

## COLOUR IDENTIFICATION OF HONEY AND METHODOLOGICAL DEVELOPMENT OF ITS INSTRUMENTAL MEASURING

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**Abstract:** Colour is one of the most important sensory traits of honey for the consumers. Honeys originating from different plant species are different in colour, but there could be variability within them as well, if originating from different geographical locations. Colour of the honey usually is determined by subjective methods. We determined colour categories by Lovibond method, and compared the results with reflectance spectrometry by Minolta Chromameter for broadening the possibilities of determining the colour of the honey and getting an objective image. First purpose of the study was to find whether the results by Lovibond method are in concordance with those got by reflectance spectrometry. The second purpose was to decide whether white or black backgrounds get the more accurate result when using the Minolta equipment. The results revealed that Minolta Chromameter is suitable to determine honey colour and the reflectance spectrometry data is comparable with the Lovibond method. Additionally white background is advised to be used by this instrument. The  $L^*$  ( $r=-0.884$ ;  $p<0.001$ ) showed close significant correlation with the Lovibond categories.

**Keywords:** honey, subjectivity, colour, Lovibond, Minolta Chromameter.

### Introduction

Honey is a natural material produced by honeybees (*Apis mellifera*). It is an over-saturated sugar solution, containing a high percentage of sugar (e.g. arabinose, fructose, galactose, glucose) and not more than 20% water (Körmendy and Rácz, 2009; Nyawali *et al.*, 2015). Honey is a natural products in which nothing is added or taken away from it (Wilczynska, 2014). Next to different carbohydrates there are minerals, amino acids, pigments, organic acids, enzymes, vitamins, aromatic and colour materials are also present in honey (Szalay, 2002; Bentoncelj *et al.*, 2007; Czipa *et al.*, 2015; Dominguez & Centurión, 2015). The colour of the honey is determined by its ingredients (e.g. mineral content), and by the type of polyphenols (Can *et al.*, 2015; Czipa *et al.*, 2015). Among the main colour materials, flavonoids are the most important (e.g.: 6-flavonol, 4-flavonol, pinocembrin, pinobanksin, galangine, luteoline) (Szalay, 2002; Turkmen *et al.*, 2005; Gheldof and Engeseth, 2002; Gheldof *et al.*, 2002). Colour spectrum of the honey can spread from colourless (light) to amber yellow or even to black (Mateo Castro *et al.*, 1992).

Colour is one of the most important feature in consumers' decisions and main attribute in food products, therefore affects the price of honey in the world market (Gonzales *et al.*, 1999; Quintas *et al.*, 2007, Dominguez & Centurión, 2015; De Silva *et al.*, 2016). The lightness of honey plays appreciable role in the preference of the consumers. In many countries, the price of the honey is related to its colour. The general acceptance of the honey's colour is very widely but generally the lightly coloured honeys have a better price (González - Miret *et al.*, 2007; De Silva *et al.*, 2016). There is a close correlation between colour and mineral content, pollen content, plant origin, geographical origin and also between colour and physical traits of the honey, such as electrical conductivity (Tuberoso *et al.*, 2004; Habib *et al.*, 2014; Czipa *et al.*, 2015; De Silva *et al.*, 2016). The colour of honey also depends on its ash content, the temperature and the storage time (Gupta *et al.*, 1992; González - Miret *et al.*, 2007; De Silva *et al.*, 2016). Different types of honey get darker with diverse speed and to different proportion which depends on acidity, sodium- and fructose content. There are natural changes in colour

during crystallisation: the honey typically gets lighter. Furthermore, processing and handling of the honey, and circumstances and duration of the storage also can have measurable effect on its colour, making it darker. Caramelisation reaction can change the colour of honey. The HMF (hydroxymethylfurfural) content alone is not able to explain colour changes through the caramelisation reaction. (Quintas *et al.*, 2007). Amino acid and mineral content is broader in darker honeys, and they also have more tyrosine and tryptophan content, while lighter honeys not (Negueruela and Perez-Arquillue, 2000; Gonzales *et al.*, 1999; Turkmen *et al.*, 2006). There is a close correlation between the colour and the antioxidant capacity of the honey. According to some researchers, darker honeys are having higher antioxidant content (Frankel *et al.*, 1998; Beretta *et al.*, 2005; Saxena *et al.*, 2010). In conclusion, a lot of facts suggest that colour being an important issue in case of honey, however, there is no officially standardised method available for its measurement (González-Miret *et al.*, 2007).

Organoleptic analysis of food from the consumer's viewpoint is rather common, as with applying that direct, immediate information can be gathered from the costumers (Stolzenbach *et al.*, 2011). The colour change kinetics is important for industrial process design and control so we have to decide which tools would be the best for measure the colour of honeys (Quintas *et al.*, 2007). Defining the colour is not an easy task and in a way a sensory perception and a subjective interpretation at the same time. Environmental circumstances have different impacts on the perception of a certain colour (Konika-Minolta, 1998), nevertheless, there are instruments available to measure the colour of the honey. The idea of classification leads to the development of several honey colour scale, e.g. Pfund or Lovibond scale (Quintas *et al.*, 2007). Pfund colour measuring is the well-known visual comparing instrument in case of honey, which results are given in

mm (Koerner, 2005). The Pfund colorimeter is a simple instrument which has a reference unit (Pfund scale) (Dominguez & Centurión, 2015). Traditionally Lovibond 2000+ equipment is also used for the visual analysis of the honey. During this process it is compare six glasses of different shades of yellow and the given honey sample. These methods do not distinguish small colour differences and depend on person observing (Dominguez & Centurión, 2015). While the methods listed above can be affected by the environmental conditions, reflectance spectrometry (Minolta Chromameter) operates always with the same light conditions and illumination, so the circumstances of the measurement are constant. The most popular colour distance is based on the CIELab method, where L\* (lightness), a\* (degree of greenness/redness) and b\* (degree of blueness/yellowness) values which is applied widespread for measuring colour of subjects and food products (Negueruela and Perez-Arquillue, 2000; Konica-Minolta, 1998, Wilczynska, 2014). This colour system is practical because any colour can be defines by a mixture of red, blue and green colours (Quintas *et al.*, 2007).

The purpose of the present study was to compare objectively the results gathered by Lovibond and Minolta equipments about the colour as consumers perceive subjectively, independently of the ingredients and other physico-chemical properties of honeys. Our aim was also compare white and black backgrounds so better background could be chosen for Minolta equipment.

## Materials and methods

### *Samples*

A total of 21 honey samples of different plant origin was analysed collected from producers, honey traders and shops (Table 1.). Majority of the samples originated from Hungary, and some of them from other countries. Have to stress, that it is always essential to work with

Table 1. Origin of honey by plants

Plant	Number of honey samples
Mixed wildflower	5
Acacia	4
Linden	3
Common milkweed	1
Lavender	1
Orange	1
Forest wildflower	1
Raspberry	1
Sycamore maple	1
Chestnut	1
Sunflower	1
Wild privet	1

Table 2. The categories of Lovibond

Values	Categories
8	Water white
17	Extra white
34	White
48	Extra light amber
83	Light amber
114	Dark amber

Table 3. Connection of the visual perception and the  $\Delta E_{ab}^*$  values according to the equation proposed by Lukács, 1982

Domain	Perceptible difference
$\Delta E_{ab}^* \leq 0,5$	non-perceptible
$0,5 < \Delta E_{ab}^* \leq 1,5$	barely perceptible
$1,5 < \Delta E_{ab}^* \leq 3$	perceptible
$3 < \Delta E_{ab}^* \leq 6$	visible
$6 < \Delta E_{ab}^*$	huge

fluid and clear honey samples, as the light scattering of crystallised honey is different. Due to that already crystallised honeys have to be melted in a water bath at a maximum of 40 °C, then cool back to room temperature. Visible physical contaminations have to be removed by filtration, sample have to be homogenised by a mixer. When filling out honey, air bubbles have to be avoided, so it is necessary to let the cuvette rest, or a real-sonic cleaner is advised for eliminating bubbles: these is how we have done in every case.

### Measuring colour and its backgrounds

First we analysed the colour of samples by Lovibond instrument, by its colour disk samples can be classified into colour categories. Three independent persons made the analysis parallel, next to natural light. The categories of Lovibond are shown in the Table 2.

Then we measured colour by the Minolta Chromameter® CR 410 type instrument as well. The same honey samples was measured as by Lovibond. Minolta Chromameter is built from anti-reflexion glass; we placed honey samples on its cuvette suitable for measuring the colour of liquids and powders. The resulting  $L^*$  value refers to the lightness of the sample (0=black; 99=white),  $a^*$  value refers to the redness of the sample (in +60 direction red, in -60 direction green) and the  $b^*$  value gives the yellowness of the sample (in +60 direction yellow, in -60 direction blue) (Wilczynska, 2014).  $\Delta E_{ab}^*$  value is necessary to be used for evaluating the colour of honey samples from the consumer's point of view based on the values measured in the  $L^*a^*b^*$  colour system, according to the following formula (Lukács, 1982):

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Differences among results can be compared and also evaluated in regard to the visual perception based on it (Table 3).

As background for the Minolta equipment white ( $L^*=63.15$ ;  $a^*=2.21$ ;  $b^*=2.44$ ) and black ( $L^*=38.04$ ;  $a^*=1.06$ ;  $b^*=-2.44$ ) colours were used. White colour means the sum of colours, while black the lack of colours (Negueruela and Perez-Arquillue, 2000). Statistical correlation between results of Lovibond and Minolta Chromameter were analysed by Pearson's test using R 3.2. 0 software.

### Results and discussion

Three of four acacia honeys belonged to the brightest categories (water and extra white) and had the highest  $L^*$  only in case of the

Table 4. Results of Lovibond

Honey	Examiner 1	Examiner 2	Examiner 3	Final result
Acacia (3)	17	17	17	17
Acacia (1)	8	8	8	8
Acacia (2)	8	8	8	8
Acacia (4)	83	83	48	83
Orange	83	83	83	83
Lavender	114	114	114	114
Raspberry	83	83	83	83
Sunflower	48	83	48	48
Forest wildflower	34	48	34	34
Chestnut	48	83	48	48
Sycamore maple	83	83	83	83
Common milkweed	34	34	17	34
Wild privet	34	34	34	34
Mixed wildflower (1)	48	48	48	48
Mixed wildflower (3)	83	83	83	83
Mixed wildflower (4)	114	114	114	114
Mixed wildflower (5)	114	114	114	114
Mixed wildflower (2)	48	34	48	48
Linden (1)	48	48	48	48
Linden (2)	48	48	48	48
Linden (3)	83	83	83	83

white background. This tendency is similar to previously reported data (Wilczynska, 2014).

Correlations between the categories of the Lovibond instrument and the results got by using Minolta Chromameter showed the following results. Table 4 and 5 show the results separately. In case of using white background, the  $L^*$  values showed close significant negative correlation with the Lovibond categories ( $r=-0.884$ ;  $p<0.001$ ). It means that honeys with higher  $a^*$  value (in +60 direction red, in -60 direction green), falls into higher Lovibond category. In case of  $b^*$  values and Lovibond categories, no significant correlation was found ( $r=-0.188$ ;  $p=0.427$ ).

If the correlation analysis was made with using black background, there was a significant negative correlation between  $L^*$  values and categories formed by Lovibond ( $r=-0.616$ ;  $p<0.01$ ). In case of black background the correlation coefficient is weaker than the coefficient of the white background. The  $a^*$

values showed close negative correlation with the Lovibond categories ( $r=0.816$ ;  $p<0.001$ ) but the  $b^*$  values not ( $r=-0.079$ ;  $p=0.741$ ). The results of  $a^*$  and  $b^*$  values of black background show similar tendency with white background values. Comparing the two different background it can be conclude that negative correlation between  $L^*$  value and Lovibond categories is stronger in case of the white background. Similarly, stronger but positive correlation was found between  $a^*$  value and Lovibond colour categories when white background was used. In case of the  $b^*$  values there were no correlations with either background, giving a reason for further studies.

The  $\Delta E^*_{ab}$  value gives the visible difference between two samples. By dint of it, honeys which belong to the same category of Lovibond can be confronted. If honeys have the least difference (non- or barely perceptible) in a same Lovibond category, the two honey colour measuring methods are related. Generally,

Table 5. Results of Minolta Chromameter

Honey	White background			Black background		
	L*	a*	b*	L*	a*	b*
Linden (1)	14.16	3.54	-7.45	35.81	0.3	7.31
Common milkweed	60.17	2.44	27.78	35.76	0.87	4.3
Lavender	36.22	17.62	9.77	30.3	4.43	-0.84
Orange	48.12	14.45	29.04	33.08	3.99	3.64
Forest wildflower	57.35	2.35	33.39	35.23	0.75	5.07
Mixed wildflower (1)	56.57	0.16	42.05	35.74	0.14	7.8
Raspberry	45.4	15.33	24.84	32.05	4.05	1.97
Sycamore maple	44.18	16.17	23.17	31.82	4.13	1.63
Chestnut	51.34	10.29	32.92	33.37	2.78	3.75
Mixed wildflower (3)	49.98	6.77	31.57	33.97	2.47	5.17
Mixed wildflower (5)	31.12	10.69	0.74	29.25	2.96	-2.62
Acacia (3)	64.37	-0.97	22.7	36.54	-0.01	3.14
Sunflower	52.06	0.81	33.67	47.74	-1.02	26.18
Mixed wildflower (4)	37.4	8.03	9.55	36.87	6.48	8.62
Wild privet	61.77	-1.31	37.2	35.81	-0.26	5.71
Acacia (1)	66.77	-1.26	15.03	36.7	0.04	1.29
Acacia (2)	65.47	-0.38	20.28	36.52	0.23	2.41
Mixed wildflower (2)	55.36	3.87	36.42	34.99	1.25	5.82
Linden (2)	55.6	5.08	33.61	34.42	1.47	4.4
Linden (3)	43.64	11.9	21.31	31.76	3.23	1.26
Acacia (4)	49.53	13.89	30.59	32.95	3.73	3.22

the Lovibond categories and the  $\Delta E^*$  values are connected. If the two backgrounds were compared, small or big variances were detected, mainly in case of raspberry, sycamore maple and chestnut honeys. In case of white background, between raspberry honey and sycamore maple honey the difference of visual perception was 'perceptible' ( $E^*=2,23$ ), between raspberry honey and orange honey was 'visible' ( $E^*=5,08$ ), between raspberry honey and linden honey (3) was 'visible' ( $E^*=5,23$ ), between raspberry honey and acacia (4) honey was 'huge' ( $E^*=7,22$ ), between raspberry honey and mixed wildflower honey (3) was 'huge' ( $E^*=11,81$ ).

If the black background was used, the difference of visual perception between raspberry honey and sycamore maple honey was 'non-perceptible' ( $E^*=0,42$ ), between raspberry honey and linden honey (3) was 'barely perceptible' ( $E^*=1,12$ ), between

raspberry honey and acacia (4) honey was 'perceptible' ( $E^*=1,57$ ), between raspberry honey and mixed wildflower honey was 'visible' (4,05), between raspberry honey and orange honey was 'perceptible' ( $E^*=1,96$ ).

In case of white background, between sycamore maple honey and linden honey (3) the difference of visual perception was 'visible' ( $E^*=4,69$ ), between sycamore maple honey and acacia (4) honey was 'huge' ( $E^*=9,43$ ), between sycamore maple honey and mixed wildflower honey (3) was 'visible' ( $E^*=13,88$ ), between sycamore maple honey and orange honey was 'huge' ( $E^*=7,28$ ).

If the black background was used, the difference of visual perception between sycamore maple honey and linden honey (3) was 'barely-perceptible' ( $E^*=0,97$ ), between sycamore maple honey and mixed wildflower honey (3) was 'visible' ( $E^*=4,46$ ), between sycamore maple honey and acacia (4)



honey was 'perceptible' ( $E^*=1,99$ ), between sycamore maple honey and orange honey was 'perceptible' ( $E^*=2,38$ ). In case of white background, between chestnut honey and linden honey (2) the difference of visual perception was 'visible' ( $E^*=6,77$ ), between chestnut honey and mixed wildflower honey (2) was 'huge' ( $E^*=8,34$ ), between chestnut honey and linden honey (1) was 'huge' ( $E^*=55,30$ ), between chestnut honey and sunflower honey was 'huge' ( $E^*=9,54$ ), between chestnut honey and mixed wildflower honey (1) was 'huge' ( $E^*=14,61$ ).

If the black background was used, the difference of visual perception between chestnut honey and linden honey (2) was 'perceptible' ( $E^*=1,8$ ), between chestnut honey and mixed wildflower honey (2) was 'visible' ( $E^*=3,04$ ), between chestnut honey and linden honey (1) was 'visible' ( $E^*=4,98$ ), between chestnut honey and sunflower honey was 'huge' ( $E^*=26,91$ ), between chestnut honey and mixed wildflower honey (1) was 'visible' ( $E^*=5,38$ ).

### Conclusions

Many type of honey can be found in the market which differs in package, prize, colour or origin. Hence estimate the preference of the costumers would be important specifically for honey color. By means of this, the beekeepers would target produce, especially in migratory beekeeping (Czipa *et al.*, 2012; Gyau *et al.*, 2014).

The colour of honey must be objectively measured to classify the product for the processing industry and the quality control.

Lovibond results showed some subjective error, if more than one people is involved in making the measures. All honey originating from different plants can be ordered to one of the Lovibond categories but minor differences between colours cannot be enlightened by using it. However, its great advantage is, that

the equipment itself is simple and portable, so measures can be made even in field condition, and also, its use does not require previous training.

Minolta Chromameter is suitable for measuring honey colour, as values resulted by using it are in concordance with colour categories developed by Lovibond. According to our result, by using it very detailed pieces of information can be gained about the colour of the honey. Due to the stability of circumstances always can get accurate and objective results. Further advantage of the equipment, that it is portable, and can also be used for determining colour of other substances. With Minolta Chromameter human errors can be reduced (Dominguez & Centurión, 2015).

The results of Lovibond and Minolta are comparable and correlate in case of white and black backgrounds. The use of the Minolta instrument was resolved but there was not enough result about different background effect. Based on the  $L^*$ ,  $a^*$ ,  $b^*$  ( $L^*=63.15$ ,  $a^*=2.21$ ,  $b^*=2.44$ ) applying a white background is advised for correct colour measurement of honey if the  $L^*$ ,  $a^*$ ,  $b^*$  parameters are separately highlighted. However if we want to measure the difference of visual perception, the use of black background ( $L^*=38.04$ ;  $a^*=1.06$ ;  $b^*=-2.44$ ) is the better choice. This result is similar to Negueruela and Perez-Arquillue, 2000.

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