



**INVESTIGATION OF FLAVOUR PERMANENCE OF STRAWBERRY AND WATERMELON FLAVOURED CHEWING GUMS BY USING RETRONASAL AROMA TRAPPING DEVICE AND SENSORY ANALYSIS TECHNIQUES**

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Received / Geliş: 18.11.2021; Accepted / Kabul: 04.04.2022; Published online / Online baskı: 07.04.2022

Uzun, P., Özünal, A. E., Başkaya, H., Doğan, N., Gülay, S. M., Hantal, B., Üner, M., Barla Demirkoz A. B. (2022). Investigation of flavour permanence of strawberry and watermelon flavoured chewing gums by using retronasal aroma trapping device and sensory analysis techniques. *GIDA* (2022) 47 (3) 408-419 doi: 10.15237/gida.GD21139

Uzun, P., Özünal, A. E., Başkaya, H., Doğan, N., Gülay, S. M., Hantal, B., Üner, M., Barla Demirkoz A. B. (2022). Çilek ve karpuz aromalı sakızlarda aroma kalıcılığının retronazal aroma-yakalama cihazı ve duyuşsal analiz teknikleri kullanılarak araştırılması. *GIDA* (2022) 47 (3) 408-419 doi: 10.15237/gida.GD21139

**ABSTRACT**

Combining in vivo instrumental analysis with sensory evaluation techniques to analyze the release of volatile components from the gum matrix is gaining importance due to the flavour expectations of consumers. Besides consumers' preferences and quality of flavourings determine the market value of chewing gums. In this study, strawberry and watermelon flavoured chewing gums were prepared and evaluated with both sensory evaluation and analytical techniques by using retronasal aroma trapping device and dynamic headspace analysis - DHA-GC/MS. Ethyl hexanoate and isoamyl acetate were chosen as flavour compounds for strawberry and watermelon flavoured gums to monitor in the study. The in vivo experiment with retronasal aroma trapping device showed that selected compounds were quite intense in the first 5 min of breath, and they were also detected in the 45<sup>th</sup> min of chewing. The flavour stability of the chewing gums was also monitored for 3 months. According to all sensory and instrumental analyzes, watermelon flavour was perceived as watermelon taste both at the 45<sup>th</sup> min and at the end of shelf life (equal to 12 months).

**Keywords:** Flavoured chewing gum, flavour permanence, retronasal aroma trapping device, sensory evaluation technics

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## ÇİLEK VE KARPUZ AROMALI SAKIZLARDA AROMA KALICILIĞININ RETRONAZAL AROMA YAKALAMA CİHAZI VE DUYUSAL ANALİZ TEKNİKLERİ KULLANILARAK ARAŞTIRILMASI

### ÖZ

Günümüzde, uçucu bileşenlerin sakız matrisinden salımını analiz etmek amacıyla *in vivo* enstrümental analiz ile duyuşal deęerlendirme tekniklerini birleřtirmek, tüketicilerin lezzet beklentilerini karřılamak aısından önem kazanmaktadır. Tüketicilerin sakızdaki tercihleri ve kullanılan aromaların kalitesi sakızların piyasa deęerini belirlemektedir. Bu alıřmada, ilek ve karpuz aromalı sakızlar, retronazal aroma yakalama cihazı ve dinamik headspace analizi-GC/MS kullanılarak hem duyuşal deęerlendirme hem de analitik tekniklerle deęerlendirilmiřtir. alıřmada ilek ve karpuz aromalı sakızlar, aroma öncülü olarak seilmiř olan etil heksanoat ve izoamil asetat üzerinden izlenmiřlerdir. Retronazal aroma yakalama cihazı ile yapılan *in vivo* deney, seilen bileřiklerin nefesin ilk 5 dakikasında oldukça yoęun olduęunu ve iğnemenin 45. dakikasında bile tespit edildięini göstermiřtir. Sakızların raf ömrü 3 ay boyunca izlenmiřtir. Tüm duyuşal ve enstrümental analizlere göre, karpuz aroması hem 45. dakika hem de raf ömrünün sonunda (12 aya eřit) yine karpuz tadı olarak algılanmıřtır.

**Anahtar kelimeler:** Aromalı sakız, aroma kalıcılıęı, retronazal aroma yakalama cihazı, duyuşal deęerlendirme teknikleri

### INTRODUCTION

Chewing gum is one of the most popular confections consumed all over the whole world. According to the Mintel market size report (2018), 14-15 thousand tons of chewing gum were put on the market for the consumption of approximately 43 million people in Turkey. Turkish Food Codex Communiqué on Chewing Gum (7/24/1999) defines chewing gum as a product that can be produced with a sugar-free or sugar-sweetened gum base, formulated with some other additives such as texture modifiers, preservatives, and flavourings. All over the world, chewing gums are produced similarly (De Roos, 2008). The gum base consists of a hydrophobic water-insoluble base and a water-soluble sugar or polyol phase in a ratio of approximately 1:3. The water-insoluble part remains in the mouth during chewing and the water-soluble substances are dissolved and swallowed (Hinderink et al., 2019).

Flavourings, which give taste and odour to foods, are the most important ingredient of chewing gum formulations that appeal to the consumer. 0.4-1g/100g flavour components are generally used in formulations (Wong et al., 2009; Menis-Henrique, 2020). However, after about 3-5 min, most chewing gums immediately lose their flavour and consumer perception (Thomas et al., 1984; Fenimore, 2008).

The perception of flavourings in foods begins with orthonasal olfaction and continues as the food is being processed in the mouth. Perception of flavourings ends with the last flavour molecule disappearing from breath. It depends on the nature of the food and how it is consumed. The chewing action does not only provide the disintegration of foods into small pieces and mixing them with saliva, but also plays an important role in the perception of flavourings by transferring flavour molecules to the pharynx (Hinderink et al., 2019; Selli and Kilic, 2020). Chewing gum is a food item in which flavour molecules have lower mobility, and the basic flavour perception occurs in the mouth. During chewing, the flavour molecules mix with saliva, thus the volatiles are perceived by the consumer (Voilley and Etievant, 2006).

The release of flavour components is very important as it also determines how pleasant and how long lasting a flavour will be perceived (Taylor et al., 2000; Potinini et al., 2008). As it is known, a long-lasting taste of chewing gum during a long chewing period is an important parameter for consumer preference. Based on previous studies, it is predicted that when the compounds are more hydrophobic, they interact better with the gum base. This interaction results in a relatively lower flavour release rate during chewing. De Roos (2008) determined that the

flavour release was diffusion controlled after 5 min of chewing. In another study, it was observed that flavour compounds were released from the chewing gum through saliva into the mouth, throat and nose cavity and these components could then be detected in the epithelium of the olfactory region (Hodgson et al., 2003).

It is also known that the permanence of the flavour, in other words, the flavour release during chewing depends on many factors such as the composition, texture and rheological properties of the matrix, the chewing power and the flow rate of saliva (Boland et al., 2006; Koliandris et al., 2008). Also, the release of flavours from the chewing gum is often limited because the flavours are lipophilic and thus largely retained in the gum base (Haar et al., 2003).

Due to the increasing demand for chewing gum confectionery, manufacturers are trying to improve their product quality by focusing on customer satisfaction. There is a challenge among manufacturers on the design of a gum matrix that releases both volatile compounds and sweeteners displaying a stable flavour during chewing up to 20 min. Therefore, studies on this subject have led to the development of reliable analytical techniques for monitoring the flavour volatiles released from the chewing gum during the production process and storage (Guichard, 2002). The headspace solid-phase microextraction (HS-SPME) method, which is one of the instrumental methods used to analyze the flavour compounds in chewing gums, was developed by Pawliszyn et al. (2001). However, one of the main limitations of this method is the difficulty in quantitative analysis of head cavity components of a complex solid matrix (eg, chewing gum). Subsequently, more advanced devices were designed to detect and analyze the release of volatile compounds from the challenging food matrix. The most used device for this purpose is the atmospheric pressure-chemical-ionization mass spectrometer (APCI-MS, MS-noise). The breath of assessors is continuously sampled by these systems, which enable precise and rapid monitoring of volatile release (Taylor, 2000; Haarh, 2003; Boland et al., 2006). Another device developed for this purpose

is the proton transfer reaction mass spectrometer (PTR-MS). In both, the basic principle is based on detecting the flavour components in the breath that the assessors exhale during chewing the gum (Buettner and Schieberle, 2000; Buettner et al., 2008). Another device designed to examine flavour release by using the nose retronasal method is retronasal aroma trapping or retronasal olfactometer-GC/MS device. In this device, the breath of assessors into the nose is placed in a glass mask, and the air sample (breath) passing through the mask is trapped in the Tenax, and these trapped molecules are analyzed through GC/MS to determine what or not the flavour components in-breath (Munoz-Gonzalez et al., 2014; Bonneau et al., 2018).

As it is known, only "sensory" features can be controlled by consumers among food quality characteristics. For this reason, sensory evaluation experiments are used in consumer preference studies to determine consumers' reactions to the appearance, taste, texture and other sensory characteristics of foods.

Among the sensory analysis tests, the affective (preferences) test is mostly used to determine consumer preferences. On the other hand, analysis of permanence, perception, and flavour release of flavours are usually hard to determine by using these instruments in food manufacturing. Since the flavour permanence of the chewing gum determines consumers' preference. Studies on flavour release have led to the development of reliable analytical techniques for monitoring the behaviour of flavour volatiles release of the chewing gum (Fenimore, 2008). However, RATD-GC/MS gives the advantage of following selected flavour pre-cursors in every flavouring (Koliandris et al., 2008).

According to the literature, there is not any previous study that analyzed flavour release both sensory and instrumental techniques in chewing gum. In this study, the permanence of strawberry and watermelon flavoured gums during chewing was analyzed *in vivo* by RATD-GC/MS combined with sensory evaluation techniques for the flavoured chewing gum. The correspondence

between analytical and sensory evaluation techniques (an affective test and descriptive sensory analysis) was also criticized.

## MATERIAL and METHODS

### Materials

The gum base was purchased from Remik Chemistry San. ve Tic. A.Ş., Pendik, Istanbul. Ethyl Hexanoate (CAS 123-66-0), isoamyl acetate (CAS 123-92-2), citric acid (CAS 77-92-9, Jungbunzlauer Suisse AG.), malic acid (CAS 6915-15-7, Tate and Lyle, Turkey), encapsulated citric acid (50%, Tastetech, Bristol, UK), sorbitol, maltitol syrup, mannitol, xylitol (Roquette Agriculture and Food LLC., Turkey), cooling agent (WS3 Type, Henan Sunlake Enterprise Corporation, Henan, China), isomalt (Beneo, Turkey), sucralose (Splenda, Turkey), encapsulated sucralose (10%, Tastetech, Bristol, UK), strawberry flavour, encapsulated strawberry flavour, extruded strawberry flavour, watermelon flavour, encapsulated watermelon flavour, extruded watermelon flavour were obtained internally (Aromsa Flavours and the Food

Additives Inc. Co., Kocaeli, Turkey). All other chemicals were of analytical grade.

### Sample preparation

According to the Mintel/Global New Product data, strawberry and watermelon flavoured chewing gums are the 4<sup>th</sup> and 5<sup>th</sup> most favored chewing gums after mint, spearmint, and peppermint flavoured gums. Thus, strawberry and watermelon flavoured chewing gums were prepared in this study (Table 1). Talk type of gums is preferred to use because citric acid causes decomposition and instability of other gum types. Artificial flavouring mixture components of strawberry and watermelon (liquid form: encapsulated form: extruded form, 1:1:1) were used. Each chewing gum was prepared to be suitable Council Regulations EU No:1330/2008 (The European Parliament and of the Council of 16 December 2008 on Food Additives) for chewing gum additives. Stick-type gums weighing  $2.2 \pm 0.2$ g were prepared. They were put in a plastic bag, wrapped in aluminum foil, and stored at  $21 \pm 1^\circ\text{C}$  at 35% relative humidity until they were used for the analysis.

Table 1. Composition (%) of strawberry and watermelon flavoured chewing gums

Ingredients	Strawberry				Watermelon			
	A	B	C	D	A	B	C	D
Flavour mix*	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Gum base	30	30	30	30	30	30	30	30
Encapsulated citric acid	1	1	1	1	-	-	-	-
Citric acid	0.4	0.4	0.4	0.4	-	-	-	-
Malic acid	0.4	0.4	0.4	0.4	-	-	-	-
Sorbitol	47.6	47.6	48.05	48.05	49.4	49.4	49.85	49.85
Maltitol syrup	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Mannitol	3	3	3	3	3	3	3	3
Encapsulated sucralose	1	1	0.5	0.5	1	1	0.5	0.5
Sucralose	-	-	0.05	0.05	-	-	0.05	0.05
Xylitol	7.5	-	7.5	-	7.5	-	7.5	-
Isomalt	-	7.5	-	7.5	-	7.5	-	7.5
Cooling agent	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

\*Flavour mix includes the same flavourings: Liquid, encapsulated and extruded forms.

### **Study design**

The study was designed as two main stages. Sensory evaluation (affective test) and subsequently, preselection were performed at the first stage. Sensory evaluation based on descriptive sensory analysis (DSA) and instrumental analysis was conducted on the selected samples at the second stage. A shelf-life study was also carried on for selected samples.

Strawberry and watermelon flavoured chewing gums were evaluated in terms of affective

(preference) and DSA tests. Thus, the optimum taste for the chewing gums was selected by trained and experienced assessors. The use of the free and encapsulated form of sucralose was compared between the groups of A-C and B-D by the presence of xylitol or isomalt. After then, the flavour profiles of selected formulas were characterized by DSA (Table 2). The flavour permanence of each selected chewing gum was monitored with both sensory evaluation analysis and in vivo retronasal aroma trapping method.

Table 2. Flavouring attributes selected for DSA by tasting the flavoured chewing gums

Flavouring	Attribute	Characteristics
Strawberry	Sour	Typical sourness in strawberry
	Fleshy	The taste of the outer seeds of strawberries
	Green	The taste of the green stem of the strawberry
	Creamy	Creamy taste in strawberry flavours
	Vanillin	Typical vanilla flavour
	Sweet	Typical sweetness in watermelon
	Juicy	The typical taste of watermelon juice
Watermelon	Soapy	The typical taste of watermelon flesh
	Fresh	Fresh watermelon flavour
	Peely	The taste of the watermelon close to the skin
	Fleshy	The taste of the fibrous meat part of the watermelon
	Green	Raw watermelon felt a green note

### **Sensory evaluation analyzes**

#### **Assessors**

The assessors were 16 male and 24 female, between 35±12 age, non-smoking, non-pregnant, no piercings in the mouth, no mastication or swallowing disorder, and healthy volunteers of AROMSA Inc. Co. They had also normal olfactory and gustatory functions. All assessors' permission was taken by their consent for this study. All assessors were selected and trained in accordance with the instructions of the International Organization for Standardization (ISO 8586:2014). The chewing gums were evaluated by affective (preference/acceptance) test done with the assessors, 7 of whom participated in the in vivo RATD-GC/MS to determine the intensities between the tasting/flavouring attributes.

#### **Affective (preference/acceptance) test**

Evaluations were performed in the personal cabins of the AROMSA Inc. Co. Sensory Analysis Laboratory. Positive pressure was applied to remove the several odors in those cabins when the cabin temperature and relative humidity were kept at 21±1°C and 50-55% during the analysis. There were no objects that could disturb the concentration of the assessors in the cabins. A five-linear scale was used to evaluate the intensity of the sample taste. Instructions about the evaluation and the scale were also demonstrated on the evaluation monitors. The samples were presented to the assessors as blank and coded with randomly selected three-digit numbers (Lawless and Heymann, 2010).

The flavoured chewing gums were evaluated in separate sessions by each assessor. The assessors participated in two evaluation sessions which were held morning and afternoon hours. Each assessor evaluated two samples in each session: A and C were one session, and B and D were other sessions. In the last session, the best sample was chosen by the assessors. Water and crackers were provided to clean the mouth of the assessors.

### ***Descriptive sensory analysis (DSA)***

Strawberry and watermelon flavoured chewing gums were evaluated by the experienced assessor group to determine the intensities between the tasting/flavouring attributes. Before DSA, the flavoured chewing gums were given to the assessors, who were informed about the studied method, analysis steps and product information (ISO/DIS 13299: 2016). Critical information like the production and the expiration dates was not given to the assessors. The evaluations were performed in the personal cabins of the Aromsa Sensory Analysis Laboratory. A five-linear scale was used to evaluate the sample taste intensities. Instructions about the evaluation and the scale were also demonstrated on the evaluation monitors (Fizz Software, Biosystems, Couternon, France). The samples were presented to the assessors blank and coded with randomly selected three-digit numbers.

Flavour lexicons are used in a sensory evaluation to determine the flavour profile of food products. In the DSA method, a consumer panel of a small group of highly educated people, known as the descriptive panel, was used. Descriptive panels used lexicons, a list of defined terms, to describe products. The assessors discussed and found out the characteristics of samples and the reference products for each attribute (Lawless and Heymann, 2010). DSA was performed for the flavouring attributes of selected flavoured chewing gums listed in Table 2.

### ***In vivo sensory analysis***

The RATD used for the in vivo experiment was performed based on an earlier study (Buettner and Schieberle, 2000; Buettner et al., 2008; Muñoz-González et al., 2014; Bonneau et al., 2018). The

device consisted of an olfactory glass port, a Tenax, and a vacuum pump. The olfactory port was combined with an adsorbent polymer conditioned in a glass tube (200 mg Tenax TA, 60–80 mesh, Gerstel, Germany). The Tenax tube was connected to the vacuum pump at the outlet of the device. The vacuum pump was provided a stable 500 mL/min flow during in vivo aroma trapping. In this part, a gas flowmeter (ADM 2000 flow meter, Agilent Technologies, Wilmington, DE) was used for measuring the stable gas flow. The assessors put their noses olfactory glass port and exhaled through the nasal cavity during mastication of flavoured chewing gum. Flavoured compounds from the assessors' breath were collected in the Tenax, and then in thermal desorption unit of dynamic head space-GC/MS (DHA-GC/MS) desorbed these compounds from the Tenax. Desorbed compounds were injected automatically into the same column used for calibration.

### ***Protocol***

The conditions of the sensory analysis by using RATD were determined based on our studies at Aromsa Research and Development Center. According to the study protocol, in vivo sensory analysis was performed in triplicate over six-session held on separate days, at the same time of day. The first gum sample was put in the mouth and chewed for 20 s by exhalation through the nose. At the end of 20 s, 3 breaths are blown into the olfactory glass port at approximately 5-s intervals and data T0 were collected. The same process was repeated for T5 and T45 min by chewing for 5 and 45 min (3 blows at 5-s intervals) and the permanence of the flavour components, which were collected into Tenax, was determined with DHA-GC/MS.

### ***Volatile compound analysis***

Ethyl hexanoate for strawberry and isoamyl acetate for watermelon flavouring's peaks were monitored easily. The peaks were more clear than other compounds. Therefore, ethyl hexanoate and isoamyl acetate were chosen as flavouring precursors. They were dissolved in ethanol for the preparation of the calibration curve (6 points, 0-2 mg/mL), separately. Solutions were put into the

vials and collected in Tenax and then in thermal desorption unit desorbed these compounds from the Tenax. Desorbed compounds were injected automatically into a DP-WAX UI GC column (60 m × 25 µm film thickness × 0.25 mm innerdia) (INNOWAX, Germany). A calibration curve was obtained for each compound ( $R=0.999$ ,  $y=1.12 \times 10^8 x + 6686.51$  for ethyl hexanoate and  $R=0.999$ ,  $y=1.26 \times 10^9 x - 1.24 \times 10^6$  for isoamyl acetate). When the calibration curve was prepared, all measurements were done with amounts of flavouring compounds. Their total ion chromatogram was obtained using a Gerstel DHS System (Germany) connected to an Agilent 7890A GC and 5975C MS equipped with an Inert MSD with Triple Axis Detector (Germany). The flow rate of the helium carrier gas was adjusted to 1.2 mL/min. Each sample in Tenax was analyzed in the splitless mode. The GC oven temperature was programmed from 40°C to 240°C at 5°C/min. The results were reported as mg/mL (Table 3).

#### **Data treatment and statistics**

RATD-GC/MS data were evaluated by one-way analysis of variance (ANOVA), and significant differences found among assessors were further evaluated by Tukey's test in the SPSS package program (Version 23.0, IBM Inc., New York, USA). The results were considered statistically significant at  $P < 0.05$ . All sensorial analyzes were done by using the Fizz Software (the non-parametric Friedman test), (Biosystems, Couternon, France) at a 95% confidence level.

#### **Accelerated shelf-life analyzes**

The flavour permanence of selected flavoured chewing gums was monitored during 3 months of storage. The samples were divided into two groups: experimental and control groups. Experimental samples were produced at the same time for both sensory and instrumental analysis. Experimental groups of chewing gums were stored under 35°C and 30-40% RH storage conditions in a cooled incubator (Nüve ES252, Istanbul, Turkey), and the control group of chewing gums was produced freshly for each time of analyzes for the shelf-life study. Sensory evaluation analyzes were performed for every

week of storage during the shelf life. The samples were analyzed with RATD-GC/MS at the end of the 12th week. The shelf life of chewing gums on the market is expected to be at least 12 months. According to the previous research results of the R&D center of the company, storage at  $35 \pm 1^\circ\text{C}$  and 30-40% RH conditions for 3 months are equal to 12 months. The flavoured chewing gums were chewed by a panel of 7 assessors. Each gum was chewed for 45 min.

## **RESULTS and DISCUSSION**

### ***The first stage of the study***

When considering strawberry flavoured chewing gums, formula C was preferred by 72.73% of the assessors in the first session, and for the second session formula B was preferred by 63.63% of assessors. As a result, in the third and final session between groups B and C, formula C was preferred by 56.25% of assessors. The assessors rated the selected formula C as having a sweeter strawberry flavour and balanced sour taste, and as a powerful, permanent and the most favorable flavour (Figure 1a).

Free and encapsulated forms of sucralose were used with xylitol in formulation C. The sweeteners used in the gum matrix influence the flavour perception and sensory properties of chewing gums. The increased amount of the sweeteners provides an increase in flavour taste. In the meantime, the loss of sweetness due to chewing gives the impression that the flavour is consumed, even if the amount of released flavour does not decrease (Davidson et al., 1999; Fisker and Nissen, 2006). It is also known that the use of liquid and encapsulated forms of the flavourings in the chewing gum, separately or in combination, increase the flavour permanence during the chewing. Encapsulation is a technology provided to control the release properties and support the strength and flavour permanence of flavourings in the products (Merritt et al., 1983; Castro and Johnson, 2006; Madene et al., 2006). Xylitol, which gives the same effect in strawberry flavouring, is known to support the flavour in the excess sucralose, like the effect of menthol (Davidson et al., 2000).

For watermelon flavoured chewing gums, 78.35% of the assessors preferred formula A in the first session and 80% preferred formula D in the second session. In the last session, 68.42% of the assessors preferred formula D. The assessors

rated the selected formula D as the juicier and more permanent product with highly intensive fruity nuances compared to other formulations (Figure 1b).

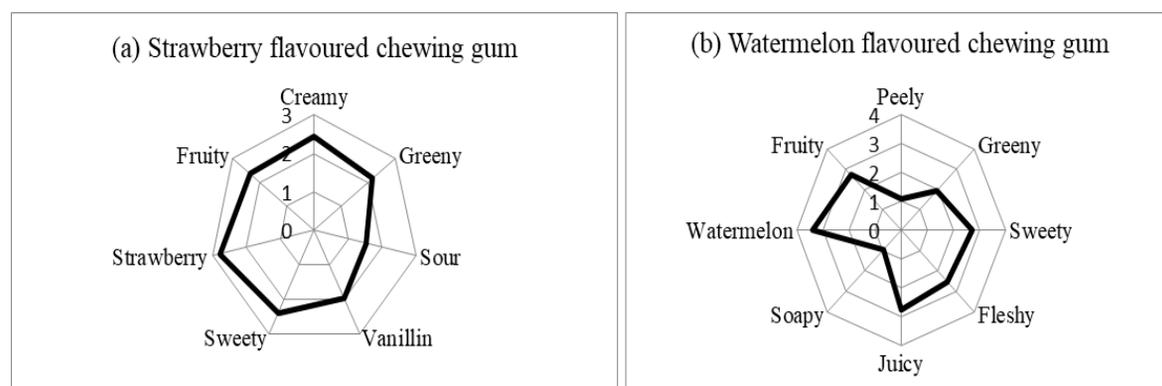


Figure 1. Radars of flavoured chewing gum sensory analysis

Formula D was prepared by using isomalt, and free and encapsulated forms of sucrose without any acidity regulator. In the sensory test, the non-sour flavour enhancing effect and sweet taste of isomalt conducted the preference of the assessors. Another advantage of isomalt is masking the bitter aftertaste of some sweeteners (Sentko and Willibald-Ettle, 2012). Watermelon flavour is a soapier and mouthful flavour than other fruit flavourings. It has also no acidity. These factors may also affect positively the preference of formula D. Xylitol was usually preferred in the

strawberry flavoured chewing gums in the market because of its cooling and better emphasising effects of fruit flavourings, especially in acidic environments.

#### *The second stage of the study*

Results for every two types of gums during 45 min are shown in Figure 2. As seen in Table 3, the permanence of both ethyl hexanoate and isoamyl acetate flavouring compounds was still observed at 45<sup>th</sup> min.

Table 3. Data from in vivo sensory analysis (RATD-GC/MS) of strawberry and watermelon flavoured chewing gums

Assessors	Strawberry flavoured chewing gum			Watermelon flavoured chewing gum		
	Ethyl hexanoate			Isoamyl acetate		
	T= 0 min	T= 5 min	T= 45 min	T= 0 min	T= 5 min	T= 45 min
1	20.67±0.11 <sup>e</sup>	19.52±0.10 <sup>b</sup>	10.97±0.10 <sup>f</sup>	14.08±0.12 <sup>b</sup>	12.92±0.10 <sup>a</sup>	6.16±0.11 <sup>d</sup>
2	3.06±0.09 <sup>a</sup>	2.79±0.11 <sup>a</sup>	0.67±0.11 <sup>a</sup>	1.99±0.12 <sup>a</sup>	1.32±0.11 <sup>b</sup>	0.13±0.10 <sup>a</sup>
3	5.99±0.10 <sup>d</sup>	5.80±0.12 <sup>e</sup>	4.32±0.10 <sup>e</sup>	4.07±0.09 <sup>c</sup>	3.89±0.11 <sup>d</sup>	2.58±0.12 <sup>e</sup>
4	4.51±0.11 <sup>c</sup>	4.35±0.10 <sup>d</sup>	2.62±0.13 <sup>c</sup>	2.92±0.11 <sup>d</sup>	2.70±0.09 <sup>c</sup>	1.11±0.09 <sup>c</sup>
5	4.77±0.12 <sup>c</sup>	4.28±0.11 <sup>bc</sup>	2.18±0.09 <sup>b</sup>	2.05±0.10 <sup>a</sup>	1.97±0.10 <sup>e</sup>	1.34±0.09 <sup>c</sup>
6	4.71±0.08 <sup>c</sup>	4.49±0.11 <sup>d</sup>	3.58±0.09 <sup>d</sup>	4.58±0.10 <sup>e</sup>	4.09±0.13 <sup>d</sup>	0.79±0.10 <sup>b</sup>
7	4.18±0.07 <sup>b</sup>	4.01±0.13 <sup>b</sup>	2.80±0.10 <sup>c</sup>	4.99±0.08 <sup>f</sup>	4.48±0.11 <sup>f</sup>	0.68±0.11 <sup>b</sup>

Results are mean± SD (10<sup>-2</sup> mg/mL)

Different letters in same column indicate significant differences at  $P < 0.05$ .

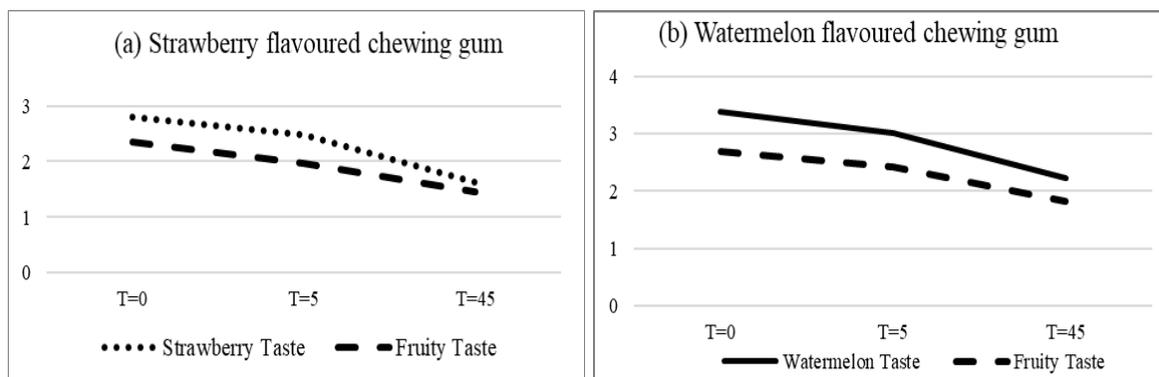


Figure 2. Sensory analysis of flavoured chewing gum

The compounds selected for strawberry and watermelon were quite intense in the first 5 min of breath, and they were still detected at the 45<sup>th</sup> min. Therefore, the sensory and DHA-GC/MS analyzes were in correspondence. According to the sensory evaluation results, the watermelon flavouring was still perceived as watermelon flavourings by the assessors at the 45<sup>th</sup> min, but the fruity nuance perception remained in the strawberry flavourings. However, the specific strawberry flavour was determined to disappear at the 30<sup>th</sup> min (Table 3). The reason for this is that the flavour components are known to be effective in the flavour release (Cook et al., 2005). Ethyl hexanoate is one of the strawberry flavouring ingredients. However, it is also frequently used as an ingredient to obtain fruity flavours. As it is known, a flavouring ingredient consists of many flavour chemicals and it releases each of these chemicals in different manner (Due et al., 2011; Sentko and Willibald-Ettle, 2012; Hinderink et al., 2019). Therefore, ethyl hexanoate was determined at the 45<sup>th</sup> min. The reason of perception of the fruity taste by the assessors instead of the sensory-specific strawberry flavouring might be the monitoring a single component in the instrument. It should be considered that working with devices with selected compounds gives parallel results with sensory evaluation studies but may show variability in specific flavour components. As a result, a single chemical is monitored with the instrument. However, in the sensory analysis, the instrument is human and evaluates a flavouring. The advantage of this device is that it requires only one vacuum pump, a special glass apparatus, and a Tenax, which is easily adapted to GC/MS.

Flavour compounds specific for various gum types can be monitored with the RATD-GC/MS to determine flavour quality parameters. This technique also gives parallel results to human perceptions. Although data from the assessors were statistically different ( $P < 0.05$ ), the permanence of the flavour was determined by all panelists after 45 min.

#### Accelerated shelf-life analyzes

Data from accelerated shelf-life analysis of flavoured chewing gums by RATD-GC/MS are given in Table 4 and Figure 3. It was confirmed that the formulations maintained their flavour permanence at the end of the shelf-life. The sensory results were in correspondence with the device. However, a deviation in the strawberry flavoured chewing gum was determined. The reason for this can be explained by the differences in the volatiles used in strawberry flavouring and their interaction with each other and with the gum base.

#### CONCLUSION

Flavour release is usually determined solely by employing sensory perception analyses to estimate the taste of foodstuffs. However, due to the human sensory nature of taste perception, there may be situations where flavour release could not be predicted specifically and the taste perception may not be clear due to some properties of food products (sugar or acidity in the food matrix, etc.). The importance of changes in instrumental measurements of flavour release should always be in correspondence to human perception due to the complexity of mixing

effects and the potential for perceptual interaction. In this study, the permanence of the chewing gum was determined by using both sensory techniques and RATD-GC/MS. For this purpose, the presence of the selected compounds

of strawberry and watermelon flavourings was monitored. It was observed that the measurements were in correspondence with the assessor's evaluations.

Table 4. Shelf-life of strawberry and watermelon flavoured chewing gums

Assessors	Strawberry		Watermelon	
	Ethyl hexanoate		Isoamyl acetate	
	T= 0 min	T = 45 min	T = 0 min	T = 45 min
1	3.10±0.09 <sup>c</sup>	3.45±0.08 <sup>f</sup>	2.82±0.08 <sup>d</sup>	0.66±0.01 <sup>e</sup>
2	0.58±0.01 <sup>a</sup>	0.53±0.02 <sup>c</sup>	0.59±0.01 <sup>a</sup>	0.31±0.01 <sup>d</sup>
3	1.55±0.10 <sup>b</sup>	0.78±0.01 <sup>e</sup>	1.02±0.08 <sup>b</sup>	0.29±0.01 <sup>d</sup>
4	1.91±0.09 <sup>b</sup>	0.67±0.01 <sup>d</sup>	0.96±0.01 <sup>b</sup>	0.22±0.01 <sup>c</sup>
5	0.55±0.01 <sup>a</sup>	0.25±0.01 <sup>b</sup>	1.09±0.08 <sup>b</sup>	0.21±0.01 <sup>bc</sup>
6	4.03±0.78 <sup>d</sup>	5.11±0.02 <sup>c</sup>	1.76±0.08 <sup>c</sup>	0.16±0.01 <sup>a</sup>
7	1.83±0.08 <sup>b</sup>	0.01±0.01 <sup>a</sup>	0.63±0.01 <sup>a</sup>	0.19±0.01 <sup>b</sup>

Results are mean± SD (10<sup>-2</sup> mg/mL)

Different letters in same column indicate significant differences at  $P < 0.05$ .

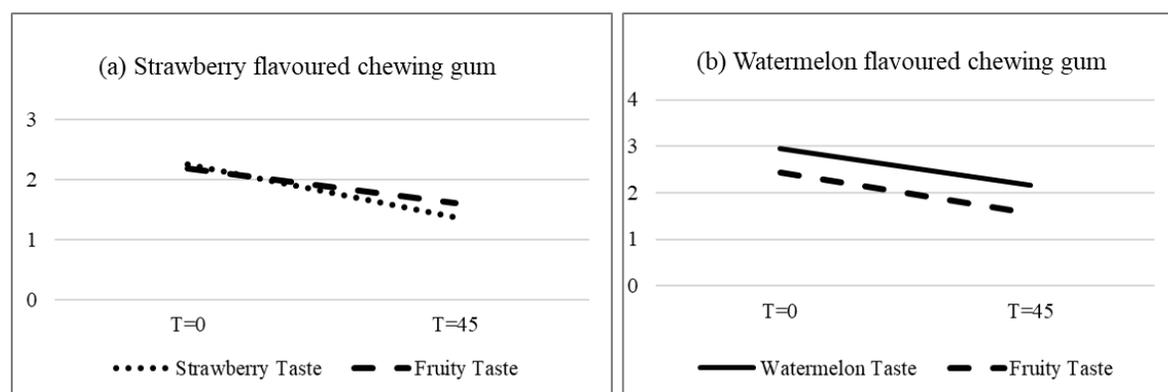


Figure 3. Shelf-Life sensory analyses result of flavoured chewing gum

This study presents important data that will contribute to the confectionery design process in the food industry that meets consumer expectations. Due to the human sensory nature of taste perception, measuring flavour permanence and estimating the taste experience of foodstuffs solely by sensory techniques could not be sufficient. Flavour permanence studies in different food matrices by using a RATD-GC/MS will be beneficial to create more permanent flavourings and may help to optimize production systems. In this study, the taste permanence of chewing gums evaluated both sensory techniques and RATD-GC/MS with the

presence of selected compounds from the flavourings of flavoured chewing gum.

#### ACKNOWLEDGMENTS

This work was supported by the TUBITAK (The Scientific and Technological Research Council of Turkey) [Grant Number TEYDEB 3180337]. The authors also thank the Aromsa Flavours and Food Additives Inc. Co., Kocaeli, Turkey and are grateful to Murat YASA and Melis YASA for their support in this study.

## DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## AUTHORS CONTRIBUTIONS

Pınar Uzun: Methodology, Writing-original draft; Ahmet Emir Özünal and Hakan Başkaya: Methodology, Formal Analyses, and Formulation of Chewing Gums; Nuray Doğan: Instrumental Analyses; Sonay Merve Gülay and Büşra Hantal: Sensory Analyses; Melike Üner: Writing review and editing; Asli Barla Demirköz; Supervisor and Writing-original draft.

## REFERENCES

Boland, A.B., Delahunty, C.M., Van Ruth, S.M. (2006). Influence of the texture of gelatin gels and pectin gels on strawberry flavour release and perception. *Food Chemistry*, 96(3), 452-460. doi:10.1016/j.foodchem.2005.02.027

Bonneau, A., Boulanger, R., Lebrun, M., Maraval, I., Valette, J., Guichard, É., Gunata, Z. (2018). Impact of fruit texture on the release and perception of aroma compounds during in vivo consumption using fresh and processed mango fruits. *Food Chemistry*, 239, 806-815. doi:10.1016/j.foodchem.2017.07.017.

Buettner, A., Schieberle, P. (2000). Exhaled odorant measurement (EXOM)-a new approach to quantify the degree of in-mouth release of food aroma compounds. *LWT-Food Science and Technology*, 33(8), 553-559. doi:10.1006/fstl.2000.0708

Buettner, A., Otto, S., Beer, A., Mestres, M., Schieberle, P., Hummel, T. (2008). Dynamics of retronasal aroma perception during consumption: Cross-linking on-line breath analysis with medico-analytical tools to elucidate a complex process. *Food Chemistry*, 108(4), 1234-1246. doi:10.1016/j.foodchem.2007.03.042

Castro, A.J., Johnson, S.S. (2006) Encapsulated Flavors and Chewing Gum Using Name. *U.S. Patent No. 7,022,352*. U.S. Patent and Trademark Office, Washington, DC.

Cook, D.J., Hollowood, T.A., Linforth, R.S., Taylor, A.J. (2005). Correlating instrumental measurements of texture and flavour release with human perception. *International Journal of Food Science and Technology*, 40(6), 631-641. doi:10.1111/j.1365-2621.2005.00973x.

Davidson, J.M., Linforth, R.S., Hollowood, T.A., Taylor, A.J. (1999). Effect of sucrose on the perceived flavor intensity of chewing gum. *Journal of Agricultural and Food Chemistry*, 47(10), 4336-4340. <https://doi.org/10.1021/jf9901082>

Davidson, J.M., Linforth, R.S., Hollowood, T.A., Taylor, A.J. (2000). Release of non-volatile flavor compounds in vivo. In: *Flavor Release*, Roberts D.D. and Taylor A.J. (eds), American Chemical Society, Washington, pp 99-111.

De Roos, K.B. (2008), Flavourings for Chewing gum. In: *Formulation and Production of Chewing and Bubble Gum*, Douglas F. (Ed.), Kennedy's Publications Limited, London, UK, pp. 205-231.

Du, X., Plotto, A., Baldwin, E., Rouseff, R. (2011). Evaluation of volatiles from two subtropical strawberry cultivars using GC-olfactometry, GC-MS odor activity values, and sensory analysis. *Journal of Agricultural and Food Chemistry*, 59(23), 12569-12577. <https://doi.org/10.1021/jf2030924>

Fenimore, E.L. (2008). Flavourings for Chewing gum. In: *Formulation and Production of Chewing and Bubble Gum*, Douglas F. (Ed.), Kennedys Book Ltd, London, UK, pp. 1-46.

Fisker, H.O., Nissen, V. (2006). Effect of the gum base and bulk sweetener on the release of specific compounds from fruit flavoured chewing gum. *Developments in Food Science*, 43, 429-432. doi:10.1016/S0167-4501(06)80101-9.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International*, 18, 49-70. <https://doi.org/10.1081/FRI-120003417>

Haahr, A.M., Madsen, H., Smedsgaard, J., Bredie, W.L., Stahnke, L.H., Refsgaard, H.H. (2003). Flavor release measurement by atmospheric pressure chemical ionization ion trap mass

- spectrometry, construction of interface and mathematical modeling of release profiles. *Analytical Chemistry*, 75(3), 655-662. <https://doi.org/10.1021/ac026035u>
- Hinderink, E.B., Avison, S., Boom, R., Bodnár, I. (2019), Dynamic flavor release from chewing gum: mechanisms of release. *Food Research International*, 116, 717-723. <https://doi.org/10.1016/j.foodres.2018.09.002>
- Hodgson, M., Linforth, R.S.T., Taylor, A.J. (2003). Simultaneous real time measurements of mastication, swallowing, nasal air flow, and aroma release. *Journal of Agricultural and Food Chemistry*, 51, 5052-5057. <https://doi.org/10.1021/jf030118+>
- Kataoka, H., Lord, H.L., Pawliszyn, J. (2000). Applications of solid phase microextraction in food analysis. *Journal of Chromatography*, 880, 35-62. doi:10.1016/j.foodres.2018.09.002.
- Koliandris, A., Lee, A., Ferry, A.L., Hill, S., Mitchell, J. (2008). Relationship between structure of hydrocolloid gels and solutions and flavour release. *Food Hydrocolloids*, 22(4), 623-630. doi:10.1016/j.foodhyd.2007.02.009.
- Lawless, H. T., Heymann, H. (2010). *Sensory Evaluation of Food: Principles and Practices*. Springer Science+Business Media LLC, New York, pp 1431-1450.
- Madene, A., Jacquot, M., Scher, J., Desobry, S. (2006). Flavour encapsulation and controlled release - a review. *International Journal of Food Science and Technology*, 41(1), 1-21. doi:10.1111/j.1365-2621.2005.00980.x.
- Menis-Henrique, M.E.C. (2020). Methodologies to advance the understanding of flavor chemistry. *Current Opinion in Food Science*, 33, 131-135. <https://doi.org/10.1016/j.cofs.2020.04.005>
- Merritt, C. G., Wingerd, W. H., Keller, D. J. (1983). Process for preparing a time delayed release flavorant and an improved flavored chewing gum composition. U.S. Patent No. 4,386,106. U.S. Patent and Trademark Office, Washington, DC.
- Muñoz-González, C., Rodríguez-Bencomo, J.J., Moreno-Arribas, M.V., Pozo-Bayón, M. Á. (2014). Feasibility and application of a retronasal aroma-trapping device to study in vivo aroma release during the consumption of model wine-derived beverages. *Food Science and Nutrition*, 2(4), 361-370. doi:10.1002/fsn3.111.
- Parliament, T.H. (1998). Applications of a microextraction class separation technique to the analysis of complex flavor mixtures. In: *Flavour Analysis*, Mussinan C.J. and Morello M.J. (eds.), ACS Symposium Series Vol. 705, pp. 8-21. DOI:10.1021/bk-1998-0705.ch002
- Pawliszyn, J. (2001). Solid phase microextraction. In: *Headspace Analysis of Foods and Flavors, Advances in Experimental Medicine and Biology*, Rouseff R.L., Cadwallader K.R. (eds.), Springer, Boston, MA, pp. 73-87. DOI: 10.1007/978-1-4615-1247-9\_6
- Potineni R.V., Peterson D.G. (2008). Mechanism of flavor release in chewing gum: Cinnamaldehyde. *Journal of Agricultural and Food Chemistry*, 56, 3260-3267. <https://doi.org/10.1021/jf072783e>
- Sentko, A., Willibald-Ettle, I. (2012). Isomalt, In: *Sweeteners and Sugar Alternatives in Food Technology*, O'Donnell, K., Kearsley M.W. (eds), John Wiley & Sons, Ltd., West Sussex, UK, pp. 243-274.
- Selli S., Kiliç Buyukkurt, Ö. (2020). Aroma maddelerinin salınımlarında etkili faktörler, *GIDA*, 45, 204-216. <https://doi.org/10.15237/gida.GD19122>
- Taylor, A.J., Linforth, R.S.T., Harvey, B.A., Blake, A. (2000). Atmospheric pressure chemical ionization mass spectrometry for in vivo analysis of volatile flavour release. *Food Chemistry*, 71(3), 327-338. doi:10.1016/S0308-8146(00)00182-5.
- Voilley A., Etievant, P. (2006). *Flavour in Food*, Woodhead Publishing Limited, Cambridge, England, 287p.
- Wong, S. W., Yu, B., Curran, P., Zhou, W. (2009). Characterising the release of flavour compounds from chewing gum through HS-SPME analysis and mathematical modelling. *Food Chemistry*, 114, 852-858. <https://doi.org/10.1016/j.foodchem.2008.10.030>