

OBTEINTION OF ROSEMARY ANTIOXIDANT OLEORESINS BY MECHANICAL EXTRACTION WITH NON VOLATILE SOLVENT AND UNDER PRESSURE

A.R. Tymoschuk and R. Mato
INGAR - Instituto de Desarrollo
y Diseño CONICET
Avellaneda 3657
3000 Santa Fe, Argentina

J. Luna
INTEC- Instituto de Desarrollo Tecnológico
para la Ingeniería Química CONICET -UNL
Güemes 3450
3000 Santa Fe, Argentina

Keywords: rosemary antioxidant oleoresin, mechanical extraction, solvent extraction

Abstract

The objective of the present work was to assess the efficiency of mechanical extraction of *Rosmarinus officinalis* antioxidant oleoresin in the laboratory. The results were compared with those obtained by volatile solvent extraction.

Oleoresin was obtained by pressing a mixture of rosemary ground dry leaves and medium chain triglycerides (MCT). The mixture was subjected to gradually increasing pressure from 50 bars to 100 - 250 bars. The oil, which was a little turbid, was filtrated and purified. Several experiences at different temperatures were carried out. The extraction with volatile solvent was accomplished by percolation of dry rosemary with ethanol in inert atmosphere. The extract obtained was filtrated and centrifuged. The content of Carnosic Acid (CA), the main antioxidant compound in rosemary, was determined by HPLC. In both volatile solvent extraction and extraction under pressure processes, oleoresins with 3 to 7% of CA were obtained. These values are in order of those of commercial oleoresins.

The antioxidant oleoresin efficiencies were tested in lard samples. The oxidation of samples was carried out at 80°C and static conditions (oven test). Peroxide value was measured during several days. A good performance of the products of both processes was observed.

1. Introduction

The work consisted in obtaining rosemary antioxidant oleoresin in laboratory by both the volatile solvent and extraction under pressure. The goal was to establish the operative and product characteristic conditions. The results would allow antioxidant production at pilot or industrial scale.

Rosemary antioxidant oleoresin was selected for its commercial interest and satisfactory efficiency in foods.

Extraction under pressure as presented by Aeschbach *et al.* (1993) is a simple procedure and an alternative to the method to conventional volatile solvent extraction. It does not require a volatile solvent recovery step, or complete elimination from the product. The quality product is compatible with market requirements. Technical complexity is not limiting in analytical controls. Small capital investment is necessary to implement the process at an industrial scale.

Volatile solvent extraction of oleoresin is a relatively conventional procedure (Aeschbach *et al.*, 1989, 1992). It consists of an extraction by volatile solvent with subsequent evaporation of the solvent and some final purification treatment of the product. It is relatively simple and easy to implement in laboratory trials and scale to industrial production.

Laboratory equipment for both obtention production processes was designed and installed. Several sample experiences were done seeking for optimal operative conditions. Sample products obtained and commercial ones were tested in lard, incubated in stove at 80°C (Bailey, 1961). Evolution of antioxidant activity was followed measuring the peroxide value (Bailey, 1961; Allen, 1994).

2. Materials and methods

2.1. Materials

Dry *Rosmarinus officinalis* provided by INTA La Consulta, Mendoza, and Platario S.A., San Juan, Argentina, was used as raw material.

Carnosic Acid (CA), major antioxidant principle in rosemary (Qinyun Chen et al., 1992 and Economou et al., 1991), was taken as reference. NESTEC S.A., Vevey, Switzerland, supplied CA pure standard. Commercial rosemary oleoresins were Herbor-O-25 and Herbor O-21 supplied by Nestec too. CA concentration in rosemary oleoresins was quantified by chromatographic methods. High performance liquid chromatography (HPLC) was done, with a Hewlett Packard 1050-chromatograph and Hypersil C18 column (Cuvelier *et al.*, 1994).

CA concentrations in raw material (dry rosemary) were computed by volatile solvent extractions until exhausting the content of CA.

Solvents were pure ethanol to extract the oleoresin and hexane to eliminate impurities. Medium Chain triglycerides (MCT) are a mixture 40:60 of eight- and ten-carbon chain fatty acids (caprylic and capric acids). They acted as antioxidant carrier in both extraction methods and as non-volatile solvents in the extraction under pressure. MCT was Delios V, supplied by the commercial company Grunau GmbH, Germany. Additional distilled water was used for mechanical extraction.

The volatile solvent extraction equipment consisted of a glass vessel, hermetically closed to work with inert nitrogen atmosphere at low above atmospheric pressures, and a magnetic agitator. A punctured polypropylene filter with filtering steel meshes and filter papers was located in the outlet nozzle of the extraction vessel. A rotavapor with heating bath was used to desolventize the oleoresins.

The mechanical extraction equipment included a piston type Carver press with a capacity of 12 ton. which permits to operate under pressures of 50 to 250 bars. Other accessories were a stainless steel cylinder with a perforated bottom (orifices of 1 mm) a set of steel meshes mounted on bottom and a set of steel disks.

In both processes cleaver windmills were used for size reduction of rosemary leaves were used. Stainless steel sieves with openings of 500, 420 and 297 microns were used to classify particles.

2.2. Extraction methods

In the solvent extraction procedure, dry ground rosemary and pure ethanol were loaded into the glass-extracting vessel, in a relationship 8:1 solvent volume/dry rosemary weight. The extraction was carried out under nitrogen atmosphere at 0.3 bars with magnetic agitation at room temperature during 2 to 3 hours. This process was repeated twice. The second extraction also was with fresh ethanol. The resulting rosemary alcoholic extract was placed into the rotavapor with MCT, previously added in a relation 1:5 MCT volume/dry rosemary weight. Ethanol evaporation was done at 40-50°C. A dark viscous liquid was obtained. Impurities such as gums and waxes were removed adding hexane in a relation 8:1 hexane volume/ MCT volume. Mixture was shaken during 1.5 to 2 hours

and was cooled to 6°C during 2 to 4 hours. After filtering this mixture, hexane was eliminated in the rotavapor. The treatment was repeated until a clear product was observed.

In the pressure extraction method, a mixture of ground dry rosemary at selected particles size and 10% of distilled water was prepared. This operation eases the transfer of CA into the liquid oil carrier. After this, 50% of MCT was added. This mixture was shaken during 1 hour approximately. The mixture was put in the cylindrical pressing chamber and compressed gradually up to 80 and 150 bar pressures. The flowing and viscous liquid oleoresin obtained was constituted by MCT and extracted materials from rosemary ground leaves. It was subjected to centrifugation and filtration, a similar treatment as in the solvent extraction procedure, to separate impurities like waxes and gums from final oleoresin.

2.3. Oleoresin performance proofs

To study antioxidant efficiency of the resulting product, an accelerated oxidation of lard samples with and without antioxidants in static conditions (stove test or Schaall methods) was implemented (Bailey, 1961; Allen, 1994). The obtained antioxidant oleoresins, similar commercial products and synthetic antioxidant were applied separately to lard samples.

Peroxide values of samples were periodically measured to establish the induction periods (time required fats to become rancid) by Cd 8-53 methods of American Oils Chemist's Society (AOCS).

3. Results

Volatile solvent extraction produced a rosemary oleoresin with 55 mg/ml of CA. The yield extraction was approximately 50% of total CA present in the plant material. This was estimated through the relationship of CA content in oleoresin and in raw material. The last was taken from an extraction of CA with ethanol in extended time (24 hours). Achieved concentration was 3.5% of CA in relationship to dry rosemary weight.

Preliminary experiences of pressure extractions were carried out at room temperature and at pressure gradually increased until 150 bars. Laboratory operative conditions like particle size, commercial oil of food grade as carrier, contact time between plant material and carrier were analyzed. In Table 1 it can be observed that grinding and MCT as carrier are process meaningful factors in relation to CA content in final product.

Other set of experiences of mechanical extractions consisted in selecting particle sizes and temperatures as response variables. The other operative conditions were the use of MCT as carrier, 1-hour contact time of rosemary and carrier, and the other conditions of previous experiences like extraction pressure, material quantity and proportions. The results shown in Table 2 demonstrate that high CA content in oleoresins was obtained with room temperature and high grinding of plant material. Higher temperatures are acceleration factors to oxidation and antioxidants undergo degradation too. Also increased temperature produces a retention of the MCT into the solid and this reduces the transfer of CA from rosemary to the carrier.

In order to test the antioxidant efficiency, commercial oleoresin and those obtained in the laboratory by mechanical extraction were applied to lard samples at different concentrations (Table 3). Lard samples were oxidized in accelerated conditions (80°C without airflow at darkness). Sample peroxide values were measured during several days and the results were compared with a control sample (lard without antioxidant). The results of replicated trials are showed in figures 1 and 2.

The slowest evolution of lard oxidation with greater content of CA in oleoresin can be appreciated in figure 1.

Another important index of inhibition of lard oxidation is induction period. This is a time when lipid oxidation reaction changes from initiation stage to propagation stage. Generally this time is reached when lipids become rancid (Hudson, 1990).

Figure 2 shows the observed induction period of lard samples during storage in oven. In the present experience, the sample control reached its induction period at 5 days, while the sample with highest CA concentration made it at 17 days approximately.

4. Concluding remarks

Both extraction methods produced antioxidant products with maximum CA concentrations between 56 and 70 mg CA/ml MCT oleoresin. Efficiency trials in lard verified good performance, similar to those of commercial ones. Lard samples with antioxidant extended their useful life more than twice in reference to lard without oleoresin.

The mechanical extraction procedure is rather simple and safe. It consists on grinding of the plant material, mixture with MCT and extraction by pressure, all of these stages without volatile solvents. It produces oleoresin with similar CA concentrations as those of solvent process. The principal advantages are small operative risk and no-pollution as compared with solvent utilization, and smaller equipment and operative costs because it does not require the solvent recovery system nor to remove residual solvent from the final product.

The volatile solvent process has the problems related to solvents like manipulation, evaporation, recovery and residual quantity in final products. However solvent extraction guarantees an effective extraction of antioxidant principle in liquid form. There is another type of product like a dry powder, which is produced without oily carrier addition. We did not make it in the present work.

Rosemary antioxidant oleoresins show a great interest as a natural additive to preserve foods with lipid composition.

5. Acknowledgements

We thank to CERIDE personnel for their technical assistance in laboratory equipment construction and analytical measures and our colleagues of INGAR and INTEC for their contributions to this work.

6. References

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Table 1 - Preliminary mechanical extraction results

Sample Name	Material plant size	Carrier	Contact time of rosemary and carrier (Hours)	CA concentration in oleoresins (mg AC/ml oleoresin)
M1	Integer rosemary leaves	MCT	24	20.0
M2	Integer rosemary leaves	MCT	48	22.0
M3	Ground rosemary leaves	Commercial Oil Sunflower	1	23.0
M4	Ground rosemary leaves	MCT	1	44.0

Table 2 – CA extraction by the mechanical procedure and results

Trial	Operative conditions during mixing		Obtained trials results	
	Particle size of ground dry rosemary	Temperature of rosemary mixture and MCT contact (°C)	CA concentration in oleoresin (mg CA/ml oleoresin)	Extraction yield in relation to MCT (%)
1	50% particles with size < 297 microns 50% particles with size between 297 and 420 microns	40	55	70
2	100% particles with size between 420 and 500 microns	40	22	26.8
3	50% particles with size < 297 microns 50% particles with size between 297 and 420 microns	60	46.1	31.7
4	100% particles with size between 420 and 500 microns	60	20	14.1
5	100% particles with size < 297 microns	20	72.6	94

Table 3 – Samples Nomenclature

Sample name	Type of antioxidant (*)	Antioxidant quantity (ppm)	CA Content in oleoresin (%)
Control			
Sample 1	Commercial (Herbor O-25, Nestlé)	200	5
Sample 2	Commercial (Herbor O-25, Nestlé)	600	5
Sample 3	M1	200	2
Sample 4	M1	600	2
Sample 5	M2	600	2.2
Sample 6	M3	600	2.3
Sample 7	M4	600	4.4

Initial peroxide value (zero day): 0.755 oxygen miliequivalent/kg lard

(*) Samples M1, M2, M3 and M4 are described in Table 1.

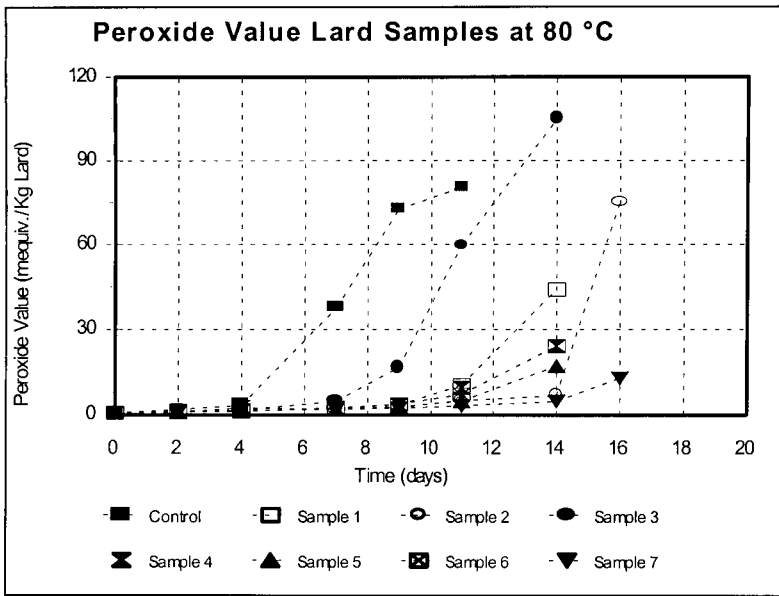


Figure 1 - Evolution of peroxide value in lard samples with antioxidants

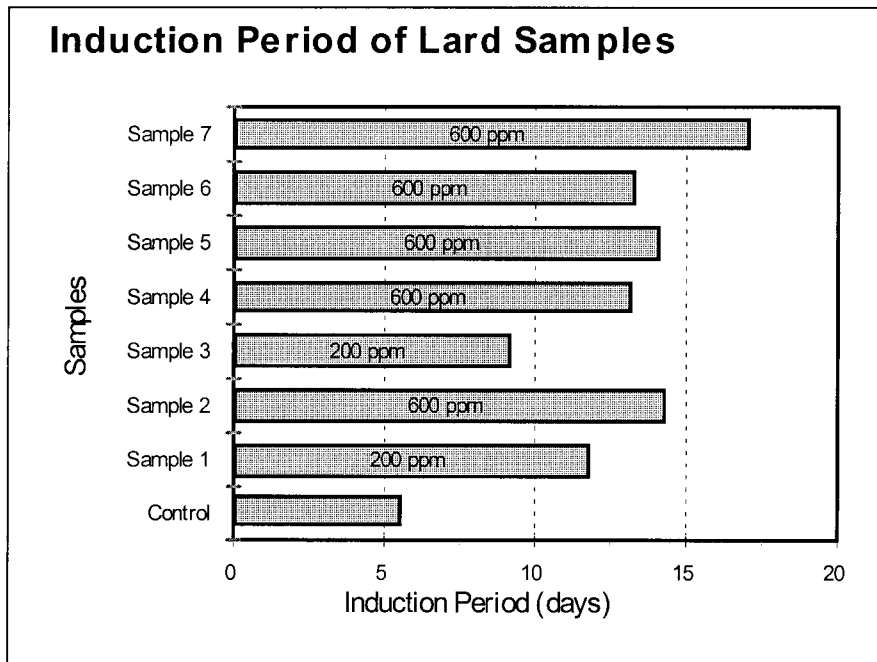


Figure 2 - Induction period of lard samples with and without antioxidant.