# Enzyme-Aided Alternative Processes for the Extraction of Oil from *Rosa rubiginosa*

Sir:

*Rosa rubiginosa* seed oil is valuable for cosmetics and pharmaceuticals as a reducer of dermal scars, color spots, and wrinkles from the skin surface. Although vast land areas in Chile are committed to this crop, the seeds are still underexploited or, in the best case, exported for processing. The value of the oil, the lack of requirements of fertile soils for growth, and the development of efficient and safe oil extraction processes will facilitate agro-industrial expansion of the producing areas.

Hexane is the usual solvent for oil extraction from low oil content seeds, but concerns about safety, health, and the environment have fostered research on substitution for sustainable options such as water and alcohols. Aqueous processing includes milling the seeds, mixing with water to carry out the emulsified oil, and centrifugal separation of the liquid and solid phases. The oil extraction efficiency can be enhanced through cell wall degradation by enzymes. Enzyme treatment can be accomplished during mixing as the operational conditions are highly compatible (1–4). Alcohols are the best alternative to hexane, particularly ethyl alcohol and isopropyl alcohol, because they can be used in the existing hexane extracting facilities (5,6). Chill-separation and reverse osmosis lower the energy requirements for oil separation and solvent recovery, but the low oil solubility and the sensitivity to the moisture content of the materials to be extracted are major disadvantages. Pressing is an environmentally friendly process; exposure of products to high temperatures, which could affect their quality, and residual oil in the meal are aspects that could be improved by the incorporation of an enzymatic treatment prior to extraction. Enzyme treatment has been successfully applied at low and intermediate moisture conditions during pressing and solvent extraction (7–10).

Three alternative hexane-free and enzyme-aided extraction processes of *R. rubiginosa* seeds were compared. *Rosa rubiginosa* seeds, supplied by Forestal Casino Ltda. (Santiago, Chile), were cracked or ground to the desired particle size and then stored at 4°C. Proximate composition of the seeds (dry basis) was 9% oil, 6% protein, 77% neutral detergent fiber, 6% pectic substances, and 2% ash. The conditions during the enzymatic treatment were defined to be compatible with the three oil extraction technologies studied (Fig. 1). Before cold batch pressing, the cracked seeds (<6 mm) were treated at 30% moisture with Finizym/Cellubrix (1:1) enzyme mixture (Novo Nordisk Bioindustries AS, Madrid, Spain) for 9 h at 45°C. Samples were subjected in a batch press to 450 kg/m$^2$ for 20 min, after adjusting the moisture content to 11.1%. The expressed oil yield was reported as the difference between the initial and residual oil, determined by Soxhlet extraction. Before ethanol extraction, the ground seeds (1–2 mm) were treated with Finizym/Cellubrix (1:1) at low

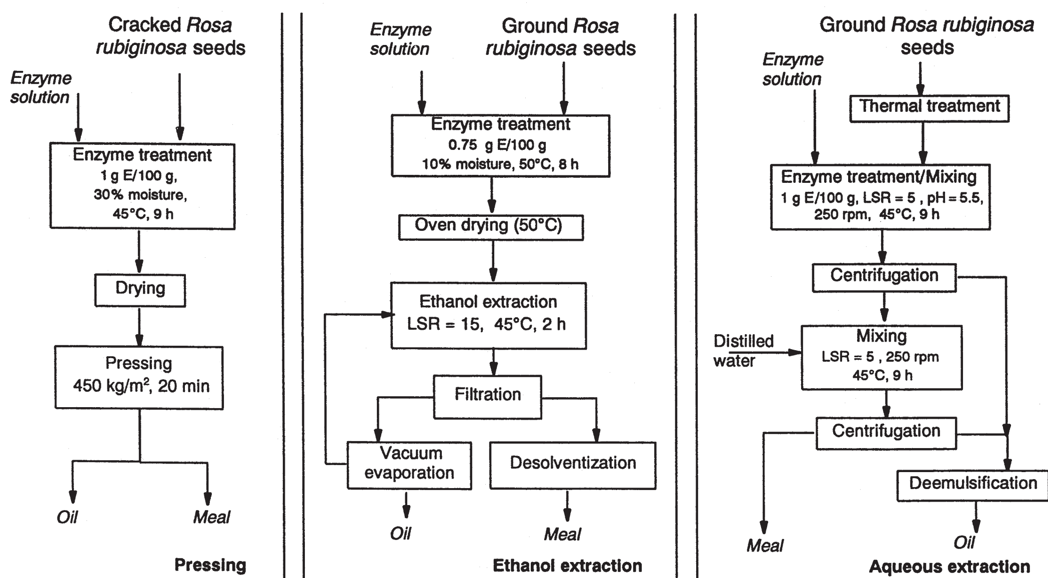


**FIG. 1.** Flow diagram of the three hexane-free processes for the oil extraction from *Rosa rubiginosa* seeds. LSR, liquid/solid ratio.

Paper no. J9814 in *JAOCS 78*, 437–439 (April 2001).

Copyright © 2001 by AOCS Press 

**TABLE 1**
**Oil Extraction Yields of *Rosa rubiginosa* Caused by the Enzymatic Treatment During Pressing, Ethanolic and Aqueous Extraction**

| | Oil extraction yield (% total oil)[a] | |
|---|---|---|
| | Control[b] | Enzyme treated |
| Cold batch pressing | 42.7 ± 0.8 | 64.1 ± 1.2 |
| Ethanolic extraction | 89.8 ± 0.2 | 92.5 ± 0.5 |
| Water extraction | | |
| 1st stage | 37.3 ± 1.5 | 47.3 ± 1.4 |
| 2nd stage | 59.6 ± 3.5 | 61.7 ± 3.2 |

[a]Mean of three experiments ± standard deviation.
[b]Control indicates incubated with water instead of enzymes.

moisture (10%) and then dried at 50°C (until 3% moisture was reached). Extraction was carried out with 96% ethanol at a liquid/solid ratio (LSR) of 15 g/g in capped bottles at 45°C under static conditions. Defatted meals were separated by filtration from the ethanolic miscella, which was then subjected to vacuum evaporation to determine the oil extraction yield by weight difference. During aqueous extraction the ground seeds (<0.5 mm) were thermally treated in order to inactivate endogenous enzymes. Then they were mixed with water (pH = 5.5) and with the Finizym/Olivex mixture at an LSR of 5 g/g and continuously stirred at 250 rpm for 9 h. Two extractions were performed, and phase separation was accomplished by centrifugation. The oil extraction yield was calculated as the difference between the initial and the residual oil in the solid, measured by Soxhlet extraction. Nitrogen content of meals and cakes was assayed by Kjeldahl, and the crude protein value was obtained by multiplying by 6.25. *In vitro* digestibility was measured with the apparent digestibility coefficient (ADC) (11). Gravimetric analyses were performed for determining detergent fiber content and soluble fiber.

Table 1 summarizes the oil yield from control and enzyme-treated seeds with the three hexane-free extraction processes. Maximal improvement was obtained during the cold batch pressing due to the low oil yield of the control. The destruction of the cell walls caused by enzymes could favor both the liberation of oil and the pressing performance. Similarly, the first stage of the aqueous extraction process was more efficient for the enzymatic process than in the absence of enzymes, whereas this difference became nonsignificant in the second stage. The ethanolic extraction, with almost 90% oil extraction yield in the control, was less improved by enzyme treatment. In all cases improvements in the extraction rates could be observed at short times (data not shown).

The hydrolytic efficiency of the enzymes, also noticeable in the extent of the cell wall degradation or fiber content reduction, depended mainly on the operational conditions during treatment (Table 2). The fiber content differed among samples, probably due to the different range of particle sizes employed. A slight but significant reduction was observed for all the enzyme-treated samples regardless of the moisture content during treatment, but did not result in a higher ADC. The more marked reduction in neutral detergent fiber was observed in samples treated at 30% moisture and extracted by pressing.

## ACKNOWLEDGMENTS

This work was funded by the INCO-DC Programme of European Commission (ERBIC 18 CT 970206).

## REFERENCES

1. Fullbrook, P.D. The Use of Enzymes in the Processing of Oilseeds, *J. Am. Oil Chem. Soc. 60*:476–478 (1983).
2. Gresch, W., Verfahren zur Gewinnug von Öle und Fette aus Naturprodukten, und Anlage zur Durchfürhrung des Verfahrens, International Patent WO89/0925 (1989).
3. Rosenthal, A., D.L. Pyle, and K. Niranjan, Aqueous and Enzymatic Processes for Edible Oil Extraction, *Enzyme Microb. Technol. 58*:402–420 (1996).
4. Sineiro, J., M.J. Núñez, H. Domínguez, and J.M. Lema, Optimization of the Enzymatic Treatment During Aqueous Oil Extraction from Sunflower Seeds, *Food Chem. 61*:467–474 (1998).
5. Chien, J.T., J.E. Hoff, M.J. Lee, H.M. Lin, Y.J. Chen, and L.F. Chen, Oil Extraction of Dried Ground Corn with Ethanol, *Chem. Eng. J. 43*:103–113 (1990).

**TABLE 2**
**Comparison of the Meal from Control and Enzyme-Treated Samples of *Rosa rubiginosa* During Pressing, Ethanolic and Aqueous Extraction[a]**

| | ADC[b] (%) | NDF[b] (%, d.d.b.) | ADF[b] (%, d.d.b.) | Soluble fiber[b] (%, d.d.b.) Water | Soluble fiber[b] (%, d.d.b.) Phosphate buffer |
|---|---|---|---|---|---|
| Hexane defatted | 77.3 ± 1.2 | 82.6 ± 1.2 | 56.6 ± 2.4 | 4.6 ± 0.1 | 3.8 ± 0.5 |
| Cold batch pressing | | | | | |
| Control | 75.4 ± 1.0 | 77.5 ± 0.9 | 55.5 ± 1.5 | 0.90 ± 0.06 | 2.1 ± 0.08 |
| Enzyme treated | 74.8 ± 0.3 | 69.5 ± 0.9 | 53.1 ± 2.4 | 0.63 ± 0.03 | 2.20 ± 0.11 |
| Ethanolic extraction | | | | | |
| Control | 71.6 ± 0.5 | 86.6 ± 2.6 | 62.2 ± 2.5 | — | — |
| Enzyme treated | 72.7 ± 0.5 | 82.1 ± 1.2 | 57.3 ± 3.1 | — | — |
| Aqueous extraction | | | | | |
| Control | 75.6 ± 0.5 | 82.1 ± 1.5 | 58.4 ± 0.64 | 1.2 ± 0.2 | 2.30 ± 0.1 |
| Enzyme treated | 76.4 ± 0.6 | 81.3 ± 0.5 | 59.9 ± 0.40 | 0.3 ± 0.1 | 2.38 ± 0.7 |

[a]ADC = apparent digestibility coefficient (Ref. 11); NDF = neutral detergent fiber (Ref. 12); ADF = acid detergent fiber (Ref. 12); d.d.b. = dry defatted basis.
[b]Mean of three experiments ± standard deviation.

6. Lusas, E.W., Final Report: IPA as an Extraction Solvent, *inform 8*:290–306 (1997).
7. Sosulski, K., F.W. Sosulski, and E. Coxworth, Carbohydrase Hydrolysis of Canola to Enhance Oil Extraction with Hexane, *J. Am. Oil Chem. Soc. 65*:357–361 (1988).
8. Smith, D.D., Y.C. Agrawal, B.C. Sarkar, and B.N.P. Singh, Enzymatic Hydrolysis Pretreatment for Mechanical Expelling of Soybeans, *Ibid. 70*:885–890 (1993).
9. Domínguez, H., J. Sineiro, M.J. Núñez, and J.M. Lema, Enzymatic Treatment of Sunflower Kernels Before Oil Extraction, *Food Res. Int. 28*:537–545 (1996).
10. Shankar, D., Y.C. Agrawal, B.C. Sarkar, and B.N.P. Singh, Enzymatic Hydrolysis in Conjunction with Conventional Pretreatments to Soybean for Enhanced Oil Availability and Recovery, *J. Am. Oil Chem. Soc. 74*:1543–1547 (1997).
11. Hsu, H.W., D. Vavak, L.D. Satterlee, and G.A. Miller, A Multienzyme Technique for Estimating Protein Digestibility, *J. Food Sci. 42*:1269–1273 (1977).
12. Goering, H.K., and P.J. van Soest, Forage Fiber Analyses, *Agriculture Handbook No. 379,* United States Department of Agriculture, Agricultural Research Service, Washington, DC, 1970, pp. 5–11.

Andrés Moure[a], Daniel Franco[b], Rosa I. Santamaría[c], Carmen Soto[d], Jorge Sineiro[a], Herminia Domínguez[a], M. Elvira Zúñiga[d], M. José Núñez[b,*], Rolando Chamy[d], Agustín López-Munguía[c], and Juan M. Lema[b]
[a]Departamento Enxeñería Química, Universidade de Vigo (Campus Ourense), Edificio Politécnico, As Lagoas, 32004 Ourense, Spain, [b]Departamento Enxeñería Química, Universidade de Santiago de Compostela, Santiago de Compostela, Spain, [c]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Morelos, México, and [d]Escuela de Ingeniería Bioquímica, Universidad Católica de Valparaíso, Valparaíso, Chile

[Received November 20, 2000; accepted November 23, 2000]

*To whom correspondence should be addressed at Departamento Enxeñería Química Universidade de Santiago de Compostela, Avda. das Ciencias s.n. 15706 Santiago de Compostela, Spain. E-mail: eqmajose@usc.es