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ANTIOXIDANT PROPERTIES OF SOME MEDITERRANEAN FLORAL HONEY

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Abstract

The floral source of nectar is responsible for the monofloral types of honey all over the world leading to specific variety. For the Mediterranean area, Eucalyptus, Lavender and Citrus honey are among the most specific type of honey. The present study investigates two samples of each names honey types originated from Spain, Italy and France related to their antioxidant properties. Total phenol content (TP) was determinate by Folin-Ciocalteu method and colour by Pfund scale and net absorbance. For antioxidant activity (AA) DPPH and FRAP test ware applied. The results show that Eucalyptus honey has the greatest TP content (119.76 mg GAE/100g) and AA (IC50% -3.47 and 322,82 µmFeSO4 FRAP) meanwhile Lavender honey has the lowest values(18,32 mg GAE/100g and (IC50% -12,73 and 17 µmFeSO4 FRAP). TP is strongly correlated to AA, especially for DPPH test. The great variety of floral sources in the case of Citrus honey was reflected by the lack of homogeneity of the result for all investigated parameters.

Key words: Mediterranean floral honey, antioxidant properties

INTRODUCTION

Bees (*Apis mellifera)* produced honey using two different sources: floral nectar and honeydew (*EU, 2002).* The floral honey is the most spread and obviously the types are directly related to geographical conditions which lead to specific flora. For example in Romania and Eastern European countries, the most predominant types of monofloral honey are acacia honey, rape honey, lime honey and sunflower honey. Heather honey is typical for Scotland even if it can be found in other countries like Poland, Slovenia, Spain or Portugal. Mediterranean region of Europe has specific flora which is responsible for some specific honey collected in Spain, Portugal, France, Italy or Greece. There are mainly Lavender, Eucalyptus, Rosemary, Chestnut and Citrus honey. These types of honey can be found in North Africa too (EL-Kalyoubi et al, 2013).

Honey, as an animal origin food, is very different from all other foodstuff from the same category. First of all the difference regard its composition with very low protein content and high sugar content which is responsible for honey nutritional value (Escudero et al, 2013). Most European regulations required a minimum of 70% reducing sugars in floral honey

(Zielinska et al., 2014, SR 784-1/2, 2009). Regarding honey benefices to health the main use is related to sugar replacement as sweetener in all kind of foods or soft drinks. Also it has from ancient time's very divers appliances in human health (Bogdanov, 2010). But the differences between different types of honey derive from its minor component responsible as well for aroma and colour as for the antioxidant activity. Many studies emphasise that antioxidant activity is correlated to the botanical origin of honey and their source (Weselowska et al, 2014, Vela et al, 2007, Baltrusaityte et al., 2007, Al-Mamary, 2002). The main components credited to be responsible for the AA are poliphenols (phenolic acids and flavonoides. Many studies were conducted in this area and Uthurry et al, reviewed in 2011 the role of honey polyphenols in human health referring to their antimicrobial, antiviral, antitumor, antiulcer and cardio protective properties.

The aim of this study was to determinate the polyphenol content, the colour and the antioxidant activity of some Mediterranean floral honey from different countries: Spain, Italy and France.

MATERIALS AND METHODS

Materials

The material consists in three floral Mediterranean honeys types, Lavender from Spain and France (LS, LF), Eucalyptus from Spain and Italy (ES, EI) and Citrus from Spain and Italy (CS, CI) which ware purchased from shops in named countries. The samples were stored at room temperature and were liquefied by gentle warming at 45^0C in a thermostatic bath. All used reagents were p.a. grade. The laboratory devices used in the present study were: Vortex Hettrich, Thermostatic Oven Nitech, spectrophotometer UVMini-1240 Shimatzu.

Methods

Total phenol content (TP) was determined by Folin-Ciocâlteu colorimetric method as described by Meda et al, 2004. Gallic acid (ROTH Germany) was used as standard for the calibration curve from 0 to 250mg.L^- TP content was expressed in mg of Gallic acid equivalents GAE/100 g of honey using the calibration curve, y=0.009x+0.159; R^2 = 0.986.

Color of honey was determinate by two methods on honey solution50% (w/v)

(1) ABS_{450} - the net absorbance was defined as the difference between A_{450} and A_{720} (Beretta et al, 2005)

(2) For colour classification according to the Pfund scale: mm Pfund = $-38.70 + 371.39 \times Abs_{635}$ (Ferreira et al, 2008).

Antioxidant activity AA

DPPH Test - The antioxidant activity by a DPPH assay first introduced by Brand-Williams et al in 1995, was determined using a combination of (Meda *et al*, 2004) and (Bobiș *et al*, 2008) methods with some modifications. RSA was calculated for each of them as %Inhibition = (A_{blank} – A_{sample})/ A_{blank} x 100. A graph of honey solution concentration against %Inhibition was prepared in order to calculate the IC50% values.

FRAP test - FRAP reagent (Benzie and Stain 1996) was freshly prepared and two calibration curves ware prepared using $FeSO_4 \cdot 7H2O$ (0-1000µM) and TROLOX (0-250µM) and results are expressed as µM of $FeSO_4 \cdot 7H2O$ (Piljak-Zegarac et al, 2009) and TROLOX (Weselowska et al, 2014) corresponding to the activity of 10% honey solution.

The experiment was performed in the food control laboratory of the Environmental Protection Faculty, University of Oradea, between September 2014 and March 2016. All tests were performed as triplicate and are presented as mean ± SD.

RESULTS AND DISCUSSIONS

The results for TP and colour determination are shown in Table 1.

Table 1

Total phenol content and colour determination

Honey type	**Sample**	**TP∓SD mgGAE/ 100g honey**	**A635 (mm Pfund)**	**A450-720 (Net Abs)**	**Colour according to Pfund scale**
Lavender	LS	27.16∓3.72	10.36±3.87	180,7±14	Extra white
	LF	18.32∓0.78	1.78±0.88	116,7±7.2	Water white
Eucalyptus	ES	92.28∓1.95	47.09±31	545,2±31	Extra light amber
	EI	119.76∓2.44	111.27±21.04	1352,6±44.8	Amber
Citrus	CS	87.6∓5.27	57.4±8.87	543,9±29.4	Light amber
	CI	59.7∓3.44	4.6±2.01	149,6±16.11	Water white

The TP content for Lavender and Eucalyptus are in accord to those reported by other researchers. So Anjos et al, 2009 found values between 14,4±1,09 mg GAE/100g and 23,77±3,23mg GAE/100g for Lavender honey and between 61,99±0.4 mg GAE/100g and 83,4±1.1 mg GAE/100g for Eucalypt honey meanwhile Ciapini and Strapani, 2014, found 106,7 mg GAE/100g and Escudero et al, 2013, 78,4±41,4 mgGAE/100g for Eucalyptus.One can notice that obtained values for Citrus honey are very different between samples collected from different countries. This aspect

could be related to the fact that nectar sources are much more variable than for Lavender or Eucalyptus. Burratti et al, 2007, found the same situation for Citrus honey originated from different Italian regions: 20,4 mg GAE/100g and 29 mg GAE/100g, honey from Sicily, 24,7 mg GAE/100g, honey from Sardinia and 60 mg GAE/100g honey from Campagna. Anjos et al, 2009 found for Portuguese Citrus honey similar values as in Italian islands respectively from 22,8±0.91 to 24,1±3,23 mg GAE/100g. Values found in the present experiment are higher than the reported ones in Europe. The correlation between TP and colour for the investigated samples is very strong ($y = 0.9676x - 26.532$, $R^2 = 0.8158$) as observed by Berlocelj et al, 2009.

The results of AA by both applied tests are presented in table 2.

Table 2

Antioxidant activity.

Honey type		**DPPH test, mean** ±SD			**FRAP test, mean** ±SD	
		RSA10%	**RSA5%**	**IC50**	μm FeSO4	μm TROLOX
Lavender	LS	40.81∓5.72	29.06%∓3.14	12.73∓2.03	28±1.15	39.4±0.39
	LF	55.17±3.22	34.11±4.44	9.84±2.15	17±0.98	33.68±0.35
Eucalyptus	ES	>100	65.83∓3.07	3.47∓1.89	159.00±1.90	136.25±1.88
	EI	>100	59.81±2.78	4.31±0.66	322.82±2.56	212.24±2.49
Citrus	CS	>100	82.42±8.07	2.89±1.05	51.53±1.72	81.84±1.74
	CI	67.67±6.12	42.5± 4.09	8.46±2.58	23±2.04	36.83±1.53

As one can see, in order to compare antiradical activity of tested samples by DPPH test we needed to work at concentrations below 10% because at this value half of the tested samples have an activity over 100%. The comparison to results already obtained is not clinching in the case of DPPH test due to many variables regarding reagent concentration, rate between sample and reagent, reaction time, etc. So the use of IC50% is more appropriated in order to compare RSA of different types of honey. The experimental values show the greatest AA for Eucalyptus honey and the lowest for Lavender honey. Escudero et al, 2013 found for Spanish Eucalyptus honey higher IC50% values, 17,8±8,1%. As for TPC and colour, the RSA of Citrus honey are very significantly different, the Spanish sample has the highest RSA of all tested samples, meanwhile the Italian sample reach the Lavender honey level. This aspect is notice in Burratti et al 2007 study who found significant differences between IC50% of Citrus honey from Italian regions: 15,1% and 6,9% for Sicily honey, 11% Sardinian honey and 5 % for Campagna honey.

The same situation was observed for AA determined by FRAP test, no matter the expression of results, with Eucalyptus having the highest activity, Lavender the lowest and great differences at Citrus. Contrary to DPPH test, for FRAP test the experimental values are less homogenous for Lavender and Eucalyptus.

IC50% is strongly correlated to TPH content ($y = -0.0995x + 12.999$ $R^2 = 0.87$) but less to colour express as Pfund units ($y = -8.1685x + 92.799$, $R^2 = 0.692$), the reason can be due to antioxidant components which does not affect colour as enzymes or vitamins.

AA determined by FRAP test is still correlated to TPH, but less then IC50% with $R^2 = 0.777$ for Trolox and $R^2 = 0.674$ for Fe^{2+}. As for colour, the correlation to FRAP is higher than in IC50% case ($R^2 = 0.8956$ and $R^2 = 0.8327$) so the pigments present in honey seem to have more influence o this test.

CONCLUSIONS

The experiment leads to some conclusions, as follows:

Referring to antioxidant content, the tested Mediterranean honey has different phenolic content. Meanwhile Eucalyptus and Citrus honey has significant TPH, Lavender honey is much poorer from this point of view. The homogeneity of experimental values for Eucalyptus and Lavender honey compared to Citrus honey can be the result of a very large nectar sources: citrus, orange, Clementine, Mineola. This aspect is emphasise by the AA of tested samples too.

Regarding the AA, no matter the used test, Lavender honey has the lowest activity and Eucalyptus the highest, among the tested samples. The differences related to AA correlation by different tests to TPH and colour point to the necessity to extend the determination to other components of honey such are enzymes and vitamin C.

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