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Water activity of fresh bee pollen and mixtures of bee pollen-honey of different botanical origin

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A b s t r a c t

A mixture of bee pollen and honey could represent a complete food supplement for human diet. In this study, for three bee pollen-honey mixtures ratios at two storage times (0 and 90 days) at room temperature, water activity (aw) were investigated. For the mixtures were employed Castanea and Eucalyptuspollen species, and acacia, chestnut and "beach" honeys. In the mixtures, Castanea and Eucalyptus pollen showed a different aw trend in relation to pollen concentration and to storage time. In Castanea-honeys mixtures and in Eucalyptus-honey mixtures (only at time 0) aw values were higher than each component of the corresponding mixtures. In Eucalyptus-honeys mixtures (only at time 90) aw values were lower than each component. This investigation highlights that honey, bee-pollen and their mixtures, stored at room temperature, could have a little change in their aw content, in time. Results shows that aw values of bee pollen mixed with honey were all lower than 0.7, although bee pollen-honey mixtures were stored at room temperature instead at freezing temperature (- 15 °C) for 90 days.

1. Introduction

Bee pollen contains a wide variety of bioactive compounds, deriving from its botanical origin, that make bee pollen a food of considerable interest (De Arruda, Pereira, De Freitas, Barth, & De Almeida-Muradian, 2013; Gabriele et al., 2015; M arg aoan et al., 2014). Bee pollen contains all the essential amino acids, in concentrations that are five to seven times higher than those found in traditional high protein foods (Campos et al., 2008; Herbert & Shimanuki, 1978). Additionally, carbohydrates, fatty acids, vitamins (A, D, K, C, E, B5 and pro-vitamin A) and bioflavonoids are contained in bee pollen (Campos et al., 2010; Costa, Morgano, Ferreira, & Milani, 2017; de Florio Almeida et al., 2016; Feas, Vazquez-Tato, Estevinho, Seijas, & Iglesias, 2012; Sagona et al., 2017; Schulte, Lingott, Panne, & Kneipp, 2008). Bee pollen is normally commercialized fresh-frozen or dried. Honey may be another natural preservative for pollen (Campos et al., 2008). Honey is a nutritious food of worldwide economic importance. It is a complex mixture of carbohydrates, proteins, enzymes, amino acids, lipids, vitamins, volatile chemicals, phenolic acids, flavonoids and minerals (Ajibola, Chamunorwa, & Erlwanger, 2012; Ball, 2007). This mixture give honey preservative capacity and storage stability but tends to be affected by weather conditions, processing, manipulation, packaging and storage time (Denisow & Denisow-Pietrzyk, 2016; Escuredo, Dobre, Fernandez-Gonzalez, & Seijo, 2014). Pollen-honey mixture has been investigated for antinociceptive, anti-inflammatory, gastroprotective and antioxidant effects (Akkol, Orhan, Gürbüz, & Yesilada, 2010). Furthermore, the addition of bee pollen in a honey-royal jelly mixture has led to an increase in the total phenolic content (€Ozk€ok & Silici, 2017). So, the investigation on storage and properties of bee pollen-honey mixture could be interesting to formulate a new possible

nutraceutical food, with both bee pollen and honey proprieties. Among the parameters that might influence food shelf life, water activity (aw) plays an important role, since it reflects the amount of water that is available to spoilage microorganisms (bacteria, fungi and yeasts). In order to grow, microorganisms require a minimum aw value which varies with each microorganism (Jay, 2007, p. 745). Yeasts and molds generally have lower aw requirements than bacteria, and growing to aw value over 0.61 (Jay, 2007, p. 745). The aw value of honey generally varies between 0.5 and 0.6 (White, Riethof, Subers, & Kushnir, 1962), and as such it is low enough to inhibit most microorganisms. Indeed, most spoilage bacteria do not grow below aw of 0.91, with 0.75 being the minimum reported value for foodborne bacteria, whereas osmophilic yeasts have been reported to growat aw values of 0.61 (Jay, 2007, p. 745). Osmophilic yeasts can pose a problem for the honey industry since they are not inhibited by sucrose and can grow in acidic condition, and as such they can ferment honey, especially in case of increased moisture (Snowdon & Cliver,1996). Hence, it is important to consider aw values when studying the shelf life of honey-based products. The legislation for commercial bee pollen, Brazil, Argentina, Switzerland and Bulgaria are the only countries to require bee pollen moisture limits (4%, 8%, 8% and 10%, respectively) and aw values lower than 0.61 (Campos et al., 2008), for the dried product. The aims of this work are:

1) the measurements of the aw of Castanea and Eucalyptus fresh bee pollens;

2) the measurements of the aw in acacia, chestnut and “beach” honeys;

3) the measurements of aw of mixtures of bee pollen and honey.

2. Material and methods

2.1. Samples

Two fresh-frozen bee pollen samples, commercialized as Castanea and Eucalyptus monofloral bee pollen and wrapped in sealed packages, were used for this investigation. Organic Castanea bee pollen was obtained from a local beekeeper in the Lucca province (Tuscany, Italy) (2015, harvest)while organic Eucalyptus bee pollen was bought from a Sardinian beekeeper (Italy) (2015, harvest).Three organic honey samples (acacia, chestnut and a particular polyfloral called “beach honey”), produced in Pisa province (Tuscany, Italy) (2015, harvest) were employed in this investigation. On all bee pollen and honey samples, a palynological analysis (Louveaux, Maurizio, & Vorwohl, 1978) was performed to confirm their botanical origin. Pollen loads were divided in groups according to the color. The frequency of each species was determined by weigh of the respective pollen on a total of 5 g of the mixture (Ricciardelli D'Albore, 2007). One microscope slide for each group was prepared by washing the pollen with distilled water and using glycerol gelatin (Sigma; St. Louis, MO, USA) for permanent preparations. For the melissopalynological analysis, 40 ml of water were added to 10 g of honey. After two centrifuges, the pellet was collected, dried and fixed in a microscope slide using glycerol gelatin. Pollen grains identification was performed by optical microscope with total magnification of 400x and 1,000x. A reference collection of Pisa University and different pollen morphology guides were used for the recognition of the pollen types. Melissopalynological profiles were compared with guidelines by Colombo et al. (2007), in order to confirm the honey samples' botanical origin. Analysis nomenclature was in agreement with Persano Oddo & Ricciardelli D'albore (1989). Three mixtures containing different pollen (Castanea or Eucalyptus) and honey (acacia, chestnut and “beach honey”) ratios 80 g/100 g pollen and 20 g/100

g honey, 80P:20H; 50 g/100 g pollen and 50 g/100 g honey, 50P:50H; 20 g/100 g pollen and 80 g/100 g honey, 20P:80H were investigated.

2.2. aw analysis

The values of aw, in bee pollens, honeys and mixtures, were measured using a Rotronic Hygropalm HP23-AW-A-SET-14®(Rotronic Italia srl, Milano, Italy). The value of aw is obtained as the ratio of partial pressure of water vapor above the surface of the product to pressure of saturated water vapor at the same temperature. The measurement started simultaneously for both probe inputs, relative humidity and temperature. All aw determinations of bee pollen and honey were carried out in triplicate at collection time of samples (t0), when sterile packages were opened for the study (within one or two months of production and commercialization) and after three months (t90). The aw content of pollen and honey mixtures was also evaluated after one month (t30). Samples were conserved at room temperature in screw-cap sealed plastic containers.

2.3. Statistical analysis

Statistical analysis was performed using SAS Institute (2008). The differences between samples were analysed with non-parametric Kruskal-Wallis Test (Rank Sums). When significant differences were found among aw values, means were ordered and differences were again tested with non-parametric Kruskal-Wallis Test. P-values <0.05 were considered statistically significant.

3. Results

3.1. Palynological analysis of bee pollen and honey samples

The Castanea monofloral bee pollen sample was composed of 84% Castanea, 15% Rosa f. and 1% Echium pollens. The Eucalyptus monofloral bee pollen sample was composed of 96% Eucalyptus f. and 4% Trifolium repens gr. pollens. Melissopalynological analysis (Table 1) confirmed the botanical origin indicated in the commercial label of honey samples. As reported in guidelines by Colombo et al. (2007), the melissopalynological analysis showed, in the Acacia honey, a presence of 16% Robinia pollen (higher than 15%, expected minimum value for Acacia monofloral honey) and, in the Cheshunt honey, 99% Castanea pollen (higher than 90%, expected minimum value for Cheshunt monofloral honey).

3.2. aw analysis of bee pollen and honey samples

The aw values of Castanea and Eucalyptus bee pollens at t0 and t90 are reported in Table 2. Only the aw value of Eucalyptus pollen samples increased after storage at room temperature for 90 days ($p < 0.05$). The two bee pollens were significantly different in aw contents at both sampling times and Eucalyptus bee pollen always showed the highest value. The aw values of the three honeys differed significantly at both sampling times (Table 3). Chestnut and “beach honey” showed always the highest and the lowest aw contents, respectively. At t90 “beach honey” aw value was significantly increased compared to t0 ($p < 0.05$) while those of chestnut and acacia honeys were not significantly modified.

3.3. aw analysis of bee pollen-honey mixtures

In all the mixtures with Eucalyptus bee pollen and honey, aw varied during the experimental period ($p < 0.05$), while those with Castanea bee pollen varied significantly only in some pollen/honey

ratios. Comparing aw t90 values to those at t0, Castanea bee pollenhoney mixtures showed a significant increase only in acacia honey and in two chestnut honey mixtures (50P:50H, 80P:20H), but not in “beach honey” mixtures (Table 4). Instead, in Eucalyptus bee pollen honey mixtures, aw t90 values decreased significantly respect to aw t0 values in all mixtures ratios (Table 4). At time t0, all the three mixtures with Castanea pollen and Acacia honey showed aw values higher than those of the Castanea pollen or Acacia honey alone (Fig.1a). The same trend was observed in chestnut honey, except in the mixture 50P:50H where the aw value was not significantly different from that of Castanea pollen. In the other hand, the mixtures of Castanea pollen with “beach honey”, aw value was significant higher only in the mixture 20P:80H compared to each individual component of the same mixture (Fig. 1a). At time t90, in the mixtures with Castanea bee pollen and acacia or chestnut honeys, aw values were significantly higher than each component alone. Instead, in “beach honey” no significant differences were present between the mixtures and each component of the corresponding mixture (Fig. 1a). The three mixtures of Eucalyptus bee pollen at time t0 with the three honeys, aw values were significantly higher than those of each component (Fig. 1b). On the other hand, at time t90 aw values of all mixtures were significantly lower compared to those of each component (Fig. 1b), except for Eucalyptus pollen and chestnut honey (80P:20H).

4. Discussion

Field harvested bee pollen shows an aw value ranges from 0.62 to 0.82 (De-Melo et al., 2015) higher than our results where fresh frozen bee pollen samples have been employed. Conversely, in our investigation, the aw values observed for both bee pollen types are higher than those reported in literature for the commercialized bee pollen; in details, the range previously reported for aw pollen is 0.20e0.55 (Carpes, Mourao, De Alencar, & Masson, 2009; Deveza et al., 2015; Estevinho, Rodrigues, Pereira, & Feas, 2012; Feas et al., 2012; Kostic et al., 2017; Nogueira, Iglesias, Feas, & Estevinho, 2012; Serra Bonvehí & Jorda, 1997). The fact that observed aw values for bee pollen are higher than those reported in literature could be because in the cited studies dried bee pollens were used, while in our study we evaluated fresh-frozen bee pollen samples. In our investigation, only Eucalyptus bee pollen showed a significant increase of aw after 90 days of storage. The obtained value 0.62 is low enough to avoid bacteria growth. Pathogen bacteria (such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa) need aw values higher than 0.7 to grow (Jay, 2007, p. 745). Furthermore, it has been prooved that bee pollen inhibits Staphylococcus aureus and Enterococcus faecalis in vitro (Fratini et al., 2014). As reported by several Authors (Anderson et al., 2014; Bang, Buntting, & Molan, 2003; Kwakman et al., 2010) increasing aw in stored bee pollen will bring to higher pH, that will lead to hydrogen peroxide production by glucose oxidase, inhibiting microbial growth (Sagona et al., 2015). Honey samples showed aw values lower than 0.7 (0.7 is the limit for pathogen bacterial growth) (Jay, 2007). This data is in agreement with those reported from several Authors that reported aw mean values for honey ranging from 0.47 to 0.64 (Ayvaz, 2017; Manzanares, García, Gald on, Rodríguez-Rodríguez, & Romero, 2017; Olaitan, Adeleke, & Ola, 2007). The values of aw resulted also lower than 0.61, critical value for osmopholic yeast growth, that ensure relatively long shelf life of the honey samples (Ayvaz, 2017; Gleiter, Horn, & Isengard, 2006; Zamora & Chirife, 2006). The aw values recorded in the bee pollen and honey mixtures were also lower than the 0.7 limit. The highest aw value (0.67) was shown in Eucalyptus bee pollen when added to chestnut honey and “beach honey” (80P:20H). All mixtures of Eucalyptus bee pollen and honeys showed a decrease of aw values from t0 to t90. Although the size and variety of samples are small, it could be hypothesized that the aw values decrease is possible due to:

1) during the storage of the mixture both pollen and honey could release water, which then evaporates following the opening of the jar;

2) both honey and pollen could form chemical links with the free water.

The mixtures with Castanea bee pollen and honeys showed different trends. The values of aw of mixtures of Castanea bee pollen and acacia or chestnut honeys generally increased at t90. The increase of the aw values in the mixture after the storage could be due to two hypotheses:

1) Pollen releases water to the honey,

2) Honey takes water, that it was bonded in chemical links, to the pollen.

It is noteworthy that bee pollen is the pollen collected from bees and mixed with bee proteins, and other unknown compounds, in order to assemble it in small balls (pollen loads) for the stocking in the hive. Indeed, other parameters should be considered while trying to explain aw changes, such as the unknown compounds, the external sculpture of pollens, their size and the possible presence of essential oils on their surface. In order to clarify the water transfer between pollen and honey in the mixture, it may be interesting to measure aw values in mixtures created by counting the number of pollen loads, because pollen grains from different botanical species have different dimensions and therefore different weights.

5. Conclusions

This work was focalized on a food conservation parameter, the aw value of fresh bee pollen and honey. At the best of our knowledge, no data were previously reported for aw values in fresh bee pollen, but only for the dried product. Furthermore, the investigation has been extended to the bee pollen-honey mixtures. Results suggest that honey, bee-pollen and their mixtures, stored at room temperature could modify their aw content in time. Although the room temperature is not the optimal storage temperature for the fresh-frozen bee pollen, it did not drastically modify the aw values, as expected. This no drastically aw values modification suggests that the organoleptic and nutritional properties of bee pollen and honey do not change over time, as they do not change in the bee product mixture (De-Melo et al., 2015; Ozkok & Silici, 2017). After three months at room temperature, the aw values are still low enough to avoid bacterial growth, but the data don't exclude the possible yeast growth, that could cause a modification on the food shelf life. It could be interesting to continue the investigation with a bigger number of samples, with a different botanical origin, counting pollen loads (instead weighing) to mix to the honey and with different times and temperatures of storage. Furthermore, it could be interesting to screen the bacteria and yeast that could growth on bee pollen-honey mixtures.

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Table 1
Melissopalynological analysis, identification of pollen species present in the tested honeys.

	Acacia honey	Chestnut honey	"Beach honey"
Astragalus/Ononis f.	–	–	1
Brassica f.	5	P	2
Carex	–	–	P
Castanea	4	99	68
Centaurea cyanus	P	–	–
Cistus ladanifer gr.	–	P	–
Clematis	–	P	2
Compositae H	–	–	P
Compositae T	–	–	P
Cornus sanguinea	1	–	–
Coronilla/Hippocrepis f.	–	P	2
Echium	–	–	P
Erica f.	–	P	1
Eucalyptus f.	–	P	2
Fraxinus	29	P	2
Genista f.	–	P	1
Graminaceae	1	P	P
Hedysarum	2	P	–
Lonicera f.	–	–	P
Lotus	3	–	1
Melilotus	–	–	P
Olea f.	3	P	1
Papaver f.	–	P	1
Parthenocissus	–	–	P
Plantago	–	P	P
Potentilla f.	–	–	P
Poterium	P	P	–
Prunus f.	–	P	P
Quercus ilex gr.	18	P	3
Quercus robur gr.	3	P	P
Robinia	16	P	1
Rubus f.	–	P	2
Salix	1	–	P
Sambucus nigra	6	–	1
Sedum f.	–	P	3
Sinapis f.	–	P	–
Tamarix	–	–	P
Tilia	–	P	P
Trifolium pratense gr.	–	P	5
Trifolium repens gr.	–	P	2
Umbelliferae	–	P	–
Verbascum f.	4	–	P
Vicia	3	–	–

Results were rounded to integer numbers, "<1" was used for values between 0.2 and 0.5%, for lower percentages only presence (P) was reported.

Table 2
a_w values (averages and standard deviation, n = 3) of bee pollen samples.

Pollen	a_w t0	a_w t90
	Average (s.d.)	Average (s.d.)
Castanea	0.52^{aB} (0.01)	0.51^{aB} (0.01)
Eucalyptus	0.59^{bA} (0.01)	0.62^{aA} (0.00)

Different lowercase letters in the same row show a statistically significant difference ($p < 0.05$); different uppercase letters in the same column show a statistically significant difference ($p < 0.05$).

Table 3
a_w values (averages and standard deviation, n = 3) of honey samples.

Honey	a_w t0	a_w t90
	Average (s.d.)	Average (s.d.)
"Beach honey"	0.54^{bA} (0.00)	0.56^{aA} (0.00)
Acacia	0.50^{aB} (0.01)	0.52^{aB} (0.00)
Chestnut	0.43^{aC} (0.01)	0.46^{aC} (0.00)

Different lowercase letters in the same row show a statistically significant difference ($p < 0.05$); different uppercase letters in the same column show a statistically significant difference ($p < 0.05$).

Table 4
a_w values (averages and standard deviation, n = 3) of bee pollen-honey mixtures.

Mixtures	a_w t0	a_w t30	a_w t90
	Average (s.d.)	Average (s.d.)	Average (s.d.)
Castanea + acacia honey (20P:80H)	0.57^{b} (0.00)	0.54^{c} (0.00)	0.59^{a} (0.01)
Castanea + acacia honey (50P:50H)	0.55^{b} (0.00)	0.54^{b} (0.00)	0.57^{a} (0.00)
Castanea + acacia honey (80P:20H)	0.56^{b} (0.00)	0.54^{c} (0.01)	0.58^{a} (0.00)
Castanea + chestnut honey (20P:80H)	0.53^{a} (0.00)	0.55^{a} (0.02)	0.56^{a} (0.01)
Castanea + chestnut honey (50P:50H)	0.51^{c} (0.00)	0.53^{b} (0.01)	0.56^{a} (0.01)
Castanea + chestnut honey (80P:20H)	0.55^{b} (0.00)	0.54^{b} (0.00)	0.58^{a} (0.00)
Castanea + beach Honey (20P:80H)	0.55^{a} (0.00)	0.52^{b} (0.01)	0.48^{c} (0.01)
Castanea + beach Honey (50P:50H)	0.54^{b} (0.00)	0.58^{a} (0.00)	0.51^{c} (0.01)
Castanea + beach Honey (80P:20H)	0.54^{a} (0.00)	0.54^{a} (0.01)	0.54^{a} (0.00)
Eucalyptus + acacia honey (20P:80H)	0.62^{a} (0.00)	0.52^{b} (0.00)	0.48^{b} (0.03)
Eucalyptus + acacia honey (50P:50H)	0.63^{a} (0.00)	0.56^{b} (0.00)	0.48^{c} (0.00)
Eucalyptus + acacia honey (80P:20H)	0.63^{a} (0.00)	0.49^{b} (0.00)	0.46^{c} (0.00)
Eucalyptus + chestnut honey (20P:80H)	0.64^{a} (0.00)	0.52^{b} (0.01)	0.50^{c} (0.00)
Eucalyptus + chestnut honey (50P:50H)	0.63^{a} (0.01)	0.54^{b} (0.00)	0.52^{c} (0.00)
Eucalyptus + chestnut honey (80P:20H)	0.67^{a} (0.01)	0.46^{b} (0.02)	0.46^{b} (0.00)
Eucalyptus + beach Honey (20P:80H)	0.66^{a} (0.03)	0.53^{b} (0.01)	0.52^{b} (0.00)
Eucalyptus + beach Honey (50P:50H)	0.66^{a} (0.00)	0.54^{b} (0.01)	0.48^{c} (0.00)
Eucalyptus + beach Honey (80P:20H)	0.67^{a} (0.00)	0.51^{b} (0.00)	0.45^{c} (0.00)

Different lowercase letters in the same row show a statistically significant difference ($p < 0.05$).
Averages and standard deviation was calculated for 3 replicates.

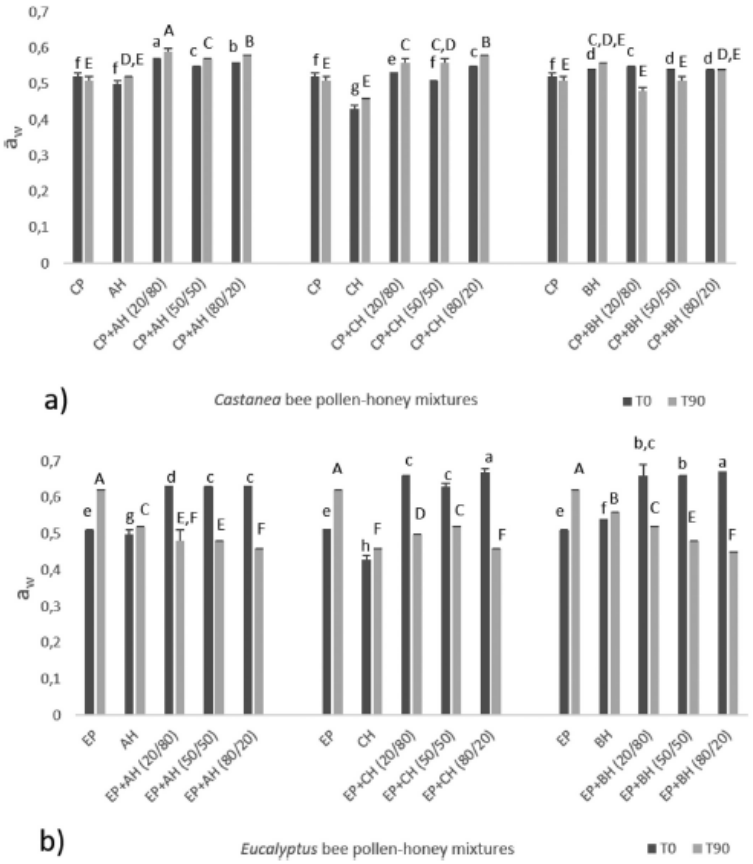

Fig. 1. Comparisons among the mixtures at the different times (T0 and T90). a) *Castanea* bee pollen with the three honeys at T0 and T90; b) *Eucalyptus* bee pollen with the three honeys at T0 and T90. Different letters above the bars (lowercase and uppercase letters to compare T0 or T90 data, respectively) in the same histogram show a statistically significant difference ($p < 0.05$). Legend: CP= Castanea bee pollen; EP = Eucalyptus bee pollen; AH = Acacia honey; CH=Chestnut honey; BH= Beach honey.