Antioxidant Activity of Malt Rootlet Extracts

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To improve the malt rootlet value, the antioxidant potentialities of this byproduct of the malting industry have been analyzed. Three extracts have been considered from the points of view of dry matter yield, level of antioxidant compounds, and efficiency and cost of the extraction protocols. They respectively contain rootlet oil, free phenolic compounds, and bound phenolic compounds. The rootlet oil contains only a low quantity of tocopherols (respectively, 20.6 and 4.2 μg of α-tocopherol and γ-tocopherol per gram of dry rootlets), and a weak antioxidant activity, evaluated in a stripped corn oil by following spectrophotometrically the conjugated dienes, has been pointed out. The bound compound extract presents a good antioxidant power mainly due to the presence of *trans*-ferulic and *trans-p*-coumaric acids, but the dry matter yield is low (2%). The free compound extract has a good antioxidant power, and the valuable dry matter, mainly composed of proteins (52%), sugars (33%), and reducing compounds (5.5%), has a yield of 12%. The mixing of bound and free compound extracts presents an antagonistic effect on the antioxidant power, but a synergistic effect has been pointed out for the mixing of α-tocopherol and free compound extract.

Keywords: *Malt rootlets; antioxidant activity; phenolic compounds; hydroxycinnamic acids; conjugated dienes; α-tocopherol; BHT; synergism; antagonism*

INTRODUCTION

To protect food and cosmetic products from oxidation, antioxidants are currently used. If synthetic antioxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) are often chosen because of their high efficiency and low cost (Duh and Yen, 1997), they are mentioned as potentially toxic (Onyeneho and Hettiarachchy, 1992; Foti et al., 1996; Duh and Yen, 1997). On the other hand, Heinonen et al. (1998) and Benavente-Garcia et al. (1997) noted that some natural polyphenols have therapeutic effects or a protective action against cardiovascular diseases and some cancers. Therefore, many investigations have been made over the past several years to find new sources of natural phenolic antioxidants, especially from byproducts. Durum wheat bran (Onyeneho and Hetteiarachchy, 1992), peanut hulls (Duh and Yen, 1997), carob seeds (Batista et al., 1998), evening primrose (Shahidi et al., 1997), or citrus peels and seeds (Bocco et al., 1998) are examples of natural matter that has been investigated for the production of antioxidant extracts. Moreover, it is well-known that rosemary, tea, and grape extracts are used as natural antioxidants in foods or food supplements.

If phenolic acids and flavonoids are often researched for their antioxidant properties (Heinonen et al., 1998), tocopherols also constitute a class of phenolic compounds that present an important antioxidant potential. Erickson (1991) described tocopherol as one of the most effective natural antioxidants. Several authors also noted that insoluble phenolic compounds such as hydroxycinnamic acids could present an interesting antioxidant potential (Maillard and Berset, 1995; Cuvelier et al., 1992).

Malt and barley have shown antioxidant properties mainly due to the presence of phenolic compounds, especially flavonoids and hydroxycinnamic acids (Maillard et al., 1996). Malt rootlets are germs appearing during the malting process of barley, which are separated before the brewing process and treated as a byproduct for animal feed. The production of an antioxidant extract from malt rootlets would add value to this byproduct and provide a potential new source of natural antioxidant.

In this study, different antioxidant extracts were prepared from malt rootlets and the main constituents were quantified by different analytical methods. The efficiency of the extractions is discussed with regard to their antioxidant power compared with those of commercial antioxidants and considering the cost and difficulties of the different extraction protocols. Moreover, synergistic and antagonistic effects obtained with different mixtures of antioxidants are described.

MATERIALS AND METHODS

Reagents. BHT, gallic acid, α-, δ-, and γ-tocopherols, 2,2,4-trimethylpentane (spectrophotometric grade), chloroform (HPLC grade), and ethyl acetate (Pestanal grade) were obtained from Sigma-Aldrich (Saint-Quentin Fallavier, France). Methanol (HPLC grade), ethanol (ACS analysis), and acetonitrile (HPLC grade) were from Carlo Erba (Val de Reuil, France). Hexane (analysis grade) was from BDH (Dorset, U.K.) and acetic acid from Fisher Scientific (Elancourt, France). Water was purified with an Elix 3 system (Millipore). *trans*-Ferulic acid, *trans-p*-coumaric acid, and (+)-catechin were obtained from Extrasynthèse (Genay, France). Glucose, galactose, raffinose, xylose,

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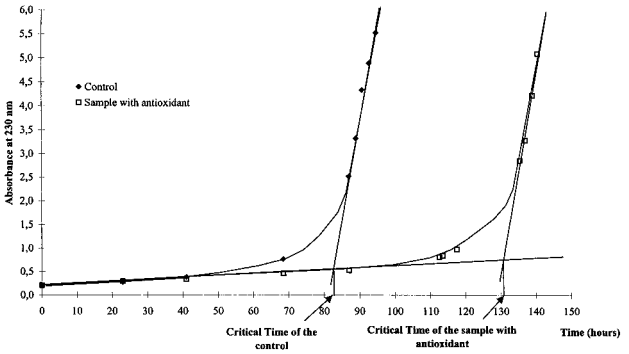

Figure 1. Graphical determination of the CT in the measurement of conjugated diene hydroperoxides.

and arabinose were from Prolabo (Fontenay-sous-Bois, France), and saccharose was from Labosi (Oulchy-le-Château, France). Corn oil stripped of tocopherols was purchased from Eastman Kodak Co. (Rochester, NY).

Plant Material. Malt rootlets issued from barley (*Hordeum vulgare* L.) var. Nevada harvested in 1997 were furnished by Grandes Malteries Modernes, Marquette-lez-Lille, France.

Antioxidant Power (AOP) Determination. The AOP was determined according to a method adapted from Huang et al. (1996). It was assessed by comparing a control oxidation kinetic of a stripped corn oil without antioxidant, with a kinetic established for a sample with synthetic or natural antioxidant. The fatty acid composition of the oil was as follows: 11% of C16:0, 1% of C18:0, 27% of C18:1, 60% of C18:2, and 1% of C18:3.

Oxidation. A total of 2.5 g of the stripped corn oil was weighed into a screw-capped 60 mL vial and oxidized at 60 °C in a shaker water bath (Grant model OLS 200) with orbital shaking (185 t min^{-1}). The same procedure was followed for samples with antioxidants. However, the antioxidants were previously dissolved in methanol (α-tocopherol, BHT, and the bound phenolic compounds extract) or in water (free compound extract) before being added at various concentrations to the stripped corn oil. The added volume of the antioxidant solution never exceeded 200 μL. The solvents were evaporated under nitrogen before the oxidation process.

Oxidative stability was determined spectrophotometrically by measuring the conjugated diene hydroperoxides at periodic intervals.

Measurement of Conjugated Dienes. Ten microliters oil samples were dissolved in 2,2,4-trimethylpentane, and the absorbance was followed at 230 nm with a spectrophotometer Lambda 9 (Perkin-Elmer). The AOP was assessed by determining a critical time (CT) on the oxidation kinetics (Figure 1) and was expressed as follows:

$$\text{AOP} = (\text{CT of the sample with antioxidant} - \text{CT of the control}) \times 100/\text{CT of the control}$$

Extraction of Tocopherols from Malt Rootlets. Twenty-five grams of malt rootlets was ground in an analysis blender IKA A10 and lixiviated in a Bolton extractor with 200 mL of hexane for 5 h at 60 °C. Hexane was then evaporated to dryness at 60 °C under vacuum, and the fatty residue was dissolved in 60 mL of chloroform/methanol (50:50, v/v).

Three oil extraction replicates were performed and analyzed by high-performance liquid chromatography (HPLC) coupled with a coulometric detector.

Extraction of Free Antioxidant Compounds from Malt Rootlets. Fifty grams of malt rootlets was ground in an analysis blender IKA A10 and extracted with 250 mL of ethanol/water (75:25, v/v) at ambient temperature for 1 h on a magnetic stirrer. The extract was then centrifuged at 20 °C during 20 min at 12000*g* with a Sorvall super T21 centrifuge. A second extraction was performed under the same conditions with 150 mL of ethanol/water (75:25, v/v).

The supernatants were combined and concentrated by distillation of the ethanol to obtain an aqueous extract of 40 mL. The residual cake was used for the extraction of bound compounds.

Extraction of Bound Antioxidant Compounds from Malt Rootlets. The cake obtained from the extraction of free compounds was hydrolyzed with 400 mL of 4 N NaOH under N_2 at 60 °C for 4 h. Fifty milliliters of the mixture was then acidified at pH 1 with HCl and extracted three times with ethyl acetate (v/v). The ethyl acetate phases were combined and evaporated to dryness at 60 °C under vacuum. The residue was dissolved in 20 mL of methanol (HPLC grade).

Separation and Quantification of Tocopherols by HPLC-ED. An aliquot of 20 μL of the malt rootlet oil was filtered through a 0.2 μm filter (Gelman) and analyzed using an HPLC 600E Waters system, an ESA Coulochem II electrochemical detector (ED) equipped with a guard cell model 5021, and an analytical cell model 5010. The electrochemical settings were as follows: guard channel, potential −250 mV; channel 1, potential 0 mV and sensitivity 1 μA; channel 2, potential 400 mV and sensitivity 1 μA. The tocopherols were separated after injection of 20 μL of the solution along an ODS Phase Sep C_{18} column (250 mm × 4.6 mm i.d.; particle size, 5 μm). The mobile phase was acetonitrile/acetic acid (90:10, v/v) at a flow rate of 1 mL min^{-1}. Three injections were performed for each of the three oil replicates. The data were therefore the average of nine results.

Peaks have been identified by comparing their retention times and electrochemical dominant potential values with those of commercial standards. Tocopherols were quantified by external standard calibration and expressed in micrograms per gram of dry malt rootlets.

Identification and Quantification of Phenolics in Bound Compound Extract by HPLC-UV. Identification of bound phenolic compounds was performed with an HPLC Gilson system, a Hewlett-Packard 1040 M photodiode array detector, and an Hypersil ODS C_{18} column (250 mm × 4.6 mm i.d.; particle size, 5 μm). The injected volume was 20 μL. The mobile phase was a mixture of acetonitrile/water/acetic acid (4.5:85.5:10, v/v/v). The solvent flow rate was 1 mL min^{-1}.

The hydroxycinnamic acids were identified by comparing their retention times and UV spectra with those of commercial standards. Their quantification was performed at their maximal absorbance values using an external standard method: *trans-p*-coumaric acid and *cis*-ferulic acid at 311 nm and *trans*-ferulic acid at 322 nm. *cis*-Isomers were prepared by photoisomerization according to the method of Maillard and Berset (1995). Results were expressed in micrograms per gram of dry malt rootlets.

All of the values were means of three injections from three different extracts. A coefficient of variation (ratio between the standard deviation and the mean) of 9% was thus obtained.

Determination of the Composition of Free Compound Extract. The protein quantification was performed using the Kjeldahl method (NF V 18-100, 1978).

Sugars were identified and quantified with an HPLC Gilson system (model 302) coupled with a differential electronic refractometer (Waters Associates R 401). Sugars were separated along a Lichrospher 100 NH_2 column (250 × 4.6 mm i.d.; particle size, 5 μm). The injected volume was 20 μL. The mobile phase was acetonitrile/water (90:10, v/v) at a flow rate of 1.5 mL min^{-1}. The chromatogram obtained was compared with the one of a standard solution prepared with glucose, arabinose, raffinose, galactose, xylose, and saccharose at a concentration of 10 g L^{-1}.

The amount of total free reducing compounds was measured with the Folin−Ciocalteu method (Folin and Ciocalteu, 1927) as adapted by Maillard et al. (1996). The results were expressed in grams of gallic acid equivalents per 100 g of dry rootlets.

RESULTS AND DISCUSSION

Measurement of the Antioxidant Activity. The antioxidant activity of pure molecules and rootlet ex-

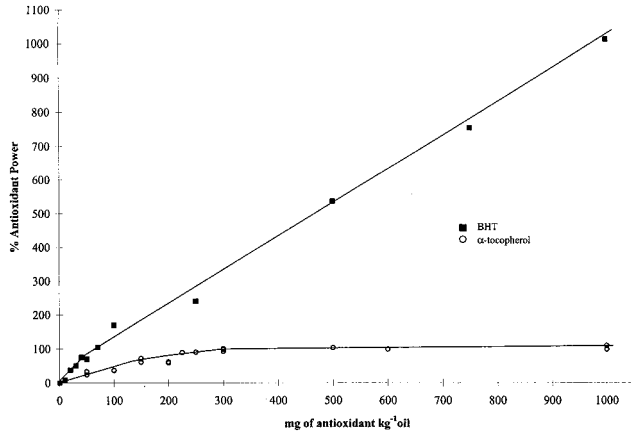

Figure 2. Relationship between the antioxidant power and the concentrations of BHT and α-tocopherol in a stripped corn oil.

tracts has been evaluated with an accelerated oxidation test using moderate thermal conditions (60 °C) and a stripped corn oil as substrate.

The oxidation kinetics, established by following the production of conjugated diene hydroperoxides at 230 nm, show two steps: a lag phase and an accelerated oxidative phase (Figure 1). From the tangent intersection of these two phases, a CT has been determined. The initial oxidation state of the oil clearly influences the control CT value: a fresh sample of oil gives a control CT value of 85 h against 61 h for an oil that has been held for few weeks at 4 °C under nitrogen. The possible presence of some free radicals that accelerate the oxidation kinetics could explain these different control CT values. This observation requires doing a control at each assay. The perfect control of the temperature and of the shaking is particularly important to obtain reproducible results. Indeed, when the oxidation of the stripped corn oil has been evaluated in an oven with magnetic stirring, the coefficient of variation of the control CT values was >10%. The use of a water bath with a simultaneous shaking of the samples and a homogeneous temperature improves the reproducibility. We should also note that the variability of the absorbance values in the accelerated oxidation phase has few consequences on the CT value.

From this CT value, the AOP can be assessed as

$$\text{AOP} = (\text{CT of the sample with antioxidant} - \text{CT of the control}) \times 100/\text{CT of the control}$$

The relationship between the concentration of antioxidant and the AOP varies according to the studied antioxidant. A linear relationship has been established between the concentration of BHT in the corn oil and its AOP from 75 to 1000 ppm (Figure 2) leading to a CT of 10 times that of the control CT. For α-tocopherol, from 0 to 300 ppm, the AOP value depends on the concentration (Figure 2) and then reaches a plateau, showing that increasing the concentration does not improve the antioxidant activity: the AOP maximum value only leads to a doubling of the control CT.

Tocopherols in Malt Rootlets. The oil extracted from rootlets represents 1.65% of the dry matter. This yield is slightly lower than that observed in barley grains: 2.1% (Wang et al., 1993).

As previously described in barley (Peterson, 1994), we found that α-tocopherol was the most abundant in

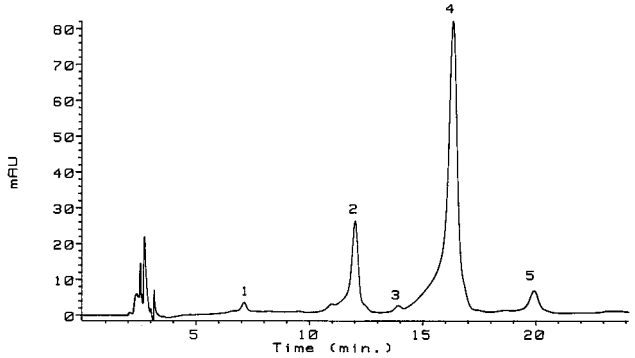

Figure 3. HPLC profile of the bound phenolic compound extract of malt rootlets: (1) hydroxycinnamic derivative; (2) *trans*-*p*-coumaric acid; (3) *cis*-*p*-coumaric acid; (4) *trans*-ferulic acid; (5) *cis*-ferulic acid.

rootlets (20.6 μg g^{-1} of dry matter), followed by γ-tocopherol (4.2 μg g^{-1} of dry matter). These values are higher than those observed in malt, for which the quantities of α- and γ-tocopherols are, respectively, 10.0 and 0.4 μg g^{-1} of dry matter (Peterson, 1994).

However, the valorization of tocopherols from rootlets does not seem valuable. In fact, considering the antioxidant activity of pure α-tocopherol (Figure 2), 120 ppm of α-tocopherol is needed to reach an AOP of 50%, corresponding to 80 g of rootlet oil/kg of stripped corn oil. Such a result does not justify the cost of a specific extraction of vitamin E to produce a natural antioxidant extract.

Bound Phenolic Compounds in Malt Rootlets. Maillard and Berset (1995) showed that barley and malt contained bound hydroxycinnamic acids, mainly *trans*-ferulic and *trans*-*p*-coumaric acids, which have antioxidant properties. These phenolic acids are respectively linked to arabinoxylans (Nordkvist et al., 1984) and to lignin (Salomonsson et al., 1980), and the interest of an extraction by an alkaline hydrolysis has been considered with regard to the extraction yield and to the antioxidant power of the extract.

A typical HPLC profile of the extract obtained by alkaline hydrolysis is shown in Figure 3. Peaks 2 and 4 have been identified, respectively, as *trans*-*p*-coumaric acid and *trans*-ferulic acid, according to their spectral characteristics and retention times, in comparison with commercial pure compounds. *cis*-*p*-Coumaric and *cis*-ferulic acids (peaks 3 and 5) were compared to standards obtained by photoisomerization. The spectral analysis of peak 1 suggested that it was a hydroxycinnamic acid derivative. However, its structure has not been further investigated.

As already shown in barley and malt (Nordkvist et al., 1984; McMurrough and Byrne, 1992; Maillard and Berset, 1995), *trans*-ferulic acid is the main bound phenolic acid of malt rootlets, followed by *trans*-*p*-coumaric and *cis*-ferulic acids (Table 1). If *trans*-isomers of cinnamic acids are generally considered to be the native molecular forms because of their higher stability, *cis*-isomers have often been reported. However, they could partially come from an isomerization of the *trans*-isomers occurring during exposure of the extracts to ultraviolet light (Hermann, 1989) and slightly during alkaline hydrolysis (Maillard and Berset, 1995).

To optimize the extraction of bound phenolic acids, two parameters of the alkaline hydrolysis have been studied: the concentration and the volume of NaOH.

Table 1. Influence of the Alkaline Hydrolysis Conditions on the Extraction of the Bound Phenolic Compounds of Malt Rootlets[a]

Influence of the Alkaline Strength

from 50 g of malt rootlets	400 mL of 1 N NaOH, 60 °C, 4 h	400 mL of 2 N NaOH, 60 °C, 4 h	400 mL of 4 N NaOH, 60 °C, 4 h
trans-ferulic acid (mg kg^{-1})	819 ± 74	892 ± 80	1027 ± 92
trans-*p*-coumaric (mg kg^{-1})	127 ± 11	163 ± 15	184 ± 17
cis-ferulic acid (mg kg^{-1})	nd	nd	nd
total phenolic acids (mg kg^{-1})	946 ± 85	1055 ± 95	1211 ± 109

Influence of the Volume of NaOH

from 50 g of malt rootlets	400 mL of 2 N NaOH, 60 °C, 4 h	600 mL of 2 N NaOH, 60 °C, 4 h	800 mL of 2 N NaOH, 60 °C, 4 h	1000 mL of 2 N NaOH, 60 °C, 4 h
trans-ferulic acid (mg kg^{-1})	892 ± 80	990 ± 89	1005 ± 90	1001 ± 90
trans-*p*-coumaric (mg kg^{-1})	163 ± 15	202 ± 18	223 ± 20	208 ± 19
cis-ferulic acid (mg kg^{-1})	nd	nd	54 ± 5	84 ± 8
total phenolic acids (mg kg^{-1})	1055 ± 95	1192 ± 107	1282 ± 115	1293 ± 117

[a] Data are means of three replications. nd, not detected.

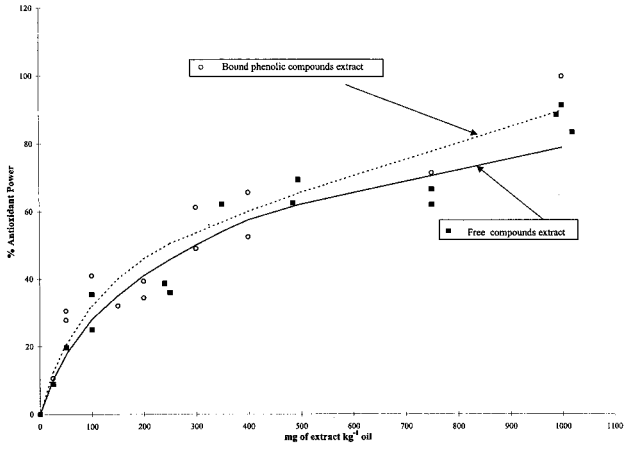

Figure 4. Relationship between the AOP and the concentration of bound and free compound extracts of malt rootlets in a stripped corn oil.

Results reported in Table 1 show that increasing the concentration of NaOH enhances the extraction of total bound hydroxycinnamic acids, thus breaking more easily the linkages between *p*-coumaric acid and lignin and between ferulic acid and arabinoxylans. Indeed, the use of 4 N NaOH improves the extraction yield by 15% compared to 2 N NaOH and by 28% compared to 1 N NaOH. The extraction of *trans*-*p*-coumaric acid appears to be more significantly increased. However, no *cis*-isomers have been detected in such conditions. In fact, *cis*-ferulic acid has been detected only for high volumes of NaOH (800 and 1000 mL). When the viscosity of the alkaline solution is lower, the stirring is easier and the extraction yield is improved. However, this slight improvement does not justify the use of high volumes of NaOH. Thus, the extraction with 400 mL of 4 N NaOH for 4 h has been retained. These extraction conditions yield a dry matter of 2%.

The antioxidant power of the bound phenolic extract from malt rootlets has been determined (Figure 4). As for α-tocopherol, the AOP increases with the antioxidant concentration in corn oil but does not seem to reach a plateau.

To compare the efficiencies of the different antioxidants, the concentration to obtain an AOP of 50% has been calculated. Two reasons explain the choice of this value: first, this AOP corresponds to a shelf life extension of the control by a factor of 1.5, which is an appreciable increase for food products; second, the concentrations corresponding to this AOP are of the same order as those authorized in the food industry.

To obtain an AOP of 50%, 250 ppm of bound phenolic compound extract is required against only 120 ppm for α-tocopherol. Compared to BHT, it is ∼5 times less efficient. Such results were expected, considering that the bound extract contains only 5.6 g of phenolic compounds for 100 g of dry extract. This means that when an AOP of 50% is obtained for 250 ppm of bound extract, only 14 ppm of phenolic compounds is introduced in the stripped corn oil. This high activity could be explained by a synergistic effect between *trans*-*p*-coumaric and *trans*-ferulic acids that has been pointed out in a recent work (Peyrat-Maillard et al., 1998). Moreover, other compounds not detected in our HPLC conditions could participate in the antioxidant activity of the bound phenolic compound extract. We should also note that the quantification of total phenolic compounds with the Folin−Ciocalteu method gives a higher value than that with HPLC: the yield of total reducing compounds assessed with the Folin−Ciocalteu method is 2.25 times higher (0.27 g of gallic acid equiv 100 g^{-1} of dry malt rootlets against 0.12 g 100 g^{-1} of dry malt rootlet with the HPLC method). The Folin−Ciocalteu method was often described as a nonspecific method for phenolics (Marigo, 1973; Deshpande et al., 1986; Macheix et al., 1990).

Therefore, these results show that the bound compound extract has a non-negligible antioxidant activity, but the low yield of valuable matter (2%) and the high number of extraction steps make this method of industrial valorization difficult.

Free Compounds in Malt Rootlets. As described for the bound extract, the extraction of free reducing compounds has been optimized (Figure 5). For a one-step extract, the best yield (0.54 g of gallic acid equiv 100 g^{-1} of dry malt rootlets) is obtained for 50 g of malt rootlets extracted with 500 mL of solvent (ethanol/water, 75:25, v/v) corresponding to a ratio of 0.1. To minimize the solvent consumption, several lower volumes have been tested. Results show that the decrease of the solvent volume leads to a linear decrease ($R^2 = 0.996$) of the extraction efficiency. Indeed, for ratios of 0.2 and 0.3, only 81 and 54% of reducing compounds are, respectively, extracted compared to the quantity obtained with a ratio of 0.1. If such extraction conditions correspond to a non-negligible cost lowering, the loss is

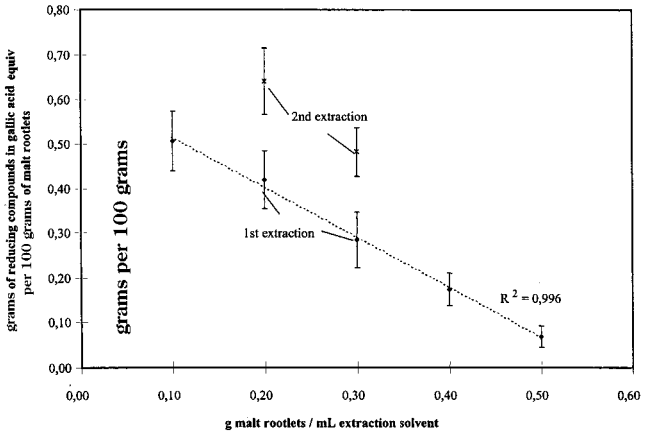

Figure 5. Relationship between the reducing compound yield in gallic acid equivalents and the ratio quantity of malt rootlets/volume of extraction solvent. Data are means of three replications.

too important. To improve the yield, a second extraction has been performed on the residue of the first extraction (Figure 5 and 6). Moreover, the extraction time and the solvent volume have been optimized for a ratio of 0.2. In all cases, the reducing compound yield is increased with a second extraction. However, three conditions allow us to obtain a maximum value (Figure 6): 200 mL/30 min, 250 mL/30 min, and 150 mL/60 min, which, respectively, increase the extraction yield by 52, 48, and 50%. These rises are not significantly different and, thus, the last conditions have been selected to minimize the used volume of ethanol. These conditions allow us to obtain a yield of 12 g of dry matter for 100 g of dry rootlets.

Free extract is mainly composed of proteins and sugars, which, respectively, represent 52 and 33% of the dry matter. Salama et al. (1997) reported that crude proteins are the main constituents of the rootlets with 32%, followed by starch, crude fiber, and ash. Considering the extraction conditions, it was expected that proteins and sugars were present in the free phenolic compound extract. Saccharose (8.0%), glucose, and galactose (9.7%) are the main sugars of the extract followed by xylose (4.3%) and arabinose (3.2%). The reducing compounds represent 5.5% of the dry extract corresponding to 0.66 g of gallic acid equiv for 100 g of dry malt rootlets. This yield is 2.8 times higher than that observed by Salama et al. (1997). The variety of barley and the harvest date, as well as the quantification method, could explain such a difference.

The antioxidant power of the free compound extract has been evaluated at different concentrations (Figure 4). The relationship between the AOP and the concentrations is similar to that obtained for the bound phenolic compound extract. A total of 300 ppm of free extract allows us to obtain an AOP of 50%. The presence of phenolic compounds could partially explain the antioxidant activity. As observed for the bound extract, when the AOP of 300 ppm of free extract is evaluated, only 16.5 ppm of reducing compounds is introduced in the corn oil and perhaps much less if we consider the lack of specificity of the Folin−Ciocalteu method. As in malt, Maillard reaction products appearing during kilning could also contribute to the antioxidant activity (Maillard, 1996).

The elimination of proteins and sugars could allow us to obtain a more efficient antioxidant extract; nevertheless, the raw free compound extract shows an interesting antioxidant activity for concentrations <1000 ppm and presents a good extraction yield (12%), proving that the production of a natural antioxidant extract from malt rootlets could be a new outlet for the malting industry.

Synergistic and Antagonistic Effects of the Mixtures. To improve the antioxidant power of the malt rootlet extracts without increasing their concentration

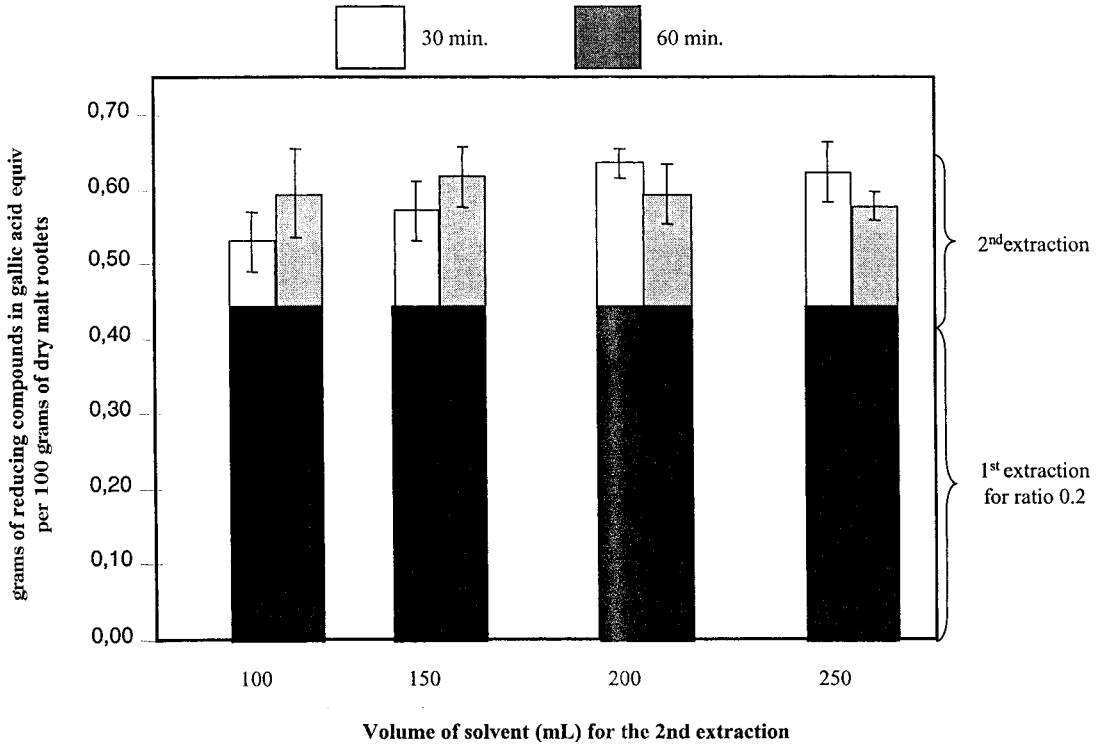

Figure 6. Efficiency of a second extraction step considering the extraction time and the volume of solvent. Data are means of three replications.

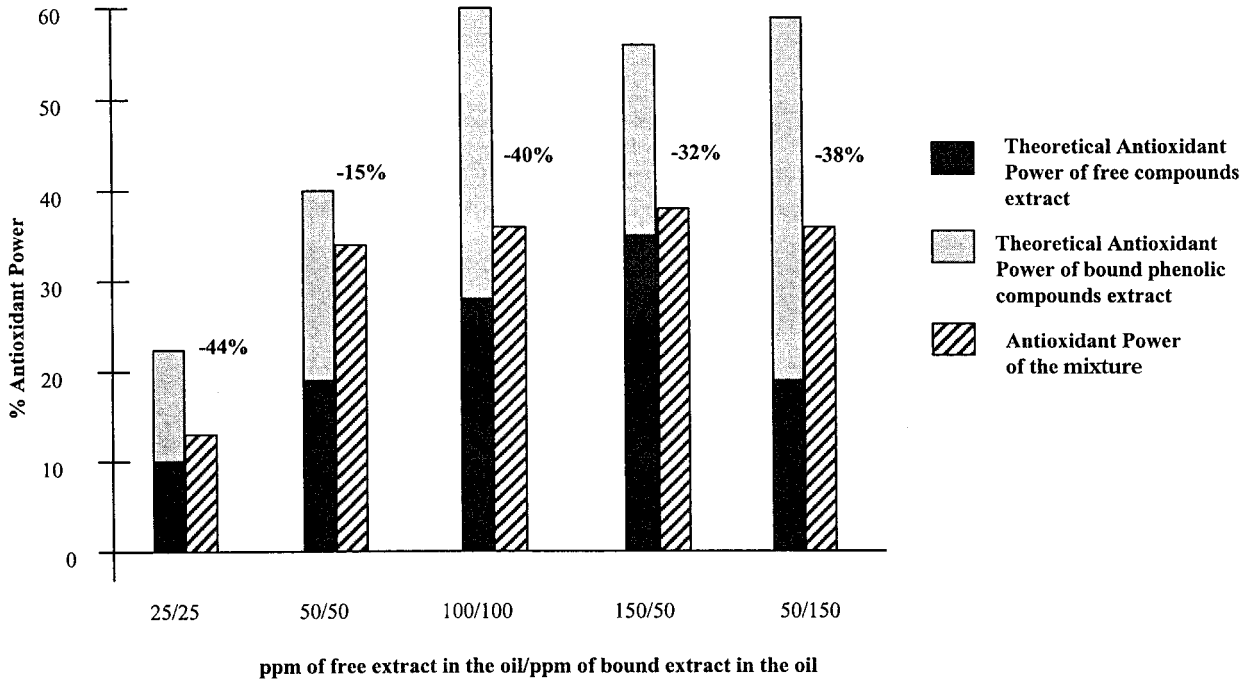

Figure 7. Comparison between the theoretical antioxidant power of the free and bound compound extracts of malt rootlets and the antioxidant power of the mixture.

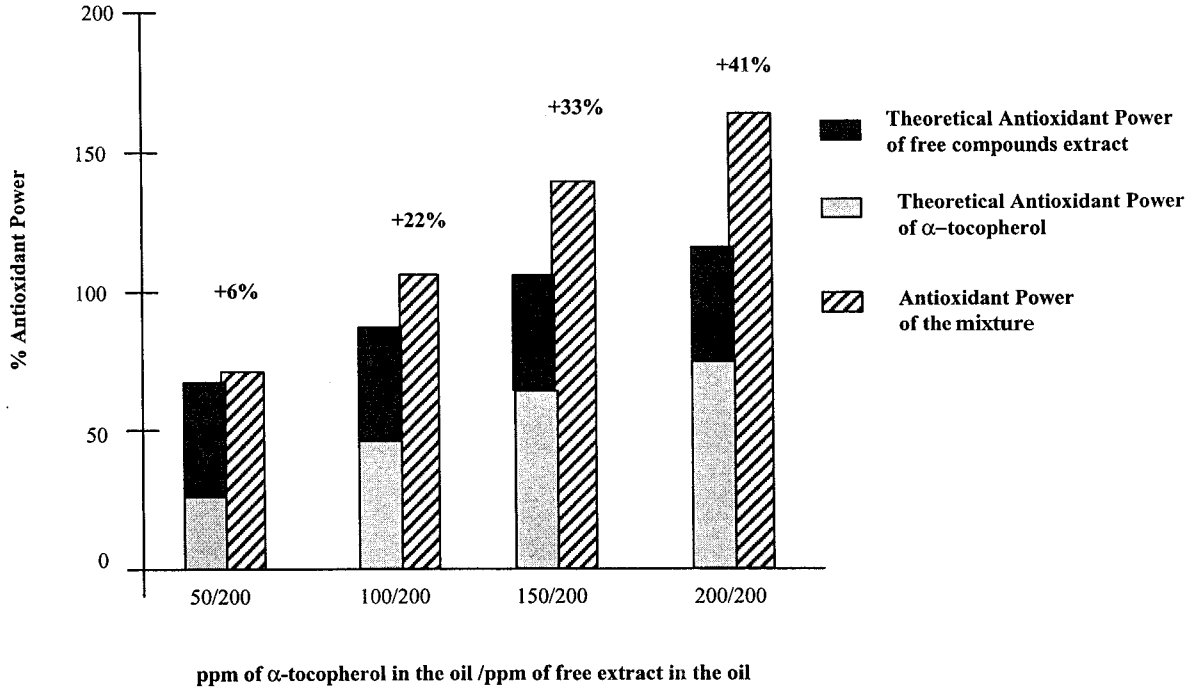

Figure 8. Comparison between the theoretical antioxidant power of the free phenolic compound extract of malt rootlets and of α-tocopherol and the antioxidant power of the mixture.

in the corn oil, a synergistic effect has been researched. Several mixtures of the bound and free extracts have been tested at different ratios and at different concentrations to point out a potential "mixing effect " between these two extracts.

Figure 7 compares the AOP measured from the mixing (AOP_m) and the theoretical calculated AOP (AOP_t), which is the sum of the AOP values of each extract. If a synergism occurs, the AOP_m value is higher than the AOP_t value; for an antagonism, the AOP_m value is inferior to the AOP_t value; and if no "mixing effect" is observed, the AOP_m value must be similar to the AOP_t value. For all of the studied ratios and concentrations, the mixing of bound compound extract and free compound extract showed an antagonistic effect (Figure 7). The intensity of this effect varied from 15 to 44%. Peyrat-Maillard et al. (1998) found an antagonistic effect between *trans*-ferulic acid and (+)-catechin. However, the bound extract contains *trans*-ferulic acid, but no flavan-3-ols, (+)-catechin in particular, have been found. More investigations of reducing compounds present in the free extract are now necessary.

The comparison between the AOP measured for the mixture of α-tocopherol/free compound extract and the theoretical AOP obtained by summing the AOP value of each antioxidant separately is shown in Figure 8. This

comparison has been made for several concentrations of α-tocopherol and a constant concentration of the free extract (200 ppm). Except for the ratio 50:200, we observed a significant synergistic effect of the mixing, which proportionally increases with the α-tocopherol quantity: from 22 to 41%. We can also note that the AOP obtained with a mixing of 200 ppm of free compound extract and 100 ppm of α-tocopherol is similar to that obtained with 300 ppm of α-tocopherol alone. From this point of view, 200 ppm of α-tocopherol could be substituted by 200 ppm of rootlet extract in a vegetable oil without losing any antioxidant power.

Such synergistic effects have already been observed with α-tocopherol. Indeed, Han et al. (1991) showed a synergistic effect between α-tocopherol and ascorbic acid, and Packer et al. (1979) suggested that ascorbic acid regenerated the α-tocopheroxyl radical to α-tocopherol by the oxidation of ascorbic acid to ascorbyl radical. Rosemary extract is also described as a regenerator of α-tocopherol, explaining the synergism observed between these antioxidants in a sardine oil system (Cuppett and Hall, 1998). However, Wong et al. (1995) did not mention any synergism between α-tocopherol and rosemary extract in a model meat system. These authors explained that the behaviors of the antioxidants are very dependent on the oxidation medium.

Therefore, the synergism between α-tocopherol and the rootlet extract could be very interesting for the formulation of a new natural antioxidant, considering the low cost of the rootlets, but further investigations are necessary to evaluate the antioxidant properties of this mixture in other media.

Conclusion. Three antioxidant extracts from malt rootlets have been produced; their yields and their main constituent compositions have been determined, and their antioxidant power has been evaluated and compared to those of BHT and α-tocopherol.

Extraction of vitamin E and insoluble bound phenolic compounds from malt rootlets is of little interest considering their low quantity. However, the extraction of free antioxidant compounds could constitute a new valorization method for this byproduct: the antioxidant properties and the yield of this extract argue for this conclusion. Moreover, this work points out a synergistic effect between α-tocopherol and the free extract. The identification of the main compounds present in this free extract is now being further investigated.

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