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Sensory Profile of Greek Islands Thyme Honey

Inmaculada Rodríguez [1], Chrysoula Tananaki [2], Hortensia Galán-Soldevilla [1], Pilar Ruiz Pérez-Cacho [1] and Salud Serrano [1,*]

[1] Food Science and Technology Department, University of Córdoba, 14071 Córdoba, Spain; v62rodem@uco.es (I.R.); bt1gasoh@uco.es (H.G.-S.); bt2rupem@uuco.es (P.R.P.-C.)
[2] Laboratory of Apiculture-Sericulture, School of Agriculture and Forestry, Aristotle University of Thessaloniki, 54636 Thessaloniki, Greece; tananaki@agro.auth.gr
* Correspondence: sserrano@uco.es; Tel.: +34-957212654

Featured Application: The application of this work is to add new data to the characterization of unifloral honeys. Recently, it has been accorded that the best way to authenticate genuine food as honey is by means of rapid non-targeted methods that need to be validated from established patterns describing each of the different unifloral honeys. Sensory profiles responding to specific botanical and geographical origins are an important tool for recognizing the authenticity of a particular honey.

Abstract: The sensory profiles of thyme honey from the Greek islands with different thymus pollen grain contents (A: >60%, B: 40–60%, and C: 18–40%) were studied. The results of the physico-chemical analyses fulfilled the criteria set by international quality standards and, specifically, Greek legislation (moisture content < 18%, hydroxymethylfurfural < 10 mg/kg, and diastase activity > 20 DN). The sensory results showed that there were significant differences between groups with different pollen grain contents ($p < 0.01$) for all attributes except for floral aroma, with the Group A samples being the lightest in color (4.9 ± 1.8) and having the highest floral odor intensity (5.0 ± 2.0) and salty taste (3.5 ± 1.1). Additionally, samples with the highest pollen grain content (i.e., Group A) had olfactory notes of wood/wax/resin and a chemical aroma.

Keywords: unifloral thyme honey; physico-chemical parameters; descriptive sensory analysis

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1. Introduction

Honey is a natural food produced by honeybees from nectar or honeydew varying not only in chemical composition but also in color, taste, and odor [1]. It mainly consists of sugars but also contains many other substances such as proteins, enzymes, minerals, acids, and volatile compounds. Its properties depend on many factors such as the botanical and geographical origin, the intensity of nectar flow, the climatic conditions, the beekeepers' manipulations, the handling and packing procedure, the time of storage, and the conditions of storage [2].

Traditionally, the botanical origin of honey is determined with the use of pollen analysis (i.e., melissopalynology). Although pollen analysis may have several limitations [3,4], the combination of pollen analysis with physico-chemical and organoleptic characteristics can overcome these limitations and provide reliable results [5].

Unfortunately, international legislation that combines these three types of analysis (i.e., pollinic, physico-chemical, and sensory analysis) necessary to authenticate honey does not exist. The Codex Alimentarius Commission [6] and European legislation [7] indicate solely physico-chemical quality criteria. The International Honey Commission (IHC) has made an effort to characterize 15 unifloral honeys that are the most representative in Europe due to their abundance and commercial importance. This work could serve as a starting point for each country to adopt additional parameters to describe domestic honey types [2].

Thyme honey is one of the main blossom honeys in Greece and represents 10% of the total annual Greek honey production. It is mainly produced on the islands and on the mainland where plants of the genus *Thymus* grow. Thyme blossoms in summer and its nectar flow depends on the weather (i.e., Mediterranean climates). Honeybees collect the nectar and produce aromatic honey that has been famous since ancient times. This type of Greek honey presents higher values of diastase activity and proline than other monofloral honeys [8], and because of its appreciated sensory characteristics, it is sold at higher prices. According to Greek legislation, thyme honey can only be labeled as unifloral if its thyme pollen percentage is equal to or greater than 18% of the total nectariferous pollen grains, and the total number of pollen grains per 10 g of honey must also be lower than 90,000.

Several papers have been published on the physico-chemical and microscopic characteristics of thyme honey [9–13]. Especially concerning Greek thyme honey, studies have been conducted on its physico-chemical characteristics, pollen spectrum, mineral content, bioactive compounds, and volatile substances [8,14–17], but no data regarding its sensory characteristics were found. Over the last few years, few studies have been conducted regarding the sensory characteristics of thyme honey [12,13,18,19].

The aim of this study was to determine the sensory profile of thyme honey from the Greek islands with different thymus pollen grain contents.

2. Materials and Methods

2.1. Material

2.1.1. Samples Used for Development of the Lexicon

Fresh thyme honey samples from the Greek islands were collected directly from producers. Instructions were given to beekeepers to collect thyme honey samples from new honeycombs as well as to apply good beekeeping practices during the nectar flow season. After pollen analyses, the thyme honey samples were classified into three classes according to their thymus pollen grain content: A: >60%, B: 40–60%, and C: 18–40%, and they were stored at −18 °C until analysis.

2.1.2. Samples Used for Sensory Profile

Nine fresh thyme honey samples, three from each pollen group (A, B, and C), were used to determine the sensory profile.

2.2. Methods

2.2.1. Pollen Analysis

A qualitative melissopalynological analysis (performed in duplicate) was carried out on all honey samples according to the working methods described by Louveaux et al. (1978) [20] to identify the pollen types and to confirm the dominance of *Thymus capitatus* L. Counts were expressed as percentages after counting a minimum of 1200 pollen grains on three slides per sample. The pollen slides were examined at 400 and 1000 under a light microscope (Nikon Labophot-2 microscope; Nikon, Tokyo, Japan) to identify the types of pollen.

2.2.2. Physico-Chemical Parameters

For the humidity, electrical conductivity, hydroxymethylfurfural (HMF), and diastase activity measurements, the recommended IHC methods were applied [21]. Humidity (moisture) was determined following Chataway (1932) [22] and Wedmore (1955) [23], a method established by the Codex Alimentarius Commission (1969) [24]. We used an Abbe-type refractometer, obtaining the corresponding percentage of water from the Chataway table. Electrical conductivity was measured at 20 °C in a 20% (w/v) solution of honey (dry matter basis) in deionized water using a Crison model 524 conductimeter (Crison Instruments, Barcelona, Spain), according to Vorwohl (1964) [25]. Hydroxymethylfurfural determination was conducted according to the Winkler method (Winkler, 1955) [26] using a Pharmacia Biotech Ultrospec-3000 spectrophotometer. The results are expressed in

HMF milligrams per kg of honey. Diastase activity was examined via the procedure of Siegenthaler (1977) [27] and modified by Bogdanov (1984) [28]. Adsorption was followed using a Pharmacia Biotech Ultrospec-3000 spectrophotometer (England). The results were calculated (as Gothe's degrees, °G) as ml of 1% starch hydrolyzed by an enzyme in 1 g honey for 1 h.

2.2.3. Sensory Analysis

Development of the Lexicon

A group of assessors, with previous experience in the sensory analysis of honeys, participated in the flavor lexicon generation (odor/aroma, basic tastes, and trigeminal sensations) using a previously defined vocabulary to describe honeys [29]. This group of assessors was composed of ten highly trained panelists from the Sensory Laboratory at the University of Córdoba (Spain). They were selected and trained following international standards (ISO). The selection of the candidates was based on detection, recognition, and discrimination tests as well as their ability to memorize and communicate sensory impressions. These panelists had prior experience in the sensory evaluation of different products, and they had undergone specific training in honeys. They were exposed in panel booths to a variety of thyme honeys to obtain a comprehensive set of descriptors. The procedure followed to obtain the vocabulary was based on ISO 13299:2016 [30].

Sensory Profile

The methodology followed was based on ISO 13299:2016 [30]. For each sample, first its odor was analyzed, then its color intensity and its fluidity, next its aroma, basic tastes, and trigeminal sensations, and last its persistence. Thirty grams of each sample was put into a glass vial and covered with a watch glass for sensory analysis. Three samples, labeled with three-digit random numbers, were served, one at a time, over a session, and mineral water was used to cleanse the palate between samples. Testing was carried out in the Sensory Laboratory, which was equipped with a round table for training sessions and individual booths, in accordance with the ISO 8589:2007 [31]. All analyses were conducted in the morning (10 a.m.–12 p.m.).

Statistical Analysis

Data obtained from physico-chemical parameters and sensory profile were processed using SPSS 17.0 software. A basic descriptive statistical analysis was performed (mean and standard deviation) and one-way ANOVA was applied for each sensory attribute to test mean differences between honeys with different pollen grains, followed by Tukey's test at a 95% confidence level ($p < 0.05$). In addition, one-way ANOVA was applied for each sensory attribute to test the mean differences between replicates.

3. Results and Discussion

3.1. Development of the Lexicon

The freshness and authenticity of the tested samples were confirmed by HMF and diastase activity analyses. We rejected samples that had a diastase activity less than 20 DN and those with a 5-(hydroxymethyl)-2-furaldehyde (HMF) of more than 10 mg/kg to ensure that the samples were fresh, unheated, and authentic [32].

The preliminary flavor vocabulary is presented in Table 1. Odor and aroma attributes were grouped into families and/or subfamilies in a roundtable session under the direction of the panel leader, and a consensus lexicon was developed: **floral**; **fruity**—acid (citric and lemon), ripe (raisin), and nutty (almond); **vegetal**—wood/resin/wax, aromatic herbs (mint); **toasty**—caramel and smoke; **chemical** (thymol). The resulting initial working list of terms was composed of nine odor/aroma attributes (i.e., overall intensity, floral, lemon, raisin, almond, wood/resin/wax, caramel, smoke, and chemical), four terms for basic tastes (i.e., sweet, acidic, salty, and bitter), and three for trigeminal sensations (i.e., fresh, astringent, and piquant). In the following panel sessions, the initial working list was reduced in accordance with ISO 13299:2016 [30]. Thus, the citric, lemon, raisin, almond, and aromatic herbs odor/aroma attributes, acidic taste, and piquancy were discarded because they did not describe the product. The final list of flavor attributes, definitions, and references for Greek islands thyme honey is presented in Table 2. In addition, color intensity (i.e., appearance), fluidity (i.e., texture), and flavor persistence were also included in the final list of the lexicons, resulting in 14 final attributes (one for appearance, one for texture, six for odor/aroma, three basic tastes, two trigeminal sensations, and flavor persistence).

Table 1. Flavor preliminary descriptors.

Odor/Aroma	Basic Taste	Trigeminal Sensation
Overall intensity Floral Fruit Acidic fruit Citric Lemon Nutty Almond Ripe fruit Raisin Wood Wax Resin Chemical Thymol Mint Aromatic herbs Smoke Caramel	Sweet Salty Bitter	Fresh Cooling Astringent Pungent Piquant

Table 2. Sensory attribute definitions and references.

Attributes	Definition	Reference Product	Bibliography
ODOR/AROMA	Sensation perceived by the olfactory organ in sniffing certain volatile substances (odor) or via the back of the nose when tasting (aroma).		ISO 5492
Overall intensity	Strength of the stimuli perceived by the nose (odor) or by olfactory receptors via the retronasal method (aroma).		ISO 5492
Floral family			
Floral	Odor associated with different flowers.	Benzyl acetate (0.1 g/L ethanol)	ISO 5496
Vegetable family			
Wood/resin/wax	Odor/aroma associated with pine trees, new furniture, sawdust, wax, and resin.	Pine shavings in a 60 mL flask	Galán et al. 2005
Toasted family			
Caramel	Characteristic odor/aroma of caramelized sugar.	Liquid caramel	Galán et al. 2005
Smoke	Characteristic odor/aroma of smoke.	No reference used	
Chemical family			
Chemical	Odor associated with drugs.	No reference used	
BASIC TASTES	Any one of the distinctive tastes: acidic, bitter, salty, and sweet.		
Sweet	Primary taste produced by diluted aqueous solutions of natural or artificial substances such as sucrose or aspartame.	Sucrose (10 g/L water)	ISO 5496

Table 2. *Cont.*

Attributes	Definition	Reference Product	Bibliography
Salty	Primary taste produced by diluted aqueous solutions of sodium chloride.	Salt (1 g/L water)	ISO 5496
Bitter	Primary taste produced by diluted aqueous solutions of several products such as quinine or caffeine.	Caffeine (0.03 g/L water)	ISO 5496
TRIGEMINAL SENSATIONS	Sensation resulting from irritation caused by chemical stimuli in the mouth, nose, or throat.		
Fresh	Sensation of freshness in the buccal cavity (as produced by eucalyptus oil).	Minty sweet	
Astringency	Organoleptic attribute of a pure substance or mixtures that produce the astringent sensation.	90% dark chocolate	

The aroma of honey is particularly specific, resulting from the combination of volatile compounds present in low concentrations. Chemical volatile composition has great importance in characterizing honey's botanical source, which directly influences its organoleptic characteristics [33–38]. In this field, Machado et al. (2020) [33] and Karabagias et al. (2016) [39] showed the volatile fingerprint of thyme honey, which exhibits several compounds that vary according to geographical origin, emphasizing the importance of the production area in the final volatile composition.

3.2. Sensory Profile

To confirm the freshness and authenticity of the samples, electrical conductivity, moisture, diastase activity, and HMF were determined (Table 3) before the sensory analysis. With respect to the moisture content of honeys, the latter varied between 14.1% and 18.2%, with a mean value of 15.4. This indicates that all samples were from ripe honey. The electrical conductivity was less than 0.6 mS/cm^3 as required by Greek legislation for thyme honey [40]. Regarding HMF and diastase activity, they ranged from 0.9 mg/kg to 10.0 mg/kg and from 20.2 to 50.6 DN, respectively, coinciding with Thrasyvoulou and Mannikis (1995) [8], who found high diastase values for thyme Greek honey.

Table 3. Physico-chemical characteristics of thyme honey samples (*n* = 9).

	M (%)	EC (mS/cm)	HMF (mg/kg)	DA (DN)
A1	15.3	0.600	0.9	50.6
	14.6	0.319	2.5	20.2
	14.7	0.376	5.5	26.8
B	14.1	0.203	3.6	20.2
	15.8	0.522	10	21
	15.8	0.522	10	21
C	15.8	0.517	7	20.4
	14.4	0.468	2.8	20.3
	18.2	0.462	8	20.2
Minimum	14.1	0.203	0.9	20.2
Maximum	18.2	0.600	10.0	50.6
Mean ± SD	**15.4** ± 1.4	**0.443** ± 0.1	**5.6** ± 3.5	**26.5** ± 11.5

M = moisture; EC = electrical conductivity; HMF = hydroxymethylfurfural; DA = diastase activity expressed as diastase number.

Table 4 presents the means, standard deviations, and the analyses of variance between honey groups with different pollen grain contents for the sensory attributes studied.

Table 4. Descriptive measures (means values and standard deviation), analysis of variance (replicates) and analysis of variance (pollen grain groups) of sensory attributes (F and probability values).

Pollen Grain Content	S	Color Int.	Fluid	Overall Odor Int.	Floral Odor	Caramel Odor	Overall Aroma Int.	Floral Aroma	Wood aroma	Caramel Aroma	Chemical Aroma	Sweet Taste	Salty Taste	Fresh	Persistence
A	1	7.1 ± 0.5 ^{a}	4.1 ± 0.3 ^{a}	4.3 ± 0.3 ^{a}	2.3 ± 0.5 ^{a}	-	5.4 ± 0.3 ^{a}	5.3 ± 0.2 ^{a}	-	-	5.2 ± 0.2 ^{a}	5.1 ± 0.1 ^{a}	5.0 ± 0.1 ^{a}	2.0 ± 0.4	3.6 ± 0.3
	2	2.9 ± 0.3 ^{b}	3.9 ± 0.3 ^{a}	3.5 ± 0.3 ^{b}	6.3 ± 0.4 ^{b}	-	4.3 ± 0.2 ^{b}	3.3 ± 0.2 ^{b}	3.4 ± 0.1	-	4.2 ± 0.2 ^{b}	5.1 ± 0.2 ^{a}	2.9 ± 0.3 ^{b}	1.4 ± 0.4	3.8 ± 0.3
	3	4.7 ± 0.5 ^{c}	4.9 ± 0.4 ^{b}	4.2 ± 0.3 ^{a}	6.5 ± 0.4 ^{b}	-	4.3 ± 0.2 ^{b}	3.3 ± 0.2 ^{b}	3.9 ± 0.2	-	4.4 ± 0.3 ^{b}	5.8 ± 0.4 ^{b}	2.6 ± 0.3 ^{b}	1.5 ± 0.4	3.9 ± 0.3
	M	**4.9 ± 1.8 ^{A}**	**4.3 ± 0.6 ^{B}**	**4.0 ± 0.4 ^{A}**	**5.0 ± 2.0 ^{B}**	**-**	**4.7 ± 0.6 ^{A}**	**3.9 ± 1.0**	**2.4 ± 1.8**	**-**	**4.6 ± 0.5**	**5.3 ± 0.4 ^{A}**	**3.5 ± 1.1 ^{A}**	**1.7 ± 0.4**	**3.8 ± 0.3 ^{A}**
	F ***p***	**109.47** **0.001**	**13.65** **0.001**	**9.27** **0.01**	**155.57** **0.001**	**-**	**38.09** **0.001**	**142.46** **0.001**	**-**	**-**	**20.51** **0.001**	**13.72** **0.001**	**123.17** **0.001**	**ns**	**ns**
B	1	4.2 ± 0.5 ^{a}	4.9 ± 0.3	4.3 ± 0.4	3.1 ± 0.2 ^{a}	-	4.1 ± 0.3	3.9 ± 0.2	-	-	-	4.9 ± 0.5 ^{a}	2.3 ± 0.3 ^{a}	-	2.8 ± 0.4 ^{a}
	2	3.8 ± 0.3 ^{a}	4.9 ± 0.3	4.6 ± 0.3	4.4 ± 0.5 ^{a}	-	3.8 ± 0.1	3.8 ± 0.2	-	-	-	4.3 ± 0.5 ^{a}	2.8 ± 0.2 ^{b}	-	1.8 ± 0.5 ^{b}
	3	6.2 ± 0.5 ^{b}	4.7 ± 09	3.7 ± 0.4	3.6 ± 0.6 ^{b}	3.5 ± 0.5	3.9 ± 0.5	3.8 ± 0.6	3.5 ± 0.7	3.0 ± 0.8	4.3 ± 0.2	6.1 ± 0.4 ^{b}	1.4 ± 0.3 ^{c}	-	3.9 ± 0.3 ^{c}
	M	**4.7 ± 1.2 ^{A}**	**4.8 ± 0.5 ^{A}**	**4.2 ± 0.5 ^{A}**	**4.1 ± 0.5 ^{A}**	**-**	**3.9 ± 0.3 ^{AB}**	**3.8 ± 0.4**	**-**	**-**	**-**	**5.1 ± 0.9 ^{A}**	**2.2 ± 0.6 ^{B}**	**-**	**2.8 ± 0.9 ^{B}**
	F ***p***	**41.60** **0.001**	**ns**	**ns**	**4.28** **0.05**	**-**	**ns**	**ns**	**-**	**-**	**-**	**16.93** **0.01**	**31.07** **0.001**	**-**	**35.81** **0.001**
C	1	7.0 ± 0.5	6.3 ± 0.5	3.7 ± 0.5 ^{a}	4.2 ± 0.3 ^{a}	3.6 ± 0.4	3.3 ± 0.5 ^{a}	1.9 ± 0.2 ^{a}	-	3.2 ± 0.6	-	5.8 ± 0.4 ^{a}	1.4 ± 0.2 ^{a}	1.5 ± 0.4	4.4 ± 0.4 ^{a}
	2	6.7 ± 0.5	5.9 ± 0.3	6.4 ± 0.5 ^{b}	3.5 ± 0.3 ^{b}	-	5.9 ± 0.6 ^{b}	5.8 ± 0.6 ^{b}	-	-	-	6.5 ± 0.4 ^{ab}	3.6 ± 04 ^{b}	-	5.4 ± 0.5 ^{ab}
	3	6.5 ± 04	5.3 ± 0.8	6.6 ± 0.3 ^{b}	4.1 ± 0.3 ^{a}	-	6.0 ± 0.3 ^{b}	5.9 ± 0.4 ^{b}	-	-	5.9 ± 0.3	6.9 ± 0.4 ^{b}	4.2 ± 0.3 ^{b}	3.4 ± 0.6	6.2 ± 0.9 ^{b}
	M	6.7 ± 0.5 ^{B}	5.8 ± 0.7 ^{B}	5.6 ± 1.4 ^{B}	3.9 ± 0.4 ^{A}	-	5.1 ± 1.3 ^{AC}	4.5 ± 1.9	-	-		6.4 ± 0.6 ^{B}	3.1 ± 1.3 ^{A}	1.6 ± 1.5	5.3 ± 1.0 ^{C}
	F ***p***	**ns**	**ns**	**75.15** **0.001**	**8.33** **0.01**	**-**	**50.33** **0.001**	**139.39** **0.001**	**-**	**-**	**-**	**9.28** **0.01**	**90.96** **0.001**	**-**	**9.43** **0.01**
F ***p***		**11.03** **<0.001**	**23.90** **<0.001**	**13.18** **<0.001**	**3.48** **<0.05**		**6.60** **<0.01**	**ns**	**-**	**-**	**-**	**16.79** **<0.001**	**6.42** **<0.01**	**-**	**37.56** **<0.001**

Superscript: different lowercase letter in the same row indicates significant statistically differences ($p < 0.05$) between samples from the same pollen grain content group (A: >60% B: 40–60% and C: 18–40%); different capital letter in the same row indicates significant statistically differences ($p < 0.05$) between samples from different pollen grain contentngroups.

The results showed that there was a single qualitative profile with all the honeys being medium in color intensity, thick, with floral olfactory notes, having a sweet and salty taste, and of a low–medium persistence. Additionally, samples from Group A had olfactory notes of wood/wax/resin and a chemical aroma. For common sensory attributes, the results showed significant differences ($p < 0.01$) between groups with different pollen grain contents for all attributes, except for floral aroma, with the Group A samples (thyme pollen content > 60%) being the lightest in color and having the highest floral odor intensity and salty taste.

If we compare our findings with other research works, we find that there are very few studies on thyme honey. Greek honeys are described in terms of woody, chemical, and floral–fresh fruit odors, with sweet and acidic tastes, medium persistence, and a medium dark color [4]. Turkish honeys are described in terms of floral, honey, bitter almond, thyme, and wax odors [12], while Spanish honeys are described in terms of aromatic herbs, citric fruit, ripened fruit, caramel, balsamic, and species-specific olfactory notes [13]. The sensory profile determined for Greek islands thyme honey in this work was similar to that defined by Persano Oddo et al. (2004) [4], except for the acidic taste. Over the last few decades, the collected reference works [13,19,29,41–45] evince that since the paper entitled "Main European Unifloral Honeys: Descriptive Sheets" was published [18], there has been an emergence of different working groups presenting honey sensory profiles and, particularly, descriptive techniques for sensory attributes and indications on unifloral honeys from specific geographical areas and their correlation with physico-chemical characteristics. These previous studies have shown that the sensory profile is capable of allowing for differentiation between honeys with different botanical and geographical origins. This may have a certain logic, as consumers' sensory perceptions are the main determinant of their willingness to consume a product. In this sense, the description of honey sensory profiles is a valuable cue for consumers when purchasing a honey type.

It is worth mentioning that the honey sensory profile changes with the pollen grain content, with honeys with a high pollen grain content presenting a floral and chemical olfactory profile (>60%, Group A).

Recently, the European Commission [46] concluded that the chemical and biological characteristics of genuine honeys should be generated and stored in a publicly available database. This process would require obtaining samples by authorized personnel from carefully selected honey producers. Moreover, in the private sector, the authenticity of a sample will have to be defined beforehand. In this sense, the sensory profiles of unifloral honeys from different botanical and geographical origins should be studied and introduced in this publicly available database.

4. Conclusions

Although there are international regulations (CODEX) and European Union norms that regulate the quality criteria of honeys, they are based solely on physico-chemical parameters (i.e., moisture content, HMF, diastatic activity, electrical conductivity, and sugars) and microscopic analysis. At present, the importance of sensory analysis in establishing authenticity criteria for honeys is more than justified. However, there is limited scientific work that combines the three techniques for the characterization of unifloral honeys. This work contributes to a better characterization of Greek islands thyme honey by determining its sensory profile based on pollen content. A single qualitative profile was obtained, with all of the honeys being medium in color intensity, thick, with floral olfactory notes, having a sweet and salty taste, and of a low–medium persistence. Within this profile, there were significant differences between groups with different pollen grain contents ($p < 0.01$) for all the attributes except for floral aroma, with the Group A samples (thyme pollen content > 60%) being the lightest in color and having the highest floral odor intensity and salty taste.

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