



Study of Volatiles from 'Pera' Sweet Orange Peel Using Karl Fischer Titration, GC-FID and GC/MS Techniques

Karine Zanella, Clairon L. Pinheiro, Edson Tomaz, Osvaldir P. Taranto*

School of Chemical Engineering, University of Campinas, Av. Albert Einstein, 500, Campinas, São Paulo, Zip Code 13083-852, Brazil
val@feq.unicamp.br

Orange is a large cultivated fruit and worldwide studied. During its industrial processing it is obtained juice and essential oil as the main products and, d-limonene, pectin and citrus pulp as by-products, where the last one is dried, stored and commercialized. However, during the drying process, volatile organic compounds (VOCs) present in the orange peel (albedo and flavedo), are issued and released to become atmospheric pollutants, causing environment problems related to the formation of tropospheric ozone, photochemical smog, olfactory discomfort of local residents. Thus, this work aims: determine the moisture content from albedo and flavedo from 'pera' sweet orange (*Citrus sinensis* L. Osbeck), as well as, quantify and qualify the VOCs released during the drying process. For that, the following steps were taken: (1) determination of water and volatile contents via oven dryer at 378 K for 24 hours; (2) determination of water content via Karl Fischer titration at 378 K and nitrogen output at 90 mL/min; (3) determination of volatile content by the difference between (1) and (2); (4) collect and make the qualitative and quantitative analysis of volatile compounds by gas chromatography coupled to mass spectrometry (GC/MS) and by gas chromatography with flame ionization detector (GC-FID), by using the response factor. The results shown that, in wet basis, the water content and the volatiles content were 66 % and 11 % for the albedo and 67 % and 6 % for the flavedo, respectively. In addition, the volatile organic compounds identified were 2-methylene-1,4-dioxane, α -pinene, 3-carene and d-limonene and the VOCs quantified were α -pinene, 3-carene and d-limonene, both of albedo as flavedo. So, we conclude that the methodology adopted in this work is adequate to determine the volatile organic compounds released during the drying of orange peel, as well as qualify each chemical compound among the volatiles.

1. Introduction

During the sweet orange industrial processing, it is obtained two essential main products, juice and essential oil, and a range of by-products, among them are d-limonene, pectin and citrus pulp, where this last one is posteriorly dried, stored and commercialized. (Braddock, 1999; Licandro and Odio, 2002; Arami et al., 2005). The orange peel contains on average of 80 % moisture (wet basis) that is distributed in different ways, and it is well known that the water content is one of the most important properties of foodstuffs, which influences, amongst other things, taste, shelf life and appearance (Isengard, 2001; Schoffski, 2001). However, the water content determination of food is very difficult, since it is a very complex material and requires very careful sample preparation (Schoffski, 2001).

The traditional drying method is not adequate because high temperatures can induce the formation and release of volatiles substances (water and others), but this method is used in many industries which need using the drying process (Heinze and Isengard, 2001; Gergen et al., 2006; Morgano et al., 2011). For example, during the orange bagasse drying process, there is the issue and release of volatile organic compounds (VOCs), since they are present in the orange peel (albedo and flavedo). These VOCs are responsible for many environmental problems, such as the formation of tropospheric ozone, photochemical smog, as well as cause olfactory discomfort of local residents (Seinfeld and Pandis, 1998; Albuquerque, 2007; Ras-Mallorquí et al., 2007; Ueda, 2010), so the study of these VOCs is very important.

One way to collect all those VOCs is by using the Karl Fischer titrator plugged with an oven. Despite the Karl Fischer is the most important direct (primary) method to determine the total water content of a sample, when connected with an oven has a range of applications (Ronkart et al., 2006).

For this reason, the aim of this work is use the Karl Fischer titrator plugged with an oven to collect and quantify all the volatile compounds which are released to the atmosphere in an orange drying process and analyze qualitatively and quantitatively this VOCs using gas chromatography coupled to mass spectrometry (GC/MS) gas chromatography with flame ionization detector (GC-FID), using the response factor, respectively.

2. Materials and methods

2.1 Raw material preparation

'Pera' sweet oranges were obtained on market in Campinas, São Paulo, Brazil. The first step of the raw material preparation was the washing process, to remove dross, avoiding modifications on the posteriors data. After washing, the oranges went to the flavedo (colored portion of the peel) peeling process, using a manual orange peeler (LUCRE). The use of this peeler was chosen since it provides a homogeneous sample. Flavedo samples were ground in a mill rotor cyclone (TE-651/2), providing particle size equal or smaller to 10 *mesh Tyler* (2.0 mm diameter), stored in airtight plastic bags and placed under cooling, at a temperature of 255 K in domestic refrigerator (CVU20, Consul), until further use.

After removal of flavedo, the oranges were processed for obtaining the albedo (white and spongy portion of the peel). This process involved the extraction of the juice through a commercial fruit squeezer (Bellagio, Britain), and the manual removal of the remaining albedo aggregate, such as juice vesicles. After this process, the albedo samples were ground into mill rotor cyclone (TE-651/2), providing particle size equal or smaller to 10 *mesh Tyler* (2.0 mm diameter), stored in airtight plastic bags and placed under cooling, at a temperature of 255 K in domestic refrigerator (CVU20, Consul), until further use. The samples of flavedo and albedo were removed from the refrigerator about 4 hours and left in desiccators prior to analysis to be performed, ensuring the thermal equilibrium with the environment.

2.2 Water and volatiles content determination

The water and volatiles content (Equation 1) were determined by gravimetric method using an oven dryer. About 3.0 grams of wet sample (flavedo or albedo) were transferred to tared aluminum dish (triplicate) and placed in the oven at a temperature of 378 K for 24 hours, to determine the quantity of water and volatile material during the drying process.

$$WV_{WB} = \frac{(m_i - m_{dm})}{m_i} \cdot 100 \quad (1)$$

Where WV_{WB} represents the water and volatiles content (wet basis), m_i represents the initial mass and m_{dm} the dry matter mass.

2.3 Water content determination

The water content was determined using the Karl Fischer titrator (915 KF, Metrohm) linked to an oven (768 KF, Metrohm), plugged with a nitrogen cylinder (carrier gas). About 0.02 grams of wet sample (albedo or flavedo) were put inside the oven, where nitrogen at 378 K and 90 mL/min carries the moisture present on the samples for the bottle where the Karl Fischer reaction occurs. After the end of the analysis, the equipment gave the water content present on the sample in percentage. This method gives only the quantity of water removed, so, by comparison between the gravimetric method and this one, were possible to determine the quantity of volatile material (except water) that were lost during the drying process. The percentage of this volatile material is given by Equation 2.

$$VC_{WB} = WV_{WB} - WC_{KF} \quad (2)$$

Where VC_{WB} represents the volatile content (wet basis) and WC_{KF} represents the water content obtained on Karl Fischer titration.

2.4 Sample collection

The equipment used to collect VOCs was the Karl Fischer oven (768 KF, Metrohm). 0.2 grams of wet sample (flavedo or albedo) were placed inside the oven with N_2 atmosphere, at 378 K and 90 mL/min. The VOCs released during the drying process were collect for 20 seconds, after 3 minutes of the wet sample inside the oven, using a gas sampler device (Figure 1.B) and a gastight microsyringe (1 mL, Hamilton). This equipment set is shown in Figure 1. The samples were analyzed in terms of VOC concentration.

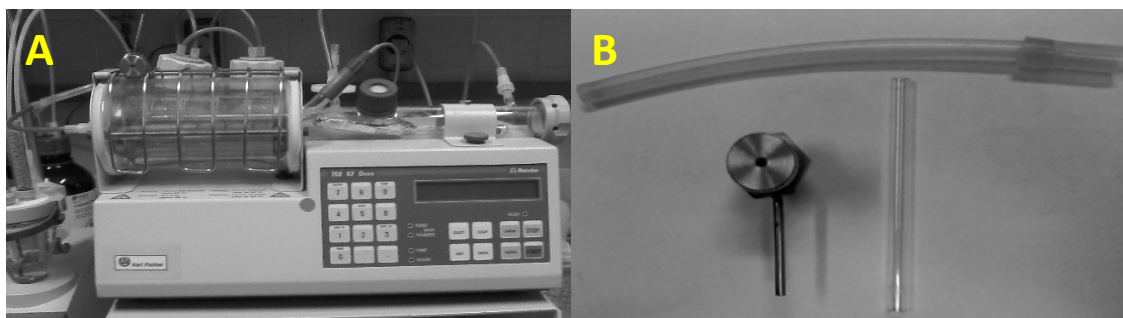


Figure 1: (A) Karl Fischer Ove; (B) gas sampler.

2.5 Analysis of the volatile organic compounds (VOCs)

Qualitative analyses of volatile organic compounds were performed using a GC/MS (Autosystem XL Gas Chromatograph and TurboMass Mass Spectrometer, Perkin Elmer). Analytical capillary column used was a NST-05 (60 m of length, 0.25 mm of internal diameter, and 0.25 μm of film thickness), where methyl-phenyl polysiloxane was used as stationary phase. Chromatographic method is summarized in Table 1. The injection temperature was 250 ° C in split mode at 1:10 with helium as carrier gas with flow rate of 10 mL/min. The interface GC-MS was maintained at 250 ° C. The mass spectrometer acquired data in scan mode with a mass interval ranging from 35 to 300 Da with a repetition rate of 0.01 scan.s⁻¹. The ion source was run in EI mode at 250 ° C and 70 eV ionization energy. The compounds were identified using the standard references, by matching the mass spectra with a mass spectrum library (NIST Library) available in the GC-MS system.

Table 1: Method of analysis by gas chromatography

	Rate (°C/min)	Temperature (°C)	Hold (min)
Initial	0	45	5.0
Step 1	2.5	70	2.5
Step 2	2.0	150	1.5
Step 3	1.9	200	1.0

Gas chromatography with flame ionization detector (GC-FID) was used to quantify VOCs previously identified by GC/MS. The chromatograph, capillary column and chromatographic method (Table 1) were the same used in GC/MS analyses. The operating conditions of FID were temperature at 250 ° C; synthetic air flow of 420 mL/min and hydrogen gas flow of 45 mL/min.

The amount of each volatile compound identified was calculated from the response factor of each one, obtained from analytical standards injections.

3. Results and discussion

3.1 Volatiles content determination

The wet basis results, as well as their respective standard deviations, of the water and volatiles content (WV_{WB}), obtained by oven drying, the water content (WC_{KF}), obtained by volumetric Karl Fischer titration and the volatiles content (VC_{WB}), obtained by Equation 2, of albedo and flavedo are summarized in Table 2.

Table 2: Summarized results of water and volatiles content, water content and volatiles content

Part of the peel	WV_{WB} (%)	WC_{KF} (%)	VC_{WB} (%)
Albedo	77.43 \pm 0.09	66.52 \pm 1.05	10.91 \pm 1.13
Flavedo	73.36 \pm 0.20	67.33 \pm 0.31	6.03 \pm 0.33

Analyzing Table 2 it is possible to see that there is a significant difference in the moisture content between the drying techniques applied, mainly to albedo. This difference was expected, since the Karl Fischer identifies only the water content of the material, while the oven drying analysis identifies both loss of water as volatiles. The difference between these two techniques gives the quantitative value of volatiles that the

material has. It is verified that the albedo has an average of 10.91 % of volatiles in its composition, and flavedo 6.03 %, both on a wet basis.

It was not found in literature results for water analysis by Karl Fischer titration, concerning to wet orange peel, making difficult a comparison with the results obtained in this work. However, the literature brings results for the analysis of moisture content made by other methods to both parts of the orange peel (flavedo and albedo) and only to the peel, allowing a brief discussion with the results obtained here. Braddock and Crandall (1981) determined the moisture content of Valencia orange flavedo by vacuum oven dryer at 333 K for 16 hours, obtaining a value of 74.1 % (wet basis), a result similar to that found in this work. This small difference is due to the different drying conditions studied and by the oranges belongs to different species.

Other authors such as Cháfer et al. (2003), determined the moisture from Valencia orange peel also at 333 K until constant weight and obtained value of $74.8 \% \pm 0.012$, a similar result found by previous authors for the same kind of orange. Drying process of the flavedo of two species of oranges, Navelina and Thomson, was studied by Lindihout (2007) at a temperature of 313 K in oven drying for 2 days, obtaining moisture content of 76.1 % and 76.5 % (wet basis), similar to the results found here. Heinze and Isengard (2001), determined the moisture content in different sugar syrups by halogen drying (at different temperatures) and by Karl Fischer titration, and found that there is a difference between methods, in which, the water content obtained by the method of Karl Fischer is smaller than the one found by halogen drying.

In general, although the species of oranges and the methods are different from each other and from the specie studied here, it is possible to ensure that the results obtained are similar, however it is difficult to make a direct comparison with the values obtained in this work.

In conclusion, it appears that all the results obtained, for different species and different methods to determining the water and volatiles content, have value results above those found by Karl Fischer titration, making it possible guarantee the quantitative values found for the total volatile content for the albedo and flavedo of "pera" sweet orange.

3.2. Qualitative and quantitative analysis of VOCs

Based on results obtained by GC/MS and GC-FID analyses, it was possible to identified four volatile organic compounds on albedo and flavedo: 2-methylene-1,4-dioxane, α -pinene, 3-carene and d-limonene. Figures 2 and 3 show the chromatogram obtained of flavedo and albedo.

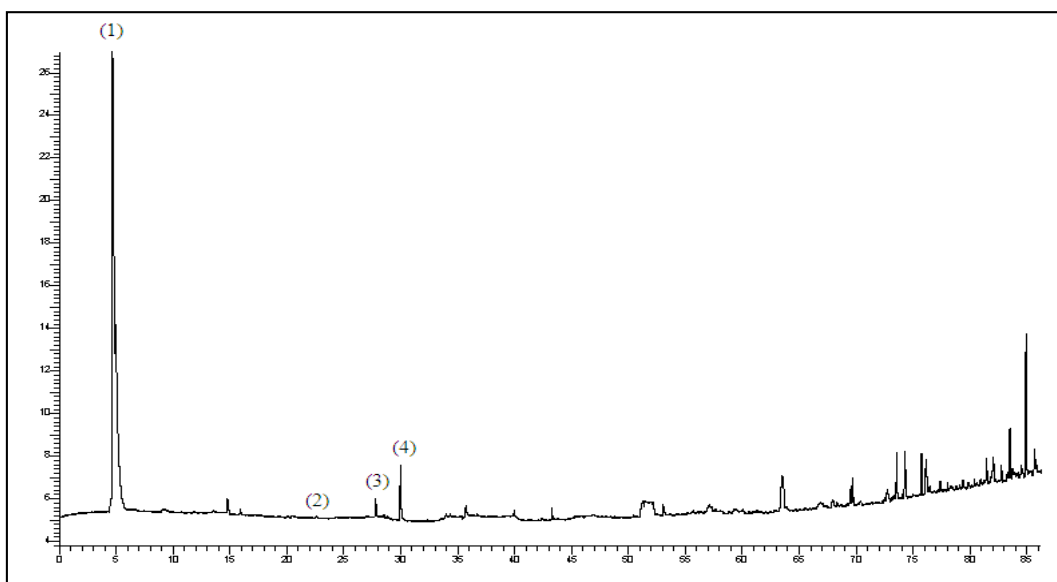


Figure 2: Chromatogram of flavedo obtained by CG-FID.

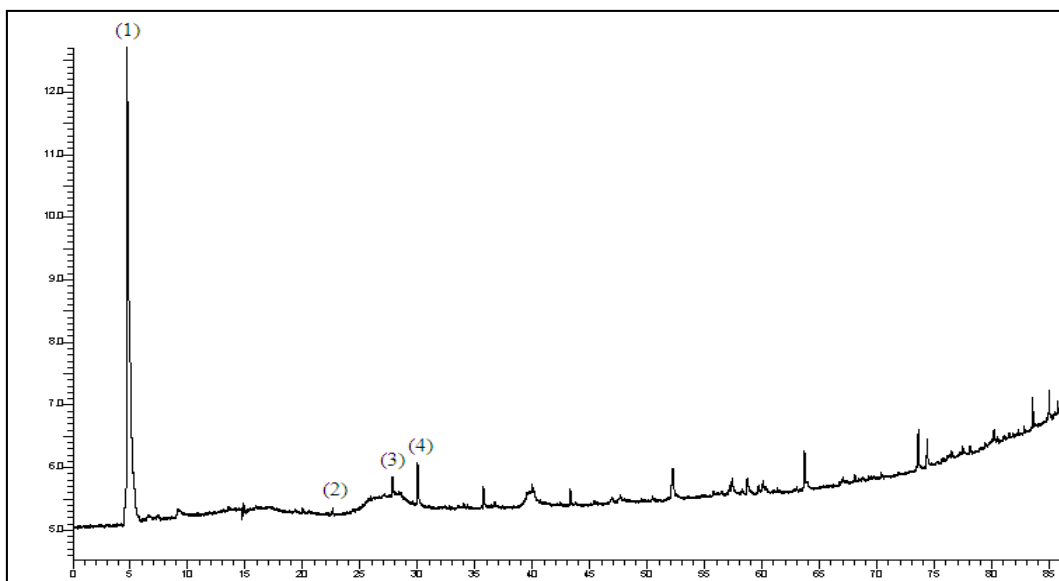


Figure 3: Chromatogram of albedo obtained by CG-FID.

It was possible to observe in Figures 2 and 3 the peaks of the volatile organic compounds that were determined by GC/MS. Peaks 1, 2, 3 and 4 represent 2-methylene-1,4-dioxane, α -pinene, 3-carene and d-limonene respectively.

The quantitative results of VOCs are shown in Table 3, which presents the retention time, response factor and amount of mass of VOCs per raw material mass. GC-FID quantified only α -pinene, 3-carene and d-limonene compounds. The analytical solution used for each VOC response factor determination was prepared with analytical standards of α -pinene (Fluka, purity 98.5 %), 3-carene (Fluka, purity 98.5 %) and d-limonene (Fluka, purity 99 %) diluted in methanol (Merck KGaA, purity 99.9 %).

Table 3: Retention time, response factor and the amount mass of volatile organic compounds

Volatile Compound	Retention time (min)	Response factor* (mV.s)	Mass of volatile compound per mass of raw material (g/g)	
			Albedo	Flavedo
α -pinene	22.619	6,563.69	0.2255	0.0855
3-carene	28.535	7,824.29	0.1478	0.0426
d-limonene	30.016	7,271.98	2.4579	0.9842

*Value of peak area from chromatogram

In literature are reported similar results. For instance, MacLeod et al. (1988) identified and quantified different VOCs (most terpenes) from orange peel: α -pinene, 3-carene and d-limonene in the amount of 0.1, 0.4 and 28.6 $\mu\text{g/g}$ (in μg of volatile compound per gram of orange peel), respectively. Hognadotirr and Rouseff (2003), used GC-MS and detected 95 volatile components in orange essential oil, including α -pinene, carene-3 and d-limonene. Qiao et al. (2008), used GC-MS to identify components of oil from *Citrus sinensis* (L.) Osbeck peel, and detected α -pinene, 3-carene and 3-d-limonene among the compounds that they identified.

It is verified that the other works have used orange oil as main raw material, while in this work we used the VOCs released from albedo and flavedo. However, this method seems to be satisfactory since the volatile compounds found in this study were the same found by other authors. It is also noted from Table 3, that the lowest values of volatiles compounds are associated flavedo and the highest to albedo. This result is suitable to the total amount of volatile found for these materials using the Karl Fischer titration method, of 6.03 % (flavedo) and 10.91 % (albedo) related to wet material (see Table 2).

4. Conclusions

In this work were determined and analyzed the volatiles from albedo and flavedo of "Pera" sweet orange through three different analyzes: oven drying, Karl Fischer titration and gas chromatography coupled to mass spectrometry (GC/MS). The difference between the methods of oven drying and Karl Fischer, shows to be suitable for the quantitative determination of volatile, for wet material. Also, the method used to analyze the volatile organic compounds (VOCs) proved to be efficient, since it brings satisfactorily results in the qualitative identification of the compounds in GC/MS.

References

- De Albuquerque E. L., 2007, Volatile organic compounds in urban atmosphere of metropolitan area of São Paulo. School of Chemical Engineering, University of Campinas, Campinas, Brazil, PhD thesis, 499 (in Portuguese).
- Arami M., Limaee A. Y., Mahmoodi N. M., Tabrizi N. S., 2005, Removal of dyes from colored textile wastewater by orange peel adsorbent: Equilibrium and kinetic studies. *Journal of Colloid and Interface Science*, 288, 2, 371-376.
- Braddock R. J., 1999, *Handbook of citrus by-products and processing technology*. John Wiley & Sons, Inc., New York, USA.
- Gergen I., Radu F., Bordean D., Isengard H. D., 2006, Determination of water content in bee's pollen samples by Karl Fischer titration. *Food Control*, 17, 3, 176-179.
- Heinze P., Isengard H. D., 2001, Determination of the water content in different sugar syrups by halogen drying. *Food Control*, 12, 7, 483-486.
- Hognadottir A., 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, 201-211.
- Licandro G., Odio C. E., 2002, Citrus by-products. In: DUGO, G. e DI GIACOMO, A. (Ed.). *Citrus: The genus Citrus*. Taylor & Francis, London.
- Macleod A. J., Macleod G., Subramanian G., 1988, Volatile aroma constituents of orange. *Phytochemistry*, 27, 7, 2185-2188.
- Morgano M. A., Milani R. F., Martins M. C. T., Rodrigues-Amaya D. B., 2011, Determination of water content in Brazilian honeybee-collected pollen by Karl Fischer titration. *Food Control*, 22, 10, 1604-1608.
- Qiao Y., Xie B. J., Zhang Y., Fan G., Yao X. L., Pan S. Y., 2008, Characterization of Aroma Active Compounds in Fruit Juice and Peel Oil of Jincheng Sweet Orange Fruit (*Citrus sinensis* (L.) Osbeck) by GC-MS and GC-O. *Molecules*, 13, 1333-1344.
- Ras-Mallorquí M. R., Marcé-Recasens R. M., Borrull-Bllarín F., 2007, Determination of volatile organic compounds in urban and industrial air from Tarragona by thermal desorption and gas chromatography-mass spectrometry. *Talanta*, 72, 3, 941-950.
- Ronkart S. N., Paquot M., Fougnyes C., Deroanne C., Van Herck J. C., Blecker C., 2006, Determination of total water content in inulin using the volumetric Karl Fischer titration. *Talanta*, 70, 5, 1006-1010.
- Seinfeld J. H., Pandis S. N., 1998, *Atmospheric Chemistry and Physics: From Air Pollution to Climate Change*, John Wiley & Sons, Inc., New York, USA.
- Schöffski K., 2001, New Karl Fischer reagents for the water determination in food. *Food Control*, 12, 7, 427-429.
- Ueda A. C., 2010, Study of volatile organic compounds in the atmosphere of metropolitan area of Campinas. School of Chemical Engineering, University of Campinas, Campinas, Brazil, PhD thesis, 256 (in Portuguese).