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HONEY – ROSEHIP PREPATIONS AND THEIR ANTIOXIDANT PROPERTIES

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Abstract

Honey is an animal origin food with sugars as main component hence it is used mostly as a healthy natural sweetener. Even if many studies emphasise on the bioactive components of honey (phenolic acids, flavonoids, vitamin C, proline) which are responsible for its antioxidant activity, vegetal origin food especially red fruits as forest berries, are known as the main antioxidant source for human diet. This study proposes to use fresh preparations of rape honey and rosehip juice or rosehippowder in different proportions, instead of traditional rosehip jelly prepared with sugar. Those mixtures combine their properties in terms of taste and at the same time they are able to provide important antioxidant content. Total phenolic content determined by Folin-Ciocalteu spectrophotometric method for tested mixtures showed a raisefrom 10 to 25 times compared to rape honey.The phenolic content and the antioxidant activity determined by FRAP test are strongly correlated to the rosehip contentof the mixtures,with R^2values over 0.96. The rosehip powder allows obtainingconsistent products useful in pasty not only as fresh product.

Key words: rape honey, rosehip, antioxidant properties

INTRODUCTION

The relationship between food and human health is well known. Food industry focuses on the production of food not only from the nutritional point of view, but also pointing more and moreto functional food. Herby antioxidants are advisable food components due to their beneficial role for human health.Vegetal origin food is known as the main antioxidant source for human diet, especially red fruits as forest berries (Sengul et al., 2014, Soare et al., 2012, Pantelidis et al, 2007), but also cocoa(Jonfia-Essien et al, 2008) or green tea (Danciu et al, 2017).Rosehips are the fruits of Rosa genus from *Rosaceae* family having many uses for foods or tea (Yldiz, 2013) or even rosehip soup in Sweden (Zhao, 2012). On Romanian markets rosehip juice extracted from fruits is sold in autumn in markets in order to prepare homemade jelly or marmalade with sugar, in variable proportion, from 1:1 to 1:0.5. Those products are also available on the market. Rosehip is known not only for its large content of vitamin C, but also for a rich content of phenolic compounds. Their antioxidant content leads to a strong antioxidant activity that makes them a valuable food ingredient. Recently, rosehip was used in probiotic drinks or yoghourt in Turkey (Demir et al., 2014).

Honey is an animal origin food with sugars as main componenthence it is used mostly as a healthy natural sweetener. Many studies emphasise on the bioactive components of honey (phenolic acids, flavonoids, vitamin C, proline)which are responsible for its antioxidant activity (Meda et al, 2005, Bertoncelj et al, 2007). Antimicrobial, antiviral, antitumor properties of honey related to phenolic component were reviewed by floral honey (Uthurry et al, 2011). Meanwhile dark floral honey as buckwheat, chestnut or heather honey are rich in antioxidant component, light coloured honey as acacia, lime or rape honey has a significant lower antioxidant content (Bertoncelj et al., 2007, Weselowska et al., 2014). Due to the availability of the floralsource, rape honey is obtained in large quantities in Central and Eastern Europe. It is characterised by a Fructose/Glucose ratio less than 1 which explains its quick granulation in small crystals (Persano-OddoetPiro, 2004).This property makes it useful as crystallisation starter, but consumers often choose to liquefy it before use.

It is well known that replacing sugar with honey assweetener is highly recommended. A number of preparations containing honey and vegetal components as cocoa, cinnamon or seabuckthorn are available onthe market but, to our knowledge not a honey/rosehip product. The aim of this study is to proposeinsteadof traditional rosehip jelly fresh preparations of honey and rosehip juice or rosehip powderwhich combine their properties in terms of taste and,at the same time, are able to provide important antioxidant content. Among lightly coloured honey we choose for the present experiment rape honey due to its creamy appearance and availability in terms of market and price.

MATERIAL AND METHOD

Material

The tested materials consist in foursamples of artisanal rape honey fromBihor county (R1, R2, R3, R4),three samples of Rosehip juice also from Bihor county (RHJ14, RHJ15, RHJ16) andtwo samples of Rosehip powder, one from a naturist shop (RHC) and the second obtained by lyophilisation from 2015 rosehip juice(RHL). Honey was liquefied prior to use by gentle warming at 45^0C in a thermostatic bath.The following mixtures (m/m), performed in porcelainmortar, were tested: rape honey: rosehip juice 2:1 (coded A); rape honey: rosehip powder2:1 (codedB); rape honey: rosehip powder, 4:1 (codedC);rape honey: rosehip powder, 5:1 (codedD); rape honey: rosehip powder 9:1 (codedE).All along the “Result and Discussions” section of this paper, we will refer to the above mentioned mixtures using the appropriate code letter, A, B, C, D or E. The mixing proportions were established by sensorial analysis. We aimed to balance the

sweet taste of honey with the sourer one of rosehip and at the same time to obtain products able to be spread on bread as jelly or to be used as stuffing in pastry.

Visible mini-1240 Shimadzu spectrophotometer was used for all analytical determinations. The used chemicals were analytical grade.The procedures were applied on fresh or frozen samples and the experiment was performed between September 2014 and February 2017.

Methods

Extraction techniques - Antioxidant componentsfrom honey were extracted with water (10% solution) and with methanol/water mixture (50/50 v/v) for rosehip and mixtures. Different ratios were used, e.g. 1:1 for rosehip juice and 1:10 for rosehip powder.

Antioxidant components- Total phenolic content (TPH) was determined by Folin-Cioc âlteuspectrophotometric method(Meda et al, 2005). Gallic acid (ROTH Germany) was used as standard for the calibration curve from 0 to 250mg.L^{-}. TPH content was expressed as mg of Gallic acid equivalents (GAE)/1 g specific sample using fresh calibration curves to each series of determination, for example $y = 0.0097x + 0.0298$, $R^2 = 0.9925$. Different dilution factors (10 – 50) were used in order to have absorbencies in the domain of the calibration curve.

Antioxidant activity(AA)- We applied FRAP assay(Berzie and Stain, 1996) using the calibration curves for $FeSO_4 \cdot 7H_2O$(0 to 1000 µM) and Trolox (0 to 250 µM. Results were expressed as the correspondent of $FeSO_4 \cdot 7H_2O$and Trolox activity for 10% honey solution activityor appropriateddiluted rosehip and mixture extracts.

RESULTS AND DISCUSSION

The first step of the experiment was to determine the initial phenolic content and antioxidant activity of the tested material. Further on we decided to express the results for TPH content (Table 1) as mg GAE/1g sampleby reason of the great difference of values between the investigated materials, e.g. rape honey versus rosehip powder. Therefore we made the conversion, where needed, for the scientific references cited in this paper. The TPH content of the tested rape honey is relatively high for a light type of honey, between 0.39 and 0.62mg GAE/1g. Only Wilzynska, in 2010 and Piljac-Zegarac et al, 2009) found values in the same range, from 0.33 to0.41mg/1g. Other studies revealed much lower values, from 0.07 to 0.11mg GAE/1g (Weselowska et al., 2014, Kaskoniene et al., 2009). So we decided to continue the experiment using the honey samples with the lowest and the highest TPH values among the one tested by us (R2 and R4),

excluding R1 because it was at the final stage of guarantee period as foodstuff.

Table1

Total phenolic content and antioxidant activity of tested row materials

Sample		TPH content, mg GAE/1 g Mean ±Sd	FRAP Mean ±Sd		
Type	Code		µM Fe^{2+}	µM Trolox	Sample conc.
Rape honey2013	R1	0.39±0.02	39.7±4.1	27.2±2.2	10%
			3.97*	***2.72****	***1%***
Rape honey2014	R2	0.62±0.03	74.2±17.1	47.4±6.6	10%
			7.42*	***4.74****	***1%***
Rape honey2015	R3	0.61±0.03	73.1±14.2	45.0±8.2	10%
			7.31	***4.5***	***1%***
Rape honey2016	R4	0.49±0.08	48.4±8.6	37.2±4.5	10%
			4.84*	***3.37****	***1%***
Rosehip juice 2014	RHJ14	15.42±0.50	970.9±62.0	406.1±33.6	1%
Rosehip juice 2015	RHJ15	19,02±0.08	980.1±43.0	421.8±23.3	1%
Rosehip juice 2016	RHJ16	9.65 ±0.11	663.1±35.2	284.0±24.8	1%
Rosehip powder From shop	RHC	63.15±4.65	949.6±47.1	397.6±35.1	0.25%
			3798*	***1590****	***1%***
Rosehip powder lyophilized	PHL	15.09±6.66	986.4±26.6	412±28.2	0.25%
			3945.6*	***1649****	***1%***

***** Value obtained by calculation towards the upper experimental values

On the other hand one can see that TPH content of tested rosehip products are very high, but different inside the same type of product. So for rosehip juice which was provided by the same producer we found values from 9.65 to 19.02 mg GAE/1g. That is not an unusual situation, Oprica and al. reported in 2016 for rosehip pulp values from 23.99 to 71.48 mg GAE/g DW. Demir et al., in his study from 2014 found for different rosehip cultivars values from 31.08 to 52.9 mg GAE/gand Roman et al. in 2013, found for frozen rosehip pulp values from 3.26to 5.75 mg GAE/1g. The differences were attributed by the authors to genetic derivation. Only Abaci et al. in 2016 with 28.326 mgGAE/1g, found for rosehip fruit values at higher level than the samples from 2015 tested by us. Then again any comparison, in our opinion, is relative as long as researchers apply different procedures for extraction and even for Folin-Cioc âlteu method.

Obviously, due to the different water content, rosehip powder had a much higher TPH content than rosehip juice, the highest value being reported for the lyophilised product in agreement withZhao, 2012, which reported in 2012 for lyophilised rosehip fruit a TPH content of 99.82 mgGAE/1g. As far as we know, there are not reported values for Romanian

rosehip powder excepted the one presented by Nechifor et al, in 2014, but the comparison is not relevant because the TPH content in this case is expressed as mg caffeic acid (1.35 g/100 g) instead of galic acid.

The comparison regarding antioxidant activityrefers to FRAP method results. Our experimental values for rape honey match those reported by Piljac-Zegarac et al, 2009 (from 52.22 to 6.14 μMFe^{2}+) and Weselowska et al., 2014 (from45.51 to 36.75 μMTrolox).In order to use the same calibration curves (Fe^{2+} andTrolox) the test was performed at different concentrations of the matrix, as the table 2 reveals: 10% for honey, 1% or 0.5% for rosehip juice and 0.25% for rosehip powder. Even in this situation it is obvious that the AA of rosehip is much higher than that of rape honey. We choose to make calculations for honey and rosehip powder of a 1% concentration, as table 1 shows, only to make the comparison between tested matrices more obvious.

The next step of the experiment was to determine the antioxidant properties of the honey-rosehip mixtures realised both with juice or powder previously tested. For rape honey/rosehip juice the ratio 2:1 was found the best regarding the balance of sweet/sour taste and a proper viscosity in order to use the mixture as a substitute ofclassic rosehip jelly obtained from rosehip juice with sugar. Table 2 shows the experimental results for R2 and R4 mixed with available rosehip juices (RHJ14, RHJ15, and RHJ16). The honey/ rosehip juice ratio was 2:1 for all mixtures, realised with R2 (R2-RHJ) or with R4 (R4-RHJ).

Table 2

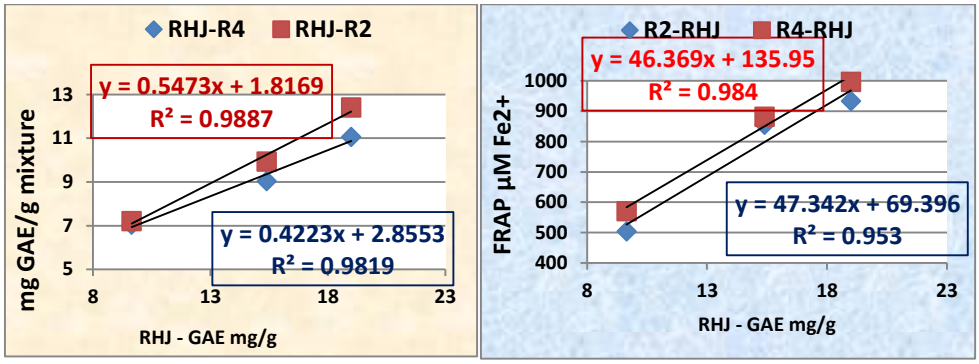
Rape honey/rosehip juice mixtures, antioxidant properties

Rosehip juice	**TPH, mgGAE/ 1g sample ±SD**		**FRAP, for 1% sample(A mixture)**			
			μM Fe^{2+} ±SD		**μM Trolox ±SD**	
	R2-RHJ	**R4-RHJ**	**R2-RHJ**	**R4-RHJ**	**R2-RHJ**	**R4-RHJ**
RHJ14 *	**9.93±0.22**	**9.05±0.9**	**855.2±64.4**	**882.7±21.0**	**359.9±15.4**	**379.7±18.3**
RHJ15 *	12.41±3.11	11.07±1.92	934±24.5	997.3±35.0	411.2±15.7	469.0±27.9
RHJ16	7.22±0.79	7.15±1.14	504.4±24.8	576.1±24.5	212.6±19.5	261.0±16.4

Note: * frozen rosehip juice, gently defrost before mixing was used for R4 mixtures

The increase of TPA and antioxidant activity for all A mixtures is significant, 11-18 time for R2-RHJ towards R2 and 14-25 time for R4-RHJ towards R4, whereasthe content of rosehip juice in the mixtures is 33%. There are non-significant differences for TPC or FRAP values between mixtures realized with R2 or R4 rape honey.

Figure 1 illustrates a strong correlation between TPH content (a) and antioxidant activity (b) of used rosehip juice and the correspondent mixture of the juice with rape honey. For FRAP express as μM Trolox (not shown in figure 1) the correlation is also very strong, R^2 = 0.998 and 0.997.



a – Correlation for TPH b- Correlation for AA

Figure 1- RHJ and RHJ/rape honey mixtures (A) correlation

For honey/rosehip powderdifferent ratio were tested, from 2:1 to 9:1 using both R2 and R4 and rosehip powder from shop (RHC). The E variant (9:1) was the first with noticeable sour taste and the B variant (2:1) represents the maximum amount of rosehip powder possible to be mixed with melted rape honey. Table 3presents the results related to TPH content and antioxidant activity of the tested mixtures.

Table 3

Rape honey/rosehip powder mixtures, antioxidant properties

Mixture Honey:RHC			TPH		FRAP, for 1% sample			
Code	Ratio	% RHC	mgGAE/1g sample		µM Fe^{2+}		µM Trolox	
			R2	R4	R2	R4	R2	R4
B	2:1	33	27.3±1.89	23.50±2.1	1250.6±45.1	1148.2±17.3	518.1±12.7	540.4±9.9
C	4:1	20	26.4±0.74	20.6±2.0	820.45±21.4	984.4±25.5	345.9±11.8	412.6±13
D	5:1	17	13.6±0.88	12.2 ±0.9	1034.1±36.1	831.4 ±19.8	431.4±17.2	366.5±15
E	9:1	10	10.1±0.09	9.92±1.6	722.7±26.9	732.1±11.0	306.9±22.0	300.1±22

The TPH content of the mixtures issignificant and even for E variant, where the rosehip powder content is the lowest,the obtained value (10.12 - 9.92 mgGAE/1g)is 16 times higher than for R2 (0.62 mgGAE/1g) respectively 20 times then for R4 (0.49 mgGAE/1g).On the other hand, B variant which has the maximum possible rosehip content leads to a recovery of 40% of the rosehip powder phenolic content.

Data from graphs (not shown) referring to correlation between rosehip content (%)of the mixtures andits phenolic contentand antioxidant properties (FRAP test) are synthesized in Table 4. As well as for mixtures with rosehip juice, high correlation is revealed, no matter if R2 or R4 honey is used. R^2 is over 0.94 for both series of mixtures.

Table 4

Correlation rosehip content (%) – antioxidant properties for honeyrosehip powder mixtures

Mixture	Antioxidant property	Regression equation	R^2
R2-RHC	TPH content	y = 0.67x	0.9400
	FRAP - μM Fe^{2+}	y = 38.919x	0.9694
	FRAP - μMTx	y = 16.284x	0.9686
R4/RHC	TPHcontent	y = 0.6574x	0.9675
	FRAP - μM Fe^{2+}	y = 38.629x	0.9749
	FRAP - μMTx	y =16.359x	0.9760

CONCLUSION

Rape honey has lower antioxidant properties compared to rosehip, whetherit is juice or powder.Mixing rosehip with rape honey leads to sweet food products which present an obvious rise of antioxidant capacitiescompared to rape honey. So rosehip juice mixed with rape honey could be used instead of traditional rosehip jelly prepared with sugar. The rosehippowderpermits obtaining consistent products useful in pastry not only as fresh product. The phenolic content and the antioxidant activity (FRAP test) are strongly correlated to the rosehip content. From the experimental point of view,it is difficult to perform direct comparison of antioxidant properties between matrix as different as rape honey and rosehip, mostly from lack of standardisation relating the specific analytical methods. Anyhow, adding rosehip, no matter its form, to rape honey conveys to healthy products “ready to eat” with pleasant mixed sweet-sour taste and significant antioxidant content. Further experiments will be focused on the effect of temperature on these mixtures.

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