PAPER

EXTRACTION YIELD AND CHARACTERIZATION OF BURUNDIAN AVOCADO OIL OBTAINED BY MEANS OF MALAXATION WITH AND WITHOUT ENZYMATIC AID

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ABSTRACT

The mechanical extraction on the yield and quality of avocado oil extracted from different fruit varieties were investigated in this study. Batches of various varieties of ripen avocado in Burundi were processed in an oil mill located in the Gitega Region. Avocado oil was extracted considering the malaxation step carried out with and without the enzymes addition. Avocado pulp achieved by malaxation at 30°C for 90 min presented the highest yield and get the lessen acidity and peroxide values. Under the conditions applied in this study (dilution ratio between avocado paste and purified water at 1: 0.5 and malaxation temperature below 36°C), the addition of pectolitic and amylolytic enzymes did not reveal to increase the yield to such extent as to justify the cost of the treatment. According to what stated for classification of olive oils in EVO and VO categories, the results of chemical and sensorial indices allowed classifying most of the avocado oils obtained from malaxation without enzyme addition in the EVO category.

Keywords: avocado oil, oil mill, malaxation, enzymatic extraction, Burundi

1. INTRODUCTION

Avocado (*Persea americana* Mill.) is a drupe-shaped fruit, more or less elongated, with a thin, pale olive-green glossy shell. It is widely consumed today as an important and energetic fruit with high nutritional value and health benefits (DUARTE *et al*., 2016) due to the compounds of the lipid fraction that varies from 13.5 to 24%, in addition to significant levels of folic acid, minerals, sulphur, silicon, vitamins E, B1, B2, and D (DEMBITSKY *et al*., 2011). The fleshy pulp is a source of high quality oil with large levels of oleic and palmitic acids, and with physicochemical properties resembling those of olive oil (DUARTE *et al*., 2016). Besides, avocado oil can be considered as functional oil (TANGO *et al*., 2004), used in pharmaceutical and cosmetic industries, and for obtaining commercial oils for human consumption.

In this regard, the avocado pulp processing can contribute to the best use of the final oil (ROCHA, 2008). The most suitable varieties for oil extraction are Hass, Fuerte and Glória (TANGO *et al*., 2004). Depending on the location of the orchard, the oil content of these fruit flesh can range from 16-17% in September to 25-30% in April depending on the fruit ripening stage (REQUEJO-TAPIA, 1999).

In the oil-bearing cells, the major part of the oil is located in the vacuoles, where it is free, and the remaining part is bound or dispersed in the cytoplasm and is, therefore, not directly accessible in the extraction process and lost in the waste. The rupturing of the cell walls and of the structure of the finely-dispersed emulsion needs the extraction being performed in different ways (LEWIS *et al*., 1978). For cost reasons, most producers started to extract oil from dried fruits by means of solvents (MARTINEZ NIETO *et al*., 1988). In order to cut energy costs and minimise the air pollution caused by organic solvents, the avocado oil can be also separated from fruits by centrifugal or pressing forces, then oil cells are submitted to mechanical and/or enzymatic destruction (MARTINEZ NIETO *et al*., 1988; BIZIMANA *et al*., 1993). In addition, Moreno *et al.* (2003) have investigated the effect of different oil extraction methods on the physical and chemical properties of avocado oil. Extraction by heating the pulp up to 95°C using microwaves, followed by either Soxhlet extraction with hexane or pressing, was studied. Moreover, a method of fruit drying on the extractability of avocado oil with hexane and supercritical CO_2 has been studied (MOSTERT *et al*., 2007), as well as the extraction yield of Fortuna avocados oil as a function of the freeze-drying was evaluated (DOS SANTOS *et al*., 2013).

On the other hand, cold pressed avocado oil, greenish in colour, is relatively new oil in the commercial culinary oil field (WOOLF *et al*., 2009). It is defined as oil extracted using mechanical or physical means at temperatures below 50°C and it is extracted using methods similar to that used for extra virgin olive oil (KIRITSAKIS *et al*., 1998; WOOLF *et al*., 2009). Several studies have been conducted to find effective methods for the recovery of the oil enclosed in the cell and the need to destroy the cell walls through the use of specific enzyme to the breakdown of the individual types of polysaccharides in the cell wall structure has often been emphasized as a workable solution (HADJ-TAIEB *et al*., 2012; VIERHUIS *et al.*, 2001). The enzyme-assisted aqueous extraction has emerged as an alternative and environmentally friendly extraction process both for olive (ALIAKBARIAN *et al.*, 2008; HADJ-TAIEB *et al.*, 2012; NAJAFIAN *et al.*, 2009; VIERHUIS *et al.*, 2001) and avocado oil (FREITAS *et al*., 1993; MORENO *et al*., 2003). This process involves addition of selected enzymes into a mixture of oleaginous material with pre-determined amount of water at a given pH value, followed by incubation of the mixture at a pre-set temperature, time, and shaking speed (MAT YUSOFF *et al*., 2017). Hydrolytic enzymes, including cellulase and pectinase, are commonly used to hydrolyse and degrade cell wall constituents and improve the release of intracellular contents (HADJ-TAIEB *et al*., 2012).

The present study was focused to obtain extra virgin avocado oil in Burundian region both for local consumption and for export to foreign markets. Firstly, different time/temperature malaxation conditions, yield of oil extraction, and oil quality parameters, were investigated. Secondly, to the avocado batch getting the best oil yield, enzymatic trials were applied. Finally, a complete characterization of the avocado oil was carried out.

2. MATERIAL AND METHODS

2.1. Batches of avocado fruits used in oil extraction trials

Five avocado (*Persea Americana* Mill.) varieties (Fuerte = FU, Hass = HA, Local Rouge = LR, Local Vert = LV, and Washington = WA), were collected from a orchard located in the Murayi area (Gitega, Burundi). The varieties of Fuerte and Hass were already demonstrated to be among of the best for the oil content (GÓMEZ-LÓPEZ, 2002; OZDEMIR and TOPUZ, 2004), while the others three varieties, named Local Rouge, Local Vert, and Washington, were autochthonous of the Burundian region of Gitega.
The Table 1 reports the percentage of fruit varieties for each batch of production used in the extraction trials. Each batch has been prepared on real scale according to a fruit composition that couldn't be either standardised or replicated because of the cultivation of individual varieties of avocado trees in the context of Burundian agricultural system. This depends on the huge number of small-scale farmers, which generally practice an avocado production with limited know-how on the choice of cultivars, on inputs, and production techniques (JACQUES and JACQUES, 2012).
Since the avocado is classified as a climacteric fruit (BARMORE, 1976), avocados used for this study were collected unripe and were allowed ripening at a temperature of 24±2°C until visible changes in peel colour (from bright green to purplish) and pulp softening occurred. The degree of ripeness determined by measuring the firmness of the fruit (WONG *et al.*, 2010) to finger pressure, as like as the days required for fruits to soften (BARMORE, 1976) were the parameters used as general guide to avocado maturity. Hence to ensure the oil content in the avocados is at the maximum for processing, the fruit should ideally be mature (WONG *et al*., 2010).

2.2. Plant and process used for oil extraction trials

According to the Table 1, the nine batches of different avocado cultivar were processed according to the process depicted in Fig. 1. The plant was a semi-continuous system (Nuova M.A.I.P. Macchine Agricole Industriali Pieralisi, Jesi, Ancona, Italy) located in Murayi (Gitega, Burundi), and its technical scheme is shown in the Fig. 2. Before processing avocados were sorted and sanitized with a 100 ppm chlorine solution. After washing, the avocado fruits were manually cut in a half by sanitized knife to eliminate the kernel. The destoned avocado fruits were transported by means of a cochlea elevator (2.25 m), complete with hopper and kickstand in stainless steel, to one hammer crusher (15 HP) at low rotation speed (1.400 rpm) with double grid of 60 cm diameter along with grid holes of 6 mm. Besides to crush the destoned avocado fruit and enabling pigment extraction from skins, the hammer crusher is preferred to minimise the emulsion in order to optimise oil extraction (DI GIOVACCHINO *et al.*, 2002). These first process steps were very close to what reported in literature with the exception of leaving skins that are normally removed by fruits (COSTAGLI and BETTI, 2015; WONG *et al.* 2013).

The homogeneous paste mass with a creamy consistency was then pumped into the section equipped with malaxers (kneading machines). The kneading machine was composed of two tanks (350 + 350 kg) along with a mono-screw pump (P 50). Each kneading machine consisted of a stainless-steel tank with a central screw stirring the paste slowly and continuously at a monitored temperature. The effect of the kneading machine on the avocado paste (COSTAGLI and BETTI, 2015) was very similar to the one already described for the olive paste (DI GIOVACCHINO *et al.*, 2002): due to the coalescence phenomena (TRAPANI *et al.*, 2017) the small oil drops released during crushing of the destoned fruits merge into large drops that can be easily separated by centrifugal extraction. The separation of oil from solid and liquid phases was done using a centrifuge system, composed firstly by a three-phases centrifugal decanter (rotation speed 5.500 rpm), and then by a vertical centrifuge (rotation speed 6.900 rpm with a drum diameter of 30.5 cm). The paste inside the three-phases centrifugal decanter was separated into oil, vegetation water and solids i.e., pomace (exhausted pulp and residual skin). This device exploits the centripetal acceleration to separate continuously a mixture of particulate solids and liquids with phases having different densities (DI GIOVACCHNO *et al.*, 2002). Into the vertical centrifuge, the avocado oil was separated from vegetation water; then it was clarified through a bag filter in order to minimize the microbial contamination of the oil (GUERRINI *et al.*, 2015). Finally, the avocado oil was stored at 20°C into stainless steel tanks.

2.3. Extraction trials

Experiments applied a scalar approach and were aimed firstly to verify whether the avocado fruits cultivated in Burundian area behave similarly to olives during kneading. Since literature outcomes on cold avocado oil extraction techniques (WONG *et al.*, 2013; COSTAGLI and BETTI, 2015) stated temperature levels lower than 50°C, two main sets of experiments were performed at malaxing temperature of 30°C and of 36°C each coupled with specific malaxation time. Further, the addition of enzymes was also tested.

Table 1. Variety composition and maturity degree of the batches of avocado fruits used in the study.

Production batches	Maturity degree	Batch variety composition (%)*
A	Medium	50% WA + 25% LV + 25% LR
B	High	50% FU + 25% LR + 25% HA
C	High	50% FU + 25% LR + 25% HA
D	Medium	50% WA + 25% LV + 25% LR
E	High	50% HA + 25% LR + 25% FU
F	High	50% HA + 25% LR + 25% FU
G	Low	75% FU + 25% HA
H	Medium	50% HA + 25% LR + 25% LV
I	Low	75% HA + 25% FU

*where: FU = Fuerte; HA = Hass; LV = Local Vert; LR = Local Rouge; WA = Washington.

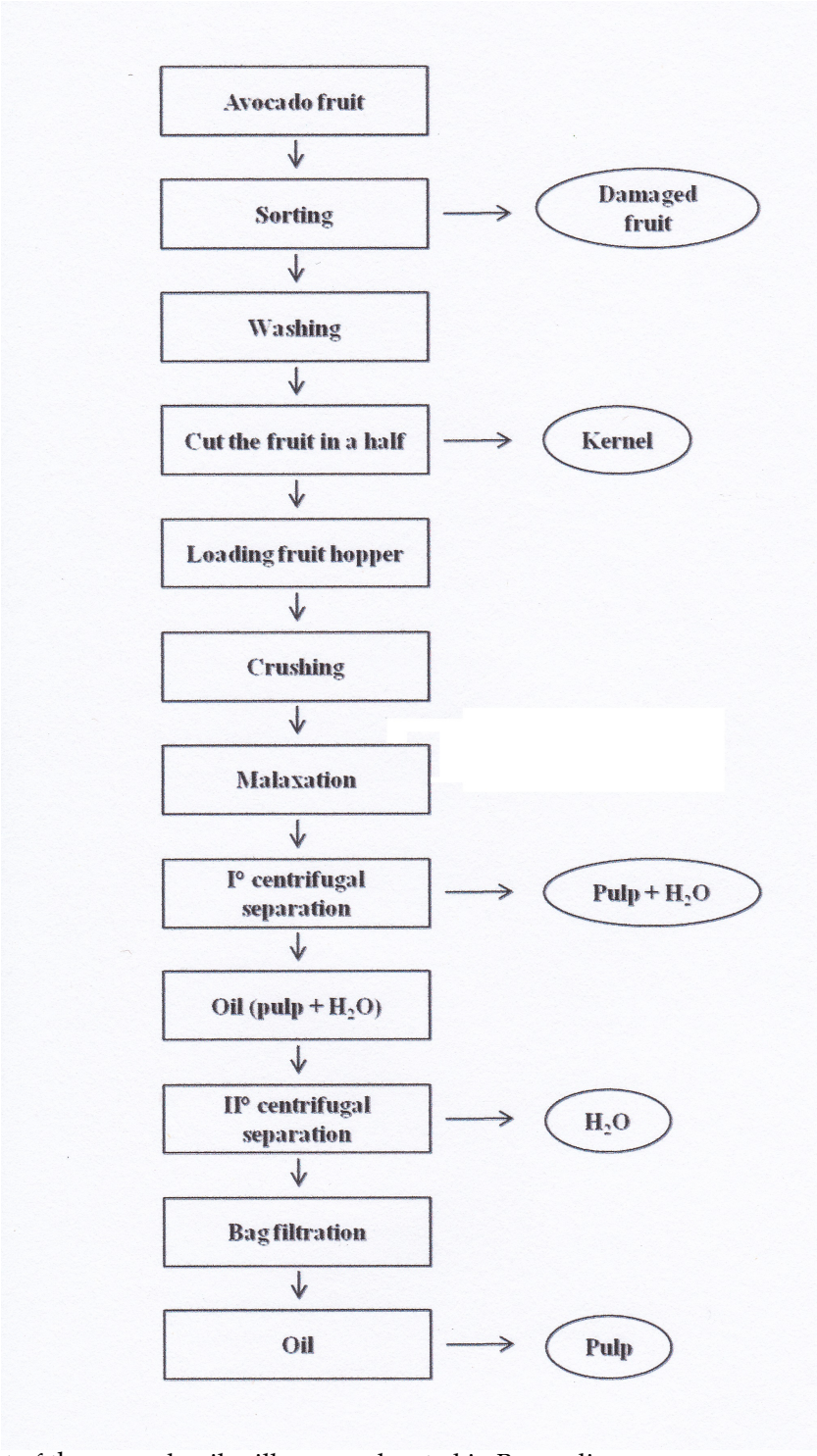

Figure 1. Flow sheet of the avocado oil mill process located in Burundi.

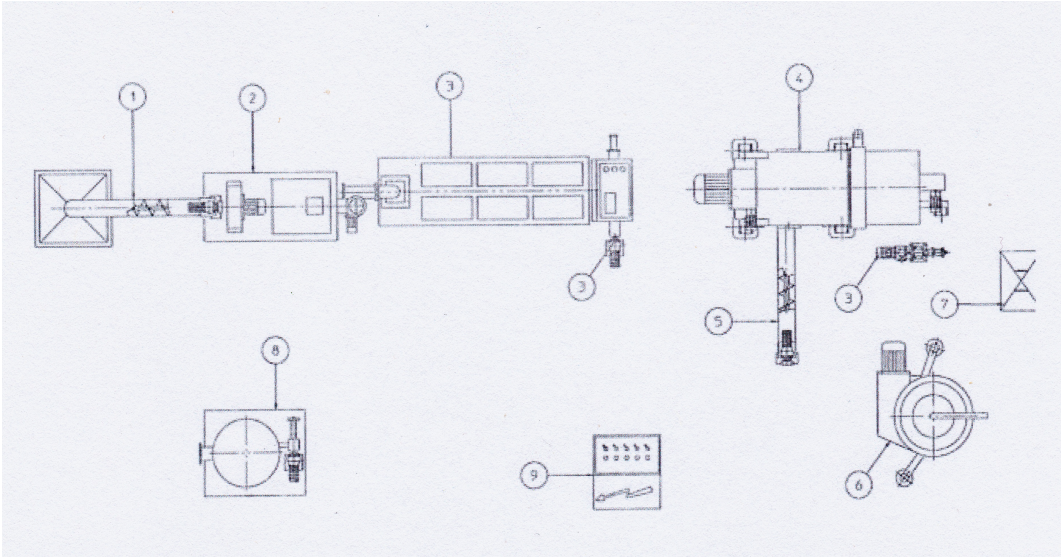

Figure 2. Technical scheme of the avocado oil mill process located in Burundi. 1. Cochlea; 2. Hammer crusher; 3. Pumps; 4. Decanter; 5. Unloading cochlea; 6. Centrifuge; 7. Decantation tank; 8. Boiler; 9. Control panel.

2.3.1 Malaxation trials

In the Table 2 the different operating conditions applied for each batch of production are shown. Noted that the crusher loading was not always constant, neither had reached the maximum level, due to the unfavourable seasonal trend which had not allowed the regular transfers/contributions of the avocado fruits in workable quantities. Moreover, as in these trials no dilution with water of the avocado paste was performed, the centrifugal decanter was used under the two-phases modality.

Table 2. Operating conditions applied to the malaxation trials.

Production batches	Pulp loading (kg)	T (°C)	t_{total} (min)
A	324	30±2	30
E	126	30±2	40
H	198	30±2	60
G	115	30±2	90
B	353	30±2	120
F	272	36±2	90
D	226	36±2	90
C	235	36±2	120
I	120	36±2	120

2.3.2 Enzymatic trials

With reference to the batch (Table 1) getting the best oil content, enzymatic extraction trials were performed by testing Maxoliva (pectinase, obtained from strains of *Aspergillus niger* and *Trichoderma longibrachiatum* belonging to the GRAS class, was supplied by DSM),

and Megazyme (α-amylase, $\geq$ 3.70 U/mg, from *Bacillus subtilis*, was supplied by Sigma-Aldrich Co., St. Louis, Mo. USA). Technical sheets outlined that: the Maxoliva enzyme is isolated specifically for the extraction of olive oil, with a balanced ratio of carbohydrate and pectinase activities, it is active between 20 and 55°C and between 3.0 and 5.0 pH. It has activity not less than 2000 units/mL. The Megazyme used in this study was an α-amylase enzyme that catalyses the hydrolysis of internal α-1.4-glucan links in polysaccharides containing 3 or more α-1.4-linked D-glucose units, yielding a mixture of maltose and glucose (TAKESHITA and HEHRE, 1975). It is active between 35 and 40°C, between pH 3.0 and 5.5, and has activity of 2000 units/mL.
The avocado paste obtained after crushing (Fig. 1) was diluted at a ratio 1:0.5 with purified water before being subjected to malaxation phase, as a consequence the centrifugal decanter was used in these trials under the three-phases modality. The temperature and time of the enzymatic hydrolysis were suggested by the previous malaxation trials (Table 2), by the condition of use of the enzymes, and considering the working pH (about 5.0). The enzymes were added at 1% w/w; the dosage was referred to each enzyme either when added individually, or in a mixture (Table 3). One unit of activity is defined as the amount of enzyme preparation that liberates 1 μmoL of reducing sugars per minute from the galacturonic acid of olive pectins (RANALLI *et al.*, 2003).

Table 3. Operating conditions applied to G batch for the enzymatic trials.

Trial	Pulp loading (kg)	T (°C)	t_{total} (min)	Enzyme
G1	191.5	30±2	90	Maxoliva
G2	190.5	30±2	120	Maxoliva
G3	230.0	30±2	90	Megazyme
G4	200.0	30±2	120	Megazyme
G5	215.0	30±2	90	Maxoliva + Megazyme
G6	393.0	30±2	120	Maxoliva + Megazyme

2.4. Oil extraction yield

For the oil content determination, the protocol was carried out following the procedure described by the EC Regulation (EEC 2568/91). Oil content in avocados was extracted from avocado paste with hexane using a Soxhlet apparatus. A cellulose thimble containing 5 g dried sample was placed in the Soxhlet device and extracted with 250 mL hexane for 6 h. The flask was removed and solvent evaporated using a rotary evaporator (Büchi Rotavapor R-3, 1000184809, Büchi Labortechnik AG, Switzerland). The oil in fruit pulp is calculated as the grams of the oil contained in 100 g of fresh fruit pulp.
The extraction yield was expressed as Business Yield (By) and as Process Yield (Py) in order to compare the different extraction steps and conditions. The By was obtained by the ratio between the weight of the oil extracted at the end of the process and the weight of the fruit pulp subjected to extraction.

$$Business\ yield\ (wt.\%) = \frac{\text{W oil extracted}}{\text{W fruit pulp}} * 100$$

The Py was calculated as the weight of oil extracted at the end of the process and the weight of the oil in the fruit pulp.

$$Process\ yield\ (wt.\%) = \frac{\text{W oil extracted}}{\text{W oil in fruit pulp}} * 100$$

2.5. Oil analysis

Filtered avocado oil was characterized for acidity value (% oleic acid/100 g avocado oil), peroxide value (mEq O_2/kg oil) and UV determinations according to the European Commission (EEC 2568/91) standard methods. Acidity value indicated the free fatty acids present in fats and oils. High degree acidity value can be related with the degree of triglyceride hydrolysis during preparation or storage. Free fatty acids are then oxidised to hydroperoxide that are measured by the Peroxide Value (PV). During the early stages of oxidation, the increase in UV absorption due to the formation of Conjugated Dienes (CDs) and Conjugated Trienes (CTs) is proportional to the uptake of oxygen and to the production of peroxides. Therefore, the content of CDs and CTs obtained measuring the oil absorbance at 232 and 270 nm (K232 and K270) also can serve as a relative measurement of oxidation. Spectrophotometric determinations were obtained using a Shimadzu UV-1601 spectrophotometer (Shimadzu Europe, Duisburg, Germany).

2.6. Determination of the phenolic fraction

Aliquots of oil (5 g) were added to 10 ml of a methanol/ water solution (80:20, v/v) in a 50-mL centrifuge tube, according to MONTEDORO *et al*. (1992). The mixture was blended (Ultraturrax, IKA, Staufen, Germany) for 5 min and then centrifuge for 5 min at 2500 *g*. The hydro-alcoholic extract was collected, and the oil phase was re-extracted with 2 x 10 mL methanol/ water solution. Finally, the hydroalcoholic fractions were combined and washed with n-hexane to remove the residual oil and then dried under vacuum at 30°C. The dry extracts were re-suspended in 1 mL methanol and the solutions were filtered through 0.2 μm regenerated cellulose filters. The absorbance of the filtered solutions was recorded at 765 nm using a Shimadzu UV-1601 spectrophotometer (Shimadzu Europe, Duisburg, Germany). The results were reported as gallic acid equivalents (mg/kg oil) based on the calibration curve (r^2 = 0.999). Folin-Ciocalteu reagent and gallic acid were obtained from Merck & Co. Inc. (Darmstadt, Germany).

2.7. Fatty acid and sterol composition

The fatty acid composition of the fatty acid methyl esters (FAME) was determined using a Shimadzu 2025 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with an auto-sampler (model AOC-20s, Shimadzu), an auto-injector (model AOC-20i, Shimadzu), a flame ionization detector, and a CP-Select CB capillary column for FAME (100 m x 0.25 mm i.d.; 0.25 μm film thickness; Chrompack, Varian, Inc., CA). The injection volume was 1 μL in split mode (split ratio 30:1) and the carrier gas was hydrogen with a constant flow of 1.5 mL/min. The injector and detector temperatures were kept at 250°C. The column oven temperature was programmed following the procedure of Prandini *et al*. (2007) with minor modification: 60°C for 2 min, from 60 to 170°C at 10°C/min for 35 min, and from 170 to 240°C at 4°C/min for 9.5 min. Peak identification was possible with the aid of reference standards (Supelco 37 component FAME mix; conjugated octadecadienoic acid; Sigma Chemical Co, St. Louis, MO). Data were expressed as a percentage of total fatty acids, calculated with peak areas corrected by factors according to AOAC 963.22

method (2000). The content of β-sitosterol was determined according to EC Regulation (EEC 2568/91).

2.8. Sensory analysis

The sensory analysis was performed on the avocado oil samples from malaxation trials without enzyme addition by a panel trained according to the International Olive Council (IOC) requirements. Indeed, some researches have already been established both the resemblance of avocado oil to olive oil (SALGADO *et al*., 2008) and the recommended standards for avocado oil tasting used to ensure its quality in terms of sensory properties (KOCHHAR and HENRY, 2009; WONG *et al.*, 2010; WOOLF *et al.*, 2009). The panel test was established using a standard profile sheet IOC method (EN ISO/IEC 17025/2005) even leaving free choice to panelists for new descriptors. Each taster analysed all samples during three different sessions. The values of the median sensory data were calculated, and the test supervisor chose a significance level of 5%.

2.9. Statistical analysis

All analytical measurements were carried out in triplicate and the results were expressed as the mean value±standard deviation of three determinations. Comparisons of mean values were performed using one-way ANOVA with a Duncan post-hoc test and p-values of < 0.05 were considered significant. The IBM SPSS Statistics21 package (IBM Corporation, New York, USA) was used.

3. RESULTS

3.1. Malaxation trials

Under the conditions reported in the Table 2, the Py from malaxation trials ranged between 48 and 95% (Table 4). The highest By and Py were achieved in longer time (up to 90 min), both at 30°C and at 36°C, with some differences. Considering the By, malaxing for 90 min at 30°C allowed to gain the 9% w/w of oil, whilst time-temperature of 120 min-36°C and 120 min-30°C achieved lower values. On the other hand, malaxing for 120 min at 30°C lead to highest Py of 95% w/w.

As for the quality parameters (Table 4) of the avocado oil obtained under malaxation trials, the acidity levels were low and ranged between 0.32 and 1.02% of oleic acid. Oil from batch F, malaxed at 30°C for 90 min, showed the lowest acidity value. The PVs were also very low: from 2.50 to 1.48 mEq O_2/kg, when all the batches are considered. The values for the CDs and CTs ranged as 1.43÷2.04 and 0.09÷0.27 absorbance units for K232 and K270, respectively. For PVs, CDs and CTs the lowest values were registered for batches A and C. The results from sensory analysis performed on avocado oils obtained in the present study are reported in Table 4: the median of defects was > 0 only for the oils C, D, and E, while the median of the positive attributes was always > 0 for the remaining trials (A, B, F, G, H, and I). The positive attributes were identified as fruity, bitter, leaves, and almonds.

Table 4. Oil extraction yields and quality parameters of avocado oil obtained in the malaxation trials.

Production batches	Fruit oil (% w/w)	Business yield (% w/w)	Process yield (% w/w)	Acidity value (% oleic acid)	Peroxide value (mEq O_2/kg)	K232	K270	Panel test		Commercial class
								Negative attributes	Positive attributes	
	Limit value* for EVO			≤ 0.80	≤ 20	≤ 2.50	≤ 0.22	0	> 0	
A	6.30±0.32 c	3.00	48.00	0.66±0.03 c	1.48±0.07 c	1,43±0.09 e	0,19±0.01 b	0	3±0.8	EVO**
E	8.30±0.42 b	5.00	61.00	1.22±0.06 a	2.49±0.12 a	1,52±0.09 d	0,12±0.01 c	3±0.2	3±0.5	VO***
H	6.90±0.35 c	5.10	74.00	0.42±0.02 d	2.50±0.13 a	1,58±0.09 d	0,12±0.01 c	0	4±0.2	EVO
G	10.20±0.51 a	9.00	88.00	0.49±0.03 d	1.94±0.10 b	1,69±0.10 cd	0,17±0.02 b	0	2±0.7	EVO
B	8.70±0.44 b	8.20	95.00	0.39±0.05 d	1.99±0.11 b	1,80±0.11 b	0,12±0.02 ab	0	5±0.2	EVO
F	8.30±0.42 b	5.00	61.00	0.32±0.02 e	1.94±0.10 b	1,68±0.10 c	0,09±0.01 d	0	4±0.3	EVO
D	6.30±0.32 c	4.60	73.00	0.83±0.04 b	2.36±0.12 a	1,86±0.11 b	0,13±0.01 c	2±0.6	2±0.9	VO
C	8.70±0.44 b	7.80	90.00	1.15±0.07 a	1.50±0.08 c	1,69±0.10 c	0,09±0.01 d	1±0.5	1±0.2	VO
I	9.30±0.47 ab	8.40	90.00	0.44±0.03 d	2.00±0.10 ab	2,04±0.10 a	0,21±0.02 a	0	3±0.3	EVO

* Reg. CEE 1531/2001. Data in column with different letters mean significantly different values according to post-hoc Duncan test at $p<0.05$. **EVO, extra virgin oil; ***VO, virgin oil.

3.2. Enzymatic trials

In the Table 5, the results of the avocado oil extraction with enzymes (Table 3) were reported. Under the experimental conditions, the single enzyme Megazyme (G3 and G4) turned out to be the more efficient both at 90 min and 120 min than Maxoliva (G1 and G2). However, the joint of the two enzymes proved to be the best solution to increase the avocado oil yield (G5 and G6), both for the By and Py. Exactly, 4.7 and 9.7% w/w By, and 46 and 88% w/w Py were achieved at 90 and 120 min malaxation time, respectively.
Considering the avocado oil quality parameters, the acidity values ranged between 0.22 and 0.79%, with a mean value of 0.41±0.21% whilst the peroxides reached values between 3.4 and 4.4 of mEq O_2/kg. The major levels both of acidity and PVs were observed in G5 and G6 where the enzyme combination was employed.

Table 5. Oil extraction yields and quality parameters obtained in the malaxation trial G with enzymes addition.

Production batches	Business yield (% w/w)	Process yield (% w/w)	Acidity value (% oleic acid)	Peroxide value (mEq O_2/kg)
G1	2.60	26.00	0.31±0.02 cd	4.40±0.27 a
G2	3.20	31.00	0.50±0.04 b	3.70±0.23 b
G3	3.00	29.00	0.22±0.01 e	3.40±0.19 b
G4	7.00	68.00	0.29±0.02 d	3.40±0.24 b
G5	4.70	46.00	0.38±0.03 c	4.10±0.26 a
G6	9.70	88.00	0.79±0.05 a	4.40±0.29 a

Data in column with different letters mean significantly different values according to post-hoc Duncan test at $p<0.05$.

3.3. Chemical profile of the final avocado oil

The Table 6 showed the most significant parameters of the final avocado oil obtained through the malaxation under the best conditions identified in this study.

Table 6. Characterization of the EV avocado oil obtained from the malaxation experiment G.

Parameters	Value
Acidity value (% of oleic acid)	0.74±0.06
Peroxide value (mEq O_2/kg)	2.02±0.16
K232	1.70±0.14
K270	0.16±0.01
Total phenols (mg/L)	40.00±3.20
Oleic acid C18:1 (% of total fatty acids)	63.30±5.06
Linoleic acid C18:2 (% of total fatty acids)	10.50±0.84
Palmitic acid C16:0 (% of total fatty acids)	18.00±1.44
Palmitoleic acid C16:1 (% of total fatty acids)	5.30±0.42
β-sitosterol (% of total sterols)	76.40±6.11
Negative attributes (panel test)	0
Positive attributes (panel test)	4±0.90

The quality parameters (acidity value, peroxide value and K232 and K270) at very low levels (EEC 2568/91), together with the absence of negative sensory attributes, bearing out the quality grade of the avocado oil obtained under this study. Considering the lipid components, oleic acid (C18:1) was the major fatty acid oil, followed by palmitic (C16:0), palmitoleic (C16:1), and linoleic (C18:2) acids (Table 6). Finally, the percentage of β-sitosterol exceeded the value of 76% of the total sterols and 40 mg/L of the total phenols were detected.

4. DISCUSSION

A number of reports have indicated that the oil content in avocado fruits and the oil composition vary according to the location of the orchard, the variety, the number of days between flowering and harvest, the dry matter contents, and even to the part of the fruit measured (REQUEJO-TAPIA, 1999; OZDEMIR and TOPUZ, 2004). Considering all the varieties taken into consideration (Table 1), the average oil content was 8.11±1.35% w/w of avocado fruit (Table 4). The G batch registered an oil content greater than 10% w/w, followed by I (9.3% w/w), B and C, both with 8.7% w/w. As expected, FU and HA avocado varieties allowed achieving the highest oil content, confirming the study of Yanty *et al*. (2011).

4.1. Malaxation trials

In the EVOO production it should be desirable to strike a balance between oil yield and oil quality characteristics, but this requires studies to check whether a time-temperature could be applied in order to predict the potential effect of malaxation on extraction yield (TRAPANI *et al.*, 2017). Under the conditions applied this study, the malaxation (Table 2) was performed at a temperature either of 30 or 36±2°C, values that are close to the environmental temperature in Burundi and which were selected in order to reduce the energy consumption and processing variable costs. The optimal malaxing time and temperature conditions to reach the best compromise between quality and quantity of extracted avocado oil have been investigated, and the results have been reported in the Table 4. Data showed that at both 30 and 36°C longer times for malaxing improved the avocado oil yield. Malaxing at 30°C by increasing time from 30 to 40, 60, 90, till 120 min (respectively for batches A, E, H, G, and B), allowed at achieving an increase in Py by several percentage points. The same was observed for malaxing at 36°C, where the Py was maximized at a time of 120 min (batch I). According to literature data, an increase in time and temperature during malaxation causes a positive influence on olive oil extraction yield (TRAPANI *et al.*, 2017), albeit the state of advancement in the Italian olive oil production pointed out that the optimal setting of the malaxation parameters should be targeted for each individual cultivar (SELVAGGINI *et al.*, 2014).
Our results (Table 4) corroborated the fact that between heat and time, the period for kneading and mixing the avocado paste into the malaxer overcomes the only thermal energy needed to activate natural degrading enzymes, diffusion, and coalescence phenomena. Since the avocado oil comes in a finely dispersed emulsion inside the cells of the fruit pulp, the extraction process requires rupturing not only the cell walls, but also the structure of the emulsion (LEWIS *et al*., 1978) in order to favour the coalescence phenomena (TRAPANI *et al.*, 2017). The emulsions are surrounded by the lipoproteic membranes or the lipophilic solids of the paste, which can absorb part of the oil itself (COSTAGLI and BETTI, 2015) and thus the malaxing time is generally longer and the temperature is higher for avocados than olives (ANGEROSA *et al*., 2001). Indeed,

experimental trials (data not reported) highlighted that malaxation with times and temperatures lower than 20 min and 30°C, respectively, did not allow the oil separation in the malaxer and, consequently, in the centrifugal decanter. This was supported by WONG *et al*. (2011) who obtained the avocado oil by malaxing the mixture for 60 min at 45-48°C. On the other hand, the experience of COSTAGLI and BETTI (2015) showed that avocado mash malaxing time should not exceed 90 min with temperature below than 50°C.
Contrary to what expected when either temperature or time are increased, the chemical and sensory parameters of the avocado oil extracted under these malaxing conditions were maintained amply below the legal requirements (EEC 2568/91) to classify the products in the "virgin" category. Extra virgin oil (EVO), as well as virgin oil (VO), is a food product for which not only chemical parameters but also sensory characteristics must comply with values established by the EU regulation (EEC 2568/91; EN ISO/IEC 17025/2005). According to what stated for classification of olive oils in EVO and VO categories, the results of chemical and sensorial indices allowed classifying most of the avocado oils (A, B, F, G, H, and I) obtained from malaxation without enzyme addition in the EVO category, whilst the oils from batches C, D, and E did not result to comply with the quality level requested for EVOs by the current legislation because their median of defect was higher than 0. Considering both the yield and the oil quality, batch G composed of the varieties richer in oil (Table 4) and more widespread in the agronomic supply chain i.e., Fuerte and Hass (TANGO *et al*., 2004), and with a low degree of fruit maturity (Table 1) was deemed to be suitable to be tested with enzymatic preparations (Table 3).

4.2. Enzymatic trials

Advances in enzyme biotechnology applications have led to economically viable processes and improved extraction yield especially for oil pastes with more tenacious emulsions as avocado gets (BUENROSTRO and LOPEZ-MUNGUIA, 1986; COSTAGLI and BETTI, 2015). Many papers have been published on the effects of enzymes on the extraction and characteristics of olive oil (ALIAKBARIAN *et al.*, 2008; HADJ-TAIEB *et al.*, 2012; NAJAFIAN *et al.*, 2009; VIERHUIS *et al.*, 2001), while fewer are the reports on the assisted-enzymatic extraction of avocado oil (Freitas *et al*., 1993; MORENO *et al*., 2003; WONG *et al.*, 2013). In the present study, the enzyme addition was applied under the optimal operating conditions (either for 90 and 120 min at 30°C) for malaxing the G batch that got extra virgin oil with a high yield (Table 4). In such case, due to the batch composition made of Hass and Fuerte varieties (Table 1) greater oil content in the avocado paste was shown (Table 4). Thus considered, together with the harder texture of the fruits because of their unripen state (Table 1), corroborated the idea to evaluate the effect of enzyme addition both on oil extraction and quality. Many researchers confirmed that the enzyme addition hydrolyzes and breaks the cotyledon cell walls (MAT YUSOFF *et al*., 2017), degrading the walls of the oil-bearing cells, making the structure more permeable and further expose the oil component (ALIAKBARIAN *et al.*, 2008; HADJ-TAIEB *et al.*, 2012; NAJAFIAN *et al.*, 2009; VIERHUIS *et al.*, 2001). The most effective enzymes used in oil extraction technology are, cellulases, xylanases, and proteases, or enzyme mixture consisting mainly of pectinases, cellulases, hemicellulases (HADJ-TAIEB *et al.*, 2012; NAJAFIAN *et al.*, 2009).
The pectolitic Maxoliva enzyme used in this study (Table 3), which is commonly applied for the olive oil extraction at the industrial level (ALIAKBARIAN *et al.*, 2008; RANALLI *et al.*, 2003; NAJAFIAN *et al.*, 2009), provided a lower yield (Table 5) than the amylolytic Megazyme enzyme (Trials G1 vs G3, and G2 vs G4). The result was in line with other studies, which added α-amilase enzymes or a mixture of α-amilase and protease during mechanical extraction of avocado paste, getting a positive effect of the treatment

(COSTAGLI and BETTI, 2015). BUENROSTRO and LOPEZ-MUNGUIA (1986) obtained better extraction yields of avocado by using α-amylase alone which resulted in an extraction of 75% of the original oil content compared to 65% with the triple enzymatic mixture of polygalacturonase, α-amylase, and protease.
Data from this study (Table 5) obtained the higher Py when the enzymes were simultaneously added (G5 and G6), in accordance with Freitas *et al*. (1993) who improved the avocado oil yield by using mixtures of commercial preparations. With few exceptions (NAJAFIAN *et al*., 2009), the enzyme mixtures with combined activity give better results than individual enzymes to improve the rate of extracted oil (ALIAKBARIAN *et al*., 2008). This positive effect was obtained also without affecting the final oil quality (Table 5) as already demonstrated by Buenrostro and Lopez-Munguia (1986). Despite a negligible increase in the PVs (Table 5) if compared with the only malaxation without enzymes (Table 4), the use of the enzymatic preparation applied in this study allowed achieving acidity values for obtaining the extra virgin denomination (Table 5). By comparing G sample from malaxation trials (Table 4) to G samples from enzymatic trials (Table 5), it was surprising to observe generally lower extraction yields with enzymatic addition than without its use. This result might be rebutted from literature where the increasing in the oil extraction yield from malaxation with enzyme addition has been widely demonstrated (BUENROSTRO and LOPEZ-MUNGUIA, 1986; COSTAGLI and BETTI, 2015; FREITAS *et al*., 1996; MORENO *et al*., 2003; WONG *et al*., 2013). Even considering variability among fruit maturity and oil content in the within of batches of the same varietal composition, the lesser yield measured in oil G when aqueous enzymes were added (Table 5 *vs* Table 4) might depend on the altered kinetics of coalescence occurring during malaxation as negatively affected by paste dilution. Under the slight dilution conditions applied in this study, avocado paste could require higher temperature to reduce its pulp-water viscosity and an increase in oil yield (FREITAS *et al*., 1996; WONG *et al*., 2013). Further, during malaxation it is common to observe that part of the oil begins to physically separate and rise towards the surface of the olive paste. Since the speed of the oil's movement towards the surface of the paste depends on the oil viscosity, TRAPANI *et al*. (2017) led to the consideration that the condition to increase the oil process yield does not only include oil droplet coalescence but also the separation and rising to the surface of the oil. Such a phenomenon could be slowed in our enzymatic trials due to the increased shear stress from water dilution combined with an inadequate heat content of the avocado paste. Further, the slight dilution ratio 1:0.5 of the paste to purified water during malaxation could affect also the separation of the phases at centrifugal decanter with a decreased oil yield.

4.3. Chemical and sensory profile of the final avocado oil

Considering all the trials of avocado oil extraction performed on real scale in the Murayi oil mill, with and without the use of the enzymes; comparing yield, quality, process costs, and operation management, the most suitable extraction technique also taking into account the oil mill location could be considered as the malaxation extraction without the addition of the enzymes. In this regard, the final EV avocado oil obtained with the batch G was completely characterized (Table 6).
Even according to Moreno *et al*. (2003), who reported acidity value between 0.65 and 1.23 mg/KOH/g, the avocado oil achieved (Table 6) could be classified as an extra virgin (EEC 2568/91). Peroxide values were very far from those reported in the range of 5.1-12.3 mEq/kg (QUIÑONES-ISLAS *et al*., 2013). This is particularly interesting if data are compared to the study of INDRIYANI *et al*. (2016), where the peroxide values of the Indonesian avocado cultivars ranged from 14.9 to 166.1 mEq/kg oil i.e., at a clearly

oxidation state (Salgado *et al*., 2008). The contents of CDs and CTs, expressed with the specific absorptivity values reported in Table 6, were significantly low compared to the values of Indonesian avocado oil varied from 2.6-3.7 (INDRIYANI *et al*., 2016).
As for fatty acid profile (Table 6), avocado oil is characterized by having high levels of monounsaturated fatty acids (oleic and palmitoleic acids), low polyunsaturated fatty acids (linoleic acid), and relatively high levels of saturated fatty acid (palmitic and stearic acids). Likewise, ROCHA (2008) has reported that avocado oil from the varieties Wagner, Fortuna, Hass and Fuerte had higher levels of monounsaturated fatty acid ranging from 59 to 72% of total fatty acids, followed by saturated fatty acids, from 17 to 23%, and polyunsaturated fatty acids to a lesser extent with levels ranging between 10 and 14%. Regarding the avocado oils from the varieties Northrop, Duke, Wagner, Quintal, and Fuerte, they are characterized by having more than 63% oleic acid, while the oils from the varieties Rincon, Barker, Waldin, Prince and Panchoy showed less than 50% of this fatty acid. Palmitic acid content ranged between 15.38 and 32.37% in oils from different varieties. Therefore, the avocado variety affects the levels of palmitic acid and oleic acid, once varieties with high oleic acid levels had low palmitic acid levels and vice versa (DUARTE *et al*., 2016). The fatty acid composition is influenced by the cultivars, maturity stage, anatomical region of the fruit, and geographic location for plant growth (TANGO *et al*., 2004). According to this, the fatty acid composition of avocado oil from this study (Table 6) was in line with the literature (KOCHHAR and HENRY, 2009), achieving a higher percentage of oleic acid than values reported by YANTY *et al*. (2011) for the local Malaysian cultivars (43.65-51.22%) and by RAMIREZ-ANAYA *et al.* (2018) for Hass cv. malaxed at higher temperature than in our study.
Avocado oil contains substantial amounts of bioactive compounds such as phytosterols, especially in the lipid fraction, and the main representative is the β-sitosterol (DOS SANTOS *et al*., 2014). As shown in the Table 6, the avocado oil achieved more than 76% of β-sitosterol, higher than the Margarida avocado oil variety, in which β-sitosterol represents 71.8% of the total sterols (DOS SANTOS *et al*., 2014), but lower compared to Fortuna avocado (87.6%) of the MOIGRADEAN *et al*.'s (2012) study. With regard to polyphenols, avocado oil showed a lower proportion (Table 6) than most of olive oils (ALIAKBARIAN *et al.,* 2008; VIERHUIS *et al*., 2001). FORERO-DORIA *et al.* (2017) reported avocado oil with a phenolic concentration of 99.8±15 mg GAE/L of oil with similar antioxidant capacity as olive oils. The low values measured in the sample G (Table 6) could be due to the no addition of enzymes (VIERHUIS *et al.,* 2001) together with long malaxation time (ALIAKBARIAN *et al.,* 2008; STEFANOUDAKI *et al.,* 2011), while the temperature applied (Table 2) should have been improved the phenolic content (SELVAGGINI *et al.,* 2014). As for sensory, the avocado oil as characterized in Table 6 showed a greenish yellow color which corroborated the use to common Burundian use to process fruits with skins (WONG *et al.,* 2011) with a pleasant distinctive flavor of avocado as like as already reported for cold pressed avocado oil recovered by mechanical extraction at temperature less than 50°C with or without the use of enzymes (KOCHHAR and HENRY, 2009; WONG *et al.,* 2009; WOOLF *et al.,* 2010).

5. CONCLUSIONS

The extraction process realized in the Murayi oil mill allowed achieving high quality grade of avocado oil mechanically extracted by means of malaxation of the avocado pulp at 30°C for 90 or 120 min. On the other hand, the study confirmed that the addition of enzymes to avocado paste during malaxation requires training and deepened knowledge on the shear-stress occurring in the paste especially when a low water dilution is applied.

Further, the use of three-phases centrifugal decanter should be optimized for improving EV avocado oil extraction yields.

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