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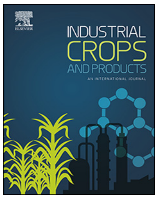

A novel analytical approach in the assessment of unprocessed Kaffir lime peel and pulp as potential raw materials for cosmetic applications

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ABSTRACT

Volatile fraction of fruits is a rich source of bioactive and aroma compounds, which can be used in the cosmetics industry after meeting relevant criteria. This is particularly evident in citrus fruits, especially in *Citrus hystrix*, in which the headspace consists mainly of terpenes. Due to the insufficient sensitivity of analytical methods, essential oils are used in investigations in contrast to fresh fruits. Therefore, a novel approach in the assessment of unprocessed *Citrus hystrix* was proposed for the first time. It was proven that the application of two-dimensional gas chromatography coupled with time-of-flight mass spectrometry combined with solid phase microextraction gives reliable results in this context. Quantitation of key aroma compounds (α-pinene, limonene, citronellal, linalool, terpinen-4-ol, myrcene, α-terpineol, and citral), in the peel and pulp of fruit after prior assessment of bioactive properties measured as total phenolic content, ferric-reducing/antioxidant power and binding to human serum albumin, gives opportunity to use *Citrus hystrix* as a raw material in the cosmetic industry. Terpinen-4-ol and citronellal appeared to be the most important constituents of *Citrus hystrix* with the highest concentrations in the peel (34.58 ± 0.75 μg/g) and pulp (66.02 ± 0.85 μg/g), respectively. Polyphenols and antioxidant activities and binding properties revealed approximately twice higher bioactivity of Kaffir lime peel than pulp. Fluorescence studies of interaction of polyphenol extracts and some volatile standards with human serum albumin (HSA) showed relatively high binding properties and the correlation between biological activity and the volatile composition. Terpenes are primarily used as components of the fragrances of new perfumes and also as additives to creams, lotions or shampoos. The natural origin of terpenes is recommended in cosmetics industry.

1. Introduction

Kaffir lime (*Citrus hysteria, Citrus hystrix*) belongs to the plants of Ruta family (*Rutaceae*). The fruit is also known as Kaffir lime, Thai lime, Makrut or Angel Wings, due to the shape of the leaves (Wongpornchai, 2012). Most often it occurs in Indonesia, Malaysia, the Philippines, Laos, Thailand and Vietnam (Tunjung et al., 2015). In this region of the world Kaffir lime leaves are, besides ginger and lemon grass, the most important spice added to almost every dish. Kaffir lime juice and pulp is not directly consumed because of their pungent taste. However, it is an excellent source of antimicrobial, antiviral, and antioxidant substances that can be used in the cosmetic industries (Fortin et al., 2002; Rafiq et al., 2016; Tunjung et al., 2015). Kaffir lime peel except for small culinary applications is also industrial waste (Shaha et al., 2013).

Citrus fruits are one of the more readily consumed fruits in the world, not only due to their flavorful, but also pro-health properties. The main part of citrus fruit, which is used on an industrial scale, is the pulp from which juices are squeezed. It is estimated that more than half of the fruit after the production process is an industrial waste. This problem is greater in the case of Kaffir lime, which juice is not consumed directly, so a significant part of the fruit is a post-production waste. For this reason, new methods of waste management are sought. However, still much of the waste remains unused. Other way to utilize fruit peel is the production of bioethanol and biogas (Negro et al., 2016; Taghizadeh-Alisaraei et al., 2017). However, this process can be disturbed by the presence of terpenes, especially limonene, in the fruit peel (Ruiz and Flotats, 2014). There are some reports on the disposal of terpenes, including mainly limonene, from citrus waste. Pourbafrani et al. (2010) proposed a method of removing limonene during the production of biogas from citrus waste. It is important to know exact

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concentrations of terpenes in the sample in order to receive fragrances, essential oils and bioactive ingredients from citrus peel or pulp. It will allow to designate which part of the fruit is more aromatic and its differences. There are no reports on the methodology of fresh Kaffir lime analysis. In general, the essential oils are analyzed. The composition of Kaffir lime peel essential oil (EO) was assessed using gas chromatography and mass spectrometry (GC–MS) (Haiyee and Winitkitcharoen, 2012; Kasuan et al., 2013). The reason of use EOs for the analysis could be too low sensitivity of the analytical devices. Analysis of fresh citrus could prevent the formation of artifacts and loss of analytes, which takes place in the extraction and distillation processes carried out to obtain EOs (Ziino and Romeo, 2004). The solution that enables the analysis of fresh fruit is the application of novel analytical techniques which are characterized by high resolution, as two-dimensional gas chromatography (GC × GC). According to the best of our knowledge, there is a lack of reports about the analysis of volatile fraction peel and pulp of C. *hysteria* using two-dimensional gas chromatography. Lubinska-Szczygieł et al. (2018) analyzed the volatile fraction of lime juice. This technique, in combination with solid phase microextraction and time of flight mass spectrometer, has been used repeatedly for fruit analysis (Dymerski et al., 2015).

The aim of the research was to develop an analytical methodology to identify, determine and compare the contents of volatile compounds present in the samples of Kaffir lime pulp and peel using the two-dimensional gas chromatography technique coupled with time-of-flight mass spectrometry (GC × GC-TOFMS). It will explain the potential causes of different taste of peel and pulp, restrictions on use of this fruit in gastronomy and provide an information, which could be a basis for further elaboration of isolation method for cosmetology applications. For this reason, two-dimensional gas chromatography couples with time-of-flight mass spectrometry was selected as a proper tool for the analysis of complex matrix of Kaffir lime fruit (Dymerski et al., 2013). In order to find correlation between the biological activity and volatile composition total polyphenols, antioxidant activities and binding properties of main volatiles to human serum albumin were carried out. Fluorescence measurements and antioxidant assays were performed.

2. Materials and methods

2.1. Samples and standards solutions

The subject matter was the peel and pulp of Kaffir lime *(Citrus hysteria, Citrus hystrix*). Kaffir lime fruits were bought in Bangkok (Thailand). Fruit samples were imported to Poland in sealed plastic bags in portable fridge maintained at between 10 and 15 °C. The procedure of sample preparation is shown in Fig. 1A. Standard solutions of terpenes: α-pinene, limonene, citronellal, linalool, terpinen-4-ol, myrcene, α-terpineol, and citral (Sigma-Aldrich, St. Louis, MO, USA) were prepared using methanol (Avantor Performance Materials Poland S.A.) as a solvent. Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid); Folin–Ciocalteu reagent (FCR); Tris, tris (hydroxymethy1)aminomethane; $FeCl_3 \times 6H_2O$; 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. Deionised and distilled water were used throughout.

2.2. Apparatus

Agilent 7980A (Agilent Technologies, Palo Alto, CA, USA) two-dimensional gas chromatograph equipped with a liquid nitrogen cooled two-stage cryogenic modulator (Zoex Co., Houston, USA) and MPS (Gerstel Co., Mülheim, Germany) configured as headspace autosampler was used. Liquid nitrogen was used as the cooling medium. The column set consisted of a 30 m × 0.25 mm i.d. × 0.25 µm – Equality 1 (Supelco Bellefonte, PA, USA) primary column and 2 m 0.1 mm i.d. × 0.1 µm – SGWAX (SGE Analytical Science Austin, TX, USA) secondary column. Applied temperature program is shown in Fig. 1B. Modulation period was 4 s (hot pulse of 0.80 s). Helium N6.0 was used as carrier gas and its volumetric flow rate was set up to 1 mL/min. The injector worked in splitless mode at a temperature of 250 °C. A Pegasus$^{\circledR}$ IV time-of-flight mass spectrometer (LECO Corp.) was used as detector. The transfer line and the ion source were maintained at 250. The detector voltage was set to – 1600 V and the MS was operated in electron impact ionization mode (70 eV). Cool time between stages was 1.20 s. In the step of isolation and enrichment of analytes a solid phase microextraction (SPME) was used. SPME 50/30 µm of thickness and 2 cm of length Carobxen/Polydimethylsiloxane/Divinylbenzene (CAR/PDMS/DVB) fiber was used (Sigma-Aldrich, St. Louis, MO, USA). The temperature of extraction was set up to 35 °C, and the time of a single extraction was 35 min. Before the extraction the samples were kept at 40 °C for 2 min and agitated with a magnetic stirrer (700 rpm). Thermal desorption was set up to 250 °C for 5 min to release the analytes from the surface of fiber. Between each analysis, the fiber was cleaned for 2 min at 250 °C.

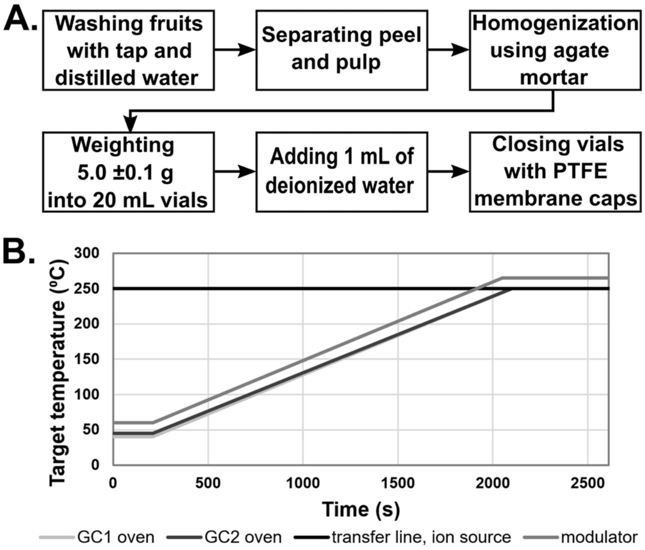

Fig. 1. A Scheme of sample preparation; B. Chromatographic temperature program applied in HS-SPME/GC × GC-TOFMS analytical procedure.

2.3. Data acquisition and qualitative analysis

ChromaTOF (LECO Corp., version 4.44.0.0) software was used to collect data. Tentative identification was done by correlation of retention times of analytes with retention times of authentic standards. The time of flight mass spectrometer (Pegasus 4D), which identified the chemicals time of flight, was produced by LECO Corp. (St. Joseph, MI, USA). Automated peak find and spectral deconvolution have been employed during data treatment. The mass range (*m/z*) from 40 u up to 500 u and the acquisition rate of 125 spectra/s were used to collect data. Major chemicals were identified by comparison of mass spectra with data included in NIST 11 and Wiley library.

2.4. Quantitative analysis

Quantitative analysis was performed using analytical procedure described above. Chemical compounds with the highest peak area in both samples were selected for quantitative determination. The standard addition method was used as a method of quantitative determination. Using this method, it was possible to compensate effects of matrix. Sample was spiked with the terpenes standards mixture to generate nine amendment levels of 0.1, 0.2, 0.5, 1.0, 5.0, 10.0, 20.0, 50.0, 100.0 µL/L (v/v). The analysis was performed in three replicates for each one of the sample and standard solutions. The concentration of tentatively identified volatile compounds was calculated using calibration curve. For each standard a calibration curve equation and determination coefficient (r^2) was calculated. The analytical parameters: limits of detection (LOD) and quantification (LOQ) were also determined. Values of LOD and LOQ were based on the standard deviation of the intersection of analytical curve (SD) and on the slope of the curve (a). The LOD as and LOQ were estimated as a 3.3·SD/a and 10·SD/a, respectively.

2.5. Determination of bioactive compounds, antioxidant and binding properties

Polyphenols were extracted from lyophilized samples of peel and pulp with water (concentration 20 mg/mL) during 1 h in a cooled ultrasonic bath and were determined by Folin-Ciocalteu method with measurement at 750 nm with spectrophotometer (Hewlett-Packard, model 8452A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW (Singleton et al., 1999).

The total antioxidant capacity (TAC) was determined by 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 extract samples as the appropriate reagent blank and absorbance was measured at 595 nm (Benzie and Strain, 1996).

Fluorometric measurements were used for the evaluation of binding properties of citrus extracts and some standards to human serum albumin (HSA). 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. The emission wavelength was recorded between 200 and 500 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 (Dymerski et al., 2015).

2.6. Odor activity value (OAV) and statistical analysis

The odor activity values (OAVs) were calculated by dividing the concentrations of aroma compounds with their sensory thresholds taken from the literature. OAV data were expressed as the mean ± standard deviation of triplicate tests. Two-way analysis of variance (ANOVA) was used to compare the significant differences among the means using STATISTICA 12 under the Fisher test at a p-level of 0.05 (StatSoft, Inc., Tulsa, Oklahoma, USA).

3. Results and discussion

3.1. Comparison of the content of selected classes of chemicals in samples of peel and pulp of Kaffir lime

Aroma of food products is a complicated mixture, sometimes consisting of several compounds (Wardencki et al., 2009). By performing chromatographic analysis, it was possible to detect about 500 chemical compounds in samples of the peel and pulp of Kaffir limes. Twenty two substances were selected as the main chemical compounds. Selected substances created a characteristic aroma profile of the tested samples, namely “fingerprint” (Fig. 2A). Based on the contour plots, it can be observed that both Kaffir lime peel and pulp was characterized by a different composition of the volatile fraction. In addition, for peel samples, selected chromatographic peaks were more intense than for pulp. On this basis, it can be deduced that Kaffir lime peel contained more terpenes. The content of these analytes was more than 70% of all chemical compounds present in the volatile fraction of sample. The heat map (Fig. 2B) shows the averaged results of the analysis of three samples of the fruit. Compound with the largest area of chromatographic peak was citronellal. Beside the citronellal, other terpenes such as terpinene, thujene and limonene in large amounts in the samples headspace were present. The major chemical compounds identified in the sample of Kaffir lime are listed in Table 1. Many studies have been conducted on the content of terpenes in Kaffir lime peel (Jantan et al., 1996; Kasuan et al., 2013; Muhammad et al., 2013; Nor, 1999; Waikedre et al., 2010). Each time the test object was not fresh peel, as in the present research, but essential oil, obtained by the use of different distillation methods. Chanthaphon et al. (2008) conducted the research of volatile fraction of ethyl acetate extract of hydrodistilled essential oil, in comparison with fresh peel used in the present experiment. The received results differ from those obtained in the reviewed study. The percentage of citronellal in the volatile fraction of Kaffir lime peel was about 17% and in case of ethyl acetate extract of hydrodistilled essential oil, 25.96% and 15.67%, respectively. The amount is therefore similar to the content in the oil. The biggest difference occurs in case of β-pinene, whose percentage in the research is about 2% and in the cited paper is about 30%. The conditions for sample preparation and analysis were influenced by the results, as well as ripeness of fruits, vegetative stage of plant, and storage conditions.

Based on the chromatographic peak area of selected compounds, the radar charts presenting the content of selected chemical classes in volatile fraction of Kaffir lime peel and pulp were prepared (Fig. 3A). In all cases the composition of volatile fraction of Kaffir lime peel is characterized by a higher content of individual compounds, than the composition of the volatile fraction of Kaffir lime pulp. The following data apply to all compounds detected in both samples.

Carboxylic acids are the smallest group of detected compounds in Kaffir lime fruits, which are present in amounts less than 0.01% of all compounds. However, when comparing the two samples it can be noticed that the fruit peel contains more carboxylic acids than the pulp. This is the reason of stronger peel smell than of pulp. In many cases, the carboxylic acids are suitable characteristics of fruit sour taste. Carboxylic acids also affect the flavor, giving the acid-fruity aroma. For Kaffir lime, because of the low content, carboxylic acids did not affect its smell. Detected carboxylic acids included caprylic, pelargonic, malic, acetic and citronellic acids.

A bigger amount of aldehydes is contained in the peel of Kaffir lime. The content of all detected compounds is 78%, while in the pulp only 22% of all aldehydes is detected. The compounds are responsilbe for fruity and fresh aroma of the fruits. The major aldehydes detected in Kaffir lime samples were dodecanal, hexanal, 2-hexenal and heptanal. Most detected aldehydes were included in both peel and pulp of the fruit, but with different concentration levels.

In case of ketones, distribution of these compounds is similar to that as in alcohols. In the volatile fraction of Kaffir lime peel 74% of the ketones was detected, while in the pulp only 26%. Acetophenone, fenchon, and 2, 6-dimethyl-3-heptanone is the most common ketones identified in samples. Most detected ketones have long carbon chains, what is associated with a pleasant fruity odor of food products.

Alcohol contents in both samples were different. The peel headspaces contained 69% of all detected compounds belonging to the alcohol class while the rest was present in the volatile fraction of fruit pulp. The most common alcohols found in the studied samples were: 1-hexanol, 2-hexen-1-ol and nonanol.

Esters were the only chemical class which in the peel and pulp occurred in approximate amounts. Among all identified esters of 54% was detected in the peel, while 46% was found in the pulp. The presence of esters in the food makes their aroma fruity. Among the esters the most popular were hexyl acetate and ethyl benzoate.

Terpenes were present in each of the research objects in a very large amount, but when comparing both fractions, it can be observed that peel of the lime fruit had higher amount of these compounds (66%) than the pulp (34%). This can be the justification for the fact that the peel has a much stronger aroma than fruit pulp.

Comparing the content of the chemical classes, it can be observed

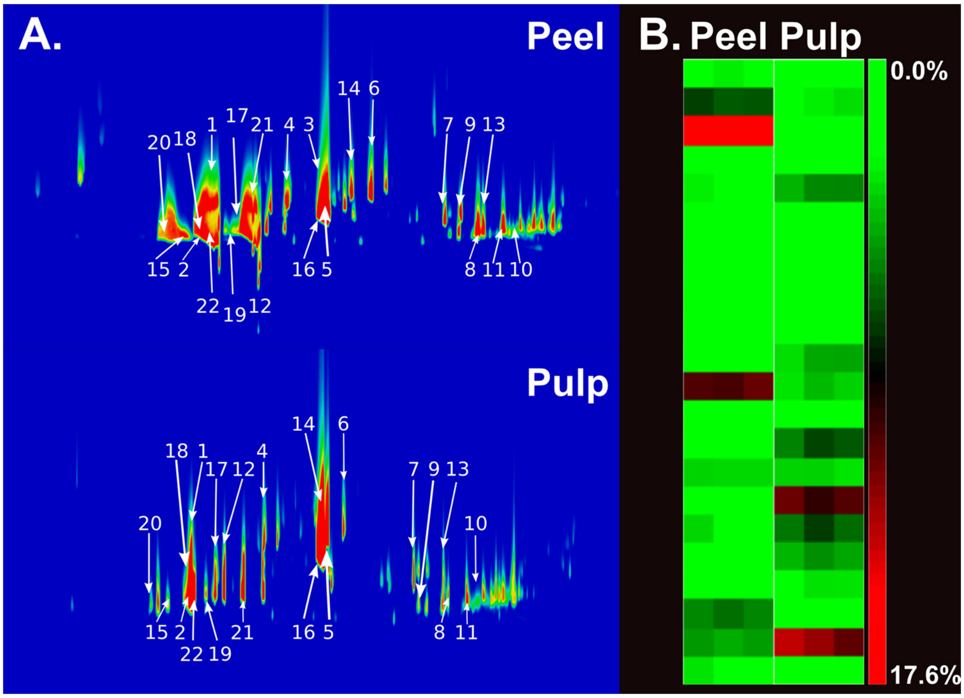

Fig. 2. A The comparison of terpene profiles by the use of GC × GC-TOFMS system (extracted ion contour plots obtained in TIC mode); B. Heat map describing a percentage distribution of selected terpenes in the Kaffir lime pulp and peel (calculated on the chromatographic peak areas); 1. β-pinene, 2. sabinene, 3. citronellal, 4. linalool, 5. α-terpineol, 6. β-citronellol, 7. citronellyl acetate, 8. α-copaene, 9. geranyl acetate, 10. α-cubebene, 11. β-caryophyllene, 12. limonene, 13. germacrene D, 14. α-pinene, 15. camphene, 16. terpinen-4-ol, 17. α-terpinene, 18. myrcene, 19. α-phellandrene, 20. α-thujene, 21. γ-terpinene, 22. β-phellandrene.

Table 1
The major compounds identified in the volatile fraction of Kaffir lime by GC × GC-TOF-MS.

No.	Chemical compound	RT1[a] [s]	Average RT2[b] [s]	Similarity	Unique mass	Aroma descriptors
1	β-Pinene	1186	1.184	854	93	green, musty, pine, resisous, sweet, turpentine, woody
2	Sabinene	1050	2.097	939	93	herbal
3	Citronellal	1310	2.204	886	69	citrus, fatty, floral, lemon, rose
4	Linalool	1332	2.208	745	71	citrus, orange, lemon, floral, waxy, aldehydic, woody
5	α-Terpineol	1402	2.408	790	59	earthy, floral, musky, spicy, woody
6	β-Citronellol	1438	2.289	928	69	citrus, floral, rose
7	Citronellyl acetate	1606	2.132	916	69	citrus, berry, floral, rose
8	α-Copaene	1682	2.070	937	161	woody
9	Geranyl acetate	1642	2.136	916	69	floral, rosy, waxy, herbal and green with a slight cooling nuance
10	α-Cubebene	1766	2.038	870	161	citrus, fruity, radish
11	β-Caryophyllene	1738	2.074	917	93	woody, spicy
12	Limonene	1170	2.026	936	93	citrus, mint, orange, terpenic, xyloid
13	Germacrene D	1694	2.012	907	161	woody, spicy
14	α-Pinene	998	2.034	861	93	pine, terpenic
15	Capmhen	1056	1.984	951	91	arborescent
16	Terpinen-4-ol	1382	2.160	864	71	woody, fruity, herbal, licorice, moldy, musty
17	α-Terpinene	1134	2.044	939	93	woody, oil, fruit, gasoline, lemon,
18	Myrcene	1050	2.188	808	93	terpenic, herbaceous, woody with a rosy celery and carrot nuance
19	α-Phellandrene	1164	2.040	915	93	citrus herbal terpene green woody peppery
20	α-Thujene	962	2.128	899	93	arborescent
21	γ-Terpinene	1272	2.156	914	93	woody, fruity, gasoline, herbal, sweet, terpenic
22	β −Phellandrene	1058	2.200	836	93	minty, terpenic

[a] RT1–first dimension retention time.
[b] RT2–second dimension retention time.

that the chemical compounds of each of these classes in higher amounts occurred in the Kaffir lime peel. The peel is therefore more aromatic part of this fruit. Moreover, peel is a natural barrier to protect the fruit against external factors and against the loss of many chemical compounds.

3.2. Comparison of the amount of key aroma compounds present in Kaffir lime peel and pulp determining the character of overall odor sensation

It is commonly known that terpenes are aroma active chemical compounds. It was shown that terpenes determinate the aroma of citrus fruits (Md Othman et al., 2016). For selected terpenes identified in samples of lime fruit corresponding aroma descriptions (Table 1) were assigned according to the AroChemBase V4 library. It can be observed that the aroma of compounds from the group of terpinene is a woody, earthy, while other chemical compounds are characterized by a citrus or fruity aroma.

The results of quantitative analysis are shown in Fig. 3B. Considering the content of individual terpenes, it can be noticed that the chemical compounds with citrus odor dominated in peel samples. While the greater the content of compounds of the Terpinene group was recorded in the fruit pulp. This explains the pungent and bitter smell of the fruit pulp and a pleasant citrus aroma of peel.

The biggest differences between the contents in the peel and pulp can be observed in the case of citronellal, which was the main chemical compound of the peel. The content was nearly one fifth of the volatile

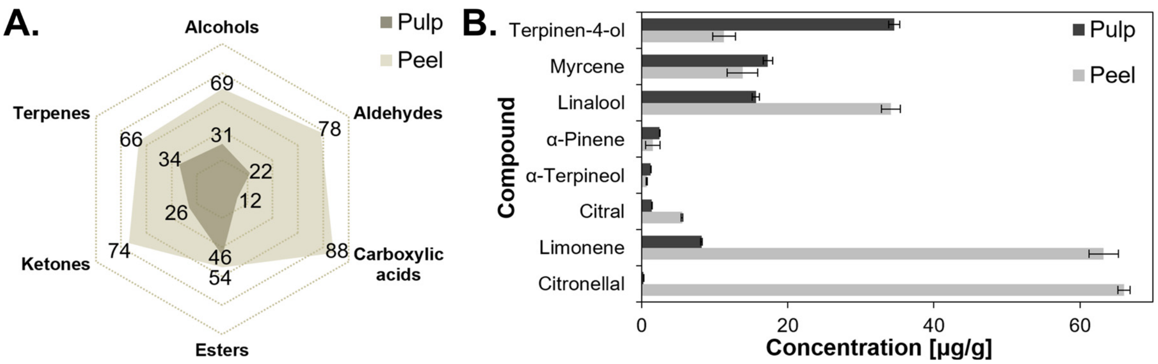

Fig. 3. A Relative amount (in percentage) of class of chemical compounds; B. Concentration of selected terpenes in Kaffir lime.

fraction of lime peel, while in the pulp it occurs in trace quantities, and not possible to be quantitative. Citronellal may be a potential marker of Kaffir lime peel aroma, especially that in other fruits of the Rutacea family (*Citrus limon*) it was at low concentrations (Nhu et al., 2006). Literature reports show that content of citronellal is also high in case of fresh Kaffir lime leaves. Citronellal, the main compounds in Kaffir lime peel, exhibits antimicrobial properties, which may hinder the production of biomass from citrus residues by destroying fermenting bacteria, therefore it has to be removed during the process. The recovered citronellal, due to its antioxidant, anti-inflammatory and redox-protective properties, can be used in the cosmetic and medicinal manufacturing. A pleasant citrus aroma makes citronellal a popular perfume ingredient and food additive. Its high content in Kaffir lime peel (66.02 ± 0.85 μg/g) shows a valuable source of this terpene. The main ingredient of the Kaffir lime pulp is terpinen-4-ol. This compound of woody aroma is used as a flavor and fragrance agent.

To investigate the cause of overall odor sensation character the concentration of aromatic compounds present in Kaffir lime peel and pulp was determined. Calculated concentrations were the basis for determining of the OVAs, which were the odor intensity indicators of these substances. The OAV can be calculated using the following equation:

$$\mathrm{OAV} = \frac{C}{C_{OT}} \tag{1}$$

where:

c – concentration of substance [μg/g],

c_{OT} – odor threshold concentration of substance [μg/g].

There are some reports presenting the value of odour threshold (Van Gemert, 2011; Averbeck and Schieberle, 2009; Pino and Mesa, 2006), which were used for OAV evaluation.

Based on Eq. (1) it can be stated that the sensory properties of studied samples were determined by two factors, namely the concentration of chemical compounds in the sample, which characterize the volatile profile of the sample, and the odor thresholds. These features are largely influenced by the structure of the chemical compounds and their physicochemical properties.

The odor activity value (OAV) in the fruit samples can be calculated as ratio of the concentration of the volatile compound in sample compound to its odor threshold in water. By calculating OAVs, it can be determined which compounds present in the fruit have the greatest effect on their aroma. Jirapakkul et al. (2013) found that citronellal, linalool and limonene were the most important contributors to Kaffir lime leaves flavor. The OAVs for the 8 selected terpenes identified in the pulp and peel of Kaffir lime samples. As it can be seen in the pulp samples, it was not possible to calculate citronellal OAV because its concentration was too low. The concentrations of the remaining selected terpenes were significantly higher than their odor threshold. This means that these compounds can be detected, and also recognized by the use of the human nose. Statistical calculations have shown that only OAV for α-terpineol in peel and pulp of Kaffir lime samples did not differ statistically.

α-Pinene has the smallest odor threshold value of 6 μg/L (m/v). Therefore, the small concentration of this substance is perceptible by the human, because OAV is inversely proportional to the odor threshold. The odor thresholds of other terpenes are similar and varies from 25 to 46 mg/L (m/v). This is caused by the fact that these substances are from the same chemical clas of terpenes, and have very similar physicochemical properties. Comparing the OAVs for peel and pulp samples, it can be concluded, that α-pinene, myrcene and α-terpineol, similarly effect on the aroma of Kaffir lime peel and pulp, giving the samples a wood aroma. More differences between OAVs were observed for terpinen-4-ol, linalool and citral. The concentration of terpinen-4-ol in the pulp was 3-fold higher than the concentration of this compound in the peel. Due to the fact that OAV is proportional to the concentration, the same dependence on terpinen-4-ol can be observed in Table 2. Threefold higher value of OAV for this compound caused that Kaffir lime pulp was characterized by intense woody aroma. In the case of linalool and citral, higher concentrations as well as higher OAVs may be observed for lime peel samples. It should be emphasized that higher value of OAV was the reason of more intense aroma. In turn, linalool and citral gave the peel samples a delicate citrus aroma, because the OAVs were not high.

The major differences in OAVs can be observed for citronellal and limonene. It can be explained by more intense and citrus aroma of Kaffir lime peel. Therefore, citronellal and limonene can be used as distinguishing parameters of Kaffir lime peel and pulp in sensory studies. Limonene, citronellal and linalool had the biggest effect on the aroma of Kaffir lime peel giving a citrus aroma, while wood aroma of the pulp was caused by the presence of terpinen-4-ol, myrcene and α-pinene. Furthermore, on the basis of Jirapakkul et al. (2013) studies, it can be concluded that Kaffir lime peel has a similar aroma to the leaves of this fruit.

3.3. *Kaffir lime health benefits*

Terpenes are the main chemical class of Kaffir lime samples. All of them have complex bioactive properties such as antioxidant, antimicrobial or antiulcer effects. The main health benefits of compounds detected in samples of Kaffir lime are shown in Table 3.

3.3.1. *Antioxidant and binding properties of volatiles and bioactive compounds of Kaffir lime*

Antioxidant activity is related to food preservation by inhibiting oxidation processes. Consuming food rich in antioxidants is essential for proper functioning of the vascular system, prevent from atherosclerosis, cancer, ischemic and heart disease. The antioxidant activity of Kaffir lime is a result of presence such chemical compounds as γ-terpinene, terpinen-4-ol or camphen. The first two of them were the main components of volatile fraction of *Citrus hystrix* peel, so that peel is a rich

Table 2
Comparison of odor intensity originating from pulp and peel of Kaffir lime.

No.	Chemical compound	r^2	LOQ[b] [μg/g]	LOD[c] [μg/g]	Odor threshold [μg/L]	OAV ± SD[d] [mg/L]	
						Pulp	Peel
1	α-pinene	0.995	1.93	0.64	6[e]	370.8 ± 3.2	256.2 ± 12.9
2	limonene	0.994	2.19	0.72	30[f]	241.4 ± 3.8	2108.0 ± 83.8
3	citronellal	0.992	2.66	0.88	25[e]	< LOQ	2640.6 ± 50.8
4	linalool	0.990	3.02	1.00	40[g]	349.8 ± 12.2	852.4 ± 42.2
5	terpinen-4-ol	0.997	1.53	0.51	41[e]	753.7 ± 18.2	274.6 ± 16.9
6	myrcene	0.994	2.22	0.73	29[g]	531.4 ± 22.4	475.5 ± 36.7
7	α-terpineol	0.996	1.75	0.58	42[e]	38.7 ± 0.2[a]	15.9 ± 0.7[a]
8	Citral	0.990	2.94	0.97	46[e]	39.6 ± 0.3	119.7 ± 0.8

[a] Averages in rows marked with the same letters not differ significantly ($P < 0.05$).
[b] LOQ– limit of quantitation.
[c] LOD–limit od detection.
[d] SD–standard deviation, Mean ± SD of 3 measurements.
[e] Van Gemert (2011).
[f] Averbeck and Schieberle (2009).
[g] Pino and Mesa (2006), Pino and Mesa (2006).

source of antioxidants and may be an important ingredient of diet. The antioxidant properties are also exhibited by linalool, whose high content was determined both in the peel and pulp of Kaffir lime (15.62 ± 0.49 μg/g and 34.10 ± 1.3 μg/g, respectively). The polyphenols and corresponding antioxidant capacities in peel were 1.80 and 1.78 times higher than in pulp (Table 4). This is in line with recent report, where similar results were shown (Park et al., 2014). Terpinen-4-ol and citronellal were similar in their bioactivity with slight higher level in citronellal. The binding properties determined by the decrease of fluorescence intensity after interaction with HSA were in direct relation with the bioactive substance (Table 4, Fig. 4). Comparison of the fluorescence intensity of HSA (Fig. 4A, peaks a, b) with the results of Fig. 4B and C showed that the binding of peel was 1.72 times higher in pulp. The binding properties of citronellal and terpinen-4-ol (Fig. 4D, E) were lower than in the citrus fruit and supported previous results (Shafreen et al., 2017).

3.3.2. Antimicrobial activity

Almost all of the volatiles of Kaffir lime are the agents that fight against microorganisms or stop their growth. Citronellal, the main chemical compound of the pulp, displays the inhibitory and bactericidal activity against *E. coli* and *S. aureus Propionibacterium acnes* (Lee et al., 2013). Kaffir lime is a component of cosmetics and medicines, especially that in recent years the interest in antimicrobial natural drugs has grown.

3.3.3. Anti-inflammatory activity

Anti-inflammatory activity of Kaffir lime is caused by the presence of volatile organic compounds such as α-pinene, β-caryophyllene, sabinene and limonene. The high content makes the consumption of Kaffir lime helps to mitigate the symptoms of such diseases as rheumatism, arthritis, edema, and gout.

3.3.4. Comparison of the composition of bioactive compounds with other citrus fruits

The comparison of content of selected chemical compounds with bioactive activity contained in the samples of Kaffir lime peel and pulp with other fruits was presented in Table 3.

For comparison, eight citrus juices were taken (Lubinska-Szczygieł et al., 2018; Moufida and Marzouk, 2003; Pérez-López and Carbonell-Barrachina, 2006) and six citurs fruit peels (Gancel et al., 2002; Vekiari et al., 2002; Asikin et al., 2012; Fan et al., 2009). All of the terpenes in the table exhibit antioxidant properties (Lu et al., 2014; Melo et al., 2011; Aggarwal et al., 2002; Rajeshwari and Andallu, 2011; Duarte et al., 2016; Dudai et al., 2005; Baik et al., 2008; El-Nekeety et al., 2011). In addition, in the case of citronellal, linalool,

In addition, in the case of citronellal, linalool, citral, camphene and terpinen-4-ol, anti-inflammatory, antimicrobial, and anticancer activities have been also found. Each of these chemical compounds was found in Kaffir lime. Kaffir lime is similar to Mexican lime and Star Ruby grapefruit, in which citronellal, is the main chemical compound of the peel and occurs in the amount of 66.02 ± 0.85 μg/g. In previous reports, the presence of this compound was not found in fruits juices and pulps, except Kaffir lime pulp and Key lime juice. Kaffir lime and bergamot are fruits with the highest content of linalool in juice and pulp comparing to the rest of citrus fruits. Terpinen-4-ol with a wide bioactive activity, in the largest amount was also noted in the samples of Kaffir lime juice and pulp. This compound is characteristic for lime peel, as well as camphene. Despite limes, only lemon and orange contain this chemical compounds, but in smaller amount that in limes. Camphene does not occur in the juice of other citrus fruits than limes. The other compound with the most extensive bioactive activity is citral. Its presence was pointed in the peels of most citrus fruits, and the highest amount was in Mexican lime. There is less of citral in the Kaffir lime peel. In the case of juice, citral was determined only in the Key lime pulp. By comparing the contents of chemical compounds with bioactive activity in the Kaffir lime peel and pulp with other fruits, it can be concluded that the activity is higher in Kaffir lime. The presence of characteristic chemical compounds as camphene, citral, linalool, terpinen-4-ol and citronellal denotes a high antioxidant effect. This is especially due to the presence of last two chemical compounds that are the main in the Kaffir lime peel and pulp and what is confirmed by determination of antioxidant and binding properties of limes (Table 4). This is consistent with the relationship between the content of terpene chemical class and the bioactive properties of Kaffir lime.

4. Conclusions

In case of Kaffir lime, despite their culinary use, the peel and pulp of this fruit is often an industrial waste. Peel and pulp contain important substances due to their potential utilization in cosmetic products. This is connected with bioactive and aroma properties of chemical compounds present in this fruit. It was proven that constituents of *Citrus hystrix* have high biological activity confirmed by indices assessment of total phenolic content, ferric-reducing/antioxidant power and binding affinities to human serum albumin. Therefore, a new analytical method was elaborated to quantify the substances, key in this context, successfully. For the first time it was possible to measure terpene content without solvent extraction or hydrodistillation step before chromatographic analysis with accordance to the roles of green analytical

Table 3

Comparison of the content and bioactive properties of selected terpenes contained in citrus fruits samples.

Health benefits	Chemical compounds									
	citronellal	limonene	linalool	camphene	citral (neral + geranial)	α-terpineol	α-pinene	myrcene	γ-terpinene	terpinen-4-ol
antioxidant	+	+	+	+	+	+	+	+	+	+
anti-inflammatory	+	+	+	+	+		+		+	+
antimicrobial	+	+	+	+	+	+		+		+
anticancer	+		+	+	+		+			+
Ref.	Lu et al. (2014); Melo et al. (2011)	Aggarwal et al. (2002); Rajeshwari and Andallu (2011)	Duarte et al. (2016)	Rajeshwari and Andallu (2011)	Dudai et al. (2005)	Rajeshwari and Andallu (2011)	Rajeshwari and Andallu (2011)	Baik et al., 2008; El-Nekeety et al. (2011)	Rajeshwari and Andallu (2011); Ramalho et al. (2015)	Rajeshwari and Andallu (2011)
Concentration [µg/g or µg/mL]										
Citrus pulps and juices										
Kaffir lime pulp	0.21	8.18	15.62	3.82	1.39	1.23	2.45	17.25	19.24	34.58
Kaffir lime juice (Lubinska-Szczygieł et al., 2018)	nd.	10.78	20.13	4.86	nd.	1.50	3.07	22.36	25.01	44.79
Blood orange juice (Moufida and Marzouk, 2003)	nd.	99.30	0.25	nd.	nd.	7.47	4.42	nd.	nd.	1.68
Sweet orange juice (Moufida and Marzouk, 2003)	nd.	301.88	0.06	nd.	nd.	2.45	22.81	nd.	nd.	nd.
Lemon juice (Moufida and Marzouk, 2003)	nd.	111.07	0.02	nd.	nd.	1.80	0.39	nd.	nd.	nd.
Bergamot juice (Moufida and Marzouk, 2003)	nd.	243.93	34.43	nd.	nd.	–	4.75	nd.	nd.	nd.
Bitter orange juice (Moufida and Marzouk, 2003)	nd.	89.41	1.39	nd.	nd.	0.58	2.65	nd.	nd.	0.16
Key lime juice (Lubinska-Szczygieł et al., 2018)	0.55	50.50	3.45	3.38	20.91	0.68	1.04	24.89	19.41	1.96
Mandarin juice (Pérez-López and Carbonell-Barrachina, 2006)	nd.	59.20	0.17	nd.	nd.	nd.	0.21	1.03	0.05	0.03
Citrus peels										
Kaffir lime	66.02	63.24	34.10	18.39	5.51	0.67	1.54	13.79	13.36	11.26
Mexican lime (Gancel et al., 2002)	10.00	8778.00	62.00	17.00	700.00	95.00	486.00	39.00	nd.	42.00
Star Ruby Grapefruit (Gancel et al., 2002)	7.00	14880.00	22.00	tr.	18.00	12.00	84.00	60.00	1.00	nd.
Mexican lime and Star Ruby Grapefruit hybrid (Gancel et al., 2002)	69.00	7934.00	56.00	nd.	204.00	15.00	61.00	88.00	1.00	1.00
Lemon (Vekiari et al., 2002)	0.98	270.90	4.60	0.44	81.90	7.36	14.90	18.90	78.30	1.04
Shikuwasa (Asikin et al., 2012)	nd.	488.20	4.40	nd.	nd.	0.70	88.00	12.20	153.70	0.50
Orange (Fan et al., 2009)	0.04	5.92	nd.	0.39	0.11	0.05	0.12	0.44	0.02	nd.

Table 4
Antioxidant and binding properties of limes and monoterpenes in water extract.

Indices	Kaffir lime pulp	Kaffir lime peel	Terpinen-4-ol	Citronellal
Polyphenols, mgGAE/g DW	22.14 ± 1.5^{b}	39.85 ± 3.1^{a}	5.75 ± 0.8 ^{d}	5.93 ± 0.7^{d}
FRAP, mMTE/g DW	27.40 ± 3.6^{b}	48.65 ± 4.6^{a}	7.11 ± 0.8^{d}	7.32 ± 0.6 ^{d}
Binding to HSA, %	32.4 ± 1.2^{b}	55.75 ± 5.5^{a}	16.1 ± 1.7 ^{b}	16.64 ± 1.3 ^{d}

Values are means ± SD of 5 measurements; Means within a raw with the different superscripts or without superscripts are statistically different ($p < 0.05$; Student's *t*-test). Abbreviations: GAE, gallic acid equivalent; FRAP, Ferric-reducing/antioxidant power; TE, trolox equivalent; HSA, human serum albumin.

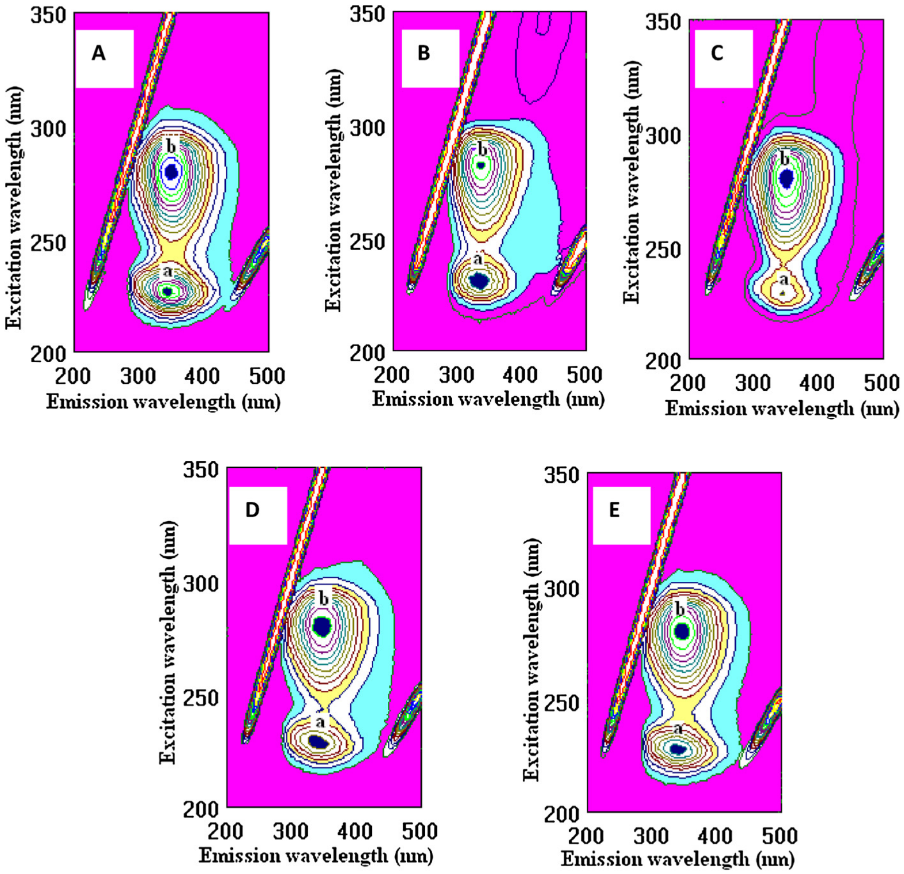

Fig. 4. Corresponding cross spectral images of water extracts of polyphenols and volatile standards in interaction with HSA, A–E, HSA, Kaffir lime peel, Kaffir lime pulp; HSA, Kaffir lime, Key lime. The values of peaks a, b were the measurement s of decreasing of fluorescence intensity during interaction. HSA, human serum albumin (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

chemistry. It was possible to assure relatively higher sensitivity of the proposed method, based on the use of HS-SPME/GC × GC-TOFMS, comparing to application of other common analytical techniques. Quantitation was also the basis for calculation of OAV, which is one of the main criteria during fragrance designing of new cosmetics. In the same time, provided investigation was useful to prove the pro-health properties of quantified substances, which is also significant in the aspect of cosmetic manufacturing. Determination of terpene concentration in *Citrus hystrix* fruit, odor activity value and bioactive activity allows for proper designing of the technological process for new cosmetics, such as creams, perfumes or lotions, which integrants origin from natural source.

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