THE EFFECT OF VARIOUS STORAGE METHODS ON ORGANOLEPTIC QUALITY OF BEE POLLEN LOADS

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S u m m a r y

The aim of this research was to evaluate the effect of storing frozen pollen loads in controlled and/or air atmosphere, on organoleptic properties of the product. Collected pollen loads were cleared of mechanical impurities and preserved as follows: frozen and stored in air atmosphere; frozen and stored in an artificial atmosphere of a carbon dioxide and nitrogen mixture (30% CO_2, 70% N_2); frozen and stored in pure nitrogen (100% N_2); frozen and stored using a vacuum system; dried out at ca. 40°C and stored in air atmosphere.

In an organoleptic study, all tested pollen samples, irrespective of their storage method, were evaluated positively. The studied storage factors did not affect the shape, specific identity or aroma of pollen loads. The average point scores obtained for these features were similar in all groups. Storage conditions did influence the color and flavor of stored pollen loads. The experiment revealed significant differences in the perception of taste and aroma of tested pollen samples depending in the sex of the testing person. The taste and aroma of pollen loads were more appealing to men than to women testers.

Keywords: bee pollen loads, sensory evaluation, conservation, storage.

INTRODUCTION

Over the last few years, consumers have shown an increased interest in products of natural origin possessing very high nutritive and dietetic value as well as products which enhance the treatment of many diseases (Wade, 1978). Bee products, including pollen loads, surely belong to this category (Campos et al., 2008, 2010).

Bee pollen consists of male reproductive cells of seminiferous plants and is produced in flower anthers. As food for bees, pollen constitutes the main source of protein necessary for bee larvae and young workers (Crailsheim and Stolberg, 1989; Crailsheim et al., 1992; Camazine et al., 1998; Hrassnigg and Crailsheim, 1998). Apart from proteins, pollen also contains fats, carbohydrates, minerals and vitamins (Szczęsna et al., 2002; Szczęsna, 2006a, 2007a, 2007b; Campos et al., 2008). Compared to protein of other food products, pollen protein is noted for its high content of exogenous amino acids (Szczęsna et al., 1995c; Szczęsna, 2006b, 2006c).

Bee foragers transport pollen to the hive in pollen baskets (or *corbicula*, i.e. depressions in the tibia of the hind pair of legs), shaping them into pollen loads, i.e. pellets of pollen moistened with a bit of honey or nectar (Schönfeld, 1955; Snograss, 1956; Skowronek, 2008). Pollen of entomophilous plants is of higher nutritive value than that of anaemophilous plants. It is also easier for foraging bees to shape pollen of entomophilous plants into pellets.

Fresh pollen loads collected by beekeepers contain ca. 20-30% water. Such a high humidity renders them susceptible to the development of microorganisms,

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mainly bacteria and molds, whose presence precipitates spoilage of pollen loads. In order to prevent spoilage, loads have to be harvested from pollen traps daily and immediately frozen (kept in a freezer for a min. of two days to destroy all vegetative forms of microorganisms and mites), or preserved by drying. Frozen pollen can also be dried, but not later than a few hours after thawing. Quick drying is done to prevent spoilage caused by the development of survival forms of the mentioned pests or drying is done to prevent spoilage caused by the accidental secondary introduction of microorganism. Yet, even if stored under optimal conditions, loss of valuable components cannot be completely prevented in the pollen loads. Madzgaraševili et al. (1982) proved that the content of carotene in loads stored for four months, in the atmosphere of carbon dioxide at various temperature regimes, dropped by 60%. Hagedorn and Burger (1968) demonstrated that during the time of storage, contents of ascorbic acid decreased by ca. 100 mg/g, both in frozen and in dried loads. Vachonina and Bodrova (1979) reported that ascorbic acid content decreased by 30-52% during a 10-11 month storage. Mačekas and Astrauskiene (1988) found the slowest decrease in ascorbic acid content - in pollen loads conserved with powdered sugar, and the fastest - in dried ones.

Youssef et al. (1978) did not find significant effects from various temperatures of storage on the total protein content in dried pollen loads. Studies by Vachonina and Jakovleva (1979) showed that protein nitrogen content in dried pollen loads decreased by ca. 16% when vacuum-stored at room temperature for twelve months, and by ca. 7% when stored in the same time frame and at the same temperature regime but in a carbon dioxide environment.

Most results refer to dried pollen loads, yet present-day beekeepers are more and more interested in selling pollen loads in a form other than dried. Thus, there has arisen a need to develop new forms of pollen load preservation. Conservation methods can bring about changes in organoleptic properties of the product as well as in its chemical composition (Szczęsna et al., 1995a). The researchers comparative study of various ways of conservation, proved freezing to be the best method of all. There is very little change in the chemical composition of stored pollen loads when freezing is used. Lyophilisation is less effective as it causes a decrease in the content of vitamin C and provitamin A. The method with the most negative effect is drying at a temperature ca. 40°C, as it causes a considerable decrease in the content of sugars, protein and vitamins. A chosen conservation method also significantly affects the chemical composition of pollen loads during their storage (Szczęsna et al., 1995b, c, d, e). The researchers demonstrated that the fastest changes occur in pollen loads with high water content, i.e. in those that were only frozen; and slowest - in highly dehydrated, i.e. lyophilisated pollen loads. The changes are also dependent on the temperature of storing. The slowest rate of decrease in the content of labile components was observed when storing at a temperature of 4°C. The most negative effect was proven for room temperatures (18-26°C) at which the rate of changes in the chemical composition of pollen loads is the fastest. The researchers' studies also point out that substituting air atmosphere with pure nitrogen considerably limits the decreases in the content of pollen load components determining nutritive value of the loads.

The aim of this study was to determine the effect of the methods for pollen load storage in air and controlled atmosphere, on organoleptic properties of the product.

MATERIALS AND METHODS

The assay was carried out in the Apiculture Division of the Warmia and Mazury University in Olsztyn, Poland. Collected pollen loads were cleared of mechanical impurities and preserved in the following ways:

Group I - frozen at the temperature -18°C, in air atmosphere;

Group II - frozen at the temperature -18°C, in air atmosphere substituted with carbon dioxide and nitrogen gases mixture (30% CO_2 and 70% N_2);

Group III - frozen at the temperature -18°C, in air atmosphere substituted with pure nitrogen (100% N_2);

Group IV - frozen at the temperature -18°C, and vacuum-stored;

Group V - dried at the temperature ca. 40°C, then for seven days frozen at the temperature -18°C, and finally stored in tight nontransparent containers at a temperature ca. 18°C.

After a four-month storage, pollen load samples were submitted for an organoleptic examination. The assessing panel consisted of ten women and eight men. Prior to the assay, the panel was tested and instructed with regard to sensory evaluation of pollen loads in accordance with the guidelines by Baryłko-Pikielna and Matuszewska (2009). All samples were offered for testing anonymously in covered containers marked with digital code symbols. Tasting was performed at room temperature (20°C). A five-point sensory evaluation of partial quality was used to rate the following qualitative indicators: shape, color, flavor, identity, intensity and desirability of flavor, intensity and desirability of aroma. In organoleptic evaluation, identity is understood as the purity of the tested product. Other features are described in Table 1. Evaluations were carried out in accordance with the Polish Standard PN-R-78893 (1996) for pollen loads and guidelines from professional

Table 1.

Characterization of pollen load evaluation criteria

Qualitative feature	Score characterization				
	5	4	3	2	1
Shape	Extremely homogenous (pellets of equal size)	Homogenous (pellets of equal size)	Single pollen grains and pellets evenly mixed	Pollen grains with single pellets	Single pollen grains
Color	Typical, specific coloring (from light yellow to black, depending on the parent plant)		Partially typical - evenly darkened		Extremely atypical, foreign
Flavor	Sweet-sour		Slightly bitter		Bitter, bland
Palatability: A. Intensity	Extremely intensive	Intensive	Fairly intensive	Perceptible	Hardly perceptible
B. Desirability	Extremely desirable, typical, distinct pellet taste	Desirable, typical, distinct pellet taste	Neutral, not marked, without a foreign aftertaste	Undesirable, with a foreign aftertaste	Absolutely undesirable, somewhat bitter
Aroma: A. Intensity	Extremely intensive	Intensive	Medium intensive	Hardly intensive	Hardly perceptible
B. Desirability	Clearly marked, typical pollen pellet aroma	Aromatic, desirable, typical	Neutral, fresh	Slightly changed, foreign odors perceptible	foreign, absolutely undesirable
Identity	No foreign bodies (sand grains, fallen bees or their body parts)		Single fallen bees or their body parts		Numerous fallen bees or their body parts and perceptible sand grains

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Table 2.

Evaluation of pollen loads' appearance, color, flavor and identity (in points)

Feature	Sex	Measure	Group				
			I	II	III	IV	V
Shape	Men	$\bar{x}$	3.3^{ab}	3.3^{ab}	3.3^{ab}	2.6^{Bb}	2.2^{Bb}
		s	1.24	1.32	0.83	1.25	1.10
	Women	$\bar{x}$	4.0^{A}	3.7^{a}	4.3^{A}	3.7^{a}	4.0^{A}
		s	0.67	1.29	0.47	1.09	0.00
Color	Men	$\bar{x}$	4.9^{AB}	4.4^{ABC}	4.4^{ABC}	3.9^{C}	4.9^{AB}
		s	0.32	0.86	0.92	1.02	0.47
	Women	$\bar{x}$	5.0^{A}	5.0^{A}	5.0^{A}	4.5^{BC}	3.0^{D}
		s	0.00	0.00	0.00	0.90	0.00
Flavor	Men	$\bar{x}$	4.6^{A}	3.9^{B}	4.6^{A}	3.5^{BCb}	3.5^{BCb}
		s	0.38	0.57	0.40	0.96	0.95
	Women	$\bar{x}$	3.9^{BC}	3.4^{CD}	3.7^{BC}	2.7^{D}	4.2^{ABa}
		s	0.54	1.09	0.77	0.89	0.30
Identity	Men	$\bar{x}$	4.9^{A}	4.2^{bc}	4.6	4.4	5.0^{Aa}
		s	0.32	1.00	0.86	0.92	0.00
	Women	$\bar{x}$	3.9^{Bc}	4.5^{ABab}	5.0^{Aa}	5.0^{Aa}	5.0^{Aa}
		s	1.01	0.90	0.00	0.00	0.00

Explanation: Significant differences: $p<0.01$ - capital letters; $p<0.05$ - small letters;
Group I - pollen loads samples frozen and stored in air atmosphere;
group II - samples frozen in air atmosphere and stored in carbon dioxide and nitrogen mixture (30% CO_2 and 70% N_2);
group III - samples frozen in air atmosphere and stored in pure nitrogen (100% N_2);
group IV - samples frozen and vacuum-stored;
group V - samples desiccated at temperature ca. 40°C and stored in air atmosphere.

literature (Baryłko-Pikielna and Matuszewska, 2009). Samples were tested three times at three testing sessions. Test results were subjected to statistical analysis by Statistica computer program. Calculations included arithmetic means ($\bar{x}$) and standard deviation (s). Significance of differences between the mean values of tested qualities in experimental groups was determined with the use of Tukey's test (Baryłko-Pikielna, 1998). In tables, the means differing significantly at the 0.05 level were marked with small letters, and those at the 0.01 level - with capital letters.

RESULTS

The results of the analysis showed a statistically high significant interaction between the evaluated pollen load samples preserved by a particular method and the sex of the examining person ($F_{1,4} = 10.49$, $p = 0.00001$). When evaluating the shape of pollen loads, women awarded the highest score to the samples from group III - frozen in natural atmosphere substituted with pure nitrogen (4.3 points, Tab. 2), and a high score to groups I and V - frozen in natural atmosphere, and dried at ca. 40°C in air atmosphere (4.0 points, Tab. 2). Men, on the other hand, evaluating the same feature, assigned the highest score to pollen loads from group I - frozen in air atmosphere (3.3 points), group II - frozen in air atmosphere substituted with carbon dioxide and nitrogen mixture, and group III - frozen in air atmosphere substituted with pure nitrogen (3.3 points, Tab. 2). Men assigned the lowest score (2.2 points, Tab. 2) to group V, while women - to group II and IV, giving them the average of 3.7 points (Tab. 2).

With regard to the color of the pollen loads, the male experts awarded the highest score - 4.9 points (Tab. 2) - to the samples

Table 3.

Evaluation of the intensity and desirability of pollen load flavor and aroma (in points)

Feature	Sex	Measure	Group				
			I	II	III	IV	V
Palatability:							
Intensity	Men	$\bar{x}$	4.1^{ABb}	3.3^{CDc}	4.1^{ABb}	2.7^{D}	4.0^{BCb}
		s	0.58	1.27	0.32	0.83	0.00
	Women	$\bar{x}$	4.4^{AB}	3.2^{D}	4.8^{Aa}	4.1^{B}	3.0^{D}
		s	0.50	0.61	0.41	0.73	0.00
Desirability	Men	$\bar{x}$	3.8^{BCDab}	3.5^{CDb}	3.2^{CD}	2.7^{D}	4.4^{ABCa}
		s	0.32	0.68	0.49	1.24	0.00
	Women	$\bar{x}$	4.8^{A}	4.5^{AB}	4.5^{AB}	2.9^{CD}	4.0^{BC}
		s	0.41	0.68	0.51	1.28	0.00
Aroma:							
Intensity	Men	$\bar{x}$	3.7	3.4^{B}	3.4^{B}	3.7	3.6^{b}
		s	0.75	1.04	0.98	0.84	0.51
	Women	$\bar{x}$	4.2^{Aa}	4.0	4.0	4.0	4.0
		s	0.41	0.79	0.00	0.00	0.00
Desirability	Men	$\bar{x}$	3.9	3.5^{b}	3.6	4.3	3.6
		s	1.02	0.92	0.85	0.75	0.61
	Women	$\bar{x}$	4.4^{a}	4.0	3.8	3.8	4.0
		s	0.50	0.81	0.41	1.35	0.00

Explanation: Significant differences: $p<0.01$ - capital letters; $p<0.05$ - small letters;
Group I - pollen load samples frozen and stored in air atmosphere;
group II - samples frozen in air atmosphere and stored in a carbon dioxide and nitrogen mixture (30% CO_2 and 70% N_2);
group III - samples frozen in air atmosphere and stored in pure nitrogen (100% N_2);
group IV - samples frozen and vacuum-stored;
group V - samples desiccated at a temperature of ca. 40°C and stored in air atmosphere.

from groups I and V, and the female experts - to the samples from groups I, II and III (the average of 5.0 points, Tab. 2). The lowest average score for color was assigned by men to loads from group IV, i.e. vacuum-stored (3.9 points, Tab. 2), and by women - to the samples from group V (3.0 points).

When evaluating identity, men gave the highest score to the samples from groups V and I - an average of 5.0 and 4.9 points, respectively. Women awarded the highest possible score (5.0 points) to the pollen loads from groups III, IV and V. The lowest score for identity was given by men to the pollen loads from group II (an average of 4.2 points), and by women - to group I (an average of 3.9 points).

For flavor intensity, pollen loads from groups I and III were appreciated at the highest rate (an average of 4.1 points) by the male testers; and from group III - by the female testers (an average of 4.8 points, Tab. 3). The lowest average score for this feature was assigned by men to the pollen loads from group IV (2.7 points), and from group II (3.3 points, Tab. 3). Women assigned the lowest score to groups II and V (3.2 and 3.0 points, respectively).

For flavor desirability, pollen loads from group V were rated most highly by the male experts (4.4 points, Tab. 3). Women awarded the highest score to the samples from group I (4.8 points) and groups II and III (4.5 points, Tab. 3). The lowest score for flavor desirability was assigned to group IV, rated at the level of 2.7 points by men, and at the level of 2.9 points by women (Tab. 3).

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Regarding the product's aroma intensity, the male experts assigned a high score (the average of 3.7 points) to groups I and IV, and the lowest score (3.4 points) to groups II and III (Tab. 3). Women most highly rated the pollen loads from group I - 4.2 points, at the same time rating all remaining groups similarly at a level of 4.0 points, on average.

With regard to the product's aroma desirability, men appreciated most highly the samples from group IV - 4.3 points (Tab. 3), assigning the lowest average score to the pollen loads from group II (3.5 points). Women awarded the highest score for aroma desirability to group I - 4.4 points, and the lowest - to the samples from groups III and IV, rating them 3.8 points on average (Tab. 3).

DISCUSSION

The Project for International Pollen Standards suggests a classification of pollen pellets based on their water content, dividing them into two categories: fresh, i.e. pollen loads not preserved in the original form, and desiccated pollen loads, i.e. submitted to the process of drying out at a temperature ca. 40°C, with water content not higher than 4% (Campos et al., 2008). At present, the only form of pollen loads authorized for sale in Poland are dried pollen loads (PN-R-78893, 1996).

The methods for conservation and storage tested in this study did not have a statistically significant effect on the product's general shape. The lower scores awarded to groups IV and V resulted from the presence of numerous separate pollen grains in dried samples, and big clods in the vacuum-stored samples. Both of these traits are regarded as undesirable according to the Polish Standard PN-R-78893 (1996). The samples distinguished by optimal shape were those stored in pure nitrogen.

The product's specific natural color was best preserved in the samples from group I - frozen in air atmosphere; group II - frozen in air atmosphere substituted with carbon dioxide and nitrogen mixture (30% CO_2, 70% N_2); and group III - frozen in air atmosphere substituted with pure nitrogen (100% N_2). All the groups were given very high average scores for the natural color trait. The much lower scores assigned for the color of dried pollen loads probably resulted from their considerable darkening. The color change is caused by adverse changes in the chemical composition of pollen, and from a loss of their characteristic glow. The loss is caused by water content decline during drying.

The best taste parameters were found in pollen loads stored in air atmosphere, in pure nitrogen, and in the atmosphere of nitrogen mixed with carbon dioxide. It seems that storing pollen loads in vacuum conditions causes a significant lowering of the taste value. During testing, men rated the flavor of pollen samples significantly higher than women. The different perception of pollen palatability most probably results from its composition, particularly its significant phytohormones content (Krala et al., 1995). Palatability, as a sum of olfactory-gustatory-tactual sensations perceived in the oral cavity, is most strongly connected with the general impression evoked by the product. Palatability also significantly affects the psycho-physiology of appetite and consumption (Fik, 1995).

The study showed a correlation between the sex of the testers and their evaluation of the taste and aroma of pollen loads as well as a significant interaction between the desirability and intensity of flavor and the method for pollen load conservation and storage. On the other hand, the study did not show a strong relationship between the method for storing and conservation, and the score awarded for intensity and/or desirability of aroma. The storage methods tested in the study caused a decrease in the quality of aroma. It is important to note, however, that all stored pollen loads retained the aroma specific for this product.

The highest scores for color and flavor given to pollen loads stored frozen in pure nitrogen correspond well with the findings of Szczęsna et al. (1995b, c, d, e) who pointed out that substituting air atmosphere

with pure nitrogen, considerably limits adverse changes in the biochemical composition of the product. The method for preserving the biological qualities of pollen loads by freezing in air atmosphere and storing in pure nitrogen was also found most effective in this study, because such organoleptic qualities as color, aroma and flavor depend on the content of chemical substances determining those traits.

The stored samples were characterized by a very high product identity. Excellent scores for this feature were awarded to the samples from group V (5.0 points, Tab. 1), which proves that desiccation makes it possible to very thoroughly clear pollen loads of impurities. The remaining sample scores for identity were also high (between 4.3 and 4.9 points, on average), though slightly lower than in group V (Tab. 1). These results can be attributed to the difficulties of effectively cleaning fresh pollen loads, suggesting that much more attention should be devoted to this task.

CONCLUSIONS

Based on the results of the study, the following conclusions can be drawn:

Organoleptic qualities of pollen loads samples preserved by both freezing and by desiccation at 40°C, and then stored for a short time (up to four months) under various conditions are considered acceptable by consumers.

Of all organoleptic features, color and flavor of pollen loads are most sensitive to the conditions of conservation and storage.

Desiccation most effectively makes it possible to clear pollen loads of various mechanical impurities.

Substituting air or inert gases with vacuum has an adverse effect on the palatability of pollen loads.

Perception of the flavor and aroma of the pollen loads depends on the sex of consumers. Both qualities are more appealing to men than to women.

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WPŁYW SPOSOBU PRZECHOWYWANIA NA WŁAŚCIWOŚCI ORGANOLEPTYCZNE OBNÓŻY PYŁKOWYCH

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S t r e s z c z e n i e

Celem pracy było określenie wpływu metod przechowywania mrożonych obnóży pyłkowych w atmosferze gazów kontrolnych i powietrza atmosferycznego na właściwości organoleptyczne tego produktu. Doświadczenie wykonano w Katedrze Pszczelnictwa UWM w Olsztynie. Pozyskane obnóża pyłkowe oczyszczono i utrwalono następującymi sposobami: zamrożono w naturalnej atmosferze, zamrożono z wymianą atmosfery na mieszaninę dwutlenku węgla i azotu (30% CO_2 i 70% N_2), zamrożono z wymianą atmosfery na czysty azot (100% N_2), zamrożono i zamknięto próżniowo, oraz wysuszono w temp. około 40^{o}C.

Wszystkie próbki obnóży niezależnie od sposobu przechowywania uzyskały pozytywne oceny podczas badań organoleptycznych. Oceniane warunki przechowywania nie wpływały na kształt, tożsamość oraz zapach obnóży pyłkowych, a uzyskane średnie oceny punktowe były podobne we wszystkich grupach. Warunki przechowywania wpłynęły na barwę oraz smak przechowywanych obnóży. Stwierdzono, istotny wpływ płci osób oceniających na odbiór wrażeń smakowych i zapachowych obnóży pyłkowych. Smak i zapach obnóży pyłkowych był bardziej akceptowany przez mężczyzn niż kobiety.

Słowa kluczowe: obnóża pyłkowe, ocena sensoryczna, konserwacja, przechowywanie.