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Rape and buckwheat honey allergy in relation to heat treatment

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Introduction. Rape and buckwheat are progressively cultured in Lithuania. Pollen of these plants can be present in honeybee products, whereas it is known that pollen can be a potential allergen.

Purpose. To explore how often and intensively the pollen of rape, buckwheat and mugwort, as well as monofloral (buckwheat and rape) and polyfloral (spring) honey can cause allergy in humans, to reveal whether there is a difference between honey allergy before and after heat treatment and to investigate the purity of monofloral honey in Lithuania.

Materials and methods. The influence of allergens was determined by a skin-prick test. The botanical origin of honey and pollen was determined by the melissopalynological method.

Results. Botanical composition studies have shown that in Lithuania where melliferous plants are cultivated, honey made by honeybees is almost homogeneous. The skin-prick test revealed no statistically significant difference between honey tested before and after heat-treatment, but proved that monofloral honey was less allergenic than polyfloral. Pollen caused allergy more often and more intensively than monofloral honey did.

Key words: pollen, honey, allergy, heat treatment, skin-prick test

INTRODUCTION

Only two grain cultures of all growing in Lithuania produce nectar, which can be gathered by honeybees (Apis *mellifera* L.) and transformed into honey, and also yield pollen and beebread. These are rape cultures (*Brassica napus*) and buckwheat (*Fagopyrum esculentum*). Their crop is important for beekeepers producing monofloral honey (in unifloral honey, pollen of a single plant species should make at least 45% of the total pollen content (1).

Rape honey very quickly crystallises after harvesting and becomes very hard to use, making problems with packing and sale. Buckwheat raw honey sometimes is more humid, because in some years buckwheat vegetation lasts up to the middle of September, and during the last honey harvesting part of honey in the comb is uncapped. It is known that if humidity in honey exceeds 18%, fermentation caused by yeasts begins in it (2–4). Moisture content below 17% is considered to be a safe level for retarding yeast activity (5). For the above reason, honey is often liquefied by heat treatment (6, 7).

For the above-mentioned reasons, throughout the last fifteen years the botanical composition of honey and the processing of honey have changed. Furthermore, people began using more widely the beebread and pollen collected by bees. Therefore, a problem has arisen concerning the use of the new composition and processed honey for food and as a folk remedy, different from the traditional ones – polyfloral honey, bee pollen and beebread.

It is known that pollen present in the environment can cause an allergic reaction and allergic rhinitis symptoms. They may manifest especially themselves severely in hay fever patients. Investigations concerning allergy caused by pollen and honey are scarce.

The skin-prick allergic test is a simple method for determining allergy to various food products and other allergens (8). Patients with allergy intolerant of honey may have the following clinical manifestations: asthma (9, 10), cough (11)

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anaphylaxis (9, 11, 12), itching in the mouth, gastrointestinal symptoms (9).

Our purpose was to explore how often and intensively the pollen of rape, buckwheat and mugwort, as well as monofloral (buckwheat and rape) and polyfloral (spring) honey can cause an allergy in humans, reveal the difference, it any, between honey allergy before and after heat-treatment and the purity of monofloral honey in Lithuania.

MATERIALS AND METHODS

Sample collection. For the diagnosis of allergy, nine samples were used: six with undiluted honey and three with pollen extracts.

Rape honey was collected in Kedainiai District, buckwheat honey in Trakai District, and spring (polyfloral) honey in Kaunas District. Buckwheat and rape pollen was collected in the same fields where the bees were collecting nectar. A 3 × 4 × 2 m isolator was put on the crop. Two bee-nuclei with a pollen collector were placed into the isolator and used. Every evening pollen was collected and refrigerated at a temperature of –18 °C.

Sample preparation. Sixth months after the sample collecting date, three honey samples of different botanical origin were liquefied using a special gear (Melitherm®) at a temperature of 70 °C for honey heat-treatment. The other three honey samples were considered to be controls and were used unchanged.

Separate samples of rape and buckwheat pollen were diluted with distilled water 1 : 5 w/v and placed into a freezer at a temperature of –18 °C for total freezing. Later on, the samples were completely defreezed and again freezed at a temperature of –18 °C to obtain ice. This procedure was repeated five times. The samples were kept for 12 h at a temperature of +5 °C; the supernatant was carefully removed, and the upper portion of the liquid was pipetted and transferred to a 10 ml glass bottle. The obtained pollen extract was used for the skin-prick allergy test. The commercial mugwort pollen extract was used for comparison in identifying the botanical origin of honey and pollen. Pollen extracts and honey samples were kept at a temperature of +5 °C.

The botanical origin of honey and pollen was determined using the melissopalynological method. 200–400 grains of pollen in a sample were counted, and the plant origin was determined in a 10 g honey sample under magnification of 400–1 200×. The number of pollen in a sample was expressed as a percentage. If honey contained more than 45% of pollen from one plant, it was considered monofloral and its name coincided with the name of the plant from which the nectar was collected (13).

Moisture content was determined by the refractometric method. In general, the refractive index increases with an increase in the solid content. The refractive index of honey samples was measured at the ambient temperature, using an Atago hand refractometer, and the readings were further corrected for the standard temperature of 20 °C by adding the correction of 0.00023/°C. The moisture content was determined in triplicate, and the moisture percentage corresponding to the corrected refractive index values was calculated using Wedmore's table (14).

Characteristics of the participating persons. Thirty-three volunteers with hay fever and hypersensitivity to pollen (23 women and 10 men, age 23.26 ± 2.915 years) took part in the study.

Allergy testing. The skin-prick test was used to determine hypersensitivity to honey and pollen. Special needles were used with a spike length of 1 mm. Anterior forearm surface was cleaned with 70° ethanol. Some minutes later, solutions were applied – one drop at a distance of 5 cm from the wrist: the control solution (0.9% NaCl), sol. histamine 0.01% (positive control), and then, at a distance of 3 cm from each other, drops of the test allergens were placed on the skin, and a sterile needle prick was made in the centre of each drop. The reaction was rated 15 min later. The biggest papule diameter was measured. The reaction was considered positive if the papule was ≥3 mm. If the histamine papule was <3 mm or the control solution papule was ≥3 mm, the skin prick test was not rated. The skin prick test was rated in accordance with the histamine papule size (diameter). In evaluating the allergy, a proportion between the study sample papule diameter and the histamine papule diameter was calculated. The ratio up to 0.25 showed a low intensity of allergy, up to 0.5 an average intensity, up to 1 intensive, up to 2 very intensive, and greater than 2 mm extreme intensity.

Statistical analysis. The data were processed using Microsoft Excel and SPSS statistical packages. To the intensity of allergy, the t test was used; the difference in the occurrence rate was estimated by the McNamara test (χ^2 criterion) and the correlation between the intensity of allergens by Spearman's spreadsheet. In estimating the intensity of hypersensitivity and correlations between allergens in individuals in whom hypersensitivity to a particular allergen had not manifested, the intensity was rated zero (0), and in calculating the means and standard errors they were included in the total number of participnts (N). The average and significantly different moisture content was counted using the confidence interval and standard deviation probability.

RESULTS

Botanical origin of samples

The botanical composition of different kinds of grain pollen and honey was analysed in five samples. The botanical composition of rape honey is presented in Fig. 1.

The data showed that the studied rape honey contained 92 ± 2.7% of rape pollen, about 6% of pulse together with pea, and about pollen of 2% of other cultures. The "rape" honey sample was relatively very pure botanically and therefore may be considered monofloral and suitable for investigating and evaluating allergic reactions to rape honey.

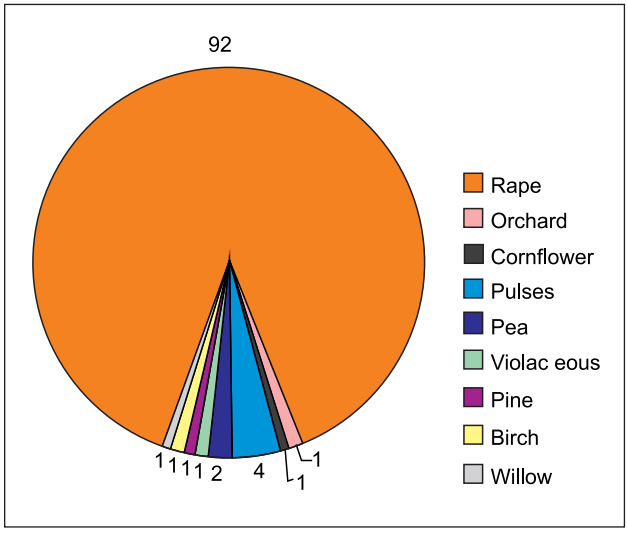

Fig. 1. Botanical composition of rape honey, %

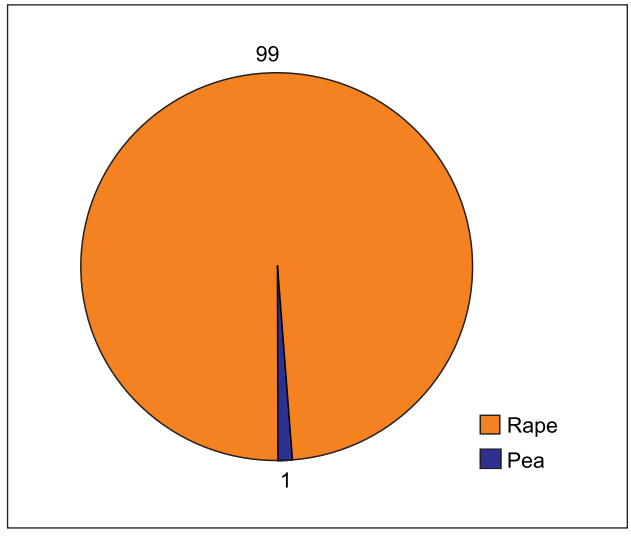

Fig. 3. Botanical composition of rape pollen, %

The botanical composition of buckwheat honey is presented in Fig. 2. Buckwheat pollen made 86 ± 1.9% in the total buckwheat honey pollen, i. e. a sufficiently high percentage to represent the quality, whereas rape pollen made 5 ± 1.4%, orchard pollen 3%, mugwort pollen 3%. Bees do not collect nectar from mugwort, but the crop of buckwheat was a bit weedy with mugwort, so there was some pollen of mugwort. Orchard pollen presented the residues of honey collected in spring when traces of honey remain in combs after honey harvest. The other sorts of pollen made 3%. So the sample of honey botanically was considered monofloral and suitable for assessing allergic reactions to buckwheat honey.

The botanical composition of rape pollen is presented in Fig. 3. One can see that the pollen mixture collected by bees placed in the isolators located on the rape field was very pure: 99% of the mixture made rape pollen, because bees were isolated in the rape crops. The sample was very suitable for revealing allergy to rape pollen.

The botanical composition of buckwheat pollen is presented in Fig. 4. It shows that this pollen mixture was very pure – it consisted in 97% of buckwheat pollen. Other pollen made only 3%, because the bees were kept in isolators placed on the buckwheat crop fields, and therefore other pollen could be brought to the isolator by wind. The extract made from this sample could objectively reveal allergy to buckwheat pollen.

The botanical composition of spring (polyfloral) honey is presented in Fig. 5. Spring honey in Lithuanian is rather heterogeneous in its pollen composition. Willow is the earliest blooming melliferous plant, and therefore its pollen made 39 ± 3.1% in spring honey pollen, whereas orchard pollen

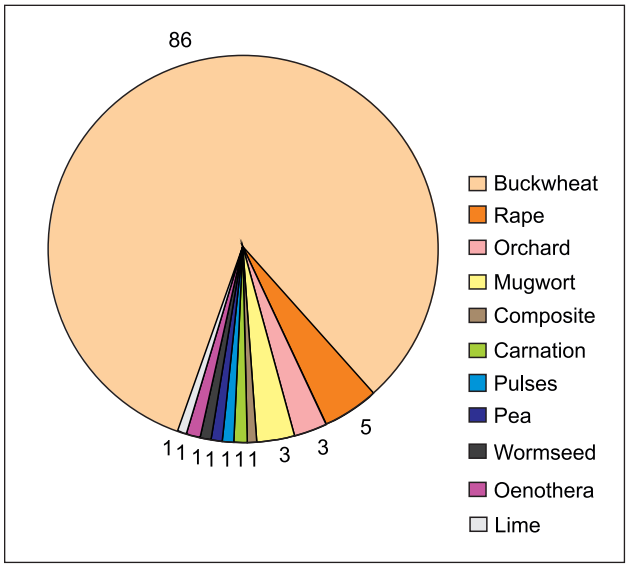

Fig. 2. Botanical composition of buckwheat honey, %

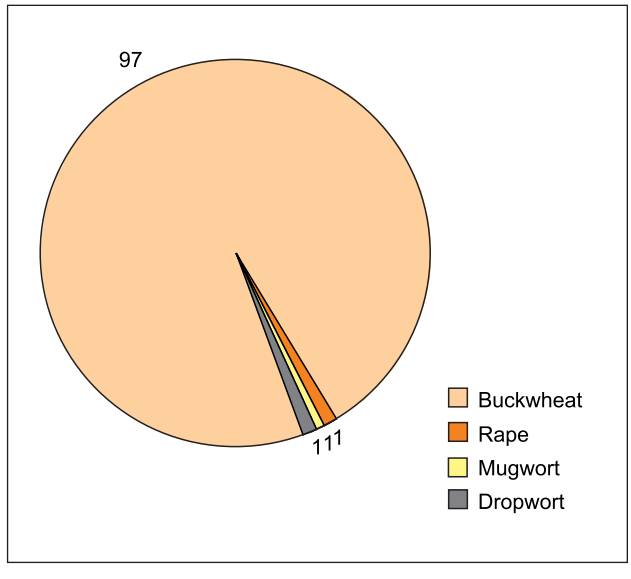

Fig. 4. Botanical composition of buckwheat pollen, %

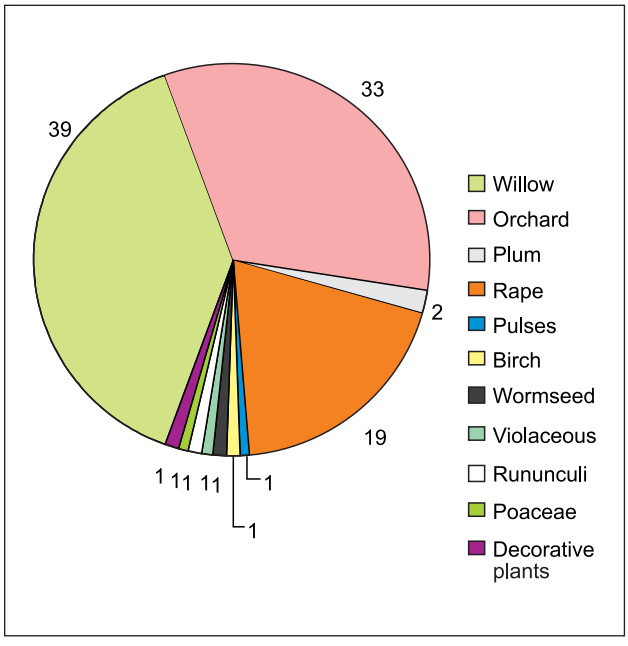

Fig. 5. Botanical composition of spring (polyfloral) honey, %

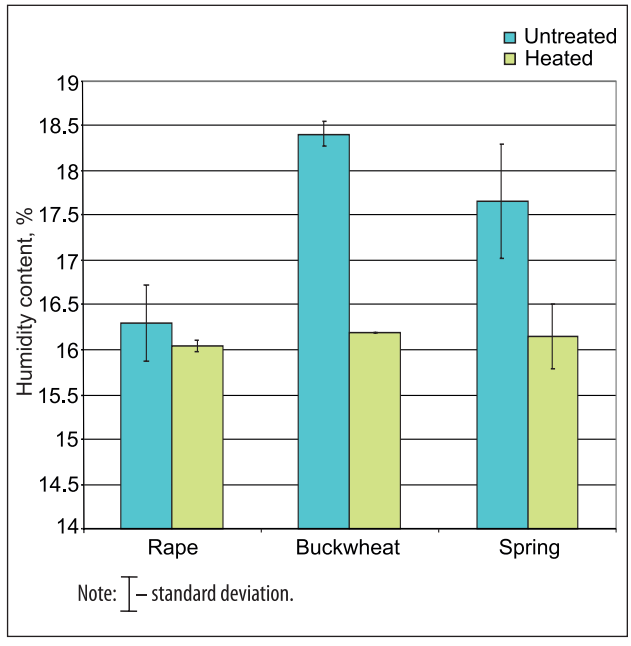

Fig. 6. Moisture content in honey, %

made 35 ± 2.7%, rape pollen 19 ± 1.9%, and 7% consisted of a mixture of other plant pollen. This sample of honey in 74% was made of nectar collected from the plants that bloom only in spring, and none of them prevailed as a source of nectar. So, this honey is to be considered polyfloral spring honey suitable for assessing the intensity and frequency of allergy to polyfloral spring honey which has been used in Lithuania for ages.

Moisture content

Moisture content in honey is shown in Fig. 6. Moisture content in rape and buckwheat honey was measured as described in Materials and Methods. We determined a 2.2 ± 0.14% decrease ($P < 0.05$) in buckwheat honey and 1.5 ± 0.49 ($p > 0.05$) in spring honey. After honey heat-treatment with a special gear "Melitherm" at 70 °C, rape honey moisture decreased only by 0.25 ± 0.24% ($p > 0.05$).

Before heat-treatment at 70 °C the average moisture of all honey samples was 17.45 ± 0.4% and after treatment 16.13 ± 0.14% ($p < 0.05$).

Allergy test results

Data on the frequency of allergy to different kinds of raw and heat-treated honey in hay fever patients are presented in Fig. 7.

Our data show that the sensitivity to different kinds of honey varied from 12.1 to 60.6% of cases among hay-fever patients. The lowest frequency was determined to buckwheat

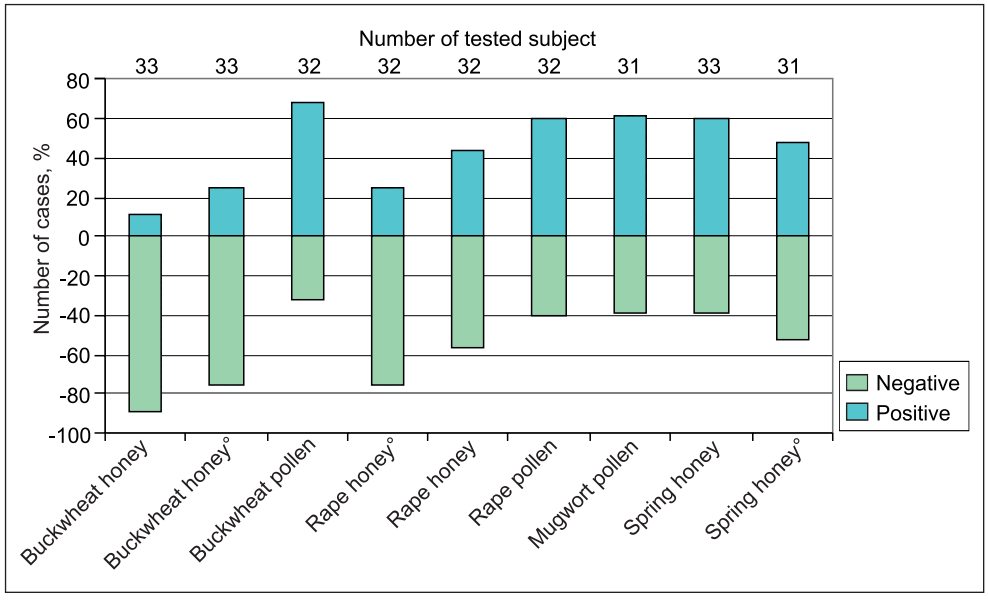

Fig. 7. Frequency of allergy to different plant pollen, raw and heat-treated honey in hay-fever patients

Note. ° – sort of heat-treated honey.

honey and the highest to spring honey. Sensitivity to pollen varied from 59.4% (rape) to 68.8% (buckwheat). While comparing the frequency of sensitivity to pollen and honey in allergy patients, statistically significant differences were found in cases of rape pollen and rape honey ($p < 0.003$), buckwheat honey and buckwheat pollen ($p < 0.001$), buckwheat pollen and heated buckwheat honey ($p < 0.001$).

Thus, sensitivity to pure rape and buckwheat pollen and to pure honey was 2.4 and 3.7 times higher, respectively, whereas the difference between buckwheat and rape pollen was not significant ($p < 0.393$).

The frequency of sensitivity differed significantly as regards rape and spring honey ($p < 0.013$), and buckwheat and spring honey ($p < 0.001$). The number of cases sensitive to spring honey was 2.9 and 2.3 times higher than the number of cases sensitive to rape and buckwheat honey. The difference between the sensitivity to heat-treated and raw honey was insignificant.

According to data presented in Fig. 7, sensitivity to none of the test allergens was determined in 9% of all the study participants. Allergy to at least one sort of honey was confirmed in 87.8% of and to at least of one sort pollen in 73% of persons. Among people not allergic to pollen (27%), even 15% were allergic to spring honey.

Data on the intensity of allergenicity are presented in Table 1. Among all the studied sorts of honey, spring honey showed the highest and buckwheat honey the lowest intensity of allergenicity. Among all the pollen types, most allergenic was mugwort pollen, whereas rape pollen was least allergenic.

Full-scale data on the intensity of various allergens are shown in Table 2.

Table 1. **Intensity of allergenicity (points)**

Allergen	Number of person	Mean	Standard deviation	Maximum value
Spring honey	33	0.355	0.3413	1.0
Buckwheat honey	33	0.129	0.3762	1.67
Rape honey	32	0.172	0.322	1.1
Spring honey°	31	0.364	0.4326	1.5
Buckwheat honey°	32	0.162	0.3028	0.94
Rape honey°	32	0.274	0.3495	1.0
Buckwheat pollen	32	0.497	0.4229	1.5
Rape pollen	32	0.420	0.4180	1.67
Mugwort pollen	31	0.924	0.9947	3.75

° – heat-treated honey.

Table 2. **Allergenicity intensity of separate allergens**

Allergens		Rape honey – rape	Rape honey – rape pollen	Rape° – rape pollen	Buckwheat honey – buckwheat°	Buckwheat honey – buckwheat pollen
McNamara test	Difference in frequency %	18.7	35.5**	16.2	15.6	59.4**
T test	Mean reaction of intensity	0.124	0.227**	0.093	0.075	0.389**
Correlation coefficient		0.013	0.358*	0.236	0.099	0.364*
Allergens		**Spring honey – spring°**	**Spring honey – rape hone**	**Spring honey – buckwheat honey**	**Buckwheat pollen – buckwheat°**	**Buckwheat pollen – rape pollen**
McNamara test	Difference in frequency %	9.7	34.4*	48.5**	45.1**	9.4
T test	Mean reaction of intensity	0.024	0.184*	0.226**	0.331**	0.077
Correlation coefficient		0.419*	–0.08	0.306	0.303	0.710**
Allergens		**Mugwort pollen – buckwheat pollen**	**Mugwort pollen – rape pollen**	**Buckwheat honey – rape honey**	**Mugwort pollen – rape honey**	**Mugwort pollen – buckwheat honey**
McNamara test	Difference in frequency %	7.5	1.9	12.9	36.3*	48.8**
T test	Mean reaction intensity	0.427**	0.504**	0.042	0.751**	0.804**
Correlation coefficient		0.662**	0.555**	0.363*	–0.064	–0.056

° – heat-treated honey.

Difference and correlation coefficient are statistically significant (* $p < 0.05$, ** $p < 0.01$).

The correlation coefficients among separate allergens and their confidence level were calculated from data on allergy intensity. A positive, statistically reliable correlation between the same sort of pollen and honey (buckwheat and rape) was determined.

DISCUSSION

Moisture data corresponded to data of other authors. It is known that when honey moisture exceeds 18–19%, honey at room temperature starts to be fermented by yeasts (2–4). Moisture content below 17% is considered to be a safe level for retarding yeast activity (5). Heat treatment at 70 °C decreases moisture to a constant level (about 16%) and protects honey from humidity and fermentation.

The crops of rape increase extensively every year in Lithuania: in 1989–2004 the area under rape increased more than 11 times. In 2004, rape was grown on the area of 100 600 ha and in 2008 on 161 559 ha (15), i. e. increased by 60%.

In 2004 the area under buckwheat was 22 000 ha, in 2005 – 28 340 ha, – (15), i. e. increased by 28% during 2005, and has little fluctuated in the last four years; furthermore, Lithuania has taken the tenth place according to the total area under buckwheat and the crop yield in the world (16).

So, we may suppose that these cultures will be marketable in the foreseeable future, and therefore more honey will be collected from them every year. We presume that the total rape and buckwheat honey produce could be 8 000 t and 1 000 t a year from rape and buckwheat crops, respectively, i. e. about two times more than the total yield of honey produced in Lithuania now, so a large part of honey collected from these sorts of plants are contained in total honey. Nowadays, people use more and more often bee pollen and beebread collected from rape.

It has been reported in the literature that rape pollen may show cross-sensitivity to pollen of other plants, such as birch, and thus worsen the state of health (17). With this in mind, we may suppose that in the future allergization can increase in spring and summer when winds disperse the pollen of plants.

Allergy to honey mostly manifests in subjects as hayfever (18). Positive allergy tests for honey in them were revealed in most of them, showing that pollen content in honey was sufficient to initiate the allergic reaction that could be determined by skin prick tests. We think that sensitivity to honey is initiated by the pollen present in honey, as the sensitizing intensity of pure buckwheat and rape pollen extract samples was considerably higher than the sensitizing intensity of buckwheat and rape honey. Of such opinion are also other authors (9). We have not found in the literature information on allergy and skin-prick testing for buckwheat and heat-treated honey and buckwheat pollen. Spring honey showed the sensitizing similar to pollen that of frequency. Such similarity could be explained by the polyflority of spring honey containing nectar collected from various plants with a pollen mixture which could condition a summed sensivity to pollen of several plants. Also we have not found in the literature any articles concerning the studies of rape and buckwheat honey in aspect of the thermal influence on their allergenicity.

CONCLUSIONS

1. The botanical composition of the studied rape and buckwheat honey was determined as very pure, with 92% of rape and 86% of buckwheat pollen content in it.

2. After the heat treatment of three kinds (rape, buckwheat and spring) of honey, the moisture content was reduced in to till 16.133 ± 0.141% of cases and remaind stable.

3. In hay-fever patients, the sensitizing intensity and frequency of rape pollen, buckwheat pollen and spring honey was several times higher than that of rape and buckwheat honey.

4. No statistically reliable difference was revealed in sensitizing intensity and frequency between raw honey and heat-treated honey with a special gear "Melitherm" at a temperature 70 °C.

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JAUTRUMAS TERMIŠKAI APDOROTAM IR NEAPDOROTAM RAPSŲ IR GRIKIŲ MEDUI

S a n t r a u k a

Įvadas. Lietuvoje vis daugiau auginama rapsų ir grikių, todėl jų išskiriamos žiedadulkės gali atsirasti ir bičių produktuose. Yra žinoma, kad žiedadulkės gali būti potencialūs alergenai.

Darbo tikslai. Ištirti, kokiu dažniu ir intensyvumu žmogaus organizmą alergizuoja monoflorinis (grikių ir rapsų) ir poliflorinis (pavasarinis) medus, rapsų, grikių, pelyno žiedadulkės; įvertinti, ar pakinta medaus alergizuojančios savybės po terminio apdorojimo; panagrinėti, kokio grynumo monoflorinis medus šiuo metu galėtų būti Lietuvoje.

Metodai ir sąlygos. Alergizuojantis minėtų alergenų poveikis buvo nustatomas atliekant odos dūrio mėginius. Medaus ir žiedadulkių botaninė kilmė tirta melisopalinologijos metodu.

Rezultatai. Medaus botaninė sudėtis rodo, kad Lietuvoje, kurioje sėjami medingi augalai, jis yra pakankamai vienarūšis. Odos dūrio jautrumo mėginių tyrimai neįrodė galimo statistiškai patikimo skirtumo tarp termiškai apdoroto ir neapdoroto medaus, bet patvirtino, kad monoflorinis medus rečiau ir mažiau alergizuoja negu mišrusis. Žiedadulkės dažniau ir intensyviau alergizavo negu monoflorinis medus.

Raktažodžiai: medus, žiedadulkės, terminis apdorojimas, odos dūrio mėginys