GDAŃSK UNIVERSITY OF TECHNOLOGY

FACULTY OF CHEMISTRY

The author of the PhD dissertation: Anna Karolina Różańska
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DOCTORAL DISSERTATION

Title of PhD dissertation: Development of new analytical methodologies for the characterization and quality assessment of fruit juices

Title of PhD dissertation (in Polish): Opracowanie nowych metodyk analitycznych do charakterystyki oraz oceny jakości soków owocowych

Supervisor *signature*
prof. dr hab. inż. Żaneta Polkowska
Auxiliary supervisor *signature*
dr inż. Tomasz Dymerski

Gdańsk, 2024

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The author of the PhD dissertation: Anna Karolina Różańska

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DESCRIPTION OF DOCTORAL DISSERTATION

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Keywords of PhD dissertation in Polish: soki owocowe; związki bioaktywne; zafałszowania żywności; aminy biogenne; alkilofurany; nowe metody analityczne; elektroniczny nos; GC-FID; GC-MS; GC×GC

Keywords of PhD dissertation in English: fruit juices; bioactive compounds; food adulteration; biogenic amines; alkylfurans; new analytical methods; electronic nose; GC-FID; GC-MS; GC×GC

Summary of PhD dissertation in Polish: Soki owocowe charakteryzują się dużą zawartością składników mineralnych, błonnika pokarmowego, witamin, czy związków bioaktywnych. Są one więc postrzegane jako żywność funkcjonalna, której spożycie może mieć pozytywny wpływ na organizm człowieka. Ze względu na duży wzrost zapotrzebowania na soki owocowe, przemysł sokowniczy stoi przed licznymi wyzwaniami związanymi z oceną jakości produktów na poziomie przemysłowym i detalicznym. Przedmiotem badań było opracowanie nowych rozwiązań metodycznych do kontroli jakości soków owocowych, z głównym naciskiem na rozwiązania mogące znaleźć zastosowanie w przetwórstwie spożywczym. Badania prowadzone były dwutorowo. Z jednej strony przeprowadzono charakterystykę profili frakcji lotnej soków owocowych przy wykorzystaniu nowoczesnej techniki analitycznej, takiej jak dwuwymiarowa chromatografia gazowa (GC×GC). Przeprowadzenie takich analiz umożliwiło wytypowanie wyróżników soków owocowych, a także potencjalnych substancji bioaktywnych charakterystycznych dla danego rodzaju soku. Z drugiej strony, opracowano metodyki analityczne użyteczne do szybkiej kontroli jakości i wykrywania zafałszowań soków przy użyciu jednowymiarowej chromatografii gazowej (GC). Opracowane metodyki dotyczyły oznaczania zawartości amin biogennych przy użyciu mikroekstrakcji ciecz-ciecz wspomaganej wysalaniem sprzężonej z GC-MS (SALLME-GC-MS) oraz zawartości furanu i alkilofuranów przy użyciu ultraszybkiej chromatografii gazowej z detektorem płomieniowo-jonizacyjnym (UFGC-FID). Dodatkowo, opracowano rozwiązanie wykorzystujące elektroniczny nos umożliwiające wykrycie zafałszowanych próbek soków. Badania były prowadzone zgodnie z zasadami Zielonej Chemii Analitycznej, a opracowane rozwiązania oceniono pod względem ich zieloności przy użyciu dwóch narzędzi analitycznych: AGREE oraz GAPI.

Summary of PhD dissertation in English: Fruit juices are characterized by a high content of minerals, dietary fiber, vitamins and bioactive compounds. Therefore, they are perceived as functional foods, the consumption of which can have a positive effect on the human body. Due to a large increase in the demand for fruit juices, the juice industry faces numerous challenges related to quality assessment at the industrial and retail levels. The subject of the research was the development of new methodological solutions for fruit juice quality control, with the main emphasis on solutions that can be used in food processing. The research was conducted in two ways. On the one hand, the volatile fraction profiles of fruit juices were characterized using a modern analytical technique, such as two-dimensional gas chromatography (GC×GC). Conducting such analyzes made it possible to select the distinguishing features of fruit juices, as well as potential bioactive substances characteristic of a given type of juice. On the other hand, analytical methodologies useful for rapid quality control and adulteration detection of juices using one-dimensional gas chromatography (GC) have been developed. The developed methodologies concerned the determination of the content of the biogenic amines using salting-out assisted liquid-liquid microextraction coupled with GC-MS (SALLME-GC-MS) and the furan and alkylfurans content using ultrafast gas chromatography with a flame ionization detector (UFGC-FID). In addition, an electronic nose solution was developed to detect adulterated juice samples. The research was conducted in accordance with the principles of Green Analytical Chemistry, and the developed solutions were assessed in terms of their greenness using two analytical tools: AGREE and GAPI.

I would like to sincerely thank

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Contents

List of publications constituting the basis of the doctoral dissertation

No	Publication	IF[1]
P1	M. Fabjanowicz, **A. Różańska**, N. S. Abdelwahab, M. Pereira-Coelho, I.C. da Silva Haas, L.A. dos Santos Madureira, J. Płotka-Wasylka, An analytical approach to determine the health benefits and health risks of consuming berry juices, *Food Chemistry* 432 (2024) 137219.	10.212
P2	M. Lubinska-Szczygeł, **A.Różańska**, J. Namieśnik, T. Dymerski, R. Shafreen, M. Weisz, A. Ezra, S. Gorinstein, Quality of limes juices based on the aroma and antioxidant properties, *Food Control* 89 (2018) 270.	4.184
P3	R. Shafreen, M. Lubinska-Szczygeł, **A. Różańska**, T. Dymerski, J. Namieśnik, E. Katrich, S. Gorinstein, Human serum interactions with phenolic and aroma substances of Kaffir (*Citrus hystrix*) and Key lime (*Citrus aurantifolia*) juices, *J. Lumin.* 201 (2018) 115.	2.595
P4	**A. Różańska**, T. Dymerski, J. Namieśnik, Novel analytical method for detection of orange juice adulteration based on ultra-fast gas chromatography, *Monatsh. Chem.* 149 (2018) 1615.	1.486
P5	**A. Różańska**, M. Fabjanowicz, K. Kalinowska, Ż. Polkowska, J. Płotka-Wasylka, Green, simple analytical method for biogenic amines determination in fruit juice samples using salting-out assisted liquid-liquid microextraction and gas chromatography-mass spectrometry, *Food Chemistry* 384 (2022) 132557.	10.212
P6	**A. Różańska**, T. Dymerski, Ż. Polkowska, A new, fast, simple, practical and environmental-friendly HS-UFGC-FID approach for furan and six alkylfurans determination in fruit juice samples, *pre-print submitted for evaluation.*	NA

[1] IF from the year of publication, NA not applicable

Abbreviations and acronyms

µSPE	micro-solid phase extraction
AAS	atomic absorption spectrometry
AGREE	analytical greenness metric approach
ANIT	1-naphthylisothiocyanate
AOT	bis [2-ethylhexyl] sulphosuccinate
BAs	biogenic amines
BAGI	blue applicability grade index
BBD	Box-Behnken design
CD	conductivity detector
CMD	conductometric detector
CSDF-ME	continuous sample drop flow microextraction
D-µ-SPE	dispersive solid-phase microextraction
DAD	diode array detector
DCM	dichloromethane
DHS	dynamic headspace sampling
DLLME	dispersive liquid-liquid microextraction
DNS-Cl	dansyl chloride
DoE	design of experiment
DVB/CAR/PDMS	divinylbenzene/carboxy/polydimethylsiloxane
ECD/NPD	nitrogen-phosphorus detection/electron capture detection
ECF	ethyl chloroformate
EFSA	European Food Safety Authority
FID	flame ionization detector
FLD	fluorescence detector
GAPI	green analytical procedure index
GC	gas chromatography
GC×GC	two dimensional gas chromatography
GFAAS	graphite furnace atomic absorption spectrometry
HCA	hierarchical cluster analysis
HFBA	heptafluorobutyric anhydride
HLLME	homogeneous liquid–liquid microextraction
HPAEC	high-performance anion-exchange chromatography
HPLC	high-performance liquid chromatography
HRTOF-MS	high resolution time-of-flight mass spectrometry
HS	headspace
HSA	human serum albumin
IARC	International Agency for Research on Cancer
IBCF	isobutyl chloroformate
IC	ion chromatography
ICP-OES	inductively coupled plasma-optical emission spectrometry

ILSFOD-LLME	ionic liquid-assisted liquid-liquid microextraction based on the solidification of floating organic droplets
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
m-PFC	multi-plug filtration cleanup
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MSPD	matrix solid phase dispersion method
NAC	n-acetyl-l-cysteine
NFC	not from concentrate
NMR	nuclear magnetic resonance
OPA	o-phthaldialdehyde
PAD	pulsed amperometric detection
PAHs	polycyclic aromatic hydrocarbons
PCA	perchloric acid
PCBs	polychlorinated biphenyls
QTOF-MS	quadrupole time-of-flight mass spectrometry
QuEChERS	quick, easy, cheap, effective, rugged, and safe
RF	random forest
SALLE	salting-out liquid-liquid extraction
SALLME	salting-out liquid-liquid microextraction
SHS	static headspace sampling
SPE	soild phase extraction
SPME	solid phase microextraction
SULLE	sugaring-out liquid-liquid extraction
TAC	total anthocyanin content
TCA	trichloroacetic acid
TEA	triethylamine
TFC	total flavonoid content
TOF-MS	time-of-flight mass spectrometry
TPC	total phenolic content
UFGC	ultrafast gas chromatography
UHPLC	ultra-high performance liquid chromatography
UPLC	ultra-performance liquid chromatography
UV	ultraviolet detector
VA	vortex-assisted

1. Introduction

One of the very important branches of the food industry is the juice industry [1]. Because the fruit spoils and rots, and their availability is seasonal, the production of juices from fresh fruits allows their consumption throughout the year. Fruit juices and fruit concentrates have become valuable products and semi-finished products [2]. Many types of fruit are used for the production of fruit juices, including apples, grapes and peaches, but oranges are the most frequently used raw material [3]. In recent years, however, it can be noted, the increase in the popularity of juices produced from berries, such as bilberries, raspberries, blueberries, blackberries and chokeberries [4]. This may be due to a significant increase in interest in a healthy daily diet. Consumers are increasingly checking the labels of food products containing lists of nutrients and additives contained in them. This allows them to make informed decisions when making food choices. Juices are characterized by a low content of carbohydrates and calories, as well as a high content of fiber, vitamins, minerals and antioxidants [5]. For this reason, juices do not have to be an addition to meals but can be treated as functional food, the consumption of which can have a positive effect on the human body. In particular, this applies to fruit juices, from which natural antioxidants: polyphenols and carotenoids, can be obtained [6].

Food authenticity assessment is of increasing importance in the food industry, where producers and suppliers can increase their profits by substituting or adding cheaper ingredients to their products [7]. The most common type of adulteration of juices is dilution with water, the addition of sugar syrup, which reduces the total amino acid value, the addition of ingredients that are not naturally present in the juice, e.g. dyes, and the addition of cheaper juices from other types of fruit [8]. Orange juices are most often adulterated by the addition of tangerine [3], [9], grapefruit [9] or lemon [10] juice. In the case of berry juices, the most common type of adulteration is the addition of dyes [11] and other berry juices [12], [13]. There are many physical, chemical or microbiological methods used to assess the quality of juices. During the tests, the content of naturally occurring substances in raw materials is determined and the presence of other decomposition products or impurities is detected [14]. The most effective methods consist in analyzing the profile of carbohydrates, phenols, carotenoids, amino acids or other organic acids using various chromatographic techniques [15], [16]. However, the use of these procedures is labour-intensive and time-consuming. For this reason, new solutions are sought for fast, simple and effective detection of adulteration of fruit juices.

In addition, there are many risks associated with improper handling of fruit juices, which is associated with the possibility of their contamination [17]. Among the contamination of fruit juices, microbiological and chemical contamination can be distinguished. However, modern analytical methodologies are still being sought to enable the determination of analytes, the presence of which may be dangerous to the health of consumers. These methods should be characterized by good greenness assessment, speed, reliability and sensitivity, enabling the determination of analytes at a trace level. Fulfilment of these assumptions enables the use of chromatographic techniques with a properly selected stage of sample preparation [15], [16].

2. Fruit juices

2.1. Fruit juices as functional beverages

The fruit juice market is one of the main markets in the food industry [1]. Due to the growing awareness of consumers, their preferences focus on increasingly healthy food products, which, in addition to good taste and smell, can additionally provide health benefits. For this reason, functional beverages have gained more and more popularity in recent years [2]. These include juices containing additional phytochemicals or bioactive compounds. Very often, these drinks are made from different types of fruit and then combined with dairy or soy-derived products [2]. Fruit juices can also be medium for probiotic strains, as they have the proper nutritional composition [18]. Citrus juices are also classified as functional juices [19]. Superfruits, such as berries and products made from them, are characterized by a high content of polyphenols, therefore these juices can also be classified as functional drinks [1].

2.2. Fruit juices – health benefits

Consuming fruit juices can provide several health benefits, as shown in Figure 1. Fruit juices contain large amounts of macro- and microelements, such as K, Mg, Na, P, or Ca and Fe [20]. These macro- and micronutrients play an important role in the transmission of nerve impulses, the control and equalization of blood pressure, and the regulation of osmotic homeostasis [21]. One glass of fruit juice can provide your daily dose of nutrients such as Co, Cr, Cu, Fe, Mn, Ni, Zn, and Se [20]. Fruit juices are also rich in B vitamins or vitamins A, C and E, so consuming them may contribute to reducing inflammation by stimulating the immune system [22]. Fruit juices, apart from the minerals and vitamins they contain, are also a source of bioactive substances, such as phenolic acids or anthocyanins. Bioactive substances contained in juices may have a beneficial effect on the human body through anti-inflammatory, cardioprotective, neuroprotective and antioxidant effects [23]. Consumption of fruit juices, and above all superfruit juices, such as berries, can improve blood pressure, glycemic and lipid profile [24].

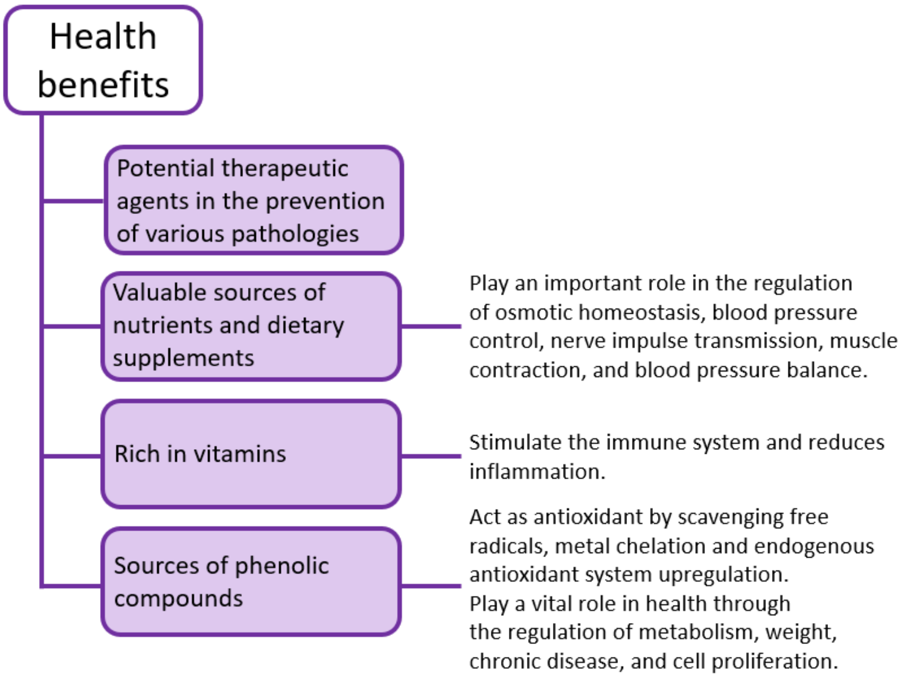

Figure 1 Health benefits related to the consumption of berry juices.

2.3. Fruit juices – risks

Including fruit juices into the daily diet can have a positive impact on the health of consumers. However, it is important to remember that there are many risks associated with consuming juices (Figure 2). These risks may be caused by contamination, such as microbiological or chemical contaminations. Microbiological contamination includes the presence of pathogenic microorganisms such as fungi, viruses and bacteria [25]. The cause of microbiological contamination is usually the lack of compliance with the sanitary regime during the production, transport and storage of juices [26]. Chemical contaminants of juices include metals, bisphenols, polycyclic aromatic hydrocarbons, pesticides, biogenic amines, furans and adulterants. Chemical contaminants in fruit juices typically come from the fruit and the processes they undergo, during cultivation as well as after harvesting. These processes cause the transfer of chemical pollutants such as pesticides, metals and biogenic amines [25]. Another factor causing the formation of impurities such as furans may be processes that extend the shelf life of juices, such as sterilization or pasteurization [27], [28]. In addition, the packaging in which the finished product is stored may also be a source of chemical contamination [25]. It should be emphasized that quality control of fruit juices is extremely important to ensure consumer safety.

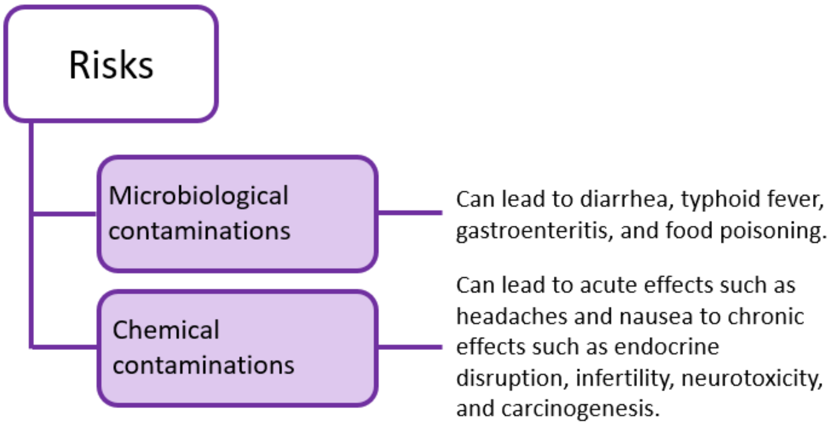

Figure 2 Risk related to the consumption of berry juices.

2.4. Analytical techniques used for fruit juices analysis

Fruit juice analysis is an extremely important issue. It makes it possible to assess the nutritional value of products, and thus determine the potential impact of juices on the human body. In addition, based on the analysis of juice samples, it is possible to control their quality, as well as detect the additives used and monitor any adulteration [16].

Due to the very complex composition of the matrix of juice samples, the analysis of bioactive substances as well as microbiological and chemical contaminations is a challenge for analytical chemists. Chromatographic techniques are the most commonly used instruments. However, due to the complex matrix, direct analysis of juice samples is often impossible. It is therefore necessary to properly prepare the juice samples [29]. This stage allows the removal of interfering substances, as well as the dilution or concentration of substances, or the separation of analytes. Table 1 lists examples of analytical methods for the determination of bioactive substances and contaminations of fruit juices [15].

Table 1 Characterization of analytical methods applied to determine the bioactive substances and contaminations in different fruit juices.

Chemical group	Pro-/Anti-health properties	Juice type	Number of analytes	Sample preparation step	Analytical technique	Targeted/ Untargeted analysis	Ref.
anthocyanins	pro-	blackcurrant	4	LLE	UHPLC	targeted	[30]
anthocyanins	pro-	blueberry	6	LLE	UPLC	targeted	[31]
anthocyanins	pro-	grape	1	-	spectrophotometry	targeted	[32]
folates	pro-	blackberry blueberry strawberry raspberry	11	SPE	UHPLC-MS/MS	targeted	[33]
organic acids	pro-	blueberry	13	SPE	IC-CD	targeted	[34]
phenols	pro-	blueberry cranberry grape	43	centrifugation, filtration	LC-MS/MS	targeted	[35]
phenols	pro-	mixed berry	1	-	fluorometry	targeted	[36]
phenols	pro-	blackberry	2	SPE	HPLC	targeted	[37]
phenols	pro-	chokeberry	-	LLE	spectrophotometry	targeted	[38]
sugars	pro-	blueberry	3	filtration	HPAEC-PAD	targeted	[39]
mycotoxins	anti-	pomegranate	3	QuEChERS	HPLC-DAD	targeted	[40]
metals	anti-	grape	7	digestion	AAS	targeted	[41]
metals	anti-	pomegranate	2	HLLME	GFAAS	targeted	[42]
metals	anti-	cherry grape pomegranate	21	digestion	ICP-OES	targeted	[43]
metals	anti-	grape	2	DLLME	FAAS	targeted	[44]
PAHs	anti-	grape	12	VA-d-μ-SPE	GC-FID	targeted	[45]
PAHs	anti-	grape	11	QuEChERS	GC-MS/MS	targeted	[46]
PCBs	anti-	grape	7	DLLME-D-μ-SPE	GC-MS/MS	targeted	[47]
pesticides	anti-	cherry grape	11	SALLE	GC-MS	targeted	[48]
pesticides	anti-	raspberry	4	SULLE	GC-FID	targeted	[49]
pesticides	anti-	grape	5	CSDF-ME	GC-MS	targeted	[50]
pesticides	anti-	blackcurrant raspberry redcurrant strawberry	160	MSPD	GC-ECD/NPD	targeted	[51]
pesticides	anti-	grape	7	ILSFOD-LLME	HPLC-UV	targeted	[52]
pesticides	anti-	grape	10	m-PFC	GC-Orbitrap	targeted	[53]
furans	anti-	pomegranate	7	SPME	GC-MS	untargeted	[54]
furans	anti-	blackcurrant	3	filtration	GC-MS	untargeted	[55]
biogenic amines	anti-	bog bilberry	7	LLE	HPLC-UV	targeted	[56]
biogenic amines	anti-	grape	31	centrifugation, derivatization	GC-MS	targeted	[57]
biogenic amines	anti-	blackcurrant	5	derivatization	HPLC-UV	targeted	[58]
biogenic amines	anti-	blackcurrant redcurrant	5	centrifugation, filtration	IC-CMD	targeted	[59]

Spectroscopic techniques are most often used for the determination of bioactive substances. These techniques are primarily used as screening tests, to determine the summary parameters, such as total phenolic content (TPC), total flavonoid content (TFC), or total anthocyanin content (TAC). Spectroscopic techniques are characterized by the simplicity and speed of measurements, and their use can even be mobile. Methods for the determination of summary parameters, however, have limitations, because reactions between analytes and reagents (e.g. Folin-Ciocalteu reagent) may not be specific, therefore the obtained results may be overestimated [16].

To obtain more reliable results, chromatographic techniques, mainly liquid chromatography (LC), are mainly used. Determination of bioactive substances using LC often precedes the liquid-liquid extraction (LLE) step, examples of used solvents are methanol with water and trifluoroacetic acid (TFA) [31], or methanol acidified with 1% acetic acid [30]. Another commonly used technique for the extraction of phenols or organic acids is the solid-phase extraction (SPE) technique [34], [37]. Both LLE and SPE are labour-intensive and high solvent usage techniques. For this reason, new analytical solutions for more ecological methods of both preparation and determination of bioactive substances are still being sought.

Among the chemical substances that may have a negative impact on human health, we can distinguish, for example, metals, PAHs, PCBs, pesticides, furans or biogenic amines.

The content of metals in fruit juices is mainly determined using spectroscopic techniques. Among them, we can distinguish various types of atomic absorption spectrometry (AAS) [41], [42], [44] and inductively coupled plasma optical emission spectroscopy (ICP-EOS) [43]. Typically, the preparation of juice samples before metal determination involves digesting with strong solvents such as nitric acid or perchloric acid. In order to search for greener methods of sample preparation, e.g. homogeneous liquid-liquid microextraction (HLLME) [42] or dispersive liquid-liquid microextraction (DLLME) [44] were used for metal extraction.

For the determination of other contaminants in fruit juices (e.g. mycotoxins, biphenyls, polycyclic aromatic hydrocarbons, biogenic amines or pesticides), chromatographic techniques, both gas chromatography (GC) and liquid chromatography (LC), are most often used. Juices can contain pesticide residues in low concentrations, therefore selective and sensitive analytical methods are required to detect these contaminants. GC with various types of detectors and various methods of analyte extraction is most often used for the determination of pesticides in fruit juices. The combination of the matrix solid-phase dispersion (MSPD) method with gas chromatography coupled with electron capture detection and nitrogen-phosphorus detection (GC-ECD/NPD) enabled the determination of 160 analytes in blueberry juices [51]. One of the solvents required for the extraction of pesticides was hexane, which was a major disadvantage of the proposed approach. A greener analytical solution was the use of continuous sample drop flow microextraction with gas chromatography coupled with mass spectrometry (CSDF-ME-GC-MS) [50]. The advantages of this methodology include high sensitivity, short analysis time and low solvent consumption. Liquid chromatography with UV detection was also used to determine pesticides in juices. During the sample preparation stage, ionic liquid-assisted liquid-liquid microextraction based on the solidification of floating organic droplets was used for analysis (ILSFOD-LLME) [52]. This methodology effectively concentrated the tested ingredients, and the solvent consumption was small, which significantly improved the assessment of the greenness of the methodology compared to conventional LLE techniques.

It should be emphasized that finding reliable, sensitive and fast methods for the detection of microbiological and chemical contaminations in fruit juices is of great importance for the assessment of food safety and quality. Information on the health benefits, as well as the risks associated with the consumption of berry juices, and analytical methodologies used to determine bioactive compounds and fruit juice contaminations, are described in a review publication, published in the journal *Food Chemistry* [60]. The work addresses the limitations of current analytical approaches as well as future trends, with a focus on green analytical chemistry.

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Review

An analytical approach to determine the health benefits and health risks of consuming berry juices

Magdalena Fabjanowicz[a,*], Anna Różańska[a], Nada S. Abdelwahab[b], Marina Pereira-Coelho[c], Isabel Cristina da Silva Haas[d], Luiz Augusto dos Santos Madureira[c], Justyna Płotka-Wasylka[a,e,*]

[a] Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, 11/12 Narutowicza Street, 80-233 Gdańsk, Poland
[b] Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt
[c] Departament of Chemistry, Federal University of Santa Catarina, Des. Vitor Lima Av., Trindade, 88040-900 Florianópolis, SC, Brazil
[d] Department of Food Science and Technology, Federal University of Santa Catarina, Admar Gonzaga Rd., 1346, Itacorubi, 88034-001 Florianópolis, SC, Brazil
[e] BioTechMed Center, Gdańsk University of Technology, 11/12 Narutowicza Street, 80-233 Gdańsk, Poland

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ABSTRACT

Food products composition analysis is a prerequisite for verification of product quality, fulfillment of regulatory enforcements, checking compliance with national and international food standards, contracting specifications, and nutrient labeling requirements and providing quality assurance for use of the product for the supplementation of other foods. These aspects also apply to the berry fruit and berry juice. It also must be noted that even though fruit juices are generally considered healthy, there are many risks associated with mishandling both fruits and juices themselves. The review gathers information related with the health benefits and risk associated with the consumption of berry fruit juices. Moreover, the focus was paid to the quality assurance of berry fruit juice. Thus, the analytical methods used for determination of compounds influencing the sensory and nutritional characteristics of fruit juice as well as potential contaminants or adulterations.

1. Introduction

Human nutrition science has developed in the last decades, turning from looking at foods as a simple source of energy to the appreciation of their role in maintaining health and in reducing disease risks. Nowadays, berry plants have become very attractive to the food industry, as it is a trend to prompt their application as replacements for synthetic nutraceuticals. In addition, the berry itself is often used to produce juice (Li et al., 2017). Due to the high content of polyphenols, antioxidants, and other bioactive compounds, berry fruit juices are often seen as one of the healthiest and the most nutritious beverages (Skrovankova et al., 2015). The mentioned groups of compounds are responsible for various health benefits, including cardiovascular diseases, prevention of inflammation disorders, or protective effects to lower the risk of various cancers (Fig. 1) (de Souza et al., 2014). Because of that general assumption, it is important to evaluate whether the amount of numerous bioactive ingredients is sufficient for them to be beneficial to human health. Moreover, it is important to assess the content of substances such as polyphenols, antioxidants, and vitamins in different juices, since their levels may differ depending not only on the type of fruit but also on its origin, processing, or storage (Arfaoui, 2021).

Even though berry fruit juices are generally considered healthy, there are many risks associated with mishandling both fruits and juices themselves (Abisso et al., 2018). It is important to collect information on the negative effects that microbial contamination, inappropriate storing condition, or the use of stale fruits may have on juices composition and safety of their consumption. While some of the risks are minimized in the case of commercially available berry juices, quality assessment is of particular importance in the case of homemade juices, since consumers are not bound to obey the same sanitary standards as food producers (Li et al., 2017).

One of the most overlooked problems associated with fruit juices, including berry fruit juices consumption is the possibility of their adulteration (Dasenaki & Thomaidis, 2019). To decrease the cost of juices production, the producer may mix in fruits that are not only cheaper but also might not have as many health benefits, as well as resorting to the addition of water. Moreover, since juices that are the richest in bioactive compounds are not always seen as the most

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* Corresponding authors at: Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, 11/12 Narutowicza Street, 80-233 Gdańsk, Poland (J. Płotka-Wasylka).

E-mail addresses: magdalenafabjanowicz@gmail.com (M. Fabjanowicz), juswasyl@pg.edu.pl, plotkajustyna@gmail.com (J. Płotka-Wasylka).

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palatable, additives are used to improve their sensory qualities leading to a further decrease of wholesomeness. Another important adulteration is the application of artificial flavors to mimic the natural aroma (Hrubá et al., 2021).

This work aims to assess the current status of analytical approaches to fruit juices analysis and quality assessment to identify both the current limitations and future trends. In the article, we critically review the up-to-date literature on fruit juices analysis, in particular on their quality assessment. The information is provided in a concise and approachable form, focusing on practical aspects and examples of implementation rather than on detailed technical aspects and principles of operation of the described methods. The focus is mainly placed on novel methods for fruit juices quality assessment in the context of green analytical chemistry, but diverse and interesting examples of studies that showcase the possibilities of future developments are presented. In addition, the practicality of the reviewed methods for end-users in and outside of analytical laboratories are given. Current trends and lines of future research are also discussed.

2. Health benefits related to consumption of berry juice fruit

The consumption of berry juice has been widely associated with a decreased risk of certain chronic diseases (Giampieri et al., 2015; Habanova et al., 2019). Several studies report that berry juice have different biological activities *in vitro* and *in vivo* systems, which are related to their bioactive composition (Bakuradze et al., 2019; Giampieri et al., 2015; Toaldo et al., 2015). These juices are excellent sources of vitamins, minerals, and phenolic compounds, especially phenolic acids and anthocyanins (Geraldi et al., 2021). The chemical composition of fruit juices depends especially on the fruit species, maturation, climate, and the treatments to which the fruit and the juices themselves are submitted. In addition, during cultivation, climatic conditions have a direct influence on the chemical quality and the polyphenolic complexity of the fruits (Coelho et al., 2021). The technology employed in juice production can provide different levels of extraction of bioactive compounds. The crushing step contributes to the extraction of phenolic compounds present in berries. It is interesting to note that during the fruit processing, some steps, such as freezing and thawing, can affect the extraction of some valuable components in the grinding and pressing steps, changing the phytochemical composition of the fruit juices (Weber & Larsen, 2017).

In addition, other processing steps affect the bioactive composition of juices, such as enzymatic treatment, filtration, clarification, and pasteurization. In enzymatic treatment, pectinolytic enzymes are used to increase productivity of juice. The use of the pectinase enzyme causes pectin degradation, which results in reduced juice viscosity and changes in physicochemical properties, such as total soluble solids, pH, and turbidity (Marsol-Vall et al., 2019). Filtering or clarification is carried out before or after pasteurization. The heat treatment (pasteurization) to which the fruit juices are subjected can also cause reductions in the content of vitamins and polyphenols (Stübler et al., 2020), however, pasteurization is still conventionally used as a procedure to preserve juices from microbial contamination (Marsol-Vall et al., 2019). Martino et al. (2013) report that polyphenol retention and antioxidant activity were significantly higher in grape juice clarified after thermal processing (pasteurization) compared to grape juice clarified before pasteurization. In the same way as pasteurization due to the use of high processing temperatures, the evaporation technique used to concentrate fruit juices can reduce the nutritional value and bioactive properties of the product (Amran & Jusoh, 2016). In contrast, low temperatures are employed in the membrane separation technique used to concentrate fruit juices preserving the most thermosensitive compounds, especially vitamins and polyphenols (Bhattacharjee et al., 2017). In addition to the reported steps, storage conditions such as exposure to heat and light have an important influence on polyphenol retention. During storage, the content of monomeric anthocyanins decreases leading to the polymerization of anthocyanins into more stable compounds (Marsol-Vall et al., 2019)..

Knowledge about the processes mentioned above is of paramount importance for the elucidation of the chemical composition of wild fruit juices and the correct correlation with the health benefits promoted by the regular consumption of these bevereges, since the concentration of vitamins, minerals and bioactive compounds directly depends on the processes to which the fruits and juices are submitted. Clinical and preclinical studies have shown that berry fruits and their juices can act as potential therapeutic agents in the prevention of various pathologies, such as diabetes, neurodegenerative and cardiovascular diseases, and cancer (Wang et al., 2021; Yang and Kortesniemi, 2015). The literature studies showed that berry fruits and their juices, alone or in combination with other functional foods or dietary interventions, can improve glycemic and lipid profiles, blood pressure, and surrogate markers of atherosclerosis (Calvano et al., 2019).

Berry fruits contain large amounts of essential and physiologically important macroelements such as P, Mg, K, and Na (Szymczycha-Madeja et al., 2014). As well as some microelements such as Ca and Fe (Toaldo et al., 2015). Both macro and microelements play an important role in

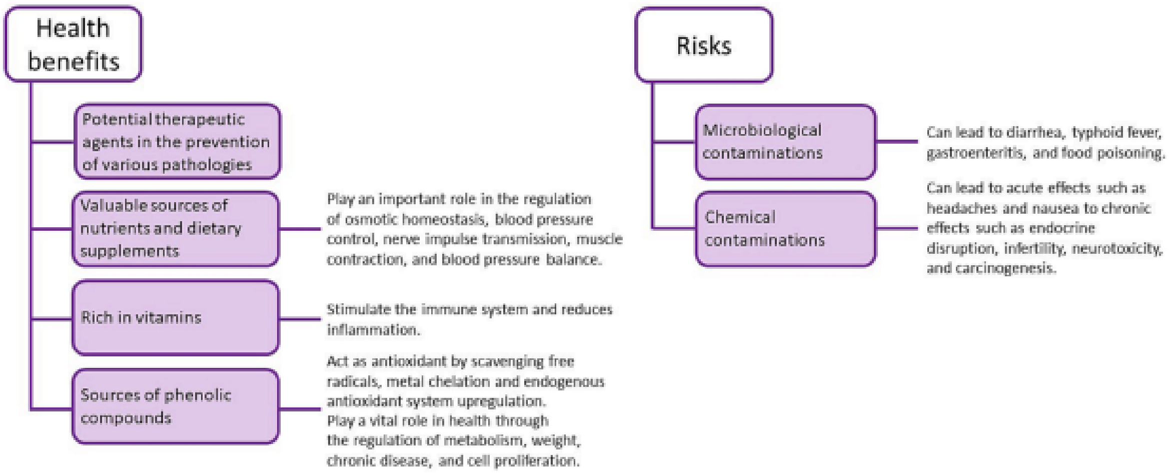

Fig. 1. Health benefits and risks related to the consumption of berry fruit juices.

the regulation of osmotic homeostasis, blood pressure control, nerve impulse transmission, muscle contraction, and blood pressure balance (Gharibzahedi & Jafari, 2017). The daily consumption of fruit juices can be a part of the recommended daily doses of some nutritionally important elements, such as Co, Cr, Cu, Fe, Mn, Ni, Zn, and Se (Szymczycha-Madeja et al., 2014).

The berry fruit juices are also rich in vitamins A, C, and E, and vitamins of the B complex, which are essential for health, as their consumption stimulates the immune system and reduces inflammation (Skrovankova et al., 2015). Since inflammation plays a key role in the development of diabetes, asthma, cardiovascular disease, and cancer, the consumption of appropriate amounts of the above-mentioned vitamins reduces the risk of those diseases (Maleki et al., 2019). Trych et al. (2020) reported that black currant contains approximately 160–285 mg/100 g of vitamin C. Zheng et al. (2009) reported vitamin C contents of 60–190 mg/100 mL in blackcurrant juices. Sapei & Hwa (2014) reported that the ascorbic acid content of fresh strawberry juices ranged from 20 to 40 mg/100 mL. The consumption of vitamin C is associated with several health benefits, as it has anti-inflammatory, antibacterial, and neuroprotective action.

We also highlight that in addition to the composition of minerals and vitamins, wild fruit juices are excellent sources of phenolic compounds, especially anthocyanins and phenolic acids (Bakuradze et al., 2019). It is known that polyphenols present in fruits and vegetables exert beneficial biological activities to the human body when consumed regularly due to their antioxidant, cardioprotective, anti-inflammatory, and neuroprotective activities. It is known that food matrix in which given compounds are present is important factor determining its release and stability while digested in a human body. To become bioavailable and subsequently bioaccessible polyphenols must be removed from the digested matrix and solubilized in the gastrointestinal fluids. Therefore, it is important to say that phenolic compounds exert their health-related properties when they reach the target tissue of the human body in biologically active concentrations (da Silva Haas et al., 2019). Anthocyanins are the most important flavonoids present in berry fruits and contribute to their high antioxidant capacity. These compounds are responsible for the flavor and the red color of the fruits (Cortez et al., 2017).

The small and aromatic berries of blackcurrant (*Ribes nigrum*) are rich in anthocyanins (delphinidin 3-glucoside, delphinidin 3-rutinoside, cyanidin 3-glucoside, and cyanidin 3-rutinoside) (Tian et al, 2023), and its consumption inhibits the activities of the dipeptidyl peptidase-enzymes IV, α-amylase, α-glucosidase, nitric oxide synthase, and cyclooxygenase-2 which are biochemical markers of type 2 diabetes and inflammation (Kowalski & Gonzalez de Mejia, 2021). Other health benefits were reported by Cortez and Gonzalez De Mejia (2019), such as improved cardiovascular, nervous, ocular, skeletal, skin, and renal systems (Cortez and Gonzalez De Mejia (2019)).

The blueberry juice (*Vaccinium ashei*) also has high concentrations of anthocyanins, such as cyanidin-3-glycoside, peonidin-3-glycoside, malvidin-3-glycoside, malvidin-3-galactoside, and malvidin-3-arabinoside (Wu et al., 2021). Yang and Kortesniemi (2015), reported an inverse association between anthocyanin intake and the incidence of chronic disease. Recent research shows that flavonoids can inhibit regulatory enzymes or transcription factors important for the control of mediators involved in inflammation, in addition to attenuating tissue damage and fibrosis (Maleki et al., 2019).

Strawberry juice (*Fragaria X ananassa, Duch.*) is one of the berry juices that has been gaining interest for its positive effect on health due to its polyphenol composition, with emphasis on phenolic acids and anthocyanins. Among the acids, ellagic acid stands out, it is a dimeric condensation product of gallic acid and is found naturally in strawberries, raspberries, and blackberries and has important anticancer, antithrombotic, and anti-inflammatory properties (Muthukumaran, Tranchant, Shi, Ye, & Xue, 2014). In addition, strawberries are rich in anthocyanins (pelargonidin-3-glucoside, cyanidin 3-glycoside, and pelargonidin 3-rutinoside) that promote benefits to human health, as they can regulate gene expression and prevent DNA damage (Giampieri et al., 2015). Preclinical and clinical investigations support the role of anthocyanins in ocular health, these polyphenols have been associated with several benefits pertinent to neurodegeneration. The anthocyanins allow the reduction of induced oxidative stress, decreasing the levels of reactive oxygen species, and malondialdehyde and increasing the levels of superoxide dismutase, catalase, and glutathione peroxidase in the pigment epithelium of the human retina (Huang et al., 2018). According to McNamara et al. (2018) supplementation with blueberry powder generated an improvement in the cognitive function of elderly people with subjective cognitive impairment, supposedly derived from a vasomodulatory effect.

On the other hand, the acute consumption of grape juice (*Vitis labrusca* L.) rich in catechin, epicatechin, *trans*-resveratrol, and anthocyanins (cyanidin 3,5-diglucoside and malvidin 3,5-diglucoside) increases the levels of antioxidants in plasma and erythrocytes in healthy individuals, reducing the lipid peroxidation (Toaldo et al., 2016). Grape juice consumption may render additional benefits for healthy adults who exercise regularly. Grape juice had great potential as an antioxidant source in improving the antioxidant status and cardiometabolic profile of healthy adults. Catechin, isoquercetin, and procyanidin B1 were the major compounds in grape juice from cultivars Isabel, Bordo, and Concord. The plasma antioxidant activity and HDL-cholesterol increased after grape juice intake, and LDL-cholesterol and systolic blood pressure decreased after grape juice consumption (Toscano et al, 2017). Renaud and De Lorgeril proposed in 1992 the French paradox which states that the consumption of polyphenols is associated with a low incidence of heart and coronary disease, despite a high-fat diet. It is noteworthy that the understanding of the "French Paradox" has stimulated the interest of further research to investigate whether polyphenols may offer protective effects beyond the cardiovascular system and whether different botanical sources may also offer beneficial effects on human health (Sun et al., 2002).

With regards to the stability of phenolic compounds, they are susceptible to several structural changes during gastrointestinal digestion, and among polyphenols, phenolic acids seem to be the most resistant compounds, being the most relevant to explain the biological activity of foods (Corrêa et al., 2017; Lingua et al., 2019). Chlorogenic and protocatechuic acids are the major phenolic acids in blueberry juice (*Vaccinium ashei*) (Wu et al., 2020) and blackberry juice (*Rubus americanus*) (Wu et al., 2021). It is known that polyphenols act in the prevention of oxidative stress, inhibiting inflammation and improving vascular health (Sinopoli et al., 2019). According to Yang and Kortesniemi (2015), regular consumption of polyphenol-rich fruit juices improves the postprandial glycemic response and the profile of circulating inflammatory markers, in addition to increasing plasma antioxidant capacity and delaying the loss of related cognitive functions the age.

Growing evidence suggests that wild fruit consumption has significant potential in preventing and treating most risks associated with a metabolic syndrome like diabetes mellitus (Hameed et al. 2020, Vendrame et al. 2016). This is probably due to the presence of polyphenols with known antioxidant and anti-inflammatory effects, such as phenolic acids and anthocyanins. In offering efficient and secure dietary therapies for diabetes mellitus prevention and control, tailored berries nutrition is compared to an individual pharmaceutical strategy (Hameed et al., 2020). In this context, the use of analytical methods to determine the bioactive composition of wild fruit juices is extremely important for the beverage industry, as this way, it is possible to report their nutritional potential and possible health benefits. We emphasize that to elucidate different perspectives on the nutritional potential of food, it is necessary to consider the effect of digestion, absorption, and metabolization of the bioactive compounds in the human body (Attri et al., 2017; Velderrain-Rodríguez et al., 2014).

3. Risks associated with consumption of fruit juice

The insightful evaluation of the quality of berry fruit juices is of great importance for consumer safety. Although the consumption of fruit juices generally has several positive health effects, it is notorious to emphasize the possible risks associated with microbiological contaminations, characterized by the presence of pathogenic microorganisms, such as bacteria, viruses, and fungi, and chemical contaminations, which mainly include the presence of pesticides, mycotoxins, illegal additives, metals, bisphenols, and organic pollutants (Mostafidi et al., 2020).

Quality inspection of fruit juices is governed by different bodies in their respective countries and regions. The European Fruit Juice Association Code of Practice (AIJN COP) brings together a collection of reference guidelines for fruits and vegetables. The AIJN COP contains parameters that a fruit or vegetable juice needs to meet in the European market, establishing criteria for evaluating juices with respect to quality, authenticity, and identity. In addition to the AIJN COP, other bodies establish drink quality criteria, such as the Polish Association of Juice Producers (KUPS) in Poland and the Food and Drug Administration (FDA) in the United States. It should be noted that the SDS requires the application of Hazard Analysis Critical Control Point (HACCP) principles that aim to ensure the safe and sanitary processing of fruit and vegetable juices.

3.1. *Microbiological contaminations*

3.1.1. *Bacteria*

The contamination of fruit juices by bacteria, these occur in places with inadequate facilities and a lack of hygienic-sanitary standards, mainly due to the lack of care when handling the fruits used during juice preparation (Nawawee et al., 2019). The bacteria *Escherichia coli, Salmonella typhi, Pseudomonas spp, Staphylococcus aureus,* and *Vibrio cholerae* are the most common in fruit juices and the consumption of drinks contaminated by these pathogens can lead to diarrhea, typhoid fever, gastroenteritis, and food poisoning (Sharma et al., 2020).

3.1.2. *Fungi*

The presence of fungi in fruit juices is an important factor to be considered since fungi are capable of producing mycotoxins, which are toxic secondary metabolites that pose a potential risk to human health (Fliszár-Nyúl et al., 2020). Recent studies have shown that a wide variety of small berries, such as strawberries, blueberries, mulberries, blackcurrants, and raspberries, due to their soft and fragile skin, are susceptible to small lesions that allow the growth of fungi, especially molds. Once present in the fruit, fungi can resist heat treatments used in the processing of fruit juices and persist in the product. In addition, products stored at room temperature are more prone to the occurrence of fungi (Jackson & Al-Taher, 2008). The main fungi found in fruits belong to the genera *Aspergillus, Penicillium,* and *Alternariae* (Guo et al., 2021).

3.1.3. *Virus*

The norovirus or hepatitis A virus is one of the most common viruses in fruit derivatives, being recognized as the cause of gastroenteritis and hepatitis in humans. Virus contamination of fruit juices is mainly related to the contamination that occurs during the various stages of raw material (fruits) production, including production, harvesting, processing, and distribution (Takahashi et al., 2018). A significant source of viruses can result from water contaminated with the viruses that are used for irrigation during planting, as it is not practical for all fruit farms to use potable water. Furthermore, bacterial indicators employed for water quality control generally cannot predict viral contamination, giving negative results for indicator tests. In addition to water, viral contamination can occur through the hands of fruit handlers without proper hygiene (Maunula et al., 2013).

3.2. *Chemical contamination*

Considering the quality and the safety of fruit juices, there is a growing interest in evaluating potential chemical contaminants such as pesticides, bisphenols or metals, as well as the presence of adulterants. Chemical contaminants present in fruit juices are usually derived from the fruit itself, which during crop production, post-harvest, and additional processes are exposed to contaminants like pesticides, metals, etc. In addition, packaging can be a potential source of chemical contaminants (Mostafidi et al., 2020).

3.2.1. *Pesticides*

In recent years agriculture has advanced rapidly and intensified the use of a huge amount of chemical inputs, mainly synthetic pesticides that play an important role in the protection of several cultivars, including pest control and disease prevention (Heidari et al., 2020). Pesticides belong to different chemical groups such as carbamates, neonicotinoids, organochlorines, organophosphorus, phenoxyacids, pyrethroids, strobilurins, triazines, triazoles etc.) and have two different modes of action (contact or systemic). Pesticides with contact action accumulate on/in the plant layer, while pesticides with systemic action penetrate deeper into plant tissues, and were definitely more difficult to reduce during juice production (Jankowska, Łozowicka, & Kaczyński, 2019). It was proven that after squeezing of blackcurrants, the pesticide residue levels in the juice were lower by more than 50% compared to raw fruits. Moreover, the contact pesticides remained on the peel and minimally penetrated into the juice (18% compared to the raw fruits) (Jankowska et al., 2019). The beverage industry is the fastest-growing food sector worldwide, especially the production of fruit juices (Food And Beverages Global Market Report 2022- *Product Image* Food And Beverages Global Market Report 2022, 2022). On the other side, in the latest sector some problems can be pointed out, especially, the application of enormous doses of pesticides used to increase productivity and product quality. As a result, from the use in agricultural activities, residual pesticides can ultimately be found in the human diet, since if they are not naturally degraded, they can penetrate plant tissues and be found in the fruit pulp and later in the juices. In addition, the use of techniques to concentrate processed juices can promote an increase in the pesticide content in the final product when compared to fruits (Jin et al., 2012).

Prolonged exposure to pesticides can generate several negative health effects, from acute effects such as headaches and nausea to chronic effects such as endocrine disruption, infertility, neurotoxicity, and carcinogenesis (de-Assis et al., 2020). Within this context, it is important to emphasize that pesticides are regulated to ensure that pesticide residues in food do not pose a risk to human health. Thus, commercialized pesticides are authorized after an intensive assessment of possible health and environmental risks (Torović et al., 2021). The maximum legally permitted concentrations of pesticide residues in specific foods, including fruit juices, are regulated by the European Union (CE/n°299/2008), but also the United States Environmental Protection Agency sets such limits. Furthermore, efforts to minimize the impact of pesticide residues can be adopted through the implementation of practices such as the rational use of pesticides, exploitation of natural pesticides, promotion of organic agriculture, and adequate application intervals (Mostafidi et al., 2020).

3.2.2. *Metals*

Fruit juices are an important source of minerals, which are essential for maintaining health (Caswell, 2009). However, in addition to the presence of elements beneficial to health, juices can contain harmful metals such as Hg, Sn, As, and Cd that can trigger serious problems for human health, even at low concentration levels. The consumption of metals through fruit juices can cause chronic diseases or mutagenesis and carcinogenesis (Bhattacharya et al., 2016). In addition to the risk of these elements to human health, the presence of some metals in excess, like Fe and Cu can reduce the shelf life of foods or possibly decrease the nutritional value of juices, since these metals are responsible for catalyzing oxidative processes, through free radicals oxidative deterioration (Mohamed et al., 2020).

The presence of unwanted metals in juices can come from the packaging or from the fruit itself. Juices stored in aluminium containers can be contaminated by the metal through leaching processes. Al (III) is highly toxic to humans due to the potential accumulation in the brain that can trigger Parkinson's and Alzheimer's disease (Hafez et al., 2019). The metals derived from fruits are commonly related to the mineral composition of the planting soil, agricultural practices with abusive use of pesticides or contaminants transported by air or water, in this sense, it is extremely important to regularly monitor the dietary intake of food sources to ensure safe food. In addition to ensuring the nutritional value of food (Anastácio et al., 2018).

3.2.3. Biogenic amines

Biogenic amines (BAs) are aliphatic or aromatic organic compounds of low molecular weights. They are generated during cellular metabolism in bacteria, plants, and animals due to microbial decarboxylation of the corresponding amino acids. The amount and kind of BAs produced are influenced significantly by the food composition, and factors that allow bacteria to flourish during food processing and storage (Gomez-Gomez et al., 2018). Low quantities of BAs in food are not thought to be dangerous, but they may have toxic effects when eaten in large doses. Their analysis in food samples is of tremendous interest not just because of their potential toxicity, but also because they can be utilized as indications of food freshness or rotting (Saaid et al., 2009). Many works have been published according to the determination of BAs in berry juice samples (Sub-section 4.2.2).

3.2.4. Adulteration

The adulteration of beverages is commonly related to dilution practices, the addition of artificial flavors to mimic the natural aroma, and the addition of flavor masking to alter specific characteristics, such as reducing or eliminating unpleasant flavors such, as bitterness. In addition, the addition of different chemical mixtures capable of masking themselves so that adulteration is not perceived also constitutes adulteration. As a result of adulterations, a lower nutritional value is expected of beverages (Xu et al., 2019).

Some examples found in the literature on fruit juice adulteration include the adulteration of grape juice with the addition of fruit juices of lesser commercial value. A good example is the addition of apple juice to whole grape juice. Apples are rich in pectin, which acts as a gelling and natural thickening agent that prevents the separation of the juice phases. Moreover, the addition of apple juice masks other adulterations, including the addition of water and other additives (Oliveira et al., 2019). Similarly, orange juice (*Citrus sinensis*), consumed worldwide, can suffer adulteration by the addition of *Citrus reticulata* (mandarins and tangerines), *Citrus aurantium* (sour orange), tangors or hybrids of sweet orange, and tangerine (Jandrić et al., 2017).

Beyond the addition of other fruit juices, one of the artificial ingredients often added is glucose-fructose syrup (Europe) or high fructose corn syrup (United States). These syrups are added as an alternative to sucrose due to their viscosity, which contributes to preventing crystallization and having a lower cost than sucrose (Wójcik & Jakubowska, 2021). Both components are associated with obesity risks when consumed in excess (Yu et al., 2013, Süli et al., 2017). Besides that, corn syrup may have levels of trace mercury resulting from syrup production technology (Wójcik & Jakubowska, 2021).

The authenticity of juices is verified by basic analytical information, such as Brix or total acidity, besides biomolecular approaches, and isotopic analysis. Also important is the application of analytical methods that assess the chemical profile of fruit juices including the quantification of sugars, anthocyanins, organic acids, carotenoids, and amino acids. Although these options are usually applied, there is a need for fast and accurate analysis methods to determine the presence of possible adulterants in fruit juices (Wójcik & Jakubowska, 2021).

3.2.5. Mycotoxins

The mycotoxins are secondary metabolites of mold and fungi, which even in low concentrations are harmful to humans. The main fungi in fruits belong to the genera *Aspergillus*, *Penicillium* and *Alternariae* and give rise to a wide range of mycotoxins, including aflatoxins, produced by *Aspergillus*, ochratoxins produced by *Aspergillus* and *Penicillium*, while citrinin and patulin are mycotoxins produced by *Penicillium*. And finally, *Alternaria* toxins are produced by *Alternariae* fungi (Guo et al., 2021).

Mycotoxins can be transferred from the fruit to the juice if the spoiled fruit is not discarded during the beverage making process. Therefore, quality control of the raw material (fruits) is essential to prevent mycotoxin contamination in fruit juices (Gil-Serna & Patiño, 2020). Prevention strategies, both during agricultural production and in beverage production, have proven to be good alternatives to inhibit mycotoxin biosynthesis, including care during the harvest, such as field management, use of biological and chemical agents, types of residence, and post-harvest care, which include improved drying, decontamination processes and care with storage conditions (Mostafidi et al., 2020). It is important to emphasize that, the occurrence of mycotoxins depends on several factors, including, the composition of the food matrix, moisture content, temperature, pH, relative humidity, and physical damage (Pallarés et al., 2021).

The great concern with mycotoxin contamination is related to adverse health effects since chronic exposure to these substances can result in neurotoxic, immunological, mutagenic, genotoxic, carcinogenic, and teratogenic problems (Guo et al., 2021, Marin et al., 2013). The Codex Alimentarius, the European Union, and countries such as the United States, Canada, and China established maximum levels for some mycotoxins in fruit juices, with a maximum of 50 μg kg^{-1} for patulin and 2 μg kg^{-1} for ochratoxin A (Guo et al., 2021).

3.2.6. Bisphenols

Bisphenols are a class of anthropogenic chemical substances widely used as modifier monomers in plastic production to improve material properties, including greater flexibility and strength (Hafez et al., 2019). A total of seventeen bisphenols have been documented for industrial applications, including bisphenol A, bisphenol B, bisphenol F, bisphenol AF and tetrabromobisphenol A. Among them, bisphenol A (BPA) is the most widely applied in plastic production, including the production of food packaging and beverage packaging. Due to its low production cost, high thermal and chemical stability, BPA is widely applied as a raw material (Khan et al., 2021).

Incomplete polymerization processes or polymer degradation can easily result in the migration of bisphenols from packaging to food and beverages during prolonged storage and at elevated temperatures (D. Yang et al., 2018). Exposure to these compounds poses a potential risk to human health since bisphenols are classified as endocrine disruptors, with a negative effect on the hormonal system. Furthermore, studies indicate that BPA can cause diseases related to the cardiovascular, metabolic, and immune systems, as well as diabetes (Hafez et al., 2019).

3.2.7. Pollutants

Fruit juices can contain trace-level contaminants belonging to different classes of organic pollutants, including polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). The PCBs are a group of synthetic organic compounds considered to be persistent organic pollutants in the environment. They are highly lipophilic compounds and for this reason, they are found in concentrations in the range of ng mL^{-1} in aqueous samples, such as fruit juices (Abujaber et al., 2019). Exposure to PCBs is associated with adverse neurobehavioral problems, endocrine disturbances, and immunological effects (Darvishnejad & Ebrahimzadeh, 2019).

The PAHs, in turn, constitute one of the largest groups of contaminants present in different matrices, including food. These compounds are derived from the incomplete combustion of organic matter and can come from natural and anthropogenic processes such as pollution, food processing, packaging, and thermal procedures such as cooking. PAHs are often found in different beverages, including fruit juices. The great concern for the scientific community and the food industry is related to harmful health properties, including carcinogenic and mutagenic activities (Rascón et al., 2018).

4. Application of analytical techniques

Food is a complex heterogeneous mixture of a wide range of chemical constituents as well as a wide array of additives and contaminants. Product composition analysis is a prerequisite for verification of product quality, fulfilment of regulatory enforcements, checking compliance with national and international food standards, contracting specifications and nutrient labelling requirements and providing quality assurance for the use of the product for the supplementation of other foods (Kumar & Gowda, 2014). These aspects also apply to the berry fruit which intends to be the sub-product for the production of juice. It also must be noted that even though fruit juices are generally considered healthy, there are many risks associated with mishandling both fruits and juices themselves (Abisso et al., 2018).

As was previously mentioned, it is important to collect information on both, the positive and negative effects connected with berry fruits and juices. While some of risks are minimized in the case of commercially available juices, quality assessment is of particular importance in the case of homemade juices, since consumers are not bound to obey the same sanitary standards as food producers (Li et al., 2017). And here, analytical chemists are coming with the specific practical knowledge of how to use analytical techniques for quality control of berry fruit juice. In this chapter, the specific techniques are described with examples of their application in the analysis of berry fruit juice. The focus is mainly placed on the novel methods for fruit juices quality assessment in the context of green analytical chemistry, but also we give diverse and interesting examples of studies that show the possibilities of future developments.

4.1. Benefits assessment

4.1.1. Spectroscopic techniques

Spectroscopic techniques are a useful analytical platform for any food screening because their application is rapid, mobile, and, in the case of some techniques, non-destructive. Spectroscopy is a popular technique commonly used at the stage of preliminary research to determine the summary parameters (Boqué & Giussani, 2021).

The spectrophotometric technique was applied for the evaluation of the effect of total phenolic concentration on the flavor of blue berry juice. The total phenolic content of blueberry juices from different cultivars was determined using the Folin-Ciocalteu method. The method showed good sensitivity (0.05–0.5 g/L) (Bett-Garber et al., 2015). The Folin-Ciocalteu method is widely applied for the analysis of total phenolic compounds, however, this method has limitations, as the Folin-Ciocalteu reagent is not specific for phenolic compounds and may also react with other oxidizable compounds present in the sample, including ascorbic acid, amino acids, sugars, ferrous ions, among others, thus, the total polyphenol content can be overestimated (Granato et al., 2016). Furthermore, the Folin-Ciocalteu method does not allow the quantification of individual phenolic compounds. Therefore, it is not possible to correlate specific compounds and their individual properties (Martins et al., 2022). Bett-Garber et al. (2015) related sensory analyzes together with physical–chemical analyzes and concluded that berries from different cultivars showed variability in their aroma and flavor. However, it was noticed that polyphenols had no significant effect on the bitter and astringent taste of berries but higher polyphenols concentrations contributed to more intense sweet taste (Bett-Garber et al., 2015). This work presents that the impact of juice composition on flavor is very complicated, and in fact, the estimating flavor with physicochemical parameters is a difficult task due to the composition of the juice. In addition to the phenolic composition, other factors can affect the flavor of fruits and juices, including natural sugars and organic acids. Furthermore, bitterness can be especially influenced by the presence of iridoids. Iridoids are monoterpenoids synthesized naturally in different plants and are characterized by a very bitter taste. However, it should be noted that iridoids present in plants have diverse biological activities, such as anti-inflammatory, antioxidative, anti-cancer, etc (Oszmiański & Kucharska, 2018). In the work presented by Tolić et al. (2017), effects of weather conditions on fruit quality attributes, phenolic compounds and antioxidant capacity of selected chokeberry juice over three consecutive years were investigated. Total phenols were determined by Folin-Ciocalteu method, while the pH differential spectroscopic method was used for total anthocyanins determination. Although quality parameters and phenolic composition vary over growing seasons, chokeberry juices from all three seasons have very high contents of phenolic substances and high values of antioxidant properties. This allows to state that weather conditions affect the concentration of antioxidative compounds. In addition, the results presented in this study showed that chokeberry juices characterized by high phenolic compound values had also high antioxidant activity. This is why it can be deduced that due to the high proportion of natural antioxidants their consumption could bring health benefits.

As the measurement of total anthocyanin value along with polymeric colour can be very useful to assess the quality of colour of anthocyanin-containing juices during heating, the knowledge on kinetic of anthocyanins degradation in specific temperature is required. Such research was performed by Danışman et al. (2016). The kinetic degradation of anthocyanins in grape juices was studied in the temperature range of 70-90C. The absorbance of diluted.

grape juice samples in buffers at pH 1.0 and 4.5 were measured at 520 nm (λmax) and 700 nm using an UV–Vis spectrophotometer. The method had simple sample preparation steps and an acceptable LOD value. In addition, the formation kinetics of percent polymeric colour (% PC) was also studied by application of the bisulphite bleaching method. High correlations were found between anthocyanin degradation and % PC formation during heating. The obtained results allow to state that due to the fact that the heat treatment had a significant effect on monomeric anthocyanins and polymeric colour, it should be carefully optimised to decrease the anthocyanin losses and polymeric colour formation in the commercial processing of grapes into juice.

4.1.2. Chromatographic techniques

Many properties as well as content of specific important compounds can be determine by application of different chromatographic methods. Such analysis allow to estimate and evaluate the quality of different berries. Furthermore, the application of chromatographic analyses is recommended for identification and quantification of specific compounds, allowing the correct correlation of such compounds and their properties. In this chapter, the specific examples of such applications are presented.

As was previously mentioned berries are commonly consumed as juice, however, the juice-processing conditions could affect their bioactive compounds. This is why many researches are published to present the impact of pasteurization conditions on the bioactive compounds content. The effect of thermal treatment on the phenolic compounds, anthocyanins and ellagitannins content as well as the antioxidant capacity of black berry was evaluated by the HPLC-PAD method (Azofeifa et al., 2015). With the use of the described method, it was possible to follow the concentration of phenolic compounds, anthocyanins and ellagitannins content. Neither of the two pasteurization conditions that were examined in this study significantly altered the concentrations of the total polyphenols or total/individual ellagitannins compared to those in the non-pasteurized juice. On the other hand, the concentration of anthocyanins significantly decreased. Over and above, the pasteurized juice was found to inhibit the peroxidation as well as non-pasteurized juice.

Another chromatographic method was used for analysis and comparison of the phenolic composition, anthocyanins, and antioxidant capacity in blackcurrant juice from ten different cultivars (Kowalski & Gonzalez de Mejia, 2021). In this work, the ultra-high performance liquid chromatography (UHPLC) method was used after sample extraction from freeze-dried black currant juice with methanol acidified with acetic acid. This method has the main advantage over the HPLC method of having shorter analysis time and hence, less solvents consumption (Azofeifa et al., 2015). It was found that anthocyanins content varied in the collected samples, moreover, anthocyanins were found predominantly in the skins of the fruit. In general, the findings of this study clearly indicate that the juices obtained from different blackcurrant cultivars differ with respect to a number of characteristics of interest.

In another work, folate vitamers and total folate in different berries were estimated using UHPLC-MS/MS (Zou et al., 2019). In addition, the changes in their concentration during handling of berries into juice were examined. In this method, a simple extraction method (boiling and centrifugation) was used followed by solid-phase extraction for further purification. According to the obtained results, the overall folate yield in the juicing fractions differed amongst berries (strawberries and blackberries had the highest total folate values (93–118 g/100g), whereas blueberries had the lowest total folate contents). The total folates in all tested were raised by 7 to 12 % after juicing which may be due to excessive release of folates from the fruit matrix during processing. In general, it can be concluded, that most of the investigated berries are good to excellent folate sources.

In case of red fruit juices, a selected method for quality and authentication is International Federation of Fruit-Juice Producers (IFU) Method No. 71 (1998), which allows to determine anthocyanin profiles by HPLC with visible detection. Despite the fact that the principle of the method is simple and the specific compounds of red fruit juice matrices or adulterations can be detected, correct interpretations of chromatograms are not as easy as expected (Obón et al., 2011), this is why the method is many often modified. Such modification was applied in the research performed in order to determine the composition of selected red fruit and vegetable juices and to evaluate their quality and authenticity. Profiles of anthocyanins, betacyanins, synthetic red pigments, hydroxycinnamic acids, hydroxybenzoic acids and catechins in these fruits were studied with the use of the HPLC-UV–VIS or HPLC-fluorescence detector methods (Obón et al., 2011). The method succeeded to separate all the studied components in 46 min analysis time, hence it can be considered as a useful technique for quality and authenticity control of fruit based products and for the detection of fraudulent mixtures with synthetic or natural red food pigments. On the other hand, as health claims of the red drinks can be related to their polyphenol contents, this method could be applied within the juice industry to label the content of components with potential health benefits.

The outcome of enzymatic processing on blackcurrant juices was studied through analysis of anthocyanins, flavonol glycosides, hydroxycinnamic acids, sugars, and acids content (Laaksonen et al., 2014). Analysis of anthocyanins, flavonol glycosides, hydroxycinnamic acids was done through developing an HPLC/DAD method, while sugars and acids were evaluated by GC/FID after derivatization with trimethylsilyl (TMS) derivatizing agent. The results show that the enzyme-aided juices were more astringent and bitter than the non-enzymatic juices. The reason was connected with lower contents of sugars, higher contents of phenolic compounds, and lower pH and sugar/acid ratio. In general, the non-enzyme-aided juices obtained higher ranking in flavour, while the enzyme-aided juices received more points in odour parameter.

In addition to evaluate the content of specific bioactive compounds in berry juices, their stability in higher temperatures is also of high importance, and thus, researchers also are focused on this aspects. The stability of polyphenols (anthocyanins, flavanols and phenolic compounds) in chokeberry and blue berried honeysuckle juices previously subjected to one of two sterilization methods (traditional thermal method and sterilization using Enbiojet® Microwave Flow Pesterizer) was tested and compared. (Piasek et al., 2011). The chemical properties verified included determinations of anthocyanins and other polyphenols by HPLC-DAD-MS, however the profiles of antioxidants were obtained by application post-column derivatization. The concentration of phenolic acids and flavonoids (except anthocyanins) did not change significantly under the influence of microwave-assisted sterilization. Moreover, it was observed that using the EnbioJet device, the decrease in anthocyanin content was lower compared to the conventional thermal method, especially in the case of blue berried honeysuckle juice. The present results allow to state that sterilization with EnbioJet® Microwave Flow Pasteurizer is highly conservative as regards bioactive phytochemicals found in examined berry juices. This conclusion could be true for other plant preparations rich in bioactive phytochemicals.

Another aspect of the application of chromatographic techniques in the context of determining compounds that have beneficial effect on human health, is their use to assess authenticity or possible adulteration of selected berry juices. In the work (Zhang et al., 2018), a metabolomic approach for authentication of berry fruit juices by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) was established. In the untargeted metabolomics analysis, obtained data were subjected to chemometric analysis such as principal component analysis-discriminant analysis. In the targeted metabolomics analysis, the 41 juice biomarkers, such as flavonoids and anthocyanins provided to separate adulterated juices from berry fruit juices. In addition, adulterants have different flavonoid glycosylation patterns as well as noteworthy differences in phenolic acids. One can conclude that the introduced LC-QTOF-MS-based metabolomic method can be used as a powerful tool to verify the quality of berry fruit juices.

In addition, the use of HPLC system in determination of specific parameters, ion chromatography (IC) is also an option while speaking about cations and anions of selected compounds. Such technique coupled to suppressed conductivity detection was applied for simultaneous analysis of organic acids and inorganic anions in different fruit juices including blueberry and grape juices (Uzhel et al., 2021). In contrast to HPLC, a simple sample pre-treatment method was applied (only filtration through 0.45 Mm membrane filter and dilution). In this method, a novel hyperbranched anion exchanger was synthetized and successfully applied to provide the baseline separation of glycolic, acetic, formic, and lactic acids, which are not resolved to baseline with modern commercially available columns when purely aqueous eluents are used. The most important factor used to improve selectivity of stationary phase was the introduction of dicarboxylic aspartic acid into the internal part of positively charged hyperbranched phase. This solution allowed to separate the selected organic acids applying KOH as an eluent without adding traditional, organic solvents. The study show, that the main organic acid present in blueberry juice was found to be citric acid (77–87% of total acids content), which improves ketosis and prevents diabetes, followed by malic acid (protects from ischemic lesions and has a positive effect on myocardium) and quinic acids (4–11% of total acids), which can be metabolized to hippuric acid, which is a strong antibacterial agent. This novel ion exchanger can be used for the estimation of organic acid profiles in food quality control to detect its deterioration during storage or authenticity assessment.

4.2. *Risk assessment*

4.2.1. *Spectroscopic techniques*

In most of the published articles dedicated to the issue of metal content in fruit juices, an atomic absorption spectroscopy (AAS) (Abbasi et al., 2020; Anastácio et al., 2018; Okhravi et al., 2020; Sorouraddin et al., 2020) and atomic emission spectroscopy (AES) (Demir et al., 2020) were applied with different sample extraction methods.

Some researchers performed, a microwave-assisted digestion with either the combination of nitric acid and hydrogen peroxide (Anastácio et al., 2018) or nitric acid and perchloric acid (Abbasi et al., 2020) followed by analysis using AAS. Microwave-assisted digestion results in shorter digestion time and avoids loss of metals by volatilization. However, the latter showed lower sensitivity.

Moreover, Co and Ni were analysed in pomegranate juice with the application of a graphite furnace atomic absorption spectrometer after complexation with 8-hydroxyquinoline and liquid–liquid micro-extraction (Okhravi et al., 2020). The most outstanding advantage of a given technique was the use of nitrogen instead of toxic chlorinated solvents. The extraction was performed within seconds and LODs on the level of 0.36 μg/L for Ni(II) and 0.20 μg/L for Co(II) were achieved. Another study shows, a green deep eutectic solvent dispersive liquid liquid extraction method (DLLE) used to extract and preconcentrate metals from the grape juice samples (Sorouraddin et al., 2020) followed by their analysis using FAAS. The most important factor in the DLLE technique is the choice of a relevant extraction and dispersion solvent. Hence, extraction solvent must have a high affinity for analytes, low solubility, high sample stability, be a liquid under standard conditions and have low vapor pressure (Makoś et al., 2020). In the study, the deep eutectic solvent acted as both a complexing agent and an extraction solvent. Application of deep eutectic solvents instead of hazardous chlorinated organic solvents is more and more popular. It may be due to their physicochemical properties: viscosity, density, acidity, basicity, polarity and good extractability. They can be designed according to the needs. Moreover, they are biodegradable, non-flammable. Thus, their use is in line with the requirements of green analytical chemistry (Makoś et al., 2020).

Several researches intended to assess the quality of fruit juices, indicated the presence of some metals like Cr, Ni, Mn above their permissible limits established by Decree-Law 306/2007 from 27th August of Portuguese Legislation for drinking water, WHO (World Health Organization) and USEPA (United States Environmental Protection Agency)(Abbasi et al., 2020; Anastácio et al., 2018; Sorouraddin et al., 2020). Moreover, the health risks index (HRI) for some metals was evaluated. HRI was calculated as a proportion between the estimated daily intake of the metal and reference oral dose for each metal and the body weight. When HRI is below 1, the exposure to metal is considered as safe. However, the results showed HRI for Cd, Cr and Pb over 1, what signalize a danger for human health. Thus, the data confirms the importance of monitoring of metal ions in fruit juices. (Abbasi et al., 2020).

Another approach of metal determination in fruit juices and nectars were done by inductively coupled plasma optical emission spectrometry (ICP-OES) method after microwave-assisted digestion (Demir et al., 2020). This method is characterized by fast extraction without the need of using organic solvents. Additionally, it showed a high sensitivity for all analyzed metals.

4.2.2. *Chromatographic techniques*

Chromatographic techniques including GC and HPLC are widely applied techniques when it comes to monitor varietal organic contaminations in juice samples, as shown in Table 1. *Alternaria* mycotoxins in pomegranate fruit and juice samples were determined with the use of HPLC-DAD method (Myresiotis et al., 2015). In this method, samples were subjected to the QuEChERS-based extraction method using acetonitrile (ACN) as organic solvent. Moreover, ACN was also used as the organic modifier in the applied mobile phase what is a big drawback. Hence the single analysis lasts 35 min the ACN is consumed in large quantities per each sample. On the other hand, high sensitivity being able to detect at targeted analysis even trace amount of toxins (LODs less than 0.02 μg/mL) was achieved. PAHs is another group of compounds being a subject of the study when quality of berry fruit juices was discussed. Analysis of a given group of compounds is very challenging, because they are present in very low concentration, so they need a pre-concentration step as well as a very sensitive analytical method of analysis (Zhao et al., 2009). Analysis of eight PAHs in grape juice samples was performed by using dispersive liquid liquid microextraction coupled with high performance liquid chromatography with fluorescence detection (DLLME-HPLC-FLD). DLLME was based on ACN (as a dispersive solvent) and methylene chloride (as an extraction solvent). Despite using toxic organic solvents in sample extraction, this method of extraction had a high enrichment factor (enrichment factors ranged from 296 to 462) leading to a wide linear range and high sensitivity as well as low detection limit.

Another method was published for extraction and pre-concentration of twelve PAHs depending on using a vortex-assisted dispersive solid-phase microextraction (VA-d-μ-SPME) using ionic liquid-modified metal–organic frameworks (ILMIL-100(Fe)) followed by GC/FID (Nasrollahpour et al., 2017). This method has some advantages over the DLLME (Zhao et al., 2009), such as shorter extraction time (only one minute) and higher extraction efficiency (due to the use of ILMIL-100(Fe)). Combination of both (i.e., ionic liquid and MOF) lead to higher sorbent capacity. The developed GC method had high sensitivity (linearity range 0.02–200 ng/mL) and a short analysis time (15 min).

Other researchers proposed GC/FID method for determination of six organic esters. In this method, sample pretreatment and pre-concentration were performed using polycarbazole/ionic liquid fiber for HS-PME. The synthesized fiber was cheap and had a long lifetime. Moreover, the extraction method showed high efficiency, however the extraction time was equal to 40 min. The developed GC method showed a wide linear range, as shown in Table.1. Total time of the analysis was 26 min (Feng et al., 2015).

Furthermore, a highly sensitive GC–MS/MS method was published for analysis of twelve phthalic acid esters in grape juice (Rodríguez-Ramos et al., 2020). In this method, extraction and pre-concentration of the target esters were carried out by a modified QuEChERS method. Results of the validation of the extraction method showed high extraction recovery (75–115%) and good repeatability. The high sensitivity of the developed method facilitated its application for analysis of the cited analytes in grape juice samples and the results confirmed the presence of some of the studied esters at different concentration levels in some of the tested samples.

As was previously mentioned, BAs are a group of compounds that are important to be monitored due to many reasons. As the BAs are usually hydrophobic, poor chromophores, and their concentration is usually low in complicated matrices, their determination in food samples and beverages is a challenging analytical task. Chemical derivatization by different reagents like dansyl chloride (for both primary and secondary amines) (Gomez-Gomez et al., 2018; Saaid et al., 2009) and O-phthaldialdehyde (specific for primary amines) is commonly used to improve methods sensitivity (Kelly et al., 2010).

BAs in different juice samples were analyzed by HPLC methods after different sample pretreatment and derivatization (Gomez-Gomez et al., 2018; Kelly et al., 2010; Saaid et al., 2009). In the method presented by Gomez-Gomez et al. (2018), BAs and phenolic compounds in grape juice were analysed to evaluate their functional and nutritional quality. Samples were homogenized with perchloric acid followed by derivatization with dansyl chloride. As well as, liquid–liquid extraction with toluene was performed. Analysis of eight BAs by HPLC-UV with a mobile phases (A) 100% ACN and (B) 50% ACN was done within 25 min. While, phenolic compounds were analysed with the use of the UPLC-UV method applying mobile phases of aqueous phosphoric acid (0.85%) and ACN (100%). Principle component analysis was then carried out and results showed that a higher phenolic compound content may be linked to a higher BAs content. The discovered association also showed that some bacteria that synthesize BAs are becoming more active at high pH levels. Some microbes' metabolism is inhibited by low pH, which prevents the synthesis of BAs (Gomez-Gomez et al., 2018). HPLC-UV method was also applied to determine BAs in blackcurrant and red grape juices after dilution in 0.1 M HCl and aqueous extraction followed by derivatization with dansyl chloride (Saaid et al., 2009). The method

Table 1
Characterization of analytical methods applied for the metals, mycotoxins, PAHs, aromatic esters, pesticides, biogenic amines and furans determination in different fruit juices.

methodology

Reference	Analyte	Sample	Number of analytes	Sample preparation	Abbreviation of the analytical technique used	Parameters of the technique	Time of the analysis [min]	Concentration range	LOD	LOQ
(Anastácio et al., 2018)	Metals	Strawberry juice	5	Microwave-assisted digestion	GFAAS	Detection at 228.8, 357.9, 283.3, 279.5, and 299.44 nm for Cd, Cr, Mn, Pb, and Ni, respectively.	————	2.29-440.09 μg L^{-1}	————	0.31-3.65 μg L^{-1}
(Abbasi et al., 2020b)	Metals	Red grape juice, strawberry Jam, blackcurrant jam, strawberry canned fruit, and cherry canned fruits.	7	Digestion	FAAS	Detection at 228.8, 240.7, 357.9, 324.8, 248.3, 217, 213.9 nm for Cd, Co, Cr, Cu, Pb, and Zn, respectively.	————	0.08-37.85 mg/kg	4-10 μg L^{-1}	————
(Okhravi et al., 2020)	Metals	Pomegranate juice	2	Liquid nitrogen induced homogenous LLE	FAAS	A Shimadzu AA-6300 FAAS. The radiation sources were cobalt and nickel hollow cathode lamps. Detection wavelengths were 240.7 and 232.0 nm, respectively. Air/acetylene flame with flow rates of 15 and 2.3 L min^{-1}, respectively.	————	0.5–20 μg L^{-1} for Co1.0–30 μg L^{-1} for Ni	0.2-0.36 μg L^{-1}	0.5-0.8 μg L^{-1}
(Sorouraddin et al., 2020)	Metals	Grape juice	2	DES-DLLME	FAAS	A Shimadzu AA-6300 FAAS. The radiation sources were cobalt and nickel hollow cathode lamps. Detection wavelengths were 240.7 and 232.0 nm, respectively. Air/acetylene flame with flow rates of 15 and 2.3 L min^{-1}, respectively.	————	0.50-50 μg L^{-1} for Co0.80-50 μg L^{-1} for Ni	0.22-0.30 μg/L	0.50-0.80 μg/L
(Demir et al., 2020)	Metals	Cherry, pomegranate and grape juice.	21	Microwave-assisted digestion	ICP-OES	Perkin-Elmer Optima 2100 DV ICP-OES. Power of 1.45 kW, plasma flow of 15.0 L min^{-1}, the auxiliary flow of 0.8 L min^{-1}, and nebulizer flow of 1 L min^{-1}.	————	0.004-1080 mg L^{-1}	0.0001-0.0063 mg L^{-1}	0.0005-0.0209 mg L^{-1}
(Myresiotis et al., 2015)	Mycotoxins	Pomegranate fruits and juices.	3	QuEChERS based extraction	HPLC-DAD	Thermo Spectra SYSTEM HPLC-DAD. Stationary phase: Hypersil BDS-C18 column (250 × 4.6 mm, 5 μm). Mobile phase: eluent (A) water with 50 μL L^{-1} trifluoroacetic acid and eluent (B) acetonitrile	35	0.05-10 μg mL^{-1}	0.02 μg mL^{-1}	<0.066 μg mL^{-1}

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Table 1 (*continued*)

methodology										
						with 50 µL L^{-1} trifluoroacetic acid. Flow rate: 1 ml min^{-1} Injection volume: 20 µL. Elution: gradient program: 90 % A and 10 % B, reaching 50 % B after 25 min and 100 % B after 30 min. 100 % B was maintained for 1 min. Thereafter the gradient was returned to 10 % B in 1 min and allowed to equilibrate for 3 min before the next analysis. Temperature: 40 °C				
(Zhao et al., 2009)	PAHs	Grape juice	8	DLLME	LC-FLD	Agilent 1200 LC system equipped with FLD Stationary phase: A Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 5-µm particle size). Mobile phase: a mixture of methanol-water (75:25, v/v). Flow rate: 0.8 mL min^{-1}. Temperature: 40 °C. Detection: Fluorescence detection was carried out as follows: 0–20 min λ ex at 256 nm and λ em at 441 nm, 20–35 min λ ex at 270 nm and λ em at 390 nm, 35–55 min λ ex at 290 nm and λ em at 410 nm.	55	0.01-100 µg L^{-1}	0.001-0.01 µg L^{-1}	————
(Nasrollahpour et al., 2017)	PAHs	Grape juice	12	VA-d-µ-SPE	GC-FID	A Chrompack CP9001 gas chromatography. Stationary phase: CP-Sil 24CB capillary column (30 m × 0.25 mm ID with 0.25 µm). Temperature program: 40 °C hold for 3 min, increasing to 100 °C at 10 °C min^{-1} and directly to 180 °C at 20 °C min^{-1} then hold for 2 min.	15	0.02–200 ng mL^{-1}	2.0-5.5 ng L^{-1}	6.0-16.8 ng L^{-1}
(Feng et al., 2015)	Aromatic esters	Grape juice	6	IL based SPME	GC-FID	Chemical Instrument SP-6890 GC-FID Stationary phase: SE-54 capillary column (30 m × 0.25 mm × 0.25 µm,). Temperature program: 50 °C held for 3 min; then increased to 190 °C at the rate of 15 °C min^{-1}, to 210 °C at the	26	0.061-500 µg L^{-1}	15.3-61 ng L^{-1}	————

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Table 1 (*continued*)

methodology										
						rate of 5 °C min^{-1} and kept 10 min at the final temperature.				
(Yang et al., 2014)	Pesticides	Grape juice	7	ILSFOD-LLME	HPLC-UV	Agilent 1200 series HPLC system. Stationary phase: Spursil C18 columns (5 µm, 4.6 × 250 mm, Dikma Limited). Mobile phase: acetonitrile–water (75:25, v/v). The flow rate: 1 mL min^{-1}. Elution: isocratic. Temperature: 25 °C. Detection: UV at 254 nm	<20	0.5-500 µg L^{-1}	0.03-0.28 µg L^{-1}	————
(Farajzadeh et al., 2021)	Pesticides	Pomegranate and grape juice.	7	DLLME	GC-FID	Shimadzu 2014 gas chromatograph equipped with FID detector. Carrier gas: He, flow rate 30 mL min^{-1}. A Zebron^{TM} capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Temperature program: 50 °C for 3 min, then 300 °C at a rate of 18 °C min^{-1} and maintained for 10 min.	27	-	45-78 ng L^{-1}	149-261 ng L^{-1}
(Wołejko et al., 2014	Pesticides and their metabolites	Strawberry and raspberry juice.	160	MSDP	GC-ECD/NPD	Agilent 7890 GC coupled to ECD/NPD-Stationary phase: HP-5 capillary column (30m × 0.32mm × 0.5 µm film thickness). Carrier gas: He, flow rate 3 mL min^{1}. Temperature program: 120 to 190 °C at a rate of 16 °C min^{-1}, increased to 230 °C at 8 °C min^{-1} and then to 285 °C at 18 °C min^{-1}, and remain for 18 min.	30.5	-	-	-
(Farajzadeh et al., 2016)	Pesticides	Grape juice	6	DES	GC-FID	Shimadzu 2014 GC coupled to FID Stationary phase: RTX-1 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Carrier gas: He. Temperature program: 80 °C hold for 3 min, ramped at 10 °C min^{-1} until 300 °C, and hold at 300 °C for 5 min	30	1.4-5000 ng mL^{-1}	0.39- 3.1 ng mL^{-1}	1.4-11 ng mL^{-1}
(Pelit et al., 2015)	Pesticides	Grape juice	5	PTh IL-Mmt SPME	GC-ECD	Agilent Model 7820A Series equipped with HP ECD detector systems. Stationary phase: DB-5-	33	0.01-50 ng mL^{-1}	0.002-0.667 ng mL^{-1}	0.025-2.224 ng mL^{-1}

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Table 1 (*continued*)

						methodology				
						MS column (30 m × 250 μm I.D. and film thickness 0.25 μm). Carrier gas: He, flow rate: 1.0 mL min^{-1}. Temperature program: 50 °C for 5 min increased to 150 °C at a rate of 25 °C min^{-1} and increased to 220 °C at a rate of 10 °C min^{-1} and increased to 280 °C at a rate of 5 °C min^{-1}.				
(Gomez-Gomez et al., 2018)	BAs	Grape juice	8	Homogenization in perchloric acid (5% v/v) and derivatization with dansyl chloride in acetone.	HPLC-UV	HPLC (Ultimate 3000 BioRS, Dionex-Thermo Fisher Scientific Inc). Column: ACE 5 C18 (5 μm, 25 cm × 4.6 mm). Mobile phase: (A) acetonitrile (100%), (B) acetonitrile (50%). Flow rate: 0.7 mL/min. Injection volume: 20 μL. Elution: gradient as follow: 0-2 min, 40 % A; 2-4 min, 60 % A; 4-8 min, 65 % A; 8-12 min, 85 % A; 12-15 min, 95 % A; 15-21 min, 85 % A; 21-22 min, 75 % A; 22-25 min, 40 % A.	25	0.00-35.25 mg L^{-1}	————	————
(Saaid et al., 2009)	BAs	Black currant juice and red grape.	5	Dilution with 0.1M HCl (ten times) and derivatization with dansyl chloride.	HPLC-UV	PU-1580 Jasco HPLC and LG-1580-04 Jasco UV/VIS detector. Column: Waters Spherisorb 5 μm ODS2 column (250 × 4.5 mm). Mobile phase: acetonitrile: water (67:33, v/v). Flow rate: 1.2 mL min^{-1}. Detection: UV at 254 nm.	30	0.1-250 mg L^{-1}	4.43 – 7.34 μg L^{-1}	14.76 -24.45 μg L^{-1}
(Kelly et al., 2010)	BAs	Grape juice	7	Dilution, filtration, in-loop derivatization with o-phthaldialdehyde and N-acetyl-l-cysteine.	HPLC-FLD	A Hewlett-Packard (Agilent Technologies Massy) 1100 series HPLC instrument and G1321A FLD. Column: CIL 250 mm × 3 mm Equisil®Mobile phase: (A) 95% 0.05 M sodium acetate buffer, pH 6.5 and 5 % methanol, (B) methanol–acetonitrile 70–30. Flow rate: 0.5 mL/min. Elution: gradient as follow: 0 min, 3% B; 0-4.5 min, 5% B; 4.5-1 0min,	39	0.25–10 mg L^{-1}	————	0.05-0.25 mg L^{-1}

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Table 1 (*continued*)

methodology										
						19% B; 10-16min, 27% B; 16-20 min, 42% B; 20-25 min, 48% B; 25-32 min, 60% B, 32-35 min, 3% B. Detection: fluorescence detection at excitation and emission wavelengths of 330 nm and 440 nm, respectively. Temperature: 25 °C.				
(Chen et al., 2019)	BAs	Bog bilberry juice	7	Sonication, heating at 70 C for 2 h, cooling down to room temperature and filtering.	HPLC-UV	Shimadzu LC-20AT LC system. Column: A Venusil XSB C18 column (4.6 × 250 mm, 5 µm (Shimadzu, Japan). Mobile phase: (A) acetonitrile:methanol (4:1, v/v), (B) 25 mM acetate buffer (0.02 % sodium azide, pH 5.8). Injection volume: 20 µL. Flow rate: 0.9 mL/min. Elution: gradient as follow: 0–20 min, 90% B isocratic; 20–30.5 min, 90% B to 83% B; 30.5–33.5 min, 83% B isocratic; 33.5–65 min, 83% B to 73% B; 65–73 min, 73% B to 28% B; 73–78 min, 28% B to 18% B; 78–82 min, 18% B to 0% B; 82–85 min, 0% B isocratic; 85–90 min, 0% B to 90% B; and 90–93 min, 90%B isocratic.	93	0.01-7.94 mg L^{-1}	———	———
(Gomez et al., 2020)	BAs	Grape juice	9	Homogenization, centrifugation, derivatization with dansyl chloride.	UPLC-UV	Agilent 1200 Series Rapid Resolution LC systemStationary phase: Agilent Zorbax Eclipse XDB – C18 column (50 mm × 4.6 mm ID, 1.8 µm particle size). Flow rate: 1.0 mL/min^{-1}. Injection volume: 5 µl. Temperature: 25 °C. Detection: 225 nm. Mobile phase: (A) acetonitrile (100%), (B) acetonitrile (50%). Elution: gradient as follows: 0–2 min, A 40 %, B 60 %, 2–3 min, A 40–80 %, B 60–20 %, 3–4 min, A 80–90 %, B 20–10 %, 4–6	12	2–150 mg kg^{-1}	0.032-0.098 µg L^{-1}	0.11-0.32 µg L^{-1}

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Table 1 (*continued*)

methodology									
						min, A 90–95 %, B 10–5 %, 6–7 min, A 95–40 %, B 5–60 %, 7–12 min, A 40 %, B 60 %.				
(Rodríguez-Ramos et al., 2020)	Phthalates	Grape juice	11	QuEChERS extraction	GC-MS/MS	Agilent 7890B GC system coupled to Agilent 7000C MS. Stationary phase: HP-5 ms capillary column (15 m × 0.25 mm, 0.25 µm film thickness). Carrier gas: He; flow rate: 1.5 mL min^{-1} and 1.7 mL min^{-1} for backflush. Temperature program: 70 °C for 2 min. Then, 200 °C at a rate of 25 °C min^{-1} and then increased to 260 °C at a rate of 3 °C min^{-1}. Finally, the temperature reached 300 °C at a rate of 30 °C min^{-1} hold for 4 min.	33	0.5-250 ug L^{-1}	————————	0.034–1.415 µg L^{-1}.
(Pelit et al., 2015)	Pesticides	Gooseberry, blackcurrant, redcurrant, raspberry, strawberry, and the concentrated juice of blackcurrant, redcurrant, raspberry, and strawberry.	5	PTh IL-Mmt SPE	GC-ECD	Agilent 7820A Series gas chromatograph equipped with HP ECD detector system.HP-5 capillary column (30 m × 0.32 mm, 0.5 µm film thickness) was used. Carrier gas: He, flow rate: 3.0 mL min^{-1}. Temperature program: 50 °C/5 min and then 150 at a rate of 25 °C min^{-1}, increased to 220 °C at 10 °C min^{-1} and then to 280 °C at 5 °C min^{-1}, and remain for 18 min.	33	0.04-0.51 ng mL^{-1}	0.002-0.667 ng mL^{-1}	0.002-2.22 ng mL^{-1}
(Timofeeva et al., 2017)	Pesticides	Raspberry juice and cherry juice.	4	IS-SULLE	HPLC-MS/MS	Shimadzu HPLC-MS/MS system LCMS-8030 Triple Quadrupole Liquid Chromatograph Mass Spectrometer. Zorbax Bonus-RP column (100 × 2.1 mm, 3.5 µm). Mobile phase: A - deionized water; B - methanol with 0.1% (v/v) formic acidFlow rate: 0.3 mL min^{-1}. Elution: gradient elution as followed: 0 – 8 min, 20 – 80 % B; 8 – 11 min, 80 % B.	11	0.01-10 mg L^{-1}	0.0003-0.03 mg L^{-1}	————————
(Farajzadeh et al., 2015)	Pesticides	Grape andsour cherry juices.	11	CCSHLLE-DLLME	GC-FID	Shimadzu 2014 gas chromatograph.CP-Sil 8CB capillary column (30	27	0.1–5 µg L^{-1}	0.34-5 µg L^{-1}	1-16 µg L^{-1}

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Table 1 (*continued*)

methodology										
						m X 0.25 mm i.d. 0.25 µm film thickness). Temperature program: 80 °C/3 min and then increased to 300 °C at a rate of 8 °C min^{-1}, and then maintained at 300 °C and remain for 10 min.				
(Farajzadeh & Afshar Mogaddam, 2016)	Pesticides	Cherry, grape, and strawberry juice.	17	Acid-base DLLME	GC-NPD	GC-1000 gas chromatograph with GLAIND-2200 hydrogen generator (H flow rate 5 mL min^{-1}). HP-5 MS capillary column (30 m × 0.25 mm i.d.). Temperature program: 80 °C hold for 3 min and then increased to 300 °C at a rate of 8 °C min^{-1}, and then maintained at 300 °C and remain for 10 min. The NPD temperature was maintained at 300 °C.	40	0.1-33 ng mL^{-1}	0.05-0.43 ng mL^{-1}	0.17-1.43 ng mL^{-1}
(Moinfar et al., 2020)	Pesticides	Grape juice	5	CSDF-ME	GC-MS	Clarus 580 GC equipped with Clarus SQ 8S quadrupole MS system. Carrier gas: He, flow rate of 1.0 mL min^{-1}. HP-5MS (30 m × 0.25 mm id, , 0.25-µm film thickness) capillary column. Temperature program: 110°C hold for 0.5 min, then increased to 195 °C with a rate of 20 °C min^{-1} and hold for 1.5 min. Next, the temperature was increased to 230 °C with a rate of 25 °C min^{-1} and hold for 3.5 min.	10	380- 500.0 µg L^{-1}	0.03-1.0 µg L^{-1}	2.0-5.0 µg L^{-1}
(Moinfar et al., 2021)	Pesticides	Grape juice	5	CSDF-ME	GC-MS	GC-MS, Clarus 580 gas chromatographyHP-5MS (30 m, 0.25-µm film thickness × 0.25 mm id) capillary column. Carrier gas: Helium, flow rate: 1.0 mL min^{-1}. Temperature programming: The oven temperature of GC was programmed for 0.5 min at 110 °C for the initial hold, then the temperature was raised by 20 °C min^{-1} to 195 °C and held for 1.5 min, then	10	1-1.2 µg L^{-1}	0.02-.0.30 µg L^{-1}	0.07-1.0 µg L^{-1}

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Table 1 (*continued*)

methodology									
						heated to 230 °C at 25 °C min^{-1} and kept at the same temperature for 3.5 min.				
(Meng et al., 2021)	Pesticides	Grape juice and strawberry juice.	350	m-PFC	GC-Orbitrap/MS	GC-Orbitrap system Thermo Scientific TG-5MS (30 m × 0.25 mm ID, 0.25 μm) column. Carrier gas: He, flow rate: 1.0 mL min^{-1}. Temperature program: 40 °C hold 1.5 min then increased to 90 °C at the rate of 25 °C min^{-1}, then increased to 180 °C at the rate of 25 °C min^{-1}, then increased to 280 °C at the rate of 5 °C min^{-1}, then increased to 310 °C at the rate of 10 °C min^{-1}, and held at this final temperature for 3 min.	34	5 to 500 μg kg^{-1}	0.3–3.0 μg kg^{-1}	1.0–10.0 μg kg^{-1}
(Dasgupta et al., 2011)	Pesticides, PCBs and PAHs	Grape juice and pomegranate juice.	165	LLME	GC-ToFMS	Pegasus 4D GC-ToFMS systemRtx®-5 capillary column (10 m × 0.18 mm, 0.20 μm) connected in series to a Varian VF-17 ms (1 m × 0.10 mm, 0.10 μm). Carrier gas: ultra-pure grade He. Temperature program: 100 °C hold for 2 min, increased to 200 °C at the rate of 20 °C min^{-1} hold for 2 min hold and finally to 285 °C at 20 °C min^{-1} hold for 2 min. The secondary oven temperature was consistently set at 10 °C higher than the primary oven.	15.25	1-500 μg L^{-1}	1-250 ng L^{-1}	0.4-1000 ng mL^{-1}
(Pérez et al., 2016)	PCBs	Grape juice	7	mSPE	GC-MS/MS	Agilent 7890A GC coupled with Agilent 7000 MS/MSCarrier gas: He, flow rate: 1 mL min^{-1}Temperature program: 150 °C hold for 1 min, then increased at 10 °C min^{-1} to 280 °C hold for 10 min..	15	7.5-90 ng mL^{-1}	1.6-2.9 ng L^{-1}	5.2-9.8 ng L^{-1}
(Shen et al., 2016)	Furan and 2-alkylfurans	Grape juice, blueberry juice, and	8	Homogenization and NaCl addition.	GC-MS	Agilent Model 7890A/5975 GC-MS. Stationary phase: HP-PLOT/Q capillary column with	43	-	0.2 ng g^{-1}	0.5 ng g^{-1}

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Table 1 (*continued*)

methodology										
		pomegranate juice.				particle trap, 30m×0.32 mm×20 µm.Carrier gas: He, flow rate: 1.5 mL min^{-1}. Temperature program:50 °C for 1 min, increased to 200 °C at a rate of 10 °C min^{-1}; held for 5 min; increased to 240 °C at a rate of 20 °C min^{-1} and held for 20 min.				
(Vázquez-Araújo et al., 2011)	Furans	Pomegranate juice	7	Headspace–SPME	Headspace GC-MS	Varian GC CP3800 coupled to Varian MS Saturn 2200. Stationary phase: VF-5MS column (30 m × 0.25 mm i.d., 1.0 µm film thickness). Carrier gas: He, flow rate: 1 mL min^{-1}. Temperature program: 40°C held for 10 min, then increased 8°C min^{-1} to 180°C, and finally increased at 10°C min^{-1} to 280°C, where was held for 10 minutes.	47.5	—	—	—
(Varming et al., 2004)	Furans	Black currant juice	3	DHS	GC-MS	Hewlett-Packard G1800A S GC-MS system. Stationary phase: DB-Wax column (30 m 0.25 mm 0.25 ím). Temperature program: 40 °C for 10 min, increased with 6°C min^{-1} to 240 °C, and kept isothermal for 25 min.	69	0.1-5 mg L^{-1}	—	—
(Furdíková et al., 2019)	Furans	Grape berries	13	SPME	GCxGC-HRTOF-MS	Pegasus GC×GC HRTOF-MS (Agilent 7890B GC). Stationary phase: DB-FFAP column (30m × 0.25 µm × 0.25 µm) and Rxi-17Sil column (1.6 m × 0.25 mm × 0.25 µm). Carrier gas: He, flow-rate: 1 ml min^{-1}. Temperature program: 40 °C kept for 10 min, then increase by 2 °C min^{-1} to final temperature 220 °C and kept for 5 min.	120	0.13-3.25 mg L^{-1}	—	—
(Fernandes & Ferreira, 2000)	BAs	Grape juice	7	Ion-pair extraction and derivatization with heptafluorobutyric anhydride.	GC-MS	A Hewlett-Packard 5890 GC coupled with Hewlett-Packard 5970B MSCarrier gas: He;Column:: A DB-5MS capillary column (30 m × 0.25 mm ID. 0.25 µm film thickness). Temperature program: 80	18	0.01-5 mg L^{-1}	<0.01 mg L^{-1}	<0.05 mg L^{-1}

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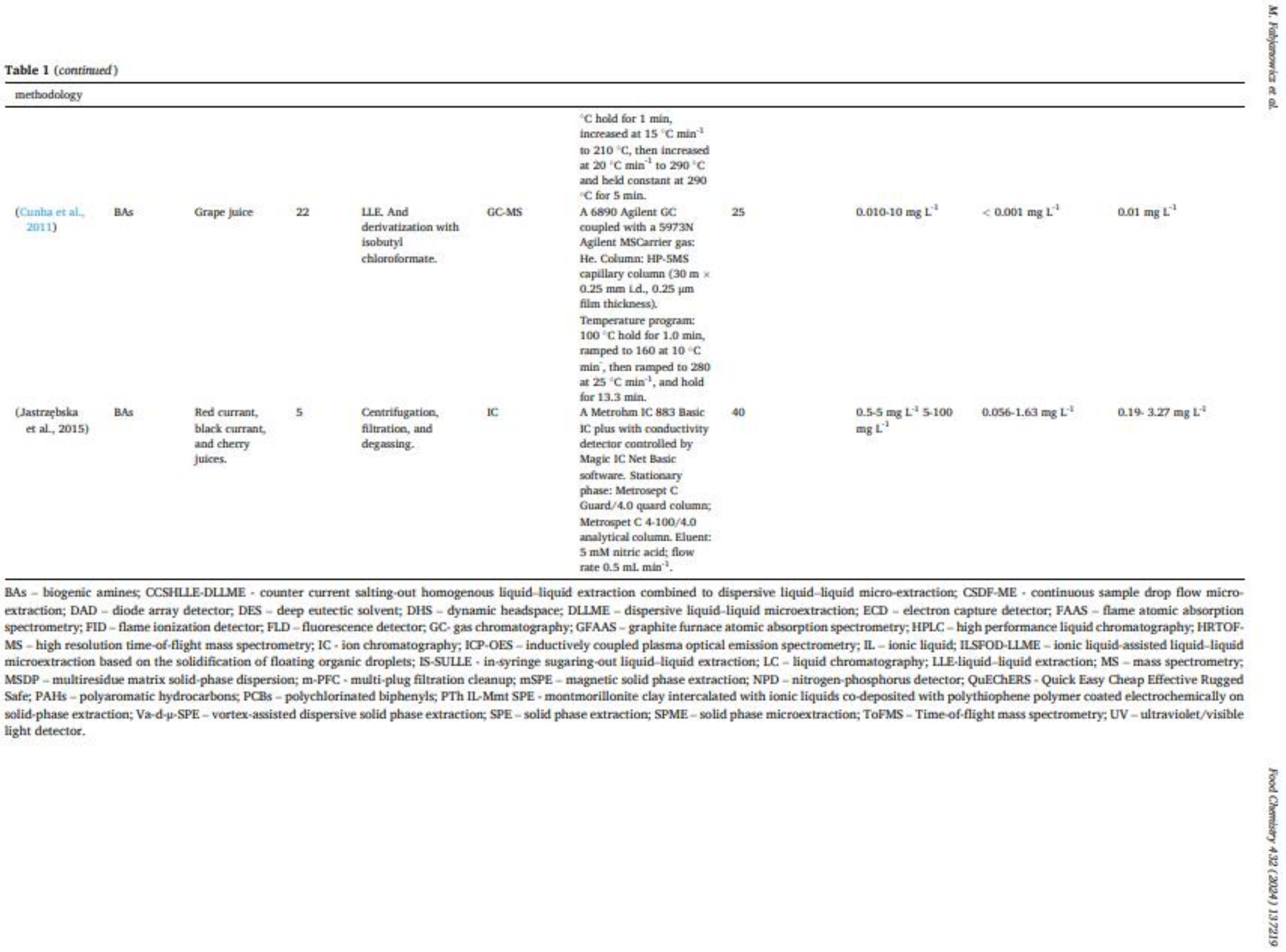

Table 1 (*continued*)

methodology										
						°C hold for 1 min, increased at 15 °C min^{-1} to 210 °C, then increased at 20 °C min^{-1} to 290 °C and held constant at 290 °C for 5 min.				
(Cunha et al., 2011)	BAs	Grape juice	22	LLE And derivatization with isobutyl chloroformate.	GC-MS	A 6890 Agilent GC coupled with a 5973N Agilent MSCarrier gas: He. Column: HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Temperature program: 100 °C hold for 1.0 min, ramped to 160 at 10 °C min^{-}, then ramped to 280 at 25 °C min^{-1}, and hold for 13.3 min.	25	0.010-10 mg L^{-1}	< 0.001 mg L^{-1}	0.01 mg L^{-1}
(Jastrzębska et al., 2015)	BAs	Red currant, black currant, and cherry juices.	5	Centrifugation, filtration, and degassing.	IC	A Metrohm IC 883 Basic IC plus with conductivity detector controlled by Magic IC Net Basic software. Stationary phase: Metrosept C Guard/4.0 quard column; Metrospet C 4-100/4.0 analytical column. Eluent: 5 mM nitric acid; flow rate 0.5 mL min^{-1}.	40	0.5-5 mg L^{-1} 5-100 mg L^{-1}	0.056-1.63 mg L^{-1}	0.19- 3.27 mg L^{-1}

BAs – biogenic amines; CCSHLLE-DLLME - counter current salting-out homogenous liquid–liquid extraction combined to dispersive liquid–liquid micro-extraction; CSDF-ME - continuous sample drop flow microextraction; DAD – diode array detector; DES – deep eutectic solvent; DHS – dynamic headspace; DLLME – dispersive liquid–liquid microextraction; ECD – electron capture detector; FAAS – flame atomic absorption spectrometry; FID – flame ionization detector; FLD – fluorescence detector; GC- gas chromatography; GFAAS – graphite furnace atomic absorption spectrometry; HPLC – high performance liquid chromatography; HRTOF-MS – high resolution time-of-flight mass spectrometry; IC - ion chromatography; ICP-OES – inductively coupled plasma optical emission spectrometry; IL – ionic liquid; ILSFOD-LLME – ionic liquid-assisted liquid–liquid microextraction based on the solidification of floating organic droplets; IS-SULLE - in-syringe sugaring-out liquid–liquid extraction; LC – liquid chromatography; LLE-liquid–liquid extraction; MS – mass spectrometry; MSDP – multiresidue matrix solid-phase dispersion; m-PFC - multi-plug filtration cleanup; mSPE – magnetic solid phase extraction; NPD – nitrogen-phosphorus detector; QuEChERS - Quick Easy Cheap Effective Rugged Safe; PAHs – polyaromatic hydrocarbons; PCBs – polychlorinated biphenyls; PTh IL-Mmt SPE - montmorillonite clay intercalated with ionic liquids co-deposited with polythiophene polymer coated electrochemically on solid-phase extraction; Va-d-µ-SPE – vortex-assisted dispersive solid phase extraction; SPE – solid phase extraction; SPME – solid phase microextraction; ToFMS – Time-of-flight mass spectrometry; UV – ultraviolet/visible light detector.

had a wide linear range (Table 1), acceptable detection and quantitation limits as well as recoveries in the range between 90.0 and 106.3%. Seven BAs in grape juice was also evaluated after automated in-loop pre-column derivatization with an O-phthaldialdehyde and *N*-acetyl-l-cysteine, followed by HPLC analysis with fluorescence detection (Kelly et al., 2010). Chromatographic analysis takes 39 min. Because of the method's great sensitivity, no sample preparation other than a straightforward dilution was needed prior to derivatization, eliminating the necessity for an internal standard.

In recent years, the application of lactic acid bacteria (LAB) inoculation to fruit and juices processing has gained popularity in the production of unique non-alcoholic fermented beverages. It is a simple and valuable biotechnological method that allows fruits to be processed into products with a longer shelf life. The effect of LAB inoculation on the chemical composition of bog bilberry juice was studied using an HPLC method (Chen et al., 2019). ACN:methanol in the ratio 4:1 (phase A) and 25 mM acetate buffer mixture were used as a mobile phase and separation of seven BAs within 93 min. The study also involved the effect of LAB on reducing sugars, organic acids, anthocyanins, and non-anthocyanins phenolic compounds. Results disclosed that inoculation with LAB resulted in significant changes in the juice composition. Sugars, anthocyanins, total phenolic acids, total flavanols, and amino acids contents decreased in the juices after incubation but no changes in organic acids were noticed. It was also observed, that the content of four biogenic amines as tyramine, cadaverine, putrescine and phenylethylamine decreased after incubation but isoleucine content increased 8 times. The findings of this study should be taken into consideration to design a fermentation process that does not result in significant losses of various health-promoting components and does not result in health risk related with the BAs content (Chen et al., 2019).

Additionally, a UPLC method was published for testing nine BAs in grape juice to be used as a quality marker for grape-derived products (Gomez et al., 2020). The derivatization process for BAs was done with the use of dansyl chloride, while the mobile phase consisted of a mixture of 100% ACN (phase A) and 50% ACN (phase B). Analysis time was shorter as compared to those obtained using HPLC-based procedures (Chen et al., 2019; Gomez-Gomez et al., 2018; Kelly et al., 2010; Saaid et al., 2009). Additionally, it had a wide linear range and good sensitivity as presented in Table 1.

Moreover, two GC–MS/MS methods were applied for the determination of BAs in grape juice samples (Cunha et al., 2011; Fernandes & Ferreira, 2000). Methods differs in the extraction techniques used. In the first one (Fernandes & Ferreira, 2000), the back-extraction with 0.1 M HCl was done after the amines have been extracted with the ion-pairing agent bis-2-ethylhexylphosphate dissolved in chloroform. Derivatization of the extracted amines was performed by using heptafluorobutyric anhydride reagent. Seven amines (β-phenylethylamine, tyramine, 1,3-diaminopropane, putrescine, cadaverine, spermidine and spermine) were quantified using this method with high sensitivity, accuracy and reproducibility. In the second one (Cunha et al., 2011), liquid–liquid extraction was used with a toluene as an extraction solvent and isobutyl chloroformate as an derivatizing agent. Application of a given method enables quantification of 22 biogenic amines in 25 min.

Pesticides residues are also often present in complex matrices such as berry juice at very small concentrations, hence for detection of these harmful components highly sensitive and selective analytical methods are needed.

The most widely used chromatographic technique for pesticide determination in fruit juice samples is GC with the application of different extraction techniques and detectors like:

- Liquid-liquid microextraction combined with gas chromatography coupled with time-of-flight mass spectrometry (LLME GC-ToFMS) which was developed for screening 165 contaminants from the group of pesticides and dioxins like PCBs and PAHs. Despite the satisfactory recoveries (76–120%) and simplicity of the extraction method, the use of chloroform and cyclohexane makes it unfavourable from the Green Analytical Chemistry requirements (Dasgupta et al., 2011).
- Multiresidue matrix solid-phase dispersion combined with GC coupled with electron capture detector (ECD) and nitrogen-phosphorus detector (NPD). The method allows to determine 160 pesticides in berry fruits and their products. The disadvantage of the proposed approach is the use of hexane as one of the extraction solvent (Wołejko, Łozowicka, & Kaczyński, 2014).
- Counter current salting-out homogenous liquid–liquid extraction combined with dispersive liquid–liquid microextraction coupled with GC/FID (CCSHLLE-DLLME GC/FID). The approach uses ACN as a coextraction/disperser, 1,2-DBE as an extraction solvent and demonstrates large linear ranges (even 1–10000 μg/L) for the target analytes under the optimum extraction conditions (Farajzadeh et al., 2015).
- Montmorillonite clay intercalated with ionic liquids co-deposited with polythiophene polymer (PTh IL-Mmt) coated electrochemically on SPME coupled to GC/ECD (PTh IL-Mmt SPME GC/ECD). The method allows determination of 5 analytes in 33 min. The imidazolium group in IL, along with the porous surface structure of the fiber, all contribute to the hybrid material's strong electrostatic contacts, hydrogen bonds, and π–π interactions, which results in a high capacity for adsorption of volatile pesticides (Pelit et al., 2015). The method is characterised by high extraction efficiency (88.7–101.7%), high sensitivity and low detection limit presented in Table 1.
- Dispersive liquid–liquid extraction coupled with GC and nitrogen – phosphorus detector (DLLME GC/NPD). The method allows to determine three classes of pesticides (triazine, triazole, and neonicotinoid) at the ng mL^{-1} range. It is based on acid-base reaction, in which the extraction solvent (p-chloroaniline) is dispersed (by deionized water) into an aqueous sample. In this study low LODs and LOQs and high extraction recoveries and enrichment factors were attained present in Table 1 (Farajzadeh et al., 2016).
- Continuous sample drop flow microextraction combined with GC–MS (CSDF-ME GC–MS) was used to determine phorate, diazinone, dimethoate, disulfoton, and chlorpyrifos from the fruit juice. The application of narrow-necked conical shaped vessel results in short analysis time, high sensitivity and low total solvent consumption (Moinfar et al., 2020).
- Continuous sample drop flow microextraction combined with GC–MS (CSDF-ME GC–MS) is a similar approach as presented in the year 2020 however, different design of extraction vessel (the extraction vessel was a conical open-end vial set in a little container filled with double-distilled water) and halogen-free organic extraction solvent were applied. Lower limit of LODs and LOQs then in previously published report were achieved and the extraction recoveries in the range between 25.5 and 48.0% (Moinfar et al., 2021).
- Dispersive liquid–liquid microextraction coupled with GC/FID. And D (DLLME GC-FID) was used to determine pesticide residues including penconazole, chlorpyrifos, ametryn, clodinafop-propargyl, diniconazole, oxadiazon, and fenpropathrin from fruit juice. The isopropanol was used as disperser and 1,2-dibromoethane as an extraction solvent. Moreover special shaped vessel (downward vaporization gas orientation) was designed and used for vaporization and ultra-preconcentration of the extract from DLLME step. The innovations of this study were good outcomes (presented in Table 1) using readily available, straightforward equipment. Recoveries from extraction were on the level of 55–89% (Farajzadeh et al., 2021).
- Multi-plug filtration clean-up combined with gas chromatography-electrostatic field orbitrap high resolution mass spectrometry (m-PFC GC-Orbitrap/MS) is a method developed for screening of 350 pesticides in grape and strawberry juice samples. This extraction method was found to be simple and time-effective. Moreover, highly efficient clean-up of all targeted samples was observed due to the fact

that the m-PFC column has the advantage of multiwall carbon nanotubes (MWCNTs). MWCNTs have superior adsorption capacities compared to other sorbents because of their extraordinarily high surface area and distinctive structure. The extraction recovery was found to be 72.8–122.4%, revealing the high performance of the used extraction method (Meng et al., 2021).

Another approach used for pesticides determination utilizes LC. Seven insecticides in grape juice samples were analysed using HPLC (M. Yang et al., 2014) after the ionic liquid-assisted LLME, which was based on the solidification of floating organic droplets utilizing a bell-shaped collection device (BSCD). The modification of the traditional LLME method increased its extraction efficiency since the use of BSCD allowed easier collection of the mixed extraction solvents (1-dodecanol and IL, which replaced commonly applied chlorinated solvents) and quicker separation after solidification. The method resulted in an efficient concentration of the studied components in the tested samples, the enrichment factor was in the range of 160 to 246 with little consumption of organic solvents.

Picó & Kozmutza (2007) developed a highly sensitive LC-MS/MS for the analysis of four pesticides and their metabolites in different grape juice samples (Picó & Kozmutza, 2007). Solid-phase extraction (SPE) was carried out before the analysis, which yield in good extraction recovery (more than 80%), high sensitivity and low quantitation limit, as shown in Table 1. Many of the pesticides are prone to degrade due to oxidative mechanisms, thus authors checked the role of antioxidant for the increase of the durability of certain of the pesticide in fruit juice. Results indicated that the degradation rate of the targeted pesticides was slower in grape juice and quercentine-containing aqueous solutions than in water. These findings suggested that natural antioxidants found in fruit juices might decrease pesticide breakdown rates and enhance their persistence.

Timofeeva et al. (2017) developed another fully-automated LC-MS/MS method for the detection of four pesticides in fruits and berry juices. Under the optimized conditions the proposed extraction procedure takes less than 2 min. Apart from that it is simple to perform, inexpensive and does not require complex equipment. However, authors suggest that combination with other pre-concentration method like SPE can improve the sensitivity (Timofeeva et al., 2017).

PCBs are another important group of compounds to be monitored in food and beverage samples. Magnetic oleate-coated Fe_3O_4 nanoparticles (Ol-coated MNPs) were used for magnetic solid-phase extraction (mSPE) of selected PCBs from grape juice samples followed by GC–MS/MS analysis (Pérez et al., 2016). Authors compared the developed method with other presented in the literature like DLLE-SFO. It was showed that recovery obtained in this research was between 52 and 85% which was lower than in DLLE-SFO (73–106%). However, the mSPE GC–MS/MS method achieved higher sensitivity (LOD in the range between 1.6 and 5.4 ng/L) than in the other work (3.7–18.5 ng/L). Although no PCB was detected in real samples, the method validation results confirmed its ability to determine targeted chemicals in different samples.

Furan and its derivatives are another group of compounds being of high interest of researchers. Furans are heterocyclic compounds, which contribute to the sensory qualities of a wide range of thermally processed foods (Shen et al., 2016). Shen, et al. (2016) proposed to determine furans in the foodstuff including grape, blueberry and pomegranate juice by static headspace GC–MS. Method was characterized by simple sample preparation (only homogenization by manual shaking and sodium chloride addition was needed). Satisfactory validation parameters were achieved for 13 furans determination (Table 1). However, the GC–MS analysis last more than 40 min. Results of this study revealed that furans were detected in trace amounts in the tested fruit juices (Shen et al., 2016).

On the other hand, the aromatic profile of pomegranate fresh and commercial juices was studied and correlated to their sensory flavors using partial least squares regression (Vázquez-Araújo et al., 2011). In this study, a headspace–SPME combined with the GC–MS method was used. This study showed that there was a significant difference between fresh and commercial juices. Results showed that there were significant changes in their chemical composition with fresh-squeezed juice being distinguished primarily by the presence of terpenes and aldehydes, whereas furans played a key role in commercial juice aroma. Moreover, different juice manufacturing processes were found to alter the aromatic profile of the fresh juice. So, companies should search for different processing methods for pomegranate juice to improve its quality without affecting its health benefits or increasing its health risks.

Varming, et al. (2004) evaluated the effect on the aroma and thus, the content of aromatic compounds including furans, of blackcurrant juice after the thermal treatment's. For the purpose of the study a headspace GC/MS method (Varming et al., 2004). In this study, blackcurrant juice samples were exposed to different temperatures (45–90 °C) for different periods (57, 80, 110, 130 s). Then the aromatic compounds were collected (samples were purged with nitrogen and target compounds were collected into the traps). Collected volatiles were thermally desorbed and determined using GC–MS. The developed analytical method was applied for the determination of 49 aroma compounds involving three furans. Results of the study proved that the concentration of several terpenoids, furans, and phenols have significantly increased after thermal treatment of 90 °C for 60 min. However, application of 60 °C and less had no influence of the juice aroma compounds composition.

Another method used for furans determination was based on the SPME combined with GC–MS designed to distinguish between healthy and noble-rotten grape berries (Furdíková et al., 2019). The concentration of 7 out of 13 significantly differs between healthy and noble-rotten grapes. It was noticed that the content of furans such as: 2-pentylfuran, dihydrofuran-2(3H)-one, 5-butyldihydrofuran-2(3H)-one, 5-pentyldihydrofuran-2(3H)-one, 5-acetyldihydrofuran-2(3H)-one 5-hexyldihydrofuran-2(3H)-one and 5-ethyldihydrofuran-2(3H)-one were higher in noble-rotten grapes than in healthy fruits.

5. Conclusions and future remarks

From year to year, the demand for fruit juices increases. Particular interest can be observed for juices produced from superfruits, which include berries. This is due to the fact that consumers pay more and more attention to the composition of food products that they include in their daily diet. A very important feature of food has become its health-promoting properties, and thus health benefits.

The presented literature review focuses on modern analytical methods that enable the determination of analytes contained in fruit juices that may have health-promoting properties, but also those analytes whose presence may be harmful to our health.

In the case of the determination of bioactive substances, spectrophotometric techniques are used for preliminary studies to determine summary parameters, such as the total polyphenols content or total anthocyanins content. In order to more accurately determine the composition of fruit juices, chromatographic techniques, mainly liquid chromatography, are the most often used. These techniques enable the determination of chemical compounds even at the trace level, and are also characterized by good selectivity, accuracy and precision. The use of high-resolution chromatographic techniques enables the detection of new potential active substances contained in fruit juices. However, analyzes often require high consumption of organic solvents and complicated sample preparation procedures for the isolation of analytes. In accordance with the principles of green organic chemistry, the aim is to replace conventional solvents with greener ones, e.g. DES and solvents of biological origin. During the research, the aim is also to miniaturize modern analytical methodologies while increasing the throughput, thus enabling the determination of as many analytes as possible in a relatively short time. It should be noted that in order to understand the nutritional potential and health-promoting properties of fruit juices, it is necessary not only to determine bioactive substances, but also to study their metabolism in the human body. Increasingly, both targeted and untargeted metabolic approaches are being used in research. When establishing a chemical fingerprint and metabolic profiling, the key element is data analysis, during which bioinformatics tools are used. Metabolomics makes it possible to find new bioactive compounds, as well as new juice biomarkers that allow them to be distinguished.

In the case of contamination of juices with microbiological and chemical agents, it is very important to find reliable methods to detect them. Due to the increase in the amount of possible food contamination caused by industrialization and globalization, food safety assessment should be at the heart of the food industry.

Finding fast, reliable and sensitive methods for detecting contaminants in fruit juices is essential for assessing food quality and ensuring consumer safety. Spectroscopic techniques (AAS and ICP-EOS) are - mainly used for metal content analysis. For the determination of other pollutants (e.g. pesticides, mycotoxins, phthalates, biogenic amines and others), chromatographic techniques (GC and LC) are most often used. During the research, the aim is to develop modern analytical methodologies enabling the determination of pollutants at lower and lower concentration levels. The ultimate goal of the new methodologies should be selective and sensitive, miniaturized, automated and lab-independent contamination determinations.

It should also be noted that metabolomics has potential as a screening tool for detecting adulteration of juices, as well as their contamination. It can be a new strategy in the food industry, enabling quick detection of any irregularities in the composition of fruit juices.

In the future, efforts should also be made to develop new analytical methods enabling the detection of impurities and quality control during in-situ juice. Different types of sensors can be used for this purpose, such as electronic noses, electronic tongues or electrochemical sensors.

In accordance with the principles of sustainable development, the industry strives to reduce the amount of waste produced. Wastes obtained during the production of juices, such as pomace, seeds, skins, etc., still contain large amounts of bioactive compounds. Future research should therefore aim at developing green methodologies for extracting bioactive compounds such as polyphenols, flavonoids or pectins from fruit pomace.

In conclusion, comprehensive specifications for fruit juices should be established in the future. It is to be hoped that modern analytical methods, as well as international cooperation between scientists, will enable the development of such analytical tools that will guarantee that fruit juices entering the market will be healthy and safe for consumers.

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CRediT authorship contribution statement

Magdalena Fabjanowicz: Conceptualization, Writing – original draft. **Anna Różańska:** Conceptualization, Writing – original draft. **Nada S. Abdelwahab:** Writing – original draft. **Marina Pereira-Coelho:** Writing – original draft. **Isabel Cristina da Silva Haas:** Writing – original draft. **Luiz Augusto dos Santos Madureira:** Writing – review & editing. **Justyna Płotka-Wasylka:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision.

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.

Data availability

No data was used for the research described in the article.

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3. The aim of the doctoral dissertation

The subject of research carried out as part of the doctoral thesis was the development of new methodological solutions for the characterization and quality control of fruit juices. The research was conducted in two ways. On the one hand, efforts were made to characterize the profile of the volatile fraction of fruit juices using modern analytical techniques. On the other hand, the focus was on the development of simple and fast analytical methodologies with the main emphasis on solutions that can be used in food processing.

New types of functional foods are still being sought. Fruit juices can fall into this category if their consumption will have beneficial health effects. For this reason, it is very important to know the exact composition of fruit juices. Characterization of the volatile fraction profiles of juice samples was carried out using the technique of two-dimensional gas chromatography with mass spectrometry in order to select markers of limes juices. In addition, the profile of volatile bioactive compounds that may have a positive effect on the human body was determined. Another aim of the research was to check the interaction between selected bioactive compounds characteristic of Key and Kaffir limes and human serum albumin. In both cases, it was decided to use the HS-SPME-GC×GC-TOFMS methodology due to its resolution capabilities and low analyte detection limits.

The scope of the research on juice quality assessment included the assessment of authenticity and detection of adulteration of fruit juice samples, as well as the determination of chemical compounds contained in them that may pose a threat to consumer health. The most common types of fruit juices adulteration are water dilution, the addition of sugar syrup which reduces the total amino acid value, the addition of ingredients that are not naturally present in the juice, such as dyes, and the addition of cheaper juices from other types of fruit. There are many chemical, physical and microbiological reference methods used to assess the quality of juices. However, the use of these procedures in the juice industry is laborious and time-consuming. For this reason, new solutions are being searched for, allowing for a fast assessment of the quality of fruit juices. The first objective was to develop an approach to detect adulteration of orange juice by adding less expensive juice to it using a commercially available electronic nose device. The non-targeted approach based on volatile fraction fingerprinting followed by the use of chemometric methods to compare them is an efficient and economical tool for assessing food quality. The use of devices such as an electronic nose would make it possible to monitor the quality of juices directly during the production process. Then, the focus was on chemical compounds, the consumption of which in excessive amounts may have negative effects on the health and life of consumers, namely biogenic amines, furan and alkylfurans. In order to determine biogenic amines, a methodology was developed that combines salting-out assisted liquid-liquid microextraction (SALLME) with a simultaneous derivatization process, followed by the determination of analytes using GC-MS. In the case of furans, an ultrafast gas chromatograph with a FID detector was used. Both methodologies were validated and evaluated in accordance with the principles of Green Analytical Chemistry.

Diagram of the concept of research carried out during the doctoral thesis is shows in Figure 3.

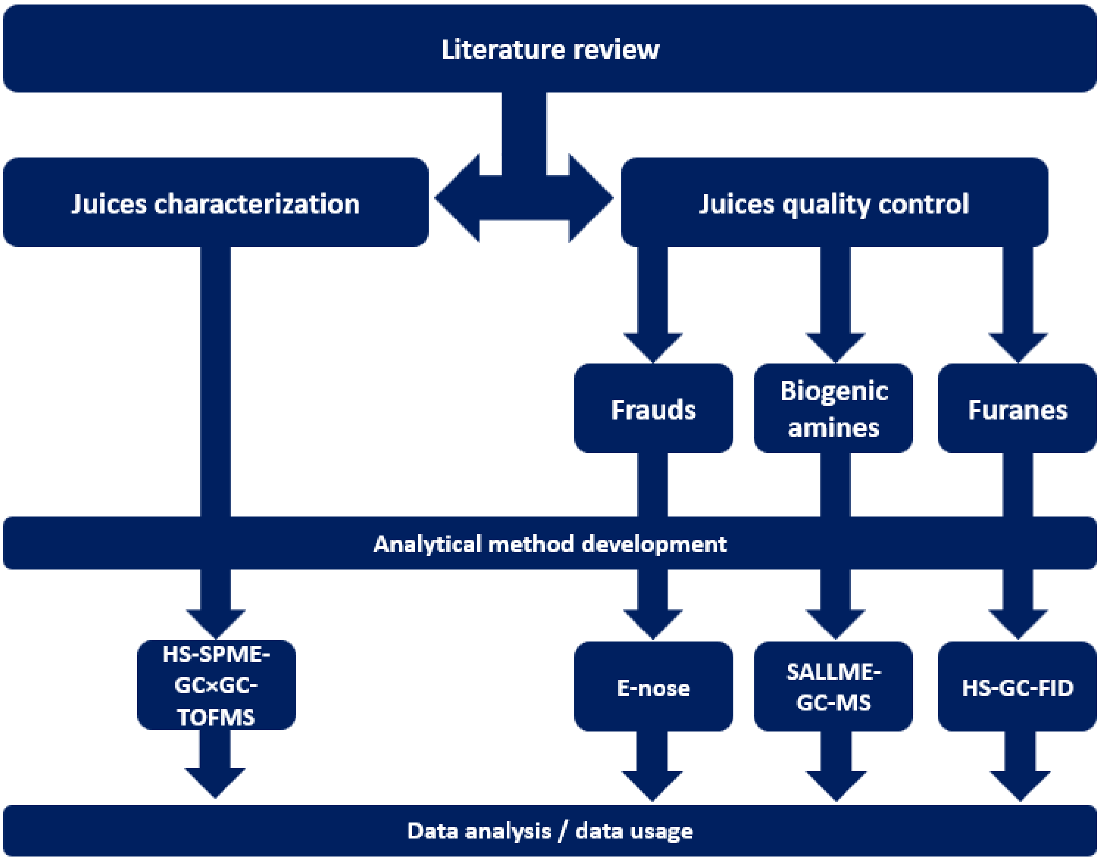

Figure 3 Diagram of the concept of research.

4. Experimental part

4.1. Characterization of fruit juice samples

4.1.1. Development of a methodology enabling the analysis of the volatile fraction of lime juices using the two-dimensional gas chromatography technique and the determination of the antioxidant properties of these juices

Hypothese

- *The use of a two-dimensional gas chromatograph made it possible to characterize the volatile fraction of juice samples with a very complex matrix*
- *The terpene profile is characteristic of different types of lime juices and allows samples to be distinguished*

Questions

- *Which one from the two types of lime juices have better antioxidant properties?*
- *Can the content of antioxidant substances be used to distinguish lime juice samples?*

The food industry is still looking for new types of fruit juices that can be classified as functional drinks, i.e. those that provide health benefits [2]. An example of functional drinks due to the high content of vitamin C are citrus juices [18], [19]. Before producers introduce new types of juices for sale, it is necessary to accurately characterize the ingredients of these juices in order to know their properties and potential pro-health abilities.

Kaffir lime juice was selected as a new type of juice for the study. This fruit is very popular in Southeast Asia, and its peel and leaves are used for culinary purposes. However, the juice of this fruit is not eaten [61]. Key lime juice, the most common type of lime in Europe, was used for comparison. In the case of functional drinks, it is very important to characterize juices in terms of their antioxidant status. In the case of the volatile fraction, terpenes are the main group of chemicals that contribute to the antioxidant properties of food [62]. Gas chromatography is the most often used to analyze the volatile fraction of food samples. The aroma of fruits and citrus juices is a very complex mixture. For this reason, the use of ultrafast GC and conventional GC does not always allow the separation of all analytes contained in the sample. The solution to this problem may be the use of two-dimensional gas chromatography technique characterized by a higher separation potential, higher sensitivity and resolution [63].

In this study, a methodology was developed for the semi-quantitative characterization of the volatile fraction of juices from two species of limes, as well as the quantitative determination of terpenes. During the study, solid phase microextraction (HS-SPME) was used for the isolation and enrichment of the analytes, and the extraction was performed using divinylbenzene/carboxy/polydimethylsiloxane (DVB/CAR/PDMS) fibers.

A two dimensional gas chromatograph coupled with a time-of-flight analyzer and mass spectrometer (GC×GC-TOF-MS) was used to separate and identify the analytes. Data on the chromatographic columns used in the research (Table 2) and the temperature programs used during the chromatographic separation of the analytes (Figure 4) are presented below. The proposed methodology enabled accurate and precise determination of selected terpenes, which is a contribution to the development of science, because so far in the literature, the content of terpenes in lime juice samples was given only as a percentage.

Non-volatile bioactive compounds in the juices of both lime varieties were determined using antioxidant tests and ^{1}H NMR spectroscopy. Finally, Kaffir and Key limes juices were compared based on the collected data in order to distinguish between them.

The research results were published in the journal *Food Control* [64].

Table 2 Parameters of the two chromatographic column used during the research.

Parameters	I dimension column	II dimension column
Type:	capillary	capillary
Lenght:	30 m	1.6 m
Internal Diameter:	250 µm	100 µm
Maximum temperature:	325°C	280°C
Trade name of stationary phase:	Equity 1	SGWAX
The film thickness of the stationary phase	0.25 µm	0.10 µm

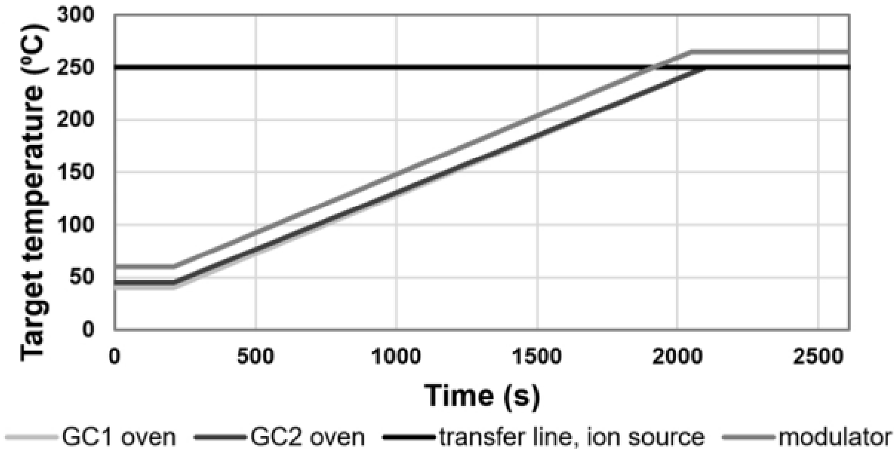

Figure 4 GC×GC-TOFMS - chromatographic temperature program applied during research.

To sum up, in this work an analytical method was developed for the determination of volatile substances in citrus juice samples using the HS-SPME-GC×GC-TOFMS technique. Comparing the composition of the volatile fractions of both juices, it was observed that the most numerous group of chemical compounds were terpenes. The proposed methodology was useful for the quantification of terpenes. Kaffir lime juice contained more terpenes compared to Key lime juice. This fruit juice is therefore characterized by a higher content of bioactive substances in the volatile fraction. Comparing the terpene profile of both types of juice allowed them to be distinguished in terms of botanical origin. In addition, by comparing the ^{1}H NMR spectra, small differences were estimated that may allow differentiation between the samples of both juices. Determination of the polyphenols content in the water extracts of the tested juices also confirmed that the Kaffir lime juice is characterized by a higher content of bioactive compounds, and thus better antioxidant properties.

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Quality of limes juices based on the aroma and antioxidant properties

Martyna Lubinska-Szczygieł [a], Anna Różańska [a], Jacek Namieśnik [a], Tomasz Dymerski [a, *], Rajamohamed Beema Shafreen [b], Moshe Weisz [c], Aviva Ezra [c], Shela Gorinstein [c, **]

[a] Gdańsk University of Technology, Faculty of Chemistry, Department of Analytical Chemistry, Gdańsk, 80-233, Poland
[b] Molecular Nanomedicine Research Unit, Centre for Nanoscience and Nanotechnology, Sathyabama University, Chennai, 600119, TN, India
[c] Institute for Drug Research, School of Pharmacy, Hadassah Medical School, The Hebrew University, Jerusalem, 9112001, Israel

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ABSTRACT

Kaffir (*Citrus hystrix*) and Key (*Citrus aurantifolia*) limes juices were investigated and compared. Two dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF-MS) was applied to assess the botanical origin of Kaffir and Key limes juices, based on volatile substances. The biggest differences in the contents of selected terpenes in Kaffir and Key limes occur in chemical compounds such as Limonene, Citral, Terpinen-4-ol. Limonene concentration is almost 8 times higher in the Key lime volatile fraction than in Kaffir lime. The difference in concentration of Citral in Kaffir lime is almost 20 mg/kg lower than in Key lime. Higher concentration of Terpinen-4-ol was noted in Kaffir lime samples and the content was almost 20 times higher. The concentrations of α-Pinene, Citronellal, Camphene, Nerol, *trans*-Geraniol and β-Pinene are at similar levels in the volatile fraction of both fruits. Bioactive substances (polyphenols, flavonoids, tannins and flavanols) and the values of antioxidant capacities by four radical scavenging assays (DPPH, CUPRAC FRAP, ABTS) were determined and compared in water and methanol extracts in Kaffir and Key limes juices. The bioactivity of Kaffir lime differ significantly in water extracts in comparison with Key lime juices. The ^{1}H NMR shifts in methanol and chloroform extracts showed some differences in aromatic region between the two varieties of lime juices. Terpinen-4-ol for Kaffir lime and Citral for Key lime were used as potential markers. The GC×GC-TOF-MS allows better separation of substances originating from complex matrices than one-dimensional chromatography, based on improved resolution, increased peak capacity and unique selectivity. The possible falsification of mentioned juices can be detected by the use of GC×GC-TOF-MS, antioxidant assays and NMR shifts.

1. Introduction

Kaffir lime (*Citrus hystrix*) is one of the most popular fruits in Thailand or Laos. Kaffir lime leaves are one of the most commonly used Thai spices. Despite the leaves, the skin is also used for culinary purposes, because of specific aroma. Both the leaves and the skin contain many chemical compounds with a healthy effect. Limonene, α-Terpineol, 2β-Pinene, Terpinen-4-ol, γ-Terpinene, α-Terpinene, and α-Terpinolene are common terpenes in leaves (Srisukh et al., 2012a,b). In turn, the content of the individual terpenes in the skin were estimated: Limonene 40.65%, Terpinen-4-ol 13.71%, α-Terpineol 13.20% (Srisukh et al., 2012a,b; Thanaboripat, Chareonsettasilp, & Pandee, 2006). Kaffir lime pulp and juice are not consumed directly (Waikedre et al., 2010). However, they also contain many bioactive substances. Kaffir limes do not grow in temperate climate, and these fruits are also not imported into European countries. In Europe, the most popular and available between lime varieties is Key lime (*Citrus aurantifolia*), which also contains many bioactive terpenes (Spadaro, Costa, Circosta, & Occhiuto, 2012).

The content of individual terpenes varies in the volatile fractions

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Abbreviations: Polyph, polyphenols; GAE, gallic acid equivalent; CE, catechin equivalent; Flavan, flavanols; Flavon, flavonoids; Vit C, vitamin C; Anthoc, anthocyanins; CGE, cyanidin-3-glucoside equivalent; Chlor, chlorophyll; Xan+Carot, xanthophylls+carotenes; ABTS, 2, 2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; FRAP, Ferric-reducing/antioxidant power; CUPRAC, Cupric reducing antioxidant capacity; 1,1-diphenyl-2-picrylhydrazyl, DPPH; TE, trolox equivalent.

* Corresponding author.

** Corresponding author.

E-mail addresses: tomasz.dymerski@gmail.com (T. Dymerski), shela.gorin@mail.huji.ac.il, gorin@cc.huji.ac.il (S. Gorinstein).

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of each above-mentioned fruits. It is extremely important to determine terpenes in fruit products, because of their health-promoting effect or on the other site their excess can cause health problems. One of the most popular food products made from limes is juice. Key lime juice is used as an additive to beverages or sauces, oppositely, Kaffir lime juice has sour and bitter taste and very often is classified as an industrial waste. In many countries for economic reasons adulteration investigations of products containing Key lime with Kaffir lime juice is provided. The major chemical compounds found in the Kaffir lime juices volatile fraction may have potential allergic effects (Rubel, Freeman, & Southwell, 1998) as well as a large number of antioxidants may induct allergic diseases (Allan, Kelly, & Devereux, 2010). Assessment of the authenticity of juices is also important for food industry. It prevents producers from material losses due to contamination of the production line. Therefore, it is extremely important to develop an analytical method to identify possible botanical origin of limes.

The applications of two-dimensional gas chromatography (GC×GC) and time of flight mass spectrometry (TOFMS) to analyze aroma of food products are shown in a number of reports (Bogusz Junior et al., 2015; Dymerski et al., 2015; 2016). Two-dimensional gas chromatography is useful tool to analyze fruit samples. Aroma profile of the volatile fraction of apples, pears, and quince fruit were performed (Schmarr & Bernhardt, 2010). In turn, 3-methylbutan-1-ol, 3-methylbutan-1-ol acetate, 2-phenylethyl acetate and phenylethyl alcohol were selected as compounds characteristic for banana smell (Capobiango et al., 2015). Using GC×GC-TOFMS technique it was also possible to quantify the volatile compounds of different kinds of berries (Dymerski et al., 2015). Untargeted analysis was also performed after the postharvest and the storage of apples (Risticevic, Deell, & Pawliszyn, 2012). It was also possible to indicate terpenes in the samples of grapes (Banerjee et al., 2008; Rocha, Coelho, Zrostlíková, Delgadillo, & Coimbra, 2007) and blueberries (Kupska, Chmiel, Jędrkiewicz, Wardencki, & Namieśnik, 2014). Strawberries growing in Australia have been distinguished due to their botanical origin (Samykanno, Pang, & Marriott, 2013) and different varieties of chili due were classified according to their species (Bogusz Junior et al., 2015). Strawberries were also examined in order to analyze profile of volatile fraction (Williams, Ryan, Olarte, Marriott, & Pang, 2005). Dymerski et al. (2016) classified samples of cranberries, blueberries and cranberries. It is also possible to determine the pesticide residues in fruit samples (Zrostlíková, Hajšlová, & Cajka, 2003).

The composition of the volatile fraction of essential oil of *C. aurantifolia* was analyzed using GC-MS by Spadaro et al. (2012). Analysis of volatile fraction of Kaffir lime was performed using GC-MS technique. It was possible to select 15 major chemical responsible for the flavor of Kaffir lime (Kasuan et al., 2013). Nevertheless, there are no literature reports about authenticity markers of above-mentioned types of limes, including also the use of two-dimensional gas chromatography.

Similarly, the situation is revealed in case of studies concerning the comparison of antioxidant activities of Kaffir and Key fruit juices. There are only a few investigations, in which a total phenolic and flavonoid contents, ferric reducing antioxidant power (FRAP) and 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity were determined (Ghafar, Prasad, Weng, & Ismai, 2009). The characterization of lime juices from the point of their antioxidant status is important. Therefore, the aim of this study was to compare Kaffir and Key lime juices regarding to their aroma and antioxidant properties. For this reason, the advanced analytical methods were elaborated, with the use of two dimensional gas chromatography coupled with time-of-flight mass spectrometry, ^{1}H NMR spectroscopy and the investigation concerning antioxidant properties, using a number of radical scavenging assays were included. According to the best of our knowledge, there are no literature reports about the quantitative determination of selected terpenes of above-mentioned juices using spectrometric methods and there is a lack of information about comparison of these matrices in respect of their bioactivities and NMR shifts in the aromatic region. Such investigations are very important for food control of the prepared limes juices.

2. Materials and methods

2.1. Chemicals

Analytical terpene standards: α-Pinene, Limonene, Citronellal, Aromadendrene, Camphene, Linalool, Nerol, *trans*-Geraniol, β-Pinene, Terpinen-4-ol, Myrcene, γ-Terpinene, α-Terpineol, Citral (Sigma-Aldrich, St. Louis, MO, USA) were used to prepare standard solutions for calibration step. Methanol (Avantor Performance Materials Poland S.A) was used as a solvent of these solutions. Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid); 2,2′-azobis-2-methyl-propanimidamide; $FeCl_3x6H_2O$; Folin—Ciocalteu reagent (FCR); Tris, tris (hydroxymethy1)aminomethane; lanthanum (III) chloride heptahydrate; $CuCl_2\times2H_2O$; and 2,9-dimethyl-1,10-phenanthroline (neocuproine), 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium persulfate, deuterated chloroform ($CDCl_3$), deuterated methanol-d4 (CH_3OH-d4), and deuterium oxide (D_2O) were obtained from Sigma Chemical Co., St. Louis, MO, USA. 2, 4, 6-tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionized and distilled water were used throughout.

2.2. Sample preparation

The objects of study were the pulps of Kaffir lime (*Citrus hysteria, Citrus hystrix*) and Key lime (*Citrus aurantifolia*). The samples of Kaffir lime fruits were imported from Thailand where they had been bought on the floating market in Taling Chan, which is located in the western part of Bangkok. Samples were transported to Poland in sealed plastic bags in portable fridge maintained at between 10 and 15 °C. Key limes were bought in local distribution point in Poland. According to the seller's information, the country of origin of the fruit was Brazil.

In order to prepare for analysis, the fruits were washed with tap water and rinsed with distilled water. The fruit peel was then separated from the pulp and then squeezed to obtain the juices (Fig. 1). The next step was to weigh out 5.0 ± 0.1 g of sample unified composition in vials of 20 mL and then 1 mL of deionized water was added to the sample. The vials were closed with caps with silicone Teflon membrane. The procedure was repeated three times for each species of lime, each time using a new fruit.

2.3. Isolation and enrichment of analytes

Solid phase microextraction was used to carry out isolation and enrichment of analytes. The extraction was conducted using the divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber with thickness of 50/30 μm and length of 2 cm (Sigma-Aldrich, St. Louis, MO, USA). The extraction was carried out at 40 °C for 35 min. After this step the thermal desorption of the analytes at temperature of 250 °C for 5 min was provided. Between each analysis fiber was desorbed at 250 °C for 5 min. Extraction step was made using a MPS autosampler (Gerstel Co., Mülheim, Germany).

2.4. Instrumentation

Two-dimensional gas chromatograph Agilent 7980 (Agilent

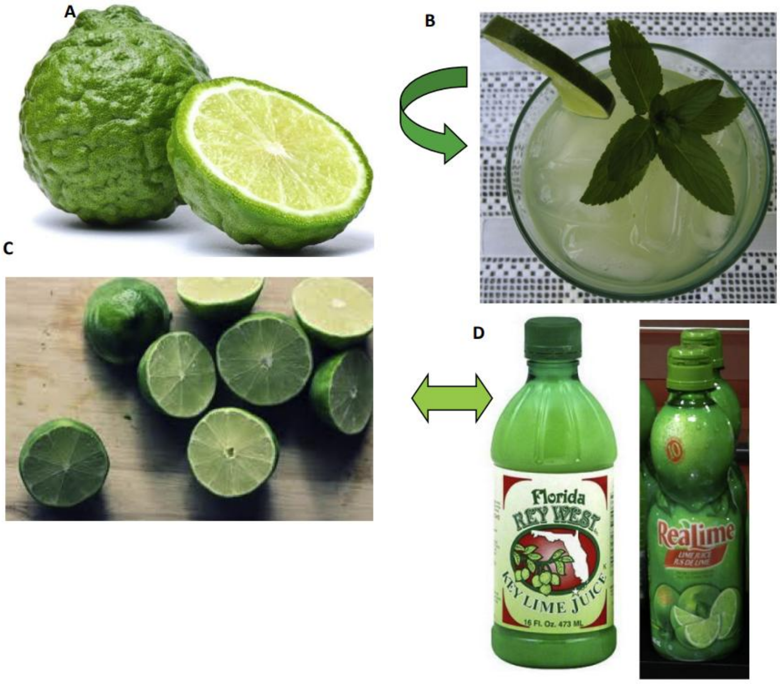


Fig. 1. A, B, C, D, Kaffir lime, Kaffir juice, Key lime, Key juice.

Technologies, Palo Alto, CA, USA) equipped with a liquid nitrogen cooled two-stage cryogenic modulator and the dispenser, working in spilt/splitless mode was used to carry out the analysis. Different types of chromatography columns were chosen to provide proper separation according to the rule of orthogonality. Table 1 shows the column parameters. Separation of substances was done by using the following temperature program: initial temperature of 40 °C was held for 3.5 min, then a linear increase of temperature to 250 °C at a rate of 5 °C/min was applied. The final temperature was held for 5 min. The temperature program applied in the secondary oven was set up with 5 °C shift. Modulation period was set up to 4 s. As the cooling medium, the liquid nitrogen was used, and hydrogen of purity N 6.0 was utilized as a carrier gas.

The volumetric flow rate was 1 mL/min. A single run time was 43.5 min. The injector worked in splitless mode at temperature of 250 °C. Temperature of transfer line and ion source was also 250 °C. The voltage of detector was set up at 1600 V. The data were collected over a mass range of m/z from 40 up to 500 with the acquisition rate of 125 spectra/s.

2.5. *Data analysis*

To identify the chemicals time of fight mass spectrometer Pegasus 4D produced by LECO (LECO Corp., St. Joseph, MI, USA) was used. Processing of data was done automatically using chromatographic peak deconvolution algorithm implemented in the software ChromaTOF (LECO Corp., version 4.44.0.0). Tentative identification of analytes was made by comparing experimental spectra with the spectra included in NIST 11 and Wiley libraries and by comparing calculated linear temperature-programmed retention indices (LTPRIs) with literature values. LTPRI values were calculated by performing analysis of C8–C20 n-alkanes. Positive identification was done using analytical terpenes standards.

2.6. *Determination of bioactive compounds and total antioxidant capacities (TACs)*

Polyphenols were extracted with methanol and water (concentration 20 mg/mL) during 1 h in a cooled ultrasonic bath. Total polyphenols (mg gallic acid equivalents (GAE)/g DW) were

Table 1
The parameters of chromatographic columns.

Parameters	I dimension column	II dimension column
Type:	capillary	capillary
Length:	30 m	1.6 m
Internal Diameter:	250 µm	100 µm
Maximum temperature:	325 °C	280 °C
Trade name of stationary phase:	Equity 1 (Supelco, Bellefonte, PA, USA)	SGWAX (SGE Analytical Science, Austin, TX, USA)
The film thickness of the stationary phase	0.25 µm	0.10 µm

determined by Folin-Ciocalteu method using spectrophotometer (Hewlett-Packard, model 8452A, Rockvile, USA) and measuring obtained absorbance after the complex reaction at wavelength of 750 nm (Singleton, Orthofer, & Lamuela-Raventos, 1999). Anthocyanins were determined by the measuring of absorbances of lime extracts (1 g of the defatted sample was extracted with 1 mL of acetonitrile containing 4% acetic acid) at 510 nm and 700 nm in buffers at pH 1.0 and 4.5, and calculated using following equation: $A = [(A_{510} - A_{700})_{pH1.0}$-$(A_{510} - A_{700})_{pH4.5}]$ with a molar extinction coefficient of cyaniding-3-glucoside of 29, 600. Results were expressed as milligrams of cyaniding-3-glucoside equivalent per 100 g dw (Cheng & Breen, 1991). Total carotenoids (xanthophylls+carotenes) were extracted with 100% acetone and determined spectrophotometrically at different absorbances (nm) such as at 661.6, 644.8, and 470, respectively (Boyer, 1990). Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3$ x H_2O and 1 M NaOH, were measured at 510 nm. Total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde method, and the absorbance was measured at 640 nm (Feucht & Polster, 2001). The extracts of condensed tannins (procyanidins) with 4% vanillin solution in MeOH were measured at 500 nm. (+)Catechin served as a standard for flavonoids, flavanols and tannins as previously was described in details (Leontowicz et al., 2016). Total ascorbic acid was determined by CUPRAC assay in water extract (100 mg of lyophilized sample and 5 mL of water). The absorbance of the formed bis (Nc)-copper (I) chelate was measured at 450 nm (Ozyurek, Guclu, Bektasoglu, & Apak, 2007).

TACs were determined using the following methods:

2, 2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method. ABTS radical cation was generated by the interaction of ABTS (7 mM/L) and $K_2S_2O_8$ (2.45 mM/L). This solution was diluted with methanol and the absorbance was measured at 734 nm (Re et al., 1999).

Ferric-reducing/antioxidant power (FRAP): FRAP reagent (2.5 mL of a 10 mmol ferric-tripiridyltriazine solution in 40 mmol HCl plus 2.5 mL of 20 mmol $FeCl_3xH_2O$ and 25 mL of 0.3 mol/L acetate buffer, pH 3.6) of 900 µL was mixed with 90 µL of distilled water and 30 µL of asparagus extract samples as the appropriate reagent blank and absorbance was measured at 595 nm (Benzie & Strain, 1996).

1, 1-Diphenyl-2-picrylhydrazyl method (DPPH) solution (3.9 mL, 25 mg/L) in methanol was mixed with the samples extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable. The scavenging rate on DPPH radicals was calculated (Brand-Williams, Cuvelier, & Berset, 1995).

Cupric reducing antioxidant capacity (CUPRAC): To the mixture of 1 mL of copper (II)-neocuproine and NH_4Ac buffer solution, acidified and non acidified methanol extracts of lime (or standard) solution (x, in mL) and H_2O [(1.1-x) mL] were added to make the final volume of 4.1 mL and the absorbance was measured at 450 nm (Apak, Guclu, Ozyurek, & Karademir, 2004).

2.7. Sample extraction and ^1H NMR analysis

Fine powder freeze dried material of 70 mg of each sample was added with either 700 µL of $CD_3OD + D_2O$ (ratio 1:1) or 700 µL of $CDCl_3$. The suspension (in a 1.5 mL Eppendorf tube) was ultrasonicated at room temperature for 30 min. And then, the suspension was centrifuged at 13.000 rpm for 10 min. The supernatant was transferred into 5 mL NMR tube and analyzed for its ^1H NMR. $CD_3OD + D_2O$ aimed to extracts polar metabolites, while $CDCl_3$ extracted non polar metabolites. All NMR experiments were recorded on Bruker 500 NMR spectrometer equipped with a 5-mm PABBO BB-probe head (499.953 for ^1H shifts) at 25 °C. NMR data processing was performed using MestReNova software (Abdul Hamid et al., 2017; Drzewiecki et al., 2016).

2.8. Statistical and classification analysis

The results of quantitative analysis were expressed as mean value and standard deviation (SD) of three measures of concentrations. Differences between groups were analyzed using two-way analysis of variance (ANOVA) followed by Duncan's new multiple range test with $\alpha = 0.05$. The analysis was carried out using STATISTICA 12 (StatSoft, Inc., Tulsa, Oklahoma, USA).

The peak areas obtained by GC×GC-TOF-MS analysis were used to sample classifications. Orange Canvas Data Mining (Bioinformatics Lab, University of Ljubljana, Slovenia) was used to perform Support Vector Machine (SVM), Tree Classification (TC), Naïve Bayes (NB) and Random Forest (RF) classifications with 2-fold cross-validation. The target class was the average over classes. All the classifiers were taken with their optimal settings.

3. Results and discussion

3.1. Composition of volatile substances

Detected chemical compounds were grouped according to their chemical classes (Fig. 2).

Comparing the volatile fractions of both species of fruits, it can be observed that the most numerous groups of chemical compounds presented in the volatile fraction, are terpenes. They represent nearly 88% of all volatile substances present in the Kaffir lime pulp, while in case of *Citrus aurantifolia* is about 53%. Kaffir lime pulp is therefore contains more aromatic compounds than Key lime. Additionally, high content of terpenes, which are considered as bioactive chemical compounds, makes this fruit as a rich source of prohealth constituents. In addition, 30% of difference in terpenes content explains the different odors of both fruits. It is well proven that the aroma of citrus fruits is composed of complex mixture of terpenes, which are chemical compounds whose main skeleton was formed by the combination of five-carbon isoprene units (Sharon-Asa et al., 2003). They are therefore the main group of Kaffir lime compounds and have a complex of bioactive properties such as antioxidant, antimicrobial or antiulcer effects (Al-Doghairi, El-Nadi, Elhag, & Al-Ayedh, 2004). Based on these properties, Kaffir limes can be classified as a superfruit, which were characterized by pro-health properties backed up by scientific research, contained bioactive compounds, stand out in exotic origin and taste. Alcohols, esters are the groups of chemical compounds with the smallest contribution in the composition of volatile fraction of *Citrus Hysteria*, which do not exceed 1%. In *Citrus aurantifolia* their content in volatile fraction is about 3%. Hydrocarbons are the next group and their percentage is more than 10%. Ketones represent 4% and aldehydes only 2% of the total content of the headspace of Kaffir lime. In case of *Citrus aurantifolia*, the content of aldehydes is similar. Such a distribution of all compounds is responsible for the smell of the fruits. Characteristic, intense scent is caused by the high content of terpenes compounds. Due to the very low content of carboxylic acids (<1%), the aroma of Kaffir lime has some irritating odor, which could cause some negative sensory perceptions during food control. To compare the content of selected terpenes, an analysis of *Citrus aurantifolia*, a fruit very popular and widely available in Europe, was also conducted. The object of the study was the juice of the above-mentioned lime fruits. The content of different terpenes for Kaffir and Key limes based on the results of Table 2 differ between them. In the volatile fraction of Kaffir lime, 119 terpenes were detected, and in case of Key lime - 87. Only chemical compounds with the mass spectral match factor, similarity >800 were considered.

The volatile fractions of both species of limes with the identified major chemical compounds are shown in Table 2. They were

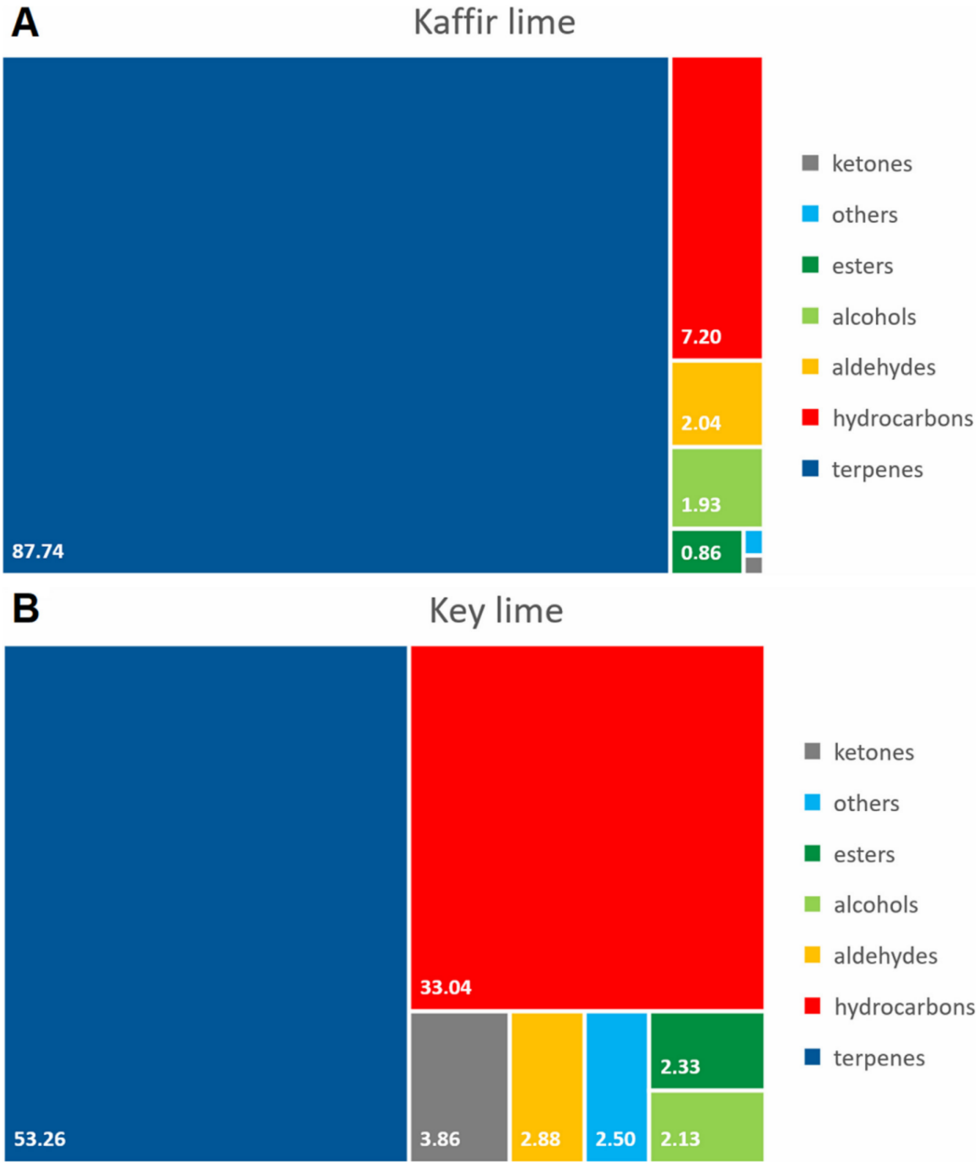


Fig. 2. Distribution of volatiles by chemical classes for: A. Kaffir lime, B. Key lime.

identified based on comparing spectra and LTPRI with literature data and the retention times were compared with retention time of internal standard. As it can be seen, all of the compounds belong to the terpenes family.

It can be observed, that the biggest differences in the content of selected terpenes in Kaffir and Key limes occur in case of chemical compounds such as Limonene, Citral, Terpinen-4-ol (Table 3). Limonene concentration is almost 8 times higher in the Key lime volatile fraction than in Kaffir lime. Extremely low content of Limonene compound in Kaffir lime was also found in citrus fruits (Waikedre et al., 2010). In the case of Citral, the difference in concentration of this compound in Kaffir lime is almost 20 mg/kg lower than in Key lime. Higher concentration of Terpinen-4-ol was noted in Kaffir lime samples and the content was almost 20 times higher. Terpinen-4-ol is the major chemical compound of volatile fraction of Kaffir lime. Terpinen-4-ol was selected as a major component of *Citrus hystrix* essential oil (Waikedre et al., 2010). For the other terpenes, differences in the contents are not statistically significant. The concentrations of α-Pinene, Citronellal, Camphene, Nerol, *trans*-Geraniol and β-Pinene are at similar levels in the volatile fraction of both fruits. In addition, in both cases the amount of the discussed analytes does not exceed 5 mg/kg. Terpenes, whose numbers in the fraction of fruits don't reach 10 ppm, are Myrcene and γ-Terpinene. The variation in the amount of the compounds explains the significant differences in taste and aroma of both fruit species. Among the determined terpenes, potential markers of Key

Table 2
The major compounds identified in the volatile fraction of Kaffir and Key limes using GC×GC-TOF-MS.

No.	Chemical compound	RT1 [s]	Average RT2 [s]	Similarity	Unique mass	$LTPRI_{lit}$	$LTPRI_{calc}$
1	β-Pinene	862	1.3	854	93	962	963
2	Sabinene	1386	2.3	939	93	958	959
3	Citronellal	1114	1.8	886	69	1132	1131
4	Linalool	1060	2.3	745	71	1082	1083
5	α-Terpineol	1294	1.4	810	59	1289	1289
6	β-Citronellol	1238	2.8	928	69	1211	1209
7	Citronellyl acetate	1402	1.7	916	69	1335	1337
8	α-Copaene	1757	1.4	937	161	1353	1354
10	α-Cubebene	1434	1.23	870	161	1345	1348
11	β-Caryophyllene	1580	1.7	917	93	1421	1420
12	Limonene	945	1.5	936	93	1022	1024
13	Germacrene D	1536	1.2	907	161	1486	1485
14	α-Pinene	796	1.3	861	93	949	950
15	Capmhene	812	1.3	951	91	953	956
16	Terpinen-4-ol	1170	2.3	864	71	1163	1060
17	α-Terpinene	930	1.4	939	93	1010	1008
18	Myrcene	857	1.4	808	93	994	993
19	α-Phellandrene	880	1.4	915	93	991	992
20	α-Thujene	1286	3.7	899	93	923	923
21	γ-Terpinene	1042	1.7	914	93	1050	1052
22	β -Phellandrene	850	1.3	836	93	1031	1030
23	Citral	1250	2.4	885	69	1240	1241
24	Nerol	1242	3.1	923	69	1228	1231
25	Geraniol	1318	3.9	890	69	1233	1232
26	Aromadendrene	1720	1.8	851	91	1455	1456

RT 1 – first dimension retention time, RT 2 – second dimension retention time, LRI_{calc} – Linear Retention Index calculated; LRI_{lit} – Linear Retention Index reported in the literature for DB 1 or equivalent stationary phase.

Table 3
Concentration of selected terpenes in the volatile fraction of Kaffir and Key limes.

No.	Chemical compound	R^2	LOQ	LOD	Concentration ± SD [mg/kg]	
					Kaffir lime	Key lime
1	α-Pinene	0.995	1.08	0.36	3.07 ± 0.03	1.04 ± 0.01
2	Limonene	0.994	1.22	0.40	10.78 ± 0.17	50.5 ± 2.1
3	Citronellal	0.992	0.25	0.49	<LOQ	0.55 ± 0.01
4	Aromadendrene	0.999	0.47	0.15	1.00 ± 0.07	<LOQ
5	Camphene	0.996	1.00	0,33	4.86 ± 0.67	3.38 ± 0.34
6	Linalool	0.990	1.69	0.56	20.13 ± 0.71	3.45 ± 0.09
7	Nerol	0.990	1.67	0.55	<LOQ	2.77 ± 0.10
8	*trans*-Geraniol	0.991	1.53	0.51	<LOQ	1.86 ± 0.10
9	β-Pinene	0.995	1.11	0.37	<LOQ	2.10 ± 0.11
10	Terpinen-4-ol	0.997	0.86	0.28	44.79 ± 1.09	1.96 ± 0.06
11	Myrcene	0.994	1.24	0.41	22.36 ± 0.95	24.89 ± 0.96
12	γ-Terpinene	0.993	1.32	0.44	25.01 ± 0.28	19.41 ± 0.69
13	α-Terpineol	0.996	0.98	0.32	1.50 ± 0.06^a	0.68 ± 0.02^a
14	Citral	0.990	1.64	0.54	<LOQ	20.91 ± 0.60

LOQ - limit of quantitation, LOD-limit od detection, SD - standard deviation, Mean ± SD of 3 measurements, Averages in rows marked with the same letters not differ significantly ($P \geq 0.05$), LOQ and LOD were calculated of the materiality level $\alpha = 0{,}05$.

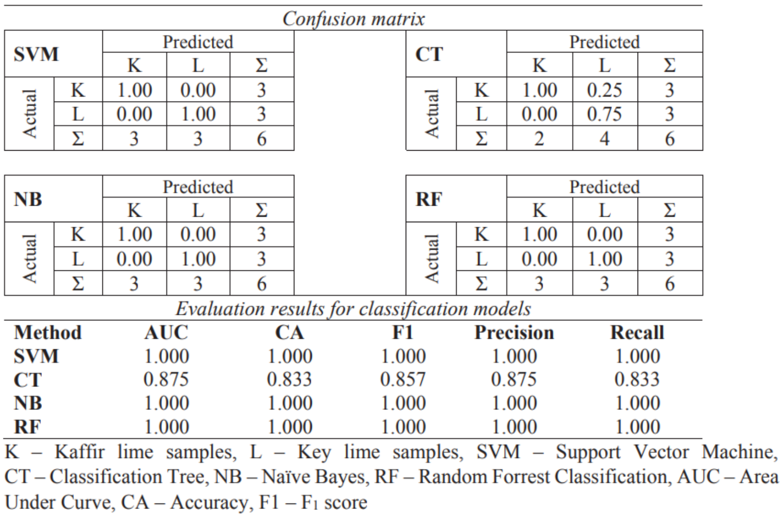
and Kaffir limes aroma were selected. It was chosen that 20-fold difference in content was used as a criterion for qualifying a chemical compound to a group of potential indicators. In the case of Kaffir lime, Terpinen-4-ol was selected as a marker, with a content of 44.79 mg/kg. This is a chemical compound with a characteristic woody aroma. In the case of Key lime, Citral is an indicator with fresh citrus scent. The presence of the two chemical compounds mainly determines the smell of the fruits. Although Limonene's most important ingredient in Key Lime was not chosen as a flavor factor, because it is the most abundant chemical compound in many citrus juices (Moufida et al., 2003). Citral was repeatedly listed as one of the Key lime component (Cruz-Valenzuela, Tapia-Rodriguez, Vazquez-Armenta, Silva-Espinoza, & Ayala-Zavala, 2015). The content of Citral in lime up to 5% was found and this refers to essential oil (Costa et al., 2014; Spadaro et al., 2012). The results of quantitative determination of these chemical compounds in lime juices have not been done so far. A quantitative analysis of 5 citrus fruits shows that in each case Limonene is a major component, which confirms the correctness of the research. Key lime and Kaffir lime were not found among the fruits which were tested and Citral content was not determined (Moufida et al., 2003).

The chromatographic peak area was used as an input data for chemometric analysis. Data set was preprocessed before classification. Preprocess was based on center by mean and scaling using standard deviation SD. Orange Canvas was used for training of four classifiers and for performance cross validation evaluation. Table 4 shows the confusion matrices and evaluation results for used classification models, for results obtained by chromatographic technique. Proportion of predicted are presented in confusion matrices. All samples were correctly classified when 3 statistical models were used: SVM, NB and RF. CT and RF models are based on simple decision trees. SVM and NB models use more complex algorithms and therefore perform better. Based on results, the superiority of the RF model over CT can be observed. This may be due to the fact that CT is a single decision tree, while RF is a collection of decision trees. Table 4 with evaluated results contains information about area under curve (AUC), accuracy (CA), F1 score, precision and recall (sensitivity). When values of all described parameters are equal 1.00, the model is perfect. These results were achieved in three cases: SVM, NB and RF. In summary, the combination of GC×GC-TOF-MS technique with chemometric analysis may be a useful tool for classifying lime juice in terms of botanical origin. The most reliable results were achieved when 3 statistical models were used (SVM, NB and RF).

3.2. Antioxidant properties of juices

The results of bioactive compounds in the juices of two lime cultivars are presented in Table 5. In order to compare the changes in the amount of bioactive compounds, polyphenols and antioxidant capacities their values were determined in the pulp of the

Table 4
Data obtained by using GC×GC-TOF-MS and four statistical models.



Confusion matrix

SVM		Predicted		
		K	L	Σ
Actual	K	1.00	0.00	3
	L	0.00	1.00	3
	Σ	3	3	6

CT		Predicted		
		K	L	Σ
Actual	K	1.00	0.25	3
	L	0.00	0.75	3
	Σ	2	4	6

NB		Predicted		
		K	L	Σ
Actual	K	1.00	0.00	3
	L	0.00	1.00	3
	Σ	3	3	6

RF		Predicted		
		K	L	Σ
Actual	K	1.00	0.00	3
	L	0.00	1.00	3
	Σ	3	3	6

Evaluation results for classification models

Method	AUC	CA	F1	Precision	Recall
SVM	1.000	1.000	1.000	1.000	1.000
CT	0.875	0.833	0.857	0.875	0.833
NB	1.000	1.000	1.000	1.000	1.000
RF	1.000	1.000	1.000	1.000	1.000

K – Kaffir lime samples, L – Key lime samples, SVM – Support Vector Machine, CT – Classification Tree, NB – Naïve Bayes, RF – Random Forrest Classification, AUC – Area Under Curve, CA – Accuracy, F1 – F_1 score

Table 5
Bioactive substances per g dry weight (DW) in (A) water (W) and methanol (M) extracts and (B) vitamin C, anthocyanins, chlorophylls and carotenoids of Kaffir and Key limes juices.

Indices	Kaffir limeW	Kaffir limeM	Key limeW	Key limeM
Polyph,mgGAE	23.16 ± 2.18^a	15.79 ± 1.34^b	11.93 ± 7.65^c	12.78 ± 1.32^{bc}
Flavan, μgCE	91.48 ± 8.54^a	83.16 ± 6.54^b	64.44 ± 5.43^{bc}	51.18 ± 5.14^c
Flavon, mgCE	0.62 ± 0.05^b	1.41 ± 0.14^a	0.36 ± 0.05^c	0.65 ± 0.04^b
Tannins,mgCE	1.92 ± 0.19^{bc}	12.91 ± 2.11^a	0.78 ± 0.07^c	9.12 ± 0.85^b
ABTS,μMTE,	161.64 ± 8.54^a	75.43 ± 6.32^{bc}	57.49 ± 5.56^c	61.05 ± 7.65^b
FRAP, μMTE,	28.50 ± 2.43^a	20.01 ± 2.23^b	14.68 ± 1.32^c	16.20 ± 1.65^{bc}
CUPRAC,μMTE	124.28 ± 5.43^a	57.13 ± 5.21^b	27.96 ± 2.67^c	30.97 ± 2.45^{bc}
DPPH, μMTE	33.29 ± 3.21^a	14.83 ± 1.32^b	12.03 ± 1.18^c	17.15 ± 1.45^{ab}

Indices	Kaffir lime	Key lime
Vit C,mgAsc	1.74 ± 0.17^a	2.44 ± 0.11^b
Anthoc, mgCGE/kg	63.45 ± 5.15^a	47.32 ± 4.11^b
Chlor a, μg	468.50 ± 12.12^a	432.16 ± 10.98^a
Chlor b, μg	260.08 ± 7.65^a	245.76 ± 5.32^a
Chlor a+b,mg	729.00 ± 16.54^a	677.92 ± 12.54^a
Xan+Carot, μg	217.11 ± 5.32^a	195.43 ± 4.43^a

Mean ± SD (standard deviation) of 5 measurements. Averages in rows marked with different letters differ significantly ($P < 0.05$).

fruits and also in the fresh prepared juices. The differences were not significant and the decrease was about 1–2%. The polyphenols in water extract of Kaffir lime were twice higher than in Key lime, but in methanol extract were nearly equal. The bioactive compounds in Kaffir lime such as polyphenols, flavanols, flavonoids, and tannins were in water extract higher than in methanol of about 1.5 times. The antioxidant values were in similar correlation in 4 applied methods and the highest values showed ABTS and CUPRAC assays in water fraction (Table 5A). It means that most of bioactive substances were in the water extract. Key lime samples showed different picture. The amount of polyphenols, flavanols, flavonoids and tannins were slightly higher in methanol extract than in water. The corresponding values of antioxidants were in direct correlation with polyphenols. Anthocyanins, chlorophylls and carotenoids were similar in two limes, accept only the amount of vitamin C in Key lime (Table 5B). The results presented in Table 5 can be compared only with a few data from cited reports such as Damian-Reyna, Gonzalez-Hernandez, Maya-Yescas, de Jesus Cortes-Penagos, and Del Carmen Chavez-Parga (2017). Total phenolics, total flavonoids, and ascorbic acid contents in Mexican sweet lime (*Citrus limetta*) juice were determined at two commercial maturity stages. The results of the above shown indices differ from the presented in this report, but the lime variety was different with polyphenols (725 ± 9.14 mg/L), flavonoids (45.91 ± 1.00 mg/L), and ascorbic acid (222 ± 16 mg/L). Results indicated that Mexican citric fruits were good sources of antioxidant agents. The results were compared with Barros, Ferreira, and Genovese (2012). Four citrus species (*C. sinensis*, cvs. Pera and Lima; *C. latifolia* Tanaka cv. Tahiti; *C. limettioides* Tanaka cv. Sweet lime and *C. reticulate*, cv. Ponkan) were characterized in relation to pulps and peels contents of ascorbic acid, total polyphenols and antioxidant capacities. The antioxidant capacity of citrus was correlated both to vitamin C and phenolics. Aside from citrus pulps, the peels are also good sources of bioactive compounds and minerals, and can be explored for their health promoting values in food products.

In our previous report (Arancibia-Avila et al., 2012) was shown the influence of different time durations of thermal processing on berries quality.

The antioxidant activity only of berries subjected to thermal processing for 10 and 20 min did not differ from the non thermally processed, showing high correlation between the total polyphenols, flavanols and the antioxidant activities (Arancibia-Avila et al., 2012). In another report for preservation of bioactive compounds long cold storage of smoothie-type 'Hayward' kiwifruit beverages was suggested (Park et al., 2016). Both of the treatments as thermal and cold can be applied in the future research on the freshly prepared kiwifruit and berries juices, but also on limes.

3.3. NMR shifts in juices

NMR spectra of two limes cultivars are shown on Fig. 3. The assignments of ^{1}H spectrum of limes chloroform and methanol extracts were obtained, where only peaks of aromatic regions appeared between 6 and 10 ppm. The spectra were similar for two

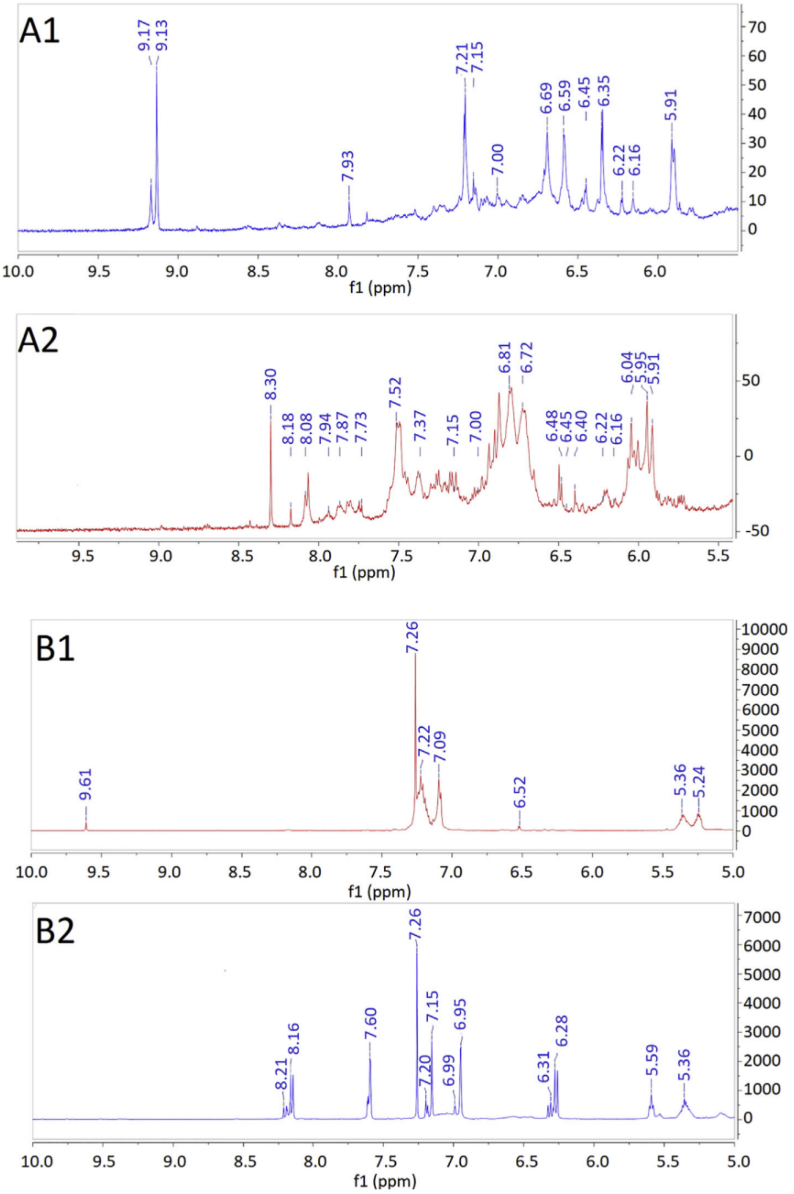


Fig. 3. Comparison of ^{1}H NMR spectra of limes juices in aromatic region: A1, A2, methanol extracts of Key and Kaffir limes; B1, B2, chloroform extracts of Key and Kaffir limes.

cultivars, but some slight differences were estimated. In the case of chloroform or methanol, mostly the sugars were the main compounds and the aromatic part was the minor (ppm). The mixture of water and methanol is usually used for extraction and subsequent NMR analysis (Fig. 3): for Key lime (Fig. 3A1: 9.17; 9.13; 7.93; 7.21; 7.15; 7.00; 6.69; 6.59; 6.45; 6.35; 6.22; 6.16; 5.91) and for Kaffir lime (Fig. 3A2: 8.30; 8.18; 8.08; 7.94; 7.87; 7.73; 7.52; 7.37; 7.15; 7.00; 6.81; 6.72; 6.48; 6.45; 6.40; 6.22; 6.16; 6.04; 5.95; 5.91) ppm. In two varieties of limes the common shifts appeared at 7.93; 7.15; 7.00; 6.45; 6.16 and 5.91 ppm. All cited literature of fruits was dealing only with aliphatic parts of juices. The aromatic range is shown in Balan, Nicolescu, Stavarache, Ciobanu, and Deleanu (2013). Most of the shifts were between 6.21 and 8.07 for orange juice: 6.65; 6.91; 7.07; 7.21; 7.32; 7.62; 7.72; and for grapefruit from 6.21 till 7.75 with the middle ones at 6.62; 6.92; 7.21; 7.38; 7.63 and 7.75 ppm. With orange and Key lime is a common shift in methanol fraction of 7.21 ppm, and for Kaffir lime at 8.08 ppm. For grapefruit juice the shifts are 6.21; 6.62; 6.91; 7.23; 7.38; 7.63; 7.75 ppm. Key lime showed a couple shifts common with grapefruit. In chloroform extract the following shift numbers appeared in Key lime (Fig. 3B1: 9.61; 7.22; 7.09; 6.52; 5.36; 5.24), Kaffir lime (Fig. 3B: 8.21; 8.16; 7.60; 7.20; 7.15; 6.99; 6.95; 6.31; 6.28; 5.59; 5.36). Common shifts for two varieties were at 5.36 ppm. It is known that phenolic compounds are poorly soluble in water, but not for lime samples (Table 5, the amount of phenolic substances). Less peaks appeared in chloroform than in methanol. This result is obtained on the basis of the amount of polypenols extracted with chloroform is less than with methanol. Previously it was described how were characterized different kiwifruit cultivars. Using the NMR approach, it has been possible to identify primary as well as secondary metabolites of different fruits such as grape, orange, apple juice, mandarin, kiwifruits, mango, black raspberry, melon, watermelon, blueberry, and peaches (Abdul Hamid et al., 2017; Balan et al., 2013; Drzewiecki et al., 2016; Sobolev et al., 2015). In NMR-based analyses, the extraction procedure is probably the most critical step aimed to the quantitative transfer of the metabolites from the solid matrix. Peaks in the aromatic region indicate naringin and hesperidin, the main flavanone glycosides in citrus fruits and from 5.10 ppm showed glucose, sucrose, fructose, and formate (Maltese, Erkelens, van der Kooy, Choi, & Verpoorte, 2012; Rosa et al., 2015).

4. Conclusions

In this paper a usefulness of two dimensional gas chromatography coupled with time-of-flight mass spectrometry and NMR shifts were proven to assess the botanical origin of Kaffir lime and Key lime juices. Regarding to this, possible falsification of mentioned juices can be detected. GC×GC-TOF-MS allows for better separation of substances originating from complex matrices than in case of using one-dimensional chromatography, because of improved resolution, increased peak capacity and unique selectivity. On the basis of obtained results potential markers of botanical origin of limes were selected, namely Terpinen-4-ol for Kaffir lime and Citral for Key lime. Presented solution can be also treated as an alternative to other analytical techniques used for determining juice adulteration, such as HPLC or isotopic measurements. Furthermore, antioxidant property assessment of these juices shows that it can be a new natural source for everyday consumption.

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4.1.2. Investigation of interactions between bioactive substances from lime juices and human serum protein

Hypothese

- *The use of two-dimensional gas chromatography with mass spectrometry as a tool to determine the terpene profile of food samples*
- *Monoterpenes as bioactive substances show good binding properties to human serum albumin*

Questions

- *Do studies of the antioxidant properties of selected juice markers reflect the properties of juices?*

Determining the pro-health properties of food is mainly based on the identification of bioactive compounds and using of conventional spectrophotometric tests for determination of the antioxidant capacity of given samples. In the case of Key lime, there are scientific reports on the determination of the antioxidant properties of these fruits, the determination of the content of polyphenols, as well as the identification of secondary metabolites that contribute to their medicinal properties [65], [66], [67]. However, there is still a lack of studies focusing on the interactions of bioactive substances with serum proteins. Determining what bioactive substances are contained in a given food sample, and then determining the affinity of this chemical compound for human protein albumin, can help understand the therapeutic use of phenols contained in food.

Key lime and Kaffir lime juices were used in the research. The first objective of the work was to determine the content of terpenes in these juices, as well as to identify substances characteristic of a given juice. Terpenes determination was performed using HS-SPME-GC×GC-TOF-MS. The binding properties of selected monoterpenes were then determined using 2D and 3D fluorescence and molecular docking. The research results were published in the *Journal of Luminescence* [68]. Based on the analysis of the terpene profile, it was shown that the greatest differences in the content of terpenes in the tested lime juices concerned limonene, citral and terpinen-4-ol. Markers of Kaffir and Key lime juices were selected as terpinen-4-ol and citral, respectively. The results of the antioxidant properties of lime juices showed that Kaffir lime juice had higher bioactive properties compared to Key lime. Results for terpinen-4-ol and citral were not proportional to Kaffir and Key lime juice data. This is because food is a complex mixture where there are synergistic antioxidant effects. Molecular docking studies have shown that HSA has active sites responsible for interactions with monoterpenes. After analyzing the results of 2D and 3D fluorescence, it was found that Kaffir lime juice showed higher binding properties to human serum albumin. Kaffir lime juice may have an important role in the development of healthy food in the future.

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Human serum interactions with phenolic and aroma substances of Kaffir (*Citrus hystrix*) and Key lime (*Citrus aurantifolia*) juices

Rajamohamed Beema Shafreen[a,*], Martyna Lubinska-Szczygeł[b], Anna Różańska[b], Tomasz Dymerski[b], Jacek Namieśnik[b], Elena Katrich[c], Shela Gorinstein[c,*]

[a] *Molecular Nanomedicine Research Unit, Centre for Nanoscience and Nanotechnology, Sathyabama Institute of Science and Technology, Chennai 600119, TN, India*
[b] *Gdańsk University of Technology, Faculty of Chemistry, Department of Analytical Chemistry, Gdańsk 80-233, Poland*
[c] *Institute for Drug Research, School of Pharmacy, Hadassah Medical School, The Hebrew University, Jerusalem 9112001, Israel*

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Lime cultivars

ABSTRACT

To understand the therapeutic application of polyphenols extracted from Kaffir (PolKaf) and Key (PolKey) limes different analytical methods were applied. Based on quantitative analysis by two dimensional gas chromatography (GC × GC) and time of flight mass spectrometry (TOFMS) it can be observed that the biggest differences in the contents of selected terpenes of Kaffir and Key limes occur in chemical compounds such as limonene, citral and terpinen-4-ol. Limonene concentration is almost 5 times higher in the volatile fraction of Key lime than in Kaffir lime. In the case of citral, the difference in concentration of this compound in Kaffir is 20 µg/g lower than in Key lime. Higher concentration of terpinen-4-ol was noted in Kaffir lime samples and the content was almost 23 times higher. Terpinen-4-ol is the major chemical compound of volatile fraction of Kaffir lime. Among the determined terpenes, potential markers of aroma were selected: terpinen-4-ol and citral for characterization of Kaffir and Key limes. Antioxidant assays revealed the highest bioactivity of Kaffir lime. Fluorescence studies between the interaction of polyphenols with human serum albumin (HSA) showed relatively high binding abilities in comparison with some antiplatelet drugs. The docking results showed that the hydrophobic residues are responsible for the interaction with the phyto-constituents. Citral is the best scored ADMET descriptor. The antioxidant strong affinity to HSA and synergism in bioactivity are the main indices in health application of citrus fruits.

1. Introduction

Juices or real pulps of some commercially grown citrus fruit (*Rutaceae*), grapefruit (*Citrus paradisi*), lemon (*Citrus limon*), lime (*Citrus x aurantiifolia*) and sweet orange (*Citrus sinensis*) were widely studied, where the phenolics and volatiles were the main antioxidant compounds found in all fruits [1,2]. Four citrus species (*C. sinensis*, *cvs.* Pera and Lima; *C. latifolia* Tanaka cv. Tahiti; *C. limettioides* Tanaka cv. Sweet lime and *C. reticulate*, cv. Ponkan) were characterized in relation to contents of ascorbic acid, total polyphenols and antioxidant capacity of pulps. The antioxidant capacity of citrus fruit was correlated both to vitamin C and phenolics. Aside from citrus pulps, the peels are also good sources of bioactive compounds and minerals, and can be explored for their health promoting values in food products [3]. There are a number of reports including Kaffir (*Citrus hystrix*) and Key (*Citrus aurantifolia*) limes. *Citrus aurantifolia* is mainly used in daily consumption and in juice production, based on antibacterial, anticancer, antidiabetic, antifungal, antihypertensive, anti-inflammatory, and antilipidemic properties. Antioxidants moreover can protect heart, liver, bone, and prevent urinary diseases. The secondary metabolites are alkaloids, carotenoids, coumarins, essential oils, flavonoids, phenolic acids, and triterpenoids. The other important constituents are apigenin, hesperetin, kaempferol, limonoids, quercetin, naringenin, nobiletin, and rutin, all of these contribute to its remedial properties [4]. The volatiles of limes have a wide application as essential oil microparticles. The use of prebiotic biopolymers can be a good option to add value to encapsulated products, thus promoting health benefits [5]. A combination of bioactive compounds makes all citrus fruits a useful source of everyday diet from a number of diseases [6–8]. As it was mentioned above there are some reports showing the composition of different kinds of limes [1,3,4], but it is a lack of research dealing with bioactive compounds and their interaction with serum proteins. The interactions between polyphenols, especially flavonoids and plasma proteins, have attracted great interest [9–13]. Few papers, however, have focused on

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* Corresponding authors.
E-mail addresses: beema.shafreen@gmail.com (R.B. Shafreen), shela.gorin@mail.huji.ac.il, gorin@cc.huji.ac.il (S. Gorinstein).

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the structure-affinity relationship of polyphenols and volatiles, on their affinities for plasma proteins, especially on human serum albumin with plant bioactive compounds [14,15] and with citrus fruits particularly [16,17]. The aim of this study was to determine the bioactive and aroma substances and their binding and antioxidant properties, using GC × GC-TOFMS, 2D- and 3D-fluorescence, spectroscopic antioxidant assays measurements and molecular docking.

2. Materials and methods

2.1. Materials

The following chemical compounds: Aromadendrene, Camphene, Citral, Citronellal, Limonene, Linalool, Myrcene, Nerol, Terpinen-4-ol, trans-Geraniol, α-Pinene, α-Terpineol, β-Pinene, γ-Terpinene, were used as standards (Sigma-Aldrich, St. Louis, MO, USA). Methanol (Avantor Performance Materials Poland S.A) was used to prepare the calibration solution mixtures. The mixture of *n*-alkanes from C8 to C20 (Sigma-Aldrich, St. Louis, MO, USA) was utilized for calculation of retention indexes. During the research deionized water of high purity from MilliQ A10 Gradient/ Elix System (Millipore, Bedford, MA, USA) was added to samples. Trolox (6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid); Folin–Ciocalteu reagent (FCR); Tris, tris (hydroxymethy1)aminomethane; lanthanum (III) chloride heptahydrate; $CuCl_2 \times 2H_2O$; and 2,9-dimethyl-1,10-phenanthroline (neocuproine), 1,1-diphenyl-2-picrylhydrazyl (DPPH), were obtained from Sigma Chemical Co., St. Louis, MO, USA. All reagents were of analytical grade.

2.2. Sample preparation

The studies were performed on juices of two lime species: Kaffir and Key limes. Kaffir lime (*Citrus hysteria, Citrus hystrix*) was bought on the floating market in Taling Chan in Bangkok. Kaffir lime samples were transported to Poland in travel fridge and the temperature was between 10^0C and 15^0C. Key lime samples (*Citrus aurantifolia*) were purchased in local market in Gdansk, where they had been imported from Brazil. During the research for each sample, three repetitions were performed. Before analysis, the fruits were washed and rinsed with distilled water. Next step was to squeeze the juice from the fruit. Samples were prepared in the proportion of 5.0 + 0.1 g of fruit pulp and 1.0 mL of deionized water. Mixtures were then transferred into 20-mL vials. All samples were sealed with caps with 20 mm thick PTFE/silicone membrane [18].

2.3. Methods

2.3.1. Two-dimensional gas chromatography (GC × GC) and time of flight mass spectrometry (TOFMS)

HS-SPME (Headspace Solidphase Microextraction) extraction was done by the GC × GC-TOFMS procedure. A Gerstel autosampler (MPS autosampler, Gerstel, Mülheim, Germany) with agitator and SPME fiber conditioning station was used to isolate and to enrich the analytes from citrus samples. Before the extraction, the samples were kept at 40 °C for 5 min. Extraction was carried out at 40 °C for 35 min using a DVB/CAR/PDMS SPME fiber of 50/30-µm thickness and 2-cm length (Sigma-Aldrich, St. Louis, MO, USA). After the extraction, the fiber was removed from the vial and transferred to the injector of a gas chromatograph for thermal desorption of the analytes at 250 °C for 5 min. The GC × GC apparatus Agilent 7890 A (Agilent Technologies, Palo Alto, CA, USA) equipped with liquid nitrogen-based quad-jet cryogenic modulator and an injector in split/splitless mode, coupled with Pegasus 4D time-of-flight mass spectrometer (LECO Corp., St. Joseph, MI, USA), were used for the analysis. Two different capillary columns were used for the analysis. The first non-polar column was Equity-1 (30 m × 0.25 mm i.d. × 0.25 µm film thickness) from Supelco (Bellefonte, PA, USA). The second column with polar stationary phase SolGel-Wax (2 m × 0.1 mm i.d. × 0.1 µm film thickness) was purchased from SGE Analytical Science (Austin, TX, USA). The chromatographic separation was performed using the following temperature program for the primary oven: Initial temperature 40 °C, kept for 3.5 min, ramped at 5 °C/min to 250 °C, and held for 5 min. The secondary oven temperature was programmed from 45 °C, kept for 3.5 min, ramped at 5 °C/min to 255 °C, and held for 5.83 min. The carrier gas was hydrogen (N6.0 class) at a constant flow rate at 1.0 mL/min. Temperature of the MS transfer line and the MS source was 250 °C. The modulation time was 4 s. The mass spectra data acquisition rate was 125 spectra/s. The data were collected over a mass range of 40–400 *m/z*. The voltage of detector was 1600 V. Analysis of the data obtained after the chromatographic analysis using GC × GC-TOFMS system was done using the algorithm for peak deconvolution implemented in the ChromaTOF software (LECO Corp., version 4.24). Analytes were tentatively identified by comparison of experimental spectra with the NIST 2011 mass spectral library. Analytes were also identified by comparing calculated linear temperature-programmed retention indices (LTPRIs) with literature values [18].

2.3.2. Determination of bioactive compounds and antioxidant activities

Polyphenols were extracted from lyophilized samples with water (concentration 20 mg/mL) during 1 h in a cooled ultrasonic bath. The extracts were filtered through the Buchner funnel. These extracts were submitted for determination of bioactive compounds. The polyphenols were determined by Folin-Ciocalteu method with measurement at 750 nm with spectrophotometer (Hewlett-Packard, model 8452 A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW [19].

The total antioxidant capacity (TAC) was determined by the following assays:

Cupric reducing antioxidant capacity (CUPRAC): To the mixture of 1 mL of copper (II)-neocuproine and NH_4Ac buffer solution, acidified and non acidified ethanol extracts of berry (or standard) solution (x, in mL) and H_2O [(1.1 − x) mL] were added to make a final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank [20].

1, 1-Diphenyl-2-picrylhydrazyl method (DPPH) solution (3.9 mL, 25 mg/L) in methanol was mixed with the samples extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable. The scavenging rate on DPPH radicals was calculated [21].

2.3.3. Fluorometric measurements and binding properties

Fluorometric measurements were used for the evaluation of binding properties of citrus extracts to human serum albumin. Two dimensional (2D–FL) and three dimensional (3D-FL) fluorescence measurements were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan. The concentrations of citrus extracts were ranged from 0 to 1.5 mg/mL, and the total accumulated volume of citrus extracts was no greater than 150 µL. The corresponding fluorescence emission spectra were then recorded in the range of 300–500 nm upon excitation at 280 nm in each case. The emission wavelength was recorded between 200 and 795 nm for three-dimensional fluorescence spectra. All solutions for protein interaction were prepared in 0.05 mol/L Tris-HCl buffer (pH 7.4), containing 0.1 mol/L NaCl [14,18].

2.3.4. Molecular docking

The Ligand Fit module of Discovery Studio (DS Version 2.5) package was used for molecular docking. The X-ray crystallographic structures of HSA, 1h9z.pdb solved at 2.5 Å and complexed with ligands was retrieved from the protein data bank (PDB) and modified for docking calculations [14]. The co-crystallized ligands and the water molecules were removed from the protein structure. The H atoms were added and side chains were fixed using the protein preparation protocol. Optimization of the atomic charges and the structure minimization was

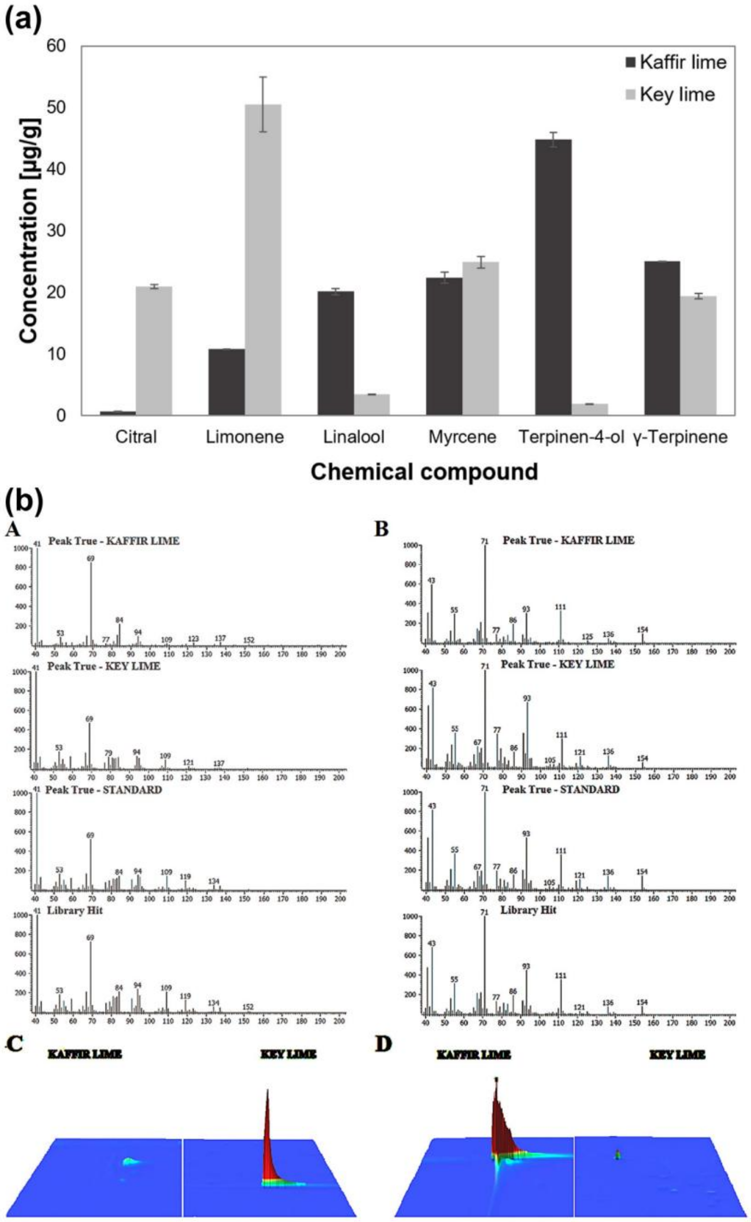

Fig. 1. **(a)** Concentrations of selected terpenes in Kaffir and Key limes juices. **(b)** Data obtained from GC × GC-TOF-MS analysis: **A**, citral mass spectrum, **B**, terpinen-4-ol mass spectrum, **C**, 3D chromatograms for citral (selected masses: 41, 69, 84, 94), **D**, 3D chromatograms for terpinen-4-ol (selected masses: 43, 77, 93, 111).

performed using CHARMM force field. To gain further insight in the interaction of Kaffir and Key lime extracts with HSA proteins, 14 active phyto-constituents detected in lime. Among which the 6 ligands based on the flavoring and aroma of the lime were selected for docking studies (Fig. 1). Two dimensional structures of these phyto-constituents were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The correct protonation states and partial charges were applied using the Ligand preparation module available with DS 2.5. Further the prepared ligands and protein structure were loaded to the docking protocol workspace. Best 10 poses were further processed and calculated [22].

2.3.4.1. In silico ADMET (absorption distribution metabolism excretion and toxicity) studies. Pharmacokinetic properties of the ligands were studied using SwissADME [23] and Molinspiration [24]. ADMET descriptor such as GI absorption, CYP1A2 inhibitor, PSA and MolLogP were predicted. The program requires the input information in SDF/MOL or SMILES file format.

2.3.5. Statistical analysis

To verify the statistical significance, means ± SD of five independent measurements were calculated. One-way analysis on variance (ANOVA) for statistical evaluation of results was used, following by Duncan's new multiple range tests to assess differences between group's means. P values of < 0.05 were considered to be significant.

3. Results and discussion

3.1. Aroma, polyphenols and antioxidant activities in lime samples

Based on Fig. 1a, it can be observed that myrcene and γ-terpinene occur in similar amounts in both types of lime juices. However, the remaining four selected terpenes show more significant differences in concentrations in the tested samples. The essential oil of *Citrus aurantifolia* [25] possesses important spasmolytic properties, which are due to their major constituents, such as limonene (58.4%), δ-pinene (15.4%), γ-terpinene (8.5%), and citral (4.4%). Limonene and citral are chemical compounds characteristic of Key lime. The concentration of limonene in Key lime is estimated to 50.5 ± 2.1 μg/g and it is higher than in Kaffir lime. In the case of citral, the concentration of this substance is more than 30-fold higher in the volatile fraction in the Key lime than in the Kaffir lime. Terpinen-4-ol and linalool are the main terpenes of the Kaffir lime volatile fraction. The content of these substances was respectively more than 20- and 5-fold higher in Kaffir lime compared to the Key lime. Above-mentioned facts can explain the variation in flavour and aroma of tested fruit species. Among the determined substances, potential indicators of Kaffir and Key limes were selected. As a criterion for qualifying a chemical compound to a group of potential markers was 20-fold difference in concentration. For Kaffir lime, terpinen-4-ol which concentration was estimated at 44.8 ± 1.1 μg/g, was selected as the indicator. This compound is characterized by a woody aroma. In the case of Key lime, the substance with fresh and citrus scent, namely citral with estimated concentration at 20.91 ± 0.60 μg/g, was chosen as a marker (Fig. 1 b). The main constituent of the volatile fraction of Key lime is limonene. This substance was not selected as a potential flavor indicator, because its presence is observed in many citrus juices.

The content of citral in lime up to 5% was found and this refers to essential oil [25,26]. The results of phenolic acids and antioxidant activities are presented in the Table 1. The amount of polyphenols in water extract in Kaffir lime was as much as twice higher than in Key lime. Citral as an indicator of Key lime was 1.5 times higher than terpinen-4-ol for Kaffir lime. The antioxidant activities were in accordance with the amount of polyphenols. There is a lack of data to compare the present results with the refereed literature. Most recent reports are connected with citrus essential oils from peels. Between four different varieties of citrus species peels *Citrus aurantifolia* contained high amounts of bioactive substances such as phenolics and flavonoids [27]. For estimation of antioxidant activity in peels the same methods as in the present research were used and correlation of total phenolics in various extracts was found [28]. Recent reports were also based on the activity of leaves from limes [29]. As it was mentioned above, there are few reports dealing with antioxidants and terpenes in lime juice and pulp. The values of bioactivity obtained in this report are in line with the recent report [30], where was concluded that the health benefits of *citrus aurantifolia* are associated with its high amounts of photochemical and bioactive compounds such as flavonoids and phenols. The phenolic content and antioxidant properties of the manually squeezed lemon juice had higher total phenol content (64.5 GAE mg/L), while lime juice had higher total flavonoid content (29.5 QE mg/L). These results were slightly lower than the found in this research. Both juices exhibited antioxidant activities as typified by their ferric reducing power, and radicals (DPPH·, ABTS·, OH·, and NO·) scavenging abilities. Lime juices showed higher antioxidant activities than lemon. The inhibition of anti-angiotensin-1-converting enzyme (ACE) activity *in vitro* and *in vivo* hypocholesterolemic effect of the juices could explain the use of the juices in the management of cardiovascular diseases [31]. Comparison of antioxidant activities of water extracts [32] of different plants showed that *Phoenix dactylifera and Citrus aurantifolia* had a significantly higher total phenol and DPPH scavenging activities than other investigated plants. The water extracts of *Phoenix dactylifera* and *Citrus aurantifolia* had the highest protective ability and this probably due to its higher antioxidant activity, total phenol content, and DPPH scavenging activity [32]. Our results are in accordance with reported in [33], where all oils showed the effects on DPPH in the range of 17.7–64.0%. The oils of Ichang lemon (64.0%, 172.2 mg TE/mL), Tahiti lime (63.2%, 170.2 mg TE/mL), and Eureka lemon (61.8%, 166.2 mg TE/mL) showed stronger radical scavengers than other citrus oils. Citrus volatile components such as geraniol (87.7%, 235.9 mg TE/mL), terpinolene (87.4%, 235.2 mg TE/mL), and γ-terpinene (84.7%, 227.9 mg TE/mL) showed marked scavenging activities on DPPH ($p < 0.05$). These numbers are in accordance with the results presented in Table 1, where terpinen-4-ol and citral showed relatively high DPPH values. The results of antioxidant activity of citral was similar to [34], where citral isolated from sweet orange possesses antioxidant activity by DPPH radical scavenging activity and cytotoxic properties, and is a potential source of active ingredients for food and pharmaceutical industry. Limonene, linalool and citral are common non-phenolic terpenoid components of essential oils, with attributed controversial antioxidant properties. Results indicate that antioxidant behavior of limonene, linalool and citral occurs by co-oxidation with the substrate, due to very fast self-termination and cross-termination of the oxidative chain [35]. Individual substances such as citral and limonene had the minimum antioxidant activities, but the antioxidant activities of their mixture were higher. The synergistic effects in the antioxidant activity and stability of the main oil components were found [36]. In another report [37] as well were discussed the similarities and differences between the antioxidant activities of some essential oils and their main components such as thymol, estragole, menthol, eugenol, carvacrol, camphor and limonene. The comparison of antioxidant values of the oils and their components shows that the

Table 1
Antioxidant and binding properties of limes and monoterpenes in water extract.

Indices	Kaffir lime	Key lime	Terpinen-4-ol	Citral
Polyphenols, mgGAE/g DW	23.65 ± 2.5^a	12.13 ± 1.1^{ab}	5.84 ± 0.6 ^{c}	8.32 ± 0.8^b
CUPRAC, μMTE/g DW	123.45 ± 10.4^a	28.34 ± 2.3^{ab}	13.43 ± 1.3^c	19.54 ± 1.8 ^{b}
DPPH, μMTE/g DW	33.87 ± 3.6^a	17.21 ± 1.6^{ab}	8.54 ± 0.8^c	12.23 ± 1.7 ^{b}
FI (peak a), A.U.	450.69 ± 9.7^b	488.90 ± 7.7^{ab}	520.80 ± 11.6^a	164.15 ± 10.3^c
FI (peak b), A.U.	709.71 ± 12.4^b	730.85 ± 5.2^{ab}	784.42 ± 6.3^a	–
FI (peak c), A.U.	168.32 ± 6.3^a	83.64 ± 2.5^b	–	–
Binding to HSA, peak a %	20.96 ± 1.5^b	19.04 ± 4.6^b	8.67 ± 0.9^c	28.79 ± 2.8^a
Binding to HSA, peak b %	16.74 ± 1.2^a	14.26 ± 0.5^{ab}	7.66 ± 0.7 ^{b}	–

Values are means ± SD of 5 measurements; Means within a row with the different superscripts are statistically different ($p < 0.05$; Student's *t*-test). Abbreviations: GAE, gallic acid equivalent; CUPRAC, Cupric reducing antioxidant capacity; DPPH, 1, 1-Diphenyl-2-picrylhydrazyl method; TE, trolox equivalent; FI, fluorescence intensity; A. U., arbitral units; per g dry weight (DW); HSA, human serum albumin; FI of HSA in water according to peak **a** is equal to 570.21 ± 9.2; peak **b is** equal to 852.40 ± 11.3.

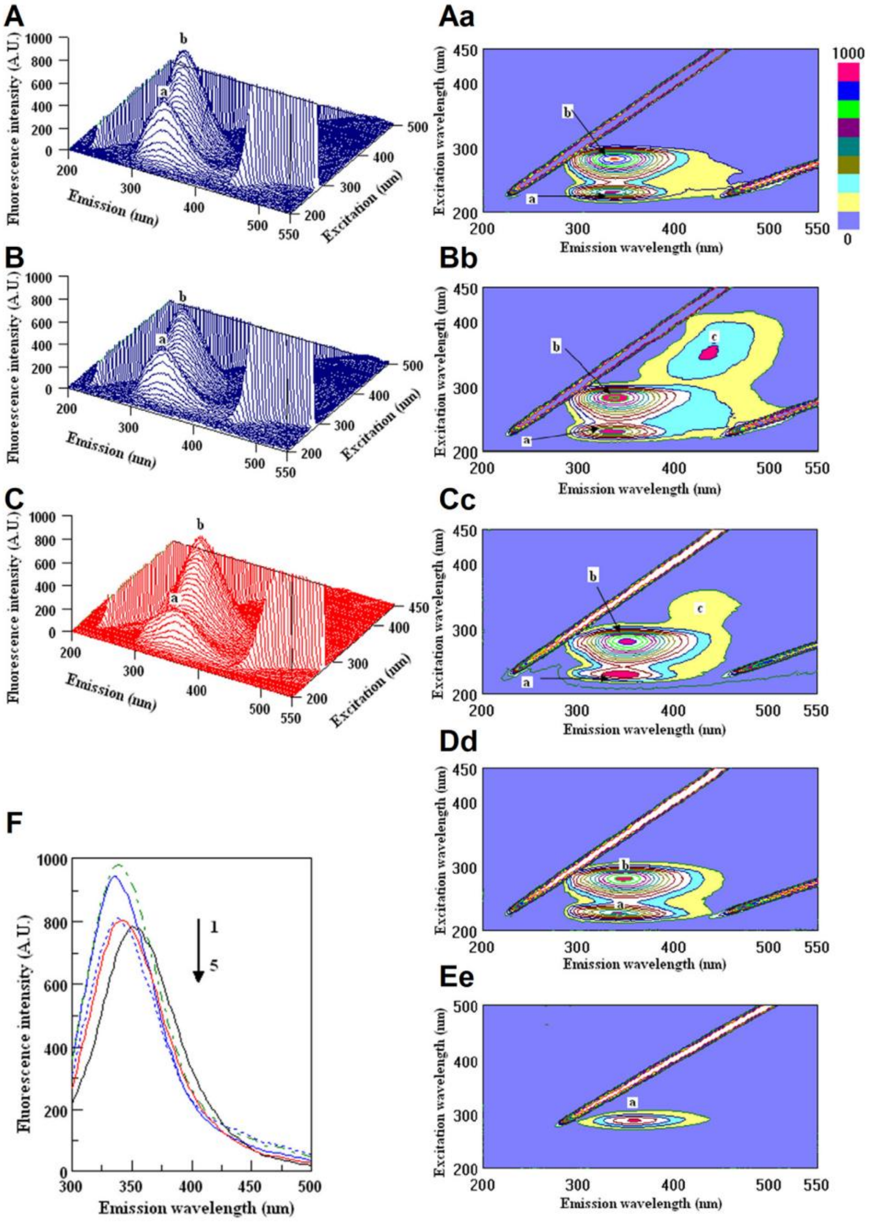

Fig. 2. 3D-fluorescence spectra of water extracts of polyphenols in interaction with HSA, **A–C**, HSA, Kaffir lime, Key lime; **Aa, Bb, Cc, Dd, Ee,** corresponding contour spectral images of HSA, Kaffir lime, Key lime, Terpinen-4-ol, Citral. The values of peaks **a**, **b** and **c** are given in Table 1. **F**, lines from the top: 1–5, HSA in buffer (FI = 980.87), HSA after interaction with water (FI = 945.87); HSA after interaction with catechin (FI = 821.00); HSA after interaction with Key lime (811.00); HSA after interaction with Kaffir lime (FI = 787.55). Measurements in 2D- FL were done in water extracts of citrus samples with concentration 0.83 mg/mL; Catechin was at concentration of 0.001 mM; λem 290 nm, λex 280 nm; HSA, human serum albumin; FI, fluorescence intensity in A.U.

antioxidant properties of essential oil do not always depend on the antioxidant activity of its main component, and that they can be modulated by their other components. The obtained results in this study support the conclusion that when comparing the antioxidant properties of essential oils and their main components, the concepts of synergism, antagonism and additivity are very relevant [34–37].

3.2. Fluorescence studies

The fluorescence properties of lime extracts and standards are shown in Table 1 and Fig. 2. The highest binding properties were in Kaffir lime (Table 1 and Fig. 2B, in two peaks). Lower binding properties in comparison with Kaffir were in Key lime (Table 1, Fig. 2C). The binding properties of the polyphenols extracted from limes were relatively high showing the correlation between the antioxidant and quenching properties of polyphenols towards human serum albumin (Table 1). Peak **b** mainly reveals the spectral behavior of Trp and Tyr residues. Peak **a** mainly exhibits the fluorescence spectral behavior of polypeptide back- bone structures, and its intensity relates to the secondary structure of the protein. Both fluorescence peaks of HSA had been quenched by limes, but to different extents (Table 1). Peak **c**, which was detected only after interaction of lime samples with HSA, did not influence the quenching and remained in the same position and with the same fluorescence intensities in two samples (Fig. 2B, C).

The results of 2D-fluorescence showed the changes in the fluorescence intensity (FI) according only to one peak, and the results of

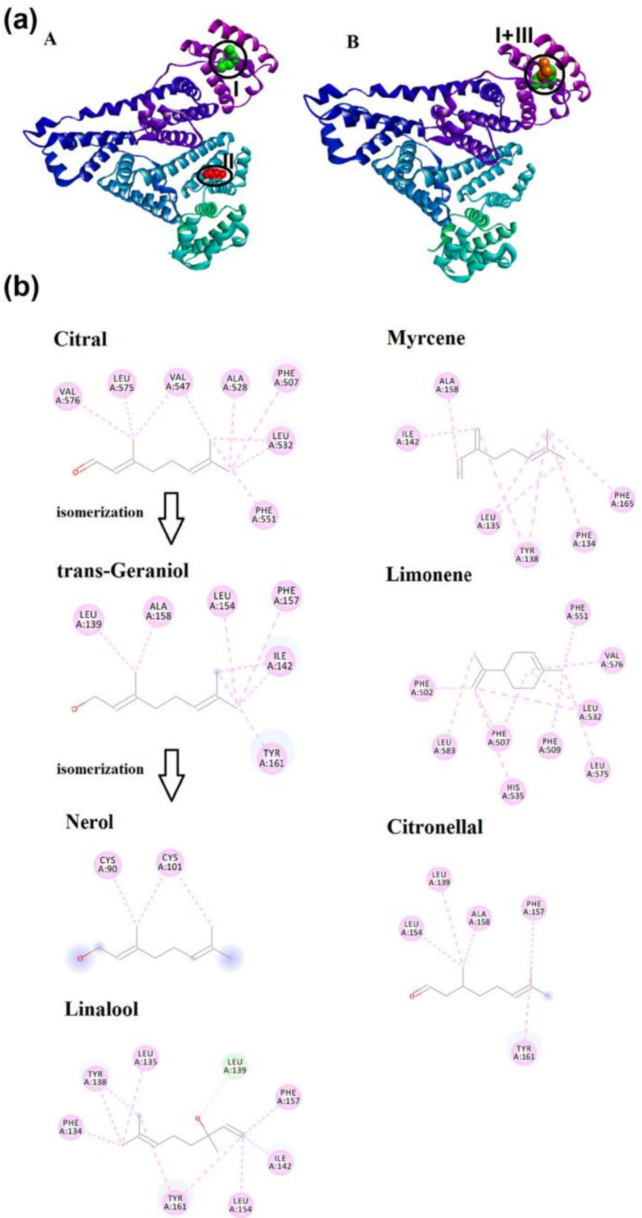

Fig. 3. **(a)** The docking images of the chemotypes. **A.** Binding ligands- citral/myrcene from the Chemotype I. **B.** Binding of the ligand - citral/limonene from chemotype II. Roman numerals represent: I-citral; II-myrcene; III-limonene. **(b)** 2D images showing the active residues in the binding site involved in the interaction of the ligands with the receptor protein (HSA).

binding (%) were the following: with catechin (13.21, Fig. 2F, line 3 from the top), Key lime (14.26, Fig. 2F, line 4 from the top) and Kaffir lime (16.74, Fig. 2F, line 5 from the top).

The present results are in agreement with [38], where the effects of three kinds of flavonoids, quercetin, rutin and baicalin, on the binding of ticagrelor to HSA were investigated using fluorescence. According to the data in [38] the binding properties of ticagrelor, a new antiplatelet drug, is about 28.8%. The addition of Kaffir lime to HSA showed increased binding of 37.7%, and with Key lime was slightly lower of 33.3%. As it was described above volatile and polyphenol substances have numerous pharmacological activities, including cardiovascular effects, antioxidant, anti-inflammatory, antiallergic, antimicrobial, antithrombotic, antiviral, antidiabetic, estrogenic and anticarcinogenic activities. Our results of interaction of volatile substances with human serum albumin are in line with other research reports. It was shown that the volatiles possess antioxidant activity similar to polyphenols, then their binding properties can be compared with polyphenols [39–41]. The present results showed that polyphenols extracted from limes quenched HSA (Table 1) similar to the drugs shown in the cited above reports.

Table 2a
Flavour profile of the phyto-constituents and interaction of the ligands with HSA.

S. No	Phyto-contituents of lime	Flavor profile	Docking Fitness	LOQ	Binding Energy (kcal/mol)	Interacting residues from HSA
1	Citral	Lemon	240.900	1.64	-89.92	VAL576;LEU575;VAL547; ALA528;PHE507;LEU532; PHE551
2	Nerol	Floral, Fruit	236.001	1.67	-9.36	CYS90; CYS101
3	Geraniol	Geranium, Lemon Peel, Passion Fruit, Peach, Rose	238.232	1.53	-66.26	LEU139; ALA158; LEU154; PHE157; ILE142; TYR161
4	Myrcene	Balsamic,Fruit, Geranium, Herb, Must	246.248	1.24	-53.75	ALA158; ILE142; LEU135;TYR138; PHE134;PHE165
5	Limonene	Citrus, orange	213.456	1.22	-56.22	VAL576;PHE551; LEU532; LEU575; PHE509;PHE507; HIS535;LEU583; PHE502
6	Citronellal	Citrus, Leaf	135.074	0.25	-64.92	LEU139; ALA158; LEU154; PHE157; TYR161
7	Linalool	Coriander, Floral, Lavender, Lemon, Rose	138.067	1.69	-61.94	TYR138;LEU135; LEU139:PHE157; ILE142;LEU154; TYR161;PHE134

Table 2b
ADMET properties of the compounds from lime.

S.no	Phyto-contituents of lime	TPSA	LogP$_{o/w}$	GI absorption	MolLogP > 4.15	Drug likeness (Lipinski)
1	Citral	17.07	2.35	High	3.49	− 1.08
2	Nerol	20.23	2.78	High	3.33	− 1.11
3	Geraniol	20.23	2.78	High	3.33	− 1.11
4	Myrcene	0.00	3.43	Low	3.91	− 1.30
5	Limonene	0.00	3.37	Low	3.83	− 1.51
6	Citronellal	17.07	2.94	High	3.37	− 0.98
7	Linalool	20.23	2.66	High	3.25	− 0.99

3.3. Molecular docking

Based on the flavoring profile, high concentration and LOQ the phyto-constituents from lime were selected and the ligands were subjected to docking with HSA. Citral, myrcene, linalool, nerol and trans-Geraniol showed good dock score compared to the other monoterpenes (Fig. 3a, b). The LOQ is also high for these compounds compared to the other volatiles. Citral at low pH and under oxidative stress can isomerize from geraniol to nerol that leads to the degradation of the citrus aroma [42]. The docking results have also shown that binding energy of the trans-geraniol and nerol is less compared to citral. The binding energy of citral was 1.44 times higher than for terpinen-4-ol [39]. This is in direct correlation with the binding properties determined by fluorescence (Table 1), because the binding of citral was 1.76 times higher than for terpinen-4-ol. This result substantiate that isomerisation of the citral from trans-geraniol to nerol can reduce the binding efficiency. Citral and limonene are major compounds available at high concentration in Key lime which gives the unique lemon odor and also makes the difference from the Kaffir lime. There are three different chemotypes reported with essential oil of lime such as Chemotype-I; citral/myrcene, Chemotype-II; citral/limonene and Chemotype-III; limonene/linalool/citronellal. Chemotypes I and II have been vastly studied as antitumor [43], antibacterial, antifungal [44] and antidepressant [45]. Citral, limonene and myrcene are the main chemical constituents of the three chemotypes. From the Ligandfit module, 24 binding sites were predicted in HSA. Citral and myrcene from chemotype-I have similar dock score and binding energy. Interestingly, citral showed interaction with amino acid residues in binding site 20 of HSA and myrcene interacts with binding site 2 of HSA. Citral/limonene under the chemotype II demonstrates interaction with the same amino acids in the binding site 2 of HSA. However, in chemotype III citronellal and linalool showed interaction with binding site 2 and limonene have interaction with residues from binding site 20 (Table 2a). Therefore from the docking results substantiate that site 2 and 20 as the active sites of HSA which is exclusively exposed with the hydrophobic residues responsible for interaction with the phyto-constituents.

3.3.1. In silico ADMET studies

In ADMET descriptors, total polar surface area (TPSA) is one of the most widely-used descriptors for predicting membrane permeability. The ligands myrcene and limonene the membrane permeability was poor compared to the other flavoring compounds of lime. Citral was detected with TPSA of 17.07. The GI absorption is used to reveal the absorption of the drug across the intestine. Intestine has a surface rich in microvilli and covers about 1000-fold of the stomach thus GI absorption of the drugs is efficient pharmacokinetic properties. Therefore among the top scoring compounds (citral, limonene and myrcene) it was observed that citral is the best scored ADMET descriptors (Table 2(b)).

4. Conclusions

Citral, myrcene and limonene are well known as plant-derived natural products finding their use in therapeutic applications in recent decades. The present investigation provides an insight into the binding properties of HSA with these pharmacologically important molecules. Chemotype II showed similar binding characteristics, with the latter have a stronger affinity to HSA. Both the compounds bind to site-2 with hydrophobic residues enclosed in the binding pocket of HSA. The data from present study can be useful in the establishment of their pharmacokinetic profiles in the process of future health food development. The binding properties of polyphenols from citrus fruits to HSA were relatively high in comparison with other plants, and it was a correlation between the binding properties and their bioactivities. This study gives evaluation of the bioactive interaction with human physiological system since HSA is the most important serum protein.

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4.2. Quality control of fruit juice samples

4.2.1. Development of an analytical method for authenticity assessment of fruit juice samples

<u>Hypothese</u>

- *Electronic nose as an effective instrument for quality control of fruit juices*
- *Volatile fingerprints combined with chemometric methods as solution for juices adulteration detection*

<u>Questions</u>

- *Which chemometric methods (unsupervised or supervised) will be more reliable?*

The authenticity of fruit juice is the preservation of its basic chemical, physical, organoleptic and nutritional properties of the fruit from which it comes [69]. Food authenticity verification is important for both food quality and safety and has become even more important due to globalization [69], [70]. In the process of food quality control, large groups of samples from several dozen to even several hundred are analyzed [69]. Therefore, methods much less time-consuming than classical analytical methods are sought.

The lack of cheap, easy, low-cost and non-destructive procedures to control the quality of fruit juices is one of the main challenges of the juice industry [71]. The solution may be the use of an electronic nose, which can be used to control the quality of food from farm to table by analyzing the fingerprints of food properties. The combination of analyses using the electronic nose with advanced chemometric methods can bring positive results. The use of e-noses is economical, high-performance and low labour compared to long and complicated conventional methods [70].

The aim of the work [72] was to combine rapid chromatographic analysis to obtain a chromatographic fingerprint with statistical analysis methods to distinguish between food products. The use of the same method for the analysis of various food products, adulterated and non-adulterated, or unprocessed and processed, enables an objective assessment of the effectiveness of the developed method, as well as determining the possibility of its practical application. The technique of ultrafast gas chromatography was used to obtain the chromatographic fingerprint. It is very important that the method of extraction of the volatile compounds does not change their profile. The solution is to obtain a profile of volatile compounds using the headspace technique, in which volatile substances migrate from the liquid or solid phase to the gaseous phase, reaching an equilibrium between the phases. During the study, an optimized HS method was used to extract volatile compounds without modifying the food matrix. The effectiveness of the applied method was assessed using chemometric methods. Similarities and differences between the analyzed samples were assessed based on the distance between the samples in the experimental space.

In the mentioned article [72], samples of NFC (*Not From Concentrate*) juices, i.e. orange juice, apple juice and mixtures of orange-apple juice, were tested. The obtained results were published in the journal *Monatshefte für Chemie - Chemical Monthly* [72].

A simplified diagram of the developed procedure is shown in Figure 5.

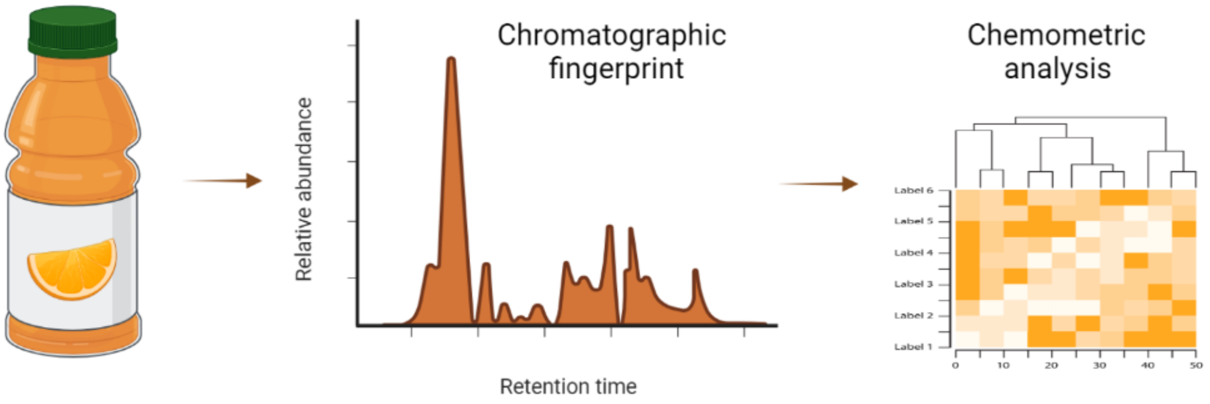

Figure 5 A simplified diagram of the methodology for assessing the authenticity of fruit juices.

The use of electronic nose along with advanced chemometric methods is an effective tool for detecting adulteration of fruit juices. Such a tool can also be useful in assessing other parameters of fruit juices or quality control of other food products. The electronic nose based on ultra-fast gas chromatography technology allow to skip the sample preparation step, and the analysis time is reduced to two minutes. This technique is characterized by a high simplicity, speed, and reliability.

In conclusion, the use of the electronic nose in combination with the unsupervised - Hierarchical Cluster Analysis (HCA) method was not able to distinguish pure orange juice samples from orange juice samples adulterated with 1% addition of apple juice. The use of the Random Forest (RF) classification algorithm ensured 100% success in classifying the samples, so all adulterated samples were detected. It can be concluded that supervised chemometric methods were more reliable for detecting adulteration of juice samples.

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ORIGINAL PAPER

Novel analytical method for detection of orange juice adulteration based on ultra-fast gas chromatography

Anna Różańska[1] · **Tomasz Dymerski**[1] · **Jacek Namieśnik**[1]

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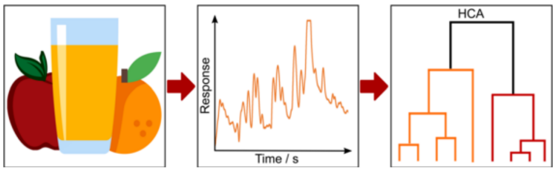
Abstract

The food authenticity assessment is an increasingly important issue in food quality and safety. The application of an electronic nose based on ultra-fast gas chromatography technique enables rapid analysis of the volatile compounds from food samples. Due to the fact that this technique provides chemical profiling of natural products, it can be a powerful tool for authentication in combination with chemometrics. In this article, a methodology for classification of Not From Concentrate (NFC) juices was presented. During research samples of 100% orange juice, 100% apple juice, as well as mixtures of these juices with known percentage of base juices were tested. Classification of juice samples was carried out using unsupervised and supervised statistical methods. As chemometric methods, Hierarchical Cluster Analysis, Classification Tree, Naïve Bayes, Neural Network, and Random Forest classifiers were used. The ultra-fast GC technique coupled with supervised statistical methods allowed to distinguish juice samples containing only 1.0% of impurities. The developed methodology is a promising analytical tool to ensure the authenticity and good quality of juices.

Graphical abstract



Keywords Clusters · Electronic nose · Fruit juices · Gas chromatography · Random Forest

Introduction

A juice manufacturing is one of the major branches which can be distinguished in the food industry. Due to the fact that the production of the fruit is seasonal, the fruit juice manufacturing allows us to consume them during the whole year. The most commonly consumed one is orange juice [1]. Its production accounts for nearly 85% of total citrus juice consumption [2].

Food quality assessment is increasingly important in the food industry. There are many types of fruit juice adulterations. The main one is dilution with water, which reduces the content of soluble solids, such as sugars or organic acids [3] or fragrance extracts and colourants [4]. Moreover, very popular type of fruit juice sophistication is the addition of cheaper fruit juices [3]. Orange juices are most often adulterated with the addition of mandarin [5, 6], tangerine [7], lemon [8], or grapefruit [9] juices.

In recent years, the interest in the healthy and balanced diet is growing. The consumption of orange juice allows not only to supply nutrients, but it can also have a positive

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✉ Anna Różańska
anna.rozanska@pg.edu.pl

[1] Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, Narutowicza 11/12, 80-233 Gdańsk, Poland

effect on the human organism. Wabner et al. proved that orange juice improved blood lipid profiles in subjects with moderate hypercholesterolemia [10]. Furthermore, orange juice intake with the high-fat, high-carbohydrate meal prevented meal-induced oxidative and inflammatory stress, and moreover, it prevents the expression of plasma endotoxin and Toll-like receptors [11]. The consumption of this type of fruit juice is beneficial in the control of calcareous and uric acid nephrolithiasis [12].

One of the healthiest juices is raw, and naturally, cloudy Not From Concentrate (NFC) juice, due to their composition, is most similar to the composition of fruits from which they are obtained. According to the European Fruit Juice Association, over the past 5 years, the demand for NFC juices has increased. Across Europe, the increase was of about 14.0%. Moreover, in Poland, consumption of NFC juices increased nearly tenfold [13]. Such an intense increase in the demand for juices can cause a decrease in the product quality. According to experts, orange juice in Poland can be diluted by the addition of apple juice, which is cheaper and more easily accessible.

There are many reference methods to assess the quality of juices. Among them, chemical, physical, and microbiological methods can be mentioned. Orange juice is a widespread subject of research regarding the analysis of the aroma profile and monitoring of processes occurring in fruit juices [14, 15]. Samples of these juices are also classified into NFC, From Concentrate (FC), and pasteurized juices using chromatographic techniques [16–18] and e-nose devices [19, 20]. For detecting adulterations of orange juices, the most effective are methods in which spectrometry and chromatographic techniques are involved [21, 22]. However, these procedures are time-consuming, labour-intensive, or expensive. For this reason, new solutions that allow for a rapid assessment of the quality of fruit juices are sought. Devices enable rapid analysis are called electronic noses [23, 24]. The electronic nose is a device which makes possible to detect and distinguish complex mixtures of fragrances. The advantage of this equipment guarantees low time consumption and low costs of single analysis, the omission of sample preparation step and the possibility of in situ measurement. The applications of e-nose to analyze aroma of food products are shown in a number of reports [25–30]. An electronic nose is a useful tool for classification fruit juice samples [31–33].

There is a lack of literature reports about the research of adulteration of orange juice by apple juice addition. This also includes electronic nose investigations. Therefore, the aim of this study was to develop a methodology for rapid evaluation of the authenticity of orange juices. For this purpose, the aroma profiles of orange juice, apple juice, and mixtures of both juices were compared by the use of e-nose based on ultra-fast gas chromatography. Moreover, e-nose analyses were combined with chemometric methods. Provided investigations can be supplementary to other control methods used for fruit-juice quality assessment.

Results and discussion

During the process of food quality control, from several dozens to several hundred samples need to be analyzed. For this reason, much less time-consuming methods are sought. In this work, the ultra-fast gas chromatography technique was used. Duration of the measurement was less than 2 min. Regarding that fact, chromatographic separation may be insufficient. This is particularly problematic when samples with a very complex matrix composition are subjected to testing. In the research, a holistic approach was used. This approach uses the fingerprint method, i.e., the entire chromatograms of the samples are compared using statistical data analysis.

Figure 1 shows the fingerprints obtained for samples of 100.0% orange juice (0.0) and a mixture of 50.0% orange

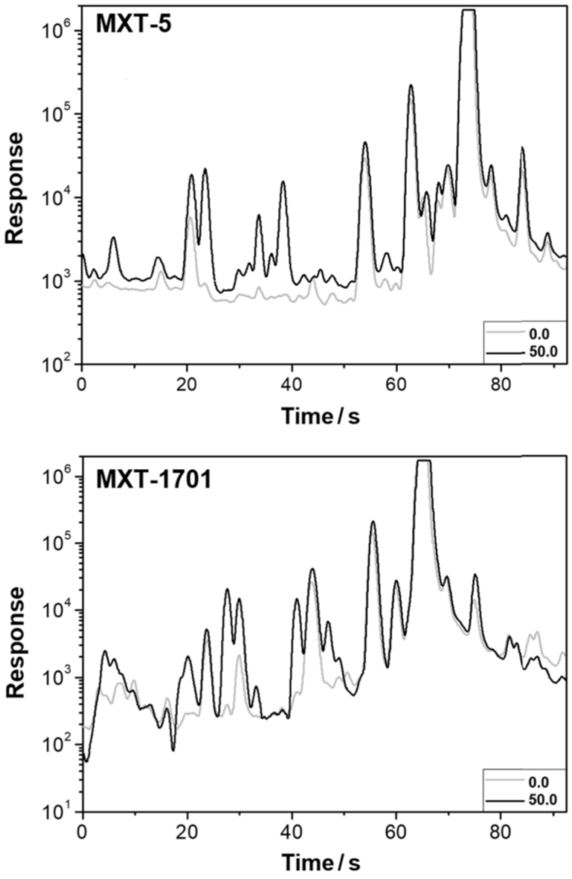

Fig. 1 Chromatographic fingerprints for 100% orange juice (0.0) and a mixture of 50.0% orange juice and 50.0% apple juice (50.0)

juice and 50.0% apple juice (50.0) for both chromatographic columns (MXT-5 and MXT-1701). The fingerprints show the differences in the composition of the headspace of unadulterated orange juice and orange juice with the addition of apple juice. As it can be seen in Fig. 1, the signals corresponding to the chemical compounds detected in the samples of the juice mixture (50.0) are much more intense compared to the samples of orange juice (0.0). Furthermore, as a result of the addition of cheaper juice, it can be observed more signals in the fingerprint. Peaks detected in adulterated juice may be characteristic of apple juice. Identification of these chemical compounds is very important due to the fact that these compounds may be potential markers of adulteration of orange juice with apple juice. Moreover, the determination of these markers in volatile fraction allows designating the quality of orange juice.

After chromatographic measurements, chemometric analyses were performed. The chromatographic peak areas corresponding to detected chemical compounds were used as an input data. All tested samples were compared based on the similarities and differences in the composition of the volatile fraction. During data processing with a large number of variables, there is a high probability of "voodoo correlation" [34]. As a general rule, to avoid this type of accidental correlation, the number of measurements should be fivefold greater than the number of variables. In the presented studies, over 120 chemical compounds (variables) were detected during each analysis. However, carrying out over 600 analyses is not easy to realize. Therefore, instead of increasing the number of measurements, it was decided to reduce the number of variables to 10.

Table 1 shows selected ten chemical compounds on the basis of the analysis of variance ANOVA. These compounds showed the greatest relative changes in concentration during testing and, therefore, had the greatest impact on the result of statistical analysis. It should be noted that these compounds are not necessarily those that have the highest concentrations in the volatile fraction of the sample, but these are the compounds for which the respective chromatographic peak areas show the greatest deviations depending on the percentage of apple juice. These compounds can be considered as potential indicators of the quality of orange juice. The Kovats retention indexes for both chromatographic columns (KI—MXT-5, KII—MXT-1701) were given for each potential quality markers of orange juice. In Table 1, aroma descriptors, which can be caused by the presence of selected chemical substances, are also listed. The six pre-selected chemical compounds listed in Table 1 (2-methylbutanol, ethyl butyrate, butyl acetate, 2-hexenal, 3-hexenol, and propan-2-one) are volatile organic compounds (VOCs) that have been identified in the volatile fraction of apples by Vrhovsek et al. and Mattheis et al. [35, 36]. The main chemical compounds affecting the aromas of these fruits are esters, mainly ethyl butyrate and butyl acetate, as well as alcohols, among others 2-methylbutanol and six-carbon compounds, such as 2-hexenol, which have been identified in various apple varieties: Redchief, Granny, or Golden [37, 38]. However, different varieties of apples are characterized by different compositions of their volatile fractions. For example, propan-2-one is the substance, which was detected only in the headspace of the Bisbee Delicious apple samples [35].

During the research, Hierarchical Cluster Analysis (HCA) was used as a chemometric model. HCA is a method that allows sorting data and binding them into natural groups based on their similarity [39]. At the beginning of the agglomeration procedure, each analyzed object is located in a separate cluster. Next, the number of clusters decreases in every step until the moment when all input data will belong to one cluster [40]. To group objects

Table 1 Selected compounds identified as potential orange juice quality markers

No.	Chemical compound	Kovats index		Aroma descriptors	Molar mass
		MXT-5	MXT-1701		
1	Propenal	450	566	Apple, fruity, sweet	56
2	2-Hexenal	854	956	Apple, cherry, fruity, green, strawberry	98
3	Butyl acetate	810	879	Banana, fruity, green, pear, pineapple, sweet	116
4	3-Hexenol	852	960	Fresh, green, leafy	100
5	Ethyl butyrate	799	864	Banana, fruity, pineapple, strawberry, sweet	116
6	2-Butanol	594	699	Alcoholic, winey	74
7	2-Methylbutanol	740	852	Fruity	88
8	*m*-Xylene	870	922	Plastic	106
9	Propan-2-one	478	586	Fruity	58
10	Methyl acetate	489	596	Blackcurrant, fruity	74

into clusters, it is necessary to define the numerical value of the similarity between objects. Usually, the Euclidean distance is used for this purpose [41]. However, in the presented research, the Ward method was used. This method is characterized by the fact that analysis of variance ANOVA is used to assess the distance between clusters [40, 42]. The application of this method allows obtaining the best results if the clusters are of equal size [41].

The purpose of the statistical analysis was a verification whether, using the proposed analytical procedure, it is possible to classify samples of unadulterated orange juice and samples of adulterated juice. Figure 2 shows the results of HCA. The composition of the aroma for 100.0% apple juice samples (marked as 100.0) forming a single cluster—C6. Subsequently, separated clusters (C5, C4, and C3) were obtained for samples of mixtures of orange and apple juice containing, respectively, 50.0, 30.0, and 10.0% of apple juice (marks as 50.0, 30.0, and 10.0). This means that the composition of the volatiles for these samples is statistically different and distinguishing them is not a problem. However, for data for samples containing from 1.0 to 5.0% of apple juice and samples of 100% orange juice (0.0), the distinction is difficult. The data create two clusters: one for the samples marked as 1.0 and 0.0 (C1) and the other for the samples marked as 3.0 and 5.0 (C2). This means that if the juice samples contain only 1% apple juice, this has a slight effect on the composition of the orange juice volatile fraction and these samples are classified as unadulterated samples. Whereas, samples marked as 3.0 and 5.0 are classified as samples of adulterated juice, but their aromas are so similar that it is difficult to distinguish them from each other.

Figure 3 shows the average of chromatographic peak areas for individual six clusters corresponding to the chemical compounds, as listed in Table 1. In the case of the first four clusters, six of ten selected substances were detected, namely: 2-hexenal, butyl acetate, 3-hexenol, ethyl butyrate, propan-2-one, and methyl acetate. The four remaining chemicals were below the limit of detection. In the samples assigned to the first cluster (C1), the chromatographic peak areas did not exceed the value of 200. For objects from the second, third, and fourth clusters (C2, C3, and C4), the peak areas reached maximum values of approximately 800, 1800, and 5000, respectively. Therefore, it can be observed that along with the increase in the apple juice content, the chromatographic peak areas also increased. On this basis, it can be concluded that these substances are characteristic of the volatile fraction of apples. In the samples classified to C5, an additional compound was detected which was 2-butanol, and the peak areas achieved value almost 9000. In the volatile fraction of the juices from the sixth cluster (C6), as many as nine of ten selected substances were detected. In these samples, the presence of 2-methylbutanol and *m*-xylene can be observed. 2-Methylbutanol is the alcohol identified in many apple varieties [36]. In contrast, *m*-xylene was detected in the headspace of apple bearing twig with leaves by Vallat et al. [38]. The presence of this compound may be caused by air pollution deposited on the surface of the fruit or residues of pesticides [43]. However, on the basis of the obtained results, it can be concluded that their concentration in the headspace of apple juice samples is small, as they were detected only in samples of 100% apple juice. In summary, the use of electronic nose in combination with the HCA method allowed the grouping of objects up to six clusters. However, it was impossible to distinguish samples of 100% orange juice (marked as 0.0) from samples adulterated with a 1% addition of apple juice (1.0).

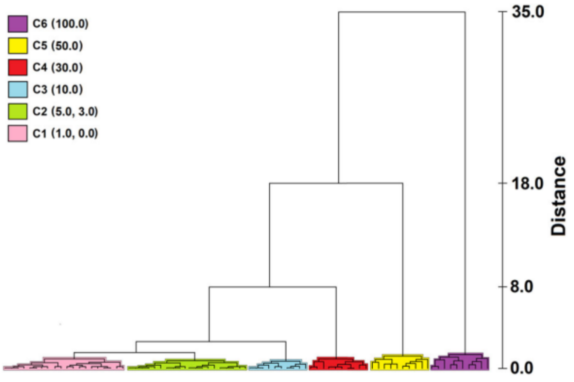

Fig. 2 Classification of orange juices according to the percentage of apple juice content using HCA method

To determine the quality of juice samples with greater precision, supervised statistical methods are used. Data obtained using the Heracles II apparatus were analyzed using several supervised algorithms to determine the use of which the more reliable results will be obtained. The effectiveness of these algorithms was evaluated using tenfold cross validation, and the results are given in Table 2.

The most reliable results were obtained for the Random Forest (RF) classification algorithm. It can be observed that the results of the evaluation of this algorithm regarding accuracy, precision, and sensitivity are 1.0. These results provide 100% effectiveness in predicting the Random Forest algorithm.

Random Forest is a kind of forecasting tools. This algorithm is a combination of decision trees [44]. Using this method, a very precise classification of the tested samples can be obtained. In addition, they are characterized by high accuracy, resistance to noise, simplicity, and speed of action [45]. These properties make Random Forest a useful tool for classifying objects even with a huge number

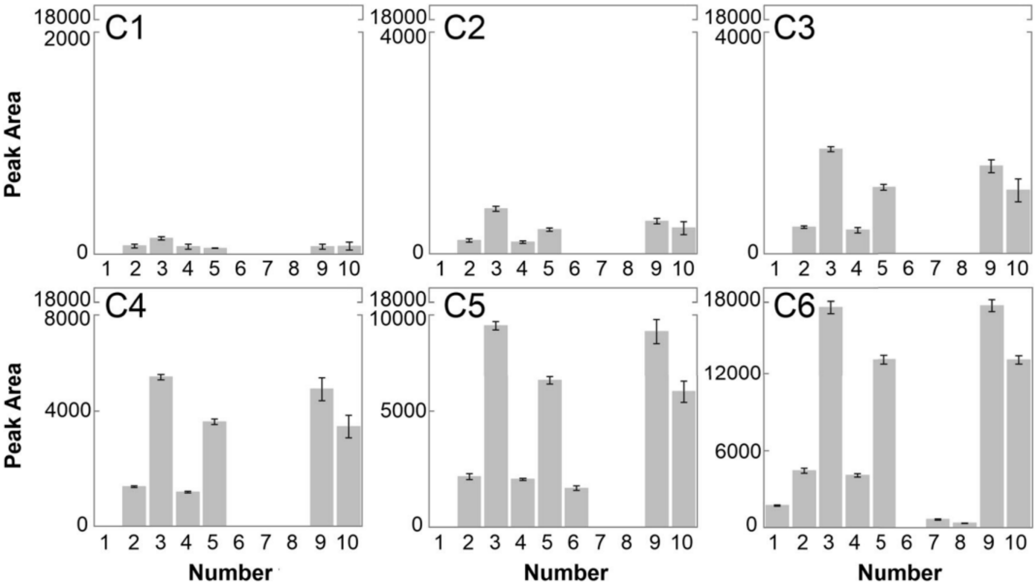

Fig. 3 Histograms depicting the mean values with a standard deviation of chromatographic peak areas for selected chemical compounds (numbers correspond to Table 1) belonging to the six clusters, as illustrated in Fig. 2

Table 2 Cross validation of supervised algorithms used for classification of data from the analysis of fruit juice samples

Method	AUC	CA	Precision	Recall
RF	1.000	1.000	1.000	1.000
NB	0.943	0.675	1.000	0.200
NN	0.857	0.762	0.667	0.200
CT	0.950	0.938	1.000	0.900

RF random forrest classification, *NB* naïve bayes, *NN* neutral network, *CT* classification tree, *AUC* area under curve, *CA* accuracy, precision, recall (sensitivity)

of features. Based on the information obtained, it was decided to use this algorithm for further research.

The Random Forest method was trained using 67% randomly selected data to avoid error. The remaining data were used to test the chosen method. To present the prediction results for the selected supervised algorithm, Table 3 contains information about the sample confusion matrix for the Random Forest classifier. This matrix compares the percentage values of the proportions of real samples that were well classified. While sampling data for classification, 100% correct classification based on the degree of adulteration of orange juice has been repeatedly obtained.

Conclusion

The use of e-nose based on ultra-fast gas chromatography equipped with unsupervised and supervised chemometric methods is an effective tool for authentication fruit juice

Table 3 Confusion matrices of fruit juice samples classification using RF; scores are given as a proportion of predicted

Actual	Predicted								
	0.0	1.0	3.0	5.0	10.0	30.0	50.0	100.0	Σ
0.0	**100%**	0%	0%	0%	0%	0%	0%	0%	5
1.0	0%	**100%**	0%	0%	0%	0%	0%	0%	4
3.0	0%	0%	**100%**	0%	0%	0%	0%	0%	2
5.0	0%	0%	0%	**100%**	0%	0%	0%	0%	3
10.0	0%	0%	0%	0%	**100%**	0%	0%	0%	5
30.0	0%	0%	0%	0%	0%	**100%**	0%	0%	1
50.0	0%	0%	0%	0%	0%	0%	**100%**	0%	2
100.0	0%	0%	0%	0%	0%	0%	0%	**100%**	4
Σ	5	4	2	3	5	1	2	4	26

samples. This technique allows to omit sample preparation step and provides a low time-consuming single analysis. Based on the obtained results, it can be concluded that using HCA methods allowed to classify orange juice samples for unadulterated and adulterated with apple juice. Unfortunately, samples of orange juice containing 1.0% of apple juice were assigned to the group of unadulterated samples. More reliable results were achieved through the use of supervised statistical methods. The combination of e-nose measurements with Random Forest classifier made it possible to distinguish between particular orange juice samples based on the added volume of apple juice. The obtained results are the basis for further investigations. In the near future, the focus should be placed on the developed methodology, in which samples of fruit nectars and juices from concentrate will be considered. They are one of the most falsified ones.

Experimental

Sample preparation

Fruit juices were obtained at local distribution centres in Gdansk. Samples were NFC juices, i.e., orange juice, apple juice, and mixtures of orange and apple juice (1/3/5/10/30/50% v/v addition of apple juice). The juice mixtures were prepared immediately after their purchase. A sample of 5.0 ± 0.1 g of each fruit juice was poured into 20 cm^3 glass vials that were then sealed with a cap with a silicone–PTFE membrane. Samples were stored for 24 h at 4 °C. For each type of samples, the analyses were performed in ten replicates.

Instrumentation

The measurements were performed using an ultra-fast gas chromatograph Heracles II (Alpha MOS, Toulouse, France) equipped with a split/splitless injector and two flame ionization detectors (μFIDs). The two parallel linked capillary chromatographic columns used for separation were non-polar MXT-5 (diphenyl dimethylpolysiloxane, 10 m × 0.18 mm × 0.40 μm) and medium-polar MXT-1701 (cyanopropylphenyl polysiloxane, 10 m × 0.18 mm × 0.40 μm). Before the headspace analysis, samples have been incubated in 40 °C by 120 s and with agitation speed 500 rpm. The samples were injected by the HS 100 autosampler (Gerstel, Mülheim, Germany) with a 5.0 cm^3 syringe and the injection volume was 2.5 cm^3. The temperature of the injector and the detector were, respectively, at 200 and 270 °C. Hydrogen was used as carrier gas and its flow was kept constant at 250 mm^3/s. The column temperature programming started at 40 °C, held for 5 s, and raised at a rate of 4 °C/s to 270 °C, maintained for the 30 s. The AlphaSoft 12.4 software was used to process the data. The aroma descriptors were obtained through the use of data collected from the AroChemBase.

Data processing

Data from the e-nose measurements were exported and further processed using statistical methods. The chemometric analysis was performed using the Orange Canvas Data Mining v. 3.3.9 software (Bioinformatics Lab, University of Ljubljana, Slovenia). To normalise the features, they were centred by mean and scaled by the standard deviation. Ten chemical compounds were then selected based on one-way analysis of variance (ANOVA). The chromatographic peak areas corresponding to the selected chemical compounds were used as input data for hierarchical cluster analysis (HCA). Ward's linkage method was applied. Clusters were created after cutting the dendrogram at a value corresponding to 3.0% of the maximum distance. In this way, six clusters were formed. The supervised statistical methods Random Forest (RF), Naïve Bayes (NB), Neural Network (NN), and Classification Tree (CT) were also used. RF method was chosen as a classifier based on the results of stratified tenfold cross validation. All the classifiers were taken with their optimal settings.

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4.2.2. Development of an analytical methodology for determination of biogenic amines in fruit juice samples

Hypothese

- *Salting-out assisted liquid-liquid extraction (SALLME) with in-situ derivatization as an effective procedure of microextraction of biogenic amines from food samples*
- *Box-Behnken Design (BBD) as a useful tool to optimize the BAs microextraction from food samples*
- *SALLME-GC-MS as an effective methodology for the biogenic amines determination in fruit juice samples*

Questions

- *Determination of biogenic amines (BAs) in juice samples - will SALLME-GC-MS be a useful tool in routine analysis and quality control?*
- *Can the developed methodology be applied to other food products?*

Biogenic amines (BAs) can be present in fresh fruit and vegetables and in unfermented beverages such as juices, as polyamines are ubiquitous compounds in plants, and their content increases during fruit ripening [73]. A low concentration of BAs in consumed fruit juices is not dangerous for human health. However, the high content of BAs in consumed products can cause toxic effects such as vomiting, nausea, headache, diarrhoea, palpitations or respiratory failure [74]. The high content of BAs in fruit drinks may be caused by the undesirable activity of microorganisms [75]. For this reason, determination of BAs content can be a tool to determine food quality and safety [76]. However, the number of scientific reports on determining BAs in fruit juices is still limited. For this reason, it is important to search for new methodologies for determining these analytes, especially those that will be characterized by high throughput, easy sample preparation step and the green nature of the method.

Prior to the development of the methodology for the determination of BAs in fruit juices, a review of the literature was carried out. In this way, information was collected on the analytical methodologies used for the determination of BAs in fruit juices, and more specifically on the preparation of samples, the method of derivatization and extraction of analytes, and the techniques used for their detection, as well as the validation parameters of the developed solutions. The information collected are summarized in Table 3.

The detection and determination of BAs in fruit juice samples can be problematic due to the complex composition of the sample matrix, the presence of other interfering substances, the low concentration of analytes, and the polar nature of BAs. It has been observed that in over 80% of cases, the determination of BAs in fruit juices uses the liquid chromatography technique, which is characterized by a time-consuming extraction process, a long separation time, and a large amount of generated liquid waste (Table 3). It is very important to choose the optimal procedure for the derivatization and extraction of analytes that will meet the principles of green analytical chemistry. During the research, it was decided to use alkyl chloroformates, because the process with their use is cheap and less time-consuming compared to other derivatizing reagents. Alkyl chloroformates are also reactive in the aqueous environment, which facilitates their use in the analysis of beverage samples.

Table 3 Information on analytical methodologies developed for biogenic amines determination in fruit juice samples.

Juice type	Number of BAs	Sample preparation	Extraction type	Extracting agent	Derivatizing agent	Determination technique	LODs [μg/L]	LOQs [μg/L]	Recovery [%]	Ref.
apple	8	protein precipitation, centrifugation	LLE	PCA	DNS-Cl	HPLC-UV	50 - 100	150 - 310	92.35 - 107.01	[77]
apple, black currant, cherry, grapefruit, pineapple, plum	5	centrifugation, filtration	-	-	-	IC-CMD	56 - 1630	190 - 3270	98 - 100	[59]
apple, black currant, lychee, mango, orange, pineapple	5	dilution	LLE	HCl	DNS-Cl	HPLC-UV	4.43 - 7.34	14.76 - 24.45	93.4 - 105.4	[58]
apple, grape, lychee, mango, orange, pineapple	7	protein precipitation, centrifugation, filtration	SALLE	$NaHCO_3$	ANIT	HPLC-UV	0.06 - 5.71	0.21 - 16.95	95.2 - 105.2	[78]
apple, orange, peach, pear, pineapple	9	centrifugation, filtration	LLE	AOT	DNS-Cl	HPLC-FLD	1.00 - 28.86	3.33 - 76.23	41.0 - 106.4	[79]
apricot, peach, pear	11	centrifugation, filtration	LLE	$HClO_4$	DNS-Cl	HPLC-FLD	2 - 112	6 - 373	96.3 - 104.2	[80]
grape	8	protein precipitation, centrifugation	LLE	Toluene	DNS-Cl	HPLC-UV	n.d.	n.d.	n.d.	[81]
grape	7	dilution, filtration	-	-	OPA-NAC	HPLC-FLD	n.d.	250	78.3 - 104.0	[82]
grape	22	centrifugation	DLLME	Toluene	IBCF	GC-MS	1	10	92 - 112	[57]
grape	8	centrifugation	LLE	DCM	HFBA	GC-MS	10	n.d.	74 - 120	[83]
orange	6	-	μSPE	ACN	DNS-Cl	HPLC-UV	3.82 - 31.30	12.70 - 104.00	71.5 - 95.0	[84]
orange	7	centrifugation, filtration	μSPE	HCl	DNS-Cl	HPLC-UV	0.02 - 0.06	0.07 - 0.19	63.2 - 99.4	[85]

The optimum pH for acylation reaction and carbamates formation is pH>10. Fruit juices are acidic, so sodium hydroxide solution was added during the sample preparation process to increase the pH to basic. After adjusting the pH, a derivatizing reagent, and triethylamine (TEA) were added to the sample. TEA was added in case to remove the by-product, i.e. hydrogen chloride, from the reaction mixture. The scheme of the acylation reaction is shown in Figure 6.

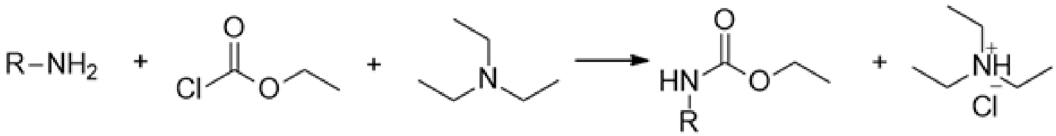

Fig. 6 Scheme of amine acylation reaction using ethyl chloroformate.

The aim of the research was to develop a simple, fast (salting-out assisted liquid-liquid microextraction) SALLME procedure for the extraction of BAs that would comply with the principles of green analytical chemistry. Another goal was to create an analytical method using GC-MS to identify and quantify the extracted analytes. Optimization of the extraction procedure was performed using the Design of Experiment (DoE) using the Box-Behnken Design plan. The influence of three independent variables was examined, namely the volume of the sample, the volume of the NaOH solution and the volume of the derivatizing agent, i.e. ethyl chloroformate (ECF). Validation parameters of the GC-MS method were also defined namely LOD, LOQ, recovery, repeatability, and matrix effect. The greenness of the proposed methodology was also assessed using two analytical tools developed by employees of the Gdańsk University of Technology: GAPI [86] and AGREE [87]. The methodology prepared in this way was used to test various fruit juices purchased from local grocery stores. To our knowledge, this was the first study to focus on the use of SALLME with in-situ derivatization followed by the determination of biogenic amines in juice samples using GC-MS. The obtained results were published in *Food Chemistry* [88].

A simplified diagram of the developed methodology is shown in Figure 7.

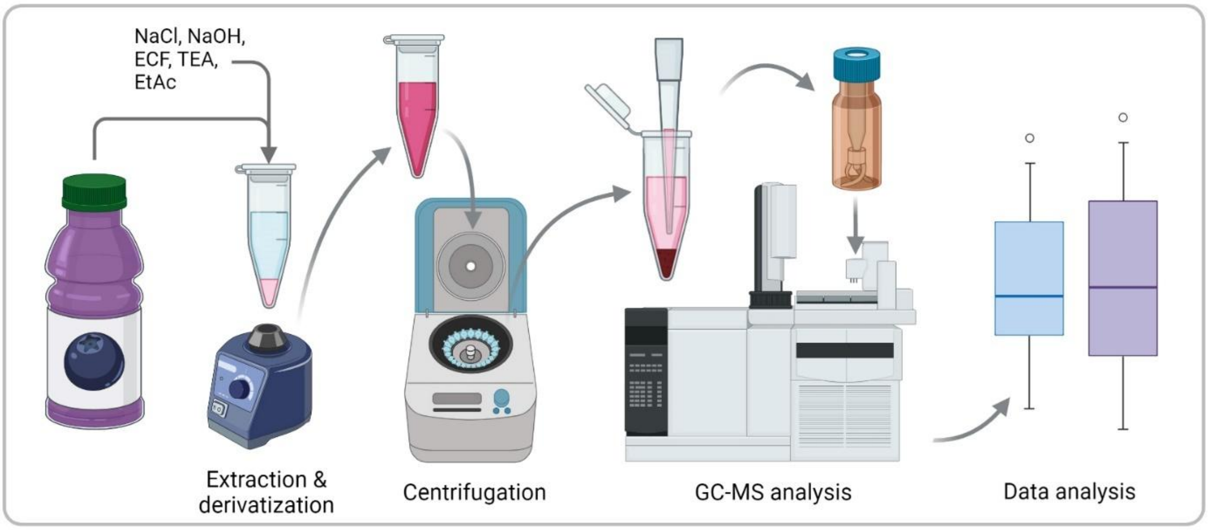

Fig. 7 Scheme of the SALLME-GC-MS procedure for the determination of biogenic amines in fruit juice samples.

In presented article [88], a new analytical method for the determination of biogenic amines in fruit juices was developed. Using the Box-Behnken Design allows us to determine the optimal conditions for microextraction of biogenic amines in the liquid-liquid system assisted by salting-out (SALLME). It has been shown that the developed method offers the potential for the determination of a large number of biogenic amines, combining selectivity, high resolution and fast analysis time with good validation parameters. SALLME has also been proven to be a simple technique in which small amounts of reagents and solvents are used in the extraction, which reduces the negative environmental impact of this analytical step. The obtained results confirmed the usefulness of the SALLME-GC-MS method for the determination of biogenic amines in trace amounts in fruit juice samples. The total content of BAs in all juice samples was relatively low (<1 mg/L), which indicated their good quality. Therefore, it has been proven that the proposed methodology can be a useful tool for juice quality control. The proposed solution was then used to analyze biogenic amines in wine samples [89], which confirms the possibility of using the developed methodology to assess the quality of other food products.

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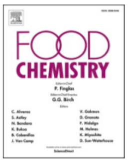

Green, simple analytical method for biogenic amines determination in fruit juice samples using salting-out assisted liquid-liquid microextraction and gas chromatography-mass spectrometry

Anna Różańska [*], Magdalena Fabjanowicz, Kaja Kalinowska, Żaneta Polkowska, Justyna Płotka-Wasylka [*]

Gdańsk University of Technology, Faculty of Chemistry, Department of Analytical Chemistry 11, 12 Gabriela Narutowicza Street, Gdańsk 80-233, Poland

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ABSTRACT

Salting-out assisted liquid-liquid microextraction (SALLME) was integrated with the derivatization procedure to establish a one-step sample pre-treatment approach for rapid analysis of 14 biogenic amines (BAs) in fruit juices. The methodology consists of salting-out of analytes, derivatization with ethyl chloroformate (ECF), extraction with ethyl acetate (EtAc), and the analysis of the derivatized BAs using gas chromatography-mass spectrometry (GC–MS). Optimization of the SALLME parameters, including the amount of sample, NaOH, and ECF was carried out through a Box-Behnken response surface design. The developed method exhibits satisfactory limits of detection (from 1.5 to 8.1 μg/L) and quantification (from 5.0 to 26.7 μg/L), and average recoveries between 84% and 108%. The developed procedure was used for BAs determination in juices of different berries with the highest determined concentrations found for cadaverine, putrescine, tryptamine, and tyramine. Both GAPI and AGREE tools were used to assess the green character of the SALLME-GC–MS procedure.

1. Introduction

Biogenic amines (BAs) are nitrogenous organic bases formed in food as a result of the activity of microorganisms capable of decarboxylating amino acids (Wójcik, Łukasiewicz, & Puppel, 2021). BAs are commonly found in food products, especially those subjected to fermentation processes (beer, wine), long maturation (cheese), rich in protein (meat, fish), but also fresh fruits and vegetables (Wójcik et al., 2021). Consumption of BAs in low concentrations is not dangerous to the consumers' health. However, consumed in excess, they can cause several toxic effects such as nausea, vomiting, diarrhoea, headache, respiratory failure, and palpitations (Doeun, Davaatseren, & Chung, 2017). In the case of non-fermented beverages, such as e.g. juices, a high BAs content may indicate undesirable activity of microorganisms (Vinci & Maddaloni, 2020). Therefore, BAs concentration can be a useful indicator of food quality and safety (Ruiz-Capillas & Herrero, 2019).

Biogenic amines determinations are commonly performed using ion chromatography (Jastrzębska, Piasta, & Szłyk, 2015), liquid chromatography (Eliassen, Reistad, Risoen, & Ronning, 2002; Saaid, Saad, Hashim, Mohamed Ali, & Saleh, 2009), and gas chromatography (Cunha, Faria, & Fernandes, 2011; Fernandes & Ferreira, 2000) coupled with various detectors. When developing new analytical methods for the determination of BAs in food, several problems should be bore in mind, namely the low concentration of analytes, the presence of many interfering substances, the complex matrix composition, and the polar nature of BAs. To obtain satisfactory results, the procedure of extraction and -derivatization of analytes should be properly selected (Płotka-Wasylka, Morrison, Biziuk, & Namieśnik, 2015).

The most commonly used reagent to derivatize BAs from fruit juice samples is dansyl chloride, since it forms very stable derivatives and can react with both primary and secondary amines (Basheer et al., 2011). The products of such derivatization are then analysed using high-performance liquid chromatography coupled with spectrophotometric or fluorometric detection. Other frequently used BAs derivatizing agents are o-phtaldialdehyde (Vieira, Theodoro, & Glória, 2007), 1-naphthylisothiocyanate (Jain, Gupta, & Verma, 2015), heptafluorobutyric acid (Fernandes & Ferreira, 2000), and isobutyl chloroformate (Cunha et al., 2011). Alkyl chloroformates are extremely useful in determining BAs using GC–MS because the derivatization step avoids the low sensitivity and the tailing of the peaks due to the high polarity of the amines (Zaikin

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[*] Corresponding authors.
E-mail addresses: anna.rozanska@pg.edu.pl (A. Różańska), juswasyl@pg.edu.pl (J. Płotka-Wasylka).

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& Halket, 2003). Moreover, the derivatization process with alkyl chloroformates is inexpensive and less time-consuming compared to derivatization with dansyl chloride. Alkyl chloroformates are reactive in an aqueous medium facilitating their use in the analysis of beverages samples.

The next critical step in the analytical process is the extraction of the analytes. To isolate BAs prior to chromatographic analysis, conventional liquid-liquid extraction (LLE) is most often used (Kelly, Blaise, & Larroque, 2010; Preti, Antonelli, Bernacchia, & Vinci, 2015). However, it is characterized by high consumption of hazardous reagents and solvents. To reduce the consumption of chemicals, as well as the produced wastes, other techniques such as dispersive liquid-liquid microextraction (DLLME) (Cunha et al., 2011) and micro-solid phase extraction (μSPE) (Tameem, Saad, Makahleh, Salhin, & Saleh, 2010) have been proposed in the literature.

However, multi-step extraction and subsequent derivatization of analytes can result in loss of analytes and increase the measurement uncertainty. The solution could be a simple and fast salting-out assisted liquid-liquid extraction (SALLE) with simultaneous derivatization. The salting-out effect consists of adding an electrolyte to an aqueous solution to change the ionic strength of the mixture, which favours the extraction of the analytes into the organic phase (Tsochatzis, Lopes, Gika, Dalsgaard, & Theodoridis, 2021). SALLE has been used for the extraction of BAs from samples of cheese (Ramos, Brandão, & Rodrigues, 2020), meat, fish (Francisco et al., 2020), and wines (Ramos, Valente, & Rodrigues, 2014).

This study was aimed at developing a simple, fast, and green SALLME procedure for the extraction of BAs from fruit juices, followed by the use of GC–MS for identification and quantification. The optimization of the method was carried out using the Design of Experiment (DoE) instead of the commonly used one-time-factor procedure. The three major parameters influencing SALLME were analysed using Box-Behnken Design to determine the optimal extraction conditions. Then, accuracy and precision of the developed method were estimated and validated. The method's greenness was assessed using two analytical tools: GAPI (Płotka-Wasylka, 2018) and AGREE (Pena-Pereira, Wojnowski, & Tobiszewski, 2020). Finally, various fruit juices from local grocery stores were analysed to demonstrate the applicability of the developed methodology for the determination of BAs in food products. To the best of our knowledge, this is the first work focused on the application of *in situ* derivatization coupled with SALLME for the determination of biogenic amines in fruit samples by GC–MS. The procedure is characterized by good validation and separation parameters, and also conforms with many criteria of Green Analytical Chemistry.

2. Materials and methods

2.1. Reagents, chemicals and standards

All the BAs, such as methylamine hydrochloride (MET, 98.0%), dimethylamine hydrochloride (DIMET, 99.0%), ethylamine hydrochloride (ET, 98.0%), diethylamine hydrochloride (DIET, 99.0%), propylamine (PROP, 98.0%), butylamine (BUT, 99.5%), isopentylamine (IPA, 98.0%), hexylamine (HEX, 99.0%), 2-phenylethylamine hydrochloride (2PEA, 98.0%), putrescine (PUT, 97.5%), cadaverine (CAD, 96.5%), histamine (HIS, 96.5%), tyramine (TYR, 96.5%) and tryptamine (TRYP, 97.5%) were purchased from Merck (Merck Life Science Sp.z.o.o, Poznań, Poland). Aniline (IS, 99.5%), ethyl chloroformate (ECF, 99.5%) and triethylamine (TEA, 99.5%) which were used as internal standard, derivatizating agent and catalyst respectively were also from Merck Life Science Sp.z.o.o (Merck, Poznań, Poland). Ultrapure water for aqueous solutions and glassware washing was prepared using HLP5 Hydrolab (Wiślina, Poland). For the salting-out assisted liquid-liquid microextraction procedure, sodium chloride (NaCl, ACS grade, POCH, Gliwice, Poland), sodium hydroxide (NaOH, ACS grade, POCH, Gliwice, Poland) together with ethyl acetate (EtAc, 99.9%, VWR International, Gdańsk, Poland) were used.

2.2. Samples and preparation procedure

The nonfiltered berry juice samples used in this work were commercially available in local supermarkets and were of different types: bilberry juice (BI), blackcurrant juice (BL), blueberry juice (BU), chokeberry juice (CH), elderberry juice (EL), honeyberry juice (HO) and raspberry juice (RA). Juice samples of the same type were purchased from several producers. All samples were stored at refrigerator temperature and were protected from light.

All samples were derivatized according to SALLME protocol. In a 2 mL Eppendorf tube, 32.5 mg of sodium chloride and 65 μL of fruit juice (enriched with 1.3 μL of IS at a concentration of 50 μg/mL) were mixed. Afterwards, ECF derivatization combined with simultaneous liquid–liquid extraction (LLE) was carried out at room temperature. More in detail, 11 μL of NaOH solution (1.0 mol/L) were added to the sample to obtain the pH appropriate for carbamate formation, followed by the addition of 1.2 μL of the derivatizing reagent (ECF) and 1.2 μL of TEA. The solution was mixed with 260 μL of EtAc and then vortexed for 1 min at 2500 rpm. Then, the mixtures in the Eppendorf tubes were centrifuged for 2 min at 3500 rpm. Finally, an aliquot of the upper organic phase was collected and analysed by GC–MS. Each sample was prepared in five replicates. A schematic representation of the derivatization and extraction process is presented in Fig. 1.

2.3. Instrumentation

All analyses were carried out on a GC–MS instrument (Agilent Technologies, Santa Clara, CA, USA) consisting of an Agilent 7890A gas chromatograph coupled to an Agilent 5975C single quadrupole mass spectrometer detector. A fused silica capillary column (0.3 m × 0.25 mm, Phenomenex, Torrance, CA, USA) was used as a guard column connected to a ZB-5MS capillary column (0.3 m × 0.25 mm × 0.25 μm, Zebron, Phenomenex, Torrance, CA). Helium (99.999% pure, Air Liquide, Kraków, Poland) was used as a carrier gas with a flow rate of 1.0 mL/min. 2 μL of the extracts were transferred into an injector which was operated in splitless mode at 240 °C. For the chromatographic separation, the GC oven temperature program was as follows: initial temperature 55 °C, held for 4 min, then increased to 280 °C at 50 °C/min and held for 7.5 min. All targeted compounds were separated within 16 min. The total time needed for analysis per sample was 20 min: 1.0 min for reagents preparing, 1.0 min for derivatization and extraction step, 2.0 min for centrifugation and 16.0 min for the chromatographic run. The MS was performed in EI mode (70 eV). The transfer line, ion source and detector temperatures were 300 °C, 230 °C and 150 °C, respectively. Synchronous scan/selected ion monitoring (SIM) mode was used for the collection of both types of data in each run (solvent delay: 4.5 min). The scan m/z range was set to 30–500 amu. In the SIM mode, one quantification and two qualifier ions were monitored for quantification purposes. Data were acquired using MSD ChemStation, Ver. E.02.00.493 software from Agilent Technologies. For the preparation step Vortex (MX-S, (Chemland, Stargard, Poland) and mini-centrifuge (FVL-2400 N Combi-Spin, Biosan, Józefów, Poland) were utilized.

2.4. Box-Behnken design

The optimization of biogenic amines extraction was performed using the response surface methodology (RSM) (Minitab 17, LLC, State College, Pennsylvania, USA). The design of experiment (DoE), namely the Box-Behnken design, was used to evaluate the optimal level and interaction effects of the three independent factors affecting the content of the BAs. The three experimental factors and factor levels were selected by preliminary studies based on the results of single-factor tests (data not shown). Factors in question were sample volume [Sample], the volume of NaOH solution (1 mol/L) [NaOH] and the volume of

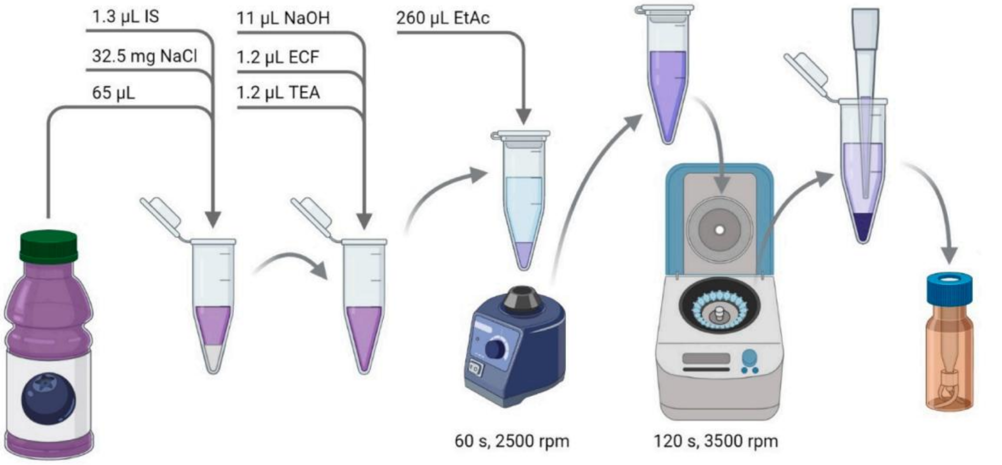

Fig. 1. Scheme of SALLME protocol for BAs analysis in juice samples.

derivatizing reagent [ECF]. A total of 18 experiments (3-level design including runs in the full three-level factorial and 6 centre points to estimate the experimental error) were carried out. During randomized runs order the juice samples enriched with BAs mix at 10 µg/mL were analysed. The response value was depicted by the total sum of the standardized peak areas for BAs. To interpret the effects of these independent variables on BAs extraction efficiency, three-dimensional response surface plots were constructed. RSM was implemented to optimize the extraction process. The regression coefficients of linear, quadratic, and interaction involved in the model and their effects were tested statistically by one-way analysis of variance (ANOVA) at probability levels ($p \leq 0.05$). Graphical and numerical analyses were used to optimize the processing conditions based on the model desirability features.

2.5. Quality assurance (QA)

The optimized method was evaluated using the following validation parameters: linearity, precision, sensitivity and accuracy according to quality assurance protocol. Linearity was examined by application of 10 different concentrations. Limits of detection (LODs) and limits of quantification (LOQs) were calculated to estimate the sensitivity of the methodology. Both LODs and LOQs were calculated from spiked samples ($n = 5$) and the minimum detectable analyte amount with a signal-to-noise ratio of 3 and 10, respectively, was established. The intra-day (RSD_r) and inter-day (RSD_R) precision were determined by the application of five replicates of juice samples spiked at two levels (0.25 and 2.5 mg/L). In addition to validation parameters, recovery rates were estimated using the ratio of the peak areas of the spiked samples of known concentration of biogenic amines to those of spiked ethyl acetate solution ($n = 5$).

2.6. Data analysis

Chromatographic data were processed using MZmine 2 (Pluskal, Castillo, Villar-Briones, & Orešič, 2010). The concentration values of the BAs determined in fruit juice samples were used as input data for multivariate statistical data analysis using a dedicated Python toolkit Orange v.3.20. Initial data processing involved standardization. Standardized values were taken as input to the cluster analysis and principal component analysis. Based on its results the heat map, loadings plot and linear projection for the three principal components were obtained.

3. Results and discussion

3.1. Analytical method development

3.1.1. Optimization of SALLME and derivatization protocol

The main parameters affecting the efficiency of SALLME are sample volume, type and volume of extractant, duration of extraction, process temperature, and type and amount of added salt (Jain et al., 2015; Ramos et al., 2014). In the proposed procedure, the extraction and derivatization processes were carried out simultaneously which significantly shortens the sample preparation step, but also introduces additional factors that can affect the efficiency of the extraction. Proper selection of the type and volume of the derivatizing agent as well as ensuring that the solution is basic, not only facilitates the extraction but also enables the formation of the products of the derivatization (Husek & Simek, 2006; Munir & Badri, 2020).

In SALLME, the salting-out effect is used to facilitate the separation of water-miscible organic solvents by adding electrolytes to the solution, which changes the ionic strength and increases extraction efficiency (Francisco et al., 2020; Tsochatzis, Lopes, Gika, Dalsgaard, & Theodoridis, 2021). The main factor responsible for the efficiency of phase separation in SALLME procedures is the type of salt anion (Ramos et al., 2020). Another important aspect that needs to be taken under consideration is the fact that the amount of added salt must be sufficient for clear separation of the two phases, but its amount should be as small as possible to avoid adsorption of the analytes on the salt crystals surface. (Tsochatzis et al., 2021). For this reason, the NaCl amount corresponding to 10% w/v of the reaction mixture was chosen. Another crucial factor is the type and volume of the extractant. Ethyl acetate was chosen as the extraction solvent because of its green nature and the fact that it is immiscible both with water and juice samples (Manca et al., 2017; Sánchez, Santos, Sappó, Pavón, & Cordero, 2014). The aim was to develop an extraction procedure performed on a micro-scale, therefore it was decided that the total volume during extraction should not exceed 1 mL. Assuming a suitable solvent to sample ratio of 4:1 (Fabio et al., 2020), 200 to 600 µL of EtAc was used.

In the case of BAs determination using GC–MS, derivatization is carried out in order to change the nature of the analytes, improve the shapes of the chromatographic peaks, increase the sensitivity and selectivity of the technique, and improve the quality of the mass spectra. The derivatization process was performed using different alkyl chloroformates, namely methyl chloroformate (MCF), ethyl chloroformate (ECF), propyl chloroformate (PCF), and isobutyl chloroformate (IBCF). The derivatization agent was chosen based on the results obtained during GC–MS analysis of four BAs, selected according to their chemical structure and derivatized with the above-mentioned chloroformates (Fig. 2).

The results for the BAs derivatized with ECF were characterized with the lowest SD, and for PUT and TRYP, the peak areas were the largest, therefore it was selected as a derivatization reagent for further stages of the research. The pH of the sample is a parameter that should be controlled before the derivatization process as it affects the time of the reaction. Moreover, in the case of reaction with chloroformates, amines must be in the deprotonated form before the derivatization step (Husek & Simek, 2006; Qiu et al., 2007; Zaikin & Halket, 2003). To this end, optimized amounts of NaOH solution (1 mol/L) were added to the samples to improve the efficiency and shorten the time of derivatization with ECF. The optimum pH of the acylation reaction and the formation of carbamates is pH >10 (Husek & Simek, 2006; Hušek, 1998). Fruit juices are acidic, therefore during the sample preparation process, an optimized volume of NaOH solution (1 mol/L) was added to increase the pH to basic. After adjusting the pH, an optimized volume of ECF was added to the sample. Both parameters, volume of NaOH and ECF were selected for optimization using DoE (Section 3.1.2). TEA was added to the samples to remove the by-product of derivatization, i.e. hydrogen chloride, from the reaction mixture (Husek & Simek, 2006).

3.1.2. Response surface methodology for SALLME and derivatization protocols

The RSM was used to evaluate the maximum efficiency of BAs extraction from food samples. The extraction yield is expressed as the standardized sum of the peak areas. The influence of three independent variables was investigated, namely the sample volume (50–150 μL), the NaOH solution volume (volume corresponding to 5.0–35.0% of the sample volume), and the volume of the derivatizing agent, i.e. ECF (volume corresponding to 0.5–2.5% of the sample volume). The codes and levels of the standardized variables along with the experiment design are listed in Table S1.

A polynomial model for estimating the BAs content in terms of sample volume, NaOH content, and ECF content is shown in Eq. (1):

$$\begin{aligned} BAs = & -1.266+0.00806[Sample]+0.07552[NaOH]+1.332[ECF]-0.000039[Sample]^2-0.001473[NaOH]^2-0.2460[ECF]^2+0.000006[Sample]\bullet[\mathrm{NaOH}] \\ & -0.001562[Sample]\bullet[\mathrm{ECF}]-0.01172[\mathrm{NaOH}]\bullet[\mathrm{ECF}] \end{aligned}$$

The results of ANOVA and model coefficients are listed in Table S2. The F-value (45.53) and the associated p-value ($p < 0.001$) indicated that the regression model was significant. The F-value (0.69) for lack of fit was negligible (LoF > 0.05) and therefore the validity of the model was confirmed. High values of R^2 (0.981), pred-R^2 (0.959), and adj-R^2 (0.891) indicated the high predictive ability of the model. The low correlation variance (CV) value indicated that the experiments were characterized by a high degree of reliability and precision. Based on the analysis of the p-values of each component of the model, it was possible to conclude that two linear coefficients ([Sample] and [ECF]), three square coefficients ($[\mathrm{Sample}]^2$, $[\mathrm{NaOH}]^2$, $[\mathrm{ECF}]^2$), and two two-way interaction coefficients ([Sample]·[ECF] and [NaOH]·[ECF]) were significant and indicative of a pattern of interactions between the studied variables.

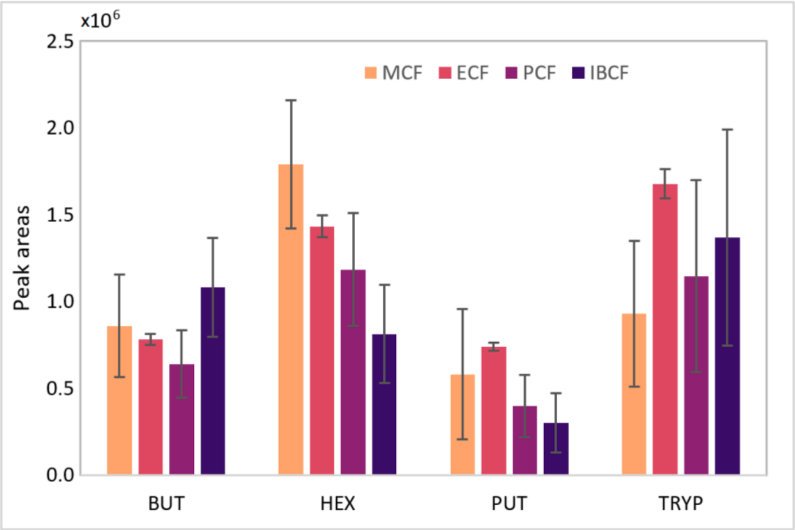

Fig. 2. Influence of the derivatization reagent on the resulting peak areas of selected biogenic amines; MCF – methyl chloroformate, ECF – ethyl chloroformate, PCF – propyl chloroformate, IBCF – isobutyl chloroformate.

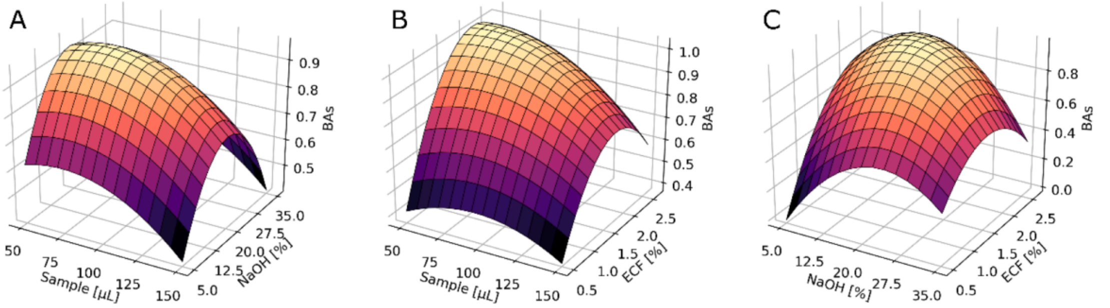

Fig. 3. Response surface plots of Box-Behnken DoE. The × and y-axis represent the variables, namely sample volume, NaOH content and ECF content while the z is the standardized sum of BAs peak areas.

The application of the Box-Behnken design resulted in three response surface plots for BAs extrction, which are graphical representations of the regression equation (Fig. 3). With the use of these plots, it is possible to visualize the relationship between the responses and the experimental parameter levels of variables, and the type of interaction between them.

In Fig. 3 it can be seen that all three optimized parameters have a large impact on the efficiency of BAs extraction. Extraction efficiency was inversely proportional to the sample volume. The sum of the BAs peak areas increased with increasing NaOH content up to approx. 20%, and then extraction efficiency decreased. There is an inconsistency in the literature about the most appropriate concentration of derivatizing reagents for BAs, as it depends on the type of sample and the solvent (Hušek, 1998; Ramos et al., 2020). Theoretically, the higher the derivatization reagent amount, the more effective the derivatization process. However, excessive volume of alkyl chloroformate can result in the formation of by-products that interfere with BAs derivatives determination and can also shorten the life of the chromatographic system (Munir & Badri, 2020; Zaikin & Halket, 2003). This is why it is important to optimize the volume of the ECF so that the derivatization can be performed efficiently whilst keeping the derivatizing agent volume as low as possible. This is consistent with the obtained results (Fig. 3), where the yield was directly proportional to the ECF content until reaching the value of 2%, beyond which point the efficiency of the process decreased. Based on the obtained results, it was found that the optimal values of the three continuous variables of the BAs extraction and derivatization procedure from juice samples are: 65 µL of the sample, 17% NaOH, and 2.0% ECF. The four experiments were conducted using optimal values and the obtained normalized sum of peak areas for BAs was 1041 ± 0014, which was comparable to the predicted value of 1037 calculated by the model.

3.1.3. Optimization of GC–MS conditions

Gas chromatography method parameters, namely temperature programme, injector temperature, and carrier gas flow, among others, were selected to obtain satisfactory separation and signals for all analysed BAs. Additionally, the goal was to obtain a high-throughput method. The chromatographic run took 16 min. The mass spectrometric conditions were also optimized to ensure the best parameters for BAs analysis. Peak identification was performed by comparing the retention times and MS spectral information with the information obtained from the analysis of standard solutions. It is worth noting that not all BAs bind to a single molecule of ECF. Based on the obtained results, it can be observed that monoamines bind to only one ECF molecule, while polyamines bind to one or two ECF molecules. This information, along with retention times and characteristic fragments obtained, can be found in Table S3. The molecular ion peaks were observed in the mass spectra of all analytes. The *m/z* 102 fragment appears in the mass spectra of most of the

Table 1
Analytical figures of merit for the developed SALLME-GC–MS methodology.

Analyte	Concentration range		Concentration level				Inter-day (%RSD)			LOD (µg/L)	LOQ (µg/L)	ME (*n* = 5) (c = 0.25 mg/L)
			0.25 mg/L		2.5 mg/L							
	0.05 to 1 mg/L	1 to 10 mg/L	Intra-day (% RSD)	Recovery (%)	Intra-day (% RSD)	Recovery (%)						
	Linearity (R^2)						Day 1	Day 2	Day 3			
MET	0.9956	0.9965	3.3	91	3.5	95	4.4	4.4	4.6	2.3	7.6	93 (4.1)
DIMET	0.9978	0.9985	6.2	93	6.8	97	6.5	6.4	6.7	5.4	17.8	95 (5.0
ET	0.9968	0.9977	4.1	96	3.3	99	4.8	4.9	4.7	2.3	7.6	96 (9.8)
DIET	0.9948	0.9956	10.4	99	10.9	100	11.3	11.5	11.6	1.9	6.3	101 (9.3)
PROP	0.9987	0.9991	2.3	84	2.6	91	2.5	2.5	2.7	4.2	13.9	83 (6.9)
BUT	0.9979	0.9989	4.7	101	4.9	99	4.3	4.5	4.6	2.8	9.2	95 (7.1)
IPA	0.9983	0.9987	3.1	92	3.6	96	3.4	3.1	3.3	5.3	17.5	96 (8.3)
HEX	0.9987	0.9992	6.0	97	5.9	102	6.3	6.6	6.4	2.7	8.9	93 (5.4)
2PEA	0.9986	0.9990	4.3	99	5.0	98	4.2	4.4	4.5	6.4	21.1	94 (7.2)
PUT	0.9980	0.9991	4.1	101	4.9	102	4.4	4.7	4.6	8.1	26.7	98 (3.9)
CAD	0.9985	0.9993	2.9	85	3.6	89	3.1	3.0	3.3	1.5	5.0	82 (4.1)
HIS	0.9989	0.9993	3.4	95	4.1	97	3.7	3.3	3.7	2.9	9.6	91 (3.7)
TYR	0.9989	0.9988	4.7	96	4.9	98	5.0	4.7	4.9	2.1	6.9	94 (9.1)
TRYP	0.9952	0.9975	10.1	106	11.3	108	10.6	9.9	10.8	3.1	10.2	99 (11.1)

MET – methylamine; DIMET – dimethylamine; ET- ethylamine; DIET – diethylamine; PROP – propylamine; BUT – butylamine; IPA- isopentylamine; HEX – hexylamine; 2PEA – 2-phenylethylamine; PUT – putrescine; CAD – cadaverine; HIS – histamine; TYR – tyramine; TRYP – tryptamine; ME – matrix effect.

analysed amines. This fragment can be related to the common presence of the $CH_3CH_2OC(O)NHCH_2$ group in the molecular structures of these compounds, i.e. (*N*-methyl)-ethyl carbamate group (Reddy, Chary, Pavankumar, & Prabhakar, 2016).

3.2. Method validation

The matrix effect (ME) is one of the main challenges when developing new analytical methods. For this reason, the ME of the optimized method was evaluated using the procedure described by Matuszewski et al. (Matuszewski, Constanzer, & Chavez-Eng, 2003). The ME was tested at a concentration level of 0.25 mg/L, and calculated by comparing the mean peak area of the analyte standards in the EtAc solution (A, $n = 5$) with the mean peak area of an analyte spiked postextraction (B, $n = 5$). The following Equation was used:

$$ME[\%] = \frac{B}{A} \cdot 100\%$$

The MEs, shown in Table 1, were ranged from 82% and 101%. In general, ME has no impact on the qualitative and quantitative results of this method and can be omitted. Additionally, it was proven that it is justified to use an internal standard (IS) for calibration.

The method's linearity and sensitivity were assessed for fourteen BAs by calibration with standard solutions in the presence of IS. Ten EtAc solutions containing all tested BAs in two separate concentration ranges: from 0.05 to 1 mg/L and from 1 to 10 mg/L, respectively, were subjected to the developed procedure. Least squares linear regression was used to calculate the equations of the calibration curves and the determination coefficients (R^2). Detailed information for each analyte is provided in Table 1. The linearity was excellent for all analytes with determination coefficients from 0.9948 to 0.9989 (for the first range) and from 0.9956 to 0.9993 (for the second range). The LODs ranged from 1.5 to 8.1 μg/L and the LOQs ranged from 26.7 to 49.5 μg/L. LOD and LOQ were the lowest for CAD and the highest for PUT.

The intra-day precision (RSD_r) was estimated based on the results of analysis of five replicates of juice samples fortified at two concentration levels (0.25 and 2.5 mg/L) on the same day. The inter-day precision (RSD_R) was determined by analysis of samples from three different days over three weeks. RSD_r ranged from 2.3 to 10.4% (for 0.25 mg/L) and from 2.6 to 11.3% (for 2.5 mg/L), while RSD_R ranged from 2.5 to 11.6% (Table 1). The stability of the analytes in the juice matrix led to satisfactory precision.

The accuracy of the method was determined by a recovery test, i.e. a comparison of the unenriched sample with the samples enriched with analytes at two concentration levels (0.25 and 2.5 mg/L) with five replicates. The recovery rates are listed in Table 1. The average recovery values ranged from 84 to 106% (for 0.25 mg/L) and from 89 to 102% (for 2.5 mg/L). These results indicate that the developed procedure of BAs determination in fruit juices samples was characterized by high accuracy.

3.3. Greenness evaluation

The developed analytical procedure for the determination of BAs in fruit juice samples was subsequently assessed in terms of 'greenness' using two different metrics, namely the Green Analytical Procedure Index (GAPI) (Płotka-Wasylka, 2018) and the Analytical Greenness Calculator (AGREE) (Pena-Pereira et al., 2020). To evaluate its environmental impact, the developed approach was juxtaposed with five other methods for BAs determination chosen from the literature. Two different analytical methodologies based on GC–MS were selected for the comparison: method denoted M2 in which the analysis proceeded with multi-stage LLE and isobutyl chloroformate derivatization (Cunha et al., 2011) and M3 in which sample preparation consists of ion-pair extraction and heptafluorobutyric anhydride derivatization (Fernandes & Ferreira, 2000). Three liquid chromatography-based methodologies were also included in the greenness evaluation, since LC is seen as the gold standard for BAs determination. In selected methods involved different sample preparation techniques: micro-solid phase extraction (μ-SPE) and dansyl chloride derivatization in M4 (Basheer et al., 2011), conventional LLE also with dansyl chloride derivatization in M5 (Preti, Bernacchia, & Vinci, 2016), and SALLE combined with 1-naphthylisothiocyanate derivatization performed prior to the determination in M6 (Jain et al., 2015).

Based on the obtained results for the greenness assessment (Fig. S1), SALLME-GC–MS method developed in this work is the greenest. Compared to other methods, its main advantage is a very short time of the derivatization and extraction step (only 4 min), while in other methodologies it ranges from 25 min (Cunha et al., 2011) to 90 min (Preti et al., 2016). To reduce the negative impact on the environment, the throughput of the methodology was also increased by reducing the analysis time (16 min) and increasing the number of analysed BAs during a single analysis (14 amines). In addition, the entire extraction procedure was miniaturized, so that only 65 μL of the sample is needed for the analysis, and the amount of waste was reduced to approx. 370 μL per analysis. Therefore, the adoption of the proposed method for the analysis of BAs in analytical laboratories would result in reducing health hazards and environmental impact.

3.4. Real samples analysis

The developed analytical method was used to determine BAs in selected nonfiltered berry juices characterized by a high content of bioactive substances (Table S4). PUT and CAD were detected in each sample which was to be expected since they are typically found in plant-based products (Ordonez & Callejon, 2020). PUT was reported to be the predominant amine in most fruit juice samples (Eliassen et al., 2002). DIMET and DIET were detected only in a few samples. The ranges of each biogenic amine content in berry juices are shown in Fig. 4A. The most abundant amines in fruit juices were TYR with an average concentration of 197 ± 70 μg/L, TRYP (129 ± 88 μg/L), PUT (91 ± 49 μg/L), and CAD (46 ± 27 μg/L). The mean concentrations of the remaining BAs ranged from 8.0 μg/L for DIET to approx. 25 μg/L for 2PEA. Jastrzębska et al. also noted that the most abundant amines in the samples of non-filtered juices were TYR, PUT, and CAD, and their concentration depended on the type of juice (Jastrzębska et al., 2015). Additionally, Saaid et. al. observed that in tropical fruit juices the most abundant amines were TRYP and HIS, while in blackcurrant juice the - most abundant amines were PUT, HIS, and spermidine (Saaid et al., 2009). Low levels of PUT in juices may also suggest that overripe fruit were not used in their production (Jastrzębska et al., 2015). The level of TYR can fluctuate during storage even at refrigerator temperatures and a high content of this amine may indicate that a long time has elapsed between the production of the juice and its purchase (Saaid et al., 2009).

The total BAs content depending on the type of juice is shown in Fig. 4B. Based on these results, it can be observed that the samples of honeyberry, blueberry, elderberry, and blackcurrant juices were characterized by a relatively small variation in BAs concentrations. On the other hand, the BAs concentration in raspberry, chokeberry, and bilberry juices had high variability. This may be caused by the fact that the amount of BAs, apart from the storage conditions of food products, also depends on the content of amino acids, the processes applied to the food products, or the production technology used (Ordonez & Callejon, 2020). However, in each tested juice, the total amount of BAs was relatively low (<1 mg/L).

Large variability was observed in the profiles (shown in Fig. 5) of BAs in fruit juices, and even within the same type of juices from different producers. Therefore, a chemometric analysis was performed to check the relationship between each biogenic amine and the berry juices. As shown in Fig. 5, some variables were not relevant for discrimination between juices, while others, e.g. DIMET, PROP, BUT, and CAD, were

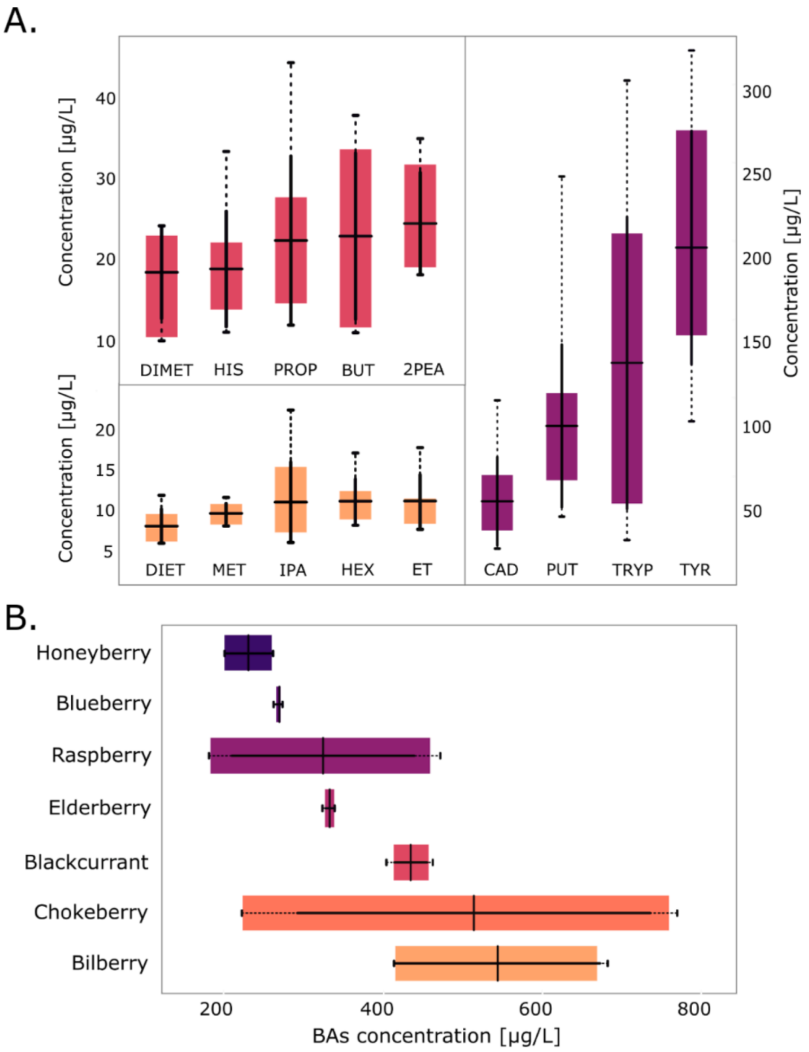

Fig. 4. Biogenic amines concentrations in fruit juices; A – box-plots for biogenic amines, B- box-plots for different juice types.

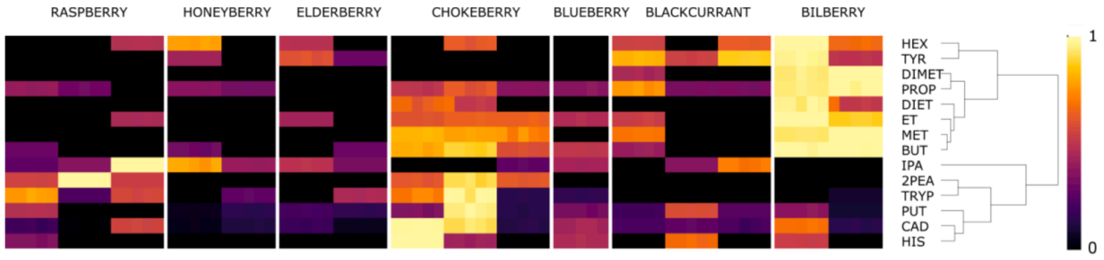

Fig. 5. Heat map depicting the normalized values grouped by class (fruit juice types), with clustered variables.

characteristic for a specific class of samples. Hierarchical clustering of the set of variables revealed that the variables form 2 clusters at h = 0.33. The first cluster contained low-concentration BAs and TYR, while the second cluster was comprised of PUT, CAD, HIS, IPA, and 2PEA.

The chemometric approach (e.g. PCA shown in Figs. S2 and S3) confirmed several observations made after preliminary analysis of the GC–MS results. Bilberry juice samples were characterized by a relatively high concentration of DIMET, PROP, ET, MET, and BUT. Based on concentrations of these BAs it was possible to distinguish bilberry juice from other juice types. Relatively high concentrations of 2PEA, TRYP, PUT, and CAD, together with moderately high concentrations of MET and BUT, was typical for chokeberry juice samples. Furthermore, the presence of both 2PEA and IPA at moderately high concentrations was specific for raspberry juice samples. The remaining juice samples have similar BAs profiles. It can be concluded that BAs profiles were characteristic for several juices types and it was possible to distinguished bilberry, chokeberry and raspberry samples from the other berry juices.

4. Conclusions

A new analytical method was developed and fully validated for the simultaneous determination of biogenic amines in fruits juices. The developed method offers the potential of the determination of a high number of compounds (14), combining selectivity, high-resolution capacity and fast analysis time (only 16 min) of GC–MS with the advantages of simple, rapid and reliable extraction procedures. SALLME is a straightforward technique in which small amounts of reagents and solvents are utilized for each extraction. The developed method is inexpensive, reduces the usage of hazardous organic solvents compared to previous approaches, and it is environmentally friendly, which was assessed using two greenness metrics for analytical procedures: GAPI and AGREE. The method has been fully validated and displays satisfactory linearity ($R2 \geq 0.9948$), low LODs (1.5–8.1 μg/L), low LOQs (5.0–26.72 μg/L), excellent accuracy (84–108%), good repeatability (2.6–11.3%) and reproducibility (2.5–11.6%). The obtained results confirmed that the SALLME-GC–MS method was suitable for the determination of BAs at trace levels (μg/L) in liquid food samples. The developed method can be a useful tool for monitoring food quality and ensuring food safety in terms of biogenic amines content.

CRediT authorship contribution statement

Anna Różańska: Conceptualization, Methodology, Writing – original draft. **Magdalena Fabjanowicz:** Methodology, Writing – review & editing. **Kaja Kalinowska:** Methodology, Writing – review & editing. **Żaneta Polkowska:** Writing – review & editing. **Justyna Płotka-Wasylka:** Writing – review & editing, Supervision.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132557.

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Supplementary Material

Green, simple analytical method for biogenic amines determination in fruit juice samples using salting-out assisted liquid-liquid microextraction and gas chromatography-mass spectrometry

Anna Różańska, Magdalena Fabjanowicz, Kaja Kalinowska, Żaneta Polkowska, Justyna Płotka-Wasylka

Gdańsk University of Technology, Faculty of Chemistry, Department of Analytical Chemistry 11/12 Gabriela Narutowicza Street, 80-233 Gdańsk, Poland

Submitted to *Food Chemistry*

Table S1 Box-Behnken response surface design and corresponding response values.

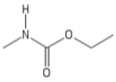
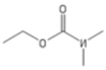
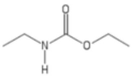
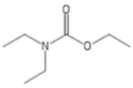
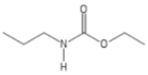
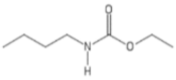
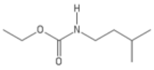
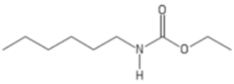
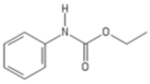
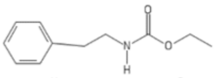
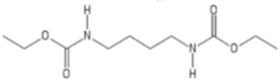
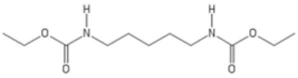
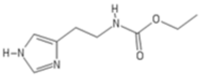
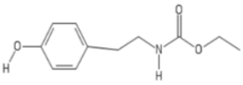
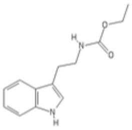
StdOrder	Run	Sample	NaOH	ECF	Sample [µL]	NaOH [%]	ECF [%]	Standardized sum of BAs peak areas		
								Actual	Predicted	Residual
10	1	0	1	-1	100	35	0,5	0,297	0,331	-0,035
9	2	0	-1	-1	100	5	0,5	0,000	-0,009	0,009
2	3	1	-1	0	150	5	1,5	0,416	0,417	-0,001
4	4	1	1	0	150	35	1,5	0,456	0,414	0,042
8	5	1	0	1	150	20	2,5	0,593	0,627	-0,034
11	6	0	-1	1	100	5	2,5	0,785	0,750	0,035
17	7	0	0	0	100	20	1,5	0,968	0,943	0,025
18	8	0	0	0	100	20	1,5	0,969	0,943	0,027
15	9	0	0	0	100	20	1,5	0,994	0,943	0,052
14	10	0	0	0	100	20	1,5	0,853	0,943	-0,090
13	11	0	0	0	100	20	1,5	0,871	0,943	-0,071
16	12	0	0	0	100	20	1,5	1,000	0,943	0,057
3	13	-1	1	0	50	35	1,5	0,602	0,601	0,001
12	14	0	1	1	100	35	2,5	0,378	0,387	-0,009
5	15	-1	0	-1	50	20	0,5	0,449	0,415	0,034
6	16	1	0	-1	150	20	0,5	0,368	0,376	-0,007
1	17	-1	-1	0	50	5	1,5	0,580	0,622	-0,042
7	18	-1	0	1	50	20	2,5	0,986	0,979	0,007

Table S2 Analysis of variance and regression coefficients of the calculated surface quadratic model for sum of BAs peak areas.

Source of variation	**SS**	**df**	**MS**	**SEM**	**F Value**	***p*-Value**
Model	1.47910	9	0.16434	0.025	45.53	<0.001
Linear	0.40896	3	0.13632		37.76	<0.001
Sample	0.07681	1	0.07681	0.021	21.28	0.002
NaOH	0.00028	1	0.00028	0.021	0.080	0.789
ECF	0.33188	1	0.33188	0.021	91.94	<0.001
Square	0.92196	3	0.30732		85.14	<0.001
Sample*Sample	0.04146	1	0.04146	0.029	11.49	0.010
NaOH*NaOH	0.47948	1	0.47948	0.029	132.83	<0.001
ECF*ECF	0.26398	1	0.26398	0.029	73.13	<0.001
2-Way Interaction	0.14818	3	0.04939		13.68	0.002
Sample*NaOH	0.00008	1	0.00008	0.030	0.020	0.883
Sample*ECF	0.02441	1	0.02441	0.030	6.76	0.032
NaOH*ECF	0.12369	1	0.12369	0.030	34.27	<0.001
Error	0.02888	8	0.00361			
Lack-of-Fit	0.00849	3	0.00283		0.69	0.594
Pure Error	0.02039	5	0.00408			
Total	1.50798	17				

SS – sums of squares; df – degrees of freedom; MS – mean square; SEM – standard error of means

Table S3 MS parameters for GC-MS analysis of BAs and IS derivatized (quantification ions in bold).

BAs	Derivatized amine stucture	Ions	t_r (min)
MET		**58** (100) 43 (58) 75 (48) 103 (33) 74 (31) 30 (28) 45 (22) 44 (18) 59 (13) 76 (8)	5.14
DIMET		**72** (100) 44 (84) 89 (72) 88 (71) 117 (56) 43 (41) 58 (37) 42 (34) 73 (13) 45 (12)	5.26
ET		**30** (100) 102 (96) 88 (84) 44 (79) 72 (75) 117 (36) 43 (27) 74 (26) 89 (23) 45 (21)	5.66
DIET		**130** (100) 58 (95) 145 (45) 72 (34) 116 (30) 100 (29) 86 (27) 44 (26) 30 (22) 42 (14)	6.17
PROP		**102** (100) 30 (55) 131 (20) 43 (20) 58 (11) 86 (10) 41 (10) 103 (7) 45 (5) 44 (5)	6.28
BUT		**102** (100) 30 (53) 145 (13) 57 (13) 41 (12) 103 (9) 116 (8) 74 (8) 58 (8) 44 (7)	6.76
IPA		**102** (100) 30 (45) 90 (33) 103 (20) 43 (16) 74 (13) 41 (13) 70 (13) 55 (8) 44 (7)	7.01
HEX		**102** (100) 30 (33) 144 (12) 90 (11) 43 (11) 103 (10) 41 (10) 173 (9) 74 (8) 58 (6)	7.45
IS		**165** (100) 93 (74) 106 (61) 65 (19) 92 (16) 120 (13) 119 (13) 166 (12) 66 (12) 77 (12)	7.81
2PEA		**102** (100) 91 (59) 104 (46) 30 (36) 193 (23) 105 (22) 65 (17) 103 (13) 164 (10) 77 (10)	8.17
PUT		**102** (100) 142 (54) 30 (50) 70 (30) 56 (25) 98 (20) 141 (19) 129 (19) 74 (18) 43 (17)	8.77
CAD		**102** (100) 30 (48) 56 (26) 84 (21) 156 (19) 43 (17) 116 (16) 74 (15) 128 (15) 41 (13)	9.01
HIS		**81** (100) 166 (75) 154 (51) 82 (36) 94 (23) 153 (21) 109 (11) 30 (10) 54 (9) 167 (8)	9.32
TYR		**120** (100) 107 (95) 102 (32) 148 (19) 30 (19) 192 (18) 135 (18) 108 (15) 121 (15) 77 (14)	9.54
TRYP		**130** (100) 143 (38) 232 (20) 131 (12) 144 (7) 77 (6) 103 (6) 186 (6) 102 (4) 129 (4)	9.95

MET – methylamine; DIMET – dimethylamine; ET- ethylamine; DIET – diethylamine; PROP – propylamine; BUT – butylamine; IPA- isopentylamine; HEX – hexylamine; IS – internal standard (Aniline); 2PEA – 2-phenylethylamine; PUT – putrescine; CAD – cadaverine; HIS – histamine; TYR – tyramine; TRYP – tryptamine

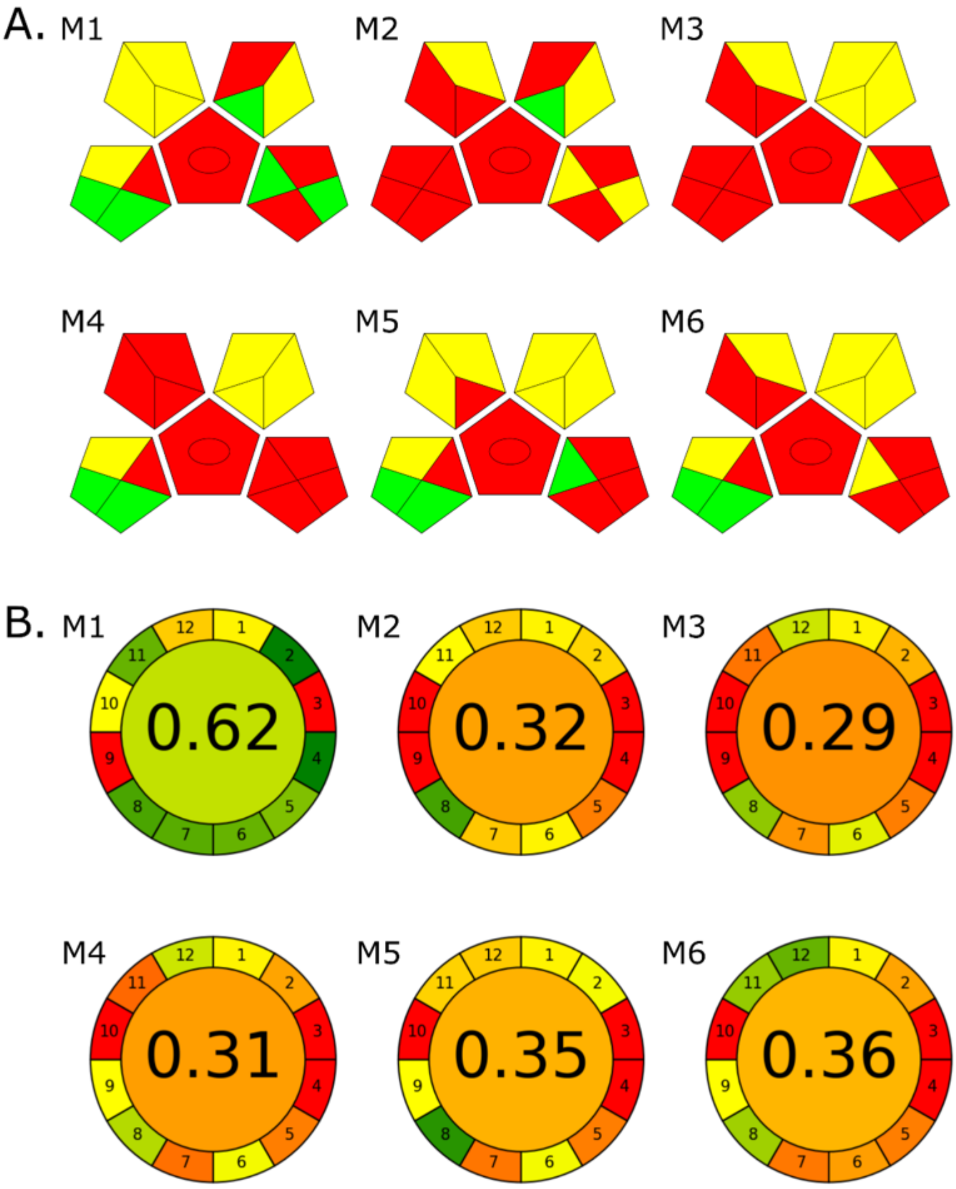


Fig. S1 Results of green index evaluation of analytical methodologies for BAs determination in fruit juice samples; A - GAPI, B – AGREE

Table S4 Concentrations (µg/L) of selected BAs detected in fruit juices using SALLME-GC-MS.

	Biogenic amines concentration [µg/L]														
	MET	**DIMET**	**ET**	**DIET**	**PROP**	**BUT**	**IPA**	**HEX**	**2-PEA**	**PUT**	**CAD**	**HIS**	**TYR**	**TRYP**	**SUM**
BI1	11.577±0.042	23.28±0.16	15.605±0.094	6.46±0.87	44.16±0.86	37.97±0.42	<LOD	11.39±0.11	<LOD	55.11±0.15	30.35±0.53	<LOD	154.63±0.90	23.56±0.41	414.1±1.8
BI2	10.846±0.045	22.18±0.31	17.772±0.081	11.47±0.31	42.28±0.44	37.50±0.45	<LOD	17.079±0.058	<LOD	113.4±1.3	77.84±0.83	17.78±0.20	305.1±5.3	<LOD	673.3±6.1
BL1	<LOD	<LOD	<LOD	<LOD	14.55±0.34	<LOD	15.65±0.28	10.722±0.066	<LOD	87.52±0.83	37.0±2.0	<LOD	274.3±1.3	<LOD	432±17
BL2	<LOD	<LOD	<LOD	<LOD	14.37±0.38	<LOD	8.041±0.041	<LOD	<LOD	155.0±1.1	41.84±0.77	22.30±0.31	170.0±1.4	<LOD	411.5±1.5
BL3	8.062±0.060	10.55±0.29	9.40±0.26	<LOD	34.17±0.71	15.61±0.21	<LOD	9.302±0.028	<LOD	76.21±0.53	37.20±0.19	<LOD	258.2±1.9	<LOD	458.7±2.3
BU	<LOD	<LOD	8.36±0.11	<LOD	16.30±0.36	19.38±0.24	9.905±0.055	<LOD	<LOD	101.3±1.5	56.2±1.5	15.44±0.26	<LOD	44.84±0.59	270.1±4.3
CH1	8.48±0.48	<LOD	11.39±0.18	<LOD	16.45±0.21	22.43±0.34	6.096±0.051	<LOD	21.64±0.19	64.1±1.2	29.49±0.85	<LOD	<LOD	41.20±0.76	221.28±0.82
CH2	9.133±0.043	<LOD	11.416±0.035	6.48±0.89	27.34±0.95	33.69±0.41	<LOD	10.64±0.21	32.02±0.60	230.9±5.1	99.1±1.4	13.98±0.25	<LOD	287.4±9.2	762.0±5.7
CH3	9.590±0.046	<LOD	10.752±0.030	7.69±0.34	22.18±0.33	30.53±0.30	<LOD	<LOD	21.45±0.27	106.1±3.0	104.9±1.1	33.31±0.35	<LOD	210.9±7.9	557.4±8.1
EL1	<LOD	<LOD	<LOD	<LOD	<LOD	11.392±0.087	7.202±0.029	<LOD	<LOD	67.49±0.35	22.71±0.15	<LOD	94.62±0.69	134.5±2.1	337.9±1.6
EL2	<LOD	<LOD	7.625±0.025	<LOD	<LOD	<LOD	11.053±0.029	8.335±0.042	<LOD	78.5±1.6	33.93±0.53	<LOD	187.0±3.8	<LOD	326.4±4.3
HO1	<LOD	<LOD	<LOD	<LOD	13.996±0.055	<LOD	8.982±0.097	<LOD	<LOD	66.63±0.87	27.99±0.54	<LOD	<LOD	80.6±1.2	198.19±0.17
HO2	<LOD	<LOD	<LOD	<LOD	16.52±0.20	11.61±0.29	17.65±0.49	13.36±0.14	<LOD	46.56±0.33	21.48±0.29	<LOD	133.6±1.7	<LOD	260.75±0.94
RA1	<LOD	<LOD	7.981±0.040	<LOD	<LOD	<LOD	22.18±0.24	8.149±0.067	18.964±0.045	37.70±0.30	66.55±0.47	<LOD	<LOD	166.2±2.7	327.7±2.7
RA2	<LOD	<LOD	<LOD	<LOD	13.732±0.034	<LOD	8.147±0.028	<LOD	35.00±0.20	37.32±0.38	18.67±0.43	<LOD	<LOD	67.44±0.87	180.31±0.87
RA3	<LOD	<LOD	<LOD	<LOD	17.71±0.26	11.32±0.10	6.111±0.035	<LOD	19.22±0.13	133.04±0.38	34.44±0.80	11.55±0.26	<LOD	229.4±4.7	462.8±5.5

BI – bilberry juice; BL – blackcurrant juice; BU – blueberry juice; CH – chokeberry juice; EL – elderberry juice; HO – honeyberry juice; RA – raspberry juice; MET – methylamine; DIMET – dimethylamine; ET- ethylamine; DIET – diethylamine; PROP – propylamine; BUT – butylamine; IPA- isopentylamine; HEX – hexylamine; 2PEA – 2-phenylethylamine; PUT – putrescine; CAD – cadaverine; HIS – histamine; TYR – tyramine; TRYP – tryptamine

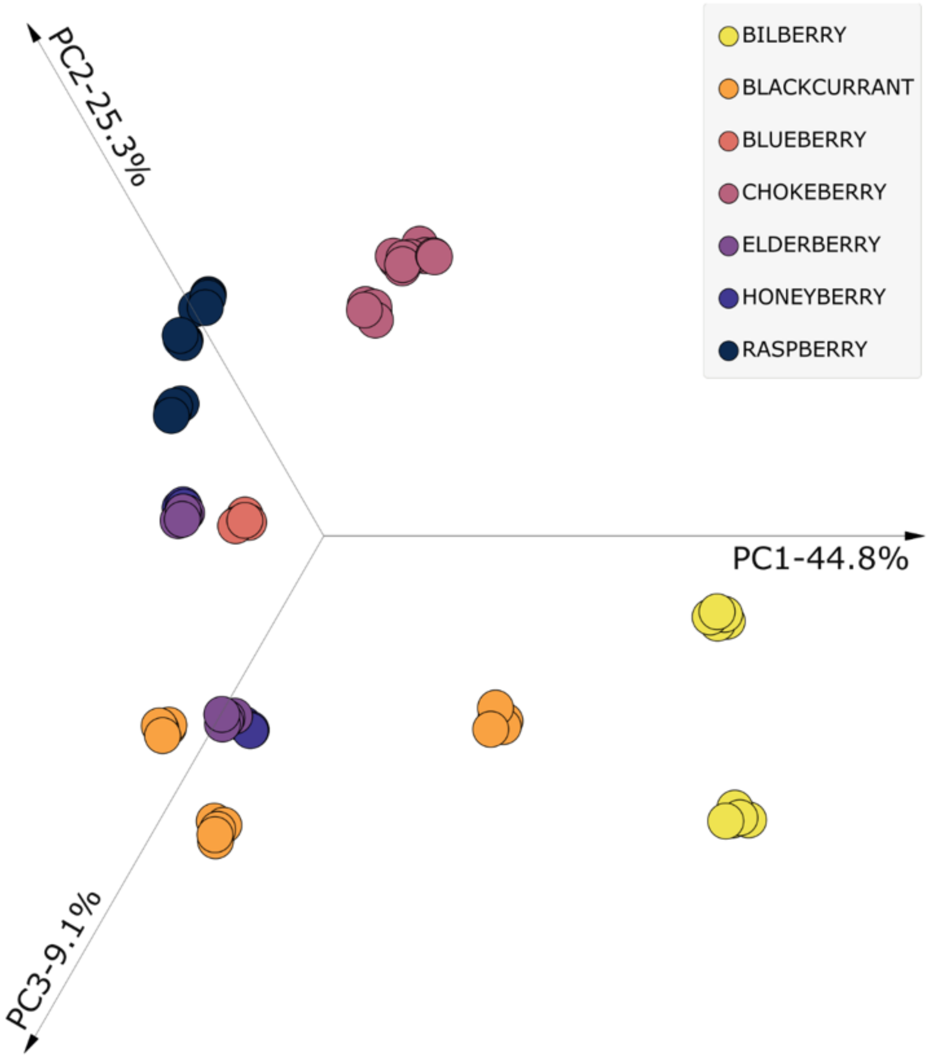


Fig. S2 Linear projection for principal component analysis of the BAs concentrations in berry fruit juice samples

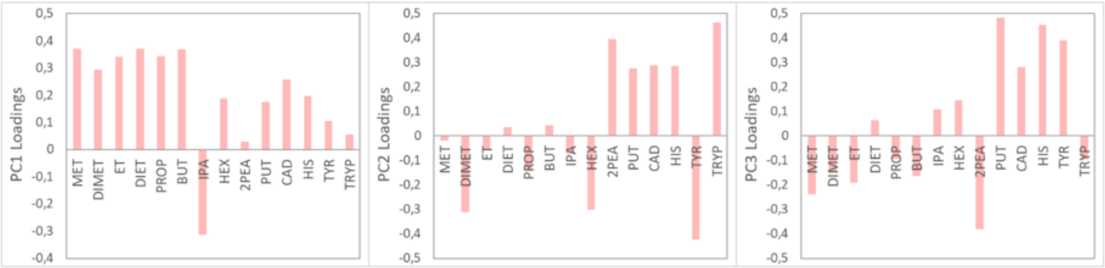


Fig. S3 Loadings for three principal components obtained after analysis of the BAs concentrations in berry fruit juice samples

4.2.3. Development of an ultrafast analytical method for determination of furans in fruit juice samples

Hypothese

- *Ultrafast gas chromatograph as an effective tool for determination of furans in food samples*

Questions

- *Determination of furans in juice samples - whether there were differences in the concentration of furans in home-made and store-bought juices?*

Furan and alkylfurans are oxidized, lipophilic heterocyclic compounds characterized by high volatility. They are present in many food products that are subjected to heat treatment, mainly in coffee, fried and baked products, cereal products, or in jars and canned products [27], [90].

Furan in food products is of concern as it is classified as a human pathogen by the National Toxicology Program [28] and also classified as "possibly carcinogenic to humans" by International Agency for Research on Cancer (IARC) [90]. Due to the toxicity of furan and alkylfurans, the European Food Safety Authority (EFSA), recommended collecting data on the occurrence and determination of furan and alkylfurans in food [91]. The main focus should be on the development of new methodologies for the determination and quantification of furan, 2-methylfuran, 3-methylfuran, 2,5-dimethylfuran, 2-ethylfuran and 2-pentyfuran in various food matrices. It is particularly important that the developed methodologies could be useful in the industry and food quality control, so they must be characterized by high throughput, simplicity and short analysis time.

Table 4 summarizes information on analytical methodologies for the determination of furans in fruit juices, presented in the literature. The information collected in Table 4 concerned the type of samples, the number of analyzed analytes, the technique used for determination and its parameters (LOD, LOQ, or analysis time).

Table 4 Information on analytical methodologies developed for furans determination in fruit juice samples.

Juice type	Number of furans	Sampling	Determination technique	Time of analysis [min]	LODs [ng/mL]	LOQs [ng/mL]	Recovery [%]	Ref.
apple	3	SHS	GC-MS	21	1	n.d.	99 - 102	[92]
apple grape raspberry, orange	7	HS-SPME	GC-MS	33	n.d	5.0	90.0 - 106.3	[93]
apple, grape, orange	1	SHS	GC-MS	25	0.2	0.6	n.d.	[94]
apple, orange	3	HS-SPME	GC-FID	22	0.042 - 0.230	0.14 - 0.76	90.2 - 110.1	[95]
black currant	3	DHS	GC-MS	69	n.d	n.d	n.d	[55]
blueberry, grape	7	SHS	GC-MS	43	<0.5	<1	80.3 - 107.2	[96]
pineapple, watermelon	1	SHS	GC-MS	30	0.37	1	n.d	[97]
grape	13	HS-SPME	GC×GC-HRTOF-MS	105	n.d	n.d	n.d	[98]
mango, orange	3	HS-SPME	GC-MS	24	n.d	n.d	n.d.	[99]
orange, tomato	10	HS-SPME	GC-MS/MS	16	0.002 - 1.071	0.006 - 3.571	84.94 – 117.24	[100]
orange	1	HS-SPME	GC-MS	15	0.079	0.26	104.79	[101]
pomegranate	7	SHS	GC-MS	48	n.d	n.d	n.d	[54]

n.d. – no data

Determination of furan and its derivatives in food is a challenge due to the high volatility and low concentration of analytes in a complex matrix. The most commonly used technique for the detection and determination of furans is gas chromatography (both one-dimensional and two-dimensional) coupled with mass spectrometry. However, different ways of furans sampling can be distinguished: dynamic headspace (DHS) or static headspace (SHS). More often the SHS technique is used, where the analytes are taken directly from the headspace or when the analytes are extracted using solid-phase microextraction fibres (HS-SPME). For both techniques, special attention should be paid to optimizing the process of extracting analytes from the samples. Very important parameters are sample type, sample volume and headspace volume, sample equilibration time and temperature, or salt addition. SPME is characterized by good selectivity, high simplicity and speed of the extraction step, and no need to use solvents. Unfortunately, the use of SPME is also associated with low reproducibility or extraction efficiency [95]. For this reason, in this research, the focus was on the development of a methodology that would enable direct headspace sampling without the isolation and enrichment of analytes.

The aim of the work was to develop a simple, fast, high-throughput and ecological methodology useful for the detection and determination of furan and alkylfurans in food samples. An ultrafast gas chromatograph with a flame ionization detector was used during the research. The developed method was validated and its greenness and blueness were assessed using two tools: AGREE [87] and BAGI [102] respectively. Homemade berry juices and berry juices purchased from grocery stores were analyzed. The following work is a response to the EFSA recommendation on obtaining data on the content of alkylfurans in food samples. The obtained results are described *in the preprint submitted for evaluation*.

A simplified scheme of the developed methodology is shown in Figure 8.

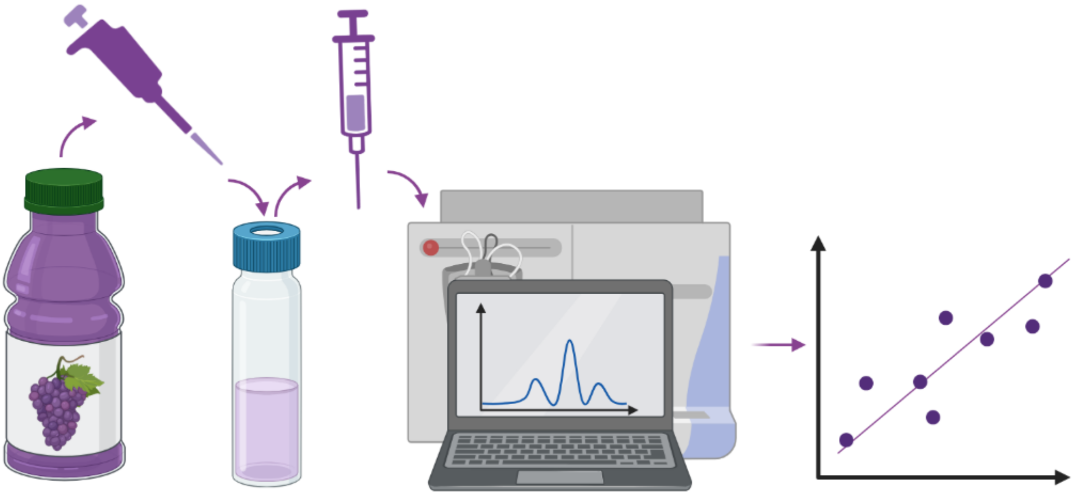


Fig. 8 Scheme of the HS-GC-FID procedure for the determination of furans in fruit juice samples.

In described research, a new analytical method for the determination of furans in fruit juices was developed. The extraction step of analytes from headspace was optimised using the one-factor-at-a-time method. It has been proven that the developed method offers the potential for the determination of furan and five alkylfurans, combining simplicity with very fast analysis time with good validation parameters. The proposed method was characterized by good greenness assessment. The obtained results confirmed the usefulness of the HS-GC-FID method for the determination of furans in trace amounts in fruit juice samples. In the most of samples, furans were not detectable, which indicated their good quality. The concentration of furans in home-made juices was higher than in the bought-juices, which can indicate that canning jars with juices at home generate more furans than industrial low-temperature pasteurization. Ultrafast gas chromatograph can be a useful tool for furans determination in food quality control.

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A new, fast, simple, practical and environmental-friendly HS-UFGC-FID approach for furan and six alkylfurans determination in fruit juice samples

Anna Różańska*, Tomasz Dymerski, Żaneta Polkowska*

Gdańsk University of Technology, Faculty of Chemistry, Department of Analytical Chemistry
11/12 Gabriela Narutowicza Street, 80-233 Gdańsk, Poland

Corresponding authors: anna.rozanska@pg.edu.pl; zanpolko@pg.edu.pl

Abstract

A simple, rapid, sensitive and robust gas chromatographic method was developed for the simultaneous determination of seven furans in fruit juice samples. The target analytes were transferred directly from the headspace of incubated samples without any preparation step. The developed method exhibits satisfactory limits of detection (from 1.0 to 2.4 μg/L) and limits of quantification (from 3.1 to 7.3 μg/L), and average recoveries between 86% and 107%. The developed procedure was used for furan and alkylfurans determination in the juices of different berries. During research, two types of juices were tested, namely store-bought and homemade juices. It should be noted that homemade elderberry juice contained the highest level of furans. The HS-UFGC-FID procedure was assessed for green and blue characters using AGREE and BADI indexes.

Keywords: *furan, alkylfurans, UFGC-FID, electronic nose, quality control, fruit juices, greenness evaluation, blueness evaluation*

1. Introduction

Furan and alkylfurans, which are oxidized, lipophilic heterocyclic compounds with high volatility, are commonly found in various food items that undergo heat processing. They are particularly prevalent in coffee, fried and baked goods, cereal products, as well as in preserved foods stored in jars and cans [1,2].

Furan found in food products raises concern due to its classification as a human pathogen by the National Toxicology Program [3], as well as its designation as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC) [1]. Furan has garnered global attention due to its carcinogenic and toxic properties, as demonstrated by numerous in vitro and in vivo experimental studies [4].

The generation of furans occurs through the interaction of amino acids and reducing sugars, leading to the formation of Maillard reaction products, as well as through the oxidation of lipids, polyunsaturated fatty acids or triglycerides, carotenoids, and ascorbic acid [1,2]. Among them, polyunsaturated lipids (such as linolenic and linoleic acid) and ascorbic acid were considered as the most effective precursors [4]. In the case of fruit juices, furan and alkylfurans may be formed during processes that extend the shelf-life of these products, i.e. temperature processes (heating, pasteurization) and even sterilization using ionizing radiation or UV light [5,6]. Additionally, furans may form during juice storage, especially for long periods in impermeable packaging and at higher temperatures. Research indicates that furan was detectable in pasteurized fruit juices (such as orange and mango) after a short storage period, ranging from a few weeks up to 32 weeks, despite being undetectable immediately after pasteurization [7].

In response to the potential toxicity of furan and alkylfurans, the European Food Safety Authority (EFSA) has advised the gathering of data regarding the occurrence and detection of these substances in food. The primary emphasis should be on devising novel methodologies to detect and measure furan, 2-methylfuran, 3-methylfuran, 2,5-dimethylfuran, 2-ethylfuran, and 2-pentylfuran in diverse food matrices [8]. These methodologies must prove beneficial in industrial settings and food quality control, necessitating attributes such as high throughput, simplicity, and swift analysis times.

Detecting furan and its derivatives in food presents a challenge because of their high volatility and low concentration within a complex matrix. Gas chromatography, both one-dimensional [5,9–13] and two-dimensional [14], coupled with mass spectrometry, stands out as the most frequently employed technique for their detection and determination. Different detectors such as flame ionization detectors as not common [6]. However, various methods of sampling furans can be identified, including dynamic headspace (DHS) [11] or static headspace (SHS). The SHS technique is more commonly utilized, where analytes are either directly extracted from the headspace [5,9,12,13,15] or extracted using solid-phase microextraction fibres (HS-SPME) [6,7,10,14,16,17]. For both techniques, optimizing the extraction process is crucial, with key parameters including sample type, sample volume, headspace volume, sample equilibration time and temperature, and salt addition. SPME offers good selectivity, simplicity, and speed in the extraction step, eliminating the need for solvents. However, its use may result in low reproducibility or extraction efficiency. Hence, this research focuses on developing a methodology for direct headspace sampling without the isolation and enrichment of analytes.

The objective of the study was to create an uncomplicated, rapid, efficient, and environmentally friendly method for detecting and quantifying furan and alkylfurans in food samples. The research utilized an ultrafast gas chromatograph (UFGC) with a flame ionization detector (FID). The developed method underwent validation, and its environmental impact and practical use were evaluated using two tools: AGREE and BAGI, respectively. Homemade berry juices and commercially available berry juices were subjected to analysis. This study responds to EFSA's recommendation to gather data on alkylfuran content in food samples.

2. Materials and methods

2.1. Reagents & standards

Furan (F, 99.0%), as well as most of the alkylfurans used, such as 2-methylfuran (2MF, 99.0%), 2-ethylfuran (2EF, 99.0%), 2,5-dimethylfuran (25DMF, 99.9%), 2,3-dimethylfuran (23DMF, 99.9%), and 2-pentylfuran (2PF, 98.0%) were purchased from Merck (Merck Life Science Sp.z.o.o., Poznań, Poland). 3-methylfuran (3MF, 97.5%) and methanol (MeOH, 99.9%) were purchased from Avantor Performance Materials (formerly VWR International, Gdańsk, Poland). Ultrapure water for aqueous solutions was prepared using HLP5 Hydrolab (Wiślina, Poland).

Furans and alkylfurans are highly volatile substances, therefore special attention should be paid to eliminate the possibility of losses of analytes during the preparation stage, which may result in a lack of accuracy of the method. Stock solutions of furan and alkylfurans were prepared at a concentration of 1000 mg/L in methanol (MeOH). Stock solutions were stored in a freezer. Various working solutions were prepared from the stock solutions by diluting them with ultrapure water. Prepared solutions were stored in the refrigerator and/or on ice until use.

2.2. Samples

Two types of berry juices were used for research: store-bought and homemade. The first type were samples of unfiltered berry juice that were commercially available in local supermarkets and were of different types: black currant juice (BC), bilberry juice (BI), blueberry juice (BL), chokeberry juice (CH), elderberry juice (EL), honeyberry juice (HO) and black mulberry juice (MUL). The second type were berry juices prepared at home and pasteurized at about 90°C for 15 minutes. The homemade juices were of different types: black currant juice (BC HM), blueberry juice (BL HM), and elderberry juice (EL HM). All samples were stored at refrigerator temperature and protected from light. A quantity of 5.0 ± 0.1 g of each fruit juice was transferred into 20 mL glass vials, which were subsequently sealed with a cap featuring a silicone–PTFE membrane. Each sample was tested in six replicates.

2.3. Instrumentation

All analyses were conducted using an ultra-fast gas chromatograph, Heracles II (Alpha MOS, Toulouse, France), featuring a split/splitless injector and two flame ionization detectors (μFIDs). Two capillary chromatographic columns were employed for analyte separation, including a non-polar MXT-

5 column (diphenyl dimethylpolysiloxane, 10 m x 0.18 mm x 0.40 µm) and a medium-polar MXT-1701 column (cyanopropylphenyl polysiloxane, 10 m x 0.18 mm x 0.40 µm), which were parallel-linked. Before analysis, samples were incubated in an HS 100 autosampler (Gerstel, Mühlheim, Germany) at 50°C for 5 minutes with an agitation speed of 500 rpm. The injector and detector temperatures were set at 200°C and 270°C, respectively. Hydrogen served as the carrier gas, maintained at a constant flow rate of 250 mm3/s. Column temperature programming was initiated at 35°C, held for 70 seconds, raised at a rate of 10°C/s to 270°C, and maintained for 56 seconds. Data processing was performed using AlphaSoft 12.4 software.

2.4. Method validation

The optimized method underwent assessment based on the following validation parameters: linearity, precision, sensitivity, and accuracy, per the quality assurance protocol. Linearity was assessed by utilizing six distinct concentrations. Limits of detection (LODs) and limits of quantification (LOQs) were determined to gauge the sensitivity of the methodology. Both Limits of Detection (LODs) and Limits of Quantification (LOQs) were computed from spiked samples (n = 5), establishing the minimum detectable analyte amount with signal-to-noise ratios of 3 and 10, respectively. Precision was evaluated by employing five replicates of juice samples spiked at 1.0 mg/L. Additionally, recovery rates were estimated by comparing the peak areas of spiked samples of known furans concentration to those of spiked water solutions (n = 5).

2.5. Statistical analysis

The peak areas during optimization and concentration values of the furans determined in fruit juice samples were used as input data for statistical data analysis. Analysis of variance, followed by Duncan's multiple comparison tests ($p < 0.05$) was carried out using XLSTAT (v.2014, Addinsoft, Paris, France).

3. Results and discussion

3.1. Optimization of sampling and GC-FID conditions

To optimize the analyte sampling procedure, the influence of incubation temperature, incubation time and injection volume on the efficiency of furan and alkylfurans determination was examined.

The concentration of substances in the headspace is intricately linked to the sample incubation temperature. Elevated incubation temperatures correspond to increased sensitivity; however, excessively high temperatures may compromise resolution. Moreover, excessively high incubation temperatures can lead to higher water concentrations, potentially adversely impacting analysis repeatability. During research, three incubation temperatures (40, 50, and 60°C) were examined, with results depicted in Figure 1A. The optimal incubation temperature for furan, 2-methylfuran, and 3-methylfuran was determined to be 50°C, with a slight decrease in concentration observed at 60°C. The increase in temperature aids in the transfer of analytes from the liquid phase to the headspace, thereby reducing the partition coefficient. In contrast, for the remaining alkylfurans (2EF, 2,5-DMF, 2,3-DMF, and 2PF), the concentration showed a direct correlation with the incubation temperature, with 60°C identified as the optimal incubation temperature. Literature suggests that high incubation temperatures may lead to additional formation of analytes, such as furan or alkylfurans, in certain food products during headspace sampling and their overestimation [1]. Therefore, considering the efficiency losses in primarily detecting furan at 60°C and the potential formation of alkylfurans at this temperature, 50°C as incubation temperature was chosen for subsequent analyses. This decision is consistent with findings reported by other researchers [10,17].

Desorption of a volatile substance from the sample to the headspace is not instantaneous and depends on various substance and sample properties such as polarity, volatility, matrix composition, interfering substances, pH, and state of matter, selecting an appropriate incubation time was crucial. The objective was to identify an incubation time while the tested substances reached sufficient concentration in the headspace, against minimizing this time to expedite analysis. During research, the concentration of analytes in the headspace was assessed after 1, 3, 5, 15, and 30 minutes of incubation, results shown in Fig. 1B. It was observed that furan and alkylfurans reached the highest concentration after 5 minutes of incubation. The decline in peak area for the tested substances beyond 5 minutes of incubation could be attributed to competition between analytes in the headspace and the matrix [3,6]. Thus, for the next analysis, 5 minutes of incubation was chosen.

The peak area, which is related to the sensitivity of the method, is related to injection volume. During studies, three injection volumes (1 mL, 2.5 mL and 5 mL) were tested. The volume of 2.5 mL was chosen as an optimal injection volume.

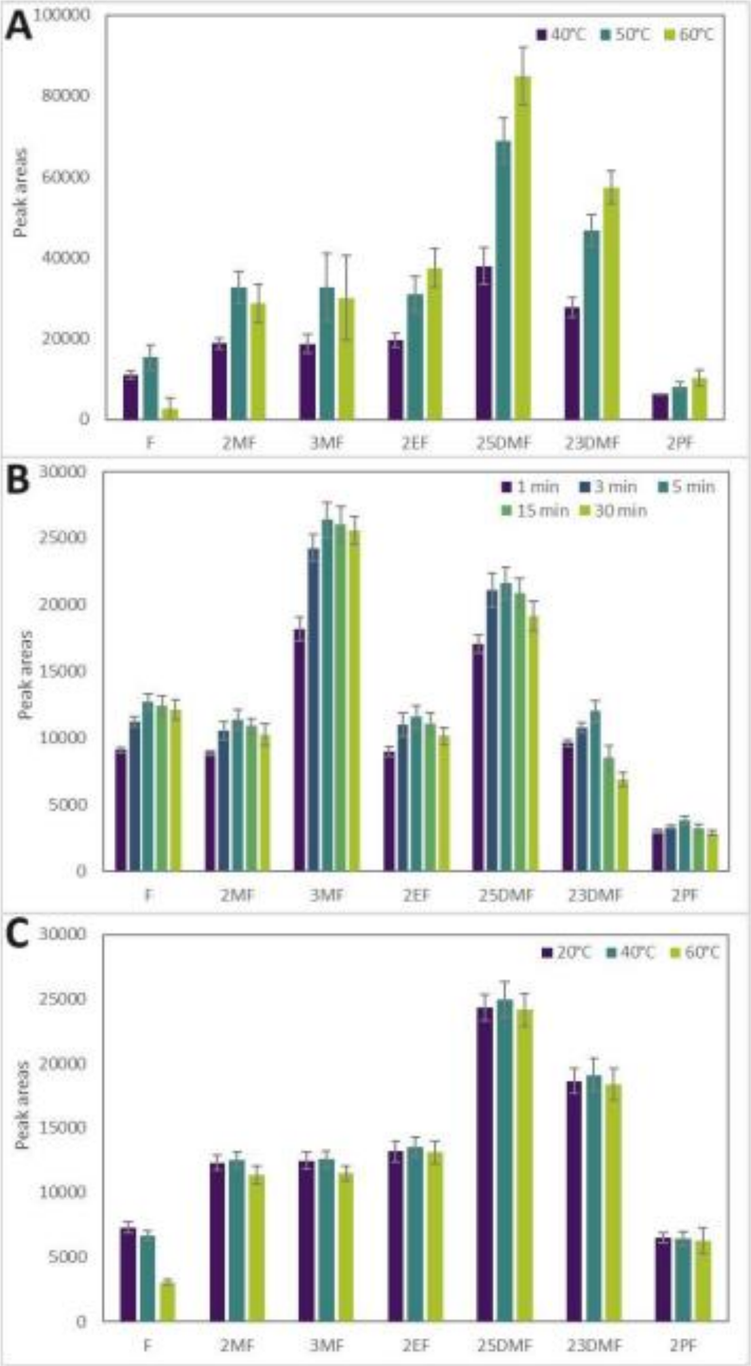

Figure 1 Influence of the sampling and gas chromatograph conditions on the resulting peak areas of selected furans (F – furan, 2MF – 2-methylfuran, 3MF – 3-methylfuran, 2EF – 2-ethylfuran, 25DMF – 2,5-dimethylfuran, 23DMF – 2,3-dimethylfuran, 2PF – 2-pentylfuran); A – incubation temperature; B – incubation time; C – trap temperature

Gas chromatography method parameters, namely temperature programme, trap temperature, and split flow ratio, among others, were selected to obtain satisfactory separation and signals for all analysed furans. Additionally, the goal was to obtain a high-throughput method. The chromatographic run took only 2.5 min. An example chromatogram obtained for a mixture of standards, a sample of mulberry juice and a sample of mulberry juice spiking with the mixture of standards is shown in Figure 2.

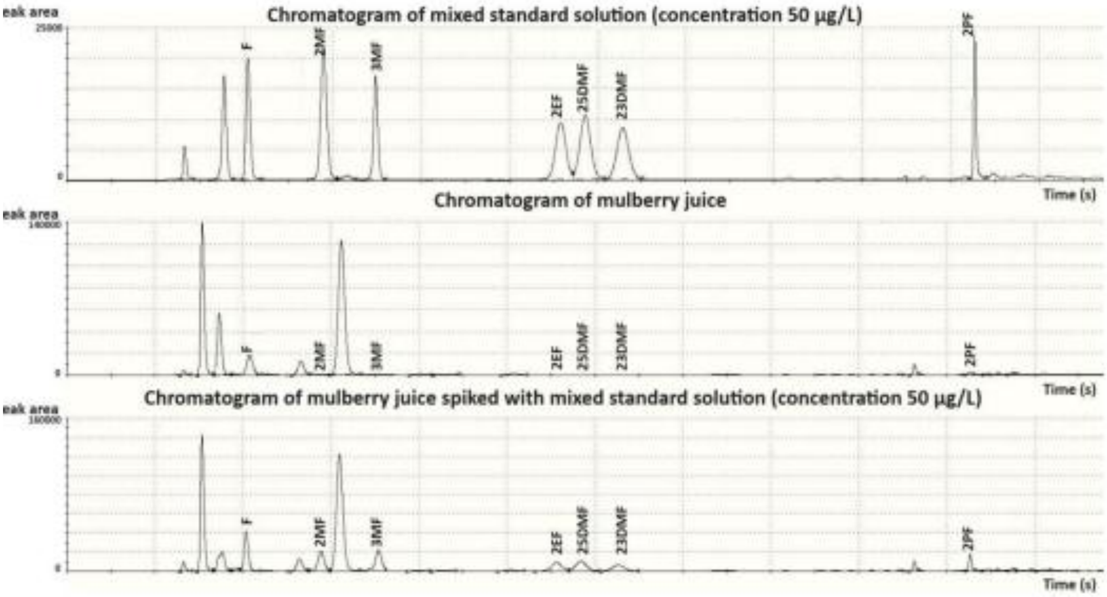

Figure 2 Chromatogram of mulberry juice and standard mixtures. (A) a mixture of standards with a concentration of 50 ppb (H2O as a solvent), (B) mulberry juice as a control sample, (C) mulberry juice with the addition of a mixture of standards with a final concentration of 50 ppb (F – furan, 2MF – 2-methylfuran, 3MF – 3-methylfuran, 2EF – 2-ethylfuran, 25DMF – 2,5-dimethylfuran, 23DMF – 2,3-dimethylfuran, 2PF – 2-pentylfuran)

The trap is used to concentrate volatile compounds and transfer these compounds very quickly to the column. Lowering the trap temperature may increase the concentration of volatile compounds, but it may also increase the efficiency of capturing low-volatile compounds (including water), and water condensation may negatively affect the sensitivity of the method for very volatile compounds as well as the repeatability of analyses. Results for different trap temperatures (20, 40 and 60°C) are depicted in Fig. 1C. In the case of furan, the most optimal trap temperature was 20°C, but for the tested alkylfurans the optimal trap temperature was 40°C. However, these results did not differ statistically significantly. For this reason, 40°C was selected as the temperature of the sorption trap for subsequent analyses.

Various split flow ratios of the stream transferred to two chromatographic columns were examined. As the split flow increases, the mass of the substance injected into the columns decreases, resulting in narrower peaks for low-volatility chemicals. This can potentially lead to decreased efficiency in detecting and determining highly volatile compounds. Among the tested split flow were 1, 3, 5, 10 and 25 mL/min. A split flow ratio of 5 ml/min was chosen as the optimal value, and this parameter was employed in subsequent tests.

3.2. Method validation

The sensitivity and linearity of the method were evaluated for furan and six alkylfurans using an external calibration method. The developed procedure was applied to six solutions containing all the furans tested within a range from 0.05 to 5 mg/L. Least squares linear regression was utilized to compute the equations of the calibration curves and determination coefficients (R^2) for both chromatographic columns. Extensive information concerning each analyte is listed in Table 1. The linearity exhibited satisfactory results for all studied analytes, with determination coefficients ranging from 0.9808 to 0.9905 (for the MTX-5) and from 0.9813 to 0.9908 (for the MTX-1701). The LODs fell within the range of 1.0 to 2.4 µg/L, while the LOQs spanned from 3.1 to 7.3 µg/L. LODs and LOQs were the lowest for furan and 2-pentylfuran and the highest for 2-ethylfuran and 2,5-dimethylfuran. Precision was determined by analyzing five replicates of juice samples fortified at a concentration of 1 mg/L. The Relative Standard Deviation (RSD) ranged from 4.5 to 9.6% (for MTX-5) and from 4.3 to 9.9% (for MTX-1701). The stability of the analytes in the juice matrix resulted in acceptable precision. The method's accuracy was assessed through a recovery test, involving the comparison of unenriched samples with samples enriched with analytes at a concentration of 1 mg/L using five replicates. The recovery rates are detailed in Table 1. Average recovery values ranged from 89 to 103% (for MTX-5) and from 86 to 107% (for MTX-1701). These results suggest that the devised method for determining furans in fruit juice samples exhibited a high level of accuracy.

Table 1 Analytical figures of merit for the developed UFGC-FID methodology for furan and alkykfurans determination.

Column MTX-5						
Analyte		Concentration range 0.05 to 5 mg/L			Concentration 1 mg/L	
	t_R (s)	Linearity (R^2)	LOD (µg/L)	LOQ (µg/L)	Precision (%RSD)	Recovery (%)
FUR	17.6	0.9857	1.1	3.4	4.5	89
2MF	28.9	0.9844	1.8	5.3	6.3	95
3MF	30.5	0.9829	1.2	3.6	6.7	97
2EF	52.7	0.9903	2.2	6.7	6.8	98
25DMF	53.9	0.9905	2.0	5.9	5.9	102
23DMF	55.5	0.9901	2.1	6.4	5.7	103
2PF	78.5	0.9808	1.1	3.3	9.6	99
Column MTX-1701						
Analyte		Concentration range 0.05 to 5 mg/L			Concentration 1 mg/L	
	t_R (s)	Linearity (R^2)	LOD (µg/L)	LOQ (µg/L)	Precision (%RSD)	Recovery (%)
FUR	19.6	0.9813	1.4	4.1	6.3	86
2MF	32.0	0.9836	2.0	5.9	5.9	93
3MF	34.8	0.9817	1.7	5.2	4.5	94
2EF	54.1	0.9908	1.9	5.7	4.7	98
25DMF	54.8	0.9907	2.4	7.3	4.3	106
23DMF	56.8	0.9901	2.1	6.4	6.6	107
2PF	77.5	0.9900	1.0	3.1	9.9	97

FUR – furan; 2MF – 2-methylfuran; 3MF – 3-methylfuran; 2ET- 2-ethylfuran; 25DMF – 2,5-dimethylfuran; 23DMF – 2,3-dimethylfuran; 2PF – 2-pentylfuran; s –second; t_R – retention time

3.3. Greenness and bluenness evaluation

Following the tenets of green analytical chemistry, the emphasis in developing novel analytical methods should be on their environmental sustainability and human safety. Moreover, practical aspects of the method, such as considerations regarding time and cost efficiency, as well as ease of use, should also be considered. The newly developed analytical method for quantifying furan and alkylfurans in fruit juices underwent evaluation for its environmental friendliness using the Analytical Greenness Calculator (AGREE) [18] and for its practicality using the Blue applicability grade index (BAGI) [19]. To gauge the environmental impact and assess the practicality of incorporating the proposed methodology into routine analyses, it was compared with five other methods for determining furans in fruit juice samples sourced from existing literature. Five gas chromatography-based analytical methods were chosen for comparison, as GC is considered the benchmark in furan determination. These selected methodologies varied in injection technique and detector type employed for analyte determination. For instance, Shen et al. utilized a static headspace injection mode following a 15-minute sample incubation [12]. The remaining four methods were based on solid-phase microextraction (SPME). Among these, the commonly utilized fiber for analysis was the carboxene/polydimethylsiloxane (CAR/PDMS) fiber, with incubation and extraction times of 10 [16], 20 [17] and 30 minutes [6], respectively. In contrast, Frank et al. devised a methodology using polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber, with a 20-minute extraction time [6]. For the quantification of furan and alkylfurans, gas chromatography was coupled with two types of detectors. While Frank et al. employed a flame ionization detector (FID) [6], most commonly, GC was coupled with mass spectrometry, either a conventional mass spectrometer [10,12,17] or a tandem mass spectrometer (MS/MS) [16].

Based on the findings from the ecological (Fig. 3) and practicality evaluation (Fig. 4), the HS-UFGC-FID method developed in this study emerges as the most environmentally friendly and offers the highest level of functionality. Its key advantages, compared to alternative methods, lie in the reduction of sample preparation and analysis time. With a sample incubation time of just 5 minutes and the elimination of the analyte extraction step, this method streamlines the analytical process significantly. In contrast, other methodologies typically require incubation and extraction times ranging from 10 to 30 minutes. This simplification of the analytical procedure results in a shortened analysis time of only 2.5 minutes, compared to the 14 to 43 minutes required by other procedures. Moreover, this method allows to determine seven substances in a single analysis, while minimizing waste generation. Consequently, this approach enhances methodology throughput, improving both, time and cost effectiveness, while also reducing negative environmental impacts and health risks.

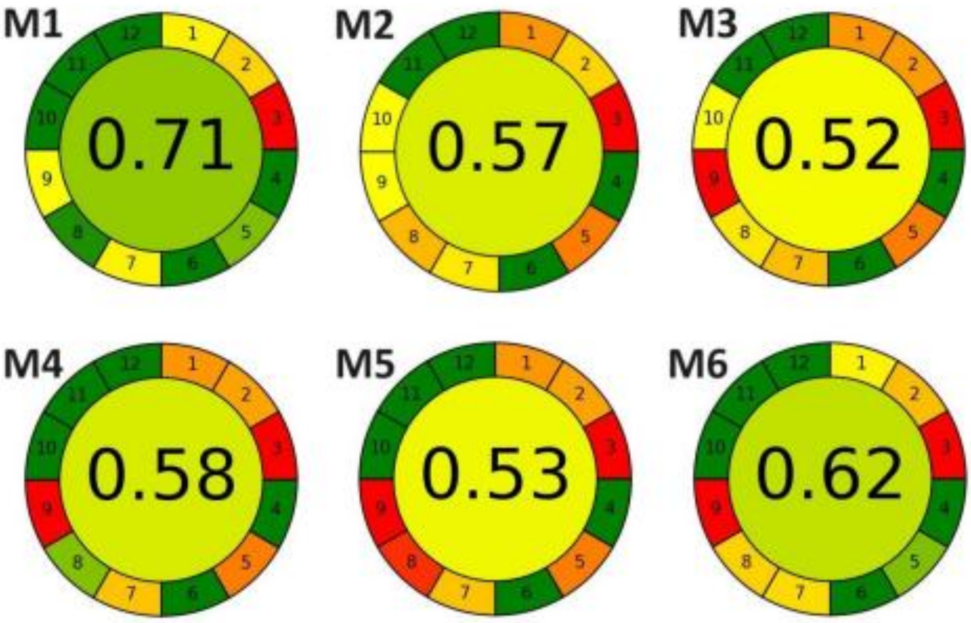

Figure 3 Results of green index evaluation of analytical methodologies for furans determination in fruit juice samples using AGREE tool; M1 – this work, M2 [6], *M3* [10], *M4* [16], *M5* [17], *M6* [12]

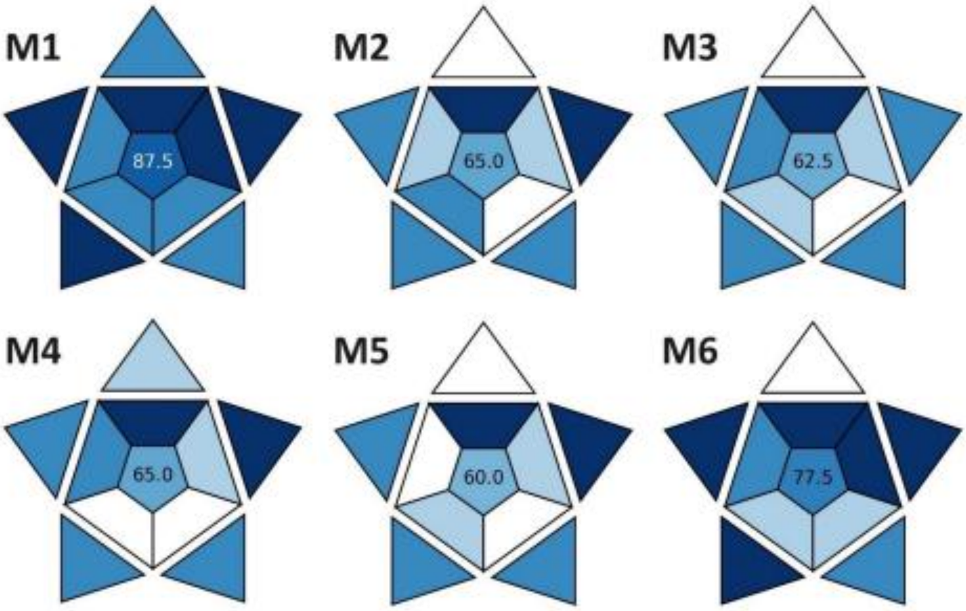

Figure 4 Results of blue index evaluation of analytical methodologies for furans determination in fruit juice samples using BAGI tool; M1 – this work, M2 [6], *M3* [10], *M4* [16], *M5* [17], *M6* [12]

3.4. Fruit juice samples

The study examined the furan and selected alkylfurans content in berry fruit juices available commercially and those made at home. The findings from the analyzed samples are outlined in Table 2.

Table 2 Concentrations (µg/L) of furan and selected alkylfurans detected in fruit juices using UFGC-FID.

	Furan and alkylfurans concentration [µg/L]						
	FUR	2MF	3MF	2EF	25DMF	23DMF	2PF
BC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BI	7.7 ± 2.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BL	4.8 ± 0.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
CH	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EL	12.14 ± 0.63	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HO	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
MUL	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BC HM	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BL HM	7.32 ± 0.44	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EL HM	34.9 ± 1.9	9.7 ± 2.4	<LOD	<LOD	<LOD	<LOD	3.95 ± 0.31

FUR – furan; 2MF – 2-methylfuran; 3MF – 3-methylfuran; 2ET- 2-ethylfuran; 25DMF – 2,5-dimethylfuran; 23DMF – 2,3-dimethylfuran; 2PF – 2-pentylfuran;BC – black currant juice; BI – bilberry juice; BL – blueberry juice; CH – chokeberry juice; EL – elderberry juice; HO – honeyberry juice; MUL – mulberry juice; BC HM – black currant homemade juice; BL HM – blueberry homemade juice; EL HM – elderberry homemade juice

According to Table 2, furan was identified in five juice types (BIL, BL, EL, BL HM, EL HM) at concentrations varying from 4.3 to 37.5 µg/L, while 2-methylfuran and 2-pentylfuran were exclusively detected in homemade elderberry juice samples with concentration from 7.5 to 13.2 µg/L and from 3.4 to 4.3 µg/L, respectively. Levels of alkylfurans other than those mentioned above, namely 3MF, 2EF, 25DMF, and 23DMF, were below the limit of detection in the examined samples. The results concerning the quantified chemical compounds are depicted in Figure 4A.

The concentration of furan in berry juices was similar to that found in commercially available orange juice samples, which varied from 0.59 to 27.39 µg/L [17]. The presence of furan in fruit juices can arise from the oxidative and hydrolytic processes of ascorbic acid during heat processing [3,4]. Research on mice and rats has confirmed that 2-methylfuran can lead to liver damage, alterations in the bronchi and lungs, and histological changes [2]. Hence, it is crucial to choose a juice pasteurization method that reduces the concentration of this substance in the final products. Indeed, the occurrence of 2-pentylfuran and 2-ethylfuran in heat-treated foods primarily stems from lipid degradation, as outlined by Kettlitz et al. [2]. The generation of these compounds arises from the oxidation of α, β-unsaturated aldehydes, originating from lipids at high temperatures, as highlighted by Kim et al. [17].

The overall furan content, depending on the juice type, is depicted in Fig. 4B. From these findings, it is evident that blueberry and bilberry juice samples exhibited relatively minor fluctuations in furan concentrations. However, there was considerable variability in the furan levels among elderberry juices. This might be attributed to factors such as amino acid content, vitamin C content, food processing methods, or production technology, in addition to the juice pasteurization conditions, as suggested by Batool et al. and Kettlitz et al. [2,4]. Nevertheless, the total furan content in each juice tested remained relatively low, not surpassing 0.06 mg/L.

Furthermore, a comparison was made between the furans content in purchased and homemade juices (Fig. 4C). The analysis revealed that homemade juices exhibited higher levels of both individual furan and the total sum of furans. In purchased juices, the total sum of furans averaged 8.2 ± 3.4 µg/L, whereas in homemade juices, it averaged 27.9 ± 21.6 µg/L. Commercial juice production typically involves exposure to lower temperatures for shorter durations, potentially reducing the formation of furan and alkylfurans.

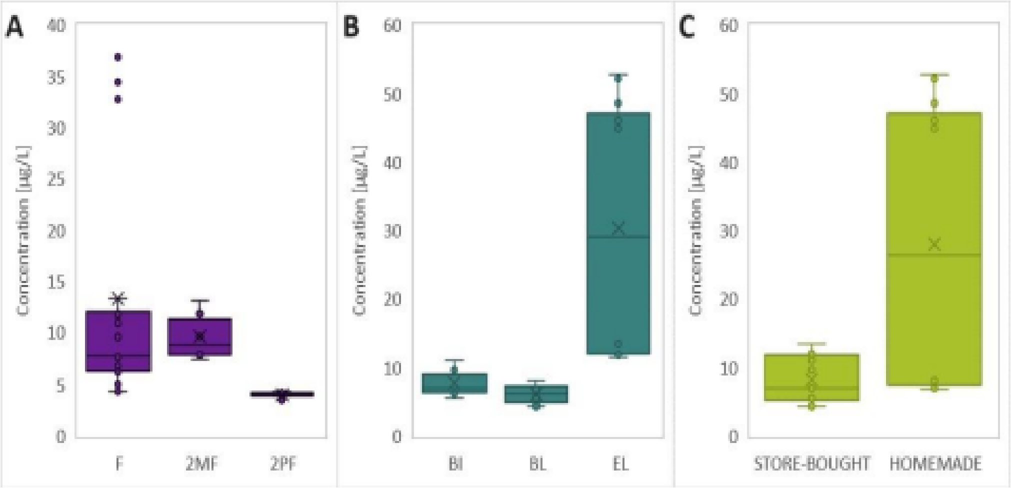

Figure 5 Furanes concentrations in fruit juices; A – box plots for furan, 2-methylfuran and 2-pentylfuran, B- box plots for different juice types (bilberry juice, blueberry juice and elderberry juice), C – box plots for store-bought and homemade berry juices

4. Conclusions

A novel analytical method has been developed and comprehensively validated for the concurrent assessment of furan and alkylfurans in fruit juices. This method presents the capability to quantify a significant number of compounds (7), merging selectivity, and short analysis time (merely 2.5 minutes) of UFGC–FID with the benefits of an uncomplicated, swift, and dependable sampling technique. The devised approach is cost-effective, devoid of solvents, efficient in time, rapid, and sturdy. Additionally, it is eco-friendly and convenient, as evaluated through the AGREE and BAGI metrics for analytical procedures, assessing environmental and practical aspects. The method has undergone comprehensive validation and demonstrates favourable linearity ($R^2 \geq 0.9808$), with low Limits of Detection (1.0–2.4 μg/L), low Limits of Quantification (3.1–7.3 μg/L), excellent accuracy (86–107%), and reliable repeatability (4.3–9.9%). The results obtained validate the applicability of the UFGC-FID method for detecting furans at very low concentrations (μg/L) in liquid food samples. This developed approach serves as an effective tool for monitoring food quality and guaranteeing food safety regarding furans content. Comparing store-bought and homemade berry juices, the concentration of furans in homemade juices was higher than in the store-bought juices, which can indicate that canning jars with juices at home generate more furans than industrial low-temperature pasteurization.

CRediT author statement

Conceptualization – A. Różańska. **Methodology** – A. Różańska. **Bibliographic research** – A. Różańska. **Writing – Original Draft** – A. Różańska. **Writing – Review & Editing** – T. Dymerski, Ż. Polkowska. **Supervision** – Ż. Polkowska.

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5. Conclusions

This doctoral dissertation focused on the development of new analytical methodologies for characterizing and the quality assessment of fruit juices. These methodologies were used to determine terpenes in lime juice samples, as well as to determine biogenic amines, furan and alkylfurans in berry juices samples.

The main achievements and novelty aspects of the dissertation include:

- development of a methodology for the determination of monoterpenes in the volatile fraction of juice samples using two-dimensional gas chromatography;
- development of an analytical approach for detecting adulteration of juices using an e-nose;
- development of salting-out assisted liquid-liquid microextraction coupled with gas chromatography and mass spectrometry for quantitative analysis of biogenic amines in fruit juice samples;
- development of a methodology for the determination of furan and alkylfurans in the volatile fraction of juice samples as a response to the EFSA recommendation regarding the collection of data on the content of furans in food products;
- new analytical methods have been developed in accordance with the requirements of Green Analytical Chemistry.

The above aspects show a broad approach to the selected topic. In addition, the research results have been published in journals devoted to analytical chemistry (*Monatshefte fur Chemie*), food science (*Food Control, Food Chemistry*), or chemistry (*Journal of Luminescence*). This demonstrates the wide thematic scope of research that had to be carried out in order to achieve the research goals.

The combination of high chromatographic resolution and analyte separation efficiency using GC×GC together with high acquisition frequencies obtained by TOFMS enabled the accurate characterization of the volatile fraction of lime juices. Determination of terpenes allowed to select markers of the tested juices. These markers were then characterized for their bioactive properties. Kaffir lime juice showed a strong binding to human serum albumin. Due to its woody aroma and taste derived from terpinen-4-ol, consuming this juice can be problematic. However, it can be used as an addition to other types of beverages to increase their health-promoting properties.

An electronic nose was used to detect adulterations of orange juice. This methodology is characterized by the lack of sample preparation step, is very fast (measurement takes less than 2 minutes) and simple. The combination of ultrafast gas chromatography with chemometric methods made it possible to detect all samples adulterated by the addition of other fruit juice. The proposed approach may be used in the future to detect other adulterations of fruit juices.

The methodology for the determination of biogenic amines (SALLME-GC-MS) and the methodology for the determination of furan and alkylfurans (HS-UFGC-FID) were characterized by good validation parameters (LOD, LOQ, linearity, recovery). These methodologies were fast, simple and characterized by a good greenness assessment, which encourages their use in industry.

The research results supplemented the knowledge about fruit juices, in particular the content of terpenes, biogenic amines and furans in these matrices. This topic is significant for the quality control of juices, as well as extremely important for the health and safety of consumers. The proposed quality control methodologies can be implemented in the food industry to replace conventional time- and labour-intensive procedures.

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7. Research achievements

7.1. JCR-listed publications

Table 3 Individual contributions to co-authored publications.

Publications	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Collections of samples	+	NA	-	-	+	-	-	+
Samples analysis	+	NA	+	+	+	+	+	+
Concept of work	+	+	+	±	+	+	±	+
Statistical analysis	+	NA	+	+	+	+	+	+
Interpretation of results	+	+	+	±	+	+	+	+
Results description	+	+	+	±	+	+	+	+
Preparation of tables and figures	+	+	+	±	+	+	+	+
Manuscript writing	+	+	+	±	+	+	+	+
Publications	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Collections of samples	+	NA	-	-	-	NA	-	-
Samples analysis	+	NA	+	+	±	NA	+	±
Concept of work	+	±	±	+	-	±	-	-
Statistical analysis	+	NA	+	+	±	NA	±	-
Interpretation of results	+	±	+	+	±	+	±	±
Results description	+	+	+	+	±	+	±	±
Preparation of tables and figures	+	±	+	+	±	+	±	-
Manuscript writing	+	+	+	+	±	+	±	-

where:
NA not applicable
± partial contribution to the action
+ full participation in action
– no involvement in the action

Citation based on the Scopus, accessed on February 25th, 2024.

[1] **A. Różańska**, T. Dymerski, Ż. Polkowska, A new, fast, simple, practical and environmental-friendly HS-UFGC-FID approach for furan and six alkylfurans determination in fruit juice samples, pre-print submitted for evaluation.

[2] M. Fabjanowicz, **A. Różańska**, N.S. Abdelwahab, M. Pereira-Coelho, I.C. da Silva Haas, L.A. dos Santos Madureira, J. Płotka-Wasylka, An analytical approach to determine the health benefits and health risks of consuming berry juices, *Food Chemistry* 432 (2024) 137219, DOI: 10.1016/j.foodchem.2023.137219, **IF=10.212**, **Q1**.

[3] **A. Różańska**, O. Bunkoed, J. Płotka-Wasylka, Development of a new green analytical methodology for the determination of phthalates in single-use babies diapers using ultrasound-assisted extraction and polypropylene porous membrane, *Microchemical Journal* 193 (2023) 109228, DOI: 10.1016/j.microc.2023.109228, **IF=5.346**, **Q1, 1 citation.**

[4] M. Fabjanowicz, **A. Różańska**, K. Kalinowska, J. Płotka-Wasylka, Miniaturized, green salting-out liquid-liquid microextraction coupled with GC-MS used to evaluate biogenic amines in wine samples, *Microchemical Journal* 180 (2022) 107616, DOI: 10.1016/j.microc.2022.107616, **IF=5.346, Q1, 9 citations.**

[5] **A. Różańska**, M. Fabjanowicz, K. Kalinowska, Ż. Polkowska, J. Płotka-Wasylka, Green, simple analytical method for biogenic amines determination in fruit juice samples using salting-out assisted liquid-liquid microextraction and gas chromatography-mass spectrometry, *Food Chemistry* 384 (2022) 132557, DOI: 10.1016/j.foodchem.2022.132557, **IF=10.212, Q1, 16 citations.**

[6] **A. Różańska**, M. Russo, F. Cacciola, F. Salafia, Ż. Polkowska, P. Dugo, L. Mondello, Concentration of Potentially Bioactive Compounds in Italian Extra Virgin Olive Oils from Various Sources by Using LC-MS and Multivariate Data Analysis, *Foods* 9 (2020) 1120, DOI: 10.3390/foods9081120, **IF=4.266, Q1, 18 citations.**

[7] M. Lubinska-Szczygeł, **A. Różańska**, J. Namieśnik, T. Dymerski, A. Szterk, P. Luksirikul, S. Vearasilp, E. Katrich, S. Gorinstein, Influence of steam cooking on pro-health properties of Small and Large variety of (*Momordica charantia*), *Food Control* 100 (2019) 335, DOI: 10.1016/j.foodcont.2019.01.027, **IF=6.492, Q1, 2 citations.**

[8] **A. Różańska**, T. Dymerski, J. Namieśnik, Novel analytical method for detection of orange juice adulteration based on ultra-fast gas chromatography, *Monatsh. Chem.* 149 (2018) 1615, DOI: 10.1007/s00706-018-2233-8, **IF=1.486, Q2, 21 citations.**

[9] **A. Różańska**, D. Sieńska, T. Dymerski, J. Namieśnik, Analysis of volatile fraction of sweetie (*Citrus maxima x Citrus paradisi*) and its parent fruit using proton transfer reaction mass spectrometry, *Monatsh. Chem.* 149 (2018) 1629, DOI: 10.1007/s00706-018-2229-4, **IF=1.486, Q2, 3 citations.**

[10] T. Majchrzak, W. Wojnowski, M. Lubinska-Szczygeł, **A. Różańska**, J. Namieśnik, T. Dymerski, PTR-MS and GC-MS as complementary techniques for analysis of volatiles: A tutorial review, Anal. Chim. Acta 1035 (2018) 1, DOI: 10.1016/j.aca.2018.06.056, **IF=5.828, Q1, 92 citations.**

[11] M. Lubinska-Szczygeł, **A.Różańska**, T. Dymerski, J. Namieśnik, E. Katrich, S. Gorinstein, A novel analytical approach in the assessment of unprocessed Kaffir lime peel and pulp as potential raw materials for cosmetic applications, *Ind. Crop. Prod.* 120 (2018) 313, DOI: 10.1016/j.indcrop.2018.04.036, **IF=4.769, Q1, 19 citations.**

[12] M. Lubinska-Szczygeł, **A.Różańska**, J. Namieśnik, T. Dymerski, R. Shafreen, M. Weisz, A. Ezra, S. Gorinstein, Quality of limes juices based on the aroma and antioxidant properties, *Food Control* 89 (2018) 270, DOI: 10.1016/j.foodcont.2018.02.005, **IF=4.184, Q1, 30 citations.**

[13] R. Shafreen, M. Lubinska-Szczygeł, **A. Różańska**, T. Dymerski, J. Namieśnik, E. Katrich, S. Gorinstein, Human serum interactions with phenolic and aroma substances of Kaffir (*Citrus hystrix*) and Key lime (*Citrus aurantifolia*) juices, *J. Lumin.* 201 (2018) 115, DOI: 10.1016/j.jlumin.2018.04.010, **IF=2.595, Q2, 14 citations.**

[14] M. Rutkowska, J. Płotka-Wasylka, M. Lubinska-Szczygeł, **A. Różańska**, J. Możejko-Ciesielska, J. Namieśnik, Birds' feathers – Suitable samples for determination of environmental pollutants, *TRAC-Trend. Anal. Chem.* 109 (2018) 97, DOI: 10.1016/j.trac.2018.09.022, **IF=9.024, Q1, 48 citations.**

[15] P. Kolasińska, **A. Różańska**, T. Dymerski, J. Gębicki, J. Namieśnik, Identification of odor substances in atmospheric air in the vicinity of industrial plants located in the area of the city agglomeration, *Przem. Chem.* 7 (2018) 1177, DOI: 10.15199/62.2018.7.29, **IF=0.299, Q3.**

[16] T. Majchrzak, M. Lubinska, **A. Różańska**, T. Dymerski, J. Gębicki, J. Namieśnik, Thermal degradation assessment of canola and olive oil using ultra-fast gas chromatography coupled with chemometrics, *Monatsh. Chem.* 148 (2017) 1625, DOI: 10.1007/s00706-017-1968-y, **IF=1.289, Q2, 22 citations.**

ΣIF: 72.834, Σcitations: 296 (274 without self-citations), h-index: 9 (Scopus)

7.2. Chapters and articles in non-JCR listed journals

[1] T. Majchrzak, K. Kalinowska, M. Lubinska-Szczygeł, **A. Różańska**, T. Dymerski, W. Wardencki, J. Namieśnik, Recent Applications of 1D GC-MS and 2D GC-MS in Foodomics Studies, Comprehensive Foodomics, Elsevier 2021, 10.1016/B978-0-08-100596-5.22773-X.

[2] M. Rutkowska, J. Płotka-Wasylka, M. Lubinska-Szczygeł, **A. Różańska**, J. Możejko-Ciesielska, J. Namieśnik, Ptasie pióra jako biomonitory do uzyskiwania informacji o stanie środowiska, *Analityka: Nauka i Praktyka* 2 (2019) 26.

[3] M. Lubinska-Szczygeł, **A. Różańska**, T. Dymerski, J. Namieśnik, Study of the effect of the hybridisation process on the content of terpenes in oroblanco fruit (*Citrus paradisi×Citrus grandis*), *Proceedings of the 14th International Students Conferennce Modern Analytical Chemistry* (2018) 236.

[4] **A. Różańska**, M. Lubinska-Szczygeł, T. Dymerski, J. Namieśnik, Classification of adulterated raspberry juice using ultra-fast gas chromatography, *Proceedings of the 14th International Students Conferennce Modern Analytical Chemistry* (2018) 253.

[5] **A. Różańska**, J. Namieśnik, Overall color parameter as a parameter determining the level of oxidation of olive oil, *World Scientific News* 75 (2017) 81.

[6] J. Myszka, M. Lubinska-Szczygeł, **A. Różańska**, J. Namieśnik, Analysis of volatile fraction selected hybrid fruits using chromatographic techniques, *World Scientific News* 75 (2017) 33.

[7] P. Michalczuk, J. Myszka, M. Lubinska-Szczygeł, **A. Różańska**, J. Namieśnik, Fast GC as a useful tool for authenticity assessment of kiwifruit, *World Scientific News* 75 (2017) 6.

[8] **A. Różańska**, J. Gromadzka, T. Dymerski, J. Namieśnik, Ocena wpływu dodatku oliwy z oliwek na degradację termiczną rafinowanego oleju rzepakowego, *Analityka: Nauka i Praktyka* 2 (2017) 8.

[9] H. Jakóbczyk, **A. Różańska**, T. Dymerski, J. Namieśnik, Characteristics of *Cucumis metuliferus*, *Actinidia deliciosa* and *Musa paradisica* fragrance profiles using a comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC×GC-TOF MS), *Proceedings of the 13th International Students Conferennce Modern Analytical Chemistry* (2017) 195.

[10] **A. Różańska**, T. Dymerski, J. Namieśnik, Detection of apple in orange juice using ultra-fast gas chromatography, *Proceedings of the 13th International Students Conference Modern Analytical Chemistry* (2017) 239.

[11] D. Sieńska, M. Lubinska-Szczygeł, **A. Różańska**, T. Dymerski, J. Namieśnik, Analysis of volatile fraction of hybrid fruit pulp using Proton Transfer Reaction - Time-Of-Flight Mass Spectrometry, *Proceedings of the 13th International Students Conference Modern Analytical Chemistry* (2017) 244.

[12] T. Majchrzak, **A. Różańska**, W. Wojnowski, T. Dymerski, J. Namieśnik, The impact of addition of olive oil on thermal degradation of refined rapeseed oil, *Proceedings of the 12th International Students Conference Modern Analytical Chemistry* (2016) 113.

7.3. Scientific conferences

Conference presentations:

- 10 authored,
- 16 co-authored.

7.4. Academic internships

- *September 2019 – March 2020:* Food Science Laboratory, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Supervisor: prof. Luigi Mondello;
- *July 2019:* School of Pharmacy, Hebrew University of Jerusalem, Supervisor: prof. Shela Gorinstein;
- *April 2019:* School of Pharmacy, Hebrew University of Jerusalem, Supervisor: prof. Shela Gorinstein.

7.5. Grants participation

- 2021: NCN OPUS 19, 2020/37/B/ST4/02886, 2021-2025, Disposable baby diapers: monitoring selected toxic compounds through the use of new analytical methods;
- 2019-2021: NCN PRELUDIUM 16, 2018/31/N/NZ9/03255, 2019-2021, Determination of the methabolic pathway of selected terpenes in citrus fruits using the PTR-TOFMS technique;
- 2019-2021: NCN PRELUDIUM 16, 2018/31/N/NZ9/02404, 2019-2021, The novel solution for real-time monitoring of secondary products of vegetable oil oxidation emitted during deep frying;
- 2019-2020: AGER, AGER2-Rif 2016-0169, 2016-2020, Valorizzazione dei prodotti italiani derivanti dall'oliva attraverso tecniche analitiche innovative;
- 2016-2020: NCN MAESTRO 6, 2014/14/ST4/00640, 2015-2021, "Antioxidant Power Series as a tool rational design and assessment of health promoting properties of functional food based on antioxidant phytochemicals.

7.6. Scholarships

- *2017-2022:* The National Centre for Research and Development Scholarship for Interdisciplinary, International PhD Studies (the PO WER 3.2 program);
- *2017-2020:* Ministry of Science and Higher Education Scholarship for best PhD Students;
- *2016-2020:* Gdańsk University of Technology Rector's Scholarship for best PhD Students.

7.7. Certificates

- Certificate - Bioacademy I module: Introduction to industrial Biotechnology;
- Certificate - Bioacademy II module: USP bioprocess – introduction to biopharmaceutical industrial;
- Certificate - Bioacademy II module: DSP bioprocess – introduction to biopharmaceutical industrial;
- Certificates of Internal Auditor:
  - Environmental Management System ISO 14001:2015;
  - Quality Management System ISO 9001:2015;
  - Health and Safety Management System OHSAS 18001:2007;
  - Laboratory Management System ISO 17025:2005.