Enzymatic Extraction of Oil from *Gevuina avellana,* the Chilean Hazelnut

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ABSTRACT: Chilean hazelnut *(Gevuina avellana)* oil is highly appreciated in the cosmetic and pharmaceutical industries. Hazelnut oil (oil content calculated on 49% dry basis) is traditionally obtained by pressing, a low-efficiency process that results in a low-quality product. In this work, the conventional process was compared with two enzymatic alternatives in which commercial enzymes were used to increase the oil extraction yield: (i) extraction in aqueous medium and (ii) extraction by pressing after an enzymatic treatment. The effect of various parameters on the extraction yield was studied to define the most satisfactory processing conditions. These included reaction time, temperature, enzyme concentration, and, in the aqueous medium extraction process, the water/seed ratio, particle size, and pH. Although pressing is the better alternative, in both processes enzyme treatment improved extraction yields (94 and 98% for aqueous medium extraction and pressing after enzyme treatment, respectively, compared to 52% obtained in the conventional process). Moreover, the quality of the oil obtained is the same as or better than that of oil obtained by the conventional process.

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KEY WORDS: Enzymatic extraction, enzyme-based oil extraction, *Gevuina avellana,* hazelnut oil.

Gevuina avellana, the hazelnut tree, is native to Austral Chile. The seed, which is the edible portion of the fruit, contains high protein and lipid contents, 12.4 and 49.3% on a dry basis, respectively (1). Owing to its high cost, hazelnut oil cannot compete with soybean or cotton oil for human consumption. However, because of its physicochemical properties, it is best suited for applications in cosmetology, as it absorbs short-wavelength UV radiation, allowing the passage of only that radiation producing a suntan without damaging the skin (2,3). Hazelnut oil contains a high percentage of unsaturated FA, so the skin readily absorbs it. For this reason, it is one of the major ingredients in the formulation of products such as suntan protective lotion and other protective creams (2,3); it is also used as a vehicle in the design of transport systems for skin nutrients and protective and regenerating substances in, e.g., creams, soaps, and shampoos (4,5).

Chilean hazelnut oil is traditionally obtained by pressing. Before pressing, hazelnuts are peeled, to eliminate the skin and the cuticle, ground, and heat-treated. Oil and the residual hazelnut paste are the products of pressing. The oil extraction yield of this process is low, and the low-quality paste so produced has a high residual oil content (6).

Enzymes have been applied to the extraction of oil from several fruits and vegetables: in aqueous processes (7–13), in a system of low humidity for a later solvent extraction (14,15), or by pressing (16). In the present work, the oil extraction yield from Chilean hazelnuts obtained in two processes involving enzymes is compared with the conventional extraction process. In the first process an aqueous treatment is proposed, whereas in the second the enzymes are added to the hazelnuts prior to pressing.

MATERIALS AND METHODS

Hazelnut seeds were obtained from the south of Chile, separated from their rinds and husks, ground in a coffee mill or a mixer, and sieved to a particle size ≤1.4 mm. Total oil in the seed was measured by the Soxhlet extraction method (17). Three processes for oil extraction were performed, and the results were compared: conventional (cold pressing), pressing with enzymatic pretreatment, and enzymatic aqueous processing. In a preliminary assay, the hazelnut mass was pretreated with enzymes to evaluate their effects on oil extraction, using three commercial enzymes: Olivex, Ultrazym, and Celluclast, all from Novozymes (Bagsvaerd, Denmark). The first two are cocktails designed to digest vegetable cell walls; they combine several activities including cellulase, pectinase, and hemicellulase; the last one contains a cellulolytic complex. Enzyme concentration is reported as vol/wt, expressed as % (where 1% = 1 mL/100 g, for example) as they are provided in liquid form (vol) and are applied to a solid (wt) or referred to the mass of substrate. The overall enzymatic activity was measured by following the release of reducing sugars from the tissue with the dinitrosalicylic acid (DNS) method (19). All experiments were carried out in at least triplicate. The SD for our results never exceeded more than 5%. The flow diagram for these processes is shown in Scheme 1.

Conventional extraction. Samples of hazelnut seeds were used in a classical oil extraction. The seeds were dried at 60°C to 3–5% humidity and were then pressed in a hydraulic press (Carver Laboratory Press; Fred S. Carver Inc., Wabash, IN) at 39.2 MPa for 15 min at room temperature. The oil extraction yield was reported as the difference between total and residual oil determined by the Soxhlet extraction method.

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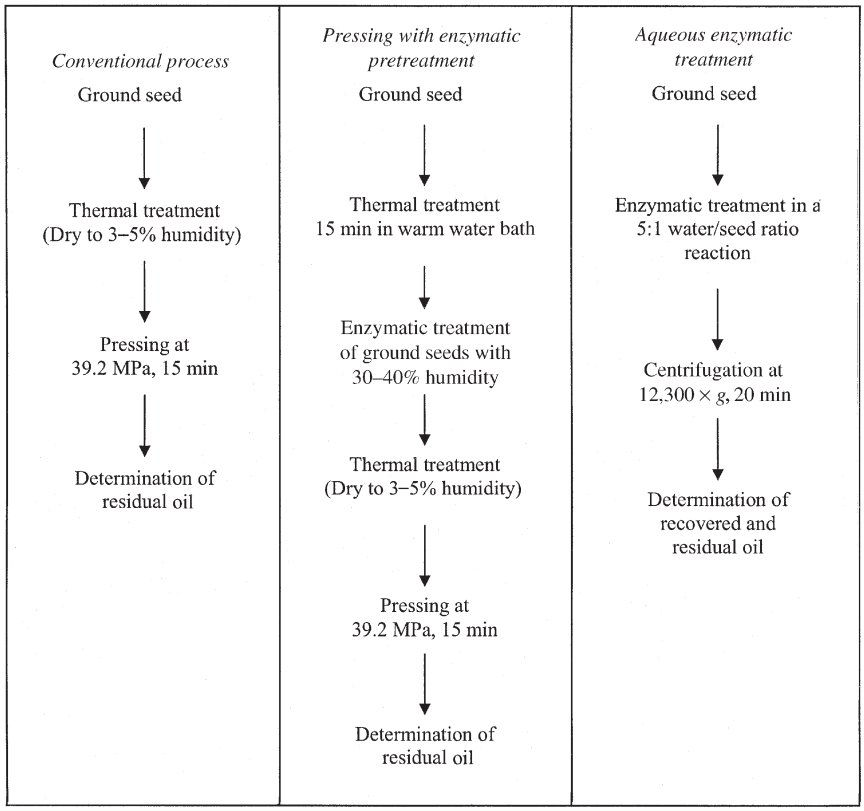

SCHEME 1

Pressing extraction. The ground seed was treated by immersion for 15 min in a boiling water bath, and the moisture content was adjusted to 35–40%. The wet mass was then treated with a mixture of enzymes (Ultrazyme and Celluclast in equal proportions—1:1 vol/vol ratio). After the enzymatic treatment, the seeds were placed in a conventional oven at 100°C for 20 min to inactivate the enzymes and dried to 3–5% humidity (18). The treated seeds were then subjected to 39.2 MPa for 15 min in the hydraulic press. The oil extraction yield was determined by comparing total and residual oil contents. Other parameters were studied, including enzyme concentration (0.1–2% vol/wt), temperature (35–60°C), and reaction time up to 12 h, as well as pressing conditions such as cold pressing, double cold pressing, warm pressing, and double warm pressing. For the cold pressing, all the process was carried out at room temperature, whereas for the warm pressing, the pieces of the press were warmed in a conventional oven and the seeds immediately pressed in order to keep the temperature at around 50°C. Double pressing was carried out discontinuously. In all cases, controls without enzymes were performed.

Aqueous extraction. Ground seeds were transferred to a reactor with water and a combination of enzymes (Olivex and Celluclast—1:1 ratio) and mixed by means of a magnetic agitator. After the enzymatic reaction, the total volume was transferred to a boiling water bath for 15 min to inactivate the enzymes and centrifuged 20 min at 12,300 × *g* at room temperature. Four phases were obtained and separated: recovered oil, an emulsion, an aqueous phase, and a pellet of insoluble material. Oil extraction was determined both by weight of the extracted oil and by comparison of the residual oil in the solid phase with the original total oil in the seed. The effects of enzyme concentration (0.1–2% vol/wt), incubation time (up to 12 h), temperature (35–60°C), pH of reaction (4 to 6.2), water/seed ratio (3–6), and particle size (0.4 to >1.4 mm) were analyzed. In all cases controls in the absence of enzymes were performed.

RESULTS AND DISCUSSION

Several enzymes are capable of improving the extraction of Chilean hazelnut oil when compared to the traditional extraction process. In initial experiments, the use of a combination of activities, such as cellulase, hemicellulase, xylanase, or pectinase, was more efficient than the application of a single enzyme. For instance, in the aqueous process maximal oil extraction was obtained with the combination of equal mass proportions of Olivex and Celluclast, whereas for pressing the best results were obtained when a mixture of Ultrazym and Celluclast was applied.

The oil yields obtained in the three processes are shown in Figure 1. The reactions were carried out at pH 6.2, the pH of the ground seed, and with average particle size of 1.4 mm. Controls without enzymes were included. For the aqueous process a water/seed ratio of 5:1 w/w was used with 1% vol/wt of an equal proportion of Olivex and Celluclast at 45°C for 12 h. In the high-humidity enzyme treatment scheme, equal proportions of Ultrazym and Celluclast were

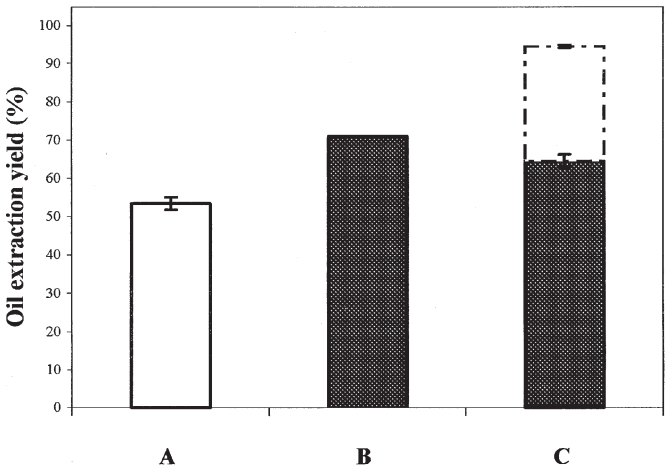

FIG. 1. Oil extraction yields obtained in (A) conventional pressing process, (B) pressing with enzymatic pretreatment, and (C) aqueous enzymatic extraction process (the dotted lines represent the total extraction yield); no oil was recovered in the aqueous process without addition of enzymes. (See text for reaction conditions.) Error bars represent SD.

used in a treatment for 6 h at 35°C at 1% vol/wt of seeds adjusted to 35–40% humidity prior to pressing. Under these pH and temperature conditions, the commercial enzymes are reportedly stable. As shown in Figure 1, the yield of recovered oil in the pressing process increased from 53 to 71% when the enzymatic treatment was included in the process. In the aqueous extraction process, the improvement was even higher, with total oil yield almost 94% when calculated based on the residual hazelnut oil content. However, only 62% was recovered after centrifugation, losing 32% oil in the process. This is probably due to the formation of an emulsion stabilized by the soluble proteins of the hazelnut. It is important to point out that the extraction of oil from hazelnuts by an aqueous process absolutely requires enzymes, as no oil was released in the control experiments without enzymes.

The dependence of the process on reaction time is obviously important. In the pressing process the effect of pretreatment with enzymes for 1, 2, 3, and 4 h was examined. Maximal extraction yield was obtained at 3 h of pretreatment with enzymes. In the aqueous process a linear correlation between extraction yield and reaction time was found out to 12 h of treatment, when 65% of the oil was recovered under the above-mentioned reaction conditions. Longer times did not result in yield improvements, while 18, 40, and 55% yield were obtained after 3, 6, and 9 h, respectively. In some experiments shorter reaction times were selected for comparison purposes.

As demonstrated in previous reports dealing with enzymatic extraction processes (8,9,12), two fundamental parameters in this type of process are the water/seed ratio and the average particle size. The effects of these two parameters are shown in Figure 2, where it may be observed that after 9 h of reaction the highest extraction yield is obtained with a 5:1 water/seed ratio using seeds ground to a particle size of approximately 0.4–0.6 mm diameter.

The effects of temperature and pH on extraction yield were also studied in the region where commercial enzymes are reportedly stable. In the aqueous process the maximal yield was obtained at pH 4.5, with lower but not significantly different yields in the range of pH 4 to 5.5. The optimal temperature was 45°C in the range of 35 to 60°C. Lower temperatures result in lower extraction yields, but higher temperatures affect enzyme stability. In the pressing process, the pH of the ground hazelnuts was not modified, and the maximal yield was observed when the extraction was carried out from seeds pretreated at temperatures in the 35–45°C range.

The effect of enzyme concentration in the processes was also studied. Experiments analyzing the concentration range of 0.1–2% were performed in both processes using similar ratios of the selected enzymes: Olivex and Celluclast for aqueous process and Ultrazym and Celluclast for pressing. The results demonstrated that in both cases a dose higher than 0.5% vol/wt of enzymes is unnecessary.

The main difficulty found in the aqueous extraction process was the formation of an emulsion after centrifugation

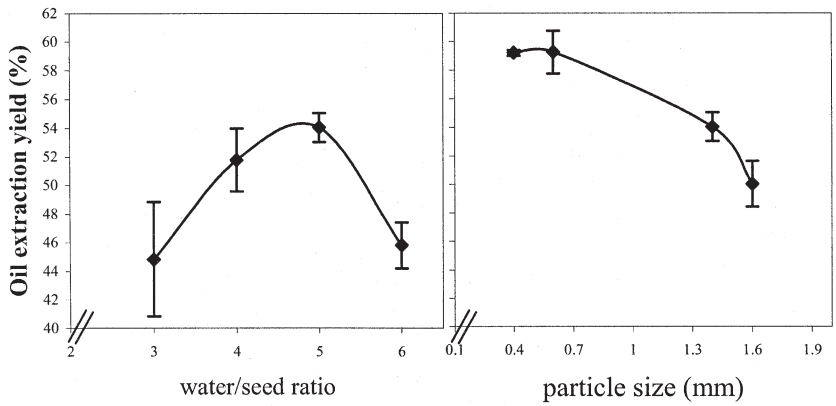

FIG. 2. Effect of water/seed ratio and particle size on extraction yield in the enzymatic aqueous extraction process of oil from the Chilean hazelnut (reaction time: 9 h at 45°C; particle size 1.4 mm for the water/seed ratio experiment and 5:1 water/seed ratio for the particle size experiment). See text for reaction conditions. Error bars represent SD. A line is drawn through the average measurement at each particle size.

in all reaction conditions explored. Under the best reaction conditions found, from a total extraction yield of 94% measured from the residual oil content in the hazelnuts, only 64% was recovered after centrifugation, and 30% (by difference) remained in the emulsion after centrifugation. This phenomenon has already been observed in fruits and other seeds (11,12). To increase the recovered yield in the aqueous extraction process, emulsion formation must be avoided. Treatments of the emulsion with organic solvents or other physical means have been proposed (11,20), but this may limit the feasibility of the enzymatic aqueous process. Owing to this limitation, in the case of the Chilean hazelnut, pressing seems the better alternative. Further optimization of the process was conducted by analyzing the effect of the pressing temperature and of a double pressing in the extraction yield. The following oil extraction yields (%) were obtained under these pressing conditions: cold, 53.0; cold with enzymes, 71.5; double cold, 80.0; double cold with enzymes, 85.7; warm, 52.9; warm with enzymes, 74.0; double warm, 89.1; and double warm with enzymes, 98.6 (pressing conditions: 39.2 MPa for 15 min. Enzymatic pretreatment for 6 h at 30°C with 0.5% vol/wt seed of Ultrazym and Celluclast. SD < 5%). In these experiments the enzymatic treatment was carried out for 6 h at the pH of the seed using Ultrazym and Celluclast, both at 0.5 % vol/wt, and a seed particle size of 1.4 mm. Enzymatic treatment improved oil extraction in all the conditions studied. Also, double pressing substantially improved yield. The extraction yield was more dependent on pressing conditions than on enzymatic treatment conditions, although the combination of both strategies resulted in extraction yields as high as 98.6%.

Concerning the oil quality, the peroxide index (7) of the oil obtained in a control extracted with ethyl acetate was actually higher (16.03 meq) than the one obtained with the aqueous enzymatic process (10.3 meq).

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