Rosmarinus officinalis L. Extract Production Antioxidant and Antimutagenic Activity

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Interest in spices and derivatives used as antioxidants in food goes back more than forty years. Literature data presents a sufficient number of works carried out in order to evidence the antioxidant properties of some spices. The results of this research were parallel to those aiming at the evaluation of bactericidal and bacteriostatic properties of the same spices.

Important experimental studies began in the fifties. However it is possible to find some patents covering the use of some spice fractions as an antioxidant, that were registered in 1938. In fact, U.S. Patent 2, 124, 706 (July 26, 1938 by D. J. Maveety) concerns the use of some fractions obtained from spices for prevention of rancidity in edible oils.

Some works between 1943-1950 also present data about experiments which demonstrate the activity of a very large series of spices in delaying the production of peroxides and free fatty acids.[1-7] At that time some experiments also demonstrated that antioxidant activity should be correlated with thermal treatment of some spices. Moreover, considering the data reported in an article by Chipault, et al.[8] on the value of a large series of ground spices and relative alcohol, soluble fractions as "Antioxidant Index" (see Table I), one realizes that among the considered spices, rosemary (*Rosmarinus officinali* L.) and sage (*Salvia officinalis* L.) have been known to have the highest antioxidant power, for some time.

In 1955, Rac and Ostric[9] also pointed out the antioxidant effect of a rosemary extract. In an additional paper by Chipault, et al.,[10] data concerning the antioxidant properties of rosemary is shown along with spices in general. In 1973, a patent for the production of a rosemary oil extract was presented to Berner and Jakobson.[11]

Subsequent works concern the optimization study of rosemary extract production processes. In 1977, a report on experiments to prepare an antioxidant extract from rosemary deodorized by molecular distillation was presented by Chang et al.[12] Extracts obtained using the following solvents were considered: methylene chloride, hexane, benzene, ethyl ether, chloroform, dioxane, methanol.

Tests on antioxidant power were carried out using the extract at 2% concentration in solid fat stored at 60°C for many days. Data obtained by S. Chang and his coworkers also showed the improved stability of the organoleptic characteristics of soy oil and potato chips when purified rosemary extract was added to these products. Experiments also were conducted by Mac Neil and coworkers[13] about the use of rosemary for chicken, and Pizzocaro and coworkers[14] performed experiments for using rosemary in sardines' muscle and oil.

In 1982, an industrial process is described in a

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Table I. "Antioxidant index" (A.I.) values of ground spices and alcohol-soluble fractions, (J.R. Chipault et al.), using active oxygen method at 98°C, employing as substrate prime steam lard with a stability of 6.5 hours.

$$\text{A.I.} = \frac{\text{Induction time with antioxidant}}{\text{Induction time in control}}$$

Spice	Antioxidant Index (A.I.)	
	ground spice	alcohol-soluble fraction
Allspice	1.8	1.0
Aniseed	1.9	3.9
Basil leaves	1.2	...
Bay leaves	2.1	2.7
Cardamom	1.3	3.9
Caraway	1.8	4.6
Celery seed	1.2	2.9
Chilli	1.5	2.6
Cinnamom	1.3	5.7
Cloves	1.8	6.2
Coriander	1.3	1.0
Cumin	1.3	3.2
Dill	1.3	1.0
Fennel	1.3	2.7
Foenugreek	1.6	4.1
Ginger	1.8	26.0
Mace	2.6	17.0
Marjoram	2.2	5.0
Mustard	2.0	6.7
Nutmeg	3.1	22.8
Oregano	3.8	14.6
Paprika	2.5	3.9
Pepper, black	1.4	...
Pepper, red	1.5	3.6
Pepper, white	1.2	...
Poppy seed	1.2	4.1
Rosemary	17.6	27.5
Sage	14.2	33.6
Savory	1.6	6.0
Thyme	3.0	23.2
Turmeric	2.9	5.8

work published by Bracco, Loliger, and Vire.[15] This report allowed for the production of natural antioxidants from spices and other vegetables by simultaneously using mechanical and physical treatments. The flow sheet on antioxidant extract from rosemary suggested by these authors is reported in Figure 1.

The results of this research demonstrated that it is actually possible to obtain rosemary derivatives, activated against rancidity, by molecular distillation.

Our study is part of a trend aiming to show the antioxidant as well as the antimutagenic activity of some fractions of vegetable extracts (spices and official plants in particular). It results from research by L. Santamaria and coworkers[16] on the photomutagenicity by 8-methoxypsoralen (8-MOP) with and without single oxygen involvement and its prevention by β-carotene (BC).

In a recent publication by L. Santamaria, F. Tateo, A. Bianchi, and L. Bianchi,[17] an extract from "*Rosmarinus officinalis* L." showed an antimutagenic activity as an antioxidant. Its efficacy was somehow less than that exerted by β-carotene (BC) in both tests with 8-methoxypsoralen (8-MOP) and benzo(a)pyrene (BP). The rosemary extract developed was derived from previous defatting of leaves by supercritical Co_2 extraction by ethyl alcohol 95° and following drying of alcoholic extract.

The experiments described in this article concern:

a) a comparison of the antioxidant power between two dry rosemary extracts obtained

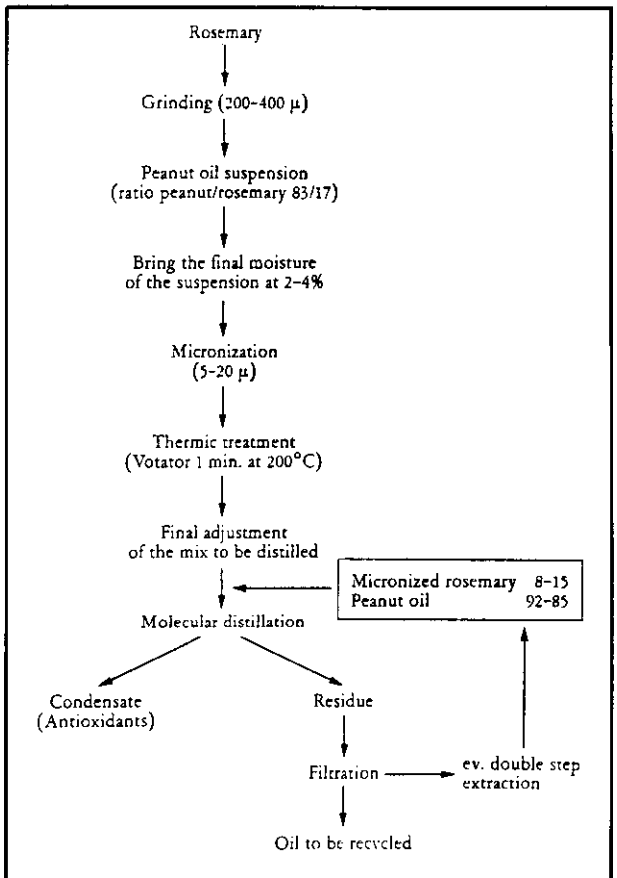

Figure 1. Recovery of antioxidant from rosemary (U. Bracco et coll).[15]

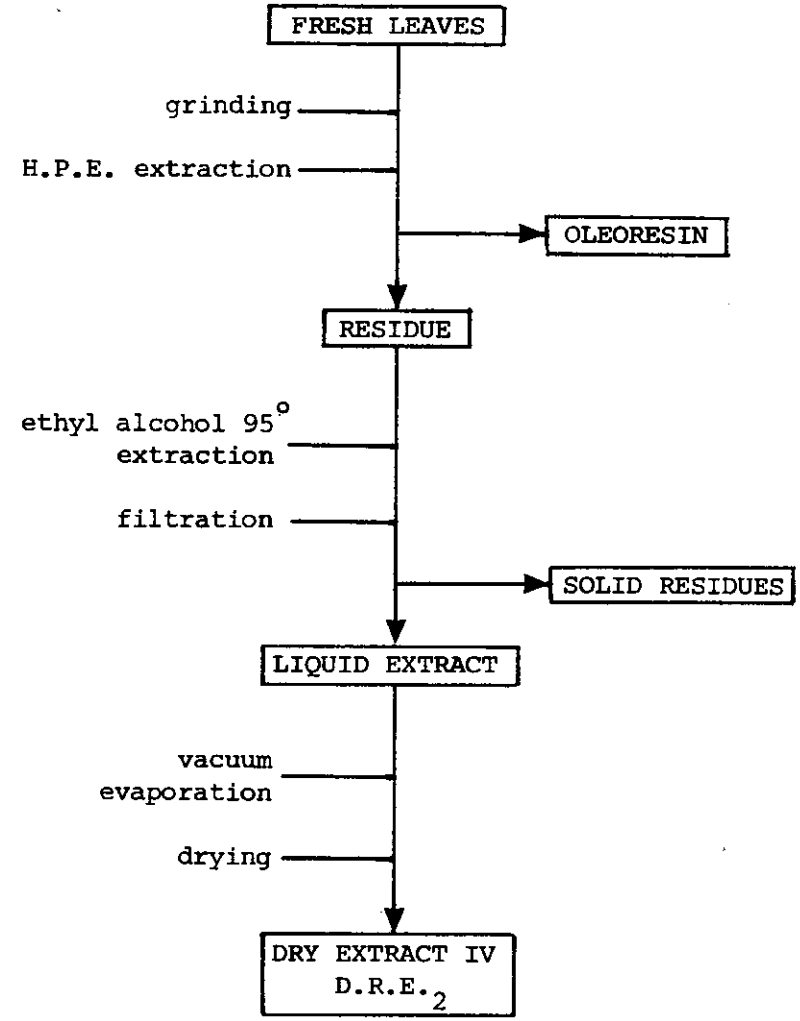

Figure 2A. Flow sheet of various extracts from "Rosmarinus officinalis L." produced for the evaluation of antimutagenic activity.

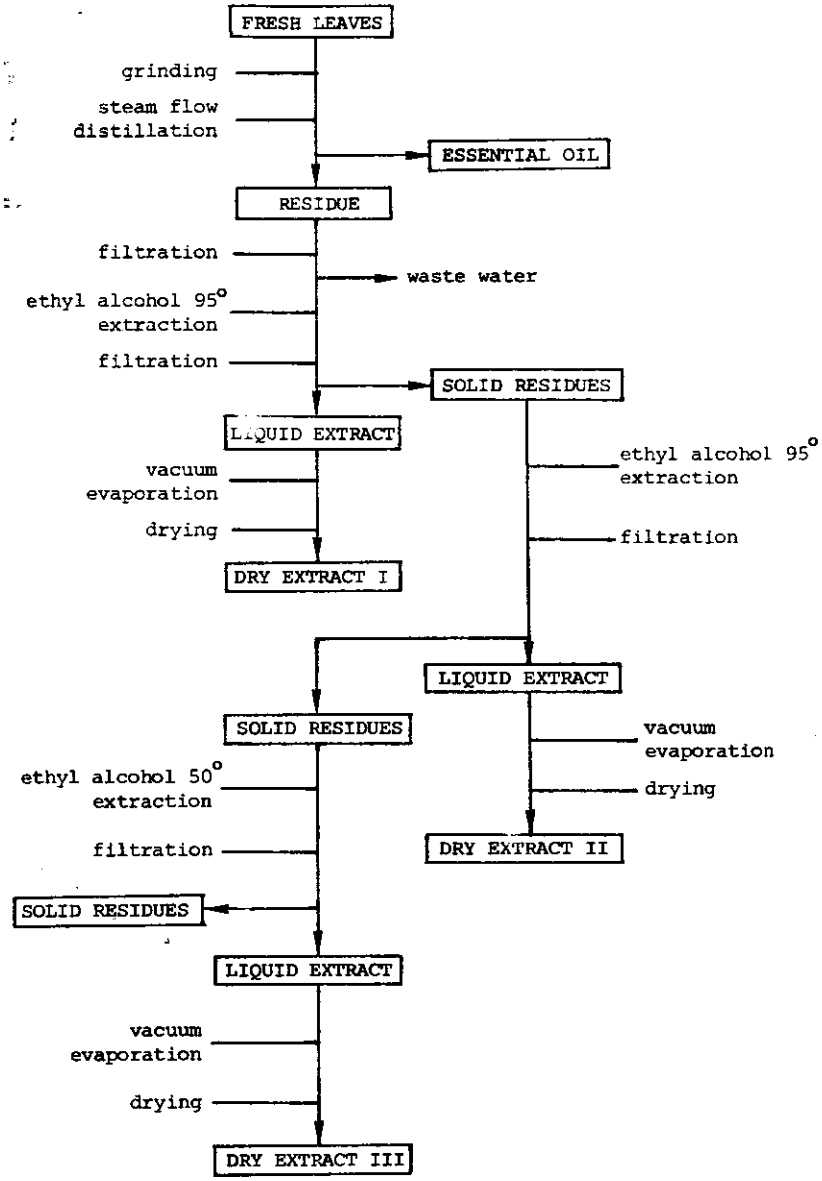

Figure 2B. Flow sheet of an H.P.E. extract from "Rosmarinus officinalis L." produced for the evaluation of antioxidant and antimutagenic activity.

by a simplified extraction process, a commercial rosemary extract (AR) and BHA,

b) an evaluation of the antimutagenic effect of four different dry rosemary extracts.

Experimental Phase

Recovery of antioxidant extracts—Three dry antioxidant extracts were prepared using fresh rosemary leaves according to the flow sheet in Figure 2A. Likewise the same process was carried out for the production of a fourth extract, but with one variation; the previous removal of essential oil from the leaves by supercritical CO_2 (300 bar/35°C/60 min), according to the flow sheet in Figure 2B.

Two reports have already been published on the composition difference of oil extracted by steam flow distillation and on oleoresin extracted by supercritical CO_2.[18,19] The criterion of previous defatting of fractions extractable by steam flow or by supercritical CO_2 seems to be preferable to others used. In fact, some authors have suggested that vacuum steam distillation of the antioxidant suspended in a vegetable oil, and molecular distillation of the suspension showed be used for the same purpose.

A leaves/ethyl alcohol 95° ratio equal to 1:4 (w/v) was used for the production of the first, second and third extracts according to the flow sheet in Figure 2A. Besides, an extract named DRE_1 (Deoleated Rosmarin Extract) was obtained by mixing an aliquot of the first and second liquid extracts, by removing the solvent by vacuum distillation at 30°C and drying the residue. A fourth dry extract (DRE2) was obtained in the same way starting, as previously mentioned, with rosemary deoleated by supercritical CO_2.

Evaluation of antioxidant power—The antioxidant activity was evaluated by a prolonged treatment (at 100°C) on solid fat samples treated with both DRE_1 and DRE_2 (0.03%) and by determining the variation in the number of peroxides. At the same time, the variation in the number of peroxides was evaluated in solid fat samples added with BHA and in solid fat samples added with a rosemary extract. This extract is commercially named AR and appears on labels of potato chips as "Rosemary Extract."

Peroxide values were determined by NDG C 35—1976.[20] The evaluation of the antioxidant effect of the same extracts related to the concentration (from 0.005% up to 0.1%) of a treatment at high temperature (100°C) and for intervals of 24 and 32 hours also was conducted. In addition, the antioxidant power was tested on soy oil, by determining the number of peroxides for different times. Analytical conditions were the same used so far for experiments on solid fat.

Table II. Antioxidant activity of two "Rosmarinus officinalis L." extracts compared with the one of BHA and AR (lard aged at 100°C). DRE_1, DRE_2, BHA and AR added at a concentration of 0.03% into prime steam lard.

Antioxidant dry extract	Peroxide value (meq.O_2/Kg)															
	h															
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
DRE_1	–	–	–	–	–	–	–	3,0	3,2	3,6	4,2	5,1	6,0	7,2	10,8	12,4
DRE_2	–	–	–	–	–	–	–	1,5	1,8	2,2	3,0	3,8	4,5	6,8	9,4	12,0
BHA	–	–	–	–	–	–	–	–	–	1,5	1,8	2,8	3,7	4,5	5,8	9,8
AR	–	–	–	–	–	–	–	–	2,5	2,8	3,0	4,0	5,2	7,0	10,5	12,1
Control 1	–	–	1,5	2,8	6,8	15,9	20,6	43,8	100,6	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Control 2	–	–	1,9	2,4	7,0	17,1	20,9	47,8	109,1	n.d	n.d	n.d	n.d	n.d	n.d	n.d

Table III. Antioxidant activity of DRE_2 extract, BHA and AR added into prime steam lard (aged at 100°C, 24 and 32 hrs).

Antioxidant \ Conc. antiox. %		Peroxide value (meq. O_2/Kg) 0,005	0,01	0,02	0,03	0,05	0,1
DRE_2	24 h	17,0	12,9	13,0	6,4	3,0	1,8
	32 h	20,1	12,3	13,5	8,9	3,4	2,1
BHA	24 h	15,0	10,0	10,0	4,4	4,0	-
	32 h	18,1	10,7	10,4	4,2	4,2	0,5
AR	24 h	14,9	10,7	9,2	4,8	4,5	0,1
	32 h	18,9	13,5	9,0	4,6	7,4	2,3

Table IV. Antioxidant activity of two "Rosmarinus officinalis L." extracts compared with the one of BHA and AR (soy oil aged at 100°C).

Antioxidant	Peroxide value (meq. O_2/Kg) h 0	2	4	8	12	16	20	24
DRE_1	2,1	3,2	5,7	7,0	9,1	12,4	16,1	17,5
DRE_2	2,3	3,2	5,1	8,0	9,5	11,5	14,6	16,9
BHA	2,0	2,5	4,9	6,8	8,5	11,0	14,3	15,9
AR	2,2	2,8	6,5	8,2	10,1	13,5	15,3	17,0
$Control_1$	2,1	3,2	9,7	14,6	20,8	28,2	35,8	42,4
$Control_2$	2,5	5,0	10,0	14,8	22,1	30,4	36,8	41,7

Study of antimutagenic activity—Referring to previous results[17] concerning the 8-MOP photomutagenesis and its inhibition by "*Rosmarinus officinalis* L." in Salmonella Typhimurium TA 102, as well as the BP mutagenesis and its inhibition by "*Rosmarinus officinalis* L." in Salmonella Typhimurium TA 98, the comparative evaluation of mutagenesis inhibition in Salmonella Typhimurium TA 102 was carried out for the four antioxidants considered in this work. It was performed in order to evaluate a possible higher activity related to the type of extracted fraction (see Figures, 2A and 2B.

The method is the same used for previous experiments in "Assay procedure for 8-MOP photomutagenesis"[17] and was similar to those described by Jose.[21] Cells from an overnight culture of S. Typhimurium TA 102, a strain sensitive to oxidative mutagens,[22] were centrifuged and resuspended in a replacing volume of sodium phosphate buffer with pH 7.4. Suspensions were transferred to glass petri dishes and all the compounds dissolved in DMSO (1%) were added to this suspension. The chemicals' concentrations were:

1) 8-MOP = 1 μg (4.6×10^{-6} M)
2) BC = 100 μg (1.86×10^{-4} M)
3) Rosmarinus off. L. extract = 100 μg

which was the highest dose. The suspension was pre-incubated for 20 minutes at room temperature and then exposed to UV-A (300-400 nm) radiation; the light source was kept at 12 cm above the culture surface.

At appropriate steps, irradiated cells (0.1 ml) were added to two ml of molten 0.6% top agar (containing 0.5 mM L-histidine and 0.5 mM biotin) kept at 45°C and poured into petri plates containing vogel minimal salt agar with glucose. After 48 hours of incubation at 37°C in the dark, revertant colonies were counted scoring for histidine reversion.

Results and Discussion

Table II shows a comparison of the "Antioxidant Activity" of DRE_1 and DRE_2 with that of BHA and AR. The antioxidant extracts DRE_1 and DRE_2 showed an excellent antioxidant activity, which was no lower than that of the two comparison products.

Table III also shows data for the evaluation of "concentration" effect for the extract DRE_2 compared to AR and BHA. Evaluation of the analytical data made it possible to deduce the following:

a) Induction time of oxidation, in the presence of the extracts DRE_1 and DRE_2, has the same order of magnitude.
b) The antioxidant extract obtained from rosemary deoleated by supercritical CO_2 seems to be slightly more active for no longer than 24 hours, and practically equal to that of the AR extract. However the difference of behavior between DRE_1, DRE_2, and AR cannot be considered really significant. In fact, BHA, at the same concentration, appears to be slightly the most active among the compared products. The activity of DRE_2, BHA and AR, at the same concentration, is practically comparable. Data concerning the antioxidant power with regard to soy oil is presented in Table IV.
c) The antioxidant activity of some vegetable active ingredients, as stated previously, may be parallel "to the antimutagenic effect."[17] The first, second and third extracts (see Figure 2A) demonstrated a considerably different antimutagenic activity, as it is shown in the graph of Figure 3. In the same graph, data relating to β-carotene are compared, which results in the most active compound at the concentrations used in the test. The comparison curve, obtained in the absence of added antioxidants, shows a similar trend with that of the second rosemary extracts. The most active extract was the third one, obtained by hydroalcoholic extraction after removing the dissolved matter in ethyl alcohol 95°, according to the flow sheet in Figure 2A. The curve corresponding to the fourth extract, obtained by defatting with supercritical CO_2, does not differentiate from the first extract, produced by previous defatting in steam flow.

Conclusion

Based on all the information gathered, we were able to reach four conclusions regarding the antioxidant and antimutagentic activity of the extract from *Rosmarinus officinalis* L.

a) The antioxidant activity of the extracts (DRE_1 and DRE_2, produced using the proposed simplified technology, were practically comparable with that of the commercial extract which was taken as term of comparison and produced using a much more complex technology (AR).
b) The extraction treatment by supercritical CO_2, which is as efficient for deodorizing as the traditional method of steam flow distillation, gives an antioxidant product (DRE_2) with an activity comparable to the product

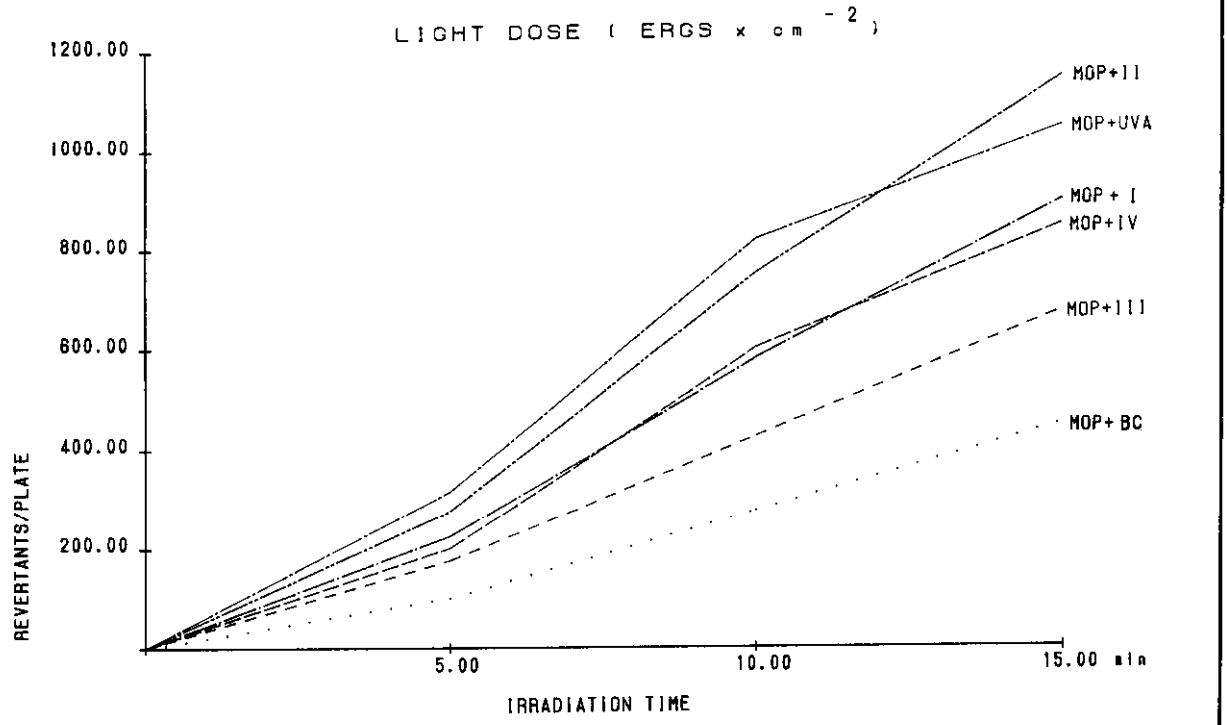

Figure 3. 8-MOP photomutagenesis and its inhibition by beta carotene (BC) and "Rosmarinus officinalis L." (I, II, III, IV) extracts in Salmonella typhimurium TA 102. Each point is the mean of three different experiments.

deoleated by steam flow distillation (DRE_t).

c) The antioxidant activity of rosemary extracts in general is less evident in regard to soy oil, even at considerably higher concentrations than those active in solid fat.

d) The antimutagenic activity is higher for the third rosemary extract obtained by hydro-alcoholic extraction (ethyl alcohol 50° v/v), according to the flow sheet in Figure 2A.

This article detailed the optimum conditions for the production of an extract from "*Rosmarinus officinalis* L." having antioxidant and antimutagenic activity. A study is now in progress on the identification and isolation of pure active molecules.

References

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