

PARTICLE CHARACTERIZATION REPORT

Characteristics of the sample delivered:

Samples code:

M1: skin and seeds (grape pomace)

M2: vitreous biomaterial obtained from M1

M3: ultrafine powder obtained from M2

Sample preparation:

The sample was deposited on a sample holder coated with conductive carbon tape.

Acquisition of micrographs

Image acquisition was performed on a High Resolution Scanning Electron Microscopy (FE-SEM), INSPECT-F50, FEI. The SE secondary electron detector (EDT) was used, with an electron acceleration of 2 and 5 kV allowing the topography of the sample to be observed.

Characterization:

1. Description of morphology and topography.

According to the micrographs obtained for each sample, it is possible to observe that in figure 1, left column (A and B, fig.1) the topography of the M1 which is the grape skin of the pomace (according to user's indications), in the middle column (C and D, fig.1), the vitreous biomaterial obtained after grape pomace treatment of sample 1 (according to user's indications) is observed, finally in figure 1 the topography of sample 3 can be seen in the right column (E and F fig. 1) which is the powder obtained, after milling the vitreous biomaterial (according to user's indications). When comparing the topography of the 3 samples (figure 1) it is possible to appreciate that at low magnifications it has a smooth surface with spheroid forms on the surface (figure 1A), which changes when passing to the vitreous biomaterial where a rough surface can be observed (figure 1C), which seems to be compacted with respect to figure 1A. This topographic difference is maintained in the higher magnification micrographs (Figure 1 B and D) where the change in roughness is evident. It is important to note that in sample 1, grape seeds were also found (figure 2), which are no longer found in sample 2 and sample 3. Finally, in the left column corresponding to sample 3 (figure 1, E and F), in which we observe the particles obtained from milling the vitreous biomaterial (sample 2), the following can be seen

amorphous particles that maintain the rough topography of sample 2, being conglomerate particles. This shows that the procedure used to process the pomace has made it possible to obtain particles with a micrometric size (see point 2).

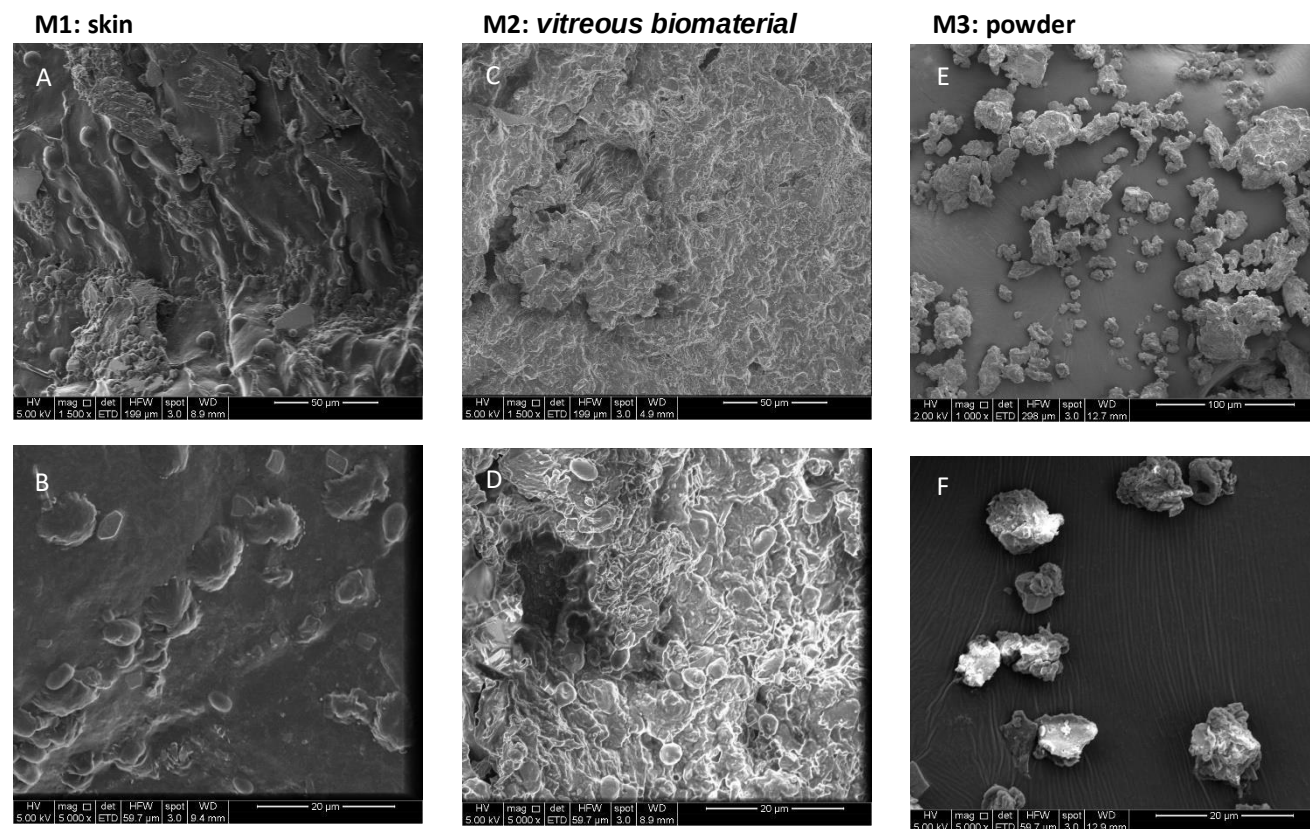


Figure 1. Representative transmission electron micrographs of sample M1, M2 and M3, obtained by FE-SEM with the secondary electron detector. Representative images of samples 1, 2 and 3. **A, B:** Left panel, M1 corresponds to grape skin; **C, D:** Center panel, M2 corresponds to the vitreous biomaterial obtained from M1; **E, F:** Right panel, M3 corresponds to the powder obtained from M2. Bars equal 20, 50 and 100 µm scale in respective micrographs.

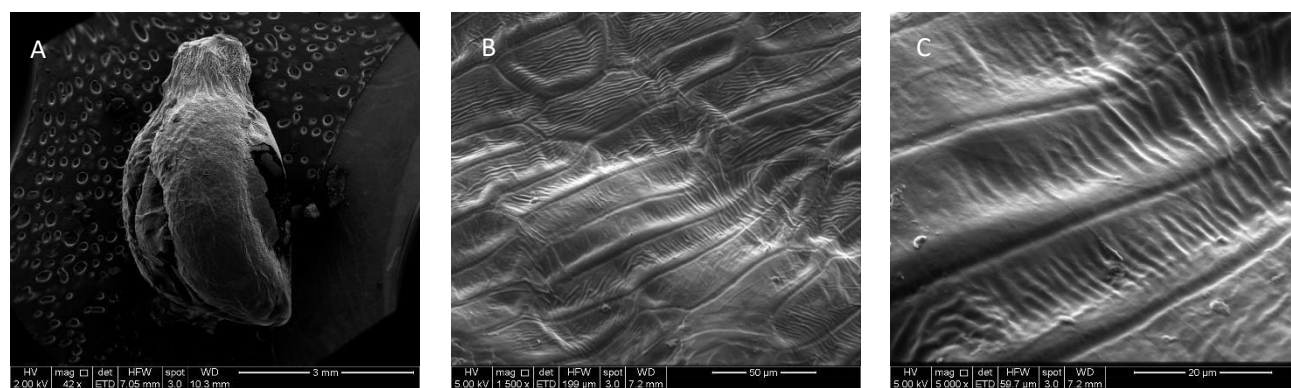


Figure 2. Representative transmission electron micrographs of M1, specifically a grape seed. It was acquired by FE-SEM with the secondary electron detector. Bar equals 3 mm, 50 µm and 20 µm scale (A, B and C) respectively.

2. Scanning Electron Microscopy size distribution

In order to determine the size of the particles observed in sample 3 (Figure 1 E, F and Figure 3), about 273 particles per sample of 5 micrographs from different areas of the sample were measured by scanning electron microscopy. With this information, a statistical analysis was performed, with a histogram of size distribution (Figure 4).

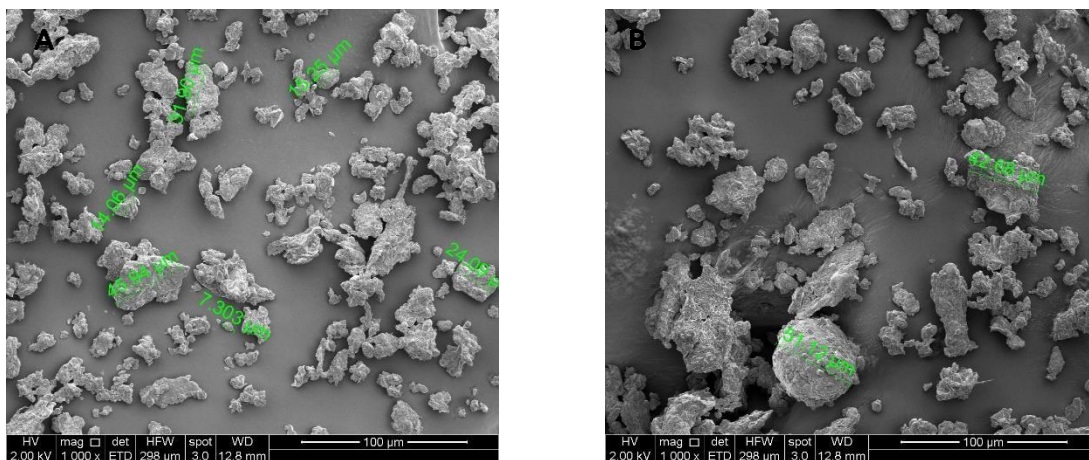


Figure 3. Representative transmission electron micrographs of sample M3 (A and B), obtained by FE-SEM with the secondary electron detector. The green lines indicate the size of some of the measured particles. Bar equals 100 μm scale.

For the size distribution analysis 273 particles were measured. The particles have amorphous type morphology (Figure 3). The distribution fits a Gaussian curve, the best fit being the 4-parameter curve with $r^2=0.9261$. The *peak* is $10 \pm 0.4 \mu\text{m}$ (Figure 4). From the statistical analysis, it can be confirmed with 95 % confidence that 50 % of the particles have a size between **8 - 19 μm** (Table 1) and that 91 % of the measured particles do not exceed 30 μm .

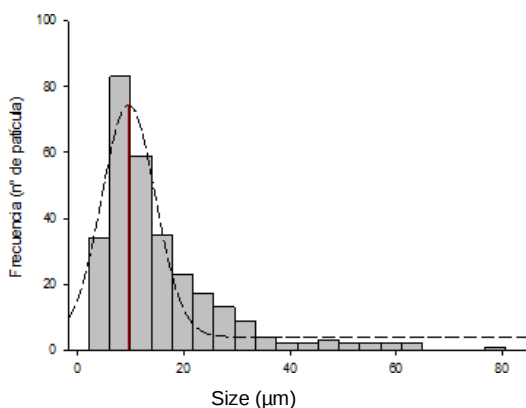


Figure 4. Histogram of size distribution of 273 particles. Segmented black line describes the best fit. Red line indicates the *peak* of the curve fit.

Size (μm)	Number of particles	% of particles
5-10	97	36
10-20	110	40
20-30	40	15
30-40	11	4
40-50	6	2
50-100	9	3
Total	273	100

Table 1. Particle diameter range and distribution percentages. The size range with the highest percentage of the population is marked in red.

3. Summary of results

From the observation of the topography by electron microscopy of the samples, the structural change after the treatment of sample 1 to obtain a vitreous biomaterial (sample 2) is appreciated, observing an increase in the roughness of sample 2 with respect to sample 1. In addition, the data obtained indicate that after treatment of sample 2, amorphous particles can be obtained and that 91 % of the particles measured do not exceed 30 μm (sample 3).

The images and data files used for the report are attached.

ImageJ version 1.5 was used to analyze the images and record particle sizes. For graphing and statistical analysis, SigmaPlot version 12 was used.



Dr. Ana Luisa Riveros Salvatierra

UICMA

Materials Research and Characterization Unit

Materials Research and Characterization Unit

Faculty of Chemical and Pharmaceutical Sciences

University of Chile servicios.hrsem@ciq.uchile.cl

Phone: 229782918-229782904