

## VIBRATIONAL AND THERMAL CHARACTERIZATION OF SEEDS, PULP, LEAVES AND SEED OIL OF *ROSA RUBIGINOSA*

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**Summary:** *Rosa rubiginosa* L. seed oil has been studied for its application in skin care products, but the chemical nature of seeds, pulp and even leaves, apart from that of oil, is also relevant with a view to the application of this weed for biodiesel production. All these vegetal materials were studied by infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) for characterisation purposes. FTIR bands at 3005, 2924, 1740, 1654 and 1456 cm<sup>-1</sup> were used to estimate the iodine index, suitable for biofuels, and the oxidation stability degree. From the viewpoint of the thermal stability, both the seed oil (for which pyrolysis occurs at 462°C), the raw seeds and the rosehip pulp (with decomposition temperatures of 373°C and 333°C, respectively) showed potential as a biomass feedstock for conversion into biofuels.

**Key words:** A-linolenic acid, DSC, FTIR, rose hip seed oil, *R. rubiginosa*.

**Resumen:** Caracterización térmica y vibracional de las semillas, pulpa, hojas y aceite de semillas de *Rosa rubiginosa*. El aceite de semillas de *R. rubiginosa* L. ha sido estudiado para su aplicación en productos para el cuidado de la piel, pero la naturaleza química de las semillas, pulpa y hojas, además de la del aceite, también es importante con miras a la aplicación de esta mala hierba para la producción de biodiesel. Los materiales vegetales mencionados se han estudiado mediante espectroscopía infrarroja (FTIR) y calorimetría diferencial de barrido (DSC) con fines de caracterización. Las bandas del espectro infrarrojo en 3005, 2924, 1740, 1654 y 1456 cm<sup>-1</sup> han sido utilizadas para estimar el índice de yodo, adecuado para los biocombustibles, y el grado de estabilidad a la oxidación. Desde el punto de vista de la estabilidad térmica, tanto el aceite de semillas (para el que la pirólisis se produce a 462°C) como las semillas crudas y la pulpa (con temperaturas de descomposición de 373°C y 333°C, respectivamente) mostraron potencial como materia prima de biomasa para su conversión en biocombustibles.

**Palabras clave:** Aceite de rosa de mosqueta, ácido  $\alpha$ -linolénico, DSC, FTIR, *R. rubiginosa*.

## INTRODUCTION

*Rosa rubiginosa* L. (= *R. eglantheria* L.) is a shrub from Great Britain that is frequent in Southern Alps and grows massively in Southern America in the shadow of the Andes Mountains. More recently, it has also started invading some of the temperate regions of Australia (Hatton, 1989; Hunter, 2004).

This member of the rose family has flowers pink in colour, and when petals fall (the flowers are ethereal, only living about 24 hours), the shrub develops enlarged floral cups (receptacles) which surround numerous small, hard dry fruits (achenes) commonly called seeds (Fig. 1). Rose hips are bright orange and oval and become fleshy but are not true fruits (Joublan *et al.*, 1996). The pulp is edible and has a high concentration of vitamin C and is often used to make jams and preserves. The leaves and the petals, with their astringent and refreshing properties, are used for baths and infusions.

The finest rosehip seed oil is derived from cold pressing hips. This oil has been used by

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local people for its soothing and moisturizing properties and is commonly known in South America as “*Rosa mosqueta*” and in Australia as “sweet briar”.

*Rosa rubiginosa* oil has a high content of essential fatty acids: over 77% in PUFA (oleic, linoleic or LA, and linolenic or  $\alpha$ LNA). The percentage of SFA (palmitic and stearic acids) is below 5%. The LA and  $\alpha$ LNA acids are considered important for the maintenance of a healthy skin. Besides non-saturated fatty acids and significant quantities of  $\alpha$ -tocopherol (300 ppm), the presence of carotenoids, flavonoids and trans-retinoic acid has also been detected and these could be responsible for some of the pharmacological and therapeutic properties of rosehip seed oil (Zielinski, 1984; Dourado *et al.*, 2000; Moure *et al.*, 2001, 2005; Franco *et al.*, 2007a, b; Romero *et al.*, 2007; Merrill *et al.*, 2008).

The characterization of *R. rubiginosa* seed oil has been of particular interest in refining processes; however, the chemical nature of plant

parts such as seeds, pulp and even leaves, apart from that of crude oil, is now also relevant with a view to the more recent application of *R. rubiginosa* for biodiesel production. In this line, the aim of this work is to investigate their physicochemical properties by FTIR spectra and their thermal behaviour by differential scanning calorimetry (DSC).

## MATERIAL AND METHODS

Fruits of *R. rubiginosa* come from Calafate, Santa Cruz, Argentina. Seeds were separated from pulp, air-dried for two weeks in the dark, grinded and sieved. High-purity commercial seed oil was supplied by Bariloche Silvestre™ (Provincia de Rio Negro, Argentina). This commercial oil contains  $1.5 \pm 0.5$  g oleic acid/100 g in FFA; 10-20 % in oleic (C18:1), 41-50% in LN (C18:2 n-6); 26-37% in LN (C18:3 n-3), 3-5% in palmitic (C16:0), and 1-3% in stearic



Fig. 1. *Rosa rubiginosa* or “rosa mosqueta” hips and leaves.

(C18:0), in agreement with the characterization carried out for rosehip oils by Concha *et al.* (2006) and Pareja & Kehl (1990). *R. rubiginosa* oil FFA content was determined by AOAC method (protocol 940.28) using a chromatograph (Hewlett-Packard 5890 series II) equipped with an FID and fitted with an SP-2330 column. After methylation, a hexane extract containing FAME was injected into the GC column. FAME were separated using a stated temperature program and its weights calculated on the basis of their relative area vs. tridecanoic acid.

The infrared spectra were recorded with a Thermo Nicolet 380 FT-IR apparatus equipped with Smart Orbit Diamond ATR system.

DSC curves were obtained using a TA Instruments Q100 under N<sub>2</sub> flow (20 mL·min<sup>-1</sup>), at a heating rate of 20°C·min<sup>-1</sup>, in 40-μL sealed aluminium capsules. After cooling of the samples down to -100°C, the scans were registered on the heating cycle from -100°C to 500 °C. For the study of the glass transition at low temperatures, samples were cooled to -100°C and then heated from -60°C to +20°C at a rate of 10°C·min<sup>-1</sup>. The data obtained were analysed using TA Instruments Universal Analysis V4.1D software.

Simultaneous analysis of TG and DSC (mW) for two samples was conducted with a Perkin-Elmer STA6000, DTA/DTG equipment, by heating the samples in a slow stream of N<sub>2</sub> (20 mL/min) from room temperature up to 800°C, with a heating rate of 20°C/min. Pyris v.11 software was used for data analysis.

## RESULTS AND DISCUSSION

### *Vibrational characterization*

Table 1 shows the frequencies and assignments of the bands observed in the vibrational spectra (Fig. 2) for the various samples of *R. rubiginosa*. To facilitate such assignments, the spectra of other plant samples with different percentages of immediate principles (lipids, proteins and carbohydrates) have been included.

The lipid characteristic band at 3009 cm<sup>-1</sup> (which only appears for oil and seeds samples) is due to C-H stretching vibration of the carbon-carbon double bond (Guillén & Cabo, 1997). The strong absorption band at 2924 cm<sup>-1</sup>, shown by

all the samples of *R. rubiginosa*, also has a lipid origin. A third lipid characteristic band at 1742 cm<sup>-1</sup> for oils and seeds (shifted at 1730 cm<sup>-1</sup> for other samples) is due to the stretching vibration of the triglyceride ester carbonyl (-C-C=O-) group. FTIR absorbance ratios  $A(3009\text{ cm}^{-1})/A(2924\text{ cm}^{-1})$ ,  $A(3009\text{ cm}^{-1})/A(2854\text{ cm}^{-1})$ , and  $A(3009\text{ cm}^{-1})/A(1740\text{ cm}^{-1})$  were considered to measure the iodine values (El-Bahy, 2005). These ratios (0.21, 0.32 and 0.24, respectively) revealed that the iodine value of rosehip oil is similar to that of corn and higher than those of, for example, olive oil or cotton oil.

The band between 1653 and 1637 cm<sup>-1</sup>, common to *Rosa moschata* oil and leaves and woody liana, should be ascribed to C=O (amide I). The peak that appears between 1543-1521 cm<sup>-1</sup> should be assigned to N-H bending vibrations (amide II). The intermediate band at 1614 cm<sup>-1</sup> is due to lipids and resins (Yoshida & Yoshida, 2003; Abdelmalik *et al.*, 2011). Bands between 1456 and 1317 cm<sup>-1</sup> are due to CH<sub>2</sub> and CH bending vibrations and are usual for vegetal fats and resins. From the above bands, one that appears at 1317 cm<sup>-1</sup> is common to *R. rubiginosa* leaves, woody liana and myrtle bark.

According to the equation deduced by Sadeghi-Jorabchi *et al.* (1990),  $v(\text{C}=\text{C})/\delta(\text{CH}) = A(1653\text{ cm}^{-1})/A(1456\text{ cm}^{-1}) = (6.845 \cdot 10^{-3})IV - (2.489 \cdot 10^{-2})$ , it was found that the iodine value of rosehip oil is ca. 115 g<sub>I</sub>/100g (again very close to that of corn, which ranges from 103 to 123).

Since the oxidative stability is dependent on the iodine value (the higher the iodine value, the less the stable the oil), a presumably moderate stability could be expected. However, in agreement with Steel *et al.* (2005), we suggest that such oxidative stability would be enhanced by the high quantity of tocopherols present (300 ppm), which act as stabilizers.

The absorption band at around 1240 cm<sup>-1</sup> is attributed to N-H bending (amide III) and the following bands (down to 1100 cm<sup>-1</sup>) are a fingerprint of methyl ester of long-chain fatty acids (Silverstein *et al.*, 2005). The FTIR band at 1097 cm<sup>-1</sup> indicates the presence of starch. The bands at wavenumbers around 1020 cm<sup>-1</sup> and 810-765 cm<sup>-1</sup> are due to the phosphate component. Finally, the band at 720 cm<sup>-1</sup> can be assigned to (CH<sub>2</sub>)<sub>n</sub> rocking.

**Table 1.** Main bands in the ATR- FTIR spectra of different parts of *R. rubiginosa* plant and of other vegetal species and natural products (for comparison purposes). All wavenumber values are in cm<sup>-1</sup>.

Rosa rubiginosa seed oil	Rosa rubiginosa seeds	Rosa rubiginosa pulp	Rosa rubiginosa leaves	Woody liana	Myrtle bark	Palm refined oil	Assignment
			3342	3288	3340		L – v(=C-H), v(C-H), arom. or olefin
			3317				
3009	3007					3004	L - v(=CH)
			2952				C
2923	2920	2918	2918	2917	2917	2914	L - asymmetrical v(C-H)
2853	2850	2850	2850	2850	2850	2850	L – symmetrical v(C-H)
1742	1730	1730	1732	1730	1734	1736	L - v(CO) ester carbonyl
1653			1633	1635			P – N-H bending (amide I), v(C=O)
	1614	1612			1616		L / R - v(C=C)
	1521		1513	1543	1510		P – N-H bending (amide II)
1456	1456		1462	1454	1463	1467	L- δ(C-H <sub>2</sub> ) <sub>2</sub> bending deform
	1439	1439					
		1414	1417	1415	1419	1389	CH <sub>2</sub> wagging, O-H in plane d.
1377	1371	1371	1356	1387	1372		L - δ(CH <sub>2</sub> ) <sub>2</sub> bending deform
			1317	1317	1316		R - δ(CH)
1238	1238	1240	1230	1238	1230	1243	P – NH bending (amide III), O-H in plane
			1205				
1160	1161	1138	1159	1149	1159	1170	L–methyl ester, v(CO)
	1115					1100	
1098	1099	1099	1097			1097	C – starch OH, cellulose
	1049	1043		1032	1032		P - ring resonance
	1012	1016	1022				M - PO <sub>4</sub> <sup>3-</sup>
	825	821					C - δ(COC)
	765	779					M - PO <sub>4</sub> <sup>3-</sup>
723	718	719	719	717	721	717	ρ(CH <sub>2</sub> ) <sub>n</sub>

L = lipids; M = minerals; P = proteins; R = resins; C = carbohydrates.

**Thermal behaviour**

*Raw seeds*

DSC curves of the heating cycle (after cooling down to -100°C) of *R. rubiginosa* raw seeds are depicted in Fig. 3a. The endotherm at around -37°C is assigned to the crystallization of α-LNA and LA polyunsaturated fatty acids as well as remaining fractions of di- and triglycerides, in good agreement with the values reported by Ueno *et al.* (2000) and Franco *et al.* (2013).

The main thermal effect at around 111°C

is related to gelatinization of starch (an order-disorder transition for the starch/moisture system). The endotherm at 242°C indicates seed protein crystallization to β-crystals accompanied by the random-coil → β-form conformational transition. The exothermic events above 270°C are related to the decomposition of hemicellulose and other fibre components (302°C) and to the decomposition of either unsaturated fatty acid triglycerides or flavonoids (373°C). Such two effects are in excellent agreement, for example, with those observed in

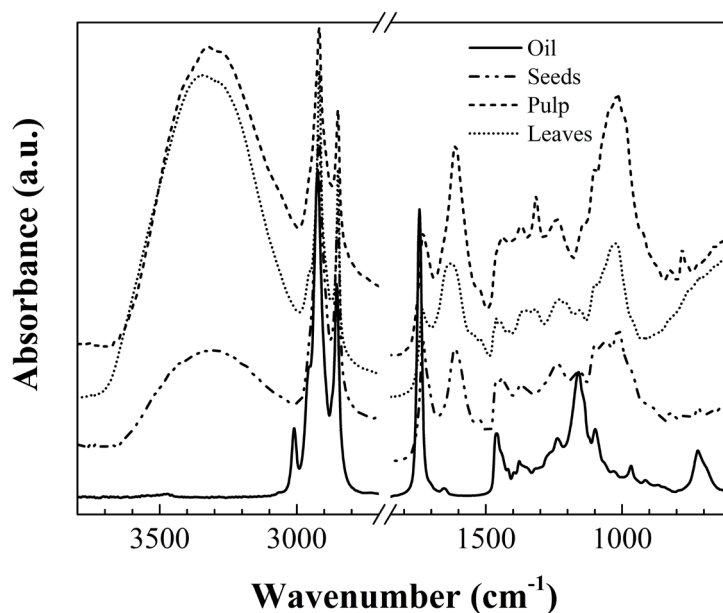


Fig. 2. ATR-FTIR spectra of different parts of *R. rubiginosa* plant.

the DSC scan of the salmon coloured bark of the myrtaceae *Luma apiculata*, rich in flavonoids, which has been included for comparison purposes (see Fig. 4).

The assignment of the last exothermic effect at 373°C to decomposition of triglycerides is in agreement with values previously reported for pure fatty acids: according to Santos *et al.* (2002), the decomposition for polyunsaturated and monounsaturated fatty acids occurs in the 300-380°C range and from 380 to 480°C, respectively. Alternatively, this same effect could be also assigned to degradation of flavonoids, according to da Costa *et al.* (2002).

#### *Rose hip pulp*

The DSC scan of pulp from *R. rubiginosa* rose hips (Fig. 5) differs significantly from the DSC of raw seeds. The thermal effects that appear during the pulp heating cycle occur 30-70°C below than those that appear for the raw seeds and have a different origin. Thus, the fine endotherm detected at around 175°C (attributed to sucrose) can be attributed to a conversion of pentahydrate into an anhydride upon temperature increase. Nevertheless, an alternative adscription to lycopene is also plausible: the melting of this red carotenoid pigment takes place

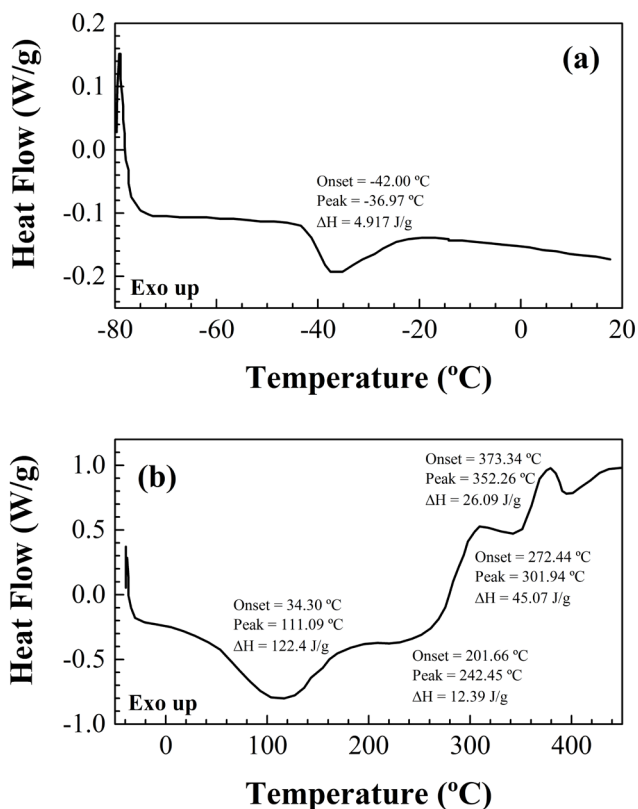
at 173°C. Even a third assignment to melting of trans retinoic acid would be possible: when this nutrient is in nanosuspension, its melting point decreases by 11°C to 173°C (Zhang *et al.*, 2006).

With regard to the exotherms at 220°C and 272°C, they may correspond to decomposition of hemicellulose and starch, respectively. Nonetheless, since an exotherm at 218°C has been reported for auto-oxidation of liposomes and other lipid systems containing water (Ayala-Zavala *et al.*, 2007), this alternative assignment to the exotherm at 220°C cannot be excluded.

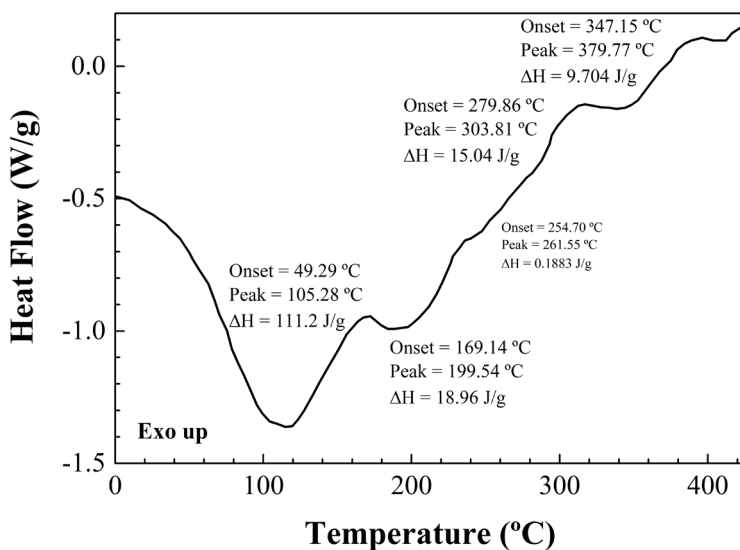
Regarding the exotherm at 333°C, it can be attributed either to decomposition of a mixture of  $\alpha$ -LNA and LA acid starch esters produced from starch (Kapuśniak *et al.*, 2003) or to the decomposition of high molecular weight lignin components. This latter hypothesis arose when comparing the exotherm temperature for pulp with the one that appears in a DSC scan of a woody liana (see Fig. 6).

#### *Leaves*

The low temperature effect at -1°C, attributed to ice melting, appears lower than the one expected for pure water (0°C) and is possibly conditioned by the temperature at which thylakoid membrane lipids



**Fig. 3.** DSC thermal effects of *R. rubiginosa* raw seeds: (a) detail of the low temperature endotherm after 5 freezing-thawing cycles; (b) details on the four main thermal effects.



**Fig. 4.** DSC scan of myrtle bark.

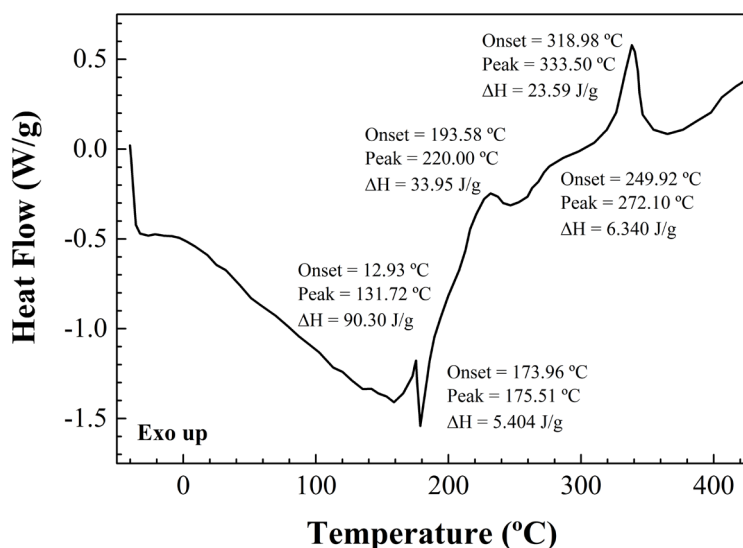


Fig. 5. DSC thermal effects of *R. rubiginosa* rose hip pulp.

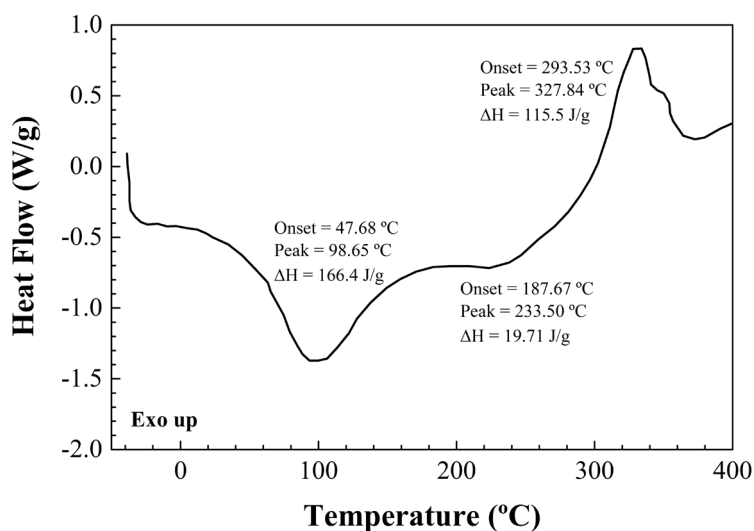


Fig. 6. DSC thermal effects of a woody liana.

undergo a phase transition (Fig. 7). The endotherm at 109°C, attributed to usual evolved water (liberation or decomposition of water molecules) in cellulose membranes, is also in agreement with Raoult's Laws.

#### Seed oil

The thermal decomposition of *R. rubiginosa* seed oil occurs in two stages. The first takes place

between 200°C and 400°C and is sensitized by endotherms at 216°C and 323°C (see Fig. 8), at temperatures similar to those found for other oils such as refined palm oil, which features endotherms at 215°C and 322°C (Fig. 9). These thermal effects are assumed to be due to partial volatilization of triacylglycerides. The other decomposition stage, due to pyrolysis, starts at around 350°C and is

characteristic from each oil: whereas for refined palm oil occurs in a single step sensitized by a peak at 440°C, for *R. rubiginosa* the decomposition takes place in two steps (peaks at 416°C and 462°C), in a similar fashion to—for example—the decomposition

of olive pomace oil (with peaks at 284°C and 429°C) (Franco *et al.*, 2013), albeit shifted to higher temperatures (by 33°C). This second stage is the most significant because it is accompanied by an almost complete weight loss.

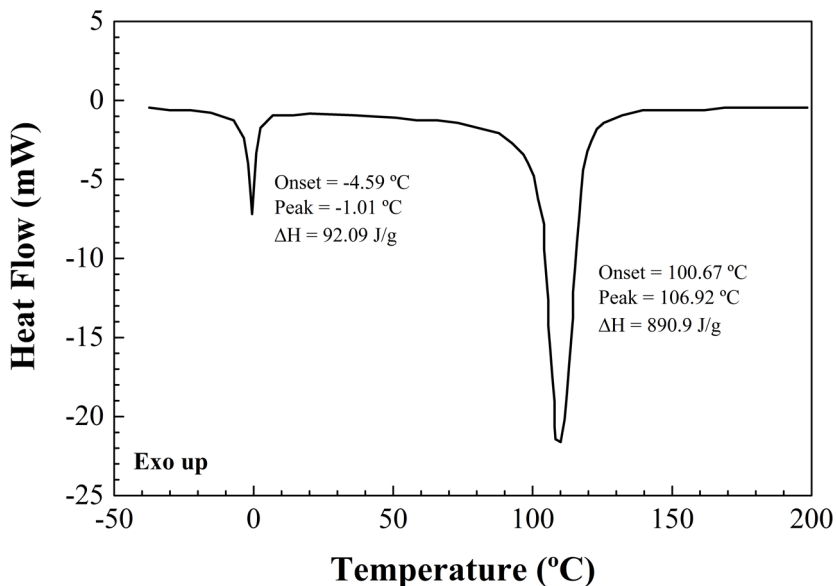


Fig. 7. DSC thermal effects of *R. rubiginosa* leaves.

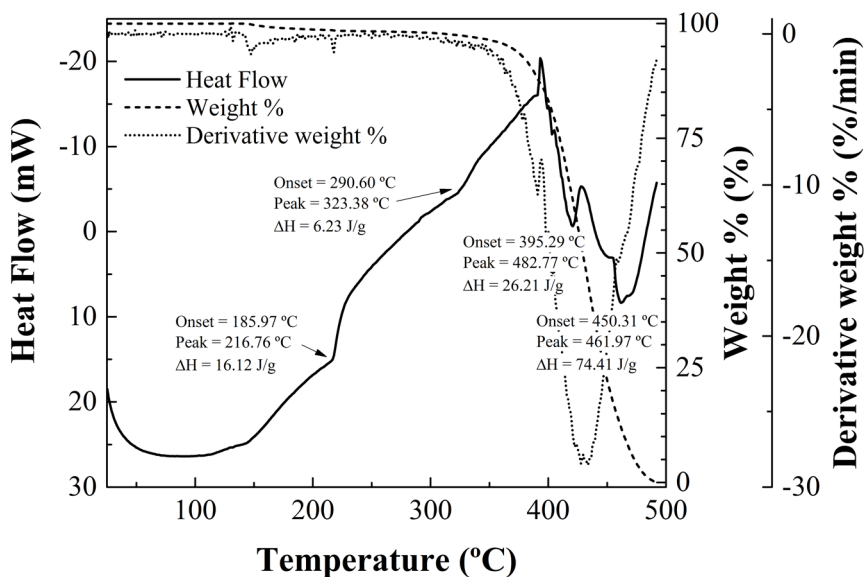


Fig. 8. TG/DSC thermal analysis of *R. rubiginosa* seed oil.



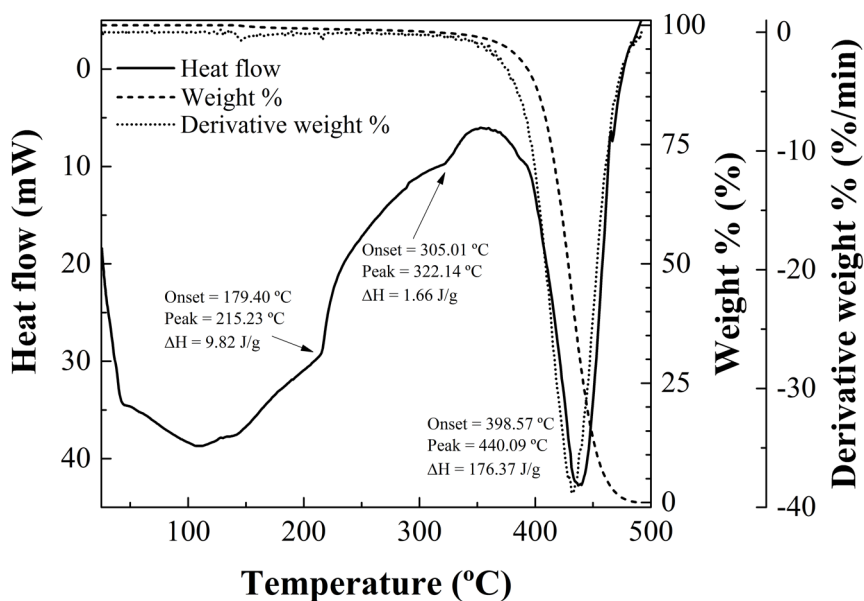


Fig. 9. TG/DSC thermal analysis of refined palm oil.

As a final remark to the overall discussion on the curves discussed above, the changes that result in endothermic and exothermic peaks must be considered as a summation of various thermal processes, out of which the main process still needs to be clarified in some cases. For example, the ambiguity in the assignation of the final decomposition thermal effects either to triglycerides or to flavonoids (in relation to the DSC of raw seeds) can be solved in favour of triglycerides because their concentration is higher than that of flavonoids, whereas in the case of rose hip pulp the elucidation of the contribution degree of polyunsaturated acid starch esters and lignin to the effect at 333°C remains unsolved and further research is still required.

## CONCLUSION

Different parts of *R. rubiginosa* plant (“*Rosa mosqueta*”) and the most well known product derived from it, rosehip seed oil, were characterized by vibrational spectroscopy and differential scanning calorimetry.

ATR-FTIR allowed to observe similarities in the absorption bands for the various components of the plant (particularly evident for seeds and oil)

and their correspondence with those of other plant species. By considering the absorbance ratios of the bands at 3005, 2923 and 1740  $\text{cm}^{-1}$ , assigned to lipids, the iodine index value for *R. rubiginosa* seed oil has been estimated to be close to 115  $\text{gI}_2/100\text{g}$ , an intermediate value for oils. Since this iodine value is within the limits fixed by EN 14111:2003 regulation, *R. rubiginosa* seeds can be deemed as suitable for biodiesel production.

From the point of view of thermal stability, both the seed oil (with a pyrolysis temperature of 462°C, higher than that of olive pomace oil or refined palm oil), the raw seeds and the rosehip pulp (which exhibit decomposition temperatures as high as 373°C and 333°C, respectively) also show a remarkable potential as biomass feedstock for biofuels. Further research on the reported raw materials (together with mill solid wastes) of *R. rubiginosa* is needed in order to explore their potential as a fuel crop, apart from their current use in skin care products.

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