekeepers were kept at -20°C in vacuumed plastic bags until further use.

Two samples of juices were freshly prepared in the days of analysis:

- *regular citrus juice* (RJ) with the following ratio between fruits orange: lemon: grapefruit, 3:1.5:1 (v/v/v);
- *citrus juice with bee pollen* (JBP) with 87% (w/v) of citrus juice (orange: lemon: grapefruit, 3:1.5:1, v/v/v), honey (8%, w/v) and bee pollen (5%, w/v). JBP was left to stand at room temperature for 15 minutes until the bee pollen was completely dissolved in the juice (the bee pollen pellets were no longer visible).

In the end, previous to any analysis, the samples were passed through a finisher with 0.5 mm holes to have a uniform appearance of the product.

The two samples were analysed for vitamin C content, antioxidant activity (DPPH method), total polyphenols (Folin Ciocalteu spectrophotometric method) and sensory evaluation by nine points hedonic test.

Evaluation of vitamin C content

Vitamin C was determined by titration with KIO_3 (0.004N) according to the method previously published by Tofana and Muresan (2011). Final results were calculated using the formula: Vitamin C (mg %) = $[(V \times V_1 \times 0.352)/M \times V_2] \times 100$, where V – the volume of KI used for titration (mL); V_1 – total volume of the extract (mL); V_2 – the volume of the extract used for titration (mL); 0.352 – the titre of KIO_3 solution; M – weight of the sample taken into analysis (g).

Determination of total polyphenols

The content of total polyphenols was estimated according to the Folin Ciocalteu method proposed by Singleton et al., 1999, using gallic acid as reference standard. Briefly, 2.5 mL Folin Ciocalteu (0.2N) reagent was added to juice sample (0.5 mL), and after 5 minutes 2 mL of Na_2CO_3 (75g/L) was added to the solution. The absorbance at 760 nm (UV-VIS 1700 Schimadzu Spectrofotometer, Japan) was then measured against a methanol blank. A standard curve of gallic acid was created using an adequately range of gallic acid solutions from 0.01 to 0.25 mg/mL (R^2=0.986). The results were expressed as Gallic Acid Equivalent (mg GAE g^{-1} dry matter sample).

Radical scavenging activity

DPPH method was used for evaluation of radical scavenging activity (RSA) (Nenadis, 2002). Methanol solution of DPPH (4 mg/100 mL) was freshly prepared in the day of the analysis. Test samples were prepared with 2.95 mL of DPPH methanol solution and 50 µl of juice extract. Methanol (50 µl) plus DPPH solution was used as negative control. Positive control was prepared using vitamin C (50 µl). After rigorous homogenisation at vortex for 10 seconds, the samples were kept in the dark for 30 minutes. Absorbance at 515 nm was read at UV-VIS 1700 Schimadzu Spectrofotometer (Japan). Radical scavenging activity of the test samples were calculated with the formula: RSA (%) = $[(A_{DPPH}-A_{sample})/A_{DPPH}] \times 100$, where A_{DPPH} was the absorbance of DPPH solution and A_{sample} was the absorbance of the sample.

Sensory analysis

Tests were conducted in Laboratory of Sensory Analysis of Foods at UASMV Cluj-Napoca, equipped with table for joint sessions and 20 computers. Samples (50 mL) were served in random order at room temperature (aprox. 20°C), in transparent glasses labelled with three-digit codes. In order to evaluate the product acceptability hedonic test was performed on consumers (N=125). Frequent consumers of citrus juices (voluntary students from 1st and 2nd year of study) were selected based on a questionnaire about their availability and willingness to participate in the test. The test design included a nine point scale hedonic test and the ranking test according to the methods previously published by Stone et al., 2010; Lawless et al., 2012. Each point of the scale had the following meaning: 1–extremely unpleasant, 2–very unpleasant, 3–unpleasant, 4–slightly unpleasant, 5–indifferent, 6–slightly pleasant, 7–pleasant, 8–very pleasant, 9–extremely pleasant (Stone et al., 2010; Lawless et al., 2012). The following sensory characteristics and attributes of the products were evaluated on the hedonic scale: colour, odour, viscosity, taste and aroma, as well as overall appreciation. Neutralisers like water and unsalted crackers were provided *ad libitum* in between samples' tastings.

Statistical analysis

Mean value and standard deviation were calculated for the three replicates of each analysis performed during the study. Hedonic score and absolute frequency of respondents were calculated for each sample on Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

On the path from ancient medical tradition to nowadays trends, honey bees and their products play significant role in the quality of life. Firstly, bees play a crucial role in insect dependent plants pollination and this has a vital impact on providing food for people and feed for animals (Delaplane et al., 2000; James et al., 2008); as a consequence, undeniable economic advantages are derived from pollination. Secondly, bee products were used as medicine or as adjuvant in treatment of different diseases and health conditions since ancient times (Boukraâ, 2013). Therefore, bee pollen is one of the many options available on market to increase the nutritional value of foods, with the advantage of being easy to use, although the knowledge about their use varies greatly depending on the local culture. For example, bee products are usually highly regarded in Eastern Europe (for their therapeutic value, therefore are mainly used as food supplements in time of sickness or during cold seasons (Bogdanov, 2014; Stangaciu, 2015). In contrast, other parts of Europe consider them food or food supplements (honey is being used regularly at breakfast, while bee pollen and propolis are almost unknown or used as food supplements). Traditional Chinese Medicine and Ayurveda (Traditional Indian Medicine – Science of Life) use honey and other bee products in many applications (Stangaciu, 2015). According to World Health Organisation (WHO, 2000), about 60% of the world's population relies on traditional medicine and in some countries it is "extensively incorporated into the public health system". Even more, a close look will reveal that the main principles of traditional medicine (WHO, 2000; Boukraâ, 2013; Stangaciu, 2015) are wisely incorporated into recent consumers' trends. For example, consumers' food trends nowadays are mainly focused on eating greener and locally, avoidance of unhealthy foods, choosing seasonal foods and cutting down food waste (Kasriel-Alexander et al., 2016).

The results of the physico-chemical parameters are presented in Table 1 and discussed in further paragraphs.

Table 1. Physico-chemical parameters of citrus juice samples

Parameter	RJ (Mean ±S.D.)	JBP (Mean ±S.D.)
Vitamic C (mg%)	38.91 ± 2.07	41.53 ± 2.94
Total polyphenols (mg $GAEg^{-1}$)	13.39 ± 1.08	16.97 ± 1.57
RSA (%)	65.24 ± 3.25	81.63 ± 4.94

Vitamin C

According to previous literature data, both citrus fruits and bee pollen are rich sources of vitamins. Fruits are rich in water-soluble vitamins (Murdock, 2002). However, bee

pollen has both fat-soluble and water-soluble vitamins (Campos et al., 2008; Campos et al., 2010; Komosinska-Vassev et al., 2015). In this study, the results of vitamin C evaluation of both citrus juices RJ and JBP was high: RJ presented 38.91±2.07 mg/100 g and 41.53±2.94 mg/100 g was determined in JBP, although no significant difference ($p<0.05$) was recorded between the samples.

Total polyphenols

Polyphenols are valuable compounds with many biological activities, like antioxidant, antimicrobial, anti-inflammatory and are abundant in fruits, tea, wine, coffee (El Gharras, 2009). Regardless the botanical or geographical origin, bee pollen is a rich source of polyphenols - flavonoids and phenolic acids (Drewnowski et al., 2000; Fernández-Vázquez et al., 2011; Bogdanov, 2014; Campos et al., 2008; Komosinska-Vassev et al., 2015).

The addition of bee pollen and honey to the citrus juice did modify significantly ($p<0.05$) the total polyphenols content (16.97 mg gallic acid/g in JBP and 13.39 mg gallic acid/g in RJ). Due to the high content in polyphenols, fruits are generally highly regarded as functional foods which contribute to prevention of many disease conditions.

Radical scavenging activity

DPPH method has many advantages for laboratory work. The method is fast and easy to apply, and although DPPH is a free radical, it is stable at room temperature (Nenadis, 2002). The radical produces a violet solution in methanol or ethanol, but in the presence of an antioxidant molecule it gives rise to an uncoloured solution.

The radical scavenging activity of JBP evaluated by DPPH was significantly ($p<0.05$) higher than RJ (81.63 ± 4.94 %). Considering that there was no significant increase in the level of vitamin C or polyphenols, it means that other compounds from bee pollen may present radical scavenging activity also (other vitamins, minerals or even proteins).

Sensory analysis

Results of sensory evaluation are presented in Table 2. There is a significant statistical difference between the two samples and the sensory attributes ($p<0.01$).

The general hedonic score of JBP was 7.29 ± 1.61 on the nine point hedonic scale, which means that the juice was highly appreciated by consumers, although slightly lower than RJ which scored 8.04 ± 0.73.

Addition of bee pollen in the juice matrix changed some sensory attributes of the product. Evaluators reported in JPB sample a orange-yellow colour, “dusty feeling on the tongue” (marked as unpleasant by some of them), decreased sweetness and sourness. In spite of textural and taste changes of the juice, more than 70% respondents declared they would buy this beverage due to the beneficial effects of bee pollen. These results stress out the importance of consumers’ education about the benefits of bee pollen.

Table 2. Sensory evaluation of citrus juices samples

Sensory attributes	RJ (Mean ±S.D.)	JBP (Mean ±S.D.)
Colour	7.97 ± 1.08	7.22 ± 1.21
Odour	7.83 ± 0.52	6.94 ± 2.03
Viscosity	7.76 ± 1.47	6.85 ± 1.92
Taste and aroma	8.58 ± 1.31	8.15 ± 0.44

Even more interesting is that only 20% of them were familiar with bee pollen taste and were regular users. Sample JBP was ranked first and these results confirm that the trend for healthy and natural foods/ingredients/food supplements goes beyond employed people.

CONCLUSIONS

Consumer trends move toward more natural food products and drinks. Usually, consumers view bee products as natural and healthy products, locally and seasonally produced and although there is a slight difference between Western and Eastern European consumers’ perception of bee products, there still are some similarities: they both acknowledge the healthy potential of bee products.

Addition of honey and bee pollen to fruit beverages positively contributes not only to sensory characteristics of the final product, but also to its functional quality. To the knowledge of the author, this is the first article which uses bee pollen as ingredient in ready-to-use citrus juice. This product could be included in a

restaurant menu and provide a new, healthy option to the consumers. According to National Honey Board (U.S.A.) honey is included more and more in the restaurants' and bars' menu. While honey provides the sweet taste and specific flavour, bee pollen adds proteins, minerals and interesting texture to the new food item. It is the job of the chefs to find new creative and attractive ways to integrate these valuable products in the menu.

The findings underscore the importance of consumer education in learning to appreciate the value of bee products in daily use, not only in time of sickness. For instance, in Romania honey consumption is about 0.4-0.6 kg/year/person, among the lowest in Europe, and this is solely due to cultural heritage – honey is not seen by most of Romanian as a regular food product, but mostly as a healthy ingredient (www.romtradeinvest.ro).

As bee population is fast decreasing in the last years, it seems that consumers' understanding is increasing about bees' role and importance of bee products in maintaining a healthy life. The use of bee products in preparing various foods will help also the beekeepers to have more success in their business and will also encourage the farmers to cultivate melliferous plants, thus providing means to maintain bee populations alive. More than that, in order to protect the consumers' safety and beekeeping market, even European Parliament adopts regulations about health and nutritional claims of bee pollen (EC 1924/2006). Other vital reasons and equally important to increase the consumption of honey and bee pollen is to develop consumers awareness about improving the quality of life with bee products.

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