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Determinación del contenido de ácido ursólico y oleanólico en la cera cuticular de frutos de diferentes especies de Rosaceae

Determinação do teor de ácido ursólico e oleanólico na cera cuticular de frutos de diferentes espécies de Rosaceae

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ABSTRACT:

Ursolic acid (UA) and oleanolic acid (OA) are two widely distributed triterpenes in fruits, especially those belonging to Rosaceae family. These triterpene isomers are of great pharmacological interest due to their multiple bioactive properties. For this reason, the objective of this study was to determine the content of UA and OA extracted from the cuticular wax of five highly edible fruits (quince, loquat, pear, peach and apple) all belonging to the Rosaceae family. The acids were analyzed by high performance liquid chromatography. Both UA and OA are present in all these fruits, however, UA is in greater quantities.

KEYWORDS: triterpenes, extraction, chromatography, ursolic acid, oleanolic acid.

RESUMEN:

El ácido ursólico (AU) y el ácido oleanólico (AO) son dos triterpenos ampliamente distribuidos en los frutos, sobre todo en aquellos que pertenecen a la familia Rosaceae. Estos isómeros triterpénicos son de gran interés farmacológico por sus múltiples propiedades bioactivas. Por esta razón, el presente trabajo tuvo como objetivo determinar el contenido de AU y AO extraídos de la cera cuticular de cinco frutos bastante comestibles (membrillo, níspero, pera, durazno y manzana), todos ellos pertenecientes a la familia Rosaceae. El método empleado para analizar estos ácidos fue cromatografía líquida de alta eficiencia. De lo anterior, se concluyó que tanto el AU como el AO están presentes en todos los frutos mencionados, sin embargo, el AU se encuentra en mayores cantidades.

PALABRAS CLAVE: triterpenos, extracción, cromatografía, ácido ursólico, ácido oleanólico.

RESUMO:

Ácido ursólico (AU) e ácido oleanólico (AO) são dois triterpenos amplamente distribuídos em frutos, especialmente aqueles pertencentes à família Rosaceae, estes isômeros triterpênicos são de grande interesse farmacológico por suas múltiplas propriedades bioativas. Por esse motivo, o objetivo deste estudo foi determinar o conteúdo de AU e AO extraídos da cera cuticular de cinco frutos altamente comestíveis (marmelo, nêspera, pêra, pêssego e maçã) pertencentes à família Rosaceae. O método utilizado para esse fim foi a cromatografia líquida de alta eficiência. O resultado mostrou que AU e AO estão presentes em todas essas frutas, sendo a primeira a que está em maior quantidade.

PALAVRAS-CHAVE: triterpenos, extração, cromatografia, ácido ursólico, ácido oleanólico.

AUTHOR NOTES

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INTRODUCTION

Rosaceae family consists of a great variety of plants with edible fruits, healthy and of great economic importance such as apple, peach, pear, quince, loquat, etc. [1]. The annual production of these fruits in the world is of millions of tons. In Peru, the production of apples, peaches/nectarines, pears, quinces, and loquats reach a total of 158, 51, 4, 7 and 2 thousand tons, respectively [2]. The surface of the fruits is covered by a lipophilic layer called cuticle, which has two main components: (i) the lipophilic compounds released by solvent extraction which are collectively designated as cuticular wax and (ii) the lipophilic component that cannot be extracted due to its polymer structure which is called cutin [3]. Cutin is a polyester-type biopolymer, composed mainly of fatty acids [4], while cuticular wax is typically a mixture of dozens of compounds with a variety of hydrocarbon chains or conjugated rings, including pentacyclic triterpenes [5].

Ursolic acid (UA) and oleanolic acid (OA) (Fig. 1) are two of the main pentacyclic triterpenes found in the cuticular wax of many fruits, vegetables, and plants [5, 6]. These molecules are of great interest because they have been shown to possess anti-oxidant, anti-microbial, anti-inflammatory, gastroprotective, and analgesic. [7-12].

The objective of the present work is to determine the content of UA and OA in the cuticular wax of fruits from plants belonging to the Rosaceae family (apple, peach, pear, quince and loquat) grown in Cusco, Peru.

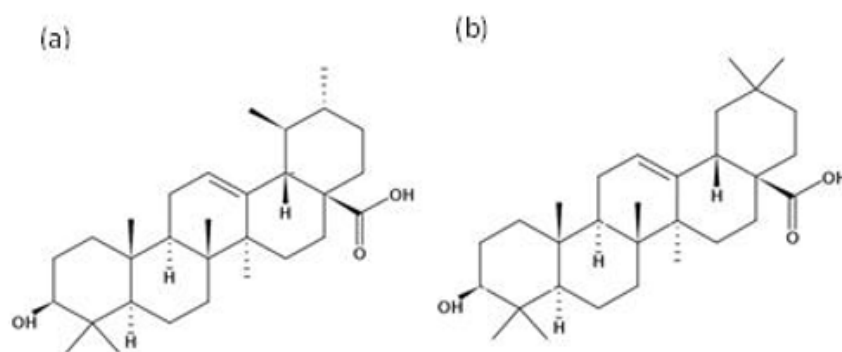


FIGURE 1
Chemical structures of ursolic acid (a) and oleanolic acid (b).

MATERIALS AND METHODS

Materials and Reagents

Five fruits: apple (*Malus domestica*), peach (*Prunus persica*), pear (*Pyrus communis*), quince (*Chaenomeles japonica*), and loquat (*Eriobotrya japonica*) were purchased at the San Pedro, Cusco-Peru market and transported directly to the laboratory. Fruits (250 g) were washed with tap water before removing the peel from the fruits. The peels were then dried at 80 °C for 12 h, which were crushed in a porcelain mortar and kept in a desiccator until being analyzed. The chemical reagents used, ethanol (Merck, Germany) and acetonitrile (Merck, Germany), were chromatographic grade, and methanol (Merck, Germany), petroleum ether 40-60 °C (Merck, Germany) together with chloroform (Merck, Germany) were of analytical grade.

Cuticular wax extraction

The extraction of cuticular wax is more efficient when using a mixture of CHCl_3 : MeOH (3:1) [13]. Therefore, the dried and crushed apple (1.04 g), peach (1.01 g), pear (1.00 g), quince (1.01 g), and loquat (1.03 g) peels were immersed in 10 mL of the solvent mixture, and then, the suspensions were extracted with ultrasound assistance under 20 °C for 3 min. This process was repeated three times. The extracts were collected and filtered to be concentrated by evaporating at 60 °C and 230 mbar (reduced pressure) in a rotary evaporator. The concentrates were defatted by washing three times with 15 mL of petroleum ether (5 mL each time), then made up to 25 mL with ethanol. Finally, all the extracts were filtered through a 0.45 μm membrane and stored at 4 °C for subsequent chromatographic analysis.

Thin layer chromatography

Thin layer chromatography was performed on a silica gel 60-F₂₅₄ plate with 10-12 μm particle size. Standard solutions of UA (1 mg/mL), OA (1 mg/mL) and test solutions were applied on silica gel plate and developed with a mixture of solvent CHCl_3 : MeOH (12:2 v:v). After developing and drying, the plate was dipped for 2 s in sulfuric acid (10% v/v) and heated on a plate heater. Triterpene acids are shown as reddish colored areas [14].

Determination of the content of UA and OA

The quantification of OA and UA was performed by HPLC, for which an UltiMate 3000 chromatograph with DAD detector (Thermo, USA), Lichrospher RP-18 column (250 x 4.6 mm, 5 μm) was used, detection was performed at 209 nm, temperature of 30 °C with a flow of 1 mL/min. The mobile phase consisted of a mixture of acetonitrile:water (8:2 v:v) with an isocratic elution mode [15]. Mixtures of UA and OA (0.5 mg/mL) were prepared in ethanol and volumes of 1, 0.5, and 0.1 μL injected for plotting the standard curves for UA and OA. The injection volume of the samples was 1 μL . The calibration curves for the determination of UA and OA were constructed and the following Ec. (1) and Ec. (2) were obtained.

$$Y = 590.02 \cdot X - 0.36 \text{ for UA} \quad (1)$$

$$Y = 780.86 \cdot X + 0.20 \text{ for OA} \quad (2)$$

Where Y is the peak area (mAU S) and X is the amount (μg). The correlation coefficient (R^2) of the regression equation of OA and UA was 0.99 for both curves.

RESULT AND DISCUSSION

Analysis of thin layer chromatography

An image of the chromatographic plate of the standards and sample test is shown in Figure 2. OA and UA travel at same rates in the chromatographic plate because of the closely related physicochemical properties of both structural isomers. The coloration that was acquired after being revealed with sulfuric acid and submitted to the heat is reddish as expected. The presence of these triterpenes can be visualized in all the samples.

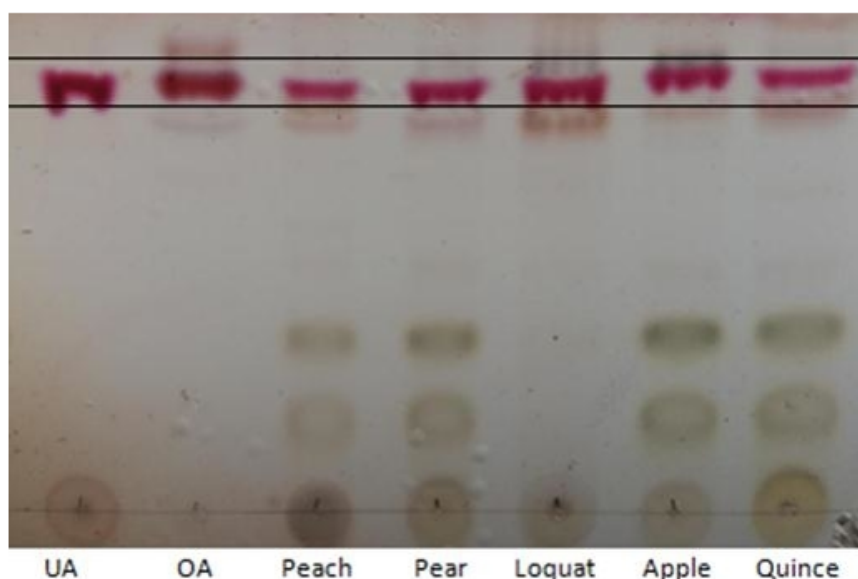


FIGURE 2.

Chromatographic plate of UA, OA and the five samples, obtained using a mobile phase of CHCl_3 : MeOH (12:2 v/v) and revealed by immersion in sulfuric acid (10% v/v) and heat application.

UA and OA contents in the cuticular wax of fruits

The results of the chromatographic analysis (Fig. 3) show that OA and UA acid were the main two active compounds in the skin of these five edible fruits. The concentration of UA ranged from 2.97 to 9.38 mg/g and that of OA from 0.25 to 1.49 mg/g (Table 1). The content of UA was significantly greater than that of OA in all cases (Fig. 4). The total content of these two triterpenes (UA+OA) is higher in the apple (10.34 mg/g) and lower in the peach (4.46 mg/g) (Fig. 5). The proportion of these two triterpenes (UA/OA) is higher in the quince (22.76) and less in the peach (1.99).

The content of UA and OA in loquat was not significantly different from those obtained by Zhou *et al.* [16]. Yin *et al.* [13] identified a series of pentacyclic triterpenes in the polar fraction of the cuticular wax of the pear, but does not give information about their concentrations of these or the type of triterpene present. Comparing the content of OA and UA in the quince peel determined by Miao *et al.* [17] and our results, the levels of UA and OA were higher in this research. Consistent with the work of Belgeel *et al.* [18], OA and UA were the main triterpenes in peach peel. However, unlike the other fruits where the UA is the triterpene in greater quantity, in the peach the difference between the amounts of OA and UA is low. The amount that was extracted from UA and OA of the apple peel was higher by the method described in this work than the one proposed by Ellgardt [19].

TABLE 1.

		mg/mL	mg/g	UA +	UA/OA
		Dp	Dp	OA	
Quince	OA	0.01	0.25		
1.01 g Dp	UA	0.23	5.69	5.94	22.76
Loquat	OA	0.06	1.46		
1.03 g Dp	UA	0.33	8.01	9.47	5.49
Pear	OA	0.05	1.25		
1.00 g Dp	UA	0.29	7.25	8.50	5.80
Peach	OA	0.06	1.49		
1.01 g Dp	UA	0.12	2.97	4.46	1.99
Apple	OA	0.04	0.96		
1.04 g Dp	UA	0.39	9.38	10.34	9.77

Results of the quantification of UA and OA.
*Dp: Dry peel.

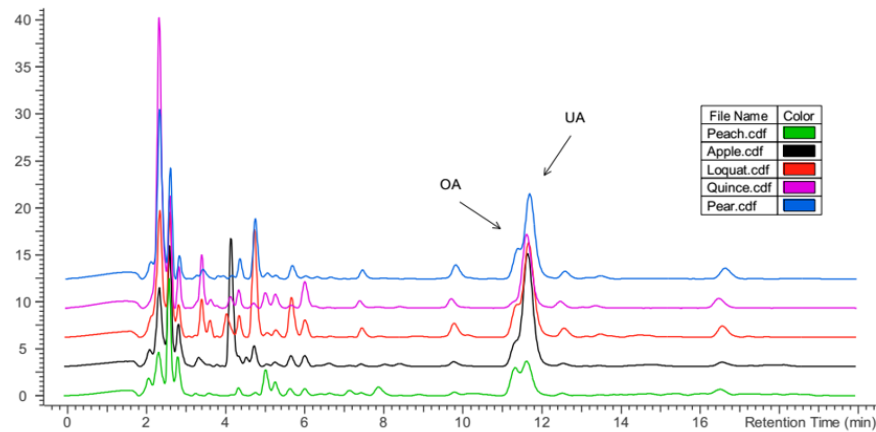


FIGURE 3

Chromatograms of the five samples, which were obtained using a mobile phase of acetonitrile: water (8:2, v:v) with a flow of 1 mL/min and detection at 209 nm.

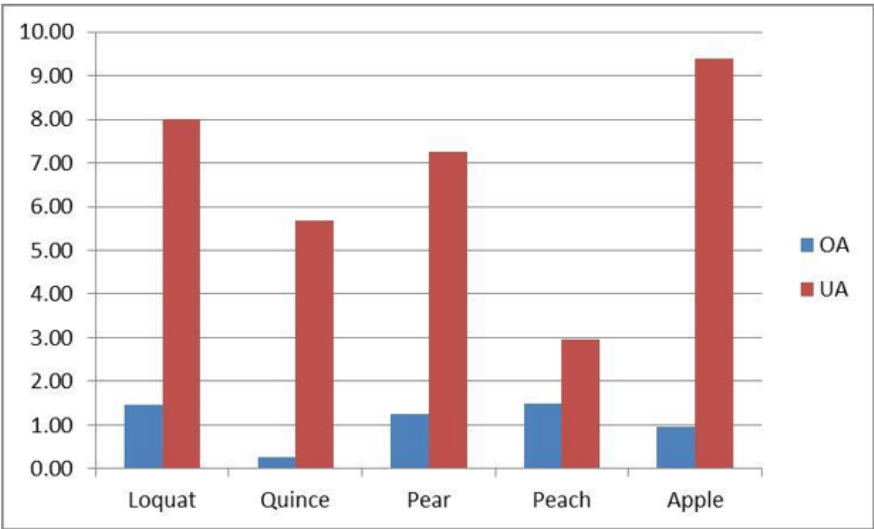


FIGURE 4

Content of UA and OA (mg/g Dp) in the different samples.

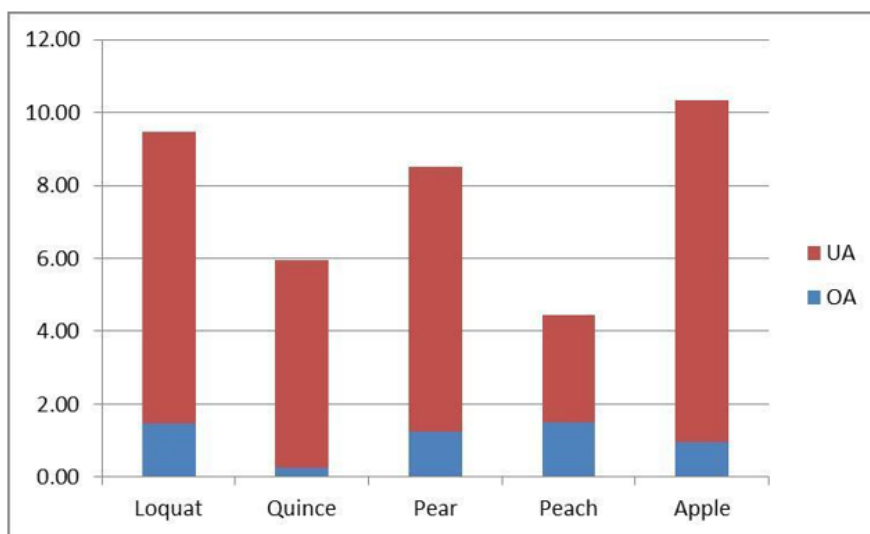


FIGURE 5

Total content (mg/g Dp) of triterpenes (UA+OA) in the different samples.

CONCLUSIONS

In this study, the content of UA and OA present in the cuticular wax of the five most edible fruits (quince, loquat, pear, peach and apple) of the family Rosaceae was determined. Ursolic and oleanolic acid were the predominant triterpenes in the cuticular wax of these fruits, and the UA content were higher than OA in all samples.

REFERENCES

- [1] S. Farinati, A. Rasori, S. Varotto, and C. Bonghi, "Rosaceae Fruit Development, Ripening and Post-harvest: An Epigenetic Perspective," *Front. Plant Sci.* vol. 8, 2017. DOI: <https://doi.org/10.3389/fpls.2017.01247>
- [2] FAOSTAT, "Food and Agriculture Organization of the United Nations," 2016. [Online]. Available: <http://www.fao.org/>. [Accessed: 06 09 2018].
- [3] C. Buschhaus and R. Jetter, "Composition differences between epicuticular and intracuticular wax substructures: How do plants seal their epidermal surfaces?," *J. Exp. Bot.*, vol. 62, no 3, pp. 841–853, 2011. DOI: <https://doi.org/10.1093/jxb/erq366>
- [4] A. Szakiel, C. Pączkowski, F. Pensec, and C. Bertsch, "Fruit cuticular waxes as a source of biologically active triterpenoids," *Phytochem. Rev.* vol. 11, no 2–3, pp. 263–284, 2012. DOI: <https://doi.org/10.1007/s11101-012-9241-9>
- [5] M. Martelanc, K. Naumoska, and I. Vovk, "Determination of common triterpenoids and phytosterols in vegetable waxes by HPTLC densitometry and HPTLC image analysis," *J. Liq. Chromatogr. Relat. Technol.* vol. 39, no 5–6, pp. 312–321, 2016. DOI: <https://doi.org/10.1080/10826076.2016.1165576>
- [6] S. Peschel, R. Franke, L. Schreiber, and M. Knoche, "Composition of the cuticle of developing sweet cherry fruit," *Phytochemistry*. vol. 68, no 7, pp. 1017–1025, 2007. DOI: <https://doi.org/10.1016/j.phytochem.2007.01.008>
- [7] J. A. Rodríguez, L. Astudillo, and G. S. Hirschmann, "Oleanolic acid promotes healing of acetic acid-induced chronic gastric lesions in rats," *Pharmacol. Res.* vol. 48, no 3, pp. 291–294, 2003. DOI: [https://doi.org/10.1016/S1043-6618\(03\)00155-5](https://doi.org/10.1016/S1043-6618(03)00155-5)
- [8] M. A. L. Vasconcelos, V. A. Rojo, D. S. Ferreira, A. E. M. Crotti, J. C. T. Carvalho, and W. R. Cunha, "In vivo Analgesic and Anti-Inflammatory Activities of Ursolic Acid and Oleanolic Acid from Miconia

- albicans (Melastomataceae),” *Z. Für Naturforschung C*. vol. 61, no 7–8, pp. 477–482, 2014. DOI: <https://doi.org/10.1515/znc-2006-7-803>
- [9] D. Kashyap, H. S. Tuli, and A. K. Sharma, “Ursolic acid (UA): A metabolite with promising therapeutic potential,” *Life Sci*. vol. 146, pp. 201–213, 2016. DOI: <https://doi.org/10.1016/j.lfs.2016.01.017>
- [10] L. H. Lopez, P. P. Larrán, M. J. G. Muñoz, E. Falqué, and H. Domínguez, “Recent developments on the extraction and application of ursolic acid. A review,” *Food Res. Int*. vol. 103, pp. 130–149, 2018. DOI: <https://doi.org/10.1016/j.foodres.2017.10.028>
- [11] D. Zhao et al., “Oleanolic acid exerts bone protective effects in ovariectomized mice by inhibiting osteoclastogenesis,” *J. Pharmacol. Sci*. vol. 137, no 1, pp. 76–85, 2018. DOI: <https://doi.org/10.1016/j.jphs.2018.03.007>
- [12] U. V. Mallavadhani, A. Mahapatra, K. Jamil, and P. S. Reddy, “Antimicrobial Activity of Some Pentacyclic Triterpenes and Their Synthesized 3-O-Lipophilic Chains,” *Biol. Pharm. Bull*. vol. 27, no 10, pp. 1576–1579, 2004. DOI: <https://doi.org/10.1248/bpb.27.1576>
- [13] Y. Yin et al., “Chemical composition and antifungal activity of cuticular wax isolated from Asian pear fruit (cv. Pingguoli),” *Sci. Hortic*. vol. 129, no 4, pp. 577–582, 2011. DOI: <https://doi.org/10.1016/j.scienta.2011.04.028>
- [14] C. A. S. Flores, B. C. Curie, A. L. T. Quispe, R. I. H. Quispe, M. L. Huaman, and E. F. R. Rodríguez, “Quantification of Oleanolic acid, Ursolic acid and Rosmarinic acid in three Peruvian species of *Clinopodium* (Lamiaceae, Nepetoideae, Mentheae),” *ARNALDOA*, vol. 23, no 1, pp. 333–350, 2016.
- [15] P. Srivastava, N. Kasoju, U. Bora, and R. Chaturvedi, “Accumulation of betulinic, oleanolic, and ursolic acids in *In vitro* cell cultures of *Lantana camara* L. and their significant cytotoxic effects on HeLa cell lines,” *Biotechnol. Bioprocess Eng*. vol. 15, no 6, pp. 1038–1046, 2011. DOI: <https://doi.org/10.1007/s12257-010-0054-7>
- [16] C.H. Zhou, X. Li, W.S. Zhang, C.D. Sun, and K.S. Chen, “Oleanolic and ursolic acid in the fruit of *Eriobotrya japonica* Lindl.” p. 6, 2011.
- [17] J. Miao, C. Zhao, X. Li, X. Chen, X. Mao, H. Huang and W. Gao, “Chemical Composition and Bioactivities of Two Common *Chaenomeles* Fruits in China: *Chaenomeles speciosa* and *Chaenomeles sinensis*,” *J. Food Sci*. vol. 81, no 8, pp. 2049–2058, 2016. DOI: <https://doi.org/10.1111/1750-3841.13377>
- [18] B. Belge, M. Llovera, E. Comabella, J. Graell, and I. Lara, “Fruit Cuticle Composition of a Melting and a Nonmelting Peach Cultivar,” *J. Agric. Food Chem*. vol. 62, n° 15, pp. 3488–3495, 2014. DOI: <https://doi.org/10.1021/jf5003528>
- [19] K. Ellgardt, “Triterpenes in apple cuticle of organically and IP cultivated apples,” Undergraduate thesis, Sveriges Lantbruksuniversitet, 2006.