e-ISSN: 2757-8836
ORIGINAL ARTICLE
J. Raw Mater. Process. Foods vol.1 (2020) 22-27

Recieved: 03/07/2020 Revised: 06/07/2020 Accepted article published: 12/07/2020 Published online: 13/07/2020

Volatile and Chemical Compositions of Freshly Squeezed Sweet Lime (*Citrus limetta*) Juices

Dritan Topi

Department of Chemistry, Faculty of Natural Sciences University of Tirana, Tirana, Albania.

Correspondence;
Dr. Dritan Topi
E-mail adress:
dritan.topi@unitir.edu.al
ORCID No: 0000-0001-7852- 5374

Abstract

Citrus limetta also known as sweet lime or sweet lemon is grown generally in tropical and subtropical regions including Turkey as one of the main producer country. In the present study, it was aimed to investigate the effects of handsqueezing methods (with or without peel of sweet lime) on volatile profile of juices. Volatile compounds were extracted with the application of liquid-liquid extraction and classified and quantified by gas chromatograpy-mass spectrometry (GC-MS). In addition to volatile compounds, total phenolic, total ascorbic acid amounts and antioxidant activity were also evaluated. Juices obtained from unpeeled sweet limes had a greater amount in total volatiles rather than the samples obtained from peeled fruits. This result displays the contribution of peel composition to the juice released due to the disruption of the peel during squeezing. Similarly, ascorbic acid amounts differed and were higher in the usage of unpeeled fruits. Aroma profile of sweet lemon juice was mainly composed of terpene compounds. Among these compounds, limonene was the major compound which stood out in both the peeled-squeezed (82%) and unpeeled-squeezed samples (52%). In contrast, acetic acid, the second compound found in higher concentrations, displayed an opposite behavior and was found in higher amounts in unpeeled-squeezed sweet lemon juices.

Keywords: *Citrus limetta, sweet lime, aroma compounds, GC-MS, antioxidant*

1. INTRODUCTION

Citrus fruits belonging the Rutaceae family and *Citrus* genus, are represented by fruits like *Citrus paradisi* (grapefruit), *Citrus reticulata* (tangerine), *Citrus sinensis* (orange), *Citrus limetta* (lime) and *Citrus lemon* (lemon) (Inan et al., 2018). Citrus fruits can be grown more easily in tropical and subtropical climatic conditions and in well drained, loose and ventilated, sandy, loamy, clayey-tin soils. Countries with these soil characteristics are also the countries where citrus production is the most common (Kafa et al., 2015). Leading countries in the world in citrus production are Brazil, USA, China, Mexico, Spain, India, Iran, Italy, Egypt, Argentina, Turkey and Pakistan. Orange is the major citrus specie in the world. Annual production of oranges in the world is over 73 million tons in 2017 (FAOSTAT, 2018). Following orange, lemons are the other important fruits in Citrus genus with a production of 17 million tons comprising 11.5% of total citrus production (FAOSTAT, 2018). Lemons are generally grown in subtropical climate regions of the northern and southern hemisphere and it is known that it is mainly studied in three groups as sour lemon, sweet lemon and different groups (lemon equivalents). Generally known as sweet lime or sweet lemon, *C. limetta* is a type of citrus that grows in tropical and subtropical regions. Specific to South and Southeast Asia and mostly grown in India, China, South Japan, Vietnam, Malaysia, Indonesia and Thailand, more than 100 million metric tons of sweet lime are produced annually worldwide. Sweet lime is a fruit rich in bioactive ingredients such as vitamin C, minerals, and phenolic compounds. Due to its rich composition, it is used in cholesterol control, inflammation and wound healing, digestive disorders (Perez et al., 2010). Although it is not widely known in Albania, sweet lime juice is an important commercial product used in fruit juice processing industry in many countries. Besides their nutritive value, the taste, unique aroma, texture and color parameters are highly effective in the wide usage of citrus fruits in the world. Within these parameters, aroma which is a combination of lots of volatile compounds plays a key role in the preference of consumers. Citrus fruits aroma is a complex mixture of terpenic hydrocarbons, alcohols, ketones, aldehydes and other volatiles (Selli, 2007). Therefore, the aim of this research was to determine the aroma profile of sweet lime juice obtained from two different squeezing types (with or without peels of limes). In addition, total phenolic compounds, antioxidant activities and ascorbic acid amounts of sweet lime juices were also investigated.

2. MATERIALS AND METHODS

2.1. Materials

The sweet limes *(Citrus limetta)* used in this study were obtained from Mediterranean region of Turkey in 2017. Immediately after harvest (20kg), sweet lime juices were obtained by using two different squeezing methods. For the first samples, fruits were halved and directly hand-squeezed and coded as JWP. For the second ones, the fruits were peeled first and then hand-squeezed which was coded as JWOP. Obtained juices were put into dark bottles (500ml) and immediately used in the analyses.

2.2. Methods

2.2.1. Extraction and Analysis of Volatile Compounds

The aroma compounds of different lime juices were determined by liquid-liquid extraction (LLE) method. 100 ml of juice samples were taken and extraction was carried out at 4-5°C with stirring in a 500 ml aliquot on a magnetic stirrer under nitrogen gas for 60 minutes with the addition of 100 ml of high purity dichloromethane solvent. After dehydration by anhydrous sodium sulfate, pooled aromatic extract was reduced to 5 mL in a Kuderna Danish concentrator fitted with a Snyder column and then a second concentration is made to 200 μL under a gentle stream of purified nitrogen. All extracts were subsequently stored at -20 °C in 2 mL glass vials (Selli and Kelebek, 2011).

2.2.2. Representativeness of aromatic extracts

Similarity and intensity tests were performed to assess the affinity of extracts and the juices in terms of aroma. The panel was composed of eleven experienced assessors (six males and five females between 28 and 49 years old). The panellists were previously trained in sensory evaluation of foods. Sensory analysis was performed with the use of cardboard smelling strips (7140 BPSI, Lyas, France) which were reported to give effective results for the representativeness test of citrus juices (Selli et al., 2008; Selli et al., 2011; Kesen, 2020). The aroma extracts were saturated to the smelling strip than placed in the brown-coded flask after 1 min waiting for solvent evaporation and the 2 ml reference sample was likewise. After that, the assessors were asked to evaluate the strips on a 100 mm scale and all of the strips were assessed at 20°C (Selli et al., 2011). Similarity test was performed to scale the closeness between the odour of extract and reference sample. Likewise, in intensity test, the panellists assessed the odour intensity of extracts.

2.2.3. GC-MS analysis of volatile compounds

The GC system consisted of an Agilent 6890 equipped with a flame ionization detector (FID), and an Agilent 5973N – mass selective detector (MSD). Aroma compounds were separated on a DB-Wax (30 m x 0.25 mm, 0.5 μm thickness; J&W Scientific, Folsom, CA). A total of 3 μL of extract was injected each time in pulsed splitless (40 psi; 0.5 min) mode. The injector and FID were set at 270 and 280 °C, respectively. The flow rate of carrier gas (helium) was 1.5 mL/min. The oven temperatures was first increased from 50 to 200 °C at a rate of 5 °C/min and then to 260 °C at 8 °C/min with a final hold at 260 °C for 5 min. The mass detector was operated in scan mode, with electronic impact ionization energy of 70 eV. The GC–MS interface and ionization source temperatures were set at 250 and 180 °C, respectively. Identification and quantification were performed in full scan mode scanning a mass range of m/z 30–300 at 2.0 scan/s. The compounds were identified by comparing their mass spectra output with those in Wiley 9 and NIST 11 mass spectral data libraries and an in-house library created from previous laboratory studies using similarity searching of MS fragmentation (Selli and Kelebek, 2011). Compounds were taken into account if they had a similarity match of at least 80%

with the MS fragmentation in the databases. The chromatogram obtained was analysed, and each peak was checked by determining the per cent area on the chromatogram, the retention time, the spectrum and the base peak and then referring to the characteristic mass spectra of compounds listed on the National Institute of Standards and Technologies (NIST) using the software of Agilent ChemStation. Each sample was analysed in triplicate. Linear Retention Indices (LRI) was calculated as a confirmation of identified compounds (Xu et al., 2017).

2.2.4. Total phenolic content

Total phenolic content was determined by Folin-Ciocalteu method. 1 ml of sweet lemon juice was taken and 60 ml of water was added and 5 ml of Folin solution was added and mixed. Then 15 ml of sodium carbonate solution was added to the mixture and the volume was completed and kept in a dark place for 2 hours. Finally, the absorbance of each solution was read on the spectrophotometer (Shimadzu UV-1201, Tokyo, Japan) at 765 nm in 10 ml cuvettes and a calibration curve was drawn. Gallic acid was used as the phenolic standard in drawing the graph (Singleton et al., 1999).

2.2.5. Total phenolic content Antioxidant activity

Total ascorbic acid values of sweet lemon juice were determined by spectrophotometric method based on the reduction of 2,6-dichloroindophenol solution (Hewitt and Dickes, 1961). 10 ml of the sample was homogenized with a 90 ml stabilizing solution (0.4% oxalic acid) and then filtered. 9 ml of pure water and 1 ml of sample were added to the first of two separate test tubes, and 9 ml of the dye solution (2,6-dichloroindophenol, Na salt) and 1 ml of the sample were measured and the absorbance value at 518 nm was measured. Calibration graph was drawn by using L-ascorbic acid standard and total ascorbic acid values in sweet lime juices were calculated.

3. RESULTS AND DISCUSSION

3.1. Volatile composition of sweet lime juices

Selecting the most appropriate extraction method is crucial in obtaining aroma compounds. The most efficient and effective procedure to make this decision is to apply similarity and intensity tests for the evaluation of representativeness of the extract. In this manner, the results of those applied tests were in acceptable levels indicating that liquid-liquid extraction is highly efficient in obtaining volatiles from sweet lime juices. According to the assessors, obtained aromatic extract had the similarity of 73% with the reference sample and had an odor intensity value of 78%. When compared to other previous studies, these results were acceptable and that the LLE was a trustable procedure (Selli et al., 2008; Kesen et al., 2013; Selli et al., 2014). The juices of sweet lemon fruits squeezed differently were examined for aroma composition and total of 21 volatile compounds were detected by GC-MS (Table 1). The volatile groups forming the composition consist of alcohols, aldehydes and acids under the abundance of terpenes. It was determined that volatile composition differed with the mode of juice extraction. Terpenes are the most important aroma compounds responsible for the characteristic odor of Citrus fruits (Perez-Cacho and Russell, 2008). DL-Limonene was found as the major terpene in both sweet lime juice samples (82,3 % in JWP and 51,6% JWOP). In literature, this compound was referred to give “citrusy” odor and found in almost all of the citrus fruits (Sawamura et al., 2006; Selli et al., 2008). Another compound found in high amounts was linalool. In some studies, it was reported that linalool is the most dominant compound among the terpene alcohols in orange peel samples (Högnadóttir and Rouseff, 2003; Qiao et al., 2008). Following linalool, β-myrcene was also determined in high amounts. It has been reported that it contributes to the odor of some tropical fruits as well as citrus fruits. It was observed that the amounts of some compounds such as limonene, linalool and β-citronellol are higher in juices squeezed with peels. This can be explained with the disruption of the peel during the squeezing and the passage of the aroma compounds in the peel into the juice. These results indicate that the increase in aroma compounds is due to volatile compounds present in the peel. Similarly to

Table 1. Aroma composition of sweet lime juices

No	LRI	Compounds	JWP	JWOP
1	1172	3-Penten-2-ol	N	4,22
2	1181	β- Myrcene	1,52	1,69
3	1205	DL-Limonene	82,3	51,6
4	1222	β-Phellandrene	0,34	N
5	1239	β-*(Z)*-Ocimene	0,14	N
6	1387	Nonanal	N	5,24
7	1427	Acetic acid	6,05	9,54
8	1474	Citronellal	0,15	N
9	1552	Linalool	1,62	0,64
10	1587	*(E)*-α-Bergamotene	0,47	N
11	1680	α-Terpineol	0,55	N
12	1676	Germacrene D	0,18	N
13	1715	β-Bisabolene	0,73	0,78
14	1782	β-Citronellol	3,17	1,97
15	1810	Hexanoic acid	0,39	3,01
16	1915	Phenylethyl alcohol	N	0,63
17	1950	Heptanoic acid	N	1,51
18	2083	Octanoic acid	0,58	5,04
19	2144	Nonanoic acid	1,32	14,04
20	2197	4-Vinylgaiacol	0,04	N
21	2279	Decanoic acid	0,37	N

**LRI: Linear retention index calculated on DB-WAX capillary column; N:Not found; The results are the means of % peak areas obtained from 3 different injections.*
***JWP: Juices obtained from squeezing with their peels; JWOP: Juices squeezed without peel.*

present study; Zhong et al. (2014) carried out the analysis of aroma compounds in lemon juice and peel and found that terpenes were dominant in the peel, similar to fruit juice. They also reported that the amount of terpenes present in the peel was significantly greater than the terpenes in lemon juice.

A total of six different organic acids were found in the Citrus limetta samples examined and it was observed that acetic acid had the abundance. One of the other identified acids, decanoic acid was found only in JWP sample. The reason for that, decanoic acid is thought to pass from the peel to water during squeezing. Reversely, heptanoic acid was detected only in lime juice squeezed without peels (JWOP). Heptanoic acid may be induced by other high amount volatiles in the peel. Regarding the alcohols in the *Citrus limetta* juices, two alcohols namely 3-penten-2-ol and phenylethyl alcohol were determined. It has been reported that 3- penten-2-ol contributes to the aroma of vegetable oils such as olive oil as well as fruits in previous studies (Kesen et al., 2014). As for the phenylethyl alcohol, it is known that this aroma compound is found in a wide range of products, including fruits, wine, honey and olive oil (Kesen et al., 2014)

Table 2. Total phenolic, total ascorbic acid and antioxidant activities of juices

Analysis	JWP	JWOP
Total phenolic content (mg/L)	109±3,8	88,9±1,2
Total ascorbic acid (mg/L)	540±7,0	510±4,1
Antioxidant activity (µM Trolox/L)	117,8±8,8	93,18±3,4

JWP: Juices obtained from squeezing with their peels; JWOP: Juices squeezed without peel.

While both alcohol compounds were not detected in JWP, they were determined after peeling. According to GC-MS results, JWP sample had more volatiles obviously and these finding are in line with previous studies. One of the earlier studies by Moshonas and Shaw (1994) reported that the concentration of volatiles in commercial orange juice was greater when the juice was obtained from whole fruits because the compounds were liberated from the aqueous phase, lipids, and from essential oils contained in the fruit peel. Similar results were also indicated in clementine and mandarin juices (Barboni et al., 2010).

3.2. Total phenolic compounds and antioxidant activities of juices

The total phenolic content of the juice obtained from fresh *Citrus limetta* peeled and unpeeled fruits was found to be 109 mg/L and 88.9 mg/L respectively, while the antioxidant activities were 118 mM Trolox/L and 93.2 mM Trolox/L respectively (Table 2). Barreca et al. (2011) determined the antioxidant activity of *Citrus limetta* juice as 261 µM Trolox by the DPPH radical quenching method. Rekha et al. (2012) determined the total phenolic content in Citrus lemon juice as 600 mg/L by Folin-Ciocalteu method. In a study performed by antioxidant activities of the extracts obtained from lemon peels with supercritical fluid extraction and Soxhlet extraction processes were 1165 and 879 µg GAE/g dry matter, and 2.415 and 23.80 µmol Trolox/g dry matter respectively. *C. limetta* juice was found to have lower values in terms of total phenol and antioxidant activity compared with Citrus varieties studied in literature. The concentrations of bioactive compounds can vary depending on the fruit culture, maturity, growth and storage conditions, and extraction processes (Hashemi et al., 2017). Thus, the mode of extraction was determined to affect both phenolic content and antioxidant activity of juices.

3.3. Total ascorbic acid amounts of juices

The amount of ascorbic acid obtained from fresh *Citrus limetta* peeled and unpeeled fruit juice was determined as 539 mg/L and 511 mg/L, respectively. Damian-Reyna et al. (2017) determined the amount of ascorbic acid of Citrus limetta juice as 112 mg/L. Rekha et al. (2012) found the total amount of ascorbic acid of Citrus limon juice 10.60 g/100 mL. In another study, the amount of ascorbic acid in *Citrus sinensis* juice was determined as 16.03 ppm. As mentioned above, the total amount of ascorbic acid in fresh *Citrus limetta* juice was found in higher amounts than the other Citrus fruits. In addition, the sweet lime juices obtained from unpeeled fruits were found to contain more ascorbic acid than the juice from peeled fruits, similar to the total phenolic content and antioxidant activity values. It is thought that this is a fragmentation of the peel reasoned from the pressing during squeezing and that the bioactive compounds in the peel pass into the juice.

4. CONCLUSIONS

The aim of this study was to investigate the volatile and general composition of sweet lime juices squeezed with and without peels. Total of 21 aroma compounds with the majority of terpenes were identified in samples. Limonene was found dominantly in all samples and the usage of peels in squeezing reasoned an increase in the amount of this compound. In the light of the findings, it was determined that the amount of volatile compounds in juices squeezed with peels was higher than the juices without peels. This is due to the corruption of the peel and the passage of volatile compounds in the peel to the juice during squeezing. In addition, higher total phenolic, total ascorbic acid and antioxidant activities were found in the juices obtained from unpeeled sweet limes.

ACKNOWLEDGEMENTS

The author would like to thank the Erasmus+ exchange program between the University of Cukurova and the University of Tirana. Dr. Topi also wish to thank Cukurova University Central Laboratory (CUMERLAB)-Turkey for the GC-MS analysis.

REFERENCES

Barboni, T., Muselli, A., Luro, F., Desjobert, J. M., & Costa, J. (2010). Influence of processing steps and fruit maturity on volatile concentrations in juices from clementine, mandarin, and their hybrids. *European Food Research and Technology*, 231(3), 379-386.

Barreca, D., Bellocco, E., Caristi, C., Leuzzi, U., & Gattuso, G. (2011). Flavonoid profile and radical-scavenging activity of Mediterranean sweet lemon (*Citrus limetta Risso*) juice. *Food Chemistry*, 129(2), 417-422.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1), 25-30.

Damián-Reyna, A. A., González-Hernández, J. C., Maya-Yescas, R., de Jesús Cortés-Penagos, C., & del Carmen Chávez-Parga, M. (2017). Polyphenolic content and bactericidal effect of Mexican *Citrus limetta* and *Citrus reticulata*. *Journal of Food Science and Technology*, 54(2), 531-537.

Ergut, M. (2015). Supercritical fluid extraction of phenolic substances in orange and lemon pulp. Mersin University, Department of Chemistry, Master Thesis.

FAOSTAT, 2018. FAOSTAT (Food and Agriculture Organization of the United Nations Statistics Division) http://www.fao.org/faostat/en/#data/QC. Accessed at 28.06.2020.

Hashemi, S. M. B., Khaneghah, A. M., Barba, F. J., Nemati,Z., Shokofti, S. S., & Alizadeh, F. (2017). Fermented sweet lemon juice (*Citrus limetta*) using *Lactobacillus plantarum* LS5: Chemical composition, antioxidant and antibacterial activities. *Journal of Functional Foods*, 38, 409-414.

Hewitt, E. J., & Dickes, G. J. (1961). Spectrophotometric measurements on ascorbic acid and their use for the estimation of ascorbic acid and dehydroascorbic acid in plant tissues. *Biochemical Journal*, 78(2), 384-391.

Högnadóttir, Á., & Rouseff, R. L. (2003). Identification of aroma active compounds in orange essence oil using gas chromatography–olfactometry and gas chromatography–mass spectrometry. *Journal of Chromatography A*, 998(1-2), 201-211.

Inan, Ö., Özcan, M. M., & Aljuhaimi, F. (2018). Effect of location and Citrus species on total phenolic, antioxidant, and radical scavenging activities of some Citrus seed and oils. *Journal of Food Processing and Preservation*, 42(3), e13555.

Kafa, G., Seday, U., Uysal, O., & Polatoz, S. (2015). Effect of budwood irradiation on seed number of 'Clementine','Nova'and 'Robinson'mandarin mutants. In Proc. XII Intl. Citrus Congress. *Acta Horticulturae* (No. 1065).

Kelebek, H., Canbas, A., & Selli, S. (2008). Determination of phenolic composition and antioxidant capacity of blood orange juices obtained from *cvs.* Moro and Sanguinello (*Citrus sinensis* (L.) Osbeck) grown in Turkey. *Food Chemistry*, 107(4), 1710-1716.

Kelebek, H., Selli, S. & Sevindik, O., (2020) Screening of phenolic content and antioxidant capacity of Okitsu mandarin (*Citrus unshui* Marc.) fruits extracted with various solvents. *Journal of Raw Materials to Processed Foods*, 1, 7-12.

Kesen, S., Kelebek, H., Sen, K., Ulas, M., Selli, S. (2013). GC–MS–olfactometric characterization of the key aroma compounds in Turkish olive oils by application of the aroma extract dilution analysis. *Food Research International*, 54, 1987–1994.

Kesen, S., Kelebek, H., & Selli, S. (2014). Characterization of the key aroma compounds in Turkish olive oils from different geographic origins by application of aroma extract dilution analysis (AEDA). *Journal of Agricultural and Food Chemistry*, 62(2), 391-401.

Kesen, S. (2020).GC-MS-Olfactometric characterization of aroma active compounds of turmeric *(Curcuma longa)* extract using aroma extract diluton analysis. *Journal of Raw Materials to Processed Foods*, 1, 13-21.

Moshonas, M. G., & Shaw, P. E. (1994). Quantitative determination of 46 volatile constituents in fresh, unpasteurized orange juices using dynamic headspace gas chromatography. *Journal of Agricultural and Food Chemistry*, 42(7), 1525-1528.

Perez-Cacho, P. R., & Rouseff, R. L. (2008). Fresh squeezed orange juice odor: a review. Critical Reviews in *Food Science and Nutrition*, 48(7), 681-695. Perez, Y. Y., Jimenez-Ferrer, E., Alonso, D., Botello- Amaro, C. A., & Zamilpa, A. (2010). Citrus limetta leaves extract antagonizes the hypertensive effect of angiotensin II. *Journal of Ethnopharmacology*, 128(3), 611-614.

Rekha, C., Poornima, G., Manasa, M., Abhipsa, V., Devi, J. P., Kumar, H. T. V., & Kekuda, T. R. P. (2012). Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chemical Science Transactions*, 1(2), 303-310.

Sawamura, M., Onishi, Y., Ikemoto, J., Tu, N. T. M., & Phi, N. T. L. (2006). Characteristic odour components of bergamot *(Citrus bergamia* Risso*)* essential oil. *Flavour and fragrance journal*, 21(4), 609-615.

Selli, S. (2007).Volatile constituents of orange wine obtained from Moro oranges *(Citrus sinensis (L.) Osbeck). Journal of Food Quality*, 30, 330-341.

Selli, S., Canbas, A., Varlet, V., Kelebek, H., Prost, C., & Serot, T. (2008). Characterization of the most odor active volatiles of orange wine made from a Turkish cv. Kozan *(Citrus sinensis L. Osbeck). Journal of Agricultural and Food Chemistry*, 56, 227–234.

Selli, S., & Kelebek, H. (2011). Aromatic profile and odouract ivity value of blood orange juices obtained from Moro and Sanguinello *(Citrus sinensis L. Osbeck). Industrial Crops and Products*, 33(3), 727-733.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology (Vol. 299, pp. 152-178). *Academic press*.

Qiao, Y., Xie, B. J., Zhang, Y., Zhang, Y., Fan, G., Yao, X. L., & Pan, S. Y. (2008). Characterization of aroma active compounds in fruit juice and peel oil of Jinchen sweet orange fruit *(Citrus sinensis (L.) Osbeck)* by GC-MS and GC-O. *Molecules*, 13(6), 1333-1344.

Xu, B. M., Baker, G. L., Sarnoski, P. J., & Goodrich- Schneider, R. M. (2017). A comparison of the volatile components of cold pressed Hamlin and Valencia *(Citrus sinensis (L.) Osbeck)* orange oils affected by Huanglongbing. *Journal of Food Quality*, 6793986.

Zhong, S., Ren, J., Chen, D., Pan, S., Wang, K., Yang, S., & Fan, G. (2014). Free and bound volatile compounds in juice and peel of Eureka lemon. *Food Science and Technology Research*, 20(1), 167-174.