Enzymatic Pretreatment on Rose-Hip Oil Extraction: Hydrolysis and Pressing Conditions

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ABSTRACT: In a previous report [Zúñiga, M.E., J. Concha, C. Soto, and R. Chamy, Effect of the Rose Hip (*Rosa aff. rubiginosa*) Oil Extraction Cold-Pressed Process, in *Proceedings of the World Conference and Exhibition on Oilseed Processing and Utilization*, edited by R.F. Wilson, AOCS Press, Champaign, 2001, pp. 210–213], the authors showed that an enzymatic pretreatment of rose-hip seeds, prior to oil extraction by cold pressing, improves the oil yield. In this work, we studied the effects of temperature and moisture during the enzymatic hydrolysis stage using two previously selected mixtures of commercial enzymes: (i) Olivex (mainly pectinase) plus Cellubrix (mainly cellulase), and (ii) Finizym (mainly β-glucanase) plus Cellubrix (mainly cellulase) (all from Novozymes A/S, Madrid, Spain). In addition, we evaluated the effect of enzymatic hydrolysis on the oil extraction pressing rate at different operational pressures. Samples hydrolyzed enzymatically by either of the two commercial enzyme mixtures at 45°C and 30–40% moisture showed oil extraction yields up to 60%, an increase of greater than 50%, as compared with control samples in which the enzyme solutions were replaced by water. Both the oil extraction rate and yield by pressing increased when enzymatic pretreatment was applied. The oil extraction yield increased slightly when the operation pressure was elevated; however, when the sample was preheated, the oil extraction yield was greatly increased, especially for enzyme-treated samples. Results confirmed the importance of temperature and moisture as enzymatic hydrolysis parameters that improve rose-hip oil extraction yields in the cold-pressing process. When pressing was carried out after preheating enzymatically treated samples, it was possible to increase the oil extraction yield to 72% compared with the control without preheating, which resulted in a 46% oil yield.

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KEY WORDS: Cold pressing, enzymatic extraction, oil extraction, *Rosa aff. rubiginosa*, rose-hip oil.

Rose-hip oil has excellent cosmetic properties. It is mainly composed of linolenic, linoleic, and *trans*-retinoic acids. The conventional oil extraction process is performed by means of organic solvents, in which the high operation temperatures (80°C or higher, reduce the content of *trans*-retinoic acid, usually known as pro-vitamin A. Cold mechanical expelling is an alternative oil extraction process, but it has a low yield, although this may be improved by an enzymatic pretreatment.

The use of enzymes in the oil extraction process has been studied by several authors (1–8). The effect of enzymatic pretreatment depends on the structure of the oilseed and the composition of the cell wall; thus, it varies according to the kind of oilseed and type of enzyme used (1). For rose-hip oil extraction, there have been reports on the effectiveness of enzymatic treatments prior to pressing in increasing the oil yield (2).

Most of the studies on enzyme-assisted oil extraction processes have been performed using aqueous extraction. This technique is frequently applied in rural oil extraction operations, as has been described by several authors (3–8). In aqueous processes, the enzymatic action improves oil recovery by degrading the seed cell wall, resulting in the rupture of the polysaccharide–protein colloid system.

In enzyme-assisted oil extraction using cold pressing, the enzyme acts only by hydrolyzing the cell wall, because in this nonaqueous system there is no polysaccharide–protein colloid. The influence of variables such as temperature, enzyme concentration, reaction time, enzyme–substrate ratio, homogeneity, and agitation, among others, is not necessarily equal to that of aqueous oil extraction. Furthermore, protective effects on enzyme reactivity and stability are provided by a low-water-activity medium (9).

A high level of moisture could reduce the reaction time required to obtain higher oil yields by facilitating the enzymatic degradation of seed cell walls. However, a high moisture content increases the energy costs involved in the subsequent drying stage (10,11), which is necessary for adequate performance of the pressing operation.

The aim of this work is to analyze the effects of enzymatic hydrolysis conditions, such as temperature and moisture, on the yield of rose-hip oil by pressing, and also the effect of enzymatic treatment on the performance of the pressing operation. These experiments were performed using two enzyme mixtures previously selected by the authors (2), Cellubrix–Olivex (CO) and Cellubrix–Finizym (CF). As reported previously (2), the main difference between these mixtures is their pectinolytic activity, which is present in only the Olivex formulation. Pectinase activity can have an important role in facilitating an enzyme's access to its respective substrates, especially cellulose and hemicellulose, which are in the inner cell wall layers.

MATERIAL AND METHODS

Enzymes. Three commercial enzymes were used: Cellubrix (mainly cellulase and hemicellulase activities), Finizym

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(mainly betaglucanase, cellulase, and hemicellulase activities), and Olivex (mainly pectinase, cellulase, and hemicellulase activities), all supplied by Novozymes A/S (Madrid, Spain). Two enzyme mixtures, CF and CO, were prepared using the commercial enzymes, as previously selected by Zúñiga *et al.* (2).

Seeds. Rosa aff. rubiginosa seeds were supplied by Loncopan S.A. (Santiago, Chile). Proximal composition of the seeds was reported previously by Zúñiga *et al.* (2).

Analytical methods. Oil and moisture determinations were conducted as reported previously (2).

Experimental procedure. The enzyme-assisted rose-hip oil extraction process is shown in Figure 1 and was explained in a previous report (2).

Hydrolysis conditions. The CF was prepared by mixing equal mass amounts of each enzyme. The mixture concentration during usage was 0.01 g enzyme on a wet basis per gram of dried substrate. The CO mixture was prepared by mixing Cellubrix and Olivex in a 1:3 mass ratio. The latter was used at 1.5% wet weight per weight of dried substrate. The enzyme combinations were previously selected by the authors (2). In this work, these two enzyme mixtures were selected to determine how pectinase activity affects the oil extraction yield.

For both enzyme mixtures, the effect of hydrolysis temperature was evaluated in the range of 35 to 55°C. The effect of moisture was analyzed in a range of 20 to 55%. The desired moisture levels were obtained by predissolving the enzyme in an appropriate amount of water and adding it to a meal sample. The hips themselves were determined to have a moisture content of 7%. In all the experiments, water was added instead of enzyme solution in control samples. The effect of the enzymatic treatment on the pressing stage was evaluated at operating pressures between 24.5 and 53.9 MPa with a hydraulic laboratory press (Carver Press, Wabash, IN). In some experiments, the samples were preheated for 5 min at 70°C before pressing.

Statistical analysis. Data are reported as means ± SD (n = 3). ANOVA (12) was used to determine significant differences between groups, considering a level of significance of less than 5% ($P < 0.05$).

RESULTS AND DISCUSSION

Figure 2 depicts the effect of enzymatic treatment temperature on the amount of oil extracted as compared with the control for both enzymatic mixtures. The effect of hydrolysis temperature was similar for both enzyme mixtures, with an optimal temperature of 45°C. This temperature was selected for subsequent experiments.

The increase in oil yield was lower at 55°C compared with the other temperatures analyzed. This result cannot be explained in terms of enzyme inactivation, because Cellubrix, Finizym, and Olivex are reportedly stable at 55°C (13). A reduction in oil yield could be caused by an increase in the content of soluble reducing sugars caused by enzymatic hydrolysis. These sugars would caramelize in the subsequent drying stage. The same effect of temperature on hydrolysis has been reported previously for oil extracted from *Guevina avellana mol* (14).

The effect of moisture during enzymatic treatment for both enzyme mixtures is presented in Figure 3. The reaction times of 6 h for the CO mixture and 9 h for the CF mixture were selected to maximize oil extraction yields. Using the CF mixture with 30% moisture resulted in a maximal oil extraction yield increase of 36% ($P < 0.05$) in comparison with the control reaction, which had 47% oil extraction. These values changed to 31 and 23% increases in oil recovery when the CF treatments were performed at 40 and 20% moisture, respectively. For seeds treated with a CO enzyme mixture, the best oil recovery was observed when hydrolysis was carried out at 30% moisture,

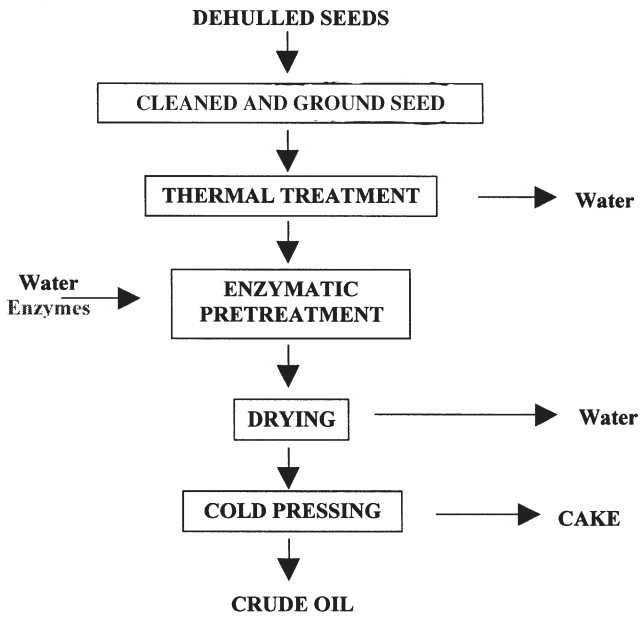

FIG. 1. The enzyme-aided oil extraction process.

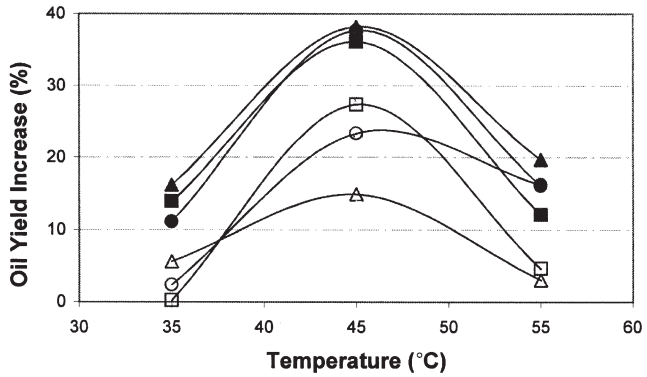

FIG. 2. Effect of enzymatic pretreatment temperature on rose-hip oil extraction yields. Hydrolysis treatments were done with an enzyme/substrate (E/S) ratio of 1.0% (w/w) and 30% moisture for a mixture of Cellubrix and Finizyme (Novozymes A/S, Madrid, Spain) (open symbols), and at an E/S ratio of 1.5% (w/w) and 30% moisture for a mixture of Cellubrix and Olivex (Novozymes A/S) (solid symbols). Pretreatment periods: 6 (△,▲), 9 (□,■), and 12 h (○,●).

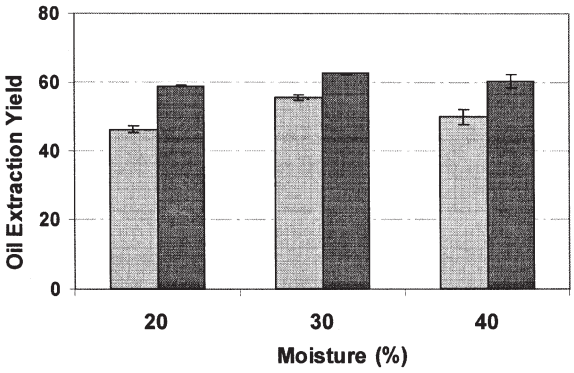

FIG. 3. Effect of moisture on rose-hip oil extraction yield by pressing after treatment with an enzyme mixture. Hydrolysis treatments were done with an E/S ratio of 1.0% (w/w) at 45°C in a 9-h reaction for the Cellubrix–Finizyme mixture, and at an E/S ratio of 1.5% (w/w) at 45°C in a 6-h reaction for the Cellubrix–Olivex mixture. Light gray columns: Cellubrix–Olivex; dark gray columns: Cellubrix–Finizyme. For abbreviation and supplier of enzymes see Figure 1.

obtaining a 17% increase in oil extraction after 6 h of treatment relative to that obtained using seeds with 20% moisture ($P < 0.001$).

Other authors (11,14,15) have reported an optimal moisture level for enzymatic hydrolysis of less than 45% when an enzyme-assisted cold-pressing process was applied. Using the same press, Smith *et al.* (15) obtained an optimal moisture of 23% in soybean oil extraction using an enzyme-aided pressing process. The cited work was carried out by using a surface-response experimental design but with pressing times lower than 7 min, which, in our experience, is insufficient to stabilize the operation of equipment. In canola oil extraction using enzymes (11), when enzymatic hydrolysis was performed at moistures between 30 and 50% (w/w), the oil extraction yield was nearly 42%. Additionally, in *G. avellana mol* oil extraction, the best yield resulted when the enzymatic reaction moisture was 45% w/w (14).

As presented in Table 1, the maximal oil extraction yield of 64%, for a 1.5% enzyme/substrate (E/S) ratio of the CO mixture (25:75, w/w) with preheating, was obtained at 53.9 MPa, as compared with the control value of 56.6% ($P < 0.05$). For a 1% E/S ratio of the CF mixture (50:50, w/w), the best oil recovery was 74% ($P < 0.001$) at 49 MPa, and a similar value of 71.2% was obtained when 39.2 MPa was applied ($P < 0.001$), and at 44.1 MPa. Figure 4 displays the effect of preheating the samples on oil extraction kinetics. Experiments with the CF mixture were performed at 44.1 MPa for cold-pressing and 39.2 MPa for preheated samples. In both experiments, i.e., pressing with and without preheating, the best results were observed at 20 min of pressing, with 70 and 50% of oil extraction, respectively, independent of the preheating treatment.

For the CO mixture, the optimal period for rose-hip oil extraction was 20 min, with an oil extraction yield of 64.2%. Nevertheless, for periods less than 20 min, there were no significant differences in the results obtained. Periods of pressing less than 10 min are not recommended because short pressing periods could produce errors in experimental performance.

With both enzyme mixtures, treatments resulted in about 70% oil extraction yield, which is comparable to industrial rose-hip oil recovery by conventional hexane extraction.

In conclusion, the enzymatic treatment is more effective when the enzymes are applied together with a preheating of the enzyme-aided samples at previously selected hydrolysis conditions. Although the hydrolysis temperature and moisture are important factors influencing the oil extraction yield, other variables, such as the type of enzyme, also could affect oil extraction.

Even though CF was a considerably more effective pretreatment, these results showed no statistically significant differences

TABLE 1
Effect of Pressure on Rose Hip Oil Extraction by Pressing[a]

	E1		E2	
MPa	Control	With enzyme	Control	With enzyme
		Cold pressing		
24.5	27.27 ± 1.37	29.62 ± 0.37	—-	—
34.3	35.67 ± 4.18	45.15 ± 1.78	—	—
39.2	—	—	47.91 ± 0.25	57.22 ± 2.54*
44.1	40.43 ± 2.21	55.67 ± 0.77**	50.36 ± 0.73	62.51 ± 0.43***
49.0	—	—	56.71 ± 1.01	57.88 ± 0.31
53.9	47.51 ± 0.82	60.54 ± 2.64*	—	—
		With preheated matter		
24.5	38.90 ± 2.38	46.76 ± 0.50*	—	—
34.3	45.22 ± 0.47	49.47 ± 0.68**	—-	—
39.2	—	—	44.10 ± 0.25	71.18 ± 3.76**
44.1	52.63 ± 0.43	60.40 ± 1.03***	51.14 ± 1.65	72.35 ± 1.87***
49.0	—	—	61.37 ± 2.35	73.96 ± 1.33**
53.9	56.61 ± 2.02	64.19 ± 0.36*	—	—

[a]Enzymatic treatment was conducted at 45°C and 30% moisture using a Cellubrix–Olivex mixture (Novozymes A/S, Madrid, Spain) at a 1.5% enzyme/substrate (E/S) ratio (E1) for 6 h, and a Cellubrix–Finizym mixture (Novozymes A/S) at a 1.0% E/S ratio for 9 h (E2). Values are presented as mean ± SD ($n = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control.

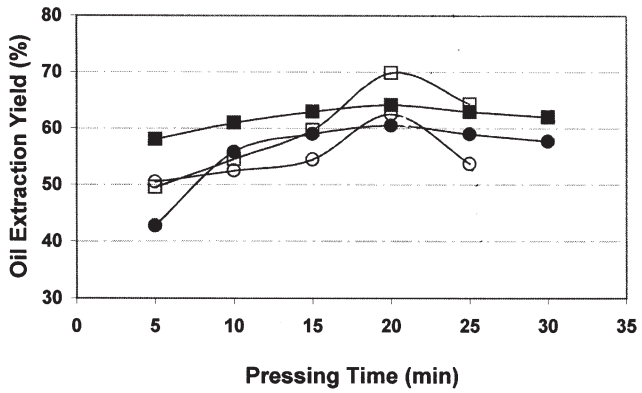

FIG. 4. Rose-hip oil extraction kinetics obtained by pressing after enzymatic pretreatment using mixtures of Cellubrix–Finizyme (open symbols) and Cellubrix–Olivex (solid symbols) at selected conditions. The pressing process was completed with samples without preheating (○,●) or using preheated samples (□,■).

between the CF and CO enzyme mixtures in improving oil extraction yields. This result confirms that pectinolytic activity is not required to enhance the action of other carbohydratases as cellulases and hemicellulases to produce greater rose-hip oil extraction yields in the enzyme-assisted cold-pressing process. This fact may be justified, because rose-hip fiber is composed mainly of cellulose and hemicellulose and contains a minor proportion of pectin.

Although enzyme-aided rose-hip oil extraction by pressing has not been applied industrially, our results show that this process allows an increase in the oil recovered, making this technology an alternative to conventional hexane oil extraction and a cleaner and less toxic option.

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