INFLUENCE OF ORGANIC ACIDS AND COMPONENTS OF ESSENTIAL OILS ON HONEY TASTE

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INTRODUCTION

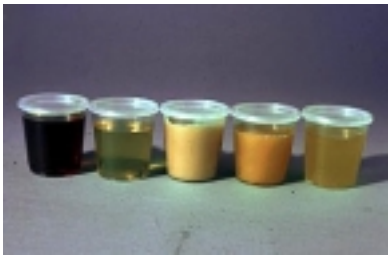
Formic, oxalic and lactic acid, as well as thymol, are used world-wide against *Varroa* as alternatives to synthetic acaricides *and* cause thus also residues in honey. However, these substances occur also naturally in honey. The quantities of these compounds accumulating after mite treatment are low and safe. The question is, whether they adulterate the taste of honey. The European and also Codex Alimentarius food legislation do not allow additives in honey adulterating its natural flavour. The present study determines the sensory thresholds for the above compounds and also for menthol and camphor, two other natural varroacides. The results are discussed in the scope of possible residues problems, arising after the use of these acaricides for *Varroa* treatments.

SENSORY TESTS

Most honey tests were carried out by a panel of about 15 FAM testing persons (see picture 1). They were trained specifically for sensory tests and performed their work according to an approved quality standard (SN-EN 45001 norm).

Photo 1
The sensory panel is working according to a quality assurance system. The sensory tests are performed in a specially equipped tasting room. The sensory abilities are practised regularly in order to assure good quality of the tests. The remaining sweet taste in the mouth after tasting honey samples is neutralised by drinking water.

Photo 2
The control honeys (here various Swiss honeys) are filled into small plastic recipients and sealed - ready for the tests. Each testing person gets her or his own test set of samples.



Samples

European acacia honey as well as Swiss rape and honeydew honeys were used for the tests. Two kinds of samples were prepared:

Adulterated samples: Defined quantities of an organic acid (lactic, formic, oxalic acid) or a component of an essential oil (thymol, camphor, menthol) were added to honey. The acids were dissolved in water, the natural substances in ethanol, and the solutions were added to honey in a proportion of 1:1000. In order to obtain a homogenous mixture the samples were stirred during 30 minutes with a glass rod.

Control samples: Water or ethanol were added to honey in a proportion of 1:1000. These samples were stirred in the same way as the standard samples.

10 to 15 g of the prepared mixtures were filled into small plastic recipients, covered with lids.

(Picture 2).

Honey Testing

The samples of each test set were compared either with paired or triangle tests. In each triangle test honeys with and without additive were compared. In the paired tests the two samples were not necessarily different. The honey recipients were coded. The testing persons compared a maximum of 10 honey samples per testing session. They followed the instructions, listed verbally below (the code numbers are examples):

Paired test according to DIN 10 954

You have a test set of two samples. Please, compare their taste.

1. Are the samples identical or different? Encircle the right answer.

2. Describe the taste of both samples

Sample No.	**Description**
379 = 695 379 ≠ 695	**379**..... **695**.....

Triangle test according to DIN 10 951

You are given a test set of three samples, two of which are identical. Please, judge their taste.

1. Encircle the number of the differing sample

2. Describe the taste of the single and the double sample

Sample No.	**Description**
125 278 698	single sample..... double sample.....

Subsequently, the test persons' ability to distinguish correctly between the honey tastes was determined by means of a statistical test according to DIN 10 954 and 10 951. Example: When 15 testing persons made the test, 12 correct answers (for a paired test) and 9 correct answers (for a triangle test) are necessary to assure, that the distinction between the standard and the adulterated sample is correct at a p level of 0.05. In the results, given in the tables the p level in all cases was < 0.05.

Taste threshold

The lowest, correctly distinguishable concentration of an additive in honey is called taste threshold. For instance: when formic acid (FA) is tested in acacia honey (table 1), the adulterated FA-taste is not distinguished correctly at 150 mg/kg, at 300 mg/kg, however, it is distinguished correctly. Therefore, the taste threshold for FA in acacia honey lies between 150 and 300 mg/kg (table: 150-300 mg/kg).

RESULTS AND DISCUSSION

Taste thresholds of added acids

The results are summarised in table 1. The distinction thresholds of acids tested in honey are ranged below, in the order of their taste effectiveness:

Formic acid (300-600 mg/kg)

Oxalic acid (400-900 mg/kg)

Lactic acid (800- 1600 mg/kg)

The taste of honey is affected mostly by formic acid and least by lactic acid. The testing persons remarked in all cases an increased acid taste in the honeys, adulterated with acid quantities above the taste threshold. The taste threshold for acacia honey is about half of the one for honeydew honey, because the latter has a much stronger aroma and can support more acid than acacia honey, having only a weak aroma. In Italy similar taste thresholds were found for formic acid (Capolongo et al., 1996). Oxalic and lactic acid have not been tested before. The taste threshold for formic acid in pure water was found to be 10 mg/kg (Handbook of Sensory Physiology, 1971) i.e. about 20 to 50 times below that in honey.

Acid	Test honey	n	taste threshold	honey	Free acidity m aeq/kg honey
Formic acid (FA)	Acacia	35*	150-300 mg/kg	control: + 300 mg AS/kg	7.5 12.5
Formic acid (FA)	Honeydew	20	300-600 mg/kg	control: + 600 mg AS/kg:	22.2 35.1
Oxalic acid (OA)	Acacia	12	300-400 mg/kg	control: + 400 mg OA/kg:	7.1 13.9
Oxalic acid (OA)	Honeydew	12	700-900 mg /kg	control: +400 mg OA/kg:	26.8 35.1
Lactic acid (LA)	Rape	17	800-1600 mg/kg	control: + 1600 mg OA/kg:	20.3 34.6

Table 1. **Taste thresholds of formic, oxalic and lactic acid in honey.** An expert test panel was used for all tests with the exception of the test for formic acid in acacia honey (*), which was carried out with people without specific sensory training.

Natural acid honey content

Honey contains naturally organic acids. Their concentration vary within a wide range, according to honey origin. The content of formic acid varies from 5 to 600 mg/kg (Capolongo et al., 1996 and Stoya et al., 1986), that of oxalic acid from 1 to 225 mg/kg and that of lactic acid from 10 to 386

mg/kg (Kary, 1987). Generally, in light honeys, e.g. acacia, much lower values are found than in dark honeys, e.g. honeydew.

Residues after treatments with organic acids

Formic acid: After application in autumn at the end of the bee season, the formic acid content in sugar feed increases significantly and may exceed by far the natural acid content. Later on the formic acid concentration decreases slowly due to evaporation to reach the original level in following spring (Capolongo et al., 1996 and Stoya et al., 1986). Therefore, formic acid treatments in autumn can be recommended without any adverse consequences for the taste of the honey, harvested the following spring. On the other hand, in spring (the beginning of the active bee season in Switzerland), only emergency treatments are advised, because residues do not evaporate in time and may affect the taste of spring and summer honey.

Oxalic acid: After treatment in autumn the concentration of oxalic acid was not increased in honey of the following year (Mutinelli et al., 1997). Therefore, application of oxalic acid in autumn can be advised without any risk for honey quality.

Lactic acid: Immediately after applications in autumn the lactic acid content in feed increases up to 1500 mg/kg, but four weeks later it decreases to a value of about 500 mg/kg (Kary, 1987). This is below the taste threshold for lactic acid. Thus, lactic acid can be used for *Varroa* control in spring until four weeks before the beginning of nectar flow.

Food legislation problems

There is no MRL (Maximum Residue Level) value for any of the above acids, because they are natural honey components. However, a maximum of 40 milli equivalents (meq) acid /kg honey is allowed world-wide. In our tests this tolerance value was not exceeded when honeys were added acids above the taste threshold (table 1).

The content of individual acids (see above) as well as the total acid content in meq/kg varies within a wide range, depending on the honey origin. Acacia, rhododenron and rosemary honeys containing 5-10 meq acid /kg tolerate theoretically additions of much more acid than honeydew, heather and lavender honeys with natural acid content of 30-50 meq/kg. During two years of correct application of formic and oxalic acid in different Swiss apiaries the value of 40 meq/kg was never exceeded in flower and honeydew honeys (Imdorf et al., 1998). Also, today's maximum value of 40 meq/kg has proved too low, because certain honeys have naturally a higher acid content. Therefore, the International Honey Commission suggests a maximum value of 50 meq/kg (Bogdanov et al., 1997).

Taste thresholds of added thymol, menthol and camphor

The results of the sensory controls are summarised in table 2. Thymol has the strongest effect on honey taste. The taste thresholds increase in the following order:

thymol (1.1-1.3 mg/kg), camphor (5-10 mg/kg) menthol (20-30 mg/kg)

The test persons marked an astringent and "medicinal" taste of the adulterated samples. The tests for camphor, carried out by trained and untrained testing persons showed the same results (table 2). It is thus evident, that consumers without special sensory training can distinguish small quantities of this substance, and probably also of the other honey additives, examined in this study.

Other investigations revealed similar distinction thresholds for thymol (between 0.5 and 2 mg/kg) (Tüshaus, 1993) and for menthol (36 mg/kg) (Li et al., 1993). We found a threshold value of 0.1 mg/kg for all three substances in aqueous solution, which is 10 to 30 times below the value for honey. As for formic acid the increase of the taste threshold is due to the neutralizing sweet sugar taste.

Tested substance,	honey	n	Taste threshold
Thymol	Rape	16	1.1-1.3 mg/kg
Thymol	Acacia	14	1.1-1.3 mg/kg
Camphor	Acacia	15	5-10 mg/kg
Camphor*	Acacia	34	5-10 mg/kg
Menthol	Acacia	18	20-30 mg/kg

Table 2: **Taste thresholds of thymol, camphor and menthol in honey.** An expert test panel was used for all tests with the exception (*) of the test with camphor, which was carried out additionally also by people without specific sensory training.

Residues after treatment with components of essential oils

Thymol, as well as camphor and menthol and camphor, have a FAO GRAS status (Generally Recognised As Safe) in concentrations up to 50 mg/kg. Thus, residues of these substances in honey are of no toxicological concern. According to EU regulation No. 2377/90, these substances in group II of the non-toxic veterinary drugs, which do not need a MRL (Maximal Residue Limit). Essential oils are, however, odor-intensive substances and very little amounts in honey can alter its taste. That is why, a MRL of 0.8 mg/kg for thymol in honey was fixed in Switzerland.

Thymol: After treatments with Apilife VAR ® (a blend of 76%thymol, 16.4% eucalyptol, 3.8% menthol and 3.8% camphor) in autumn a maximum of 0.5 mg thymol/kg was measured in next year's spring honey (Bogdanov et al., 1998a). However, if thymol is permanently used during the whole active bee season (in Switzerland between March and September), the Swiss MRL value, and also the taste threshold value can be exceeded (Bogdanov et al., 1998b). Therefore, applications of thymol and thymol blends should be carried out at the end of the active bee season, best in August and September.

Menthol and camphor: Menthol is used above all against the tracheal mite. Laboratory tests have proved a good varroacidal effect for menthol and for camphor (Imdorf et al., 1995). The taste thresholds for both substances exceed by far that of thymol. This fact favours application in bee hives. However, to our knowledge, no scientific information about the efficacy of menthol and camphor applications has been published.

Conclusions

The taste thresholds for three organic acids and for three components of essential oils in honey were investigated with sensory tests.

- For organic acids the thresholds were:
  formic acid (150-600 mg/kg), oxalic acid (300-900 mg/kg), lactic acid (800-1600 mg/kg).
  The honeys with weak aroma (e.g. acacia honey) tolerate lesser amounts of acid than more aromatic honeys (e.g. honeydew honey).
- For components of essential oils the taste thresholds were:
  thymol (1.1-1.3 mg/kg), camphor (5-10 mg/kg), menthol (20-30 mg/kg)

Most of these substances are used in bee hives for alternative *Varroa* control. Although they are also natural honey constituents, the international food legislation prohibits honey additives, adulterating its taste. Therefore, the residues of these substances in honey have to remain below their taste threshold. This means, that in apicultural practice only application methods assuring this requirement should be chosen.

We thank Barbara Bogdanov for the translation into English.

After: Bogdanov S., Kilchenmann V., Fluri P, Bühler U., Lavanchy P (1999) Influence of organic acids and components of essential oils on honey taste. American Bee Journal 139 (1) 61-6

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