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Determination of Chemical, Physical and Biological Characteristics of Some Pekmez (Molasses) From Turkey

REZZAN ALIYAZICIOGLU*, SEVGI KOLAYLI†, MERYEM KARA‡, OKTAY YILDIZ†, ALI OSMAN SARIKAYA†, SEVIL CENGIZ¶ and FATIH ER‡

Vocational School of Health Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey
Fax: (90)(462) 3252933; Tel: (90)(462)3775619
E-mail: rezzanaoglu@mynet.com

Antioxidant capacity, chemical and physical properties of 6 certificated and 1 uncertificated pekmez (molasses) samples of Turkish varieties were analyzed. All pekmez samples had similar chemical compositions. Total phenolic contents varied from 138 to 243 mg of gallic acid equivalents/100 g samples. The apricot pekmez had the highest phenolic content (243 ± 16), while the carob pekmez exhibited the highest DPPH (0.08 ± 0.01 mg g^{-1}) radical scavenging activity. DPPH radical scavenging activity was also found to be related to concentrations of the samples. Especially, selenium and other minerals content were found slightly higher in grape pekmez than the others. Present results showed that all the certificated and uncertificated pekmez samples had no any risk in public health and besides their high content of sugar. Each pekmez had antioxidant and high amount of polyphenols and minerals.

Key Words: Antioxidant, Minerals, Pekmez, Polyphenol.

INTRODUCTION

Viticulture is one of the major agricultural sectors in the world. In 2004, Turkey had a viticulture plantation area of 565 000 ha with only grape production of 3 650 000 tons and ranked in the fourth and fifth places, in the world, respectively. It is estimated that approximately 18 % of grapes produced in Turkey is processed in to pekmez. Various pekmez types are produced in Turkey, which can be differentiated by their colours, constituents and tastes. They are also named by the geographic locations where they are produced, *e.g.*, Malatya, Zile, Gaziantep, Gümüshane[1,2].

†Sebinkarahisar High College of Food Technology, Giresun University, 28400, Giresun, Turkey.
‡Department of Sugar Technology, Çumra Vocational High School, Selçuk University, 42500 Konya, Turkey.
¶Department of Biochemistry, Faculty of Medicine, Karadeniz Technical University, 61080 Trabzon, Turkey.

Pekmez is one of the most common and known concentrated fruit juices in Turkey. It has been produced for a long time and is a traditional food especially in Gümüshane, Malatya, Gaziantep, *etc.* It is consumed mainly for breakfast instead of jam and marmalade[3]. Pekmez might be considered as functional food ingredients and nutraceuticals[4]. This is nutritionally important, especially for babies, children, sportmen and in situations demanding urgent energy and supplying mineral, especially iron deficiency[5]. Pekmez is produced from fresh or dried several fruits, such as grapes, mulberry, harnup, carob, date, apricot, apple, juniperus. Fresh fruits are squeezed directly, however dried fruits are subjected to extraction in an aqueous medium then pressed and derived extract called as must is homogenized. The acidity of extract is removed with a calcareous substance called 'Pekmez Earth' depending on type of fruit[1]. After deacidification extract is filtered with plain linen and special filter setups such as drum, kieselguhr, press, vacuum and concentrated by boiling. This boiling process is performed under low pressure at industrial scale. Liquid pekmez is produced by classical and vacuum evaporation methods in Turkey. But most of the manufacturing plants, particularly in rural region, use classical production methods[6]. In present research, all pekmez samples were produced by vacuum evaporation method except village (hand made) pekmez. Since pekmez contains high amount of sugar, mineral and organic acid, it is very important food products in human nutrition[7]. Major constituents of pekmez are carbohydrates, minerals and organic acids[8]. Pekmez is becoming increasingly popular because of its potential and perceived benefits.

There are number of researches about rheologic and chemical composition of pekmez[3,5,8-11]. However, there is little study about antioxidant properties of pekmez. Therefore, the purpose of this study was to determined chemical composition and antioxidant properties of some pekmez samples in Turkey to assess health risks for consumers. In addition, the purity of the pekmez is important to conform to the safety based on national and international standards.

EXPERIMENTAL

The chemicals for determination of ascorbic acid, gallic acid (GA), 5-hydroxymethyl-2-furfuraldehyde (HMF), 2,2-dipehynl-2-picrylhydrazly (DPPH), sucrose, glucose were purchased from Sigma Chemical Co. (Steinheim, Germany). Methanol, ferric chloride, glacial acetic acid, Folin-Ciocalteu's phenol reagent were supplied from Merck (Darmstadt, Germany) and butylated hydroxytoluene (BHT) were supplied from Applichem (Darmstadt). C_{18} sorbent phase (octadecyl) was purchased from Supelco (Bellefonte, PA, USA). All the chemicals used were of analytical grade.

Pekmez samples: Total 7 pekmez samples were studied in this study and out which, 6 pekmez samples were certificated and 1 pekmez was uncertificated. Certificated pekmez samples were produced by a factory, uncertificated one was by traditional method. Five commercial pekmez samples were selected from local supermarkets and the others from Gümüshane in Turkey. The origin of these pekmez samples were grape, date, apricot, mulberry and carob.

Chemical composition analysis: Total dry matter, pH, ash, invert sugar, glucose, sucrose, total lipid were determined according to standard method AOAC[12]. HMF was determined by a method in which the UV absorbance of a clarified aqueous pekmez samples solution is determined against a reference solution of the same samples in which the 284 nm chromophore of HMF is destroyed by disulphide[13]. Ascorbic acid contents were determined according to method of Farajzadeh[14].

Minerals were analyzed by using an ATI-Unicam 929 model atomic absorption spectrometer (Cambridge, U.K.) and Jenway PFP-7 flame photometer (England). The samples were digested by the wet oxidation method, by treating the samples with HNO_3 and H_2O_2. The minerals were quantified against standard solutions of known mineral concentrations that were analyzed concurrently. The amounts of Na, K, Ca, Fe, Cu, Zn and Mn metal ions were determined at the mg kg^{-1} (ppm) level. Phosphorous was colorimetrically analyzed in the form of vanadium phosphomolybdate[15].

Rheological properties: The viscosity (Pa s) of blends were determined at 30 ºC using a Viskotester HAAKE Viscotester Model: 6L/R (Made in EEC) equipped with spindle 2 at the speed of 5, 10, 20, 30, 60, 100 and 200 rpm. Enough samples in a 200 mL beaker was used to immerse the groove on the spindle with guard leg. Temperature was maintained using a thermostatically controlled water bath. All readings were recorded after 30 s.

Preparation of extracts for antioxidant activities: The pekmez samples were extracted in water and the phenolic compounds was separated by solid phase extraction using SPE (C_{18}-colon).The phenolic compounds adsorbed on the column were eluted with methanol. The methanolic extracts were concentrated to dryness under reduced pressure in a rotary evaporator at 40 ºC. The dried residue was dissolved in methanol to be analyzed.

Estimation of total phenolic compounds: Total phenolic composition was determined using Folin-Ciocalteu reagent and expressed as gallic acid equivalent (GAE)[16]. The samples and standard gallic acid were diluted to 2-20 µg in 2.0 mL distilled water and 2.0 mL of commercial Folin-Ciocalteu reagent was added. The content was mixed well and kept for 5 min at room temperature followed by addition of 2.0 mL of 10 % aqueous sodium carbonate and incubated at room temperature for 1 h. Absorbance of the developed blue colour was read at 760 nm (Shimadzu UV-2450, Shimadzu Corporation, Kyoto, Japan) against a reagent blank.

DPPH radical scavenging activity: DPPH free radical scavenging activity of the pekmez extracts were measured with a slight modification[17]. Briefly, various concentrations 0.75 mL of pekmez extracts were mixed with 0.75 mL of a 0.1 mM of DPPH in methanol. Antioxidant activity was measured spectrophotometrically at 517 nm, using catechin and BHT as standards and the values are expressed as SC_{50} (mg or µg sample per mL), the concentration of the samples that cause 50 % scavenging of DPPH radical. In the presence of an antioxidant, the purple colour of DPPH decays and the change of absorbance can be followed spectrophotometrically at 517 nm.

RESULTS AND DISCUSSION

Table-1 shows the botanical, common, vernacular and source of the 7 pekmez samples studied. The chemical compositions of the pekmez samples are given in Table-2A and 2B. When each parameter of the chemical composition was compared to the values set of by Turkish Standards Institute (TSE), most of the values were found to be within accepted ranges. pH values of all pekmez samples were close to each other. Total dry matter of the pekmez was found 65-72 % brix values, that these values are called liquid pekmez[18]. All of the pekmez samples were found not to contain ascorbic acid. This may be attributed that ascorbic acid is degraded at high temperatures or deacidification may have been a reason for destruction of ascorbic acid[19]. Ascorbic acid, water soluble vitamin, is sensitive to heating, oxygen, light and alkalines but resistance to acids. It can be oxidized at the presence of heavy metals and can be stabilized with acids. Especially in thermally preserved juices, oxidation of ascorbic acid may occur[20-22]. Sugars represent the main components of all the pekmez and invert sugars (or reducing sugar), mainly, glucose and

TABLE-1
BOTANICAL, COMMON, NAMES, VERNACULAR NAME AND SOURCE OF THE SEVEN PEKMEZ SAMPLES FROM TURKEY

Herbarium code	Botanical	Common name	Vernacular	Source
PK1	*Vinis vinifera L*	Grape	Üzüm	Factory of Malatya
PK2	*Phoenix dactylifera*	Date	Hurma	Factory of Malatya
PK3	*Prunus armenia*	Apricot	Kayisi	Factory of Malatya
PK4	*Morus nigra*	Mulberry	Dut	Factory of Malatya
PK5	*Carotonia siliqua*	Carob	Keçiboynuzu	Factory of Malatya
PK6	*Morus nigra*	Mulberry	Dut	Factory of Gümüshane
PK7	*Morus nigra*	Mulberry	Dut	Village / Gümüshane (Hand made) Uncertificated

TABLE-2A
CHEMICAL COMPOSITION OF THE PEKMEZ SAMPLES

	PK1	PK2	PK3	PK4
Total dry matter (%)	73.1 ± 0.5	70.4 ± 1.1	69.8 ± 0.9	70.6 ± 0.8
pH	5.0 ± 0.4	5.1 ± 0.5	5.1 ± 0.4	5.4 ± 0.3
Ash (%)	2.1 ± 0.2	1.9 ± 0.1	1.8 ± 0.1	2.2 ± 0.2
Total lipid (mg 10^{-2} g^{-1})	6.6 ± 3.6	8.6 ± 4.2	7.9 ± 5.2	11.5 ± 4.4
Invert sugar (g 10^{-2} g^{-1})	59.0 ± 4.4	77.0 ± 3.8	56.0 ± 4.8	46.0 ± 5.2
Glucose (g 10^{-2} g^{-1})	56.6 ± 4.2	66.0 ± 3.7	45.1 ± 4.2	41.0 ± 3.3
Sucrose (g 10^{-2} g^{-1})	2.6 ± 0.7	11.0 ± 2.5	11.0 ± 1.5	5.3 ± 0.9
Ascorbic acid (mg kg^{-1})	ND	ND	ND	ND
HMF (mg kg^{-1})	46.0 ± 2.6	149.0 ± 4.1	77.3 ± 2.5	96.9 ± 3.1

All results are given as mean ± SD (standard deviation). PK1, grape; PK2, date; PK3, apricot; PK4, mulberry from Malatya; PK5, carob; PK6, mulberry from Gümüshane; PK7, mulberry from village; HMF, Hydroxymethylfurfural; ND, not detectable.

TABLE-2B
CHEMICAL COMPOSITION OF THE PEKMEZ SAMPLES

	PK5	PK6	PK7	TSE
Total dry matter (%)	67.8 ± 0.7	70.2 ± 0.5	70.8 ± 0.8	65-72
pH	5.6 ± 0.4	5.4 ± 0.2	4.9 ± 0.3	5.0-6.0
Ash (%)	1.3 ± 0.3	2.3 ± 0.4	1.2 ± 0.2	Max: 4
Total lipid (mg 10^{-2} g^{-1})	2.8 ± 1.1	2.9 ± 2.1	6.5 ± 3.2	–
Invert sugar (g 10^{-2} g^{-1})	48.0 ± 6.4	82.0 ± 7.8	65.0 ± 7.2	60-70
Glucose (g 10^{-2} g^{-1})	41.7 ± 5.1	65.0 ± 5.4	51.0 ± 6.1	36-54 for Mulberry
Sucrose (g 10^{-2} g^{-1})	6.3 ± 2.4	17.0 ± 2.2	14.0 ± 2.7	Max: 14-17 % for mulberry, 5 % for grape
Ascorbic acid (mg kg^{-1})	ND	ND	ND	–
HMF (mg kg^{-1})	90.3 ± 4.1	152.3 ± 5.2	18.0 ± 1.2	Max: Type I: 75 Max: Type II: 150

All results are given as mean ± SD (standard deviation). PK1, grape; PK2, date; PK3, apricot; PK4, mulberry from Malatya; PK5, carob; PK6, mulberry from Gümüshane; PK7, mulberry from village; HMF, Hydroxymethylfurfural; ND, not detectable.

fructose have been found to be the major component of pekmez. High sucrose levels of the pekmez were belonging to mulberry, date and apricot pekmez samples, grape pekmez didn't contain sucrose. However, sucrose levels were measured at 2.6 ± 0.7 g 10^{-2} g^{-1}. Some may be added during the pekmez preparation. Especially, people in the village sometimes add sugar to the pekmez to reach desired brix value immediately. But sucrose percentage of grape pekmez was below the maximum allowable limit 5 % proposed by TSE.

The most common quality problem in all types of pekmez is browning reaction. Caramelization or Maillard reactions are the non-enzymatic browning reactions and HMF is an indicator of Maillard reaction[23]. HMF contents are widely used as an indicator of the Maillard reaction and potential browning of some foods, such as pekmez and honeys[24]. HMF can be formed by hexose dehydration in an acid media. Browning reactions can be followed by measuring the intermediates and/or final products. Generally, following early intermediates with the final products is preferable to get an idea of how the reaction is proceeding at different stages. The formation of HMF depends on pH, temperature, duration of processing, storage, sugar and amino acid types[25]. In present study, the formation of HMF is used as a one of quality indicator. Maximum HMF limit for the first quality pekmez is 75 mg kg^{-1} and the second quality[18] is 150 mg kg^{-1}. The pekmez samples showed the variable HMF levels. Two pekmez, grapes (PK1) and hand made mulberry pekmez samples (PK7) showed low HMF levels and another 5 samples showed the second quality HMF levels. HMF concentration in golden delicious apple juice concentrates was between 0.52-963 mg kg^{-1} and between 0.52-190 mg kg^{-1} in Amasya apple juice concentrates[26]. The amount of HMF was 21.1 and 19.1 (mg kg^{-1}) for grape and mulberry pekmez samples, respectively[27]. HMF concentration of present hand made village mulberry pekmez was close to parameter presented in their study. According to HMF results, it can be concluded that 5 pekmez samples were exposed to high boiling temperatures. It was expected to find the HMF content of hand made village pekmez higher than the others since most of the pekmez production in Turkey is done in households without controlling temperature, time and other process parameters. But in present study, HMF level of village pekmez was low (18 mg kg^{-1}). This may have been due to degradation of HMF to other end products such as melanoidins. Melanoidins are the final products of non-enzymatic browning and HMF is one of the undesirable intermediates of the Maillard reaction[28].

The average mineral contents of pekmez (mg kg^{-1} of pekmez) are shown in Tables 3A and 3B. Each pekmez tested in present study had different mineral composition perhaps depending on their raw materials. Seven different origins of pekmez samples have showed variable mineral contents and in general, mineral contents of the pekmez samples were found to be higher in PK1 than the others, especially richer in selenium, zinc and copper. The carob pekmez having the highest sodium and potassium content. Of all the tested samples hand made village and grape pekmez samples had the highest iron content. Grape and carob pekmez samples gave the

TABLE-3A
MINERAL CONTENT (mg kg^{-1}) OF THE PEKMEZ SAMPLES

Samples	Na	K	Ca	Fe	Mn
PK1	1353 ± 124	831 ± 82	186 ± 21	3.4 ± 0.5	0.7 ± 0.03
PK2	508 ± 62	327 ± 33	124 ± 16	0.7 ± 0.1	0.2 ± 0.05
PK3	489 ± 35	470 ± 25	232 ± 26	2.5 ± 0.5	0.4 ± 0.01
PK4	944 ± 71	469 ± 38	164 ± 21	1.5 ± 0.3	0.7 ± 0.04
PK5	1775 ± 247	1039 ± 126	157 ± 14	0.7 ± 0.1	0.2 ± 0.01
PK6	818 ± 110	202 ± 17	146 ± 11	2.3 ± 0.2	0.2 ± 0.02
PK7	680 ± 89	670 ± 31	216 ± 32	7.0 ± 0.6	0.4 ± 0.01

All results are given as mean ± SD (standard deviation). PK1, grape; PK2, date; PK3, apricot; PK4, mulberry from Malatya; PK5, carob; PK6, mulberry from Gümüshane; PK7, mulberry from village.

TABLE-3B
MINERAL CONTENT (mg kg^{-1}) OF THE PEKMEZ SAMPLES

Samples	Ni[a]	Zn	Cu	Se[a]	P
PK1	0.9 ± 0.05	32.7 ± 6.7	23.20 ± 5.1	18.2 ± 2.4	48 ± 6
PK2	0.9 ± 0.04	9.7 ± 1.1	7.50 ± 1.5	ND	28 ± 3
PK3	0.5 ± 0.06	25.5 ± 4.9	7.00 ± 1.4	ND	42 ± 9
PK4	0.1 ± 0.04	14.7 ± 3.7	6.50 ± 1.1	ND	44 ± 5
PK5	1.6 ± 0.07	2.3 ± 0.9	4.05 ± 0.9	ND	48 ± 7
PK6	0.9 ± 0.02	10.2 ± 2.4	3.70 ± 0.8	ND	18 ± 3
PK7	1.4 ± 0.04	43.5 ± 3.8	2.30 ± 0.9	ND	42 ± 6

All results are given as mean ± SD (standard deviation). PK1, grape; PK2, date; PK3, apricot; PK4, mulberry from Malatya; PK5, carob; PK6, mulberry from Gümüshane; PK7, mulberry from village; a, μg kg^{-1}; ND, not detectable.

highest phosphorus content. Sodium content of grape pekmez was approximately 2 times more than that of date pekmez. In present study, pekmez samples obtained from the same fruit showed differences in mineral contents. This may be due to growing conditions such as soil and process conditions (extraction, adding pekmez earth). The Pb, Co and Cr levels were in undetectable quantities in the samples, which are considered a good food quality, which proves pekmez to be a safe or free of these toxic elements[29].

The rheological properties of the liquid pekmez were studied and Fig. 1 shows the plot of viscosity *versus* speed (rpm) for all pekmez samples at 30 °C. As shown from the figure, viscosity of pekmez samples decreased as speed increased. Sengul *et al.*[9] obtained similar diagrams for mulberry pekmez. Flow behaviour index (n) was calculated using power law model. N values of 5 pekmez (PK1, PK2, PK3, PK5, PK6) samples ranged from 0.78-0.96 and two (PK4, PK7) ranged from 1.00-1.01. Thus, 5 pekmez samples exhibited non-Newtonian behaviour because their n values (showing deviation from the Newtonian character) were less than 1 and 2 exhibited Newtonian behaviour. Mulberry pekmez studied by Sengul *et al.*[9] showed pseudoplastic behaviour and results of Newtonian behaviour of present two pekmez samples were similar to that of diluted solid pekmez studied by Kaya and Belibagli[1].

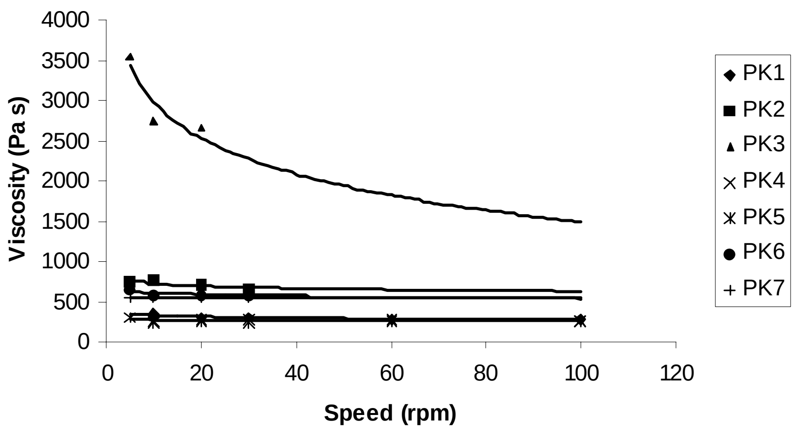

Fig. 1. The flow behaviour of the pekmez samples at 30 ºC

The total phenolic compounds of aqueous pekmez samples were determined and the values are given in Fig. 2. The pekmez samples were rich in polyphenols and the polyphenol contents were variable. Karababa and Isikli specified that protein, amino acids, phenolic compounds and flavonoids are minor components in pekmez and due to their antioxidant and antimutagenic properties, phenolics and flavanoids have positive effects on health[27]. In present study, apricot and date pekmez showed the highest polyphenols content. The amounts of phenolic contents were variable in the samples (ranging from 138 to 243 mg 10^{-2} g^{-1} sample). For example, only mulberry pekmez, obtained by three different ways showed three different polyphenols content (values were 138, 189 and 199 mg 10^{-2} g^{-1} sample). The difference may be depending on the floral origin of the pekmez and style of pekmez preparation.

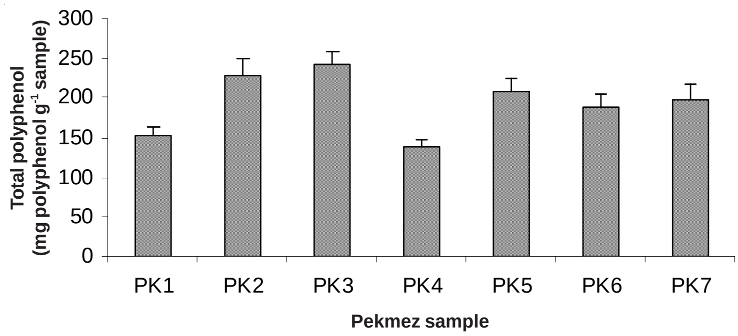

Fig. 2. Total polyphenols content of the pekmez. All results are given as mean ± SD (standard deviation). PK1, grape; PK2, date; PK3, apricot; PK4, mulberry from Malatya; PK5, carob; PK6, mulberry from Gümüshane; PK7, mulberry from village

DPPH free radical scavenging activity of the samples is given in Fig. 3. All the samples efficiently were scavenging of DPPH radicals and when compared to reference antioxidants BHT and catechine. DPPH radical scavenging activity was the highest in carob pekmez. This may be that of polyphenols in the samples may vary highly as do their scavenging capacity. Thus, present phenolic compounds may have acted as free radical scavengers by virtue of their hydrogen-donating ability[30].

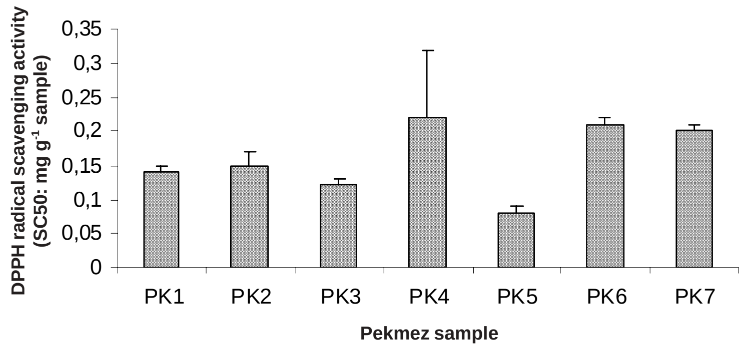

Fig. 3. DPPH radical scavenging activity of the pekmez. All results are given as mean ± SD (standard deviation). PK1, grape; PK2, date; PK3, apricot; PK4, mulberry from Malatya; PK5, carob; PK6, mulberry from Gümüshane; PK7, mulberry from village; DPPH, 2,2-dipehynl 2-picrylhydrazly; standard BHT, Butylated hydroxytoluene (10 µg mL^{-1}, 10 ± 0.1); standard catechin (2 µg mL^{-1}, 2 ± 0.1)

Although all the pekmez samples showed similar, chemical, physical and biological characteristics, small differences in their quality parameters may be due to the differences in production style, raw material and shelf life etc. Thus, it can be concluded that all the certificated and uncertificated pekmez samples had no any risk in public health and besides their high content of sugar, each pekmez had antioxidant and high amount of polyphenols and minerals.

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