SUPPLEMENTRAY MATERIAL

Short communication: Investigating the effect of saffron (*Crocus sativus L.*) nano-sizing on its colour extraction efficiency: A preliminary study

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Abstract: This study investigated the effect of saffron nano-sizing on its the color extraction yield. The whole stigma was ball-milled at three different times (10, 20 and 100 h), immediately or with a 24-h delay was submitted to absorption test, and then the color extraction efficiency was determined. When stigma was milled for 100 h, its particle size was reduced to less than 20 nm, as shown by SEM and TEM images, and its extraction efficiency was considerably increased by 19.8% as compared with the stigma blended for 10 min. However with a 24-h delay between the end of milling and absorption test, the yield of color extraction significantly decreased. The recommended milling conditions resulting in extraction efficiency of 16.2% (in comparison with stigma blended for 10 min) were determined to be the milling for 10 h with initial tendering prior to milling operation.

Keywords: Saffron, Color extraction, Ball milling, SEM, TEM

Experimental

Sample preparation

The saffron stigma (*C. sativus L.*) collected from Ghaen, located in Khorasan province, Northeast of Iran, was purchased from Bahraman Co. (whole stigma) in an air-tight package (9.2% moisture).

Ball milling of saffron stigma

Stigma samples were ball-milled using a ball mill (Fritsch, Germany). Before milling the stigmas, a tendering process was performed on samples using liquid nitrogen. The experimental treatments applied in current experiment are given in Table 1.

Scanning electron microscopy (SEM) and transmittance electron microscopy (TEM)

The range of particle size of stigma prior to and after milling was determined by SEM and TEM. Due to the non-conductivity of stigmas particles after ball milling, the powders were sputter-coated by a thin layer of gold using a sputter coater (Fisons Instruments, UK). SEM images were taken at magnifications of 100–1000 times at 20 kV using a scanning electron microscope (Philips XI-30) at the Department of Material Science and Engineering, Isfahan University of Technology. The TEM images were obtained at Department of Material Science and Engineering, Ferdowsi University of Mashhad (Iran).

Coloring intensity measurement

The coloring intensity of saffron stigma was measured by UV-Vis spectrometry at 440 nm, according to ISO/TS 3632–2 (2003).

Reference

ISO. Technical specification. Saffron (Crocus sativus L.) Part 1. (Specification). Part 2. (Test methods). Geneva, Switzerland: International Organization of Standardization; 2003.

Table S1. The various treatment conditions of saffron stigma on its color extraction and stability

Milling duration	Initial tendering	Time point for absorption test	Absorption at 440 nm
Stigma blended for 15 min	No	–	201.5
Stigma milled for 10 h	No	Immediately after milling	227.6
Stigma milled for 10 h	No	24 h after milling	216.3
Stigma milled for 10 h	Yes	Immediately after milling	234.2
Stigma milled for 10 h	Yes	24 h after milling	221.6
Stigma milled for 20 h	Yes	Immediately after milling	238.4
Stigma milled for 20 h	Yes	24 h after milling	216.8
Stigma milled for 100 h	Yes	Immediately after milling	241.4
Stigma milled for 100 h	Yes	24 h after milling	196.2

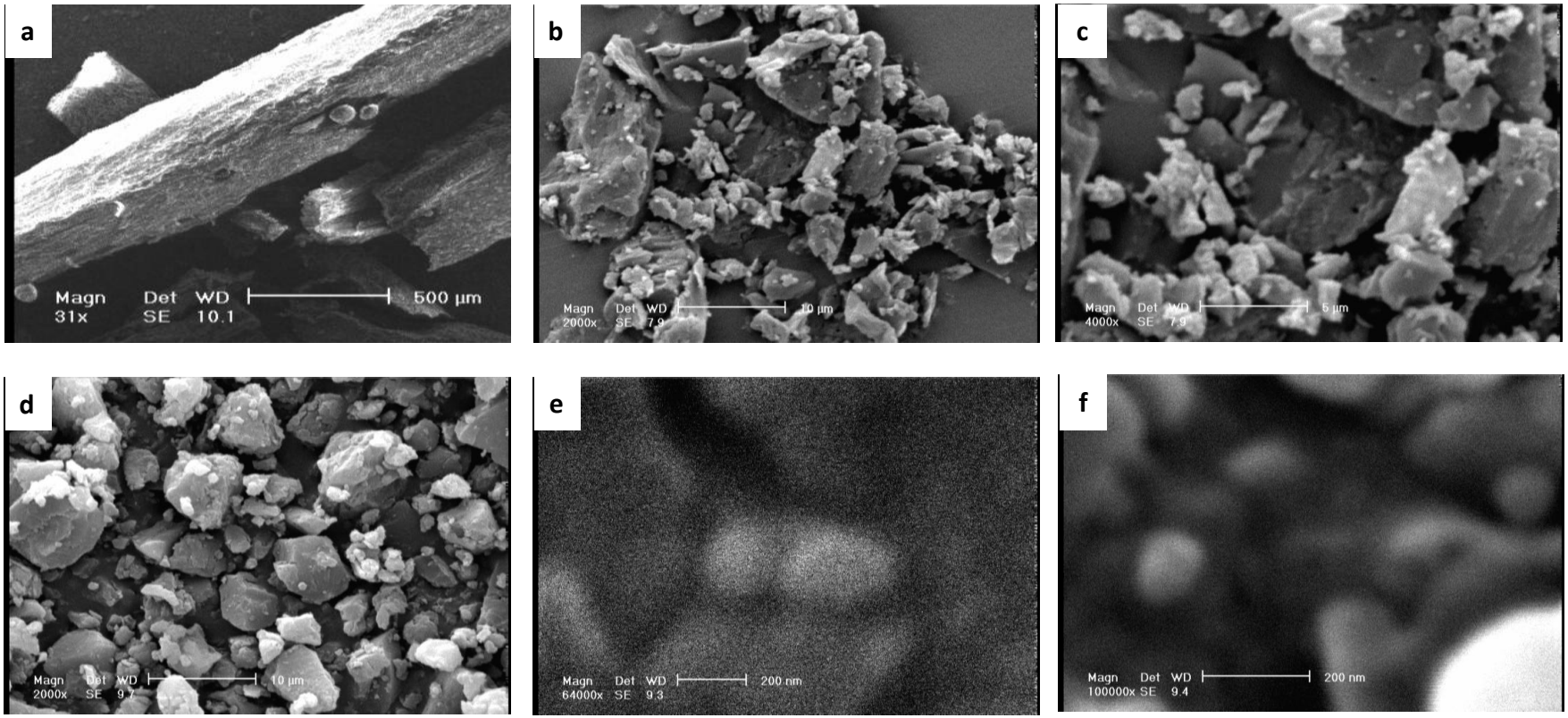

Figure S1. SEM images of a) whole (control) stigma; b and c) stigma milled for 10 h, and d–f) stigma with initial tendering milled for 10 h.

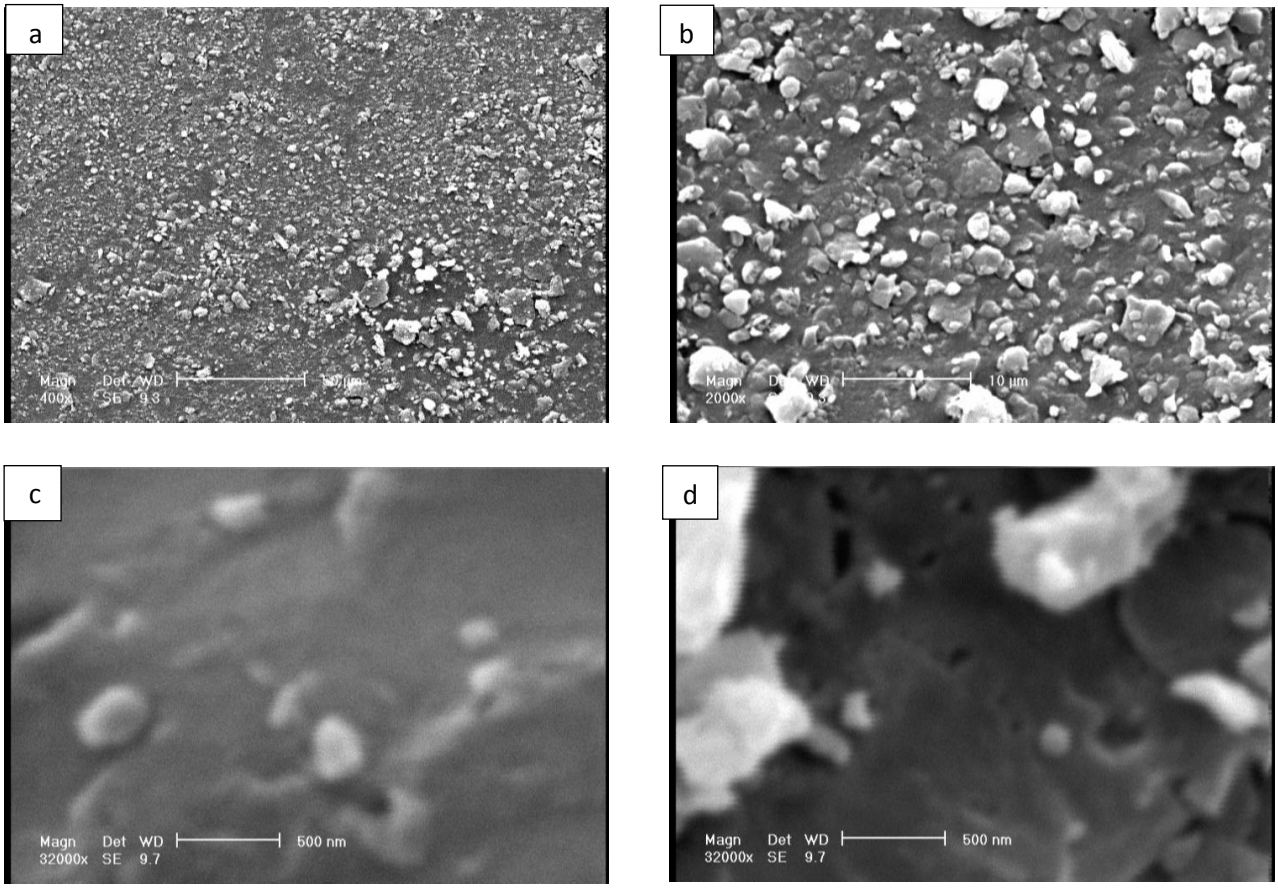

Figure S2. SEM images of stigma milled for 20 h with initial tendering (a–d).

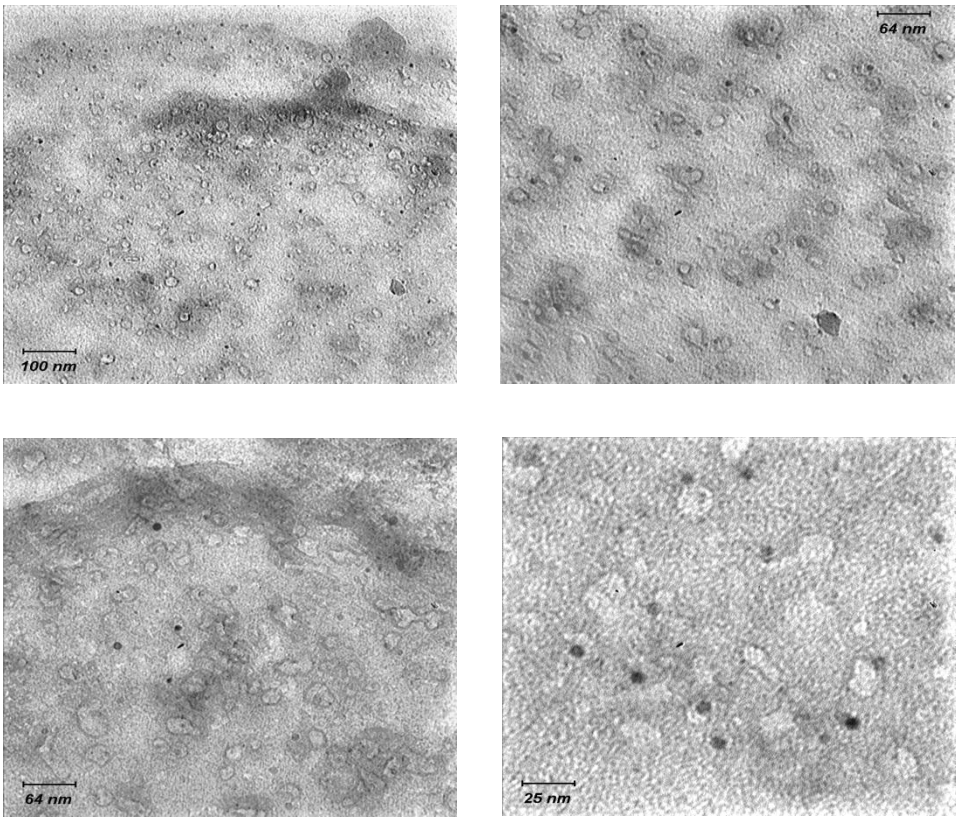

Figure S3. TEM images of stigma milled for 100 h showing the particle size of typically below 20 nm

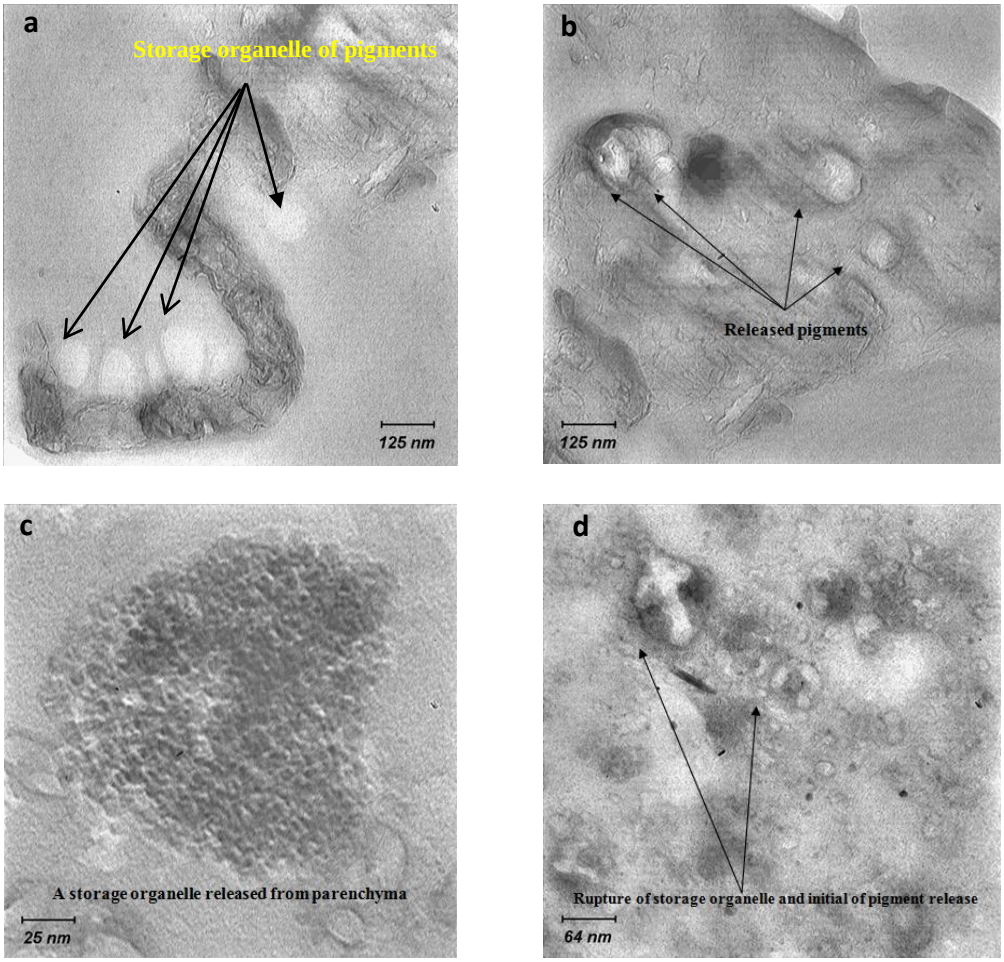

Figure S4. TEM images showing the rupture of parenchyma cell walls and release of storage organelles