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Enhancement of grape seed oil extraction using a cell wall degrading enzyme cocktail

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ABSTRACT

The application of an enzymatic pre-treatment to increase the yield of grape seed oil extraction was studied. Experiments were carried out to measure the effects that reaction time, temperature, pH, particle size and enzyme concentration have upon the enzymatic activity.

The following set of parameters was optimised: time = 24 h, pH 4, temperature 30–40 °C, particle diameters 1.0–1.4 mm, and cocktail concentration of: cellulase = 29, protease = 1191, xylanase = 21, and pectinase = 569 U/g of seed sample. The extraction yield was 13.7%, which represents an increment of 106% over non-treated samples. For 120 h the yield achieved was 17.5%, and the increment reached 163%. Such results indicate that a prolonged enzymatic treatment may certainly be used to enhance oil extraction.

1. Introduction

Grape seed (*Vitis vinifera L.*) is a worldwide well known oilseed crop containing 8–15% oil (Gomez, Lopez, & De la Ossa, 1996; Rice, 1976; Sovova, Kucera, & Jez, 1994). This oil is becoming increasingly popular for culinary, pharmaceutical, cosmetic, and medical purposes primarily due to its high levels of unsaturated fatty acids, namely oleic and linoleic acids (Crews et al., 2006; Horrobin & Manku, 1983; Kummerow, 1975). The conversion of this oil into other valuable products, such as biofuel, is also a potential prospective for future applications (Metzger & Bornscheuer, 2006). Such topics are particularly interesting for countries where the production and trade of wine products play an important role in the economy.

Grape seed oil has been traditionally recovered by hydraulic pressing and solvent extraction, mainly with *n*-hexane (Gomez et al., 1996; Rosenthal, Pyle, Niranjan, Gilmour, & Trinca, 2001). The yield of the process is significantly increased by mechanical or thermal conditioning (Dominguez, Sineiro, Nunez, & Lema, 1995; Gomez et al., 1996; Sineiro, Dominguez, Nunez, & Lema, 1998a). Milling of the seeds greatly improves efficiency, since this operation breaks down the vegetable cells and augments interfacial area for mass transfer (Gomez et al., 1996).

An alternative approach to favour oil release is the partial hydrolysis of the cell walls by means of appropriate enzymes (Fullbrook, 1983). Previous researches, with various seeds, have shown slightly encouraging results using enzyme-assisted pre-treatments, namely enhancements around 2–12% when extraction yield was compared to that obtained with untreated samples (Dominguez, Nunez, & Lema, 1994; Dominguez, Sineiro et al., 1995; Kashyap, Agrawal, Sarkar, & Singh, 1997; Sarkar, Pandey, Kumbhar, & Agrawal, 2004; Shankar, Agrawal, Sarkar, & Singh, 1997). Such small improvements have induced researchers to give up this line of investigation. However, previous studies have only considered short reaction times (generally 15–120 min) (Dominguez et al., 1994; Rosenthal, Pyle, & Niranjan, 1996; Sineiro, Dominguez, Nunez, & Lema, 1998b). Long time use of enzymes was never tried.

The faith in enzymes is inherently dependent on knowledge of seed composition itself. The oil found inside the plant cells is confined to discrete spherical organelles called oil bodies (0.6–2 μm), which consist of a triglyceride matrix surrounded by a monolayer of phospholipids linked together with proteins. They completely cover the oil bodies' surface in such a compressed way that it will never coalesce or aggregate. This stability is attributed to the steric hindrance and electronegative repulsion of hydrophobic proteins, mostly structural proteins, termed oleosins and caleosin, on the surface of the oil bodies (Capuano, Beaudoin, Napier, & Shewry, 2007; Dominguez, Sineiro et al., 1995; Frandsen, Mundy, & Tzen,

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Nomenclature

C	concentration of the enzymatic cocktail
C_1 and C_2	concentration levels 1 and 2 (see experimental design in Section 2)
d_p	particle size
Exp.	experiment
t	time
T	temperature
η	extraction yield
σ	standard deviation
Δ	increment over control case

2001; Lin, Tai, Peng, & Tzen, 2002; Tzen, Cao, Laurent, Ratnayake, & Huang, 1993). Cotyledon cells store the oil bodies in hazelnut (Dourado et al., 2003), almond (Dourado, Barros, Mota, Coimbra, & Gama, 2004) and Rosa Mosqueta (Dourado, Vasco, Gama, Coimbra, & Mota, 2000) seeds. Sineiro et al. (1998a) not only presented micrographs of cotyledon cells of soybean seeds, but also described and illustrated their evolution during an enzymatic treatment process, which indicated that treated samples presented smaller particle size, with a degraded surface, resulting from the enzymatic action, and separated cells, caused by the degradation of the middle lamellae. Hence, cell tissues slowly and gradually lose cellular and sub-cellular organisation, as the walls and cytoplasm become disrupted (Sineiro et al., 1998a).

The plant cells are surrounded by a complex cell wall matrix composed of carbohydrate molecules (cellulose, hemicelluloses, and pectic polysaccharides) as well as proteins. In order to achieve higher oil extraction, enzyme preparations should have a broad spectrum of activity to disrupt the cell wall structure. Additionally, the dissolution of proteins, at a pH far from the isoelectric point, also destabilizes the matrix and facilitates oil removal into the solvent phase (Rosenthal et al., 1996). The existence of fibre, preventing access of the enzyme to protein, has also been reported (Dominguez, Sineiro et al., 1995). The inclusion of cellulases and pectinases in the enzymatic hydrolysis, for longer periods, may allow easier access of proteases to proteins, improving digestibility. Summarily, it is possible to predict better extraction yields when a synergistic activity by several enzyme types is considered.

In this work, the topic of assisted oil extraction with enzymes is revisited by testing cocktails of cellulase, hemicellulase, pectinase, and protease on milled grape seed. Since the price of enzymes may constitute one of the major costs of any treatment, the experimental conditions adopted should be optimised. Different studies have employed dissimilar conditions, such as pH, concentration, temperature, reaction time, particles size, without analysing the effect of each one on the global results (e.g., see Rosenthal et al. (1996)). Hence, those conclusions may be highly specific to the experimental conditions run. Choosing the best enzymes and the best operating conditions becomes a difficult task. Our target is the study of the enzymatic treatment of grape seed, which will be incorporated as a preceding step of oil extraction. The effects of the key parameters cited above on the enhancement of the extraction yield are quantified and simultaneously discriminated in this work.

2. Materials and methods

2.1. Materials and reagents

Seeds were collected from grapes (*Vitis vinifera* L.) of the red variety, *Touriga Nacional*, harvested in Bairrada Appellation (Anadia, Portugal) during September, 2006. Four different types of enzymes have been selected on the basis of the structural composition of grape seeds: cellulase, hemicellulase, pectinase, and protease. They were purchased from Fluka Sigma–Aldrich Co. (St. Louis, MO). Other reagents were of analytical grade or higher available purity.

Cellulase (commercial Code No. 22178) was produced from *Aspergillus niger*. The declared activity at pH 5 and 37 °C is 1.44 U/mg of solids, where 1 U corresponds to the amount of enzyme which produces 1 μmol min^{-1} of glucose from carboxymethylcellulose.

Hemicellulase (xyloglucan) (commercial Code No. X2753) was produced from *Thermomyces lanuginosus*. The declared activity is 2750 FXUW/g of solids, where FXUW stands for fungal xylanase units wheat. The pH range recommended is 4–6 at temperatures up to around 75 °C.

Pectinase (commercial Code No. P2611) was produced from *Aspergillus aculeatus*. The declared activity at pH 3.5 (optimum) and 20 °C is 28472 PG/ml of suspension. The standard activity is determined by measuring viscosity reduction of a pectic acid solution.

Protease (commercial Code No. 93614) was produced from porcine pancreas. The declared activity at pH 7.6 and 25 °C is 11,909 U/mg of solids, where 1 U corresponds to the amount of enzyme which increases the absorbance at 253 nm by 0.001 per minute (substrate: *N*-benzoyl-L-arginine ethyl ester).

2.2. Experimental procedure and methods

2.2.1. Seed preparation, size reduction, and screening

Seeds were collected during transfer of the musts in wine fermentation, and separated from pulp and skins by decantation and sieving. A first wash removed immature grains, floating at the water surface. Subsequently, the seeds were subjected to several washes with water (200 g/l) under gentle stirring with a magnetic bar at 4 °C during a minimum of 3 days, using two water exchanges per day, until a minimum constant turbidity was observed. The purified seeds were finally washed with ethanol, air-dried at room temperature (ca 25 °C), and stored at 4 °C prior to use. Finally, milling was carried out on a domestic coffee mill, and the particles were classified in a standard sifter with several mesh sizes (<0.50, 0.50–0.60, 0.60–0.71, 0.71–1.0, 1.0–1.4, 1.4–2.0, >2.0 mm).

2.2.2. Enzymatic treatment

The enzymatic suspension to seed ratio was kept equal to 4 ml/g (dry basis) through all experiments, a value based on the earlier studies (Rosenthal et al., 1996). The pH was fixed with a buffer solution of citric acid and sodium hydrogenphosphate. Ten gram of milled seed were treated with enzymatic cocktails of cellulase, protease, xylanase, and pectinase. The reaction proceeded isothermally under continuous stirring at 200 rpm, and was stopped by freezing the suspension with liquid nitrogen. Then, the water was removed by freeze-drying the content of the flasks.

2.2.3. Oil extraction

Conventional extraction was carried out using 150 ml of *n*-hexane in a Soxhlet apparatus (50 ml capacity; 23 × 100 mm cartridge) during 4 h. Previous tests showed that 4 h was sufficient

Table 1
Operating conditions and results obtained.

Exp. No.	Variable under study	Operating conditions					Results		
		t (h)	T (°C)	pH	d_p (mm)	C	η (%)	σ (%)	Δ (%)
Control 1	–	No enzymatic treatment			1.0–1.4	–	6.66	0.2	–
Control 2	–				< 0.5	–	15.3	0.2	–
1	Base case[a]	24	40	6	1.0–1.4	C_1	9.76	0.2	46.5
2	t	8	40	6	1.0–1.4	C_1	7.25	0.3	8.86
3	t	16	40	6	1.0–1.4	C_1	7.96	0.8	19.5
4	t	48	40	6	1.0–1.4	C_1	10.7	0.7	60.7
5	t	120	40	6	1.0–1.4	C_1	15.7	0.1	136
6	pH	24	40	3	1.0–1.4	C_1	13.8	1.0	107
7	pH	24	40	4	1.0–1.4	C_1	13.7	0.7	106
8	pH	24	40	5	1.0–1.4	C_1	10.5	0.4	57.7
9	pH	24	40	7	1.0–1.4	C_1	8.65	1.1	29.9
10	t, pH	120	40	4	1.0–1.4	C_1	17.5	0.8	163
11	T	24	30	6	1.0–1.4	C_1	9.67	0.8	45.2
12	T	24	50	6	1.0–1.4	C_1	6.71	0.9	0.751
13	d_P, pH	24	40	4	<0.5	C_1	19.5	0.2	193/27.5[a]
14	C	24	40	6	1.0–1.4	C_2	12.0	2.0	80.2
15	C, pH	24	40	3	1.0–1.4	C_2	14.0	2.0	110
16	C, pH	24	40	4	1.0–1.4	C_2	15.8	0.8	137
17	C, pH	24	40	5	1.0–1.4	C_2	14.1	1.4	112
18	C, pH	24	40	7	1.0–1.4	C_2	9.57	1.4	43.7

[a] Δ = 193% in relation to control 1; Δ = 27.5% in relation to control 2.

to ensure maximum extraction. Actually, some runs were accomplished during 72 h in order to evaluate the extraction efficiency, but no measurable increment was assessed. The mass of extracted oil was determined gravimetrically after solvent evaporation. Furthermore, to ensure that the resultant oil carried no water, the extracted samples were passed over anhydrous sodium sulphate under vacuum in a G1 sintered glass filter, and evaporated in a rotary-evaporator at 30 °C. The oil was then transferred to speed-vacuum tubes and dried by centrifugal evaporation. The yield of the process is expressed as the mass of oil extracted from 100 g of dried grape seed.

2.2.4. Experimental design

The influences of several variables upon extraction yield were analyzed, namely: reaction time (t), temperature (T), pH, particle size (d_p), and the concentration of the enzymatic cocktail (C). In Table 1 the experimental conditions adopted are listed. The reaction times span 8, 16, 24, 48 and 120 h; temperatures are 30, 40 and 50 °C; pH ranges from 3 to 7; particle diameters are from 1.0 to 1.4 mm and less than 0.5 mm; two sets of concentrations are studied: C_1 (cellulase = 29, protease = 1191, xylanase = 21, and pectinase = 569 U/g sample) and C_2 (cellulase = 72, protease = 2977, xylanase = 55, and pectinase = 1708 U/g sample).

Exp. 1 in Table 1 corresponds to our base case: t = 24 h, T = 40 °C, pH 6, $d_p \in [1.0; 1.4]$ and $C = C_1$. Experiments were conducted by changing one variable whilst fixing the remaining ones, exception for the concentration and pH where simultaneous studies were accomplished.

Control experiments were established to quantify the increment of the yield due to the enzymatic action. They consist in simple Soxhlet extractions of non-treated samples with $d_p \in [1.0; 1.4]$ mm and $d_p < 0.5$ mm, hereafter denoted by controls 1 and 2, respectively (see Table 1).

2.3. Statistical analysis

Each result presents the mean and the standard deviation for a minimum of four experiments. Statistical analysis was carried out using Student's t-test and outlier analyses (Miller & Miller, 2000). Significance was defined at $P < 0.025$. All final results involved at least three replicates.

3. Results and discussion

3.1. General

In Table 1, the results obtained (yield, η; standard deviation, σ; and increment over control, Δ) are listed along with experimental conditions. These may be found graphically in Figs. 1–5. In the following, the effects of the reaction time, pH, temperature, enzymes concentration, and particle size upon extraction yield are discussed individually.

3.2. Time effect

In Exps 1–5 (see Table 1 and Fig. 1), one observes an increasing trend of η against time, as increments of 8.9, 19.5, 46.5, 60.2 and 136% have been measured for t = 8, 16, 24, 48 and 120 h of reaction. These results show a continuous increase in the oil extraction yield with time. For shorter times, it was not effective to detect observable enhancements in extraction yield (data not shown). It is important to note that most studies of pre-enzymatic treatments only consider small reaction times (generally 0.25–2 h) (Dominguez et al., 1994; Rosenthal et al., 1996; Sineiro et al., 1998b). Researches where both the enzymatic treatment and the extraction were performed simultaneously exhibit quite disappointing results in general, as enhancements were below 15% (Dominguez, Nunez, & Lema, 1995; Dominguez, Sineiro et al., 1995; Sarkar et al., 2004; Sineiro et al., 1998b; Soto, Chamy, & Zuniga, 2007). In our case, after 8 h (Exp. 2), only a small increment Δ = 8.86% was achieved in comparison to control 1 conditions but, for 24 h, this value reached 46.5%, which clearly surpassed most earlier studies.

The yield improvement reached 163% when time was stretched to 120 h (Exp. 5), in an attempt to exhaust grape seed oil content. GC-FID analysis confirmed that the increment observed was due to triacylglycerides only. Although it is an astonishing value, in practice such a long duration time is not acceptable. Nonetheless, it emphasises the milestone role that enzymes play on grape seed if they are left to act. Since, up to 24 h, the extraction increments vary quadratically with time and then inflect, 24 h (Exp. 1) has been assumed as a good compromise between time length and yield improvement (46.5%), despite other parameters having not yet been considered and optimised.

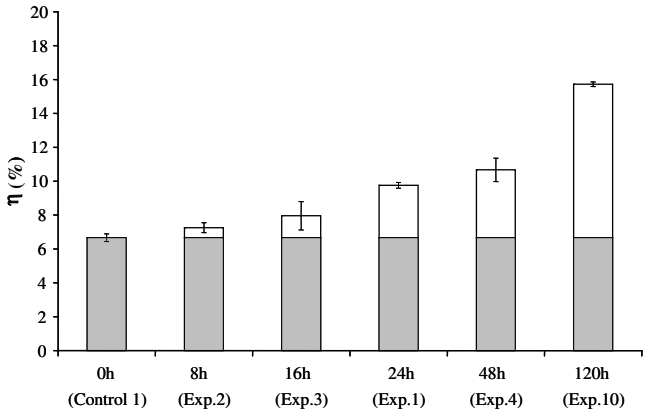

Fig. 1. Extraction yield (%) against pre-enzymatic treatment time. Control 1 refers to untreated samples; remaining parameters (see Table 1): $T = 40$ °C, pH 6, $C = C_1$, and $d_p \in [1.0; 1.4]$.

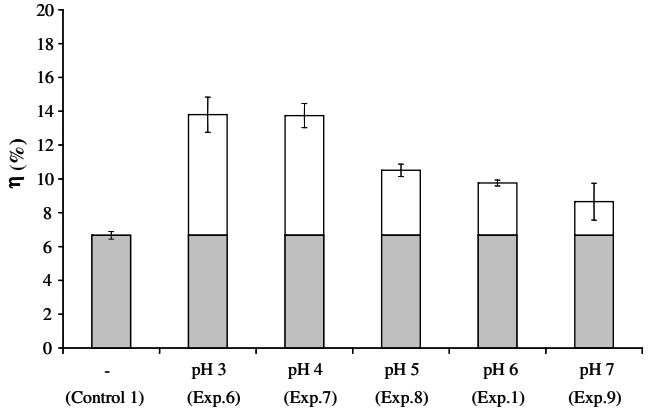

Fig. 2. Extraction yield (%) as function of the pH of the enzymatic treatment. Control 1 refers to untreated samples; remaining parameters (see Table 1): $t = 24$ h, $T = 40$ °C, $C = C_1$, and $d_p \in [1.0; 1.4]$.

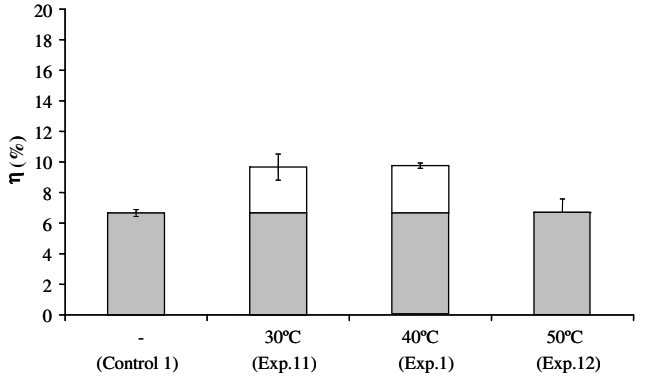

Fig. 3. Extraction yield (%) versus temperature of the enzymatic pre-treatment. Control 1 refers to untreated samples; remaining parameters (see Table 1): $t = 24$ h, pH 6, $C = C_1$, and $d_p \in [1.0; 1.4]$.

3.3. pH effect

The tests carried out to determine how pH affects extraction yield correspond to Exps. 6–9 in Table 1. The remaining parameters have been fixed at $t = 24$ h (already analysed above), $T = 40$ °C, $d_p = 1.0$–1.4 mm, and $C = C_1$. Results may be found in Table 1 and Fig. 2.

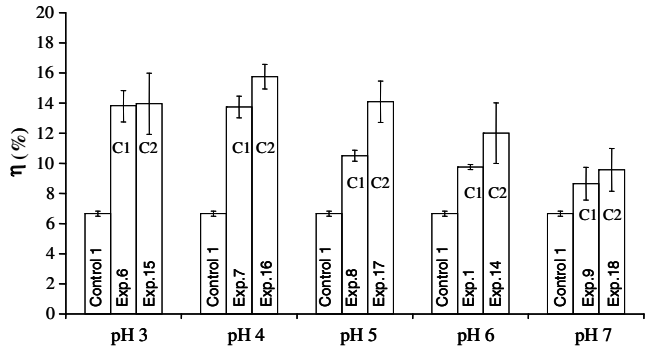

Fig. 4. Extraction yield (%) as function of pH and concentration level (C_1 and C_2) of the pre-enzymatic treatment. Control 1 refers to untreated samples; remaining parameters (see Table 1): $t = 24$ h, $T = 40$ °C, and $d_p \in [1.0; 1.4]$.

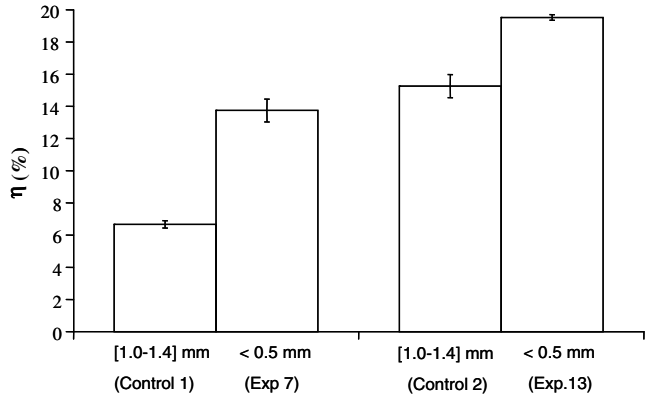

Fig. 5. Extraction yield (%) against granulometry of seed particles. Controls 1 and 2 refer to untreated samples; remaining parameters (see Table 1): $t = 24$ h, $T = 40$ °C, pH 6, and $C = C_1$.

Each enzyme has an optimum pH where its performance is the highest. When several enzymes act together under the same conditions, different behaviours may result. As has been cited above in the Section 2, the advisable individual pHs are: pectinase, 3.5; cellulase, 5; hemicellulase, 4–6; and protease, 7.6. Because it appears somewhat puzzling to establish the proper pH, several values have been investigated.

According to Table 1 and Fig. 2, the smallest increment was assessed for the highest pH tested (pH = 7, Exp. 9) for which $\eta = 8.65\%$ and $\Delta = 29.9\%$. For the remaining pHs, much better results were measured, namely $\Delta = 57.7\%$ at pH 5, $\Delta = 106\%$ at pH 4 and $\Delta = 107\%$ at pH 3. One may conclude that Δ stabilizes around pH 4, where it reaches three digits and starts to approximate the value achieved at pH 6 for the extreme $t = 120$ h ($\Delta = 136\%$).

At pH 4, pectinase, cellulase, and hemicellulase were clearly favoured, whilst protease was out of the optimum pH. Nonetheless, proteins themselves suffer from such pH adjustment, as values far from the isoelectric point destabilize the proteomic matrix and facilitate seed oil removal (Rosenthal et al., 1996). The synergistic effect of the cell wall matrix distortion, along with the enzymatic activity, is responsible for the significant increment cited above.

An extra experiment (Exp. 10, Table 1), combining the best previous conditions, pH 4 and $t = 120$ h, gave $\eta = 17.5\%$, 163% greater than in the control 1 extraction. This result corroborates the expectation, as it surmounts the values of both Exps. 6 and 7.

3.4. Temperature effect

The experiments carried out to evaluate the temperature effect upon extraction correspond to Exps. 11 and 12 in Table 1. Results may be found graphically in Fig. 3.

On the whole, literature reports do not agree with respect to the temperature impact. Although thoughtfully reported as an essential parameter in some oilseeds cases (Dominguez et al., 1994), it has also been shown to exhibit no considerable effect in others (Sarkar et al., 2004). In this work, considerable increments have been found, namely: $\Delta = 45.2\%$ at 30 °C (Exp. 11), $\Delta = 46.5\%$ at 40 °C (Exp. 1, base case), and $\Delta = 0.751\%$ at 50 °C (Exp. 12).

Since a higher temperature requires more energy, the lower value is preferable. Nonetheless, as both 30 °C and 40 °C gave rise to similar results, the optimum may lie in between. In contrast, no visible profit has been observed at 50 °C in relation to control 1 ($\Delta = 0.751\%$), which reveals that some enzymatic activity could have been lost under these conditions.

Regarding time and pH parameters, attempts were made to test a broad spectrum of possibilities. By contrast, concerning temperature, superior values have been excluded. Although higher temperatures would not compromise the quality of the oil itself, high temperatures will inactivate the enzymes, thus excluding the possibility of their recuperation and recycling. For instance, Fullbrook (1983) used only 13 min at 80 °C to achieve inactivation of the enzymes.

3.5. Concentration effect

The effect of the enzymes concentration was studied in Exp. 14 (see Table 1). The remaining parameters were the same as for the base case (Exp. 1): $t = 24$ h, $T = 40$ °C, pH 6, and $d_p \in [1.0; 1.4]$. For the concentration level C_2, the results showed a significant improvement over control 1: $\eta = 12.0\%$ and $\Delta = 80.2\%$, which contrasted with the enhancement of only $\Delta = 46.5\%$ when $C = C_1$, (Exp. 1 in Table 1).

Additional experiments were conducted to analyse the effect of pH upon extraction yield for $C = C_2$, namely Exps. 14–18. Once more, all other parameters (reaction time, temperature, average particle diameter, water/seed ratio and agitation rate) were settled equal to the base case. Experimental conditions are listed in Table 1; results are given numerically in Table 1 and plotted in Fig. 4. The trend of $\eta = \eta(\text{pH})$ for this concentration ($C = C_2$) follows approximately that for $C = C_1$ between pH 4 and 7. At pH 3 no increment due to concentration was detected, as $\Delta(C_1) = 107\% \cong \Delta(C_2) = 110\%$. This result marks pH 4 as an optimum pH, with maximum extractability gain of 137% over non-treated material (Exp. 16).

It is important to emphasise that the concentration may disguise other parameters. Actually, high concentrations may counterbalance the tenuous effect of other non-optimised variables. For instance, higher reaction times require lower enzymatic concentrations and vice-versa. Hence, a compromise should always be proposed, as the cost of the enzymes is fundamental for the economical viability of an enzymatic treatment. (Moreover, their recovery without losing their properties should also be considered). Taking into account that the individual concentrations of C_2 were nearly double or triple those of C_1, and that Δ (C_1; pH 4) = 106% and $\Delta(C_2$; pH 4) = 137%, the lower level C_1 was preferred in this work.

3.6. Particle size effect

The effect of d_p upon the extraction yield was first measured using non-treated milled samples (i.e., control experiments). Accordingly, for $d_p \in [1.0; 1.4]$ mm, $\eta = 6.66\%$ (control 1, Table 1, Fig. 5), when $d_p < 0.5$ mm the yield was 15.3% (control 2, Table 1, Fig. 5), a value 130% higher than the former, highlighting the role of particle size upon oil seed extractability.

To study the impact of d_p upon the enzymatic treatment, the experimental conditions were those from previous analyses, i.e. $t = 24$ h, $T = 40$ °C, pH 4, and C_1. Experiments with particle diameters less than 0.5 mm (see Exp. 13 in Table 1) have also been performed, which implies that control 2 should be considered for comparison.

It is well known that mechanical treatment of grape seed has a large impact on the oil removal, since it breaks plant cell walls, augmenting oil accessibility. Hence, the lower the particle size, the higher is the extraction yield. However, it is not recommendable and sometimes it is almost impossible to process such fine powders industrially. Materials with high oil content and weak structuring collapse when exposed to the flow of solvents and lose their macroporosity, which prevents a uniform and convenient percolation. Furthermore, frequently there appear to be wettability problems, also.

When particles with $d_p < 0.5$ mm were treated under conditions of Exp. 13, a $\eta = 19.5\%$ was achieved, which means an increment $\Delta = 193\%$ over control 1. The calculated enhancement due to the enzymatic action stands clearly behind this value, as $\eta = 19.5\%$ surmounts control 2 by only 27.5% (Fig. 5). This result is due to the fact that many oil bodies of the smaller particles have already been exposed after milling. Nonetheless, the enzymatic treatment still holds its significance for $d_p < 0.5$ mm.

Most of the earlier studies on enzymatic treatment did not take particle diameter into account. When aqueous treatments are being accomplished, intact seeds are frequently immersed in the aqueous medium and ground *in situ*, with no further classification. However, the present research underlines that this parameter is absolutely unavoidable, and that misleading conclusions can be drawn when comparisons between results obtained with treated/untreated samples are being made.

4. Conclusions

The yield of oil seed extraction may be enhanced with combinations of mechanical and/or enzymatic pre-treatments. Although mechanical processing gives rise to faster and cheaper results, there is a limit to handling small particle diameters at the industrial level. This fact induced us to study the effect of an enzymatic treatment to increase the extraction of grape seed oil.

Experiments have shown that the output increases after increasing both concentration and treatment times. By contrast, pH and temperature give rise to opposite behaviour. Regarding the concentration of the enzymatic cocktail, the lowest level studied (i.e., C_1: cellulase = 29, protease = 1191, xylanase = 21, and pectinase = 569 U/g seed sample) accomplished very promising results. Nonetheless, a precise economical evaluation must be performed in order to select an appropriate concentration due to the cost of the enzymes. Concerning particle size, the enzymatic treatment has a greater impact on larger diameters. On the whole, the following optimised set of parameters for grape seed oil extraction is proposed: $t = 24$ h, pH 4, temperature 30–40 °C, particle diameters in the range 1.0–1.4 mm, and concentration C_1. Under these conditions, the extraction yield was 13.7%, which represents an increment of 106% over non-treated samples. The best result was assessed for $t = 120$ h, pH 4, $T = 40$ °C, $d_p = 1.0$–1.4 mm, and concentration C_1: yield was 17.5% and increment over control was 163%.

This demonstrates that an enzymatic pre-treatment may certainly be adopted, with quantitative advantage, to enhance grape seed oil extraction. This conclusion is different from the results of many previous publications, in view of the fact that they present quite disappointing yields, generally 2–15%.

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