

EXPERIMENTAL PAPER

Physico-chemical properties of Rhododendron honey produced in Turkey

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S u m m a r y

Rhododendron honey is a monofloral honey and it is collected from the flowers of Rhododendron species widely spreading over many countries, mainly in America, Turkey, Indonesia, Australia. It is believed that Rhododendron honey treats several disorders and is used traditionally as an alternative medicine. The determination of the chemical characteristics of the Rhododendron honey is essential for public health. In this research, the physico-chemical characteristics of Rhododendron, chestnut and flower honey samples collected from beekeepers in Turkey were determined and compared. Physico-chemical parameters such as moisture, acidity, sucrose, invert sugar, fructose/glucose, conductivity, diastase and hydroxymethylfurfural were analysed in each honey sample. According to the results obtained, no differences were found in physico-chemical properties of Rhododendron, honey samples, except from moisture and acidity compared with those of chestnut and flower honeys. In order to differentiate Rhododendron honeys, new methods should be developed. This honey should be reintroduced to medicine and used in pharmaceutical industry.

Key words: *Honey, physico-chemical analysis, Rhododendron, chestnut, flower, Turkey*

INTRODUCTION

Honey is a natural complex food consisting of carbohydrates, amino acids, proteins, organic acids, vitamins and minerals. Chemically, honey contains sugar (70–80%), water (10–20%) and minor compounds (1%) such as organic acids, minerals, proteins, phenolic compounds and amino acids [1]. The chemical composition of honey depends on many factors such as honey maturity, the nectar source, climatic conditions, processing and storage conditions [2, 3].

Honey is a very important nutraceutical product that has been consumed as a nutritional food and an alternative medicine. Many authors have demonstrated that honey is a therapeutical agent which curing for wound and burns [4-6], asthma [7]. It has been reported that the therapeutic properties of honey are variable and depend on the type of honey used [8]. Honey may be categorised according to its origin as blossom honey, honeydew honey or monofloral and multifloral honey [9].

Rhododendron honey, locally known as a mad or wild honey, is a monofloral honey and is collected from the flowers of Rhododendron species in Black Sea Region of Turkey. The endemic species in Turkey are *Rhododendron ponticum* and *Rhododendron luteum*. The most frequent species of Rhododendron in America are *R. occidentale*, *R. macrophyllum*, *R. albiflorum*, *Kalmia latifolia* and *Kalmia angustifolia* [10]. Furthermore, some species of Rhododendron spreads over a large areas of the Alps in Europe, the Caucasus and Himalayan mountains in the East, the Papua New Guinea in Indonesia and Bellendenker mountain in Austuralia [11].

Rhododendron honey contains acetylandromedol, formerly called grayanotoxin and andromedotoxin, which originates from *R. ponticum* as an active agent. Grayanotoxin causes many symptoms such as sudden severe vertigo, arterial hypotension, and bradycardia owing to the consumption of large amounts of this honey. That is why official sale of this honey in Turkey is forbidden. However, some studies demonstrate that Rhododendron honey decreases the blood glucose and lipid levels in rats with diabetes mellitus [12], it has no effects on biochemical parameters in mice [13]. Also its antibacterial, antifungal, antioxidant and antimicrobial effects have been determined [14, 15]. In addition, it is belived to treat several disorders and is used traditionally as an alternative medicine. Therefore, this honey is highly expensive and many consumers prefer to buy it for medical uses. We wanted to know if we were able to differentiate Rhododendron honeys from other honeys using common analyses. The aim of this study is to determine the physico-chemical properties of Rhododendron honey samples collected from beekeepers in Western Blacksea region (Turkey) and to compare these samples with those of chestnut and flower as well as to obtain the literature for subsequent searches.

MATERIALS AND METHODS

Samples

Total number of 60 honey samples were obtained from different beekeepers in different parts of Western Blacksea Region of Turkey. All samples collected were put in glass jars, approximately 500 g in each. After the pollen analyses, the physico-chemical properties such as moisture, free acidity, invert sugar, fructose/glucose ratio, conductivity, diastase and HMF were studied in honey samples. Moisture, free acidity, diastase and HMF of samples were analysed by standard methods of AOAC [16]. All physico-chemical tests were performed in duplicate.

Physico-chemical analyses

Moisture

Moisture content in honey was measured with a Abbe WYA refractometer (Zhejiang, China). All measurements were carried out at 20°C, after waiting for 6 min for equilibrium, and obtaining the corresponding % moisture (g/100 g honey) from the refractive index of the honey sample by consulting a standard table to this end.

Free acidity

Free acidity was determined by potentiometric titration. In order to obtain this, 10 g samples were dissolved in 75 ml with CO₂-free distilled water and subsequently the alcoholic solution of phenolphthalein was added. The solution was titrated with 0.1 N NaOH.

Sugar

Sugar values were determined using Agilent 1100 Series, HPLC Value System (Waldbronn, Germany). The mobile phase consisted of deionized filter water at a flow rate of 1.2 ml/min.

Electrical conductivity

Electrical conductivity of samples were determined according to the method described by Lasakova et al. [17]. The conductivity of a honey solution at 20% (dry matter basis) in CO₂-free deionized distilled water was measured at 20°C in a Mettler Toledo SG7 conductimeter (Zürich, Switzerland).

Diastase and HMF

To measure the diastase activity and HMF, Shimadzu UV-1201V spectrophotometer was used (Tokyo, Japan). HMF was determined following dilution with distilled water and addition of p- toluidine (Merck) solution. The absorbance of the solution was determined at 550 nm using 1 cm cells in an UV-spectrophotometer.

Statistical analysis

All statistical analyses were performed with the SPSS 16.0 for Windows (SPSS Inc., Chicago). Results are shown as mean values and standard error. In each parameter, the differences between honey samples were analyzed using t-test of independent sample.

RESULTS AND DISCUSSION

The honey samples were studied in terms of physico-chemical characteristics and all tests were performed in duplicate. The means of physico-chemical results obtained in honey samples are summarized in table 1 and figure 1. The results were evaluated according to the means of honey samples. The results were not compared with the honey standards of TSE [18], CODEX [19] and EU [19] due to nonnotification of official sale of Rhododendron honeys in Turkey. It was only showed that all of analyses were in line with honey standards shown in table 2.

Honey moisture content alters depending on the climatic factors, season production and the maturity of honey. The process crystallisation in some kinds of honey is sped up due to increase of the moisture content. Thus, due to the fact that it determines the quality and the conservation of honey, it is an essential parameter. The moisture values of all the honey samples were lower than required limits. In this study, the moisture content of Rhododendron honey was significantly lower than those of chestnut and flower honeys. Kucuk et al. [20] have demonstrated that moisture contents of Rhododendron and chestnut honey samples in their studies were higher than ours. This is in disagreement with our findings. In their studies, chestnut and Rhododendron honeys were collected from East Blacksea Region of Turkey, heterofloral honey was collected from Erzincan province of Turkey, whereas all three honey samples in our study were obtained from Western Blacksea Region of Turkey. These differences in results may be owing to the climatic and geographical factors.

Table 1.

The results of physico-chemical analysis of honey samples studied.

Components	Mean	SD	Min	Max
Rhododendron honey (n=20)				
Moisture %	16.63*	1.36	15.4	18.10
Free Acidity [meq.kg ⁻¹]	16.33*	3.40	12.5	19.00
Invert sugar (%)	62.15	5.83	55.5	66.40
Sucrose	2.90	0.01	2.50	3.95
Fructose/Glucose	1.15*	0.08	1.07	1.24
Conductivity [mS.cm ⁻¹]	0.68*	0.01	0.67	0.70
Diastase (units)	19.60	2.94	17.90	23.00
HMF [mg.kg ⁻¹]	5.59	4.42	1.91	10.50
Flower honey (n=20)				
Moisture %	18.07	1.17	16.50	20.00
Free Acidity [meq.kg ⁻¹]	25.83	6.22	13.80	33.00
Invert sugar (%)	63.72	6.84	55.01	73.87
Sucrose	3.01	0.04	2.94	3.68
Fructose/Glucose	1.15*	0.07	1.08	1.27
Conductivity [mS.cm ⁻¹]	0.53*	0.24	0.19	1.02
Diastase (units)	23.62	4.75	17.9	29.4
HMF [mg.kg ⁻¹]	8.16	8.78	1.93	30.68
Chestnut honey (n=20)				
Moisture %	18.05	1.50	16.40	20.00
Free acidity [meq.kg ⁻¹]	27.35	3.55	22.50	31.00
Invert sugar (%)	60.45	4.93	55.79	67.37
Sucrose	4.29	0.06	3.52	8.34
Fructose/Glucose	1.46	0.15	1.25	1.63
Conductivity [mS.cm ⁻¹]	1.20	0.53	0.63	1.81
Diastase (units)	22.65	4.95	17.9	29.4
HMF [mg.kg ⁻¹]	5.59	2.47	2.13	7.95

Min – minimum; Max – maximum; SD – standard deviation

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 2.

The results of physico-chemical analysis of honey samples studied and honey standards of TSE, CODEX and EU

Components	Rhododendron honey			Chestnut honey		Standards	
	Rhododendron honey	Flower honey	Chestnut honey	TSE	CODEX	EU	EU
Moisture %	16.63*	18.07	18.05	<20 g/100g	≤20%	≤20%	≤20%
Free acidity [meq·kg ⁻¹]	16.33*	25.83	27.35	≤50 meq kg ⁻¹	≤50 meq kg ⁻¹	≤50 meq kg ⁻¹	≤50 meq kg ⁻¹
Invert sugar (%)	62.15	63.72	60.45	≥60 (blossom) ≥45 (honeydew)	≥60 (blossom) ≥45 (honeydew)	≥60(blossom) ≥45 (honeydew)	≥60(blossom) ≥45 (honeydew)
Sucrose	2.90	3.01	4.29	≤5 (blossom) ≤10 (honeydew)	≤5 (blossom) ≤10 (honeydew)	≤5 (blossom) ≤10 (honeydew)	≤5 (blossom) ≤10 (honeydew)
Fructose/Glucose	1.15*	1.15*	1.46	0.9 – 1.4 (blossom) 1.0–1.85 (chestnut)	–	–	–
Conductivity [mS·cm ⁻¹]	0.68*	0.53*	1.20	≤0.8 (blossom) ≥0.8 (honeydew, chestnut)	≤0.8 (blossom) ≥0.8 (honeydew, chestnut)	≤0.8 (blossom) ≥0.8(honeydew, chestnut)	≤0.8 (blossom) ≥0.8(honeydew, chestnut)
Diastase (units)	19.60	23.62	22.65	≥8	≥8	≥8	≥8
HMF [mg·kg ⁻¹]	5.59	8.16	5.59	≤40 mg kg ⁻¹	≤80 mg kg ⁻¹ (tropical honey)	≤40 mg kg ⁻¹ ≤80 mg kg ⁻¹ (tropical honey)	≤40 mg kg ⁻¹ ≤80 mg kg ⁻¹ (tropical honey)

Free acidity represents the organic acids content in honey. High acidity may be determined by fermentation of sugars into organic acids by yeasts, so the acidity of honey is of a greater importance. Like moisture, acidity of Rhododendron honey was significantly lower than those of chestnut and flower honeys in our study. These results are in agreement with free acidity of Rhododendron honey from different countries reported by different investigators [21, 22]. This is in disagreement with the findings of Kucuk et al. [20]. Differences between the results obtained from many studies may be due to harvesting method, storage conditions and different geographical location.

Sugars such as fructose and glucose are the main components of every type of honey. The fructose–glucose ratio shows alterations depending on the source of the nectar. White [23] has reported that an average fructose/glucose ratio was 1.2 and honey with high fructose/glucose ratio would remain liquid for longer periods [24]. The concentration of fructose and glucose as well as their ratio are useful indicators for the classification of unifloral honeys [21, 25]. The mean value for invert sugar was found to be from 55.01 to 73.87 % and also fructose/glucose ratio of the Rhododendron, flower and chestnut honey were determined as 1.15, 1.15 and 1.46, respectively. Fructose/glucose ratios of Rhododendron and flower honey were significantly lower than these of chestnut honey. The sucrose contents in all honey samples were lower than the limit allowed in Turkish requirements ($\leq 5\%$).

The electrical conductivity (EC) is one of the most important characteristics of honey. The EC is a good standard to determine the botanical origin of honey. Therefore, this parameter is very often used in routine honey control. In our study, it was found that conductivity of Rhododendron honey was similar to those of flower honeys and they were significantly lower than those of chestnut honey. These findings are in disagreement with the findings of Oddo and Piro [21]. It has been reported that the differences in conductivity of honeys of different botanical origin has had a major discriminatory power [26].

Diastase is the common name for α -amylase enzyme. Enzyme activity in honey depends on the intensity of the nectar flow and the amount of nectar the bee processes in each period [27]. In addition, the diastase activity and the HMF content are widely recognized as parameters indicating the freshness of honey [28, 29]. In the present study, Rhododendron honey had the lowest diastase value among other honey samples. Lower [21, 30] and higher [20] results than ours have been reported. On the basis of the results, it can be said that all samples are fresh.

The content of HMF is considered to be one of the main parameters in order to evaluate the freshness and quality of honey. HMF values may increase due to overheating during processing, prolonged storage or adulteration with invert sugar [31, 32]. In our study, HMF values of Rhododendron and flower honey samples were very close and lower than chestnut honey. It has been reported that HMF levels were higher than ours [20].

CONCLUSIONS

In this study, the physico-chemical properties of Rhododendron honey were compared with those of chestnut and flower honeys collected from Western Black-sea Region of Turkey. No differences were found in physico-chemical properties of Rhododendron honey samples, with exception of moisture and free acidity. Furthermore free acidity, conductivity and fructose/glucose ratio of Rhododendron honey were lower than the chestnut honey. There are no differences between physicochemical properties of Rhododendron and flower honey.

It was understood that the classical methods used to analyses physico-chemical properties of flower and chestnut honeys are not useful for analyses of Rhododendron honey. In order to differentiate Rhododendron honeys, new methods should be developed, this honey should be reintroduced to medicine and made usable in pharmaceutical industry.

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WŁAŚCIWOŚCI FIZYKOCHEMICZNE MIODU RODODENDRONOWEGO PRODUKOWANEGO W TURCJI

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Streszczenie

Miód rododendronowy jest miodem jednokwiatowym zbieranym z kwiatów roślin z rodzaju *Rhododendron*. Jest rozpowszechniony w wielu krajach Ameryki, a także w Turcji, Indonezji i Australii. Uważa się, że miód rododendronowy leczy niektóre zaburzenia i jest używany w medycynie alternatywnej, zatem określenie składu chemicznego miodu rododendronowego jest ważne dla zdrowia społeczeństwa. W pracy określono i porównano cechy fizykochemiczne próbek miodu rododendronowego, kasztanowego i kwiatowego otrzymanego z pasiek w Turcji. W każdej próbce oznaczano cechy fizykochemiczne takie jak wilgotność, kwasowość, poziom sacharozy i cukru inwertowanego, stosunek fruktozy/glukozy, przewodność elektryczna, diastaza i hydroksymetylofurfural. Zgodnie z otrzymanymi wynikami, nie znaleziono różnic w cechach fizykochemicznych próbek miodu z rododendronu, za wyjątkiem wilgotności i kwasowości w porównaniu z miodem z kasztanowca i kwiatowym. W celu wychwycenia cech charakteryzujących tylko miód rododendronowy potrzeba zastosowania nowych metod. Miód ten powinien zostać ponownie wprowadzony do użycia w medycynie i przemyśle farmaceutycznym.

Słowa kluczowe: miód, analiza fizykochemiczna, rododendron, kasztanowiec, kwiat, Turcja