JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Ground Red Peppers: Capsaicinoids Content, Scoville Scores, and Discrimination by an Electronic Nose

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High-pressure liquid chromatography (HPLC) was used to determine the capsaicin, dihydrocapsaicin, and total capsaicinoids levels of different ground red pepper samples obtained from local retail markets in İzmir, Turkey. Scoville scores were determined using sensory tests. An electronic nose (EN) was used to discriminate ground red pepper samples by headspace volatiles. EN data were analyzed using discriminant function analysis (DFA). An overall correct classification rate of pepper varieties by EN of 91% was obtained. A linear correlation between capsaicin, dihydrocapsaicin, and total capsaicinoids and Scoville scores was also observed, and R^2 values of 0.89, 0.85, and 0.91 were obtained, respectively.

KEYWORDS: Capsaicin; dihydrocapsaicin; pungency; Scoville score; electronic nose

INTRODUCTION

Red peppers (*Capsicum annuum* L.) are popular spices in many parts of the world, valued for their sensory attributes of color, pungency, and aroma. In the southeastern Anatolian region of Turkey peppers represent a culinary tradition and cultural identity. Peppers are economically important because of the vast quantity and the diverse varieties used. The genus capsicum comprises more than 200 varieties depending on the environmental conditions (*1*). The fruits vary widely in size, shape, flavor, and sensory heat. The food industry is the largest user of capsicums, where the spice is used as a coloring and flavoring agent in sauces, soups, processed meats, snacks, candies, soft drinks, and alcoholic beverages either in the ground form, or as an oleoresin (concentrated extract). The popularity of hot pepper products has been growing (*2*). Traditionally, in the southeastern Anatolian region commercial products are obtained by sun-drying the peppers. This form of drying requires an extensive processing area and takes about two weeks.

The pungency of capsicum fruit is due to a group of compounds called capsaicinoids which are present in paprika varieties in different amounts (*3*). Pure capsaicin is a white crystalline compound that melts at 65 °C and is volatile above this temperature. It produces a dangerous and irritating vapor. In commercial capsicums, capsaicin generally comprises 33−59%, dihydrocapsaicin accounts for 30−51%, nordihydrocapsaicin is 7−15%, and the remainder is less than 5% of the capsaicinoids (*4*). Capsaicin content of red pepper is one of the main parameters that determine its commercial quality. The concentration of capsaicinoids in fresh red pepper varieties, especially in paprika, ranges from 0.001 to 0.01%, and in strong chili varieties the concentration ranges from 0.1 to <1% (*5*).

The level of capsaicinoids can be determined using either chemical, instrumental, or sensory methods. Numerous papers have been published regarding sensory methods for determining pungency and instrumental methods for determining capsaicinoids (*6, 7*). Problems associated with these methods are long extraction time, poor reproducibility, and difficulty in sample preparation (*8*). Determination of the level of capsaicinoids by sensory methods could cause buildup of heat, fatigue, inconsistencies, and discomfort. Other methods are needed in order to eliminate these problems. One possibility would be to indirectly associate the pungency with the species of red pepper. Electronic nose (EN) can then be a rapid, simple, and objective method to determine the species of red pepper, and thus hint at the level of capsaicinoids.

EN technology is based on an array of sensors having partial specificity, and this array produces a fingerprint of the sample odor. This is used by an appropriate pattern recognition technique to identify the odor through comparison with previously obtained measurements of known samples (*9, 10*). An EN can only classify unknown samples after it is properly trained. The training data can be obtained from sensory panels, chromatography analyses, or some other chemical or microbiological analyses. Once the system is trained, it can be run with minimal training (*11*).

There is growing interest in the use of EN in analytical laboratories in the food, flavor, and fragrance area as a simple,

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fast, and reliable method of aroma/headspace analysis. In the food industry, electronic noses are used for quality control, process monitoring, freshness evaluation, and shelf life investigation (*12*).

Research to identify the origins of some spices using EN was conducted, and sensory and chemical data confirmed the findings (*11*). Different cultivars of apples and oranges were analyzed by EN, and it was reported that the discrimination rate for pairs of cultivars of the same produce type was approximately 76% (*13*). Rancidity in virgin olive oil was detected by an EN equipped with 32 conducting polymer sensors (*14*). EN was also used to monitor sausage fermentation by following changes in volatiles, and the EN results were compared with sensory panel results. It was concluded that the EN could predict fermentation time (*15*). The quality of ground meat was determined using an EN with a good possibility of predicting storage time (*16*). Discrimination of red pepper varieties by an EN was not found in the literature.

The overall objective of this study was to determine the capsaicinoid composition in ground red peppers by HPLC, and to correlate these with pungency as measured by Scoville scores. Also, the precision of EN in discriminating between red pepper varieties by headspace volatiles has been evaluated.

MATERIALS AND METHODS

Ground Red Pepper Samples. Nine ground red pepper samples were purchased from local retail markets in İzmir, Turkey. They were harvested from southeastern regions of Turkey, especially from the city of Maraş, at the same harvest time in 1999. They were dried, ground, and packed in polyethylene bags in the same region and transported and distributed to retail markets in other regions of Turkey. Nine samples were chosen according to the pungency levels stated on their labels. Some labels stated that the ground red peppers were "very hot" and some were "mild". They were stored unopened in their original bags under refrigeration temperatures (4 °C) up to one week. The water activity (a_w) of the samples was measured using a Rotronic Hygroskop DT (Rotronic Instrument Corp., Huntington, NY).

HPLC Analysis. Extracts were obtained from 10 g of ground red peppers by recirculating 250 mL of HPLC grade acetone (Merck, Darmstadt, Germany) in a Soxhlet extractor for 5 h. The extract was evaporated under vacuum to 5 mL at room temperature. Bisphenol A (Aldrich, Milwaukee, WI), a common antioxidant, was used as an internal standard. One g of oleoresin and 30 mg of Bisphenol A were dissolved in 5 mL of acetonitrile (Merck). A 2-mL aliquot was filtered through a Sep-pak C-18 cartridge (Alltech, Deerfield, IL) moistened with acetonitrile (*6*). The sample (10 μL) was then injected directly to the HPLC system (Waters Associates model ALC/GPC equipped with a M.6000A pump, a U6K injection). The column was μ-Bondapak C-18 (300 × 4 mm).

Detection was accomplished via a variable wavelength UV detector (Waters Associates) set at 280 nm. The isocratic mobile phase was methanol/water (60:40 v/v) with a flow rate of 0.8 mL/min (*17*). Two sub-samples were measured for each one of the 9 samples. Capsaicin (Sigma, St. Louis, MO; 98%) and dihydrocapsaicin (Sigma, 90%) were identified using standards of both compounds. Standard curves were prepared using serial dilutions of 50, 100, 200, and 400 ppm.

Electronic Nose Analysis. An electronic nose (e-NOSE 4000 model, EEV Inc., Amsford, NJ) equipped with twelve conducting polymer sensors (sensor types: 483, 478, 464, 463, 462, 461, 460, 459, 458, 401, 298, and 297) was used to quantify the sensor responses to the volatiles of ground red pepper samples. The EN was calibrated using 75% v/v propylene glycol solution (100% solution from Fisher Scientific, P-355-20, Fair Lawn, NJ) before starting the experiment. The headspace volatiles of 5 sub-samples for each variety were analyzed. The samples were kept at room temperature for 60 min prior to analysis. The ground red pepper samples, each weighing about 7 g, were placed in 100-mL beakers and placed in the glass sampling vessel of the EN. The vessel was purged with compressed air for 2 min to eliminate any foreign odor present in the vessel, and the sensor head was purged for 4 min. During these 4 min, the sample volatiles were equilibrating in the headspace of the vessel. Sensor response data were acquired for 4 min. Total analysis time for each ground red pepper sample was 10 min. Readings at 4 min exposure of the sensors to the ground red pepper samples were used for data analysis.

Scoville Test. The Scoville test method was used to determine the heat of the red peppers (*18*). A 4-g sample was weighed into a 200-mL volumetric flask, and 95% ethanol was added and well shaken. A standard solution was prepared by diluting 0.10 mL of the test solution to 100 mL with a 5% sucrose solution. A 5-mL portion of the standard solution was presented to each of five panel members with instructions to swallow it all at once and to note the pungent sensation in the throat. A series of dilutions was made, from the weakest to the strongest, and presented to the panelists until they indicated a pungency was detected. Perceptible positive sensation in the throats of three out of five panelists was recorded. At least a 30-min lapse between the pungency tests was allowed. Results were recorded as Scoville units.

Data Analysis. For the discrimination of the red pepper varieties using the EN, discriminant function analysis (DFA) was performed using Statistica for Windows (98 edition, StatSoft Inc., Tulsa, OK). Red pepper varieties were used as grouping variables, and 12 EN sensor outputs were used as independent variables. The separation between groups was maximized by DFA, and resulted in two linear functions of the form described in eq 1.

$$Function = \sum_{i=1}^{12} a_i x_i + a_o \tag{1}$$

Each EN reading consisting of 12 sensor data (x_i in eq 1) is multiplied with these parameters (a_i in eq 1) to find the values of Function 1 and Function 2. These functions were used to map the data in two-dimensional plots and observe separation between groups. Correct classification rates and the coefficients for each function were also calculated using Statistica.

The correlations of capsaicin, dihydrocapsaicin, and total capsaicinoids against Scoville scores were done by linear regression using Statistica. The equations for the linear regression models were obtained.

RESULTS AND DISCUSSION

The a_w values of the nine ground red pepper samples were approximately 0.70. ANOVA results showed that the a_w did not differ significantly among the samples ($p < 0.05$). Therefore, a_w did not have an effect on EN readings of the nine different samples.

HPLC Analysis. Capsaicinoids are eluted from the reverse-phase column according to the length of their aliphatic side chain or the degree of saturation. Reverse-phase column and water–methanol system offered the best potential for separating the capsaicinoids. Only the major capsaicinoids present in red peppers were identified in the samples. In **Figure 1**, peaks 1 and 2 were indicated as capsaicin and dihydrocapsaicin, respectively. A few peaks eluted earlier or later than these were thought to be capsaicinoids. It is possible that peak 3 could be nordihydrocapsaicin (*17*). Capsaicin and dihydrocapsaicin are responsible for 90% of the pungent stimuli.

Capsicums are used for their unique flavor and pungency. They include paprikas (ranging in pungency from 0 to 30 mg/kg capsaicinoids), chili peppers (30–600 mg/kg capsaicinoids), and red peppers (600–13000 mg/kg capsaicinoids) (*18*). Recoveries of capsaicin and dihydrocapsaicin were 90 and 92%, respectively. Capsaicin (21.88–53.05 mg/100 g) and dihydrocapsaicin (11.53–40.63 mg/100 g) showed a wide range (**Table 1**). Although the samples were obtained from the same region, during the same season, the exact harvest period could have been different. Some of the samples might have been harvested at the beginning of the season and others at the end of the
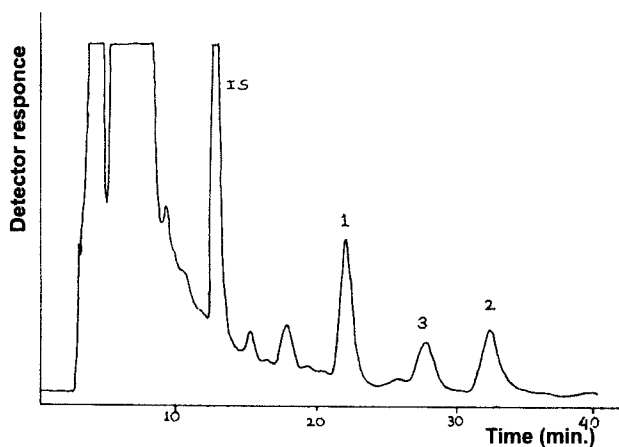
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Figure 1. HPLC chromatogram of capsaicinoids in red pepper extract. IS, internal standard; 1, capsaicin; 2, dihydrocapsaicin.

Table 1. Capsaicinoid Content and Scoville Scores in Ground Red Pepper

sample	capsaicin (mg/100 g)	dihydro-capsaicin (mg/100 g)	total capsaicinoids (mg/100 g)	Scoville test result
1	27.13	18.31	45.44	5000
2	29.31	16.75	46.06	5000
3	38.00	26.00	64.00	5500
4	38.75	24.00	62.75	5500
5	40.25	26.83	67.08	5500
6	44.50	30.13	74.63	6000
7	45.18	29.15	74.33	6500
8	45.78	25.25	71.03	6000
9	53.05	40.63	93.68	7000

season. Other reasons for these wide ranges could be varieties, growing conditions such as soil, watering, etc.

Correlation of Capsaicinoid Levels With Scoville Scores. Capsaicin, dihydrocapsaicin, and total capsaicinoid levels were used to predict the Scoville scores using the linear regression models (**Figure 2**). The equations were as follows:

$$\text{Scoville score} = 76.8 \times (\text{capsaicin level, mg/100 g}) + 2691.0$$

$$\text{Scoville score} = 88.2 \times (\text{dihydrocapsaicin level, mg/100 g}) + 3453.6$$

$$\text{Scoville score} = 42.7 \times (\text{total capsaicinoid level, mg/100 g}) + 2935.1$$

The regression coefficients (R^2) for capsaicin, dihydrocapsaicin, and total capsaicinoid levels vs Scoville scores were 0.89, 0.85, and 0.91, respectively. It could be concluded that total capsaicinoids levels gave the best fit.

Electronic Nose Analysis. EN data for ground red peppers were analyzed using DFA. The various species were chosen as the grouping variable, and 12 EN sensor outputs were used as independent variables. When grouping by species, the correct classification rate for the discriminant functions was 91% (**Table 2**). The scatter plot obtained with the discriminant functions for species separation is given in **Figure 3**. DFA coefficients (a_o and a_i) for ground red pepper species correlated to EN sensor readings are given in **Table 3**. Only 4 out of the 45 EN readings were classified incorrectly, and these are given in **Table 4**.

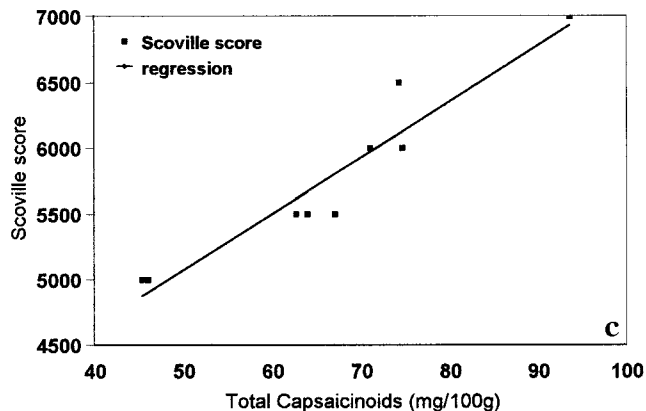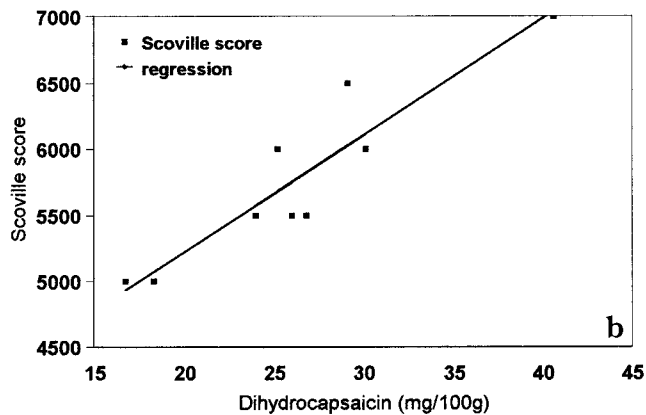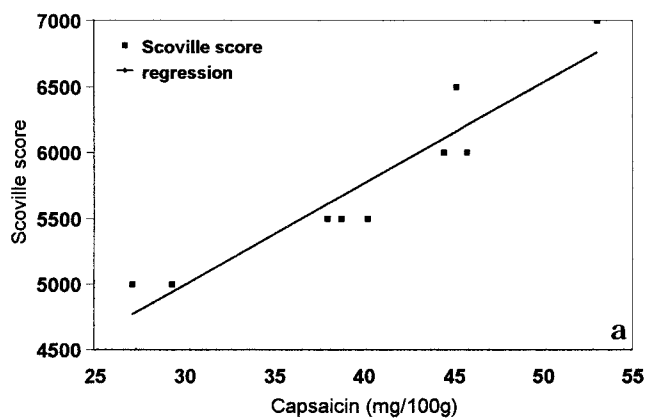This study showed that EN was able to group ground red peppers having different capsaicin, dihydrocapsaicin, and total

Figure 2. Regression of Scoville scores vs (a) capsaicin, (b) dihydrocapsaicin, and (c) total capsaicinoids levels.

Table 2. Correct Classification Rates from DFA of EN Sensor Readings Compared with Ground Red Pepper Species

sample	correct classification rates (%)
1	100
2	100
3	100
4	80
5	100
6	80
7	100
8	80
9	80
total	91

capsaicinoids levels, using DFA as a pattern recognition technique. The EN has potential to be used in a rapid and easy method for determining the pungency of ground red peppers, with no sample preparation. However, further studies are needed.
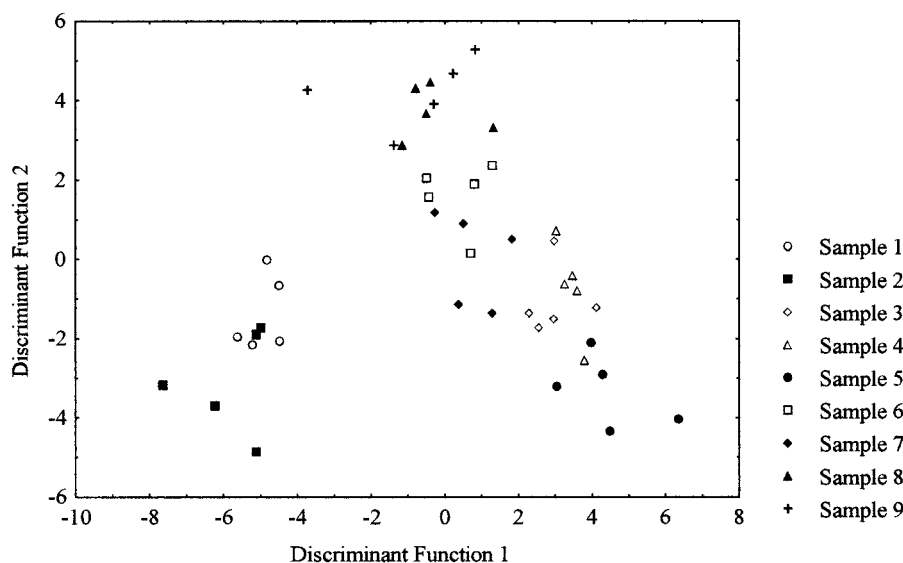
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Figure 3. DFA of odor of ground red peppers based on varieties and EN sensor readings.

Table 3. Coefficients for Discriminant Functions To Predict Pepper Species

| | coefficients for discriminant functions | |
| | red pepper species | |
sensor type	function 1	function 2
constant	−74.93	28.98
T_{483}	−19.46	−9.33
T_{478}	−2.84	−1.20
T_{464}	1.73	−1.30
T_{463}	9.65	8.18
T_{462}	25.04	−19.36
T_{461}	5.11	11.02
T_{460}	8.77	−9.82
T_{459}	−0.54	−4.05
T_{458}	−47.15	−22.16
T_{401}	8.99	4.16
T_{298}	3.93	11.76
T_{297}	−1.81	1.72

Table 4. Number of EN Sensor Readings Classified in the Same Cluster for Each Ground Red Pepper Sample

sample	number of EN sensor readings
1	5
2	5
3	6[a]
4	4
5	5
6	4
7	6[b]
8	5[c]
9	5[d]
total	45

[a] In sample 3, one of the sensor readings belongs to sample 4. [b] In sample 7, one of the sensor readings belongs to sample 6. [c] In sample 8, one of the sensor readings belongs to sample 9. [d] In sample 9, one of the sensor readings belongs to sample 8.

Developing a comprehensive database using many different red pepper samples (in the order of 100) will increase the reliability, and eventually the use of, electronic noses in this field.

ABBREVIATIONS USED

EN, electronic nose; HPLC, high-pressure liquid chromatography; DFA, discriminant function analysis.

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