INTRODUCTION

Although trading and consumption of bee pollen is an old practice (Kroyer and Hegedus, 2001), only in recent years has the growing knowledge of its composition increased its use as a nutritional supplement for the human diet (Orzáez Villanueva et al., 2002; Funari et al., 2003; Szczęsna et al., 2006 a,b,c; Szczęsna et al., 2007 a,b). Nonetheless, to date no consensus has been reached regarding the role of the bee pollen in the human diet. From a legal perspective, not all countries consider the product a nutritional supplement nor do all countries have its quality standards and limits established (Almeida-Muradian, 2006).

The composition of bee pollen varies according to the plant species visited by the bees (Serra Bonvehi, 1988), the environmental conditions, age and nutritional status of the plants when the pollen is being produced (Herbert and Shimazu, 1978), collection site, season and year of production (O’Rourke and Buchmann, 1991; Szczęsna et al., 2002), methods of preservation (Szczęsna et al., 1995a) and storage conditions (Szczęsna et al., 1995b,c,d,e). The harvesting of pollen from a variety of plant species assures bees a balanced diet.

PHYSICOCHEMICAL COMPOSITION OF BEE POLLEN FROM ELEVEN BRAZILIAN STATES*

Marcia C. T. Martins1,2, Marcelo A. Morgano3, Eduardo Vicente3, Sueli R. Baggio3, Delia B. Rodriguez-Amaya2

1Adventist University Center of Sao Paulo (UNASP), Estr. Itapecerica, 5898, 05858-001 São Paulo, SP, Brazil
2Faculty of Food Engineering, University of Campinas - UNICAMP, P.O. Box 6121, 13083-862 Campinas, SP, Brazil
3Food Science and Quality Center (CCQA), Food Technology Institute (ITAL), Av. Brasil, 2880, 13070-178 Campinas, SP, Brazil
e-mail: marciactm@yahoo.com.br

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Summary

The physicochemical composition (ash, lipid, protein, glucose, fructose and free acidity) of Brazilian bee pollen (154 samples) from 23 production sites of 11 Brazilian states was analyzed. The data showed wide within-state and between-state variations: 1.33 to 4.13 g/100 g for ash, 4.01 to 13.32 g/100 g for lipid, 12.28 to 27.07 g/100 g for protein, 6.99 to 21.85 g/100 g for glucose, 12.59 to 23.62 g/100 g for fructose, and 105.3 to 609.9 meq/kg for free acidity. The results are compared to data obtained in previous studies and the regulatory quality standards and the nutritive value for the human diet are discussed.

Keywords: bee pollen, physicochemical composition, ash, lipid, protein, sugars, free acidity, Brazil.
and reduction of the toxic potential from alkaloids and other toxins (Schimdt and Buchmann, 1993).

The composition of Brazilian bee pollen has been shown to vary in terms of macronutrients (Funari et al., 2003; Marchini et al., 2006; Modro et al., 2007) and minerals (Funari et al. 2003) under controlled production conditions. The composition and quality of Brazilian bee pollen produced for commercial purposes have also been evaluated (Bastos et al., 2003; Almeida-Muradian et al., 2005; Barreto et al., 2005; Carpes, 2008), but in the majority of these investigations, the samples analyzed were from the Southern and Southeastern regions and the number of samples ranged from 8 to 42.

Official data for the production of Brazilian bee pollen are not available, but it is known that the current production is not sufficient to meet the national demand (SEBRAE, 2007). Brazil has a great potential for supplying this product because the weather and flora are highly favorable to its production. Brazilian apiary products are being increasingly exported to North America and Europe (Resende, 2010). However, there is lack of national compositional data, which would contribute to the characterization of the national bee pollen.

In the present work, determination of the Brazilian bee pollen composition (ash, lipid, protein, glucose, fructose, and free acidity) was carried out. The results are discussed in relation to regulatory quality standards and the nutritive value for the human diet, pointing to a need for reviewing such standards and of controlling the production of bee pollen.

**MATERIALS AND METHODS**

A total of 154 samples of dried, granulated Brazilian bee pollen ready for commercialization (dry, clean, packed products) was acquired directly from apiculture producers from 23 sites (Tab. 1). The samples, weighing 200-330 g, were mostly packed in plastic bags, with a few in glass containers, simulating commercialization practices. The samples arrived at our laboratory within a month of production; this process included collection, cleaning, dehydration, packaging, and transportation.

On arrival, the samples were packed under vacuum, in polyamide-polyethylene bags to avoid absorption of moisture and oxygen, and stored in a freezer at -16°C until the analyses were carried out (maximum of 3 months). On the day of analysis, the samples were quartered and then ground in a refrigerated mill (M20, IKA Labortechnik, Staufen, Germany). The samples were then sieved using 30-mesh sieves to standardize pollen particle size.

Analyses were carried out in triplicate for free acidity, and in duplicate for ash, lipid, protein, glucose, and fructose.

For ash determination, 2 g of the ground sample were weighed in a porcelain crucible. The sample was incinerated on a hotplate and in a muffle furnace (550°C) (Zenebon and Pascuet, 2005). Total lipid was determined after thermal hydrolysis of 5 g of the ground sample with hydrochloric acid. Lipids were extracted with petroleum ether in a Butt extractor and after removal of the solvent, the lipid content was determined by weight difference (Zenebon and Pascuet, 2005).

Nitrogen content was determined through acid hydrolysis and Kjeldahl distillation of 0.5 g of sample. Ammonia was distilled and collected in boric acid solution, and later titrated with a standard solution of hydrochloric acid (Zenebon and Pascuet, 2005). For the conversion of nitrogen levels to protein, the factor 5.60 was used (Rabie et al., 1983). The use of factor N x 6.25 would overestimate the protein content (Serra Bonvehí and Escolà Jordá, 1997). In order to compare our results with those of other studies which used the factor 6.25 for a nitrogen to protein conversion, we recalculated their values using factor 5.60. Some studies did not mention the factor used; their results were not included in the comparisons.
Glucose and fructose were quantified by HPLC, after extraction of sugars (Burgner and Feinberg, 1992). A sample of 2.5 g was mixed with 25 mL of distilled and deionized water, using an orbital agitator for 2 h, clarified with zinc acetate and potassium ferricyanide and filtered in qualitative filter paper. The sugar solution was then filtered in a 0.45 µm diameter membrane and injected into a Varian liquid chromatograph (model Pro Star, Mulgrave, Australia), equipped with a refractive index detector, model 350 RI, and a normal phase column (Luna NH2, 250 x 4.6 mm, 5 µm) (Phenomenex Inc., Torrance, USA), thermostated at 40°C. The mobile phase consisted of acetonitrile and water (85:15 v/v), the flow rate was 1 mL/min, and the injection volume was 20 µL.

Free acidity was determined by potentiometric titration of the pollen samples with a standard alkali solution (0.05 M NaOH), employing an automatic potentiometric titrator (model 785 DPM Titritro, Metrohm AG, Herisau, Switzerland), until titration endpoint, which occurred at pH 8.5 (Horwitz, 2006).

In order to verify if the means obtained from the analyses of samples from different states could be considered statistically different, Tukey’s multiple comparisons test was applied according to the approach method with different repetition numbers per state at a significance level of p<0.05, using the software Statistica for Windows 5.5 (StatSoft Inc., Tulsa, USA).

RESULTS

The composition of the samples analyzed is presented in Table 2. Ash level varied from 1.33 to 4.13 g/100 g. The highest levels being found in samples from Sergipe and the lowest in samples from Piauí.

Total lipids ranged from 4.01 to 13.32 g/100 g and total protein from 12.28 and 27.07 g/100 g. Samples from Paraná and Santa Catarina had higher lipid and lower protein contents, while those from Rio Grande do Sul had higher lipid. Minas Gerais and Espírito Santo had lower protein levels.

The results for sugars confirm the predominance of reducing sugars in bee pollen. Fructose had higher levels (12.59 to 23.62 g/100 g) than glucose (6.99 to 21.85 g/100 g) in all samples.
The free acidity ranged from 105.3 to 619.9 meq/kg, with samples from Bahia and Santa Catarina having the highest levels and those from Minas Gerais and the Federal District had lower levels.

**DISCUSSION**

The average levels of ash were similar to those found by Bastos et al. (2003) (1.5 to 4.8%) in pollens from the states of São Paulo and Minas Gerais, by Barreto et al. (2005) (1.62 to 3.97%) in samples from seven Brazilian states, and by Carpes (2008) (1.90 to 3.91%) in samples from Paraná, Santa Catarina, and Rio Grande do Sul. Brazilian and Argentinian regulations establish a maximum ash level of 4% w/w, dry basis, in bee pollen (Codigo Alimentario Argentino, 1998; MAPA, 2001). Only one sample from Sergipe would be above this limit. Campos et al. (2008) suggested a maximum value of 6% w/w. Considering this recommended limit, all samples in the present study would be adequate.

The ash content is influenced by soil type, geographical origin, floral species and the plant’s capacity to accumulate...
minerals in pollen (Serra Bonvehí et al., 1986). Compared to our results, Baldi Coronel et al. (2004) found greater variation (0.96 to 6.70%) of the ash levels in 37 samples of Argentinian pollen, 17% of which was above the regulation limit. The authors claimed that the samples were free from impurities of a mineral origin. The presence of mineral impurities, due to inefficient cleaning procedures, may increase the ash content (Baldi Coronel et al., 2004), which makes this analysis an important quality index for pollen (Orzáez Villanueva et al., 2002).

Spanish samples presented smaller variations in ash content. The range obtained by Serra Bonvehí and Escolà Jordà (1997) was 1.63 - 2.20% and that of Orzáez Villanueva et al., (2002) was 1.54 - 1.95%.

All 154 samples are within the limit stipulated by the Brazilian regulations for lipids, a minimum of 1.8 g/100 g, w/w lipids, dry basis (MAPA, 2001). The Argentinian regulations do not establish minimum lipid values for bee pollen, while Campos et al. (2008) suggested a minimum of 1.5 g/100 g lipids.

The lipid results of the present study are close to those of Bastos et al. (2003) (6.1 to 14.0 g/100 g). Barreto et al. (2005) obtained 2.56 to 6.17 g/100 g and Carpes (2008) 3.72 to 6.47 g/100 g.

Baldi Coronel et al. (2004) found lipid levels varying from 1.76 to 6.76 g/100 g in Argentinian pollen samples. In pollen from Spain, Serra Bonvehí and Escolà Jordà (1997) reported lipid levels which ranged from 4.8 to 7.2 g/100 g, while Orzáez Villanueva et al. (2002) obtained 3.60 to 8.96 g/100 g. Levels similar to those obtained in the present study were found by Szczęsna (2006a) in samples from Poland (13 samples), South Korea (9 samples) and China (5 samples), with results ranging from 6.74 to 10.99 g/100 g.

Proteins constitute the second most abundant group in bee pollen, after carbohydrates. Thus, some authors point out the value of pollen as source of this nutrient for human food (Orzáez Villanueva et al., 2002; Szczęsna, 2006b,c). The values obtained in our work are within the requirement of the Brazilian regulations, which establish a minimum value of 8 g/100 g, dry basis (MAPA, 2001). However, 11 samples (7%) had protein levels inferior to the lower limit established by the Argentinian law: 15 to 28 g/100 g, dry basis (Código Alimentario Argentino, 1998). Similar results were obtained by Bastos et al. (2003), who found protein levels between 15.8 and 27.8 g/100 g in 21 Brazilian commercial samples. Carpes (2008) obtained protein levels between 13.47 and 24.81 g/100 g in 36 samples from Southeastern and Southern Brazil. Almeida-Muradian et al. (2005) obtained an average protein level of 18.82 g/100 g in 10 samples from the Southern region of Brazil.

In studies carried out with samples from other countries, protein levels of 18.46 and 25.00 g/100 g were obtained in two dried pollen samples from different species of eucalyptus (Bell et al., 1983). Protein levels were between 12.6 and 18.2 g/100 g (Serra Bonvehí and Escolà Jordà, 1997) and between 9.89 and 16.56 g/100 g (Orzáez Villanueva et al., 2002), in dried pollen samples from Spain. Szczęsna (2006b) found that in bee pollen from Poland (13 samples), South Korea (9 samples) and China (5 samples) protein values ranged from 15.80 to 24.14 g/100 g, 17.63 to 24.51 g/100 g and 17.83 to 26.13 g/100 g, respectively.

The great variability of protein contents found in bee pollen can be partly explained by the natural compositional variation. Such variation is influenced by floral origin, biological, ecological, and geographic factors during production as well as handling and storage conditions (Orzáez Villanueva et al., 2002).

The water contents of the samples analyzed in the present study were published in a previous paper (Morgano et al., 2011). These water contents are also
shown in Table 2 to complete the data for physicochemical composition. Moisture content ranged from 3.00 to 9.39%, with samples from Mato Grosso, Sergipe, Bahia, Piauí, Ceará and Federal District, showing statistically higher levels. Most samples (92%) do not comply with the current Brazilian regulation, which allows a maximum limit of 4% for the moisture content of dried bee pollen (MAPA, 2001). This result indicates how difficult it has been for the Brazilian bee pollen producers to keep the product within this standard. Some authors (Serra Bonvehí and Escolà Jordà, 1997) recommend that the drying process should result in a final moisture content between 4 and 8% to ensure bee pollen quality and stability during storage. Orzáez Villanueva et al. (2002) assert that a moisture level of 4.59 to 9.96 indicates adequate processing, while Baldi Coronel et al. (2004) consider a moisture level from 6 to 7% safe to prevent bacterial and fungal growth. Many countries have more tolerant moisture limits for dried bee pollen: Switzerland and Poland (6%), Argentina and Uruguay (8%) and Bulgaria (10%) (Baldi Coronel et al., 2004; Campos et al., 2008).

In the present study, the predominance of reducing sugars in bee pollen where the fructose levels surpass those of glucose, is in accordance with previous papers. Serra Bonvehí and Escolà Jordà (1997) found fructose values ranging from 15.20 to 22.40 g/100 g and glucose from 10.86 and 17.90 g/100 g for bee pollen samples from Spain. In a study with bee pollen samples from Poland, South Korea, and China (Szczęsna, 2007), the ranges were 15.51-19.22, 12.09-22.06, and 9.74-17.90 g/100 g for bee pollen samples from Spain. In a study with bee pollen samples from Poland, South Korea, and China (Szczęsna, 2007), the ranges were 15.51-19.22, 12.09-22.06, and 9.74-17.90 g/100 g, respectively, for fructose. The corresponding values for glucose were 9.85-14.29, 9.94-20.06, and 8.45-15.10 g/100 g. Qian et al. (2008), analyzing five bee pollen samples from Israel, China, Romania, and Spain found the following mean values: 15.9; 17.5; 16.0; 18.6, and 19.6 g/100 g, respectively, for fructose and 8.2; 13.1; 11.0; 12.1 and 12.2 g/100 g, respectively, for glucose.

The carbohydrate group, which includes sugars, starch and dietary fiber, represents the main component of the bee pollen samples. According to Human and Nicholson (2006), carbohydrates are derived from floral pollen and from nectar, with which the floral pollen is mixed by bees to form bee-collected pollen.

Fructose, glucose and sucrose are the principal free sugars in bee pollen. Smaller amounts of the disaccharides maltose, isomaltose, trehalose and the trisaccharides melezitose, erlose and raffinose are also found (Serra Bonvehí and Escolà Jordà, 1997). A recent study also reported the presence of the tetrasaccharide stachyose in pollen samples from Israel, China, Romania and Spain (Qian et al., 2008).

Serra Bonvehí and Escolà Jordà (1997) found a fructose/glucose index (F/G) of 1.13 to 1.53 in samples of Spanish pollen, while Qian et al. (2008) obtained 1.53 and 1.66 for two Spanish pollen samples and 1.94, 1.33, 1.45 for samples collected from Israel, China and Romania, respectively. In the current work, this index ranged from 1.01 to 2.24, the mean value being 1.31. The F/G index has been related to the glycemic index in honey samples (Ishayek and Kern, 2006). It is believed that an elevated F/G index is one of the reasons for the low to moderate glycemic index for honey (Foster-Powell et al., 2002). Future studies are necessary to verify the glycemic index values of Brazilian pollen, especially those with higher F/G indexes. High indexes were found in the present work in two samples, one from Sergipe (2.24) and the other from Santa Catarina (2.10).

Bee pollen is naturally acidic, with the pH ranging from 4 to 6 (Herbert and Shimanuki, 1978; Código Alimentario Argentino, 1998; MAPA, 2001; Bastos et al., 2003; Barreto et al., 2005; Marchini et al., 2006). Brazilian regulation stipulates a maximum limit of 300 meq/kg (MAPA, 2001). The percentage of samples that surpasses this limit is 50% in the present
study. In Barreto et al. (2005), high free acidity levels were found in samples from the state of Bahia (430 meq/kg) and Santa Catarina (380 meq/kg). The Argentinian regulations do not require determination of free acidity in bee pollen (Codigo Alimentario Argentino, 1998). Campos et al. (2008) do not recommend this analysis as a quality index of the product.

CONCLUSIONS

1. The physicochemical composition of dried bee pollen samples shows great variation among the samples from the different states.

2. Substantial amounts of proteins in bee pollen may justify its use in human food, provided that an effective quality control is assured.

3. Since the bee pollen has a fructose/glucose ratio equal or superior to honey, indicative of products of moderate to low glycemic index, it is recommended that its glycemic index be investigated.

4. The results of the present work can contribute towards updating Brazilian and international regulation standards for bee pollen.

REFERENCES


FIZYKOCHEMICZNY SKŁAD PYŁKU PSZCZELEGO POCHODZĄCEGO Z JEDENASTU STANÓW BRAZYLII


Streszczenie
Pyłek pszczeli, często określany jako naturalny suplement diety, jest wysoko cenionym produktem dzięki dużej zawartości składników odżywczych i innych związków prozdrowotnych. W niniejszej pracy przeprowadzono badania 154 próbek pyłku pszczego pozyskanych w 23 pasiekach pochodzących z 11 stanów Brazylii. Próbki te przebadano pod względem zawartości popiołu, tłuszcza, białka, glukozy, fruktozy oraz wolnej kwasowości.

Wyniki wykazały duże różnice w zawartości wymienionych składników w próbkach pyłku pochodzących zarówno z poszczególnych stanów jak i pomiędzy stanami: 1,33 do 4,13 g/100 g popiołu; 4,01 do 13,32 g/100 g tłuszcza; 12,28 do 27,07 g/100 g białka; 6,99 do 21,85 g/100 g glukozy; 12,59 do 23,62 g/100 g fruktozy oraz wolnej kwasowości w zakresie od 105,3 do 609,9 meq/kg. Według rozporządzenia obowiązującego w Brazylii, poziom popiołu w próbkach pyłku pszczego nie może przekraczać 4,00 g/100 g suchej masy. Zgodnie z tym wymaganiem, jedna próbka ze stanu Sergipe posiadała nieco wyższą zawartość tego składnika niż rekomenowana wartość (4,13 g/100 g). W odniesieniu do lipidów, wszystkie badane próbki mieściły się w granicach określonych przez brazylijskie rozporządzenia, wg którego minimalna wartość to 1,8 g/100 g suchej masy. Poziom białka również mieścił się w wymaganiach obowiązującego rozporządzenia, które określa minimalną zawartość tego składnika na poziomie 8 g/100 g suchej masy. Wysoka zawartość białka i lipidów w badanych próbkach pyłku pszczego może uzasadniać jego wykorzystanie w diecie człowieka, pod warunkiem zapewnienia skutecznej kontroli jakości produktu. Pyłek kwiatowy zawiera wysokie ilości cukrów redukujących; średnia łączna zawartość glukozy i fruktozy w badanych próbkach wynosiła 34 g/100 g pyłku. Zawartość fruktozy we wszystkich próbkach była na wyższym poziomie niż glukozy. W badanych próbach pyłku stosunek fruktozy do glukozy był równy lub wyższy niż w miodzie i zgodny z produktami o umiarkowanym lub niskim indeksie glikemicznym. Badania w tym kierunku powinny być kontynuowane. Mimo, że naturalny pyłek kwiatowy ma odczyn kwaśny, brazylijskie rozporządzenie określa maksymalny limit na poziomie 300 meq/kg wolnej kwasowości. W niniejszych badaniach 50% próbek przekraczała ten limit. Dyskusyjnym jest zatem, czy omawiany parametr może być wykorzystywany jako wskaźnik jakości produktu.

Sklad chemiczny wysuszonych próbek pyłku pszczego uzyskany w niniejszych badaniach wskazuje na duże zróżnicowanie wśród próbek z różnych stanów, co może być wynikiem oddziaływania wielu czynników, które wpływają na skład produktu. Uzyskane wyniki mogą być wykorzystane do uzupełnienia wymagań dla pyłku pszczego określonych w brazylijskim oraz międzynarodowych standardach dla tego produktu.

Słowa kluczowe: pyłek pszczeli, skład fizykochemiczny, popiół, lipidy, białko, cukry, wolna kwasowość, Brazylia.