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ORIGINAL RESEARCH ARTICLE

Characterization of physicochemical and antioxidant properties of Bayburt honey from the North-east part of Turkey

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The aim of this study was to determine the physicochemical properties, bioactive substance content, and microbiological quality of sixty different honey samples collected from twelve different regions of Bayburt, Turkey. The samples were analyzed for their sugar, moisture, total phenolic, total flavonoid contents, and water activity, conductivity, pH values and colour, antiradical activity, and DSC properties. As a result of physicochemical analyses, it was determined that the samples examined complied with the standard values defined in the Turkish Food Codex in terms of the parameters examined. The results of the study showed that the total phenolic content (219.43-768.82 mg GAE kg^{-1}) , total flavonoid content (31.29-118.7 mg CAE kg^{-1}) and DPPH (12.98%-94.79%) parameters differ widely among the honey samples. A principal component analysis (PCA) was applied to correlate the characteristics of honey with honey samples collected from different regions. This is the first comprehensive and original report about the physicochemical properties of honey produced in Bayburt, a region close to the Anzer region where the most expensive honey, Anzer honey, is produced.

Keywords: Bayburt Honey; bioactive properties; microbiological quality

Introduction

Honey, which is known as a healthier food choice than pure sugar (Solayman et al., 2016), is a sweet and tasty inartificial product that has been consumed by people for centuries because of its high nutritional value and positive effects on human health (Kropf et al., 2010). The composition of honey can change according to the floral origin, climatic, environmental, and processing conditions (da Silva et al., 2016). The sugars in honey are formed by the activity of various enzymes on nectar and are responsible for the honey's viscosity, hygroscopy, and granulation properties and energy value. Even though honey mostly consists of glucose and fructose (60-85%), it also comprises at least 22 different carbohydrates, aroma compounds (hydrocarbons, ketones, benzene derivatives, terpenes and its derivatives, furan derivatives, pyran derivatives, and cyclic compounds), proteins, enzymes, phenolic acids, flavonoids, vitamins, minerals, organic acids, carotenoids and various amino acids (Blasa et al., 2006; Rahman et al., 2017). The mineral composition of honey and the amount of trace elements can be used to determine its geographical origin(da Silva et al., 2016). There is a positive relationship between the total amount of phenolic compounds and the antioxidant capacity of honey (Yao et al., 2003). The detection of the total amount of phenolic compounds in honey is a good parameter in determining its quality and medical properties. Honey contains various phenolics with antioxidant properties such as p-hydroxibenzoic acid, protocatequic acid, chlorogenic acid, caffeic acid, ellagic acid, p-coumaric acid, cinnamic acid, kaempferol, pinocembrin, naringenin and chrysin (Estevinho et al., 2008).

Recently there is a great interest in the use of natural products with potential health benefits in the human diet as consumers tend not to use processed foods (Can et al., 2015). People all over the world consume honey as one of the important natural products with potential health benefits and it is necessary to have standards that determine its identity and quality for the safety of consumers (da Silva et al., 2016, Tornuk et al., 2013). The sensorial, chemical, physical, and microbiological properties of honey determine its final quality (do Nascimento et al., 2018). Depending on these criteria, there are national and international legal regulations that provide information on the quality of honey. In Turkey, which is the second biggest honey producer in the world, many parameters such as the moisture content of honey, sucrose, fructose/glucose fructose + glucose levels, water-insoluble content, diastase number, free acidity, HMF, electrical conductivity, and proline

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are considered among the quality parameters (Turkish Food Codex, 2012). In addition to the physicochemical characterizations, sensory analyses (colour, smell, taste) should be carried out on honey samples. Sensory analysis, used in many fields, allows the establishment of the organoleptic profile of different products and can be useful in monitoring how consumers perceive products (Carpenter et al., 2012). The colour of honey can vary from light to dark. The compounds that affect the colour of honey are different plant compounds such as β-carotene, xanthophyll pigments, chlorophyll, and its derivatives, flavonoids, and anthocyanins. While these properties differ according to the honey's plant source and geographical origin, they are also influenced by certain external conditions such as seasons, processing, packaging, and storage (Escuredo et al., 2014).

Many different honey varieties (*Thymus* spp., *Calluna vulgaris*, *Erica* spp., *Brassica napus*, *Medicago sativa*, *Helianthus annuus*, *Trifolium* spp., *Castanea sativa*, *Eucalyptus* spp., *Robinia pseudoacacia*, *Citrus* spp. etc.) with various properties are produced in Turkey asa result of its rich flora. Therefore, revealing the quality features of the honey produced in different provinces of Turkey is of great importance and one of these provinces is Bayburt, covering a small part of Turkey's surface area with an altitude of starting from 1550 m. This study aimed to determine a detailed profile of Bayburt honey in terms of the parameters tested.

Materials and methods

Collection of honey samples

In total 60 honey samples of honey bee (*Apis mellifera* L.) were collected from the 12 different regions (R1-R12) of the Bayburt province of Turkey in 2015to determine quality parameters. All samples were stored at room temperature protected from UV light prior to the analyses. In all analyses, except for the determination of the sugar content, five different samples from each region (R1-R12) were tested (S1-S5). Details of the sampling are shown in Table S1.

Sugar content

Sugar content analyses were carried out using the method described by the International Honey Commission (IHC),) (2009). 5 g of each honey sample was weighed and dissolved with 45 mL of pure water and transferred into 100 mL volumetric flasks. Then, 25 mL of methanol was added and the flasks were filled up to the 100 mL mark with pure water. The final mixture was filtered with a membrane filter (0.45 μm). The filtrate was analyzed to determined the sugar content using a refractive index detector (RID) with HPLC (Agilent Technologies 1200 Series, Gemany) with a carbohydrate column (Agilent Technologies Carbonhydrate 5 μm,4,6 × 250 mm, USA).

Moisture content and water activity

The moisture of each honey sample was measured according to the IHC (2009) by using a portable refractometer (RHB-32 ATC 0-32). In addition, the water activity of each sample was measured at 20 °C after holding at 55 °C for 10 min (Gleiter et al., 2006).

pH

10 g of each honey sample was dissolved in 75 mL of carbon dioxide-free water in 250 mL glass bottles. Then the electrodes of the pH meter (HANNA-HI 8314) were immersed in the solution and the pH was recorded (IHC, 2009).

Electrical conductivity

The conductivity measurement of each honey sample was performed according to the IHC (2009). 20 g of each honey sample was dissolved in 60 mL distilled water and the total volume was completed to 100 mL with distilled water. The conductivity of the 20% (w/v) honey solutions was measured with a conductometer (CDM230, Meterlab, Turkey).

Colour analysis

The colour analysis of each honey sample was carried out according to the method of Özcan and Ölmez (2014). Accordingly, 50 g honey was weighed into a glass bottle for colour measurement and L* (100: white, 0: black), a* (+: red; -:green) and b* (+: yellow; -: blue) values were determined using a colorimeter (CR-400 Chroma Meter, Japan).

Total phenolic content

The Folin–Ciocalteu method was used to determine the total phenolic content of honey samples (Singleton et al., 1999). 1 g of each honey sample was diluted with 4 mL of methanol and filtered through Whatman No. 1 paper. The solutions (0.5 mL) were then mixed with 2.5 ml of 0.2 N Folin–Ciocalteu reagent for 5 min and 2 ml of 75 gL^{-1} sodium carbonate (Na_2CO_3) was then added. After a 2 h incubation period at room temperature, the absorbance of the reaction mixtures was measured at 760 nm using a spectrophotometer (Shimadzu UV–visible 1700, Tokyo-Japan) against a methanol blank. The total phenolic content was determined as gallic acid equivalents and expressed as mg $GAEkg^{-1}$ per honey sample.

Total flavonoid content

Total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand et al. (1994). 5 mL of aluminium trichloride (2%) in methanol was mixed with the same volume of honey solution

Table 1. Sugar and DCS/TG valuesof honey samples.

	Sugar Analysis*						DSC/TG*	
Geographical origin	Sucrose (%)	Glucose (%)	Xylose (%)	Fructose(%)	F/G	F + G (%)	Onset	Midpoint
R1	2.22^{B}	34.59^{CDE}	0.28^{BC}	40.90^{ABC}	1.18^{C}	75.49^{C}	43.39^{ABC}	-38.39^{AB}
R2	2.38^{B}	34.94^{BCD}	0.37^{ABC}	40.72^{BC}	1.16^{C}	76.67^{C}	-41.51^{A}	-36.55^{A}
R3	1.72^{BCD}	35.11^{BC}	0.34^{ABC}	40.677^{BC}	1.15^{C}	75.78^{BC}	-41.57^{A}	-36.81^{A}
R4	2.34^{B}	36.35^{AB}	0.37^{ABC}	42.59^{A}	1.17^{C}	78.94^{AB}	-41.18^{A}	-36.37^{A}
R5	3.46^{A}	35.58^{ABC}	0.24^{C}	41.22^{ABC}	1.15^{C}	76.80^{ABC}	-42.53^{A}	-37.42^{A}
R6	1.19^{D}	33.38^{DEF}	0.28^{BC}	41.58^{ABC}	1.24^{AB}	74.97^{C}	-46.29^{BC}	-41.84^{B}
R7	1.88^{BC}	32.92^{F}	0.24^{C}	41.50^{ABC}	1.26^{A}	74.67^{C}	-46.45^{C}	-41.84^{B}
R8	1.90^{BC}	35.32^{ABC}	0.49^{A}	41.47^{ABC}	1.17^{C}	76.80^{ABC}	-42.73^{AB}	-37.88^{A}
R9	2.13^{B}	34.65^{CDE}	0.27^{C}	40.10^{C}	1.15^{C}	74.76^{C}	-43.02^{ABC}	-38.13^{AB}
R10	1.28^{CD}	33.14^{EF}	0.28^{BC}	41.52^{ABC}	1.25^{AB}	74.67^{C}	-41.85^{A}	-37.26^{A}
R11	3.17^{A}	36.78^{A}	0.44^{AB}	42.67^{A}	1.16^{C}	79.45^{A}	-43.11^{ABC}	-38.56^{AB}
R12	1.74^{BCD}	34.36^{CDEF}	0.36^{ABC}	41.98^{AB}	1.22^{B}	76.34^{ABC}	-41.72^{A}	-37.03^{A}

*Mean values for 5 honey samples from each region.
In each column, difference (A–F) between regions ($p < 0.01$).

(0.02 mg mL^{-1}). Absorption at 415 nm using a spectrophotometer (Shimadzu UV–vis 1700, Japan) was recorded after 10 min against a blank sample consisting of a 5 mL honey solution with 5 mL methanol without aluminium trichloride. The concentration was determined by catechin equivalents and the results were expressed as mg CAE kg^{-1}per honey sample.

DPPH radical scavenging activity

The scavenging activity of the honey samples for the DPPH (radical 1,1-diphenyl-2-picrylhydrazyl) was measured as described by Hussein et al. (2011). Firstly, the honey solution (0.75 mL, 0.1–0.4 gmL^{-1}) in methanol was mixed with a 0.09 $mgmL^{-1}$ solution of DPPH in methanol (1.5 mL). Then, the mixture was incubated in the dark for 30 min at room temperature and the absorbance was measured at 517 nm (Spectrophotometer, Shimadzu UV–visible 1700, Tokyo-Japan). Radical scavenging activity was expressed as the inhibition percentage of free radical and was calculated according to the formula below:

Percentage of DPPH assay $= [(Ac - As)/Ac] \times 100$ where Ac is the absorbance of the control and As is the absorbance of the sample.

Microbiological analysis

For the determination of mould-yeast counts in honey samples, 10 grams of each honey sample collected from different regions were homogenized with 90 mL of phosphate buffered saline and serial dilutions were conducted into PBS to count the mould-yeast numbers using the protocol of ISO 21527-2:2008. The mould-yeast numbers were expressed as $CFUg^{-1}$ following the three independent experiments.

Differential scanning calorimetry

The thermal characteristics of honey samples were determined by Differential Scanning Calorimeter (DSC) analysis using a TA Q100 Differential Scanning Calorimeter which was attached to a refrigerated cooling system to control and monitor the temperature up to $-90\,^{\circ}C$. Nitrogen was used as the purge gas at a flow rate of 50 $mLmin^{-1}$. Honey samples were weighed accurately into polymer coated aluminium pans, which was used as a reference. Runs were conducted from -80 to $260\,^{\circ}C$ with a scanning rate of $5\,^{\circ}Cmin^{-1}$ to obtain the complete thermal behavior of pure honeys from low to high temperatures. The glass transition temperature was calculated using the TA Universal analysis 2000 software (Version 3.6 C) and the onset and mid-point glass transition temperatures were reported.

Statistical analysis and multivariate data analysis

Data were analysed using one way analysis of variance (ANOVA) through the student's t-test procedure of the statistical analysis software (JMP) in order to determine the statistical differences between ($p < 0.05$) each group.

Multivariate data analysis was performed to discriminate regions (R1-R12) by applying PCA (principal component analysis) to TPC (Total Phenolic Content), TCA (Total Flavonoid Content), DPPH, for antioxidant activity, L* (lightness), a*(redness), b* (yellowness), F/G (fructose/glucose ratio), F + G (total fructose + glucose) , EC (Electrical conductivity), TG (glass transition temperature), Log 10 (yeast and mold count), aw (water activity), % moisture, as variables. Data analyses were performed by the JMP ® software.

Results

The fructose, glucose, sucrose, and xylose concentrations of the honey samples are presented in Table 1. In this study, the F/G ratio of the honey samples was found to be in the range between $1.15 - 1.26$ and in general, no significant differences were observed among the F/G ratio for the honey samples from the twelve

Table 2. Some physicochemical parameters (moisture and water activity) of honey samples.

	Moisture content (%)*					Water activity*				
Geographical origin	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
R1	15.72^{Ec}	15.48^{Fc}	16.79^{Aa}	16.94^{Ba}	16.33^{CDEb}	0.517^{He}	0.534^{Dd}	0.575^{Aa}	0.560^{Cb}	$0.546B^{Cc}$
R2	15.68^{Ec}	17.09^{Ba}	15.71^{DEc}	16.29^{CDb}	16.52^{CDb}	0.555^{Ca}	0.521^{Ec}	0.553^{Ba}	0.513^{Gd}	0.524^{DEFb}
R3	16.54^{CDb}	16.53^{Cb}	15.13^{FGd}	17.76^{Aa}	15.86^{FGc}	0.561^{Ba}	0.535^{Dc}	0.503^{Ja}	0.483^{Jd}	0.512^{Fb}
R4	15.71^{Ecd}	16.19^{Db}	16.60^{ABCa}	16.07^{Dbc}	15.64^{Gd}	0.521^{Gb}	0.539^{Ca}	0.512^{Ic}	0.513^{Gc}	0.522^{EFb}
R5	16.55^{CDab}	16.59^{Ba}	16.17^{BCDab}	16.91^{BCa}	15.93^{EFGb}	0.544^{Db}	0.536^{Dc}	0.524^{Gd}	0.552^{Da}	0.536^{CDc}
R6	18.46^{Aa}	18.39^{Aa}	15.83^{DEc}	18.07^{Ab}	18.06^{Ab}	0.543^{Db}	0.544^{Bb}	0.530^{Fc}	0.511^{Hd}	0.574^{Aa}
R7	15.94^{DEa}	16.22^{Da}	14.79^{Gb}	14.95^{Eb}	16.22^{DEFa}	0.517^{Hd}	0.543^{Ba}	0.538^{Eb}	0.536^{Ec}	0.539^{Cb}
R8	16.08^{DEb}	17.02^{Ba}	$16.14C^{Db}$	$16.97B^{a}$	16.72^{BCa}	0.523^{Fc}	0.538^{Cb}	0.541^{Da}	0.506^{Ie}	0.520^{EFd}
R9	16.92^{BCa}	16.87^{Ba}	16.68^{ABa}	16.48^{BCDa}	16.46^{CDa}	0.538^{Eab}	0.522^{Eb}	0.554^{Ba}	0.524^{Fb}	0.552^{Ba}
R10	15.76^{Ea}	15.84^{Ea}	15.88^{DEa}	16.15^{Da}	15.76^{Ga}	0.499^{Ic}	0.597^{Ab}	0.451^{Ke}	0.604^{Aa}	0.483^{Gd}
R11	16.01^{DEc}	16.28^{CDbc}	16.64^{ABCab}	16.95^{Ba}	15.95^{EFGc}	0.501^{Ie}	0.508^{Fd}	0.544^{Cb}	0.552^{Da}	0.525^{DEc}
R12	17.39^{Ba}	15.67^{EFb}	15.52^{EFb}	17.69^{Aa}	16.14^{Ba}	0.564^{Ab}	0.523^{Ec}	0.521^{Hd}	0.565^{Bb}	0.566^{Aa}

*Mean values after three repetitions.
In each column, difference (A–J) between regions ($p < 0.01$).
In each row, difference (a–e) between samples in the same region ($p < 0.01$).

Table 3. Electrical conductivity and pH of Bayburt honey.

	Electrical conductivity (mS cm^{-1})*					pH*				
Geographical origin/Sample Code	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
R1	0.400^{Ga}	0.288^{Hc}	0.372^{Gb}	0.377^{Hb}	0.286^{Jc}	3.86^{Dc}	3.83^{Fd}	3.93^{Ba}	3.91^{Cb}	3.80^{De}
R2	0.508^{Ca}	0.418^{Fd}	0.472^{Eb}	0.446^{EFc}	0.433^{Fcd}	3.90^{Ca}	3.90^{Ea}	3.88^{Db}	3.79^{Gd}	3.79^{Dc}
R3	0.452^{Ea}	0.450^{Ea}	0.422^{Fb}	0.364^{Hc}	0.350^{Id}	3.99^{Aa}	3.99^{Dab}	3.98^{Ac}	3.98^{Babc}	3.98^{Bbc}
R4	0.411^{Gb}	0.414^{Fb}	0.470^{Ea}	0.408^{Gb}	0.406^{Gb}	3.81^{Fb}	3.75^{Id}	3.79^{Hc}	3.85^{DEa}	3.74^{Ed}
R5	0.433^{Fc}	0.706^{Aa}	0.504^{Db}	0.441^{Fc}	0.497^{Eb}	3.84^{Ee}	4.10^{Bb}	3.97^{Ad}	4.17^{Aa}	4.05^{Ac}
R6	0.547^{Ba}	0.548^{Ca}	0.548^{Ba}	0.546^{Ba}	0.535^{Ca}	3.80^{Gc}	4.19^{Aa}	3.81^{FGc}	3.85^{Db}	3.85^{Cb}
R7	0.536^{Ba}	0.373^{Gd}	0.459^{Eb}	0.465^{Db}	0.389^{Hc}	3.87^{Ga}	3.81^{Hb}	3.85^{Ea}	3.81^{Fb}	3.85^{Ca}
R8	0.590^{Ab}	0.717^{Aa}	0.513^{CDd}	0.565^{Abc}	0.562^{Bc}	3.91^{Ba}	3.82^{Gc}	3.91^{Ca}	3.84^{Eb}	3.75^{Ed}
R9	0.454^{Ec}	0.620^{Ba}	0.655^{Aa}	0.456^{DEc}	0.446^{Fc}	3.80^{Gc}	4.08^{Ca}	3.87^{Db}	3.80^{FGc}	3.79^{Dc}
R10	0.500^{Cbc}	0.489^{Dc}	$0.530B^{Ca}$	0.521^{Ca}	0.513^{Dab}	3.69^{Hc}	3.66^{Jd}	3.73^{Ib}	3.65^{Id}	3.84^{Ca}
R11	0.481^{Da}	0.475^{Da}	0.418^{Fc}	0.404^{Gd}	0.444^{Fb}	3.80^{Gd}	3.80^{Hd}	3.82^{Fc}	3.92^{Ca}	3.84^{Cb}
R12	0.576^{Aa}	0.553^{Cb}	0.541^{Bb}	0.551^{ABb}	0.589^{Aa}	3.81^{FGb}	3.83^{Fa}	3.80^{GHb}	3.76^{Hc}	3.84^{Ca}

*Mean values after three repetitions.
In each column, difference (A–J) between regions ($p < 0.01$).
In each row, difference (a–e) between samples in the same region ($p < 0.01$).

regions analyzed. Similarly, the F + G ratio was found to be in the range between 74.67% −79.45%. In all of the samples, fructose was determined as the major sugar. The sucrose rate was between 1.28% −3.46%, the xylose ratio was between 0.24% −0.49% and the level of sugars varied depending on the collection region within Bayburt (Table 1).

The moisture content of Bayburt honey varied between 15.0% −18.5% and the water activity varied between 0.451-0.604 (Table 2). In terms of moisture content and water activity, as can be seen in Table 2, significant differences ($p < 0.01$) among the samples (S1-S5) from the same region and the different regions were observed depending on the collected samples.

All of the honey samples examined in the study showed acidic pH values(pH < 4.5) and the pH values of the samples ranged between 3.73 and 4.17 (Table 3). The electrical conductivity values of Bayburt honey varied between 0.286-0.717 $mScm^{-1}$ (Table 3).

The colour characteristic of the honey samples is also another important physicochemical characteristic of honey that can be mainly affected by the chemical content of honey samples (Blasa et al., 2006). In this study, the L^*,a^* and b^*values of honey samples collected from the different regions of Turkey were to be between 20.06-29.73, 0.85-3.25 and −3.67-7.80, respectively (Table 4).

The total phenolic content of honey is another characteristic related to the beneficial effect of honey on human health (Ertürk, 2014). In our study, we found a high level of variation in the total phenolic content of honey samples collected from the different regions of Bayburt (Table 5). The phenolic content of honey samples varied from 219.43 to 768.82 mg GAE kg^{-1}. The lowest value was on average 219.43 mg GAE $kg^{-1}$for the sample (S1) of the 5th region whereas the highest value was 768.82 mg GAE $kg^{-1}$for the sample (S1) of the 12th region.

Table 4. Colour variations of Bayburt honey.

Geographical origin	*L* value*					*a* value*					*b* value*				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
R1	24.55^{Cb}	24.38^{Cb}	29.73^{Aa}	25.98^{Cab}	26.19^{Aab}	1.01^{Fb}	0.97^{Fb}	2.00^{CDEa}	2.01^{Ea}	1.96^{CDa}	2.09^{Da}	1.63^{Eb}	-0.22^{Dc}	-0.22^{Dc}	-0.73^{Bd}
R2	$25.02B^{Ca}$	23.00^{Dd}	24.23^{Bb}	24.74^{Da}	23.64^{Cc}	1.07^{Fc}	1.20^{Da}	0.95^{Hd}	1.12^{Hb}	1.16^{Eab}	$2.26C^{Da}$	1.53^{Ec}	1.85^{Cb}	2.16^{Ca}	1.73^{Ab}
R3	26.74^{Aa}	20.95^{Ecd}	20.92^{Dd}	21.27^{EFbc}	21.38^{Db}	1.96^{Bb}	2.05^{Ba}	2.05^{CDa}	1.97^{EFb}	2.06^{BCDa}	-0.92^{Fa}	-2.98^{Gbc}	-3.04^{Hc}	-2.99^{Gc}	-2.83^{Fb}
R4	25.49^{Ba}	24.46^{Cb}	25.21^{Ba}	24.63^{Db}	20.30^{FGHc}	1.03^{Fb}	0.99^{Fb}	0.99^{Hb}	1.04^{Ib}	1.94^{CDa}	1.63^{Eb}	2.16^{CDa}	1.70^{Cb}	2.11^{Ca}	-2.93^{Fc}
R5	26.97^{Aa}	25.93^{Bb}	25.76^{Bb}	24.24^{Dc}	25.50^{Bb}	1.99^{Be}	2.41^{Ab}	2.07^{Cd}	3.25^{Aa}	2.14^{BCc}	-1.70^{Gd}	0.03^{Fa}	-1.03^{Ec}	-1.17^{Ec}	-0.68^{Bb}
R6	23.50^{Db}	24.18^{Ca}	20.28^{Dcd}	20.15^{Hd}	20.78^{EFc}	1.50^{Db}	1.04^{EFc}	1.96^{DEFa}	1.94^{EFa}	1.92^{Da}	2.53^{ABCb}	3.07^{Ba}	-2.77^{Gc}	-3.10^{Gd}	-2.74^{EFc}
R7	24.83^{Cd}	26.73^{Ac}	29.34^{Ab}	33.65^{Aa}	20.17^{GHe}	1.06^{Fc}	1.16^{DEc}	2.18^{Bb}	2.49^{Ba}	2.05^{BCDb}	2.55^{ABd}	3.58^{Ac}	5.51^{Ab}	7.80^{Aa}	-2.47^{CDe}
R8	21.58^{Ea}	21.34^{Ea}	21.30^{CDa}	21.45^{EFa}	20.82^{Eb}	2.17^{Ac}	2.28^{Aa}	2.25^{ABab}	2.28^{Ca}	2.21^{Bbc}	-3.30^{Ha}	-3.46^{Hab}	-3.62^{Ib}	-3.46^{Hab}	-3.45^{Gab}
R9	22.99^{Db}	24.12^{Ca}	20.87^{Dc}	$20.98F^{Gc}$	20.62^{EFGc}	1.80^{Cc}	1.60^{Cd}	1.87^{Fb}	1.92^{Fab}	1.97^{CDa}	2.30^{BCDb}	2.90^{Ba}	-2.35^{Fd}	-2.18^{Fc}	-2.25^{Ccd}
R10	24.65^{Cb}	24.47^{Cb}	23.88^{BCc}	28.18^{Ba}	20.06^{Hd}	0.85^{Gd}	1.17^{DEc}	1.18^{Gc}	1.60^{Gb}	1.89^{Da}	1.76^{Ec}	1.97^{Dc}	2.57^{Bb}	3.54^{Ba}	-2.80^{Fd}
R11	24.60^{Ca}	23.12^{Db}	20.90^{Dc}	20.59^{GHc}	20.76^{EFc}	1.32^{Ec}	1.64^{Cb}	1.90^{EFa}	1.93^{Fa}	1.86^{Da}	2.61^{Aa}	2.29^{Cb}	-2.46^{Fc}	-2.43^{Fc}	-2.51^{DEc}
R12	21.76^{Ea}	20.99^{Eb}	20.97^{Db}	21.61^{Ea}	21.83^{Da}	2.24^{Aa}	2.29^{Aa}	2.30^{Aa}	2.21^{Da}	2.43^{Aa}	-3.36^{Hb}	-3.61^{Hc}	-3.67^{Id}	-3.24^{GHa}	-3.45^{Ga}

*Mean values after three repetitions.
In each column, difference (A–H) between regions ($p < 0.01$).
In each row, difference (a–e) between samples in the same region ($p < 0.01$).

Table 5. Bioactive properties of honey samples.

Geographical origin/Sample Code	Total phenolic content (mg GAE kg^{-1})*					Total flavonoid content (mg CAE kg^{-1})*					DPPH radical scavenging activity* (% Inhibition)				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
R1	304.11^{Id}	317.02^{Id}	533.24^{Cb}	547.95^{Da}	369.27^{Gc}	97.49^{Aa}	70.70^{DEb}	101.53^{Aa}	74.24^{DEb}	58.58^{Dc}	19.93^{Id}	24.63^{Ec}	54.50^{Ba}	26.63^{Fc}	45.33^{Eb}
R2	408.01^{Ga}	293.30^{Ic}	279.19^{Gd}	288.80^{Ic}	308.02^{Ib}	39.37^{Fd}	46.95^{Gc}	$62.11D^{Eb}$	90.92^{Ba}	52.51^{Dc}	38.25^{Cb}	20.63^{Fd}	16.60^{Ie}	41.04^{Ca}	26.09^{HIc}
R3	347.06^{Hc}	329.04^{Hd}	368.08^{Fb}	338.04^{Hcd}	402.01^{Fa}	41.39^{EFa}	35.33^{Hb}	35.33^{Hb}	31.29^{Hb}	35.33^{EFb}	28.92^{Fc}	26.38^{Ed}	33.74^{Fb}	30.06^{Ec}	41.30^{Fa}
R4	299.61^{Ie}	353.66^{Gc}	486.69^{Da}	385.79^{Fb}	335.04^{Hd}	58.57^{Dab}	64.64^{EFa}	56.05^{EFbc}	55.54^{Fbc}	51.50^{Dc}	12.98^{Je}	15.96^{Gd}	18.85^{Hc}	22.60^{Gb}	26.95^{Ha}
R5	219.43^{Kd}	619.13^{Aa}	232.94^{Hc}	610.72^{Ca}	286.99^{Ib}	29.77^{Gd}	104.06^{Ba}	26.74^{Id}	82.83^{Cb}	39.37^{Ec}	20.44^{Id}	58.63^{Ba}	22.25^{Gc}	59.30^{Ba}	25.20^{Ib}
R6	532.34^{Db}	370.48^{Fd}	736.84^{Aa}	749.16^{Aa}	517.03^{Cc}	80.31^{Bc}	63.12^{Fd}	94.45^{Bab}	104.56^{Aa}	83.33^{Abc}	31.36^{Ed}	40.60^{Cc}	92.22^{Aa}	94.41^{Aa}	46.89^{Db}
R7	635.04^{Bc}	588.5^{Bd}	577.09^{Bd}	662.97^{Bb}	741.35^{Aa}	98.50^{Aa}	74.75^{Dc}	$49.98F^{Gd}$	58.58^{Fd}	85.86^{Ab}	41.62^{Bd}	56.60^{Bc}	90.92^{Ab}	94.79^{Aa}	93.30^{Aa}
R8	267.47^{Jc}	295.70^{Ib}	287.29^{Gb}	286.99^{Ib}	547.95^{Ba}	47.96^{Eb}	39.88^{Hb}	44.93^{Gb}	41.39^{Gb}	72.72^{BCa}	23.33^{Hbc}	21.68^{Fc}	22.12^{Gbc}	23.45^{Gb}	55.14^{Ba}
R9	612.82^{Ca}	541.05^{Cb}	357.86^{Fd}	355.76^{Gd}	375.58^{Gc}	81.82^{Bb}	118.71^{Aa}	49.98^{FGc}	56.05^{Fc}	52.51^{Dc}	61.43^{Ab}	65.58^{Aa}	38.22^{DEc}	33.39^{Dd}	38.70^{Gc}
R10	500.81^{Eb}	380.39^{Fc}	564.77^{Ba}	284.59^{Id}	502.31^{Db}	72.22^{Cb}	49.98^{Gd}	57.06^{Ec}	41.39^{Ge}	80.81^{ABa}	27.11^{Gd}	17.58^{Ge}	39.33^{Db}	29.27^{Ec}	52.44^{Ca}
R11	435.34^{Fc}	418.23^{Ed}	451.56^{Eb}	462.07^{Ea}	232.04^{Ke}	92.43^{Aa}	84.35^{Cb}	68.18^{Dc}	68.18^{Ec}	30.78^{Fd}	26.16^{Gd}	30.22^{Dc}	36.98^{Eb}	40.92^{Ca}	21.04^{Je}
R12	768.82^{Aa}	472.58^{Db}	444.03^{Ec}	455.46^{Ec}	447.66^{Ec}	70.20^{Cb}	74.24^{Dab}	79.30^{Ca}	78.79^{CDa}	67.67^{Cb}	35.65^{Dd}	39.87^{Cc}	42.19^{Cb}	42.54^{Cab}	44.06^{Ea}

* Mean values after three repetitions.
In each column, difference (A–K) between regions ($p < 0.01$).
In each row, difference (a–e) between samples in the same region ($p < 0.01$).

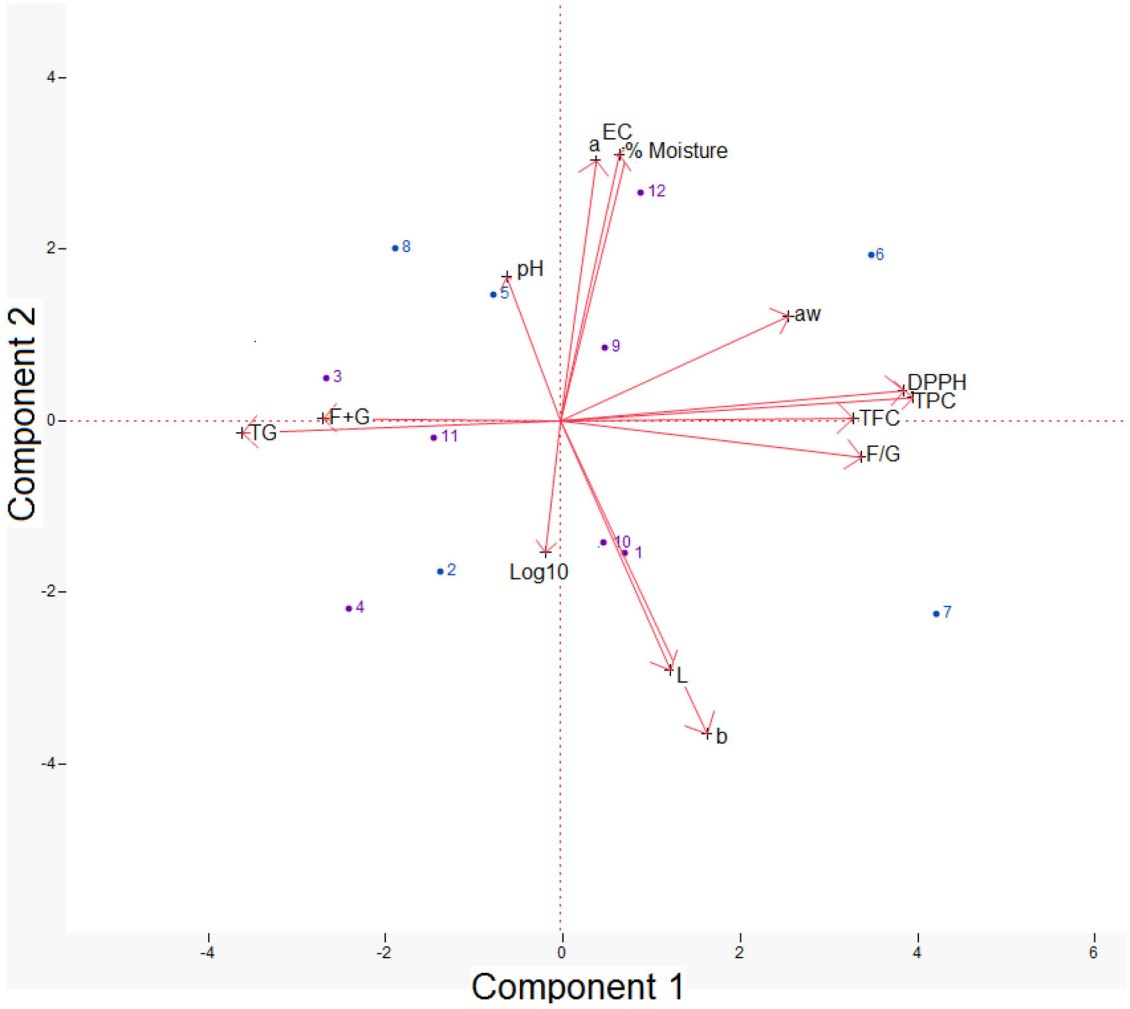

Figure 1. Principal component analysis (PCA) biplot for the honey samples collected from different regions of Bayburt. Variables were TPC (Total Phenolic Content), TCA (Total Flavonoid Content), DPPH, for antioxidant activity, L (lightness), a(redness), b (yellowness), F/G (fructose/glucose ratio), F + G (total fructose + glucose), EC (Electrical conductivity), TG (glass transition temperature), Log 10 (yeast and mold count), aw (water activity), % moisture.

The total flavonoid content of the honey samples was observed to be between 31.29-118.71 mg CAE kg^{-1} (Table 5). Total flavonoid content was determined in the S3 from the 5th region at the lowest value (26.74 mg CAE kg^{-1}) in accordance with the total phenolic content and the highest value was determined as 118.71 mg CAE kg^{-1} in the S2 from the 9th region.

We also tested the DPPH radical scavenging capacity of honey samples as this analysisis a very common method for measuring the antioxidant capacity of natural extracts(Silici et al., 2010). The percentage of the DPPH inhibition of the honey samples was found to be in the range of 12.98% and 94.79%(Table 5) and significantly varied within samples from different regions ($p < 0.01$).

The yeast-mould numbers in the honey samples collected from 12 regions were found to be between 2.69 ± 0.05 and 3.398 ± 0.08 $\log_{10}CFUg^{-1}$ in which the lowest and the highest yeast-mould numbers were detected in 3rd and 4th regions, respectively.

Together with the analysis of the phycicohemical characteristics of the honey samples collected from the different regions of Bayburt,a PCA was conducted to reduce the number of dimensions and to obtain a small number of factors that contain the maximum of variability between the samples. PC1 revealed the most variation, the differences among samples along the PC1 axis explained more compared to the similar distances along the PC2 axis. Five principal components (PCs) with eigenvalues >1 represent92.74% of total variance, PC1, PC2, PC3, PC4 and PC5 described 34.1%, 22.9%, 11.69%, 11.16% and 7.99% of total variance, respectively. According to biplot in Figure 1, the regions R1, R6, R7, R9, R10 and R12 were located on the right side of the plot while R2, R3, R4, R5, R8 and R11 were located on the left side of the biplot which illustrated that they possess approximately opposite responses. The biplot graph showed that the regions of R1 and R10 were closely related to each other. The colour L and b values were also in the same group and negatively

correlated to the a values. The variables, including TPC, TFC and antioxidant activity values (DPPH) were closely correlated to each other and showed similar information on PC1. It was also seen that there was a correlation between moisture content and water activity values. A negative correlation was also found between the F/G and the F + G values. When one compared the angles between variables, it could be seen that none of the evaluated properties were closely related to pH and $\log_{10}$ values in the same location of the biplot.

Adulteration in honey can be a major problem in many ways, including sectoral, legal and economic aspects. Although many methods have been applied to detect adulteration in honey in recent years, the most important point is the rapid and reliable detection of this adulteration. Among these methods, compared to other methods, DSC is faster and environmentally friendly, as it does not require solvents. In this study, the thermal properties of the honey samples collected from the 12 different regions of Bayburt province were recorded by DSC analysis. Figure S1 shows the DSC curves recorded during thermal scanning of the honey samples from different regions and the glass transition temperature (*Tg*) of honey samples monitored as onset and mid point *Tg* values are given in Table 1. The mid (inflection) point *Tg* values, as the most considered point for the glass transition temperature (ASTM-Standard, 1995)ranged between-36.37 °C - −41.8 °C and the lowest *Tg* values were recorded for the R5 and R6 which was also significantly lower ($p < 0.01$) compared to the *Tg* values of the other regions (Table 1).

Discussion

The geographical region of honey can affect its physico-chemical and other characteristics and in this study, the physical, chemical, and microbiological properties of honey samples collected from different regions of Bayburt, which is one of the important cities for the production of honey in Turkey were determined. The botanical-geographical origin of honey, storage conditions, and also frauds and adulterations are among the factors that affect the sugar composition of honey (Escuredo et al., 2014, Rodríguez-Flores et al., 2019). Fructose and glucose are major sugars in all honey types. In addition, disaccharides, trisaccharides and other oligosaccharides are present in honey in small concentrations. The concentration of fructose and glucose as well as their ratio are useful indicators for the classification of monofloral honeys (da Silva et al., 2016). The amount of sucrose in honey is a very important parameter used to evaluate the maturity of the honey. The sucrose content is analyzed to detect any adulteration in the honey and high levels of sucrose may indicate various adulterations such as the addition of cheap sweeteners like cane sugar or refined beet sugar (da Silva et al., 2016). Early harvest indicates that the sucrose has not been completely converted into glucose and fructose or it might reflect that honey bees have been artificially fed with sucrose syrup for a long period of time (da Silva et al., 2016; Tornuk et al., 2013). In addition, in the Turkish Food Codex (2012), it has been reported that the F/G ratio of blossom honey should be between 0.9 and 1.4 and the maximum amount of sucrose should be 5% (w/w). The results obtained in this study were in accordance with the legislation of the Turkish Food Codex (2012) and were similar to the previous studies conducted with different honey types (Can et al., 2015; Tezcan et al., 2011). Fructose is the most abundant carbohydrate in almost all types of honey, but in some honey types (rapeseed, dandelion and blue curls), which crystallize faster, the glucose ratio can be higher than the fructose ratio (da Silva et al., 2016). According to our findings, it can be said that the crystallization rate of Bayburt honey is slow and more importantly no artificial feeding was observed.

Water content is an important parameter that affects the shelf life of honey and affects the physical properties of honey such as viscosity and crystallization. It is also important for the detection of both improper storage conditions and honey adulteration. A high moisture content decreases the shelf life of honey due to microbial decomposition and the crystallization of honey causes changes in taste and aroma (Costa et al., 1999). It has also been noted that crystallization increases water activity, due to the decrease for glucose dissolved in the aqueous phase of honey and with the increased water activity, yeast cells may cause fermentation by their growth in honey. Our results revealed the appropriate composition of honey in terms of water activity and moisture content in Bayburt honey indicating that all of the honey samples meet the standards of both the Turkish Food Codex (2012) and Codex Alimentarius Committee on Sugars (2001) as the maximum moisture content for honey is determined to be 20%. These findings suggest that honey samples were stored in good conditions during their shelf life.

Another important chemical characteristic of honey is pH that can be affected by many factors especially the chemical composition of honey. In the present study, the pH of honey samples collected from different regions of Bayburt were below pH 4.5 which is a typical characteristic of floral honey (Piazza et al., 1986) and were within the range reported for honey samples from Turkey (pH 3.67 and 4.57) (Kayacier & Karaman, 2008, Sahinler et al., 2004), but higher than those obtained for Brazilian honeys (pH 3.2 − 4.2) (Costa et al., 2013), South East Asia honeys (pH 3.3-3.9) (Chuttong et al., 2016), and Amazon melipona honeys (pH 3.41 − 4.06) (de Almeida-Muradian et al. 2007). The variation in pH values of honeys among regions can be associated with differences in the pH of the nectar of the plants visited by the bees in different regions, as well as to variations in the pH of the soil, temperature and rainfall (Bandeira et al., 2018; Gheldof et al., 2002).

Electrical conductivity is a property that varies depending on the source of nectar, mineral, organic acid and protein content of the honey and is known as an important criterion in determining the botanical origin of the honey (Singh and Bath, 1997). While this ratio is less than 0.8 mS cm^{-1} in blossom honey and honeydew honey mixtures, it can be more than 0.8 mS cm^{-1} depending on the honey type (Ouchemoukh et al., 2007). According to the results of this study, it can be said that Bayburt honey shows the characteristics of blossom honey. Similarly, its electrical conductivity values closely match the results determined by Bayram and Demir (2018) for honey samples collected from the same region previously (between 0.36-0.69 mS cm^{-1}) whereas the electrical conductivity of Hatay honey from the South part of Turkey was determined to be between 0.48-1.88 $mScm^{-1}$ on average (Sahinler et al., 2004).

It has been reported in different studies that the colour of honey is associated with its botanical source and there is a high correlation between the antioxidant activity of honey and its colour and total phenolic content (Mohamed et al., 2010; Castiglioni et al., 2018; Bandeira et al., 2018). Compared to our findings, the colour values of honeys were reported to be between 24.56-41.21, 0.02-1.00 and 0.02-9.84 for L^*, a^* and for b^* values, respectively (Özcan & Ölmez, 2014). The differences in the colour values of the honey samples reveal that the region that the honey samples are produced can be a determinant factor for the colour properties of honey samples. Honeys with a L value lower than 50 are considered as dark whereas those with a L value higher than 50 are considered to be lighter (Tornuk et al., 2013). According to this, Bayburt honey can be defined as a dark colored honey and it is generally reported that dark colored honey is richer in pigments, phenolic compounds, pollen and mineral contents (Can et al., 2015). Therefore, our findings suggested that Bayburt honey might have these characteristics.

Honey is one of the main products that consumers prefer due to its potential health benefits originating from the presence of bioactive compounds that can be phenolic and flavonoid components. Previously, average phenolic content of honey from the Gümüşhane and Ordu provinces of Turkey, which are geographically close to the province of Bayburt, was determined as 308 ± 0.02 mg GAE kg^{-1} and 360 ± 0.02 mg GAE kg^{-1} respectively (Tezcan et al., 2011). In this study, the authors also determined the phenolic content of two honey samples collected from Turkey's Anzer region as 900 ± 0.04 mg GAE kg^{-1} and 880 ± 0.06 mg GAE kg^{-1}, respectively (Tezcan et al., 2011). Anzer honey is currently being sold as the most valuable honey on the Turkish markets and is considered medically beneficial (Gok et al., 2015). We should note that, in our study, there were honey samples (eg, R12-S1, R6-S4, R7-S5) with a total phenolic content similar to the values obtained for Anzer honey, indicating that the bioactive properties of Bayburt honey might be high, but detailed studies are required in terms the of characterization of these components in Bayburt honey. Similarly, Can et al. (2015) reported that the total phenolic content in different honey samples from Turkey showed a wide variation between 160.2 and 1200.4 mg GAE kg^{-1}. They reported that the phenolic content of acacia and multifloral honey samples were the lowest. The average phenolic content in the East Black Sea Region was determined to be 224 mg GAE kg^{-1} (Ertürk, 2014). The phenolic content of honey is affected by regional differences and our findings revealed that, the total phenolic content of Bayburt honey is within a good range compared to the other studies and close to the level of Anzer honey, especially in some regions.

Similar to the total phenolic content, total flavonoid content of honey samples is other important characteristics for honey as flavonoids are low molecular weight phenolic compounds that are vital components for the aroma and antioxidant properties of honey. Importantly, in recent years, there has been an increased interest in natural antioxidants such as flavonoids, due to their protective effects against oxidative damage (Blasa et al., 2006). Similar to the total phenolic content of Bayburt honey, the total flavonoid content of honey samples observed to be higher compared to the previous observations reported for multifloral honey and monofloral honey samples (Temizer et al., 2016) and within a similar range with the total flavonoid content of pine honey and flower honey which were reported to be in a range between $48.0 - 547.8$ mg QE kg^{-1} (Özkök et al., 2010) and 60.0- 267.5 mg QE kg^{-1} (Tornuk et al., 2013), respectively, suggesting a high antioxidant capacity for Bayburt honey. In the DPPH radical scavenging activity, we observed significant variances among the samples and these results can be originated from the fact that the components forming the content of the honey differ. Our findings are important, as honey has also been shown to have a broad antiradical scavenging activity (Mohamed et al., 2010; Silici et al., 2010). The antiradical activity varies between different honey samples depending on the geographical location of the different floral sources such as Turkey and Burkina Faso (Temizer et al., 2016; Meda et al., 2005;) and also within the same regions, as we noted.

Honey is a product with a minimal type and level of microorganisms, thanks to its natural properties (Snowdon & Cliver, 1996), although the presence of yeast, mold and spore-forming bacteria in honey can be a worrying situation, which might have a significant impact on the shelf life and leads to the deterioration of honey (Finola et al., 2007). Importantly, the microbiological quality of honey determines its acceptability for human consumption. The yeast and mould numbers in the honey samples collected in this study were in

accordance with previous observations (Aydın et al., 2008)suggesting that honey samples in the Bayburt region are within an acceptable level for the yeast-mould counts.

In terms of the thermal characteristics, the DSC analysis of the honey samples collected from the different regions revealed some significant differences among the tested samples as noted above. One of the main factors determining the *Tg* values of the honey samples is the level of glucose and fructose (Ouchemoukh et al., 2007) and in our study, no clear differences were observed in terms of glucose-fructose levels of the honey samples with low and high *Tg* values. This can be attributed to the fact that other factors such as process conditions of honey samples, heating/cooling rate, sample holding time, moisture content, etc. can alter the *Tg* values of honey samples as previously discussed (Ahmed et al., 2007), which can be also the reason for our observation. Nevertheless, the *Tg* values of the honey samples were in good agreement with the previous findings (Ahmed et al., 2007; Kántor et al., 1999) and no adulteration was performed in honey samples collected from twelve different regions.

Conclusions

In conclusion, this study showed that some physicochemical properties of honey samples collected from different regions of a small area, as Bayburt province in this study, can be significantly different and PCA analysis also confirmed the role of the region for the characteristics of honey samples. This study also showed the characteristics of honey samples in Bayburt region of Turkey. As the F/G ratio of Bayburt honey was found to be higher than 1.0, it can be said that the crystallization property of this honey is relatively slow, which can increase customer preference by providing convenience in the processing, transportation and storage of honey. In addition, a good level of bioactive compounds content was found for Bayburt honey depending on the location and in some regions, the content of the bioactive compound was importantly high. The microbiological quality of Bayburt honey was also within the acceptable range for the yeast-mould counts.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplementary material

Supplementary Figure S1 and Table S1 are available via the 'Supplementary' tab on the article's online page (http://dx.doi.org/10.1080/00218839.2020.1812806).

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