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Effect of crushing temperature on virgin olive oil quality and composition

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SUMMARY: The objective of the current study was to assess the influence of a modified crushing process and kneading operation on the quality parameters, volatile compounds, and the fatty acid and sterol profiles of virgin olive oil from the Edremit yaglik variety. In the study, olive oil samples were produced in two different processes. The first one was produced without malaxation and the second one was produced with the malaxing process. During crushing, the effect of different temperatures was tested. The results demonstrate that different crushing temperatures generally did not affect the amount of free fatty acids, or peroxide value. Total phenol contents were positively affected by the additional malaxation process. Fatty acids and sterol composition were not significantly altered at different crushing temperatures or during the subsequent malaxation application. PCA enabled a clear classification of the oils obtained from different processing techniques.

KEYWORDS*: Crushing; Fatty acid; Malaxation; Quality; Sterol; Volatile*

RESUMEN: ***Efecto de la temperatura de molturación sobre la composición y calidad del aceite de oliva virgen.*** El objetivo del presente estudio fue evaluar la influencia de un proceso de molturación modificado y de la etapa de batido sobre los parámetros de calidad, los componentes volátiles, los ácidos grasos y el perfil de esteroles de aceites de oliva virgen de la variedad Edremit yaglik. En este estudio, las muestras de aceite de oliva se obtuvieron mediante dos procesos diferentes con y sin malaxación. Durante la trituración, se probó el efecto de diferentes temperaturas. Los resultados demostraron que las diferentes temperaturas de trituración, en general, no mostraron tener efecto sobre la cantidad de ácidos grasos libres o el índice de peróxido. El contenido total de fenoles se vio afectado positivamente por el empleo de malaxación. La composición de ácidos grasos y de esteroles no se vieron afectados significativamente por el aumento de la temperatura en la trituración ni durante el proceso de malaxación. PCA permitió una clasificación clara de los aceites obtenidos mediante las diferentes técnicas de procesamiento.

PALABRAS CLAVE: *Ácidos grasos; Calidad; Esteroles; Malaxación; Molturación; Volátiles*

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1. INTRODUCTION

Virgin olive oil is produced from olive fruits through mechanical extraction and has a fundamental importance both for the food industry and human diet (Veillet *et al*., 2009). Since virgin olive oil is extracted from olive fruit only by means of physical extraction methods which comprise crushing the olives, malaxation of olive paste and separation of the resulting oily phase, the end product contains a number of antioxidant compounds which prevent lipids from autoxidation, as well as volatile compounds which affect the flavor of olive oil (Veillet *et al*., 2009). In addition, owing to the consumption of virgin olive oil without refining, it maintains its sensory and nutritional characteristics and has more resistance to oxidative degradation (Aparicio *et al*., 1999; Lercker *et al*., 1994). Because the efficiency of the extraction process is directly related with the rheological properties of olive paste and with the operation parameters used (Giovacchino *et al*., 2002; Fiori *et al*., 2014), many studies have been carried out in order to identify the most appropriate operating conditions which affect the quality as well as the product yield of olive oil. Malaxation and crushing are two crucial processes of virgin olive oil production (Servili *et al*., 1993) and if proper time and temperatures are applied, the extraction can be implemented effectively (Gallina-Toschi *et al*., 2005). During crushing, the olives are ground in order to liberate the oily fraction; this is followed by a malaxation step, during which oil/water emulsion is broken down to form larger droplets and consequently to enhance the amount of extracted oil (Gallina-Toschi *et al*., 2005). In many reports, the effect of malaxation conditions such as time, temperature, oxygen concentration of the malaxer atmosphere (Amirante *et al*., 2008a; Amirante *et al*., 2008b), on the composition and general quality as well as the product yield of olive oil were investigated. Yet, the influence of crushing is less considered compared to malaxation, although it is also a fundamental step of olive oil production, during which small oil droplets are formed to be separated more easily in the following malaxation step by means of various types of crushers (Angerosa *et al*., 2001). There are many studies related to crushing which focused on comparing the effects of different crushers, such as stone mills and metallic crushers which involve disks, hammers and blades, on the composition and overall quality of olive oil (Caponio *et al*., 1999; Koutsaftakis *et al*., 2000; Preziuso *et al*., 2010; Inarejos-García *et al*., 2011; Leone *et al*., 2016; Fregapane and Salvador, 2017). There are also a number of studies in the literature focusing on the effects of temperature changes during crushing. For instance, Caponio and Catalano, (2001) investigated the effects of different crushers and temperature on quality characteristics (free fatty acids, peroxide value, K_{232} and K_{270}, total phenol and chlorophyll contents) and the preservation period of virgin olive oil. Caponio and Gomes, (2001) studied the effects of crushing temperature on the diffusion of phenolic compounds in olive oil. Caponio *et al*., (2002) evaluated the influence of crushing temperature by analyzing the polar compounds in virgin olive oil. Caponio *et al*., (2003) determined the impact of crushing temperature on degree of oxidative degradation, along with the polar compounds and quality parameters of virgin olive oil. However there is no information available about the effects of crushing temperature on the fatty acid, or the sterol and volatile compositions of the resulting olive oil.

In this sense, the objective of this study was to investigate the effect of different temperatures separately during crushing and the effect of the following malaxation process on the overall quality and composition of virgin olive oil obtained from the Edremit yaglik variety, which is qualified as one of the most outstanding cultivated plants grown in the Aegean Region of Turkey. Since the development of novel processes is fundamental in order to fulfill the requirements to produce a high-quality final product, in the current study a new cooler was designed to determine the effect of the cooling process during crushing on the quality and compositional characteristics of virgin olive oil. Additionally, after crushing, the effect of the malaxation operation on the aforementioned parameters was also assessed.

2. MATERIALS AND METHODS

2.1. Reagents and standards

Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), hexane, methanol, toluen, chloroform, acetic acid, acetone, hydrogen peroxide, potassium iodine, pyridine, sodium thiosulphate, sodium carbonate, β-sitosterol and Folin-Ciocalteu reagent were purchased from Merck (Germany); gallic acid, guaiacol, 2,2-dipyridine and catechol were supplied from Sigma-Aldrich; 37 fatty acid methyl ester mix and N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMSC) were purchased from Supelco (Sigma-Aldrich Corporation).

2.2. Virgin olive oil extraction

Olive fruits (*Olea Europaea* L.) of the Edremit yaglik variety were harvested from the Edremit district (Balıkesir/Turkey) in December 2015. A representative 2 kg sample of healthy olive drupes

were processed to oil using a laboratory scale mill (HAUS, Turkey) equipped with a disc crusher, a vertical malaxer and a hydraulic press. After leaf removal and washing, the olives were crushed at different temperatures (13 °C, 18 °C, 24 °C, 30 °C) using a temperature-control system. The system consisted of a basic refrigeration system including a compressor, an expansion valve, an evaporator, a condenser and tubes. The sieve of the crusher was surrounded by the tubes, which contained the refrigerant R600. The refrigeration system was built based on the change of state. The refrigerant in vapor form was compressed in a condenser within the system and thus evaporation temperature was increased. Consequently, the refrigerant released its heat and turned to liquid. Later, the liquid refrigerant reached the evaporator by passing through the expansion valve which was responsible for pressure formation. In the evaporator the liquid refrigerant was absorbed by the compressor. Intrinsically the pressure of the evaporator was too low and therefore, the evaporation temperature was decreased and as a result, the refrigerant turned into vapor form by absorbing heat from the external environment. The compressor allowed the system to work continuously at a set value throughout the process. During the operation, the temperatures of the crusher and olive paste were controlled simultaneously.

After crushing, each batch was divided into two homogeneous portions. The first batch of olive paste was pressed by a laboratory scale discontinuous hydraulic press. The crushed paste was put in the three bags and metal discs were placed between them. The second batch was malaxed at 27 °C for 40 minutes. The resulting malaxed paste was pressed with the same hydraulic press under the same conditions. The oily liquid phase was decanted and the virgin olive oil samples were obtained. Briefly, the olives were processed in eight different ways: i) crushed at 13 °C and pressed (CO 13 °C), ii) crushed at 18 °C and pressed (CO 18 °C), iii) crushed at 24 °C and pressed (CO 24 °C), iv) crushed at 30 °C and pressed (CO 30 °C), v) crushed at 13 °C, malaxed and pressed (CMO 13 °C), vi) crushed at 18 °C, malaxed and pressed (CMO 18 °C), vii) crushed at 24 °C, malaxed and pressed (CMO 24 °C), viii) crushed at 30 °C, malaxed and pressed (CMO 30 °C). Each process was duplicated. Virgin olive oil samples of 50 mL were kept in dark brown glass bottles at 4 °C under nitrogen atmosphere until analyzed. All analyses were performed twice.

2.3. Chemical analysis

Free fatty acids, peroxide value, UV spectrophotometric indices (K_{232} and K_{270}). Free fatty acids, peroxide value, K_{232} and K_{270} values were identified according to AOCS Official Methods Ca 5a-40, Cd 8–53, Ch 5–91 (AOCS 2003), respectively.

Chlorophyll and carotenoid contents. The concentration of carotenoids and chlorophylls were determined in reference to the method of Ceballos *et al*., (2003). The absorbances were measured spectrophotometrically at 470 and 670 nm and calculated using the formulas given below:

$$\text{Chlorophylls} = (A_{670} \times 10^6)/ (613 \times 100 \times d) \text{ (mg/kg)}$$

$$\text{Carotenoids} = (A_{470} \times 10^6)/ (2000 \times 100 \times d) \text{ (mg/kg)}$$

Where, A corresponds to absorbance value and d is the cuvette thickness.

Sterol composition. The analysis of sterols was performed using the official method of AOCS Ch 6–91 (2003). The sterol fractions were silanized and subsequently analyzed with a gas chromatograph apparatus (GC 2010, Shimadzu, Japan) equipped with flame ionization detector. The carrier gas was nitrogen at a flow rate of 0.8 mL/min and the split ratio was 50:1. The column used for the chromatographic separation was a HP-5 fused silica capillary column (30 m, 0.25 mm i.d. and 0.25 mm film thickness) (Chrom Tech., Apple Walley, MN, USA). The temperatures of the column, injector and detector were 260 °C, 280 °C and 290 °C, respectively.

Total phenol content. The phenolic compounds were extracted from the olive oils according to the procedure described by Murkovic *et al*., (2004), with some modifications. 2.5 grams of virgin olive oil were extracted with 2.5 mL of methanol in reaction tubes and vigorously shaken. The tubes were then centrifuged at 6000 rpm for 5 minutes. The upper methanolic supernatant was used to determine the concentration of phenolic compounds by measuring the absorbances at 765 nm using Folin-Ciocalteu reagent (Gutfinger, 1981), and a spectrophotometer. Total amount of phenolic compounds was expressed as gallic acid equivalents.

Volatile compound analysis. Solid phase microextraction-gas chromatography mass spectrometry (SPME-GCMS) was used to identify the composition of volatile compounds. The procedure was as follows: (i) weighing 3 g of virgin olive oil into a 20 mL headspace vial and enclosing it with poly (tetrafluoroethylene) (PTFE)/silicone septum; (ii) stabilization of vials at 40 °C for 10 minutes; (iii) extraction of headspace of olive oil samples at 40 °C for 40 minutes by a manual SPME device and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) solid-phase microextraction (SPME) fiber; (iv) desorption of volatile compounds by inserting the fiber for 10 minutes into the injection port of the gas chromatograph. The chromatographic elution was

performed in splitless mode and the inlet temperature was 250 °C. The gas chromatograph used for the analysis was Agilent 7820 (Agilent Technologies, Santa Clara, CA, USA) and it was connected to a mass selective detector (Agilent 5975 model, Agilent Technologies, USA). The column used for separation was HP-5MS column (30 m, 0.25 mm i.d. and 0.25 mm film thickness) (Chrom Tech., Apple Walley, MN, USA) and the temperature program was as follows: the samples were kept for 5 min at 40 °C, 3 °C/min up to 110 °C, 4 °C/min up to 150 °C, 10 °C/min up to 210 °C for 12 minutes. The carrier gas was helium with a flow rate of 1.5 ml/min. The temperatures of MS source and MS quadrupole were adjusted to 230 °C and 150 °C, respectively. The volatile compounds were determined by comparing their mass spectra in reference to the library of Wiley.

Fatty acid composition. Fatty acids were initially converted to their methyl esters in reference to IUPAC (1987). The analysis were performed using a gas chromatography instrument GC 2010 (Shimadzu, Kyoto, Japan) with a DB-23 fused silica capillary column (60 m x 0.25 mm i.d. and 0.25 μm film thickness) (J&W Scientific). The carrier gas was nitrogen at a 1.0 mL/min flow rate and the split ratio was 80:1. The temperatures of the column, injector and detector were 195 °C, 230 °C and 240 °C, respectively. The detector used during the analysis was a flame ionization detector. The results were presented as percentage of total fatty acids.

2.4. Statistical analysis

The results reported in Tables 1–5, are the mean values of 4 analytical determinations. The analytical results were evaluated statistically using the SPSS 15 statistical software (SPSS Inc., Chicago, USA). The data were expressed by one-way ANOVA. The results were subjected to Duncan's test to determine any significant differences among the analyzed parameters. The level of significance was set at $p < 0.05$. The data were also evaluated by the principal component analysis (PCA) and hierarchical cluster analysis (HCA) using XLSTAT 2016 version (Addinsoft, New York, NY).

3. RESULTS AND DISCUSSION

The quality parameters of virgin olive oils obtained from pastes crushed at different temperatures are given in Table 1. The free fatty acid values of both CO and CMO were found to be lowest at a crushing temperature of 30 °C. Peroxide values, a sign of primary oxidation products, remained statistically unchanged. Ultraviolet spectrophotometric indices at 232 nm, indicators for the conjugation of unsaturated fatty acids, were determined to decrease for CO, while no change was observed for CMO. K_{270} values, which are markers for secondary oxidation products, were found to be below the legal limits established for extra virgin olive oil and did not have significant responses to increasing temperatures. The total phenolic contents of

TABLE 1. Quality parameters of virgin olive oils obtained from pastes crushed at different temperatures

		13 °C	18 °C	24 °C	30 °C
Free fatty acids (%)	CO	0.71 ± 0.01^{c}	0.62 ± 0.02^{b}	0.71 ± 0.00^{c}	0.52 ± 0.01^{a}
	CMO	0.70 ± 0.07^{b}	0.66 ± 0.01^{b}	0.72 ± 0.04^{b}	0.48 ± 0.04^{a}
Peroxide value (meq O_2/kg oil)	CO	5.30 ± 1.29^{a}	4.41 ± 1.32^{a}	4.00 ± 0.70^{a}	4.27 ± 0.27^{a}
	CMO	6.42 ± 2.38^{a}	7.17 ± 1.25^{a}	4.34 ± 0.16^{a}	5.14 ± 1.00^{a}
K_{232}	CO	1.53 ± 0.04^{c}	1.44 ± 0.02^{bc}	1.41 ± 0.05^{ab}	$1.34 \pm 0.02^{a,*}$
	CMO	1.61 ± 0.03^{a}	1.40 ± 0.35^{a}	1.50 ± 0.07^{a}	1.61 ± 0.09^{a}
K_{270}	CO	$0.15 \pm 0.00^{a,*}$	$0.15 \pm 0.00^{a,*}$	$0.15 \pm 0.01^{a,*}$	$0.14 \pm 0.00^{a,*}$
	CMO	0.18 ± 0.00^{a}	0.18 ± 0.00^{a}	0.18 ± 0.01^{a}	0.18 ± 0.00^{a}
Total phenol contents (mg/kg)	CO	$121.0 \pm 4.20^{a,*}$	$206.4 \pm 41.1^{b,*}$	250.0 ± 18.3^{b}	$150.7 \pm 39.9^{a,*}$
	CMO	252.6 ± 13.7^{a}	266.0 ± 23.9^{a}	234.0 ± 17.6^{a}	250.6 ± 23.5^{a}
Carotenoids (mg/kg)	CO	9.93 ± 0.85^{b}	9.00 ± 0.42^{a}	10.3 ± 0.47^{b}	8.87 ± 0.15^{a}
	CMO	9.98 ± 0.18^{b}	8.87 ± 0.26^{a}	10.4 ± 0.39^{c}	9.57 ± 0.20^{b}
Chlorophyll (mg/kg)	CO	15.2 ± 2.50^{b}	$12.4 \pm 1.20^{a,*}$	16.4 ± 1.26^{c}	13.22 ± 0.39^{b}
	CMO	11.9 ± 0.86^{b}	10.1 ± 0.61^{a}	14.6 ± 0.54^{d}	13.14 ± 0.37^{c}

CO: Virgin olive oil obtained from crushed paste, CMO: Virgin olive oil obtained from crushed-malaxed paste, Different superscript letters in the same line indicate significant differences ($p < 0.05$) among different crushing temperatures (a-d); and between crushing and crushing + malaxation applications (*)

TABLE 2. Fatty acid composition of virgin olive oils obtained from pastes crushed at different temperatures (%)

		13 °C	18 °C	24 °C	30 °C
C 16:0	CO	12.3 ± 0.10[a]	11.7 ± 0.67[a]	12.2 ± 0.72[a]	12.6 ± 0.32[a,*]
	CMO	11.8 ± 0.25[ab]	12.9 ± 0.81[ab]	13.0 ± 0.00[b]	11.2 ± 0.36[a]
C 16:1	CO	0.80 ± 0.02[a,*]	1.95 ± 0.22[b,*]	1.15 ± 0.51[a]	0.85 ± 0.04[a,*]
	CMO	1.40 ± 0.14[a]	1.10 ± 0.27[a]	1.10 ± 0.00[a]	2.15 ± 0.32[b]
C 17:0	CO	0.10 ± 0.01[a]	-	0.10 ± 0.03[a]	0.10 ± 0.01[a]
	CMO	0.10 ± 0.02[a]	0.10 ± 0.01[a]	0.10 ± 0.00[a]	-
C 17:1	CO	0.20 ± 0.03[a]	0.10 ± 0.01[a]	0.15 ± 0.05[a]	0.20 ± 0.00[a]
	CMO	0.10 ± 0.02[a]	0.15 ± 0.02[a]	0.20 ± 0.00[a]	0.10 ± 0.00[a]
C 18:0	CO	2.85 ± 0.07[b,*]	1.30 ± 0.72[a]	2.20 ± 0.86[ab]	2.75 ± 0.04[b,*]
	CMO	1.90 ± 0.16[a]	1.85 ± 0.28[a]	2.20 ± 0.00[a]	1.65 ± 0.06[a]
C 18:1	CO	72.9 ± 0.07[a,*]	68.7 ± 2.14[a]	72.5 ± 1.45[a]	72.7 ± 0.42[a]
	CMO	70.2 ± 0.10[b]	71.4 ± 0.45[b]	71.3 ± 0.00[b]	67.9 ± 2.00[a]
C 18:2	CO	9.80 ± 0.04[a,*]	15.7 ± 2.27[b]	10.5 ± 1.68[a]	9.9 ± 0.03[a,*]
	CMO	14.1 ± 0.43[ab]	11.8 ± 1.20[a]	11.3 ± 0.00[a]	16.4 ± 1.98[b]
C 18:3	CO	0.50 ± 0.02[a]	0.35 ± 0.11[a]	0.55 ± 0.11[ab]	0.50 ± 0.02[a]
	CMO	0.30 ± 0.00[a]	0.50 ± 0.12[a]	0.50 ± 0.00[a]	0.45 ± 0.17[a]
C 20:0	CO	0.30 ± 0.01[a,*]	0.15 ± 0.06[a]	0.30 ± 0.28[a]	0.30 ± 0.02[a,*]
	CMO	0.05 ± 0.01[a]	0.15 ± 0.15[a]	0.10 ± 0.00[a]	0.05 ± 0.05[a]
C 20:1	CO	0.15 ± 0.03[a]	-	0.30 ± 0.14[a]	0.10 ± 0.02[a]
	CMO	0.10 ± 0.03[a]	0.10 ± 0.10[a]	0.10 ± 0.00[a]	0.05 ± 0.01[a]

CO: Virgin olive oil obtained from crushed paste, CMO: Virgin olive oil obtained from crushed-malaxed paste, Different superscript letters in the same line indicate significant differences ($p < 0.05$) among different crushing temperatures (a-b); and between crushing and crushing + malaxation applications (*)

the olive oils from crushed paste were between 121.0–250.0 mg/kg, while the same values were found to differ from 234.0–266.0 mg/kg for the olive oils obtained from the crushed-malaxed olive paste. The malaxation process clearly increased the total phenol contents of the resulting olive oil. When the amounts of carotenoids and chlorophylls were considered, maximum quantities were obtained at 18 °C for the two processing techniques and malaxation did not cause any significant changes in the pigment concentrations of the oils. In the study by Luaces *et al*., (2005), the effect of heat treatment during crushing on the pigment composition of olive oil was evaluated and the results indicated an increase in the contents of chlorophyll and pheophytin especially when the temperature was adjusted to above 20 °C.

The fatty acid distribution of virgin olive oils obtained from the pastes crushed at different temperatures is given in Table 2. The major fatty acid, namely oleic acid was over 67% of the fatty acids in all samples, and did not vary statistically according to crushing temperature except for the sample obtained by crushing performed at 30 °C and kneading afterwards. Linoleic acid fluctuated in both processes and malaxation mainly led to an increase in its ratio. Stearic acid was found to differ by increasing crushing temperatures. Additionally, palmitoleic, margaric, heptadecenoic, linolenic, arachidic and gadoleic acids were determined in small amounts. The fatty acid compositions mainly remained unchanged by both crushing temperatures and additional malaxation process.

The changes in sterol composition with regard to various crushing temperatures are given in Table 3. The predominant sterols were β-sitosterol, Δ-5-avenasterol and campesterol ranging from 82.4–86.0%, 7.59–9.45% and 2.83–3.21%, respectively. In addition, sitostanol, clerosterol, Δ-7-campesterol, 24-methylene-cholesterol, Δ-5,24-stigmastadienol, Δ-7-avenasterol, stigmasterol, campestanol and Δ-7-stigmastenol were identified in smaller ratios. β-sitosterol was not affected by temperature changes during crushing, although the additional malaxing process caused a slight increase in its percentage. Campesterol, campestanol, sitostanol, Δ-5-avenasterol, Δ-7-stigmastanol and Δ-7-avenasterol did not change due to the temperature effect either in crushed or malaxed samples.

The change in volatiles with respect to different crushing temperatures is given in Table 4, and the variation in the volatiles of the olive oils from

TABLE 3. Sterol composition of virgin olive oils obtained from pastes crushed at different temperatures (%)

		13 °C	18 °C	24 °C	30 °C
24-methylene-cholesterol	CO	0.13 ± 0.19^{a}	0.43 ± 0.25^{b}	0.48 ± 0.03^{b}	0.37 ± 0.06^{ab}
	CMO	0.45 ± 0.00^{b}	0.32 ± 0.04^{b}	0.50 ± 0.00^{b}	0.12 ± 0.18^{a}
Campesterol	CO	3.19 ± 0.22^{a}	3.18 ± 0.13^{a}	3.21 ± 0.02^{a,*}	3.19 ± 0.13^{a}
	CMO	3.00 ± 0.02^{a}	3.13 ± 0.33^{a}	3.10 ± 0.00^{a}	2.83 ± 1.94^{a}
Campestanol	CO	0.01 ± 0.02^{a}	0.10 ± 0.02^{a}	0.08 ± 0.03^{a}	0.06 ± 0.05^{a}
	CMO	0.02 ± 0.00^{a}	0.07 ± 0.03^{a}	0.06 ± 0.00^{a}	0.11 ± 0.13^{a}
Stigmasterol	CO	0.23 ± 0.03^{a}	0.25 ± 0.04^{b}	0.26 ± 0.02^{b}	0.20 ± 0.01^{a}
	CMO	0.22 ± 0.01^{a}	0.24 ± 0.09^{a}	0.24 ± 0.00^{a}	0.12 ± 0.14^{a}
Δ-7-campesterol	CO	-	0.01 ± 0.01^{a}	-	-
	CMO	0.04 ± 0.03^{a}	0.05 ± 0.08^{a}	0.02 ± 0.00^{a}	0.20 ± 0.28^{a}
Clerosterol	CO	0.80 ± 0.13^{a,*}	1.67 ± 0.19^{ab}	1.96 ± 0.04^{b,*}	1.15 ± 0.68^{ab}
	CMO	1.96 ± 0.08^{a}	1.75 ± 0.07^{a}	1.85 ± 0.00^{a}	1.73 ± 0.17^{a}
β-sitosterol	CO	85.8 ± 0.02^{a}	83.6 ± 1.25^{a}	83.9 ± 0.34^{a}	83.8 ± 2.21^{a}
	CMO	82.6 ± 0.77^{a}	83.8 ± 0.68^{ab}	83.8 ± 0.00^{ab}	86.0 ± 0.08^{b}
Sitostanol	C	0.56 ± 0.29^{a,*}	2.00 ± 0.12^{a}	1.31 ± 0.25^{a}	1.27 ± 1.53^{a}
	CMO	1.31 ± 0.54^{a}	1.24 ± 0.74^{a}	1.01 ± 0.00^{a}	2.30 ± 0.44^{a}
Δ-5-avenasterol	C	8.37 ± 0.31^{a,*}	7.59 ± 1.47^{a}	8.03 ± 0.25^{a,*}	8.87 ± 0.50^{a}
	CMO	9.36 ± 0.05^{a}	8.48 ± 0.73^{a}	8.67 ± 0.00^{a}	8.64 ± 1.55^{a}
Δ-5,24-stigmastadienol	C	0.52 ± 0.19^{a}	0.59 ± 0.01^{a}	0.56 ± 0.12^{a}	0.62 ± 0.14^{a}
	CMO	0.71 ± 0.01^{c}	0.52 ± 0.10^{ab}	0.64 ± 0.00^{bc}	0.43 ± 0.09^{a}
Δ-7-stigmastenol	C	0.13 ± 0.01^{a}	0.08 ± 0.01^{a}	0.11 ± 0.01^{a}	0.11 ± 0.03^{a}
	CMO	0.10 ± 0.04^{a}	0.14 ± 0.08^{a}	0.11 ± 0.00^{a}	0.11 ± 0.01^{a}
Δ-7-avenasterol	C	0.26 ± 0.03^{a}	0.50 ± 0.41^{a}	0.19 ± 0.02^{a,*}	0.28 ± 0.04^{a}
	CMO	0.16 ± 0.23^{a}	0.23 ± 0.10^{a}	-	0.16 ± 0.08^{a}

CO: Virgin olive oil obtained from crushed paste, CMO: Virgin olive oil obtained from crushed-malaxed paste, Different superscript letters in the same line indicate significant differences ($p < 0.05$) among different crushing temperatures (a-c); and between crushing and crushing + malaxation applications (*)

crushed and malaxed paste is given in Table 5. The major volatile compound was hexanal, which is the main C6 volatile compound, which occurs because of the autoxidation of linoleic acid in the lipoxygenase pathway (Kalua *et al*., 2007; Raffo *et al*., 2015). There were significant hexanal reductions throughout the crushing process at different temperatures and the maximum hexanal amount was attained at 13 °C. The second dominating volatile compound was *cis*-3-hexen-1-ol, which accounts for the elicitation of bitter sensations, and did not change statistically throughout the increasing temperatures, although the malaxation process caused a fluctuation in the oil samples. The amount of *cis*-3-hexen-1-ol was followed by 1-hexanol and 3-hexen-1-ol, acetate, respectively. Outstanding increases were observed at 18 °C for 1-hexanol and at 30 °C for 3-hexen-1-ol acetate for both types of processes. *Trans*-2-hexenal, which is known to be responsible for the pleasant freshly cutgrass flavor, was not affected statistically by the increases in temperature. 2,4-hexadienal and nonanal were the other aldehydes detected in the oil samples.

The group of aldehydes was followed by alcohols including *cis*-2-penten-1-ol; 1-methyl-2-cyclopenten-1-ol; 1-octanol and phenylethyl alcohol. The alcohols did not follow a regular pattern of change upon different crushing temperatures and additional malaxation progress. The following group of volatiles was found to be hydrocarbons which were comprised of 1,3-pentadiene, toluen, 3-ethyl-1,5-octadiene, alpha-copaene, zingiberene, bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-, benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl. The amounts of bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl) were not affected statistically by temperature changes during crushing.

Considering acids, acetic acid, responsible for the pungent flavor (Morales *et al*., 2005), was the dominating one; whereas acetic acid hexyl ester was also detected.

A principal component analysis (PCA) was carried out to determine differences among the oils obtained from olive pastes processed in different ways. A factor score plot is given in Figure 1. The

TABLE 4. Headspace volatile compounds of virgin olive oils obtained from pastes crushed at different temperatures (%)

	13 °C	18 °C	24 °C	30 °C
1,3-pentadiene	0.24 ± 0.07^{a}	0.30 ± 0.02^{ab}	0.36 ± 0.02^{b}	0.34 ± 0.03^{ab}
Acetic acid	0.34 ± 0.12^{a}	0.23 ± 0.25^{a}	0.38 ± 0.47^{a}	0.64 ± 0.49^{a}
Furan-2-ethyl	0.26 ± 0.10^{ab}	0.17 ± 0.01^{a}	0.21 ± 0.05^{ab}	0.35 ± 0.04^{b}
cis-2-penten-1-ol	0.36 ± 0.08^{a}	0.57 ± 0.03^{b}	0.68 ± 0.01^{b}	0.93 ± 0.02^{c}
Hexanal	36.9 ± 5.45^{a}	34.0 ± 2.01^{a}	33.0 ± 0.11^{a}	31.8 ± 3.59^{a}
Cyclotrisiloxane, hexamethyl-	0.42 ± 0.20^{a}	0.37 ± 0.00^{a}	0.57 ± 0.04^{a}	0.75 ± 0.33^{a}
1-methyl-2-cyclopenten-1-ol	2.55 ± 0.06^{a}	1.15 ± 1.62^{a}	1.21 ± 1.71^{a}	3.23 ± 0.79^{a}
Trans-2-hexenal	2.77 ± 0.23^{a}	2.28 ± 0.06^{a}	2.69 ± 0.33^{a}	1.78 ± 2.35^{a}
Cis-3-hexen-1-ol	15.7 ± 9.65^{a}	13.8 ± 6.49^{a}	9.94 ± 3.00^{a}	9.36 ± 0.03^{a}
1-hexanol	11.3 ± 1.16^{b}	16.1 ± 2.35^{c}	14.5 ± 0.06^{ab}	6.85 ± 0.47^{a}
2,4-hexadienal	1.11 ± 1.48^{a}	1.84 ± 0.48^{a}	2.82 ± 0.13^{a}	1.71 ± 1.63^{a}
3-ethyl-1,5-octadiene	1.69 ± 0.23^{a}	2.86 ± 0.30^{b}	4.23 ± 0.19^{c}	5.65 ± 0.31^{d}
3-Hexen-1-ol, acetate	4.02 ± 0.35^{a}	4.46 ± 0.32^{a}	5.84 ± 0.06^{b}	8.39 ± 0.57^{c}
Acetic acid, hexyl ester	2.45 ± 0.44^{a}	2.31 ± 0.21^{a}	3.03 ± 0.11^{a}	4.90 ± 0.41^{b}
Nonanal	1.52 ± 0.30^{a}	1.38 ± 0.16^{a}	1.44 ± 0.09^{a}	0.60 ± 0.85^{a}
Phenylethyl alcohol	0.27 ± 0.06^{ab}	0.36 ± 0.04^{b}	0.31 ± 0.03^{ab}	0.21 ± 0.06^{a}
Cyclopentasiloxane, decamethyl-	0.30 ± 0.00^{a}	0.26 ± 0.02^{a}	0.33 ± 0.04^{a}	0.45 ± 0.13^{a}
Cyclohexasiloxane, dodecamethyl-	0.12 ± 0.01^{a}	0.11 ± 0.00^{a}	0.13 ± 0.03^{a}	0.17 ± 0.06^{a}
alpha-copaene	0.38 ± 0.06^{a}	0.35 ± 0.04^{a}	0.35 ± 0.02^{a}	0.44 ± 0.08^{a}
Zingiberene	0.52 ± 0.07^{a}	0.49 ± 0.02^{a}	0.48 ± 0.02^{a}	0.67 ± 0.12^{a}
Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	0.37 ± 0.04^{a}	0.36 ± 0.02^{a}	0.37 ± 0.04^{a}	0.49 ± 0.08^{a}
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	0.28 ± 0.02^{a}	0.29 ± 0.02^{a}	0.26 ± 0.01^{a}	0.40 ± 0.05^{b}

Different superscript letters in the same line indicate significant differences ($p < 0.05$) among different crushing temperatures

first (F1), second (F2) and third (F3) principal components had eigen values of 21.2, 12.2, 10.1 and explained 30.7%, 17.7% and 14.6% of the variance, respectively. F1 showed high and positive correlations with zingiberene, bicyclo[3.1.1] hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl) and C 20:0; high and negative correlations with K_{270}. F2 had high and positive correlations with *trans*-2-hexenal and free fatty acids; whereas it had high and negative correlations with sitostanol. The score plot revealing the relationships among the oils processed in various ways showed that oils obtained from crushed pastes (CO) had positive scores for F1; whereas the oils obtained from crushed and malaxed paste (CMO) had positive scores for F2. A clear discrimination between the two processing techniques is illustrated in Fig 1.

Hierarchical cluster analysis (HCA) was performed to examine the differentiation of oils obtained by various processing techniques. Euclidean distance and Ward algorithm were used to cluster the samples. Figure 2 shows the dendogram obtained from HCA, where 3 main groups can be identified. The first group contained 24 CO, 13 CO and 18 CO, where 13 CO and 18 CO had high similarities. The second group consisted of 18 CMO and 24 CMO. The third group was formed by 30 CMO, 30 CO and 13 CMO.

4. CONCLUSIONS

The influence of a modified crushing process and subsequent malaxation application was ascertained on the quality characteristics as well as fatty acid and sterol profiles and volatile compositions of virgin olive oil. The results reported herein exhibit the clear effect of malaxation on oil quality and compositional parameters. Also, the findings provide basic information about the impact of the cooling process during crushing on olive oil compositional characteristics, and they will provide an insight into future research projects.

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TABLE 5. Headspace volatile compounds of virgin olive oils obtained from pastes crushed at different temperatures and malaxed (%)

	13 °C	18 °C	24 °C	30 °C
1,3-pentadiene	0.33 ± 0.05^{a}	0.53 ± 0.03^{b}	0.48 ± 0.09^{ab}	0.47 ± 0.08^{ab}
Acetic acid	0.38 ± 0.02^{a}	0.63 ± 0.19^{a}	0.68 ± 0.43^{a}	0.59 ± 0.24^{a}
Furan-2-ethyl	0.25 ± 0.35^{a}	0.06 ± 0.02^{a}	0.15 ± 0.02^{a}	0.11 ± 0.00^{a}
cis-2-penten-1-ol	0.83 ± 0.17^{ab}	0.65 ± 0.09^{a}	0.80 ± 0.08^{ab}	1.09 ± 0.13^{b}
Hexanal	34.7 ± 0.89^{c}	17.7 ± 2.45^{a}	22.6 ± 0.78^{b}	23.9 ± 2.15^{b}
Cyclotrisiloxane, hexamethyl-	0.47 ± 0.28^{a}	0.61 ± 0.12^{a}	0.69 ± 0.11^{a}	0.68 ± 0.04^{a}
1-Methyl-2-cyclopenten-1-ol	1.18 ± 1.60^{a}	1.03 ± 0.33^{a}	1.55 ± 0.25^{a}	2.03 ± 0.19^{a}
Trans-2-hexenal	5.51 ± 1.52^{a}	3.36 ± 1.80^{a}	3.59 ± 0.72^{a}	1.72 ± 2.43^{a}
Cis-3-hexen-1-ol	15.9 ± 3.72^{b}	23.1 ± 0.46^{c}	18.7 ± 1.78^{bc}	7.30 ± 0.83^{a}
1-hexanol	7.73 ± 1.02^{a}	16.4 ± 4.40^{b}	9.38 ± 1.49^{a}	9.26 ± 0.40^{a}
2,4-hexadienal	2.40 ± 2.92^{a}	0.77 ± 0.84^{a}	2.33 ± 0.03^{a}	1.71 ± 0.45^{a}
3-Ethyl-1,5-octadiene	1.15 ± 0.23^{a}	2.91 ± 0.12^{ab}	4.36 ± 0.09^{b}	4.08 ± 2.04^{b}
3-Hexen-1-ol, acetate	9.83 ± 1.32^{a}	8.33 ± 0.03^{a}	9.56 ± 0.32^{a}	15.1 ± 0.31^{b}
Acetic acid, hexyl ester	1.81 ± 0.56^{a}	2.06 ± 0.03^{ab}	2.24 ± 0.33^{ab}	3.02 ± 0.26^{b}
Nonanal	1.23 ± 0.30^{a}	1.46 ± 0.15^{a}	1.55 ± 0.13^{a}	2.20 ± 0.21^{b}
Phenylethyl alcohol	0.26 ± 0.04^{a}	0.51 ± 0.04^{b}	0.38 ± 0.00^{ab}	0.43 ± 0.08^{b}
Cyclopentasiloxane, decamethyl-	0.40 ± 0.07^{a}	0.39 ± 0.08^{a}	0.47 ± 0.15^{a}	0.45 ± 0.07^{a}
Cyclohexasiloxane, dodecamethyl-	0.23 ± 0.00^{a}	0.19 ± 0.03^{a}	0.28 ± 0.08^{a}	0.17 ± 0.04^{a}
alpha-copaene	0.33 ± 0.12^{a}	0.39 ± 0.00^{a}	0.43 ± 0.07^{a}	0.50 ± 0.00^{a}
Zingiberene	0.50 ± 0.06^{a}	0.53 ± 0.08^{a}	0.59 ± 0.15^{a}	0.61 ± 0.07^{a}
Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	0.33 ± 0.06^{a}	0.41 ± 0.03^{a}	0.46 ± 0.09^{a}	0.45 ± 0.04^{a}
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	0.26 ± 0.00^{a}	0.28 ± 0.06^{a}	0.32 ± 0.07^{a}	0.38 ± 0.07^{a}

Different superscript letters in the same line indicate significant differences ($p < 0.05$) among different crushing temperatures

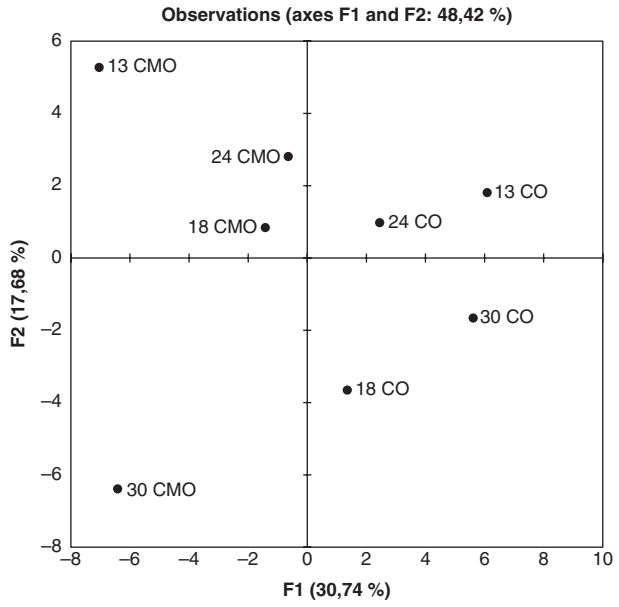

FIGURE 1. Factor score plot on the first two principal components based on oil parameters obtained from pastes crushed at different temperatures

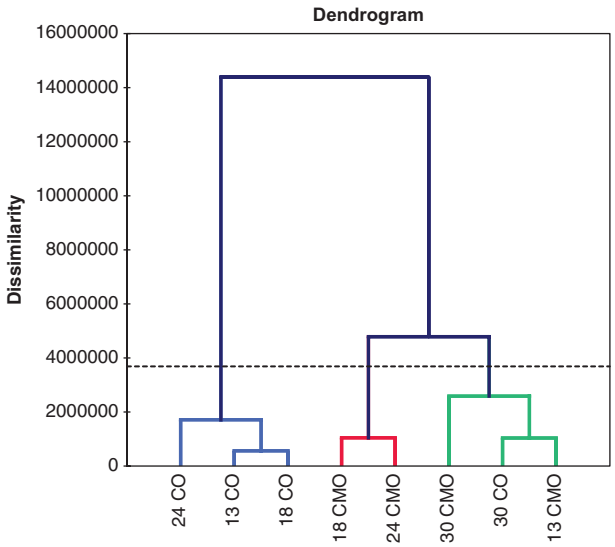

FIGURE 2. Dendogram based on oil parameters obtained from pastes crushed at different temperatures

REFERENCES

Amirante P, Clodoveo ML, Tamborrino A, Leone A, Dugo G. 2008a. Oxygen concentration control during olive oil extraction process: A New System to emphasize the organoleptic and healthy properties of virgin olive oil. *Acta Hort.* **949**, 473–480. https://doi.org/10.17660/ActaHortic.2012.949.70

Amirante P, Clodoveo ML, Tamborrino A, Leone A. 2008b. New designer malaxer to improve thermal exchange enhancing virgin olive oil quality. *Acta Hort*. **949**, 455–462. https://doi.org/10.17660/ActaHortic.2012.949.67

Angerosa F, Mostallino R, Basti C, Vito R. 2001. Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chem.* **72**, 19–28. https://doi.org/10.1016/S0308-8146(00)00194-1

AOCS 2003. Official methods and recommended practices of the American Oil Chemists' Society, AOCS Press, Champaign, IL (USA).

Aparicio R, Roda L, Albi MA, Gutiérrez F. 1999. Effect of various compounds on virgin olive oil stability measured by Rancimat. *J. Agric. Food Chem.* **47**, 4150–4155. https://doi.org/10.1021/jf9812230

Caponio F, Alloggio V, Gomes T. 1999. Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chem.* **64**, 203–209. https://doi.org/10.1016/S0308-8146(98)00146-0

Caponio F, Catalano P. 2001. Hammer crushers vs disk crushers: the influence of working temperature on the quality and preservation of virgin olive oil. *Eur. Food Res. Technol.* **213**, 219–224. https://doi.org/10.1007/s002170100364

Caponio F, Gomes T. 2001. Influence of olive crushing temperature on phenols in olive oils. *Eur. Food Res. Technol.* **212**, 156–159. https://doi.org/10.1007/s002170000225

Caponio F, Pasqualone A, Gomes T, Catalano P. 2002. Use of HPSEC analysis of polar compounds to assess the influence of crushing temperature on virgin olive oil's quality. *Eur. Food Res. Technol.* **215**, 534–537. https://doi.org/10.1007/s00217-002-0620-0

Caponio F, Gomes T, Summo C, Pasqualone A. 2003. Influence of the type of olive-crusher used on the quality of extra virgin olive oils. *Eur. J. Lipid Sci. Technol.* **105**, 201–206. https://doi.org/10.1002/ejlt.200390041

Ceballos C, Moyano M J, Vicario IM, Alba J, Heredia FJ. 2003. Chromatic evolution of virgin olive oils submitted to an accelerated oxidation test. *J. Am. Oil Chem. Soc.* **80**, 257–262. https://doi.org/10.1007/s11746-003-0686-0

Fiori F, Di Lecce G, Boselli E, Pieralisi G, Frega NG. 2014. Effects of olive paste fast preheating on the quality of extra virgin olive oil during storage. *LWT-Food Sci. Technol.* **58**, 511–518. https://doi.org/10.1016/j.lwt.2014.03.021

Gallina-Toschi T, Cerretani L, Bendini A, Bonoli-Carbognin M, Lercker G. 2005. Oxidative stability and phenolic content of virgin olive oil: an analytical approach by traditional and high resolution techniques. *J. Sep. Sci.* **28**, 859–870. https://doi.org/10.1002/jssc.200500044

García AMI, Fregapane G, Salvador MD. 2011. Effect of crushing on olive paste and virgin olive oil minor components. *Eur. Food Res. Technol*. **232**, 441–451.

Giovacchino DL, Sestili S, Di Vincenzo D. 2002. Influence of olive processing on virgin olive oil quality. *Eur. J. Lipid Sci. Tech.* **104**, 587–601. https://doi.org/10.1002/1438-9312(200210)104:9/10<587::AID-EJLT587>3.0.CO;2-M

Gutfinger T. 1981. Polyphenols in olive oils. *J. Am. Oil Chem. Soc.* **58**, 966–968. https://doi.org/10.1007/BF02659771

International Union of Pure and Applied Chemistry 1987. Standard Methods for Analysis of Oils, Fats and Derivates, 7th Edn, IUPAC Method 2.301, Blackwell Scientific Publications, Palo Alto, Calif., USA.

Kalua CM, Allen MS, Bedgood Jr, DR, Bishop AG, Prenzler PD, Robards K. 2007. Olive oil volatile compounds, flavour development and quality: A critical review. *Food Chem.* **100**, 273–286. https://doi.org/10.1016/j.foodchem.2005.09.059

Koutsaftakis A, Kotsifaki F, Papamanolioudaki A, Stefanoudaki E. 2000. Effect of olive crushing parameters on the qualitative characteristics of virgin olive oil. *Acta Hort.* **586**, 645–648. https://doi.org/10.17660/ActaHortic.2002.586.137

Leone A, Esposto S, Tamborrino A, Romaniello R, Taticchi A, Urbani S, Servili M. 2016. Using a tubular heat exchanger to improve the conditioning process of the olivepaste: Evaluation of yield and olive oil quality. *Eur. J. Lipid Sci. Tech.* **118**, 308–317. https://doi.org/10.1002/ejlt.201400616

Lercker G, Frega N, Bocci F, Servidio G. 1994. "Veiled" extra-virgin olive oils: Dispersion response related to oil quality. *J. Am. Oil Chem. Soc.* **71**, 657–658. https://doi.org/10.1007/BF02540597

Luaces P, Pérez GP, García JM, Sanz C. 2005. Effects of heat treatments of olive fruit on pigment composition of virgin olive oil. *Food Chem.* **90**, 169–174. https://doi.org/10.1016/j.foodchem.2004.03.035

Morales MT, Luna G, Aparicio R. 2005. Comparative study of virgin olive oil sensory defects. *Food Chem.* **91**, 293–301. https://doi.org/10.1016/j.foodchem.2004.06.011

Murkovic M, Lechner S, Pietzka A, Bratacos M, Katzogiannos E. 2004. Analysis of minor components in olive oil. *J. Biochem. Bioph. Meth.* **61**, 155–160. https://doi.org/10.1016/j.jbbm.2004.04.002

Ortega-García F, Blanco S, Peinado MÁ, Peragón J. 2008. Polyphenol oxidase and its relationship with oleuropein concentration in fruits and leaves of olive (*Olea europaea*) cv. 'Picual' trees during fruit ripening. *Tree Physiol.* **28**, 45–54. https://doi.org/10.1093/treephys/28.1.45

Preziuso SM, Serio MGD, Biasone A., Vito R, Mucciarella MR, Giovacchino LD. 2010. Influence of olive crushing methods on the yields and oil characteristics. *Eur. J. Lipid Sci. Tech.* **112**, 1345–1355. https://doi.org/10.1002/ejlt.201000303

Raffo A, Bucci R, D'Aloise A, Pastore G. 2015. Combined effects of reduced malaxation oxygen levels and storage time on extra-virgin olive oil volatiles investigated by a novel chemometric approach. *Food Chem.* **182**, 257–267. https://doi.org/10.1016/j.foodchem.2015.02.128

Servili M, Baldioli M, Montedoro GF. 1993. Phenolic composition of virgin olive oil in relationship to some chemical and physical aspects of malaxation in: II International Symposium on Olive Growing **356**, 331–336. https://doi.org/10.17660/ActaHortic.1994.356.69

Veillet S, Tomao V, Bornard I, Ruiz K, Chemat F. 2009. Chemical changes in virgin olive oils as a function of crushing systems: Stone mill and hammer crusher. *Cr. Chim.* **12**, 895–904. https://doi.org/10.1016/j.crci.2009.01.003