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Quality of limes juices based on the aroma and antioxidant properties

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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

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.

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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