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## Analysis of volatile fraction selected hybrid fruits using chromatographic techniques

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### ABSTRACT

At present, "healthy eating" is gaining in popularity. Many people associate this term with eating plenty of vegetables and fruits. The basic elements influencing the selection of fruit are their appearance and smell. Of course, their origin and impact on human health are also very important. In recent years, exotic fruits have become increasingly accessible, making them gaining popularity, even among Poles. You can also meet plants created by crossing two varieties or species - so-called hybrids. They arise as a result of intersection of inbred lines created by multiple self-pollination of beneficial individuals. Although it is known that many of the hybrids created so far, With higher crop yields and better pro-health properties, these plants have not yet been thoroughly investigated. The purpose of the research was to compare the volatile fraction of hybrid fruit with the fruits which they came from. Thanks to such comparison, it was possible to classify fruits in terms of their quality, freshness and suitability for consumption. The chemical compounds that occur in the volatile fruit fraction in humans produce in people the aroma characteristic of each of them. By observing differences and similarities, it is possible to distinguish between them. To carry out the experiment, a comprehensive two-dimensional gas chromatography technique coupled with a mass spectrometer with time of flight mass spectrometer was used. In addition, samples were analyzed using an electronic nose, which uses ultrasonic gas chromatography.

**Keywords:** hybrid fruits, volatile organic compounds, VOC, two-dimensional gas chromatography, electronic nose, thermal desorption

## **1. INTRODUCTION**

In recent years consumers have become more and more attached to the quality of their products [1]. Facing the high availability of food products on the market, it is very important to consume the highest quality products. Before the product goes to market, it must be checked for all quality requirements [2]. It is particularly important that food products are produced in accordance with Good Manufacturing Practice and that their characteristics do not diverge from the requirements of the standards. The use of human olfactory is insufficient to distinguish discrete differences in hedonic quality of odors. A proven solution to this problem may be the use of instrumental analytical techniques [3].

Fruits are important element of our diet. Due to their widespread presence in the human diet, it is necessary to determine the quality of the fruits we eat. The quality of the fruit is above all the fulfillment of sensory requirements such as taste, smell or appearance [4]. High quality fruits and vegetables are also those that do not adversely affect human health [5]. Fruit aroma creates a complex mixture of volatile, organic compounds whose composition is characteristic for the species of fruit. Unique scent is created by the presence in the volatile fraction of different groups of chemical compounds of various concentrations. The most important groups of chemicals that affect the smell are: terpenes, alcohols, ketones, aldehydes, carboxylic acids and esters [6].

There are more and more new fruits appearing in shops. These are for instance hybrid fruits. Kiwano is an example of hybrid fruit, which was formed during artificial insemination using banana and kiwifruit. The purpose of the research was to determine whether the fruit formed by crossing two other species, kiwano, is in terms of the composition of the volatile fraction similar to kiwi and banana. Thanks to such tests we can get to know more about hybrid fruits, which have not been thoroughly tested yet. On the basis of the results of such tests it is also possible to distinguish fragrances which, to a greater or lesser degree, affect the fragrances that humans experience while eating a given fruit. Thanks to the results we can also draw conclusions about the impact of the tested fruit on the health of people consuming them.

The volatile fractions of banana, kiwifruit and kiwano were determined by thermal desorption technique coupled with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. One dimensional GC has a limited possibility to achieve satisfactory separation [7]. It can be overcome by using of two dimensional approaches, in which two independent GC ovens equipped with proper switching system and column setup are used [8]. Two-dimensional Gas Chromatography (GC×GC) is a modern analytical technique mainly used for the analysis of samples with complex matrix composition [9]. The distinction of the GC×GC technique with one-dimensional gas chromatography is the ability to separate the components of the chemical test mixture exhibiting similar physicochemical properties.

The GC×GC system is based on two chromatographic columns, in which the separation of analytes occurs as a result of different retention mechanisms. Analytes eluting from the first chromatographic column are collected continuously and periodically introduced into a second column chromatography of a different stationary phase type. Based on the results of the analysis, the volatile fractions of fruits were compared for the present volatile groups of chemicals and their concentrations. Some of the compounds were found for the first time, but a significant number had already been reported in many other papers [10].



These instrumental analytical techniques allow to confirm the authenticity of the purchased fruit. The human nose is insufficiently sensible to accurately determine the composition of the volatile product fraction and detect its misrepresentation. The solution to this problem may be the application of modern analytical techniques, which will enable the selection of foods of good quality, allowing for maintaining health and energy for longer.

## 2. EXPERIMENTAL

### 2. 1. Tested fruits

Hybrid fruits are plants created by crossing two varieties or species. Usually they are not created with genetic engineering. This type of crossbreeding is sometimes formed naturally, with a greater or lesser people contribution: artificial insemination or controlled pollination. Some plants, such as triticale, most of wheat cultivated in Poland, are hybrids, not GMOs. This cereal was created to combine the high yield potential and good grain quality of wheat with the disease resistance and environmental tolerance of rye [11]. Many of hybrids created so far have higher crop yields and better pro-health properties, although these plants have not yet been thoroughly investigated. For instance hybrid melon, unlike its male parent, is rich in ascorbic acid and  $\beta$ -carotene both of which are considered to be critically low in the current American diet [12].

Fruits tested during the reseach were banana, kiwano and kiwi.

1) Banana - *Musa acuminata* is a fruit, whose properties and nutritional values are appreciated not from today. Bananas were probably grown over 7000 years ago in Malaysia, from where they reached India and Africa, where they are grown today. The nutritional value of bananas is unique because it contains bioactive compounds, which are highly desirable in the diet as they exert many positive effects on human health and well-being [13]. There is also a lot of fiber and minerals - mainly potassium.

2) Kiwifruit (often shortened to kiwi) - the fruit of *Actinidia*, that comes from China [14] and was originally called Chinese gooseberry. As the fruit resembles a New Zealand kiwi bird, which is also rounded, brown and hairy. There is plenty of vitamins (especially C), minerals, and also beneficial for the aging processes of terpenes or fiber.

3) Kiwano - *Cucumis metuliferus* (also known as horned melon, African horned cucumber or melon) is a plant of the cucurbits family, originating in Africa [15]. The name given in New Zealand, where the earliest attempts to cultivate this species have been started, is a stick of kiwi and banana. Consume a yellowish-green flesh with refreshing and aromatic, sour and tart taste. Kiwano are oval and have yellow-orange skin with small, thick spines, rounded at the ends. Fruit pulp is green, juicy seeds with gelatinous consistency. The aroma of African cucumber is very pleasant and it is a combination of bananas and kiwi.

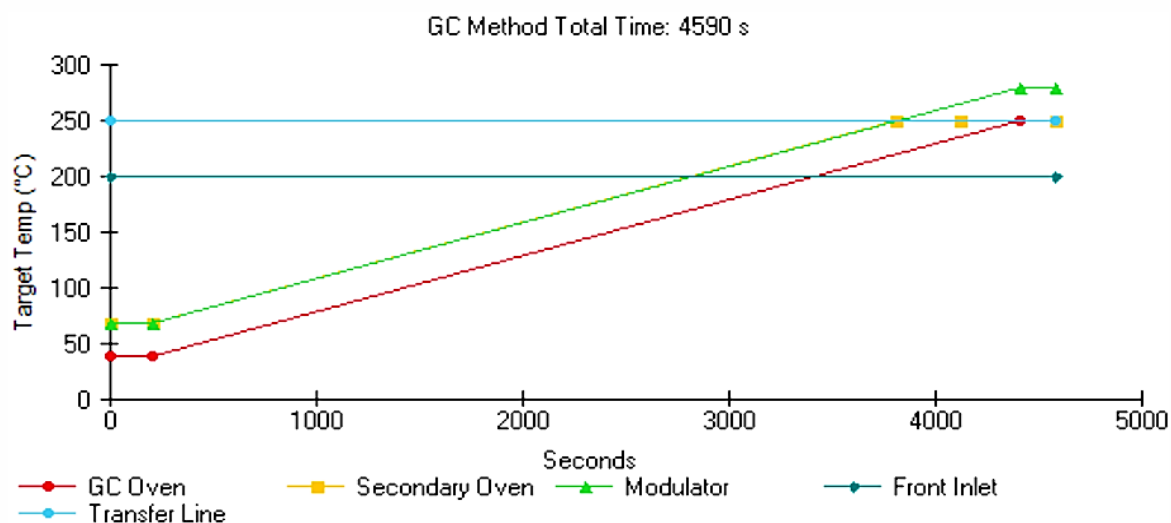
### 2. 2. Analytical procedure

All fruits were purchased in local distribution points in Gdansk, Poland. Before each analysis they were washed (firstly with use of washing-up liquid and then with distilled water), peeled and homogenized - fruits were finely chopped by stainless steel knife. 5 g of fruit were weighed into 20 ml glass vials. The vials were tightly locked with a teflon seal.



### 2. 2. 1. Two-dimensional gas chromatography technique coupled with thermal desorption

During the study, a complete two-dimensional gas chromatography technique coupled with thermal desorption was used. Two-dimensional gas chromatograph (Agilent 7980A), equipped with a cryogenic modulator coupled to the LECO Pegasus 4D Mass Spectrometry Spectrometer, was used for this analysis. The sorption trap is a glass tube with a sorbent (Tenax TA). Tenax TA is a porous polymer resin based on 2,6-diphenylene-oxide [16]. It is the most widely used adsorbent resins for use with Purge and Trap Thermal Desorption for applications such as trapping VOC's in air and liquids. Thermal desorption is a relatively new sample introduction technique [17]. Temperature conditions are shown in Figure 1.



**Figure 1.** Conditions of GC×GC - TOFMS analysis.

During the analysis the sorbent is heated, and the chemical on its surface is then desorbed to the gas phase. This gas, together with the carrier gas stream, flows to the chromatographic column where it is separated into components and analyzed. A great advantage of thermal desorption is the large surface area of the sorbent, as well as a significant amount of desorbed compounds, which contributes to the total absorption into the column. This makes it possible to denote chemical compounds with great accuracy. There are two series of chromatographic columns in the apparatus used by us. Their distinctive feature is the difference in the polarity of stationary phases. It can be very helpful in separating co-eluting peaks in the complex matrices under investigation [18]. The heart of the system is the modulator. Its task is to combine the two chromatographic columns and to move the individual components from one column to another. The eluate, together with the analyte, is scrubbed with the carrier gas stream from the first column every few seconds, and each chromatographic peak obtained is divided into fragments. Each such fragment is passed to the second chromatographic column. During modulation, the mass of the analyte does not change, so the height of the peak increases, balancing the decrease in its width, thus increasing the sensitivity of the analysis. At the end of the system there is a detector that reacts to the



presence of the dissolved chemicals producing an electronic signal that is sent to the recording system. Chemical compounds eluting from the chromatography column enter the ion source where they ionize due to collisions with high kinetic energy electrons. All chemicals are ionized with the same kinetic energy. As a result of the collisions, the compounds are fragmented. In this form, positive ions are accelerated by the external electric field 5000 times per second. They fly through the time-of-flight (TOF) analyzer and reach the detector where the flight time is recorded from the moment the external electric field is applied to the moment of impact of the fragment in the micro channel plate. Flight time is a characteristic value for a chemical. On this basis it is possible to identify individual chemical compounds. All of the parameters used during the analysis are presented below.

**Table 1.** Parameters of the instrument cluster.

Component set		Parameter	Description
Release of the sample		Sample desorption	Desorption from the tube (sorbent: Tenax TA)
Carrier gas		Type of carrier gas	Hydrogen
		Volume flow rate	1 ml/min
Injector		Operating mode	splitless
		Volume of gaseous samples taken for analysis	2,5 ml
Chromatographic column	No. 1	Type	Capillary
		Length	30 m
		Inner diameter	250 $\mu\text{m}$
		Thickness of stationary phase	0,25 $\mu\text{m}$
	No. 2	Type	Capillary
		Length	2 m
		Inner diameter	100 $\mu\text{m}$
		Thickness of stationary phase	0,1 $\mu\text{m}$
Modulator		Modulation time	6 s
Chromatographic oven		Initial temperature	$T_0 = 40\text{ }^\circ\text{C}$
		Maintenance time $T_0$	3 min

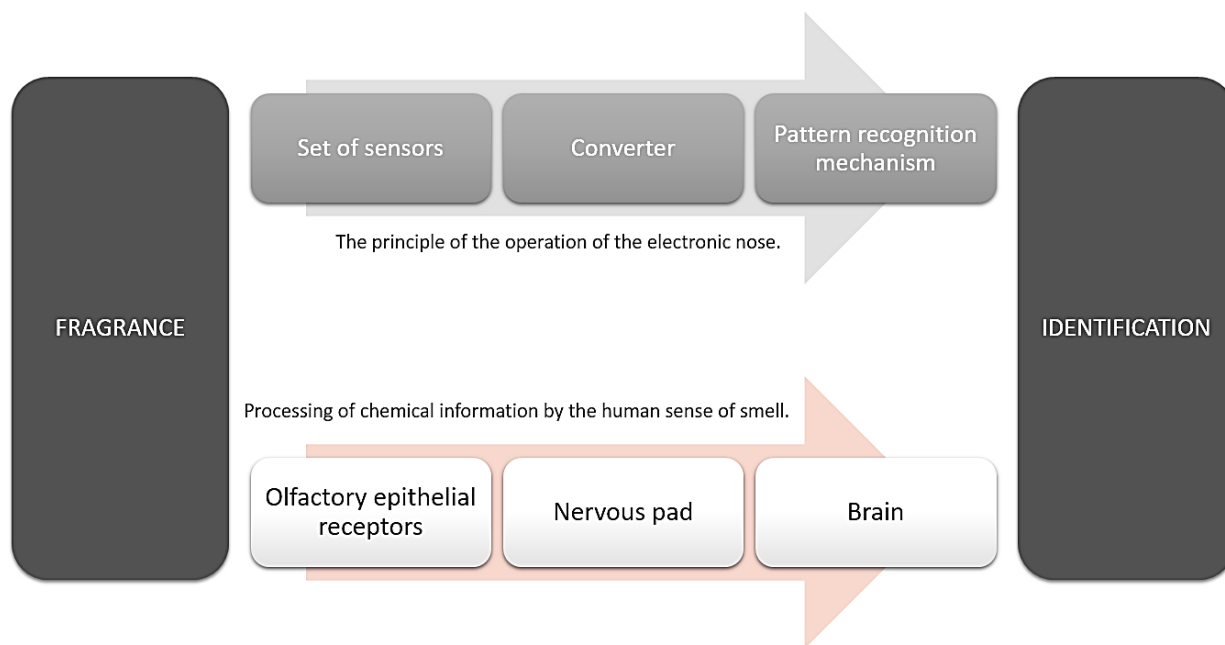


	Accretion	5 °C/min
	Final temperature	$T_k = 255\text{ °C}$
	Maintenance time $T_k$	5 min
<b>Detector</b>	Tension	1600 V
	Ionisation temperature	250 °C

### 2. 2. 2. Ultrafast gas chromatography technique with use of electronic nose

The samples were also analyzed using an electronic nose Heracles II from Alpha M.O.S., which uses ultrafast gas chromatography. The advantages of this measurement method include simple and rapid sampling as well as short and reliable analysis [19]. Interpretation of results is done with use of chemometric methods.

An electronic nose is a device that use is convergent to the function performed by the human sense of smell [20]. In many cases, the use of electronic noses coincides with the human sense of smell in experimental studies. Its use makes it possible to detect and distinguish complex mixtures of fragrances. The basic differences between the mechanism of action of the human sense of smell and the effect of e-nose are presented in Figure 2.



**Figure 2.** A diagram comparing the principle of the operation of the electronic nose with the processing of chemical information by the human sense of smell.

In Heracles II the sample is inserted into the device with a gas-tight syringe to the chromatography system. The syringe was directed to the injector, set to the no-gas carrier gas distribution mode. The sample is transferred from a dispenser to a sorption trap filled with 10 mg of Tenax TA sorbent. In this trap, the temperature is controlled from 0 °C to 280 °C. Then, using thermal desorption, the sample components were disassembled into two 10 m long chromatography columns and an internal diameter of 0.18 mm. The gradient of the temperature gradient in the chromatographic column can be up to 10 °C/s (600 °C/min). The chromatography columns are equipped with two flame ionisation detectors (FIDs) that can operate at temperatures up to 300 °C. Detector is monitored by computer software.

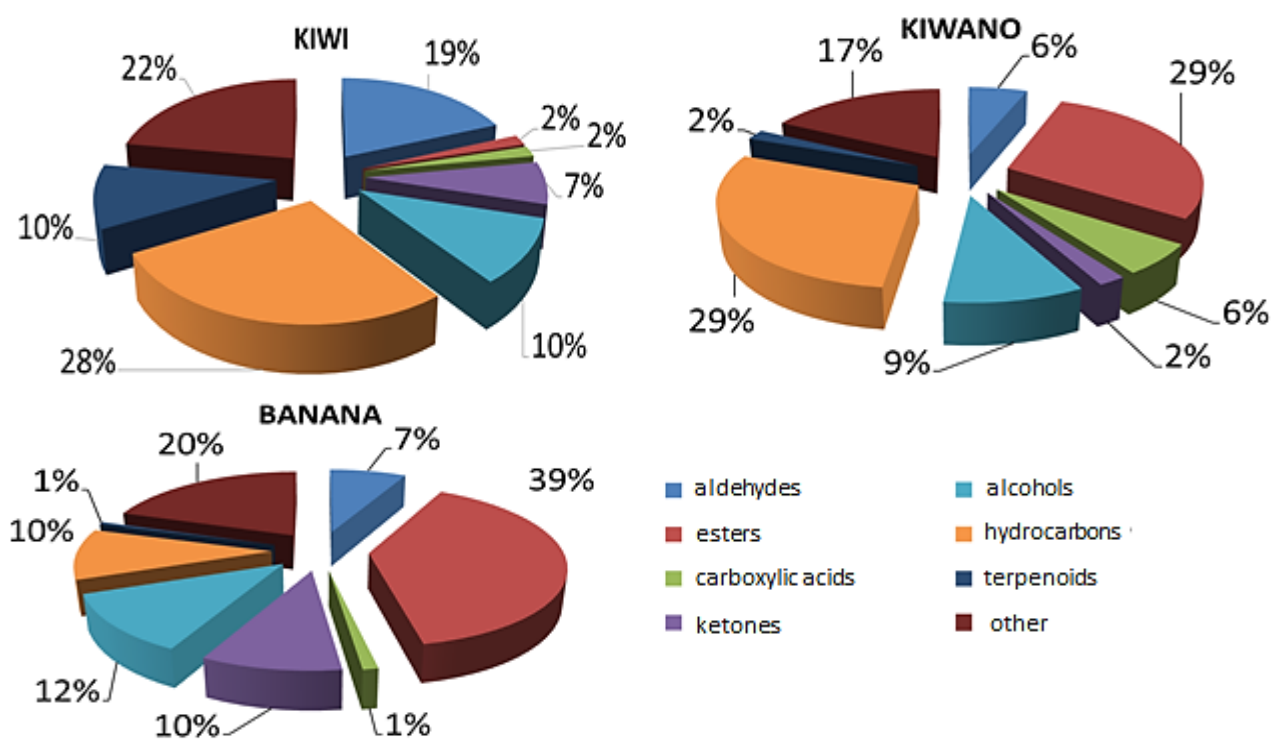
**Table 2.** Conditions for carrying out analyzes with use of Alpha M.O.S. Heracles II.

Device	Action	Adjustable parameters
<b>HS-100 Automatic Sample Feeder</b>	Drying	Temperature thermostat: 80 °C
		Thermostat time: 300 s
	Rinsing	Rinse time: 90 s
	Mixing	Mixing speed: 500 rpm
<b>Heracles II</b>	Introduction of the sample to the dispenser	Volume of gas sample: 2500 µL
		Sample rate: 250 µL/s
		Carrier gas pressure: 250 kPa
		Injector temperature: 200 °C
		Time of sample entry into sorption trap: 15 s
	Adsorption and desorption of analytes	Initial temperature of the sorption trap: 40 °C
		Initial pressure in sorption trap: 80 kPa
		Time of desorption of analytes: 20 s
Detecting analytes	Detector temperature: 270 °C	

### 3. RESULTS

#### 3. 1. Two-dimensional gas chromatography technique coupled with thermal desorption

In each sample many chemicals were detected. Small-area chromatography peaks were discarded. After initial data processing, chemical compounds were identified and assigned to one of the chemical classes. Identified substances were divided into: hydrocarbons, carboxylic acids, aldehydes, ketones, alcohols, terpenes and other compounds. With this approach, it was possible to develop diagrams showing the percentages of chemical classes in the fruit samples tested.



**Figure 3.** Diagrams showing the percentages of chemical classes in the fruit samples tested.

The unique smell of fruits and other products is created by the presence in the volatile fraction of various groups of chemical compounds of different concentrations. Terpenes for example are a huge family of natural organic compounds. They are characterized by a variety of chemical structures - apart from the basic isoprene unit, additional functional groups are present in most of them. Terpenes are also characterized by biological significance. Thanks to their ingestion, we can supply important antioxidants to the body, which can lead, for example, to delay aging [21]. Antioxidants are substances that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species [22]. Alcohols, ketones and aldehydes determine the smell, characterized by a sensory impression of "green freshness". Their presence in the fruity fraction of the fruit makes for a pleasant, intense aroma such as mown grass scent. The presence of large amounts of carboxylic acids compound in the fruit produces an acidic, fresh and slightly spicy aroma [23]. Sometimes the



scent impression can be irritating and penetrating. Therefore, the lower the carboxylic acids presence in the fruit volatile fraction is, the less their aroma is sweet but more intense. Esters are also found in most fragrant fruits. Most are short-chain and branched esters with pleasant, fresh scents. They determine the sweet fruit-flower scent [24].

By using the pattern recognition method analysis for the dominant volatile fractions of the fruit test compounds, you can determine what the chemical compound is and how it affects the perception of the fruit by humans.

The fruits tested differ in the number of classes of compounds in the volatile fraction. Banana and kiwano contain a lot of esters, often described similarly by fragrance descriptors, as fruity, strong, banana. These esters are responsible for feeling the sweet smell. Most carboxylic acids were detected in the sample of kiwano, which may suggest that the smell of this fruit is quite intense. The content of aldehydes and ketones is the highest in the banana sample. This is in line with the expectations, because they feel the smell is very pleasant and high intensity.

**Table 3.** Main chemical compound of volatile fraction of banana volatile fraction (the darkened fields show the main representatives of each class of chemical compounds).

	Name of compound	Retention time	Class	Percentage of compound in the volatile fraction
1	butanoic acid, 2-methylpropyl ester	1126,3	ester	13,47
2	butanoic acid, butyl ester	1246,2	ester	9,46
3	2-hexenal	821	aldehyde	8,05
4	3-hexen-1-ol, acetate, (Z)-	1326,7	ester	6,03
5	3-fluoro-5-hexen-2-one	1398	other	5,97
6	3-hexen-1-ol, acetate, (Z)-	1271,5	ester	5,13
7	acetic acid, propyl ester	461,1	ester	4,63
8	5-methyloctene-1	1400,9	hydrocarbon	3,63
9	2-hexanol, acetate	1094,3	ester	3,01
10	cis-hept-3-enyl acetate	1370	ester	3,00
11	butanoic acid, 3-methyl-, 3-methylbutyl ester	1589,6	ester	2,54
12	acetic acid, hexyl ester	1316,8	ester	2,38
13	acetic acid, pentyl ester	995,3	ester	2,37
14	butanoic acid, 3-methylbutyl ester	1444,8	ester	2,21

15	isoamylbutyrate	1344,8	ester	2,17
16	1-butanol, 3-methyl-, acetate	931,6	ester	1,53
17	dihydrocarvyl acetate	2384,1	ester	1,40
18	acetic acid, butyl ester	720,1	ester	1,24
19	4-methyl-5-hexen-2-ol	944,6	alcohol	1,06
20	5-hexen-2-one, 5-methyl-	943,5	ketone	0,97
...				
61	hexadecanoic acid	3680,5	carboxylic acid	0,16
...				
121	d-limonene	1373,2	terpene	0,03

**Table 4.** Main chemical compound of volatile fraction of kiwano volatile fraction (the darkened fields show the main representatives of each class of chemical compounds).

	Name of compound	Retention time	Class	Percentage of compound in the volatile fraction
1	acetic acid, butyl ester	685,6	ester	31,89
2	acetic acid, 2-methylpropyl ester	586	ester	23,43
3	2-pentanol, acetate	790,4	ester	8,41
4	2-pentanone	407,7	ketone	7,39
5	acetic acid, hexyl ester	1287,1	ester	5,71
6	1-butanol, 3-methyl-, acetate	870,5	ester	5,31
7	2-hexen-1-ol, (E)-	846,2	alcohol	4,83
8	hexanal	631,7	aldehyde	1,79
9	Hexane	320,8	hydrocarbon	1,70
10	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	3431,4	ester	1,21

11	isobutyl isopentanoic acid ester	1423,6	ester	0,96
12	Toluene	573,8	hydrocarbon	0,87
13	cyclohexene, 1-methyl-4-(1-methylethenyl)-, (s)-	1362,3	hydrocarbon	0,56
14	2-heptanone	893,9	alcohol	0,53
15	butanoic acid, 2-methylpropyl ester	1110,5	ester	0,52
16	pentadecane	2934,1	hydrocarbon	0,42
17	2-hexyl-1-decanol	4107,1	alcohol	0,38
18	tetradecane	2448,1	hydrocarbon	0,37
19	butanoic acid, butyl ester	1233,1	ester	0,32
...				
23	hexadecanoic acid	3682,7	carboxylic acid	0,31
...				
40	$\alpha$ -pinene, (-)-	1074,5	terpene	0,05

**Table 5.** Main chemical compound of volatile fraction of kiwi volatile fraction (the darkened fields show the main representatives of each class of chemical compounds).

	Name of compound	Retention time	Class	Percentage of compound in the volatile fraction
1	2-hexenal	778,2	aldehyde	34,79
2	hexanal	635,4	aldehyde	13,86
3	pentadecane	2935,6	hydrocarbon	5,41
4	$\beta$ -myrcene	1245	terpene	4,29
5	eicosane	3372,4	hydrocarbon	3,93
6	1-hexanol	855,6	alcohol	3,39

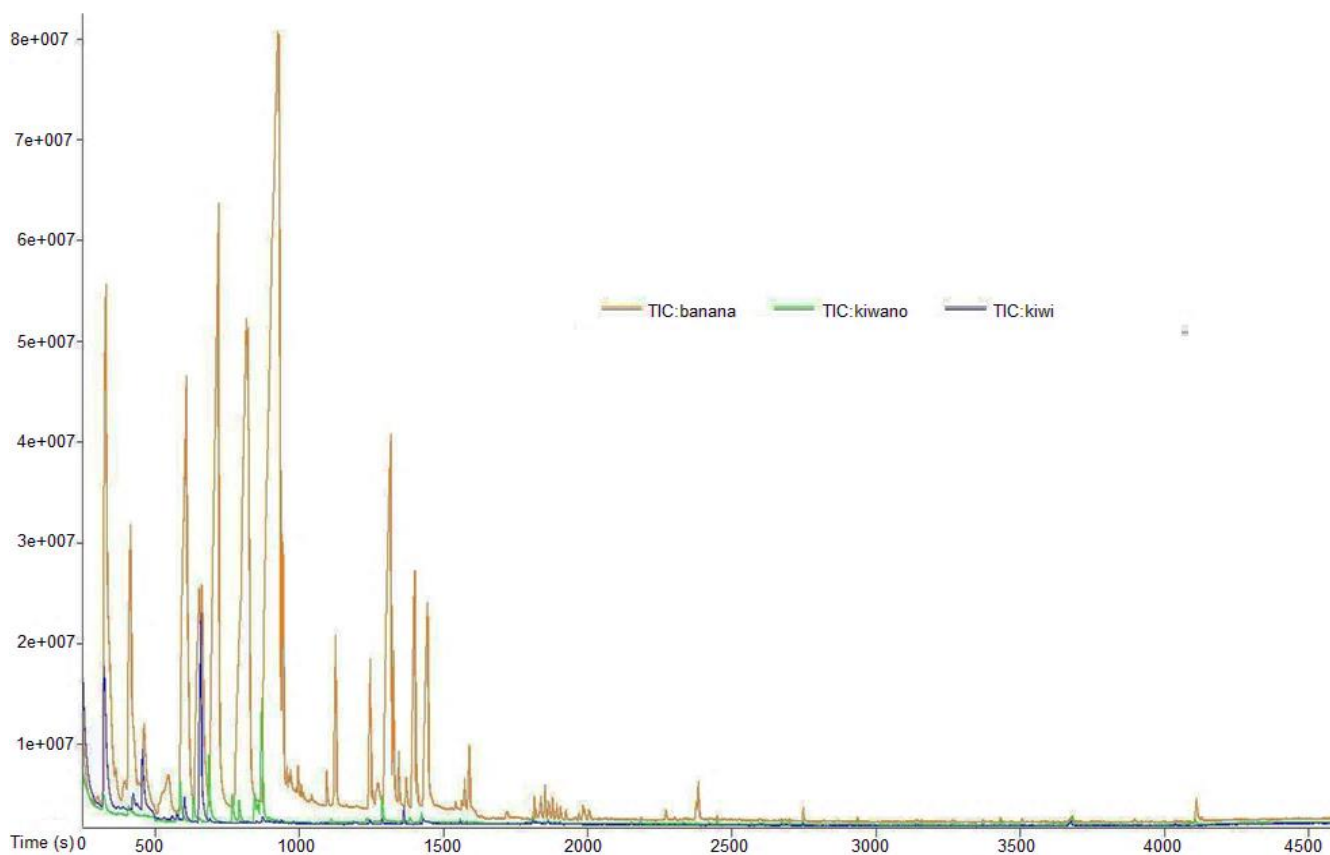
7	eicosane	3160,1	hydrocarbon	3,13
8	pentadecane	2698,4	hydrocarbon	2,78
9	limonene	1381,1	terpene	2,18
10	Nonanal	1558,8	aldehyde	2,05
11	tetradecane	2448,7	hydrocarbon	2,04
12	dodecane	1903,9	hydrocarbon	1,96
13	undecane	1607,5	hydrocarbon	1,91
14	tetradecane	2184,1	hydrocarbon	1,82
15	decanal	1863,4	aldehyde	1,81
16	1-iodo-2-methylundecane	3574,6	other	1,63
17	trans-caryophyllene	2494,2	terpene	1,59
18	decane	1297,1	other	1,52
19	2-hexenal, (E)-	634,2	aldehyde	1,43
...				
27	oxalic acid, allyl octyl ester	3653,4	ester	0,42
28	2-tridecanone	2885,5	ketone	0,38
...				
43	acetic acid	398,3	carboxylic acid	0,12

From the obtained chromatograms shown in Figure 4, it can be concluded that a much more intense odor is characteristic of the banana fruit. This is demonstrated by the height of the peaks as well as the amount of peaks. High peak signifies that the signal reaching the detector was stronger than others. In the case of kiwi and kiwano, the signals received in the detector were much weaker, which does not change the fact that the volatile compounds contained in their volatile fractions were distinguished. In order to determine the percentage of the individual compounds in the headspace of samples of the fruit normalization method without taking into account the correlation coefficients was used.

The results are shown in tables 3-5. Based on this data you can draw very interesting conclusions. In the banana sample, the biggest amount of chemical compounds was detected

(up to 186). Percentages are then much lower, but it was clearly visible that the ester content is the higher. In the sample of kiwano more than 50% of the volatile fraction are esters of acetic acid.

For kiwifruit, almost half of the volatile fraction contains aldehydes: 2-hexenal and hexanal, which makes odor of kiwifruit more fresh and “green” than in case of other samples. For this fruit, the largest amount of terpenes is detected, which is not surprising, considering that one of them constitutes 4.29% of the volatile fraction of this fruit and is already in the 4th position among the representatives of the various classes of compounds in kiwi. Also considerably lower is the content of esters. In all the samples one can see a similarity: often in high positions there are compounds with 6 carbon atoms in the chain.



**Figure 4.** The chromatogram obtained by GC×GC-TOF-MS analysis for the samples: banana (orange), kiwano (green) and kiwi (blue).

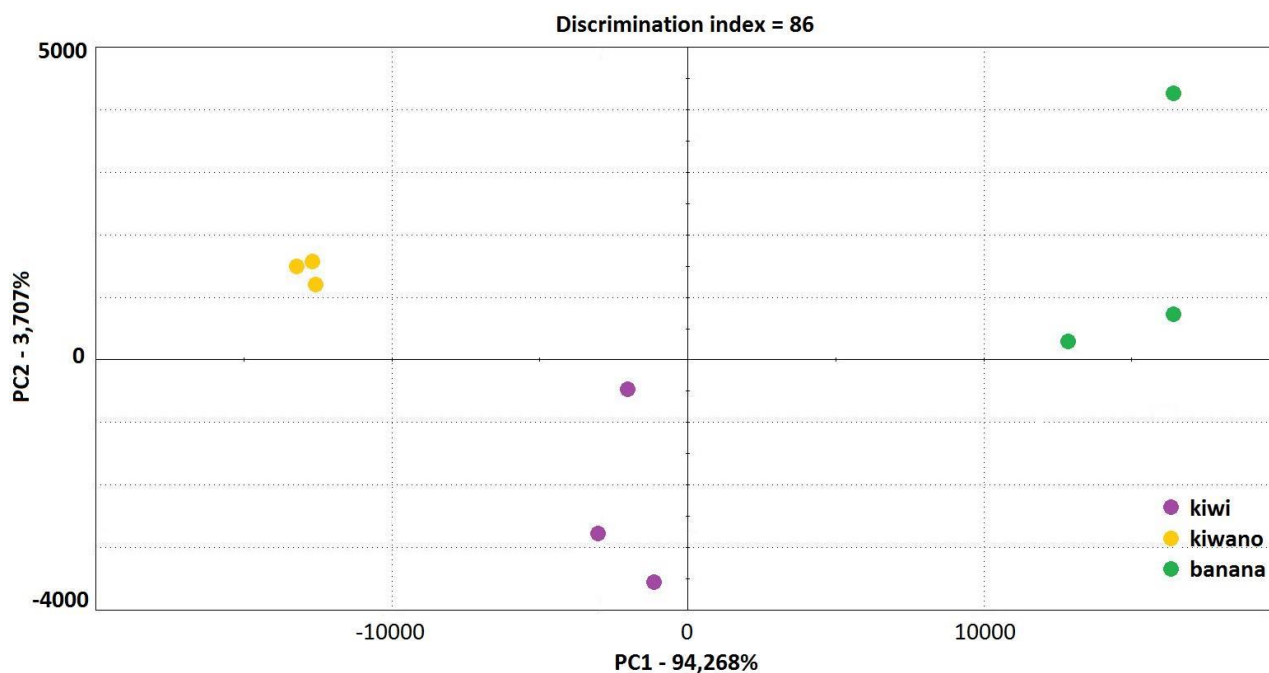
There are some other similarities between the fruits. There are many overlapping peaks at retention in given time, which is a testimony to the presence of the same or very similar chemical compounds in the odors of the tested fruits. It can be stated that the composition of the volatile fraction of kiwano is similar to both volatile fractions of kiwifruit and banana, but also kiwifruit and banana share common elements. In addition, there are peaks that allow the fruit to be distinguished from one another. Peaks occurring at a given retention time as alone in a given sample indicate compounds that occur only in one sample.

On the basis of the detection of this particular compound in the sample it can be later possible to distinguish the fruit from the other.

### 3. 2. Ultrafast gas chromatography technique with use of electronic nose

The organoleptic properties of the fruits tested are closely related. Using human senses such as smell or taste to distinguish between the test fruit is very difficult, often impossible. Based on the results, a distribution of the volatile fraction for each fruit was obtained, which made it possible to distinguish between the fruits. Despite the presence in the volatile fractions of the fruits of the same chemical groups, these groups differ in their specific chemical compounds.

In order to graphically illustrate the differences between the samples and thus to show the similarities and differences between the compositions of the volatile fractions of the tested fruits, the main components (PCA) analysis was used [25].



**Figure 5.** PCA graph for samples of banana, kiwi and kiwano.

On the basis of the obtained PCA graph of banana, kiwi and kiwano samples, it can be concluded that the higher the similarity to the volatile fraction shows kiwi and kiwano. What is more interesting kiwifruit and banana samples are more similar. Kiwano and banana are much different because the corresponding points on the chart are far apart. In addition, their separation took place with 94,268%, which means that banana and kiwano have very different odors.

#### 4. CONCLUSIONS

Use of two-dimensional gas chromatography technique coupled with mass spectrometry with time-zone fragmentation analyzer and using the electronic nose made it possible to identify and characterize the volatile compounds present in fruit samples. The high content of esters in banana samples are the reason of its sweeter smell. Interestingly, kiwi contains less esters in its aroma fraction. In this case hydrocarbons are the main components. The amounts of esters as well as hydrocarbons detected in the sample of kiwano were comparable as expected. Alcohols content in all these fruits maintained at a constant level. The relatively high content of terpenes in a sample of kiwi makes it possible to distinguish it from the other fruits tested. As a result kiwi, as a fruit containing these bioactive compounds, will work profitably on the human organism. Consumption of kiwi can contribute to delay the aging process because of the antioxidant properties of terpenes [26]. The comparison of the chromatograms allowed us to determine that the banana scent had a more intense odor than kiwi and kiwano. It is evidenced by the amount of peaks received. It can be said that the smell of banana is also the most complex one, because the chromatogram obtained after testing its volatile fraction, contains the highest number of peaks, corresponding to the largest number of volatile organic compounds present in the sample. The methods used in the studies are environmentally friendly. Tubes for chemical desorption and other components of the apparatus are (after proper preparation) ready for further use, what also reduces the cost of analysis. The carrier gas is the hydrogen generated in the tooling, so there is no need to store the hydrogen cylinder. The analysis was carried keeping the rules of the so-called "green chemistry".

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