Effect of Rosehip Extraction Process on Oil and Defatted Meal Physicochemical Properties

J. Concha[a,b], C. Soto[a], R. Chamy[a], and M.E. Zúñiga[a,*]

[a]Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile, and [b]Facultad de Farmacia, Universidad de Valparaíso, Playa Ancha, Valparaíso, Chile

ABSTRACT: The physicochemical properties of oil from *Rosa affinis rubiginosa* seeds were analyzed after extraction by (i) organic solvent, (ii) cold pressing, and (iii) cold pressing assisted by enzymatic pretreatment using a mixture of the Novo-Nordisk A/S products Cellubrix (cellulase and hemicellulase activities) and Olivex (pectinase, cellulase, and hemicellulase activities). There were no significant differences in oil quality parameters, such as iodine value, refractive index, saponification value, unsaponifiable matter, and FA profile, when applying any of the three extraction processes. Although significant variations were observed in FFA content (acid value) and PV of the oil obtained by both of the cold-pressing oil extraction processes, these results were lower than the maximum value established from the *Codex Alimentarius* Commission. All-*trans*-retinoic acid content improved by 700% in rosehip oil obtained through cold pressing, with and without enzymatic pretreatment, in comparison with organic solvent extraction. This result is quite important for cosmetic oil because all-*trans*-retinoic acid is the main bioactive component responsible for the regenerative properties of this oil.

Paper no. J11343 in *JAOCS 83*, 771–775 (September 2006).

KEY WORDS: All-*trans*-retinoic acid, defatted meal quality, oil extraction, *Rosa affinis rubiginosa*, rosehip.

Rosa affinis rubiginosa L. (rosehip) seeds contain less than 10% oil on a dry basis. Thus, the conventional process of oil extraction is basically carried out using organic solvents. However, this process has drawbacks, such as (i) the need to use volatile organic compounds and (ii) the high temperature applied during the procedure, which can diminish product quality (1). Such limitations can be reduced by using cold pressing as the oil extraction procedure; however, this process generates a rather low oil yield (30-40%). Oil extraction yield has been increased (up to 72%) by applying enzymes in the extraction process (2,3).

Rosehip seed oil contains over 77% PUFA, which are very susceptible to chemical reactions, causing its rapid deterioration. This issue is relevant to cosmetic and therapeutic applications because free radical species from rosehip oil lipoperoxidation are known to be the cause of photohemolytic effect (4). The main bioactive component of rosehip oil is all-*trans*-retinoic acid (or tretinoin), a natural precursor of vitamin A that is known to be responsible for restoring and rebuilding tissue (5).

To preserve rosehip oil's cosmetic and therapeutic properties, low-temperature oil extraction processes could become a basic requirement, allowing the active compounds to remain undamaged and maintaining the ratio between saturated and unsaturated, which is very important. Cold-pressing the seeds, i.e., never exceeding 40°C, to obtain the oil is the safest, most natural extraction process. Applying enzymes during the oil extraction process increases product qualities, and cell wall polymer degradation enhances oil extractability and improves the nutritional quality of residual meal (6–8).

The attributes of oil extracted by aqueous methods, both with and without enzymes, have been analyzed, and little change in FFA content as well as in oxidative stability was shown between the two. However, differences became more conspicuous when the extracted oil was compared with that obtained through organic solvent extraction (9–12). The type of extraction procedure used, whether aqueous, solvent (hexane), or enzyme-assisted, will have an effect on the physicochemical properties of the oil, especially on its FFA and PV.

Enzyme-assisted oil extraction using cold pressing has been studied with the goal of enhancing the oil extraction yield of canola and Chilean hazelnut oil, but only the physicochemical properties have been evaluated (8), not the bioactive compounds of the resultant oil. The objective of this work is to evaluate the effect of three different oil extraction processes on rosehip oil and residual meal properties, paying special attention to the oil tretinoin content.

MATERIALS AND METHODS

Materials. Rosa aff. rubiginosa seeds were supplied by Loncopan S.A. (Santiago, Chile), almost husk-free (6% moisture). The approximate composition (g/100 g dry matter) was 9% oil, 3% protein, 56% crude fiber, and 2% ash (3).

Enzymes. The commercial enzymes Olivex (mainly pectinase, cellulase and hemicellulase activities) and Cellubrix (mainly cellulase and hemicellulase activities) were supplied by Novozymes (Novo-Nordisk A/S, Madrid, Spain).

Oil extraction process. Enzymatic treatment was carried out with 30% moisture (w/w) using a 1:3 Cellubrix/Olivex mixture (g/g) of 1.5 g of enzyme per 100 g of substrate (1.5% E/S), for 6 h at a hydrolysis temperature of 45°C. This enzyme mixture was determined previously (3) to be optimal because it resulted

*To whom correspondence should be addressed at Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, General Cruz 34, Valparaíso, Chile. E-mail: mzuniga@ucv.cl

in the highest oil yield. Specific details pertaining to the equipment and methodology used in the extraction process have been published (3).

Analytical methods. Oil content was analyzed by Soxhlet extraction according to Chilean Standard Method NCh 485 (13). Physicochemical properties were determined by AOAC methods (14): Free Fatty Acid Content by protocol 940.28; Saponification Value by procedure 920.160; Peroxide Value (PV) by technique 965.53, and Iodine Value, according to Hanus, by method 920.158. The Refractive Index was determined in a Universal Abbé refractometer. All experiments were carried out in triplicate.

Rosehip oil FA were analyzed using a gas chromatograph (Hewlett-Packard 5890 series II) equipped with an FID and fitted with an SP-2330 column (30 m, 0.25 mm i.d., 0.2 μm film thickness; Supelco, Bellefonte, PA), using helium as the carrier gas. FA were methylated with 14% BF_3-methanol solution and extracted with hexane. Then, 0.5 μL of the upper phase, containing FAME, was injected into the GC column. The temperatures of the injector and detector ports were 220 and 250°C, respectively. FAME were separated using a temperature program starting at 170°C, ramped to 190°C at 1°C/min, then to 215°C at 4°C/min, and finally held at 215°C for 30 min. The weights of the individual FAME were calculated on the basis of their relative peak area compared with that of tridecanoic acid, which is used as an internal standard, and then they were corrected using the corresponding GC response factors for each FA (15).

The tretinoin composition was analyzed by HPLC. The procedure involved a one-step extraction of the all-*trans*-retinoic acid from rosehip oil. Then, the tretinoin was analyzed by isocratic elution on a reversed-phase column (LiChrosorb RP-18, 0.5 μm particle size; Merck, Santiago, Chile), with UV detection at 340 nm. The mobile phase used was acetonitrile/water/methanol/ammonium acetate (15:5:4:1, by vol) at a flow rate of 1.2 mL/min (16).

Residual meal was analyzed by AOAC procedures: Protein was determined by the micro-Kjeldahl AOAC 960.52 protocol, using the %N × 6.25 conversion factor; Crude Fiber by the AOAC 962.09 procedure; and Ash according to the AOAC 923.03 protocol. Moisture was measured by vacuum drying at 60°C to a constant weight. Detergent fibers were measured by Van Soest methods (17,18) and pectin according to the procedure of Carbonell *et al.* (19). Reducing sugars, once extracted from the defatted meal with 80% ethanol, were determined by dinitrosalicylic acid method (20).

Statistical analysis. Mean values and SD were calculated. One-way ANOVA was applied to compare the mean values, using the GraphPad version 3.05 software (GraphPad Software Inc., San Diego, CA).

RESULTS AND DISCUSSION

Table 1 shows the physicochemical characteristics of *Rosa aff. rubiginosa* oil extracted through different processes. The color of rosehip oil extracted by cold pressing, with and without enzymes, is characterized by its reddish pigmentation, which can be associated with carotenoid content (21). In contrast, the solvent-extracted oil has a yellowish color, which could be due to the ability of the organic solvent to extract pigments and several other substances from the seeds, and/or to the degradation of the red pigment owing to the high temperature of the oil extraction process (5).

The oils obtained through organic solvent extraction and cold-pressing, whether with or without enzymatic treatment, showed no significant differences of iodine value, refractive index, specific gravity, unsaponifiable matter or saponification value ($P > 0.05$). However, the FFA content, acid value, and PV were significantly higher ($P < 0.05$) when the oil extraction was done by cold pressing.

FFA in oil recovered through cold pressing extraction, with or without enzymatic pretreatment, showed significant differences in comparison with organic solvent extraction ($P < 0.05$). This difference could be associated with the content of dark-red pigment in oil extracted by cold pressing, which could interfere

TABLE 1
Physicochemical Characteristics of *Rosa affinis rubiginosa* Oil[a]

	Cold pressing		
Characteristic	Control	Enzyme-assisted	Organic solvent
Iodine value (Wijs) (g iodine/100 g)	179	179	179
Refractive index (20°C)	1.481	1.481	1.479
Specific gravity (20°C)	0.927	0.927	0.927
Saponifiable matter (mg KOH/g)	187.4	187.2	189.3
Unsaponifiable matter (g/100 g)	1.4	1.4	1.2
FFA (g oleic acid/100 g)	1.72	2.16	0.40
PV (mequiv O2/kg)	1.7	1.7	0.3
Tretinoin (mg/L)	0.357	0.324	0.051

[a]Replicated values with ≤5% relative SD.

TABLE 2
FA Composition of *Rosa aff. rubiginosa* Oil[a]

Component	Cold pressing		
	Control	Enzyme-assisted	Organic solvent
Palmitic (C16:0)	4.52 ± 0.45	4.97 ± 0.49	3.33 ± 0.96
Stearic (C18:0)	0.11 ± 0.03	Traces	1.75 ± 1.21
Oleic (C18:1)	14.82 ± 1.86	12.36 ± 1.97	14.43 ± 1.56
Linoleic (C18:2n-6)	47.87 ± 2.18	46.09 ± 3.31	42.20 ± 4.92
Linolenic (C18:3n-3)	26.41 ± 3.22	30.12 ± 2.62	31.09 ± 3.44
Total saturated	4.63	4.97	5.08
Total monounsaturated	14.82	12.36	14.43
Total polyunsaturated	74.28	76.21	73.29
∑Saturated/∑unsaturated	0.052	0.056	0.058

[a]All the analyses were done in triplicate and are reported as mean ± SD.

with the analysis. The possibility of an increase in FFA content, caused by hydrolytic action of natural enzymes of seeds, is ruled out because of the thermal inactivation that occurs owing to heat treatment after crushing the seeds, as it is explained in our experimental methodology of Conca *et al.* (3).

The PV was higher when the oil was recovered by cold pressing, both with and without enzymes, than that for oil obtained through solvent extraction, although all of the oil PV were below the threshold value established by the *Codex Alimentarius* Standard (10 mequiv O_2/kg).

Differences of FFA content and PV for oil obtained by cold pressing do not reflect a deterioration of the oil quality. Results obtained in this work agree with a previous report, where the use of enzymes did not affect FFA content, PV, or refractive index (9). It was expected that oil extracted with and without enzymes would produce similar values for oil parameters. Indeed, in addition to previously mentioned canola oil studies, several reports of aqueous oil extraction support this hypothesis: The product of the extraction of coconut oil by aqueous enzyme-assisted processes had quality parameters similar to those of oil treated with conventional processes (22). Olsen (23) established that coconut oil obtained through enzyme-assisted extraction had lower acidity and better organoleptic properties than oil extracted by conventional methods. Bocevska *et al.* (6) established that FFA levels of corn oil extracted through an aqueous process, with enzymatic pretreatment, were slightly higher than in control oil samples.

Table 1 also shows the all-*trans*-retinoic acid content of *Rosa aff. rubiginosa* oil extracted through different processes. Significant differences in all-*trans*-retinoic acid content are observed for oil recovered through cold pressing processes, which is seven times higher than for oil obtained by chemical extraction. This parameter is very important when rosehip oil is used in the manufacture of superior quality skin care and cosmetic products. Rosehip oil produces the same benefits of synthetic tretinoin, but it is free of secondary side effects (5).

The FA profile of *Rosa aff. rubiginosa* oil, presented in Table 2, is not modified when using any of the three oil extraction processes. This result is similar to one in which organic solvent and supercritical fluid extraction treatments were compared (24).

Figure 1 shows the approximate composition of rosehip residual meal obtained by cold pressing with and without enzymatic

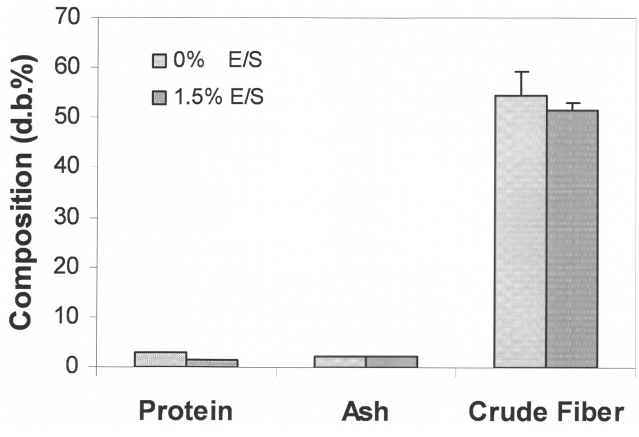

FIG. 1. Enzymatic pretreatment effect on rosehip residual meal obtained by cold-pressed oil extraction process. Enzyme treatment was carried out for 6 h. Cold-pressing oil extraction was carried out with a Carver Hydraulic Press at 53.9 MPa for 10 min. % E/S: g enzyme per 100 g of substrate. Error bars represent SD (n = 3).

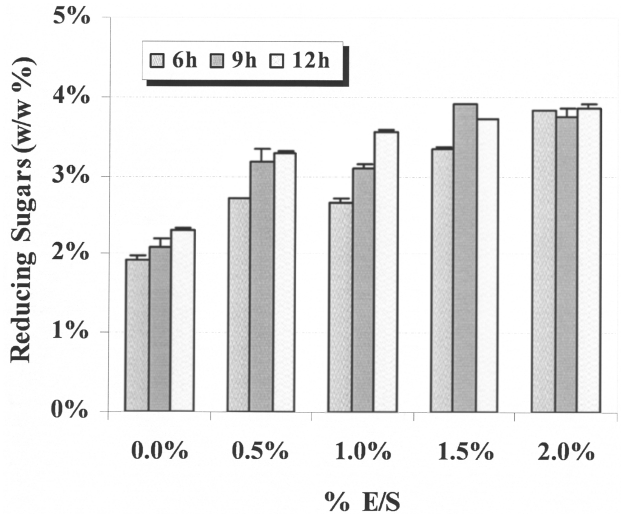

FIG. 2. Production of reducing sugars, in % (w/w, dry basis), during enzymatic hydrolysis of *Rosa affinis rubiginosa* seeds for 6, 9, and 12 h. Error bars represent SD (n = 3).

TABLE 3
Defatted Rose Hip Meal Detergent Fiber Composition[a]

	Control (%, w/w)	Enzyme-assisted (%, w/w)
Neutral detergent fiber (NDF)	74.44 ± 0.04	76.95 ± 2.57
Acid detergent fiber (ADF)	72.98 ± 4.38	73.93 ± 1.74
Acid detergent lignin (ADL)	27.54 ± 0.33	26.58 ± 0.45
Pectin	4.79 ± 0.02	4.65 ± 0.08
Hemicellulose (NFD – ADF)	1.46	3.02
Cellulose (ADF – ADL)	45.44	47.35
Lignin (ADL)	27.54 ± 0.33	26.58 ± 0.45

[a]Mean ± SD (n = 6).

pretreatment. These samples were analyzed after oil extraction by the Soxhlet procedure. The values of protein, crude fiber, and ash did not vary when enzymatic treatment was applied. These results do not agree with a previous report in which the use of enzymes diminished crude fiber, perhaps owing to the enzymatic hydrolysis applied in the aqueous oil extraction process (9).

Figure 2 shows a proportional increase in reducing soluble sugars with enzyme concentration and/or enzymatic treatment incubation period ($P < 0.05$) in comparison with control samples, suggesting degradation of seed cell wall polysaccharides by enzymatic action. However, enzyme hydrolysis is not reflected by the detergent fiber content presented in Table 3. This fact could be due to the different sensitivity of the (neutral and acid) detergent fiber measurements in comparison with the reducing sugars methodology (17,18).

ACKNOWLEDGMENTS

This research was funded by the Fondo Nacional de Desarollo Científico y Tecnológico (FONDECYT) no 1.000.300, Comisión Nacional de Investigación Científica y Tecnologica (CONICYT), Chile and, the International Cooperation with Developing Countries (INCO-DC) Programme of the European Commission (ERBIC, Environmental Risks of Biological Control in Europe, 18 CT 970206).

REFERENCES

1. Rosenthal, A., D.L. Pyle, and K.Niranjan, Aqueous and Enzymatic Processes for Edible Oil Extraction, *Enzyme Microb. Technol. 19*:402–420 (1996).
2. Moure, A., J. Sineiro, D. Franco, R.I. Santamaría, C. Soto, H. Domínguez, M.J. Núñez, M.E. Zúñiga, R. Chamy, J.M. Lema, and A. López-Munguía, Enzyme-Aided Alternative Processes for the Extraction of Oil from *Rosa rubiginosa*, *J. Am. Oil Chem. Soc. 78*:437–439 (2001).
3. Concha, J., C. Soto, R. Chamy, and M.E. Zúñiga, Effect of Enzymatic Pretreatment on Rose Hip Oil Extraction: Hydrolysis and Pressing Conditions, *Ibid. 81*:550–552 (2004).
4. Barros, S., X. Ling, M. Galea, F. Carvajal, and E. Fernández, Estudio Fotobiologico del Aceite de Rosa Mosqueta (*Rosa aff. rubiginosa* L.), *Acta Farm. Bonaerense 7*:3–8 (1988).
5. Pareja, B., and H. Kehl, Contribution to the Identification of *Rosa aff. rubiginosa* L. Oil Rose Active Principles, *An. Real Acad. Farm. 56*:283–294 (1990).
6. Bocevska, M., D. Karlovic, J. Turkulov, and D. Pericin, Quality of Corn Germ Obtained by Aqueous Enzymatic Extraction, *J. Am. Oil Chem. Soc. 70*:1273–1277 (1993).
7. Domínguez, H., M.J. Núñez, and J.M. Lema, Aqueous Processing of Sunflower Kernels with Enzymatic Technology, *Food Chem. 53*:427–434 (1995).
8. Zúñiga, M.E., R. Chamy, and J.M. Lema, Canola and Chilean Hazelnut Products Obtained by Enzyme-Assisted Cold-Pressed Oil Extraction, in *Proceedings of the World Conference and Exhibition on Oilseed Processing and Utilization*, edited by R.F. Wilson, AOCS Press. Champaign, IL, 2001, pp. 203–209.
9. Domínguez, H., M.J. Núñez, and J.M. Lema, Enzyme-Assisted Hexane Extraction of Soya Bean Oil, *Food Chem. 54*:223–231 (1995).
10. Sosulski, K., and F.W. Sosulski, Enzyme-Aided vs. Two-Stage Processing of Canola: Technology, Product Quality and Cost Evaluation, *J. Am. Oil Chem. Soc. 70*:825–829 (1993).
11. Tano-Debrah, K., and Y. Ohta, Enzyme-Assisted Aqueous Extraction of Shea Fat: A Rural Approach, *Ibid. 72*:251–256 (1995).
12. Sitohy, M.Z., F.H. Badr, M. Perifanova-Nemska, and T.S. Khadjiski, Characterization of Enzymatically Extracted Sunflower Seed Oil as Well as the Protein Residues, *Grasas Aceites 44*:345–347 (1993).
13. Instituto Nacional de Normalización, Nch 485. Granos o Semillas Oleaginosas. Determinación del Extracto al Éter de Petróleo Denominado Contenido de Aceite. Santiago de Chile, Chile, 1988, 5 pp.
14. Association of Official Analytical Chemists, *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th edn., edited by K. Helrich, AOAC, Arlington, VA, 1990, pp. 776–782.
15. Metcalfe, L.D., A.A. Schmitz, and J.R. Peika, Rapid Preparation of Fatty Acid Esters from Lipids for Gas Chromatographic Analysis, *Anal. Chem. 38*:514–515 (1966).
16. Guiso, G., A. Rambaldi, B. Dimitrova, A. Biondi, and S. Caccia, Determination of Orally Administered All-*trans*-retinoic Acid in Human Plasma by High-Performance Liquid Chromatography, *J. Chromatogr. B. Biomed. Sci. Appl. 656*:239–244 (1994).
17. Van Soest, P.J., and R.H. Wine, Determination of Lignin and Cellulose in Acid Detergent Fiber with Permanganate, *J. Assoc. Off. Anal. Chem. 51*:780–785 (1968).
18. Van Soest, P.J., J.B. Robertson, and B.A. Lewis, Methods for Dietary Fiber, Neutral Detergent Fiber, and Non Starch Polysaccharides in Relation to Animal Nutrition, *J. Dairy Sci. 74*:3583–3597 (1991).
19. Carbonell, E., E. Costell, and L. Durán, Determination of Pectin Content in Vegetable Products, *Rev. Agroquim. Aliment. 30*:1–9 (1990).

20. Miller, G.L., Use of DNS Reagent for Determination of Reducing Sugars, *Anal. Chem. 31*:426–428 (1959).
21. Hornero-Mendez, D., and M.L. Minguez-Mosquera, Carotenoid Pigments in Rosa Mosqueta Hips, an Alternative Carotenoid Source for Foods, *J. Agric. Food Chem. 48*:825–828 (2000).
22. Tano-Debrah, K., and Y. Ohta, Aqueous Extraction of Coconut Oil by an Enzyme-Assisted Process, *J. Sci. Food Agric. 74*:497–502 (1997).
23. Olsen, H.S., Aqueous Enzymatic Extraction of Oil from Seeds, in *Asian Food Conference Proceedings*, Bangkok, Thailand. Reprinted by Novo Industry A/S, A-06041a, 1988.
24. Szentmihályi, K., P. Vinkler, B. Lakatos, V. Illés, and M. Then, Rose Hip (*Rosa canina* L.) Oil Obtained from Waste Hip Seeds by Different Extraction Methods, *Bioresour. Technol. 82*:195–201 (2002).

[Received February 17, 2006; accepted June 12, 2006]