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TECHNICAL REPORT

EFFECT OF POWDER HOUSE'S VITREOUS TRANSFORMATION PROCESS OF GRAPE POMACE ON THE DEGRADATION OF "BINDER" COMPOUNDS

Report prepared by the Polymer Physicochemistry Laboratory, Faculty of Science,
University of Chile for the company Biograpes Spa.

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TECHNICAL REPORT: EFFECT OF POWDER HOUSE'S VITREOUS TRANSFORMATION PROCESS OF GRAPE POMACE ON THE DEGRADATION OF "BINDER" COMPOUNDS

Objective

The objective of this report is to determine the possible effect of the Powder House's Vitreous Transformation Process on the degradation of "binder" compounds present in grape pomace.

Hypothesis

The degradation of "binder" compounds due to the Vitreous Transformation Process of grape pomace will allow obtaining a material with granular characteristics, necessary for its use in powdered form and as a food, cosmetic and nutraceutical ingredient.

Scientific basis

The Vitreous Transformation Process of grape pomace allows obtaining a vitreous biomaterial, however, during this process also occurs the degradation of binder compounds that would act as binding agents between the different components. Thus, the absence or decrease of these binding compounds would decrease the interfacial adhesion between the microparticles present in the vitreous biomaterial. Finally, the decrease of the interfacial adhesion between the microparticles will allow obtaining a material with granular characteristics that will facilitate subsequent milling processes.

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Experimental procedure

1. Infrared spectroscopy with Fourier transform and diffuse reflectance (ATR-FTIR)

Samples of grape pomace, vitreous biomaterial and a powder - obtained through the Powder House size reduction process of this vitreous biomaterial - were taken and deposited on the detector of the IRSpirit equipment, Shimadzu. Spectra were recorded by scanning from 400 to 4000 $cm^{(-1)}$ in order to observe the signals associated with the vibrational modes of certain functional groups.

2. Ultraviolet-visible spectroscopy (UV-vis)

A total of 6 50% m/v solutions of pomace, vitreous biomaterial and powder were prepared. The solutions were prepared in ethanol and water separately. The samples were left to soak for 3 hours and then filtered with 0.45 micron porosity membranes. Subsequently, 1 mL of the filtered sample was deposited in a quartz cuvette for measurements in a Shimadzu UV-1900 equipment.

3. Thermo-gravimetric Analysis (TGA)

Samples of approximately 10 mg were taken and deposited in the aluminum capsules of a TA Instruments model Q50 equipment. The thermogram was obtained by performing sweeps from room temperature to 800 °C at a rate of 10°C/min.

4. Water uptake (Water uptake)

The pomace and vitreous biomaterial samples were massaged and placed in a vial with approximately 10 mL of deionized water. Subsequently, they were removed from the vials and placed on filter paper for 1 minute in order to remove unbound moisture from the samples. Finally, the samples were massaged again to calculate the percentage of water uptake using equation 1 [1].

$$\text{Water uptake } (\%) = (W_{wet} - W_{o)} / W_o \times 100 \quad (1)$$

Where W_{wet} corresponds to the wet mass of the sample while W_o is the initial mass of the sample. The measurements were performed in triplicate.

5. Contact angle measurements

Pomace and vitreous biomaterial samples were taken, deposited and fixed on an object holder. The measurements were performed using a contact angle instrument (Drop Shape Analyzer DSA25S, KRUSS) controlled by ADVANCE software. The measurements were set to take 2 pictures per second over 20 seconds.

6. Determination of porosity percentage

The porosity was determined using the liquid displacement method (Salehi et al., 2015) [2] for this, 3 samples of pomace and vitreous biomaterial were taken, each of which was immersed for one hour in 8 mL of ethanol in a 10 mL test tube, after this time the increased volume change was measured, then the sample was carefully removed from the liquid in order to determine the decreased volume change. The porosity change was calculated from equation 2.

$$\varepsilon=(v_1\times v_3)/(v_2\times v_3)\times 100 \quad (2)$$

Where v_1 corresponds to the initial volume (8 mL), v_2 volume of liquid displaced by the sample and v_3 residual volume after sample removal.

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Results

1. Fourier transform infrared spectroscopy with attenuated reflectance (ATR-FTIR)

Figure 1 shows the ATR-FTIR spectra of the grape pomace (A), vitreous biomaterial (B) and powder (C) samples. In all three spectra it is possible to identify a signal at 2856 cm^{-1} for associated with the symmetric stretching of the methylene group ($-CH_{(2)}-$) present in the aliphatic fatty acid chain, while in spectra (B) and (C) a signal at 1748 cm^{-1} associated with the stretching of the carbonyl group (C=O) present in triglycerides is observed, on the other hand, an intense signal at 1050-1100 cm^{-1} is observed for the spectrum of the powder sample (C) associated with the C-O and C-C group present in sugars. [2] An intense band present in the pomace spectrum at 1600 cm^{-1} is also observed associated with the stretching of the carboxylate group (C=OO-) and the C=C double bond of aromatic groups present in pectins and phenolic compounds, but observed with less intensity in the vitreous biomaterial and powder spectra.

When comparing the pomace, vitreous biomaterial and powder spectra it is possible to observe that the band at 1600 $cm^{-1}$for the pomace spectrum is particularly intense in relation to the vitreous biomaterial and powder spectra, this result would suggest that the main component present in the pomace would be phenolic compounds which would be altered after the Vitreous Transformation Process.

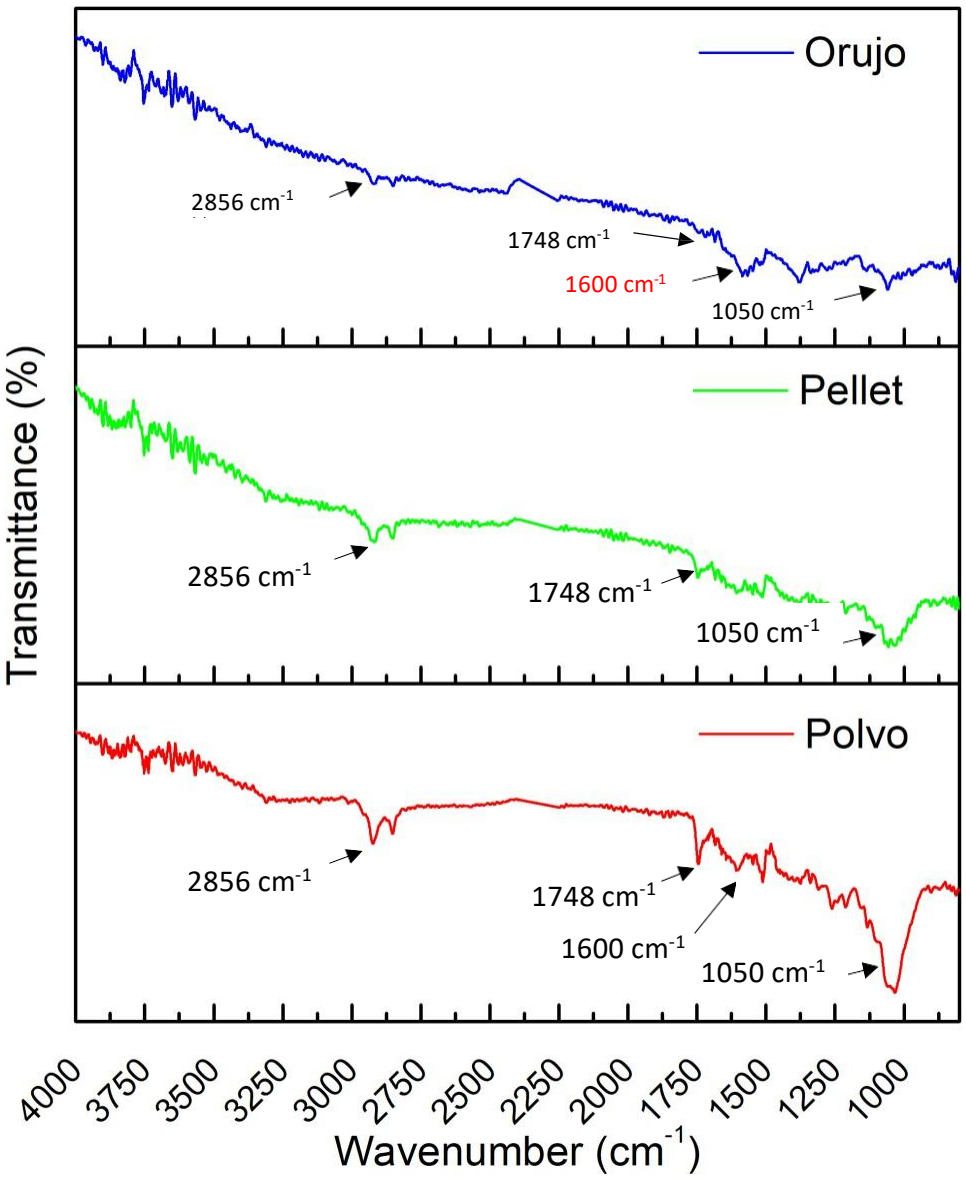

Figure 1. ATR-FTIR spectra of samples of (A) grape pomace, (B) vitreous biomaterial and (C) powder.

2. *Thermogravimetric analysis*

Grape pomace, vitreous biomaterial and powder samples were also analyzed thermogravimetrically. Figure 2 shows the mass loss curve as a function of temperature for the pomace sample, while Figures 3 and 4 show the mass loss for the vitreous biomaterial and powder sample. For all three samples a first mass loss (change of slope in green curve) is observed before 150°C, in the case of pomace the mass loss was 7% of the total sample, while for vitreous biomaterial and powder it was 5 and 3%, respectively. For both pomace and vitreous biomaterial, the mass loss corresponds to water (moisture) together with some components specific to the sample, while for the powder sample it would correspond mainly to moisture loss. This result can be confirmed from the curves of the first derivative (blue curve). In the case of the powder sample, the first mass loss occurs before 100 °C (water evaporation temperature), while for the pomace and vitreous biomaterial sample curves it occurs up to 150°C, this temperature being higher than the water evaporation temperature. According to Larrauri et al. there is a loss of polyphenols between 18.6 to 32.6% in red grape pomace samples when they are subjected to drying treatment between 100 to 140°C. [4] The mass loss associated with the degradation of polyphenolic compounds is consistent with the ATR-FTIR spectra whose results indicated a decrease in signals for vitreous biomaterial and powder samples associated with the presence of this type of compounds.

An additional observation is that when comparing the curve of the vitreous biomaterial sample with the powder sample, the low mass variation with temperature in the latter suggests the decomposition of certain components during grinding or pulverizing, therefore, the degradation of compounds by mechanical effects would also be possible and thus also the decrease of binding compounds.

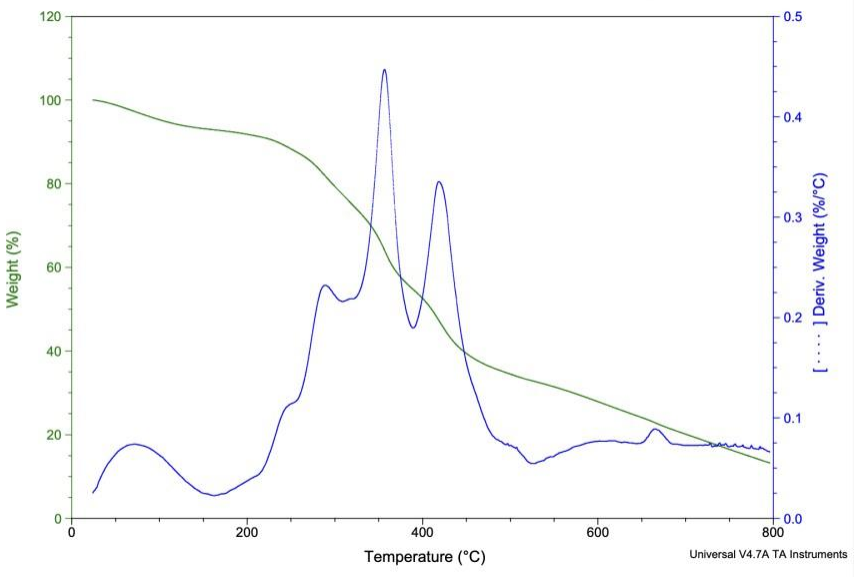

Figure 2. Thermogravimetry curve (TGA) for a grape pomace sample.

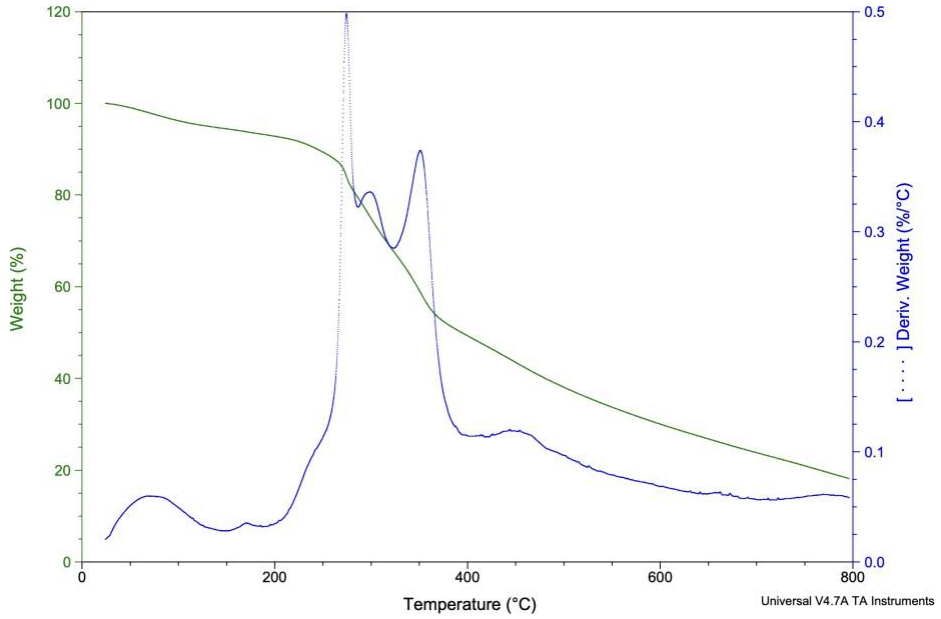

Figure 3. Thermogravimetry curve (TGA) for a vitreous biomaterial sample.

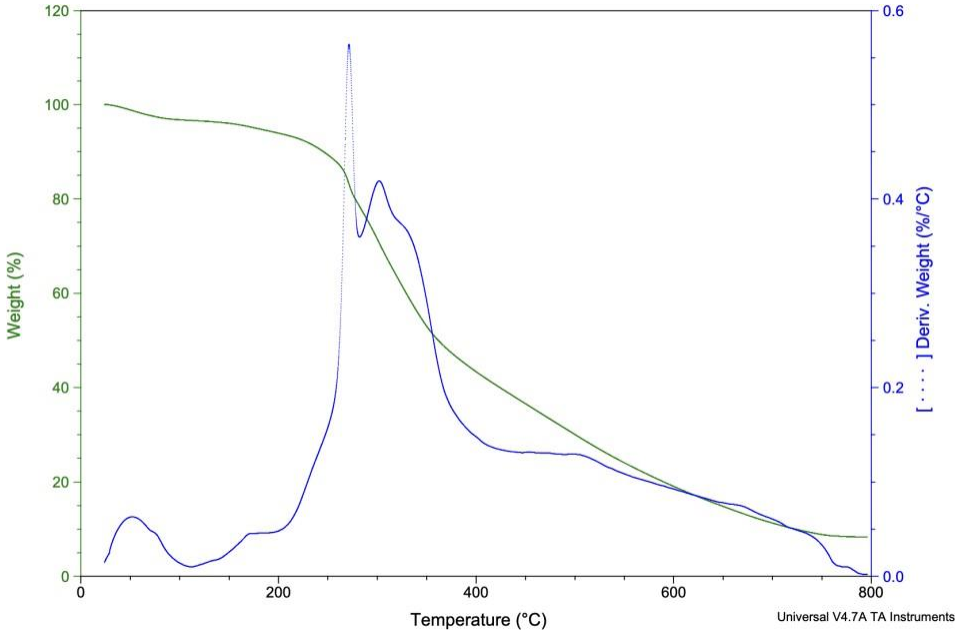

Figure 4. Thermogravimetry curve (TGA) for a **powder** sample.

3. UV-Vis spectroscopy

The pomace, vitreous biomaterial and powder samples were also analyzed by UV-vis spectroscopy. In this case, the samples were immersed in ethanol for 3 hours in order to extract the soluble compounds in this medium, and then the extracted components were analyzed by UV-vis spectroscopy.

Figure 5 shows the UV-vis spectra for the ethanol extraction products for the pomace, vitreous biomaterial and powder samples. In all three cases the presence of polyphenols is observed, with bands at 550 nm associated with the presence of anthocyanins [4] and bands at 370 nm associated with the presence of flavonoids. [5] The blue spectrum (powdered sample) presents a more intense band associated with the presence of anthocyanins, which is congruent with the larger exposed surface of this type of sample. This result is relevant in terms of food quality, considering that, although the Vitreous Transformation Process degrades a percentage of polyphenols, these are more available in the powdered product format. On the other hand, the intensity of the band associated with flavonoids is more intense for the spectrum of the pomace sample, which is consistent with the results of the ATR-FTIR spectra, which confirm the greater presence of certain types of polyphenols in the sample prior to the Vitreous Transformation Process.

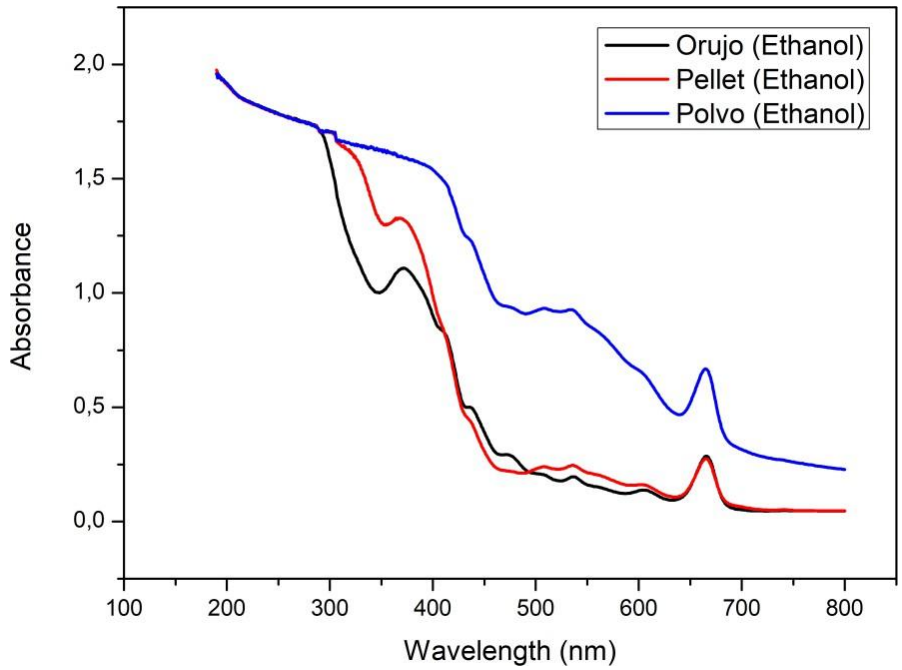

Figure 5. UV-vis spectra of extraction samples in pomace ethanol (black line), vitreous biomaterial (red line) and powder (blue line).

4. Contact angle and water absorption of pomace and vitreous biomaterial samples.

Figure 6 shows sessile droplet images obtained from the contact angle measurements, the images were taken every 4 s for a total time of 20 seconds, the time it takes for the vitreous biomaterial to completely absorb a water droplet of 8 uL volume. The sequence in Figure 6 indicates a higher water absorption capacity for the vitreous biomaterial sample than for the pomace, behavior that could be governed by changes in the composition of both samples as well as by the surface morphology-geometry. However, considering that the mass loss demonstrated by the thermal degradation curves is low (Figure 3), it is likely that the main feature promoting water absorption would be the porosity of the vitreous biomaterial over the changes in its composition. This result is confirmed by the water absorption curves over time for both samples (Figure 7) where the amount of water absorbed during 5 hours of immersion is similar except for the first point corresponding to the first hour of measurement, thus the water absorption is fast for the vitreous biomaterial sample during the first hour due to its porosity, but is similar during longer immersion times.

Based on these results, it is also possible to infer that the presence of fatty acids and/or triglycerides present in the grape pomace is not altered by the Vitreous Transformation Process, and therefore the unaltered presence of these components in both samples is a limiting step for higher water absorption. This is understandable in the knowledge that the presence of apolar components such as fatty acids and/or triglycerides decrease the ability of the materials to absorb water.

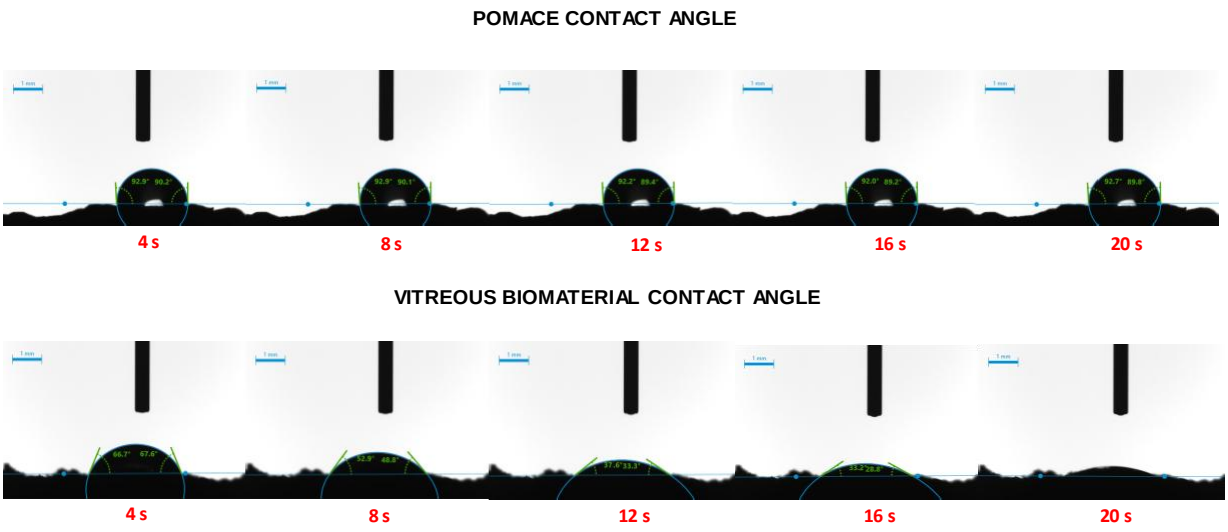

Figure 6. Sessile drop of contact angle measurements for grape pomace and vitreous biomaterial samples.

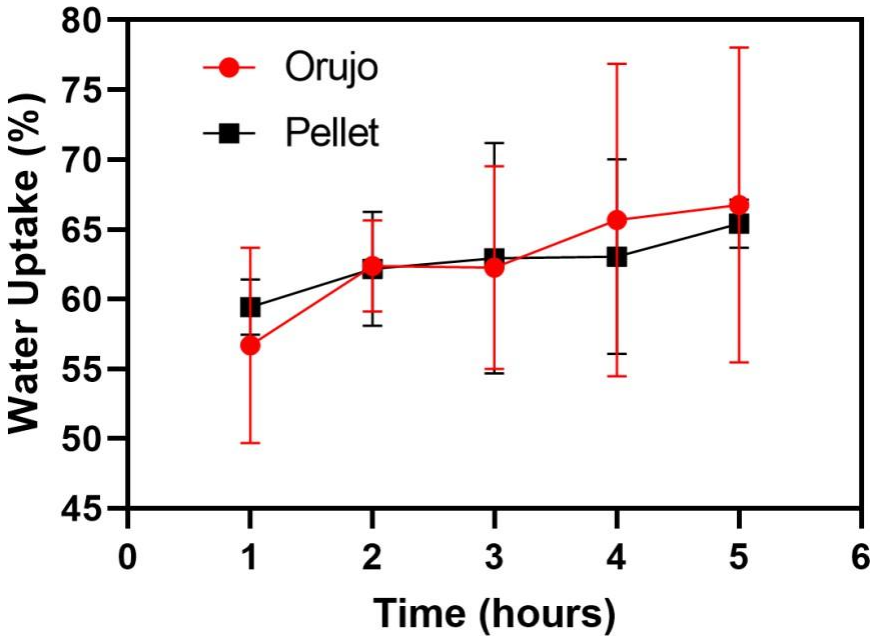

Figure 7. Water absorption profiles for grape pomace (red profile) and vitreous biomaterial (black profile) samples.

5. Porosity measurement

The porosity of the pomace and vitreous biomaterial samples were determined from liquid displacement measurements. According to the formula indicated in the materials and methods section the porosity value for the pomace was 28.9±3.4% while for the vitreous biomaterial the porosity was 39.8±3.3%. These results indicate that for pomace 28.9% of the geometric volume is occupied by pores or spaces used by air, while in the vitreous biomaterial 39.8 of the geometric volume of a vitreous biomaterial is used by pores or spaces with air.

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Conclusions

From the results obtained it is possible to conclude that the Vitreous Transformation Process to which the pomace is subjected could degrade mainly polyphenolic compounds such as tannins, anthocyanins and flavonoids, among others. This is based mainly on the results of ATR-FTIR spectroscopy and thermal degradation by TGA.

Regarding the binding role of these components, it is important to highlight that the presence of -hydroxyl, -carboxylic acid and aromatic groups present in polyphenols allow hydrogen bridge, dipole-dipole, π-π interactions and cation-π interactions. Within the group of intermolecular interactions, the aforementioned interactions are the ones that present the greatest strength and therefore the greatest binding effect.

On the other hand, according to the contact angle, water absorption and ATR-FTIR spectroscopy analyses, the fact of a possible degradation of fatty acids and/or triglycerides is ruled out, firstly because the infrared spectra did not reveal compositional changes in relation to that type of compounds, and secondly because no significant changes in water absorption capacity were observed at longer immersion times, but only during the first hour of immersion due to the porous characteristic of the vitreous biomaterial.

Finally, the binding role of polyphenols has been previously demonstrated by Ping et al. who have reported the use of grape pomace biomass as an adhesive component in wood, where the adhesive role of pomace has been specifically associated to the presence of polyphenols as tannins. [6]

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