Journal of Food Protection, Vol. 65, No. 10, 2002, Pages 1614–1622
Copyright ©, International Association for Food Protection

Antioxidant Activity of Edible Fungi (Truffles and Mushrooms): Losses during Industrial Processing

M. ANTONIA MURCIA,[1]* MAGDALENA MARTÍNEZ-TOMÉ,[1] ANTONIA M. JIMÉNEZ,[1] ANA M. VERA,[1] MARIO HONRUBIA,[2] AND PILAR PARRAS[1]

[1]*Department of Food Science, Veterinary Faculty, and* [2]*Department of Vegetable Biology, Biology Faculty, Campus de Espinardo, University of Murcia, Apartado de Correos 4021, E-30008-Murcia, Spain*

MS 01-467: Received 13 December 2001/Accepted 14 May 2002

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/65/10/1614/1672364/0362-028x-65_10_1614.pdf by University of Murcia user on 02 December 2021

ABSTRACT

The antioxidant properties of two raw truffles (*Terfezia claveryi* Chatin and *Picoa juniperi* Vittadini) and five raw mushrooms (*Lepista nuda, Lentinus edodes, Agrocybe cylindracea, Cantharellus lutescens,* and *Hydnum repandum*) were tested by subjecting these truffles and mushrooms to different industrial processes (freezing and canning) and comparing them with common food antioxidants (α-tocopherol [E-307], BHA [E-320], BHT [E-321], and propyl gallate [E-310]) with regard to their ability to inhibit lipid oxidation. All of the truffles and mushrooms analyzed exhibited higher percentages of oxidation inhibition than did the food antioxidants according to assays based on lipid peroxidation ($LOO^{\cdot}$), deoxyribose ($OH^{\cdot}$), and peroxidase (H_2O_2). Frozen samples exhibited a small reduction in free radical scavenger activity, but the results did not show a significant difference ($P < 0.05$) with respect to the raw samples, while canned truffles and mushrooms lost some antioxidant activity as a consequence of industrial processing. All of the raw and frozen truffles and mushrooms except frozen *Cantharellus* improved the stability of oil against oxidation (100°C Rancimat), while canned samples accelerated oil degradation. Antioxidant activity during 30 days of storage was measured by the linoleic acid assay, and all of the samples except canned *Terfezia, Picoa,* and *Hydnum* showed high or medium antioxidant activity. The Trolox equivalent antioxidant capacity assay was used to provide a ranking order of antioxidant activity as measured against that of Trolox (a standard solution used to evaluate equivalent antioxidant capacity). The order of raw samples with regard to antioxidant capacity was as follows (in decreasing order): *Cantharellus, Agrocybe, Lentinus, Terfezia, Picoa, Lepista,* and *Hydnum.* Losses of antioxidant activity were detected in the processed samples of these truffles and mushrooms.

Although numerous types of truffles and mushrooms exist in nature, fewer than 25 mushroom species are widely used as food, and even fewer can be considered items of commerce *(26).* The world's most widely cultivated mushroom is *Agaricus bisporus,* which accounts for 56% of the total world production, while the next most important species is *Lentinus edodes,* accounting for 14% *(9).*

Truffles and mushrooms are healthy foods that are low in calories and fat and rich in vegetable proteins. Their protein content is higher than that of most vegetables and their amino acid composition is comparable to that of animal proteins *(11).* They appear to be a good source of vitamin C, the B vitamins, and minerals. Mushrooms are the only non–animal-based food containing vitamin D, and hence they are the only natural source of vitamin D for vegetarians *(32).* Most edible fungi are rich in nonstarch polysaccharides, beta glucans that are a good source of soluble and insoluble dietary fiber for humans *(7, 29, 37).*

The lipid composition of mushrooms includes neutral lipids (high monoglyceride and low triglyceride levels), fatty acids (unsaturated fatty acids such as oleic and linoleic acids) *(39),* and phospholipids (such as phosphatidylethanolamine and cardiolipin) *(13, 26).* Truffles (*Terfezia claveryi* and *Picoa juniperi*) contain large quantities of unsaturated fatty acids, among which linoleic acid predominates *(37).*

Among the constituents of truffles and mushrooms are vitamins A, C, and β-carotene, all of which have protective effects because of their antioxidant and antiradical properties. Truffles and mushrooms also contain many phenols, which are very efficient scavengers of peroxyl radicals. Moreover, the action of phenolic compounds is related to their capacity to reduce and chelate ferric iron, which catalyzes lipid peroxidation *(16).* Several authors have observed the blocking effects of *Hydnum* mushrooms on induced liver lipid peroxidation *(24).*

Many medicinal properties have been attributed to mushrooms *(8),* such as the inhibition of platelet aggregation *(20)* and blood cholesterol *(2),* the prevention or alleviation of heart disease and the reduction of blood glucose levels *(29),* and the prevention or alleviation of infections caused by bacterial, viral, fungal, and parasitic diseases *(9, 29).* The large amount of potassium in mushrooms suggests that they could be part of an antihypertensive diet *(28).* Immunomodulation and anticancer effects have also been described *(41).*

The quality of truffles and mushrooms, which depends on, among other things, their delicate flavor and postharvest conservation, influences their value in the marketplace. After they are harvested, mushrooms change in texture and

* Author for correspondence. Fax: +34 968 364792;
E-mail: mamurcia@um.es.

lose weight and whiteness, becoming increasingly brown in appearance. This change in color, which also represents a deterioration in quality, is a result of the enzymatic oxidation of polyphenols. Most recent research on mushroom quality has examined the postharvest technology used to maintain quality, including canning, modified-atmosphere packaging, cooling, and γ-irradiation *(10).* The effect of such processing techniques on food antioxidant properties has also been extensively described and is a matter of controversy because of the presence of thermolabile and thermostable chemicals with prooxidant or antioxidant activity and because of the resultant effect of the prooxidant enzymes present in larger and smaller amounts *(17).*

There is little information on the effect of temperature on the free radical–scavenging capacity of natural antioxidant extracts *(23).* Therefore, the aim of the present study was to use established assays to evaluate the antioxidant effects of truffles and mushrooms in order to assess their suitability for the nutritional enrichment of foods. In addition, our aim was to determine the effect of industrial processing (freezing and canning) on the antioxidant activity of truffles and mushrooms, since they are widely processed in this way.

MATERIALS AND METHODS

Materials. All chemicals were of chromatography grade quality and were purchased from Sigma Chemical Co. (Poole, Dorset, UK). Two types of desert truffles, *T. claveryi* Chatin and *P. juniperi* Vittadini, were collected in March and April 2001 in southeast Spain (Zarzadilla de Totana, Lorca, Murcia), where they grow naturally in mycorrhizal association with shrubs such as *Helianthemum almeriense.* Their geographic distribution is limited to arid and semiarid areas *(21),* so they are looked upon as a potential agroforestry alternative in these areas, as they require low water input.

The mushrooms *Lepista nuda, L. edodes, Agrocybe cylindracea, Cantharellus lutescens,* and *Hydnum repandum* were purchased from a local supermarket. Both truffles and mushrooms were lyophilized and pulverized and analyzed as 100-mg samples. The widely used food antioxidants α-tocopherol (E-307), BHA (E-320), BHT (E-321), and propyl gallate (E-310) were analyzed at a concentration of 100 μg/g *(14)* as antioxidant standards.

The truffles and mushrooms were harvested, transferred to a truck within 20 min, and later transported to the processing plant at a temperature of 0 to 5°C. The trucks used complied with EC legislation regarding refrigerated trucks.

Industrial processing. Once in the processing plant, the samples were placed in prechilling chambers at 0 to 2°C and at a relative humidity of 90 to 95%, and they were kept in these chambers for 24 h on average. The samples were passed through shakers to remove any debris. The truffles and mushrooms were washed several times in water (18°C) containing decreasing concentrations of chlorine to eliminate foreign bodies (stones, insects, etc.). From this point on, two production processes were carried out, one for freezing and the other for canning.

Freezing treatment. For the freezing treatment, a 10-m tunnel of showers was used for blanching, with the samples being conveyed at 2.15 m/min. The water temperature was 96 to 98°C, and the samples were blanched for 90 s. The water volume was 15,000 ml/min. The samples were then washed with showers of cold water (5 to 10°C, 3.5 m) on a wickerwork platform and then cooled by forced air. Next, individual samples were frozen in a fluid bed tunnel (individual quick-frozen model Agacigoscandia) that was programmed to hold them for 4 min at −30°C. Samples were conveyed at 1.05 m/min. The exit temperature of the truffles and mushrooms was below −20°C. Samples were then packed into plastic bags before being placed in cardboard boxes, which were stored at −20°C in a Slos Freeze model 26 B.

Canning treatment. For the canning treatment, instead of being blanched, the truffles and mushrooms were introduced into glass jars, which were then filled with hot (85°C) filling medium (20 g of NaCl per liter of water). The jars were closed and then heated at 121°C for 30 min before being cooled in water.

Sample preparation. Raw, frozen, and canned samples were sent to our laboratory under suitable conditions. They were then lyophilized in a FTSSYSTEMS (GIRALT) and pulverized to a fine powder with a Moulinex model 505 mincer.

Peroxidation of phospholipid liposomes. The ability of samples to inhibit lipid peroxidation at pH 7.4 was tested by using ox brain phospholipid liposomes essentially as described in Aruoma et al. *(6).* The experiments were conducted with a physiological saline buffer (phosphate-buffered saline [PBS]; 3.4 mM Na_2HPO_4-NaH_2PO_4, 0.15 M NaCl) (pH 7.4). In a final volume of 1 ml, the assay mixtures were made up with PBS, 0.5 mg of phospholipid liposomes per ml, 100 μM $FeCl_3$, 100 mg of samples (or 100 μl of common food antioxidants dissolved in water), and 100 μM ascorbate (added last to start the reaction). BHT is not fully soluble in aqueous solution, and its emulsion is not homogeneous. In order to dissolve it, deionized water with a conductivity of not more than 4 μS/cm was used *(43).* Incubations were carried out at 37°C for 60 min. At the end of the incubation period, 1 ml of 1% (wt/vol) thiobarbituric acid (TBA) and 1 ml of 2.8% (wt/vol) trichloroacetic acid were added to each mixture. The solutions were heated in a water bath at 80°C for 20 min to develop the malondialdehyde thiobarbituric adduct ($(TBA)_2$-MDA). The $(TBA)_2$-MDA chromogen was extracted into 2 ml of butan-1-ol, and the extent of peroxidation was measured in the organic layer as absorbance at 532 nm.

Hydroxyl radical scavenging. In a final volume of 1.2 ml, the reaction mixtures contained the following reagents: 10 mM KH_2PO_4-KOH buffer (pH 7.4), 2.8 mM H_2O_2, 2.8 mM deoxyribose (when used), 50 μM $FeCl_3$ premixed with 100 μM EDTA before addition to the reaction mixture, and 100 mg of the tested truffles and mushrooms (or 100 μl of common food antioxidants dissolved in water). Ascorbate (100 μM), when used, was added to start the reaction. The tubes were incubated at 37°C for 1 h. The products of the hydroxyl radical ($OH^{\cdot}$) attack on deoxyribose were measured as described in Aruoma et al. *(5).*

Scavenging of hydrogen peroxide. The samples (100 mg) (or 100 μl of common food antioxidants dissolved in water) to be tested with H_2O_2 were incubated with 0.84 mM H_2O_2 for 10 min at 25°C. Aliquots of these compounds were then assayed for remaining H_2O_2 with the peroxidase system *(31).* The remaining H_2O_2 was measured as the formation of a chromophore recorded at 436 nm in reaction mixtures containing, in a final volume of 1 ml, 0.150 M KH_2PO_4-KOH buffer (pH 7.4), 50 μl of guaiacol solution (created by adding 100 μl of pure guaiacol to 100 ml of water), and 10 μl of Sigma type IV horseradish peroxidase (5 mg/ml in the same phosphate buffer).

Rancimat test for oxidative stability. Sample preparation for the Rancimat test consisted of the maceration of 3 g of refined

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/65/10/1614/1672364/0362-028x-65_10_1614.pdf by University of Murcia user on 02 December 2021

olive oil (provided by the manufacturing company and free of added antioxidants or preservatives) with 10% of the tested truffles and mushrooms (or 100 μg of common food antioxidants per g) for 1 h at room temperature prior to analysis. The Rancimat method (Metrohm model 743, Herisan, Switzerland) determines the induction period by measuring the increase in the volatile acidic by-products released from the oxidizing oil at 120°C. The concentration of the degradation products, which are transferred into distilled water, is assessed by measuring the conductivity. Longer induction periods suggest stronger activity for the added antioxidants. The relative activity of the antioxidants is expressed by the protection factor, which is calculated by dividing the induction period of the oil with added antioxidants by the induction period of the control (olive oil alone) *(44)*.

This technique has been questioned by some authors *(15)*, but, in agreement with Martínez-Tomé et al. *(31)* and Murcia et al. *(36)*, we decided to use it in this study because it is a procedure that is commonly used in the food industry and in governmental analytic laboratories.

Determination of antioxidant activity in the linoleic acid system. To a solution of 10 ml of linoleic acid (11.7 g/liter in 99.8% ethanol) and 10 ml of phosphate buffer (200 mM; pH 7.0), 5 ml of the analyzed sample (100 mg of the tested truffle or mushroom sample dissolved in 20 ml of deionized water, or 100 μg of common food antioxidants per g of water) was added. The total volume was adjusted to 25 ml with deionized water. This solution was incubated at 40°C, and the degree of oxidation was measured. For this procedure, 10 ml of ethanol (75%), 0.2 ml of an aqueous solution of ammonium thiocyanate (30%), 0.2 ml of the sample (solution mixture), and 0.2 ml of ferrous chloride solution (20 mM in 3.5% HCl) were stirred for 3 min. The absorption values of the mixtures at 500 nm were taken to indicate their peroxide contents. The percentage of inhibition of linoleic acid peroxidation, 100 − [(absorption increase of sample/absorption increase of control) × 100], was calculated to express antioxidative activity *(49)*.

Measurement of total antioxidative activity by the TEAC assay. The $ABTS^{\cdot-}$ radical solution was generated from 2.5 mM ABAP and 20 mM $ABTS^{2-}$ stock solution in PBS (containing 100 mM phosphate and 150 mM NaCl [pH 7.4]). The radical solution was protected from light during incubation at 60°C for 12 min and later stored at room temperature. Absorbance at 734 nm was measured to check $ABTS^{\cdot-}$ formation (the results must be between 0.35 and 0.45 nm) *(48)*. Fifty milligrams of truffles or mushrooms (or 100 μg of common food antioxidants per g, except for propyl gallate [25 μg/g to fit the calibration curve]) was dissolved in 3 ml of PBS. The antioxidant activity of the samples analyzed (40 μl mixed with 1,960 μl of the radical solution) was measured at 734 nm for a period of 6 min. The decrease in absorption at 734 nm observed 6 min after the addition of each compound was used to calculate the Trolox equivalent antioxidant capacity (TEAC).

A calibration curve was prepared with different concentrations of Trolox (a standard solution used to evaluate equivalent antioxidant capacity). By measuring the increase in absorption (ΔAbs) over 6 min (with a standard range of 0 to 10 μM), absorbance values were corrected for the solvent.

$$\Delta Abs_{Trolox} = Abs_{t=6\ min\ Trolox} - Abs_{t=6\ min\ solvent}$$

The regression coefficient (rc) is calculated from the calibration curve.

$$\Delta Abs_{Trolox} = rc \times [Trolox]$$

To establish the TEAC values of commercial antioxidants or analyzed samples, the increase in absorption was measured in the same way. The TEAC was calculated as follows:

$$TEAC_{sample} = \Delta Abs_{sample}/rc$$

The TEAC represents the concentration of a Trolox solution that has the same antioxidant capacity as the analyzed sample.

TABLE 1. *Inhibition of peroxidation in the lipid system with ox brain phospholipids by raw and industrially processed (frozen and canned) truffles and mushrooms compared with the activity of common food antioxidants*[a]

Substance added to reaction mixtures	Processing	% inhibition
None (control)		—
Truffles		
Terfezia	Raw	95.7 ± 1 B
	Frozen	94.1 ± 2 B
	Canned	72.9 ± 2 FG
Picoa	Raw	95.3 ± 1 B
	Frozen	94.5 ± 2 B
	Canned	74.1 ± 3 F
Mushrooms		
Lepista	Raw	95.3 ± 2 B
	Frozen	93.3 ± 1 BC
	Canned	79.0 ± 2 E
Lentinus	Raw	99.2 ± 3 AB
	Frozen	98.0 ± 2 AB
	Canned	84.7 ± 1 DE
Agrocybe	Raw	92.1 ± 2 BC
	Frozen	90.6 ± 1 BC
	Canned	76.3 ± 3 EF
Cantharellus	Raw	95.7 ± 1 B
	Frozen	94.5 ± 1 B
	Canned	89.9 ± 2 D
Hydnum	Raw	92.5 ± 1 BC
	Frozen	76.9 ± 2 EF
	Canned	76.9 ± 1 EF
α-Tocopherol		15.3 ± 1 I
BHA		71.4 ± 1 FG
BHT		22.3 ± 2 H
Propyl gallate		52.5 ± 1 G

[a] Statistical differences were analyzed by ANOVA ($P < 0.05$). Values with the same letter are not significantly different.

Statistical analysis. All experiments were carried out in triplicate. The results were analyzed with the Statistical Package for Social Sciences (version 9.0) for Windows and the analysis of variance (ANOVA) procedure. Fisher's least significant difference multiple-range test was used to discriminate between means.

RESULTS

Inhibition of phospholipid peroxidation. One way to test the antioxidant ability of a substance directly is to examine whether it inhibits the peroxidation of artificial lipid systems (liposomes) by scavenging peroxyl radicals. The formation of peroxyl radicals is the most important step in lipid peroxidation, although peroxyl radicals can also be formed in nonlipid systems. These radicals can be generated in foods and in the human body *(1)*.

Table 1 shows the inhibition of lipid peroxidation

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/65/10/1614/1672364/0362-028x-65_10_1614.pdf by University of Murcia user on 02 December 2021

TABLE 2. *Deoxyribose damage (in absorbance units) caused by the OH· radical in the presence of raw and industrially processed (frozen and canned) truffles and mushrooms compared with the activity of common food antioxidants*[a]

Substance added to reaction mixtures	Processing	Damage to deoxyribose		
		For RM + DR	% inhibition	Without ASC[b]
None (control)		1.226 ± 0.01 F	—	0.257
Truffles				
Terfezia	Raw	0.149 ± 0.03 AB	87.8	0.110
	Frozen	0.179 ± 0.02 AB	85.4	0.105
	Canned	0.371 ± 0.04 BC	69.7	0.193
Picoa	Raw	0.070 ± 0.02 A	94.3	0.067
	Frozen	0.080 ± 0.02 A	93.5	0.061
	Canned	0.455 ± 0.01 CD	62.9	0.174
Mushrooms				
Lepista	Raw	0.249 ± 0.05 B	79.7	0.509
	Frozen	0.279 ± 0.01 B	77.2	0.259
	Canned	0.408 ± 0.04 C	66.7	0.258
Lentinus	Raw	0.080 ± 0.01 A	93.5	0.067
	Frozen	0.109 ± 0.02 A	91.1	0.090
	Canned	0.419 ± 0.01 C	65.8	0.189
Agrocybe	Raw	0.249 ± 0.03 B	79.7	0.308
	Frozen	0.359 ± 0.05 BC	70.7	0.379
	Canned	0.501 ± 0.04 D	59.1	0.334
Cantharellus	Raw	0.080 ± 0.01 A	93.5	0.406
	Frozen	0.359 ± 0.02 BC	70.7	0.482
	Canned	0.419 ± 0.03 C	65.8	0.491
Hydnum	Raw	0.359 ± 0.02 BC	70.7	0.627
	Frozen	0.509 ± 0.02 D	58.5	0.505
	Canned	0.548 ± 0.05 D	55.3	0.399
α-Tocopherol		1.186 ± 0.03 F	3.2	0.240
BHA		0.914 ± 0.02 E	25.4	0.201
BHT		1.116 ± 0.05 F	8.9	0.559
Propyl gallate		2.070 ± 0.01 G	—	1.537

[a] RM, reaction mixtures; DR, deoxyribose; ASC, ascorbate. Statistical differences were analyzed by ANOVA ($P < 0.05$). Values with the same letter are not significantly different.

[b] When deoxyribose was omitted, values ranged from 0.001 to 0.006 absorbance units.

achieved in the presence of several raw truffles and mushrooms after industrial processing (freezing and canning) compared with that achieved with common food antioxidants. The truffles and mushrooms exhibiting the highest percentages of peroxidation inhibition were as follows (in decreasing order): raw *Lentinus* > frozen *Lentinus* > raw *Cantharellus* ≡ raw *Terfezia* > raw *Picoa* ≡ raw *Lepista* > frozen *Cantharellus* ≡ frozen *Picoa* > frozen *Terfezia* > frozen *Lepista* > raw *Hydnum* > raw *Agrocybe* > frozen *Agrocybe*. The inhibition percentages varied from 99.2 to 90.6% at the concentration tested. It can be seen that freezing led to a slight decrease in inhibition capacity, with raw and frozen samples (except for *Hydnum*) showing no significant differences ($P < 0.05$) in peroxyl radical inhibition.

However, when truffles and mushrooms were subjected to a canning process, their inhibition percentages significantly decreased with respect to those of the raw samples ($P < 0.05$), with their remaining inhibition percentages being in the following order: canned *Cantharellus* > canned *Lentinus* > canned *Lepista* > frozen and canned *Hydnum* > canned *Agrocybe* > canned *Picoa* > canned *Terfezia*. The antioxidant activity of frozen *Hydnum* decreased considerably with freezing and provided results similar to those for the canned sample.

All of the truffles and mushrooms analyzed exhibited higher inhibition percentages than did the common food antioxidants analyzed (α-tocopherol, BHA, BHT, and propyl gallate) even after being subjected to the different industrial processes.

Assessment of the antioxidant action of truffles and mushrooms by the deoxyribose assay. The deoxyribose assay evaluates whether a compound is a scavenger of the hydroxyl radicals (OH·) generated in the human body under physiological conditions, although the compound can also be generated from peroxyl radicals, in which case it will compete with deoxyribose for the OH· and inhibit deoxyribose degradation. Highly reactive radicals are generated by a mixture of ascorbate and $FeCl_3$-EDTA *(4)*.

The deoxyribose damage caused by the OH· radical in the presence of raw truffles and mushrooms subjected to industrial processing (freezing and canning), along with the activity of common food antioxidants, is shown in Table 2.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/65/10/1614/1672364/0362-028x-65_10_1614.pdf by University of Murcia user on 02 December 2021

On the basis of these results, these substances could be divided into two groups.

The first group comprised the substances showing the highest percentages of inhibition (94.3 to 85.4%) ($P < 0.05$). These substances were, in decreasing order, raw *Picoa* > frozen *Picoa* ≡ raw *Lentinus* > frozen *Lentinus* > raw *Terfezia* > frozen *Terfezia.* However, after these truffles and mushrooms had been subjected to the canning process, the level of $OH^{\cdot}$ scavenging was lower, resulting in the following order: *Terfezia* > canned *Lentinus* > canned *Picoa* ($P < 0.05$). When ascorbate was omitted, the attack on deoxyribose was less intense, because the absence of ascorbate decreases the concentration of $OH^{\cdot}$ in the reaction mixture *(30).* The samples lacking ascorbate exhibited lower absorbance levels than did the control sample, since *Terfezia, Picoa,* and *Lentinus* scavenged $OH^{\cdot}$ radicals. All samples exhibited better performance than did the common food antioxidants studied.

The second group comprised those substances that produced high inhibition percentages: raw *Cantharellus* > raw *Lepista* ≡ raw *Agrocybe* > frozen *Lepista* > frozen *Agrocybe* ≡ frozen *Cantharellus.* Raw and frozen *Hydnum* exhibited the lowest inhibition percentage in the deoxyribose assay. However, this group does not scavenge $OH^{\cdot}$, because when ascorbate was omitted, the level of the pink chromogen exceeded that of the control. These compounds probably react with ascorbate, decreasing the amount of $OH^{\cdot}$ generated *(30).*

The canned samples showed lower percentages of inhibition than did their raw and frozen counterparts (Table 2). The antioxidant activity of *Cantharellus* and *Hydnum* declined with both industrial processes. Propyl gallate showed prooxidant activity in this assay.

Hydrogen peroxide scavenging. Hydrogen peroxide is generated in vivo by several oxidase enzymes and by activated phagocytes, and it is known to play an important role in the killing of several bacterial and fungal strains *(19).* There is increasing evidence that H_2O_2, either directly or indirectly via its reduction product, $OH^{\cdot}$, can act as a messenger molecule in the synthesis and activation of several inflammatory mediators *(46).* When a scavenger is incubated with H_2O_2 using a peroxidase assay system, the loss of H_2O_2 can be measured.

Table 3 shows the reaction of hydrogen peroxide with raw truffles and mushrooms subjected to industrial processing (freezing and canning) and with common food antioxidants (BHT, BHA, propyl gallate, and α-tocopherol). The inhibition percentages exhibited are as follows: raw *Cantharellus,* 86%; raw *Lepista,* 81%; frozen *Lepista,* 79%; raw *Agrocybe,* 76%; raw *Terfezia,* 74%; raw *Lentinus,* 74%; frozen *Terfezia,* 73%; frozen *Lentinus,* 73%; raw *Picoa,* 73%; raw *Hydnum,* 68%; frozen *Agrocybe,* 67%; frozen *Hydnum,* 59%; frozen *Cantharellus,* 58%. All of the processed truffles and mushrooms exhibited better capacities for reaction with H_2O_2 than did BHA, BHT, α-tocopherol, or propyl gallate.

Canned truffles and mushrooms were less effective at scavenging H_2O_2; their percentages of inhibition were as follows: *Lepista,* 59%; *Cantharellus,* 56%; *Lentinus,* 54%; *Agrocybe,* 45%; *Terfezia,* 44%; *Hydnum,* 37%; *Picoa,* 35%.

TABLE 3. *Scavenging of hydrogen peroxide by raw and industrially processed (frozen and canned) truffles and mushrooms compared with the activity of common food antioxidants with peroxidase-based assays*[a]

Substance added to reaction mixtures	Processing	Absorbance (A_{436})
None (control)		0.622 ± 0.02 F
Truffles		
Terfezia	Raw	0.162 ± 0.03 AB
	Frozen	0.165 ± 0.05 AB
	Canned	0.350 ± 0.01 D
Picoa	Raw	0.168 ± 0.04 AB
	Frozen	0.321 ± 0.03 D
	Canned	0.400 ± 0.01 C
Mushrooms		
Lepista	Raw	0.119 ± 0.02 A
	Frozen	0.131 ± 0.05 AB
	Canned	0.252 ± 0.02 C
Lentinus	Raw	0.164 ± 0.02 AB
	Frozen	0.166 ± 0.04 AB
	Canned	0.288 ± 0.01 C
Agrocybe	Raw	0.151 ± 0.03 AB
	Frozen	0.207 ± 0.01 B
	Canned	0.341 ± 0.02 D
Cantharellus	Raw	0.085 ± 0.05 A
	Frozen	0.272 ± 0.02 C
	Canned	0.275 ± 0.04 C
Hydnum	Raw	0.196 ± 0.03 B
	Frozen	0.254 ± 0.04 C
	Canned	0.388 ± 0.02 DE
α-Tocopherol		0.711 ± 0.02 F
BHA		0.770 ± 0.03 F
BHT		0.700 ± 0.04 F
Propyl gallate		0.400 ± 0.02 E

[a] Statistical differences were analyzed by ANOVA ($P < 0.05$). Values with the same letter are not significantly different.

Rancimat results. To assess oxidative stability, the food industry uses the Rancimat test, in which the scavenger to be tested is added to a lipidic food and the degree of protection is evaluated *(45).* Table 4 shows the induction period and the protection factor obtained by the Rancimat method for refined olive oil with raw truffles and mushrooms after the freezing and canning processes and for refined olive oil with common food antioxidants. Refined olive oil alone (control) started the radical chain reactions of the propagation phase of autoxidation after 7.58 h. The time required for the formation of a sufficient concentration of initiating radicals (initiation phase) was slightly longer when food antioxidants or raw or frozen truffles or mushrooms were added, delaying the onset of the propagation phase of the radical chain reaction and showing the protection factors of these products. Raw *Lepista, Terfezia,* and *Lentinus* provided the greatest protection, with induction periods of 9.28, 9.26, and 9.24 h, respectively.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/65/10/1614/1672364/0362-028x-65_10_1614.pdf by University of Murcia user on 02 December 2021

TABLE 4. *Effects of raw and industrially processed (frozen and canned) truffles and mushrooms and of common food antioxidants on the oxidative stability of olive oil, expressed as induction period (IP) and protection factor (PF), as determined by the Rancimat method*

Additive	Processing	IP (h)[a]	PF[b]
None		7.58	
Truffles			
Terfezia	Raw	9.26	1.22 ± 0.1 C
	Frozen	8.00	1.05 ± 0.2 B
	Canned	2.35	0.31 ± 0.1 A
Picoa	Raw	8.75	1.15 ± 0.1 BC
	Frozen	8.51	1.12 ± 0.1 BC
	Canned	3.19	0.42 ± 0.2 A
Mushrooms			
Lepista	Raw	9.28	1.22 ± 0.1 C
	Frozen	9.10	1.20 ± 0.2 C
	Canned	1.22	0.16 ± 0.2 A
Lentinus	Raw	9.24	1.22 ± 0.1 C
	Frozen	8.91	1.17 ± 0.3 BC
	Canned	3.76	0.49 ± 0.3 A
Agrocybe	Raw	8.83	1.16 ± 0.2 BC
	Frozen	8.33	1.09 ± 0.1 B
	Canned	1.89	0.25 ± 0.2 A
Cantharellus	Raw	8.21	1.08 ± 0.1 B
	Frozen	7.26	0.96 ± 0.2 AB
	Canned	3.84	0.51 ± 0.2 A
Hydnum	Raw	8.79	1.15 ± 0.2 BC
	Frozen	8.56	1.13 ± 0.1 BC
	Canned	3.53	0.46 ± 0.3 A
α-Tocopherol		20.10	2.65 ± 0.2 E
BHA		8.68	1.14 ± 0.1 BC
BHT		7.18	0.95 ± 0.2 AB
Propyl gallate		14.21	1.87 ± 0.1 D

[a] Rancimat-tested at 120°C.

[b] PF = IP (oil + truffles-mushrooms)/IP (oil). Values with the same letter are not significantly different.

A second group of samples increased the induction period and the protection factor of olive oil. These samples were, in decreasing order, frozen *Lepista* > frozen *Lentinus* > raw *Agrocybe* > raw *Picoa* > raw *Hydnum* > frozen *Hydnum* > frozen *Picoa* > frozen *Agrocybe* > raw *Cantharellus* > frozen *Terfezia,* with induction periods that varied from 9.10 to 8.00 h.

However, when canned truffles or mushrooms were added, the time required for the formation of a sufficient concentration of initiating radicals was reduced. This result indicates that these samples did not delay the onset of the propagation phase of the radical chain reaction but degraded the oil more rapidly. The protection losses relative to raw samples ranged from 53 to 87% in lipidic foods.

Linoleic acid system assay. The linoleic system assay, which is used for determining antioxidant activity during storage at unfavorable temperatures (40°C), measures the inhibition of linoleic acid autoxidation. Figure 1 shows the absorbance values obtained during the autoxidation of linoleic acid in the presence of raw and processed truffles and

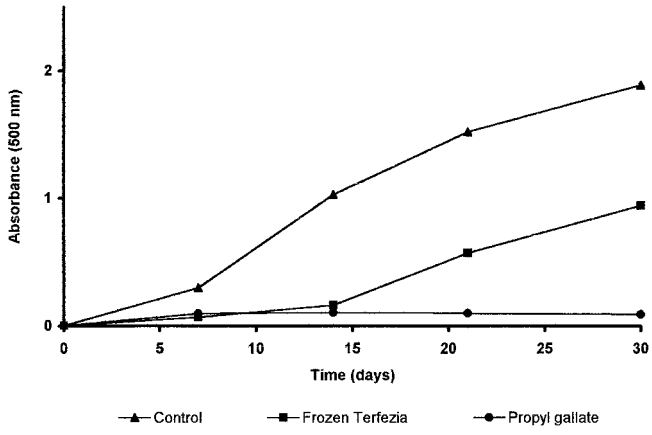

FIGURE 1. *Evolution of the absorbance at 500 nm for the oxidation of linoleic acid in the presence of raw and industrially processed (frozen and canned) truffles and mushrooms and common food antioxidants during 30 days of storage.* ●*, propyl gallate and samples with high antioxidant activity;* ■*, samples with medium antioxidant activity;* ▲*, control and samples with no antioxidant activity.*

mushrooms over 30 days of storage, along with the values for common food antioxidants. On the basis of these results, substances were divided into three groups ($P < 0.05$) according to their linoleic acid autoxidation inhibition percentages.

The first group included substances with high antioxidant activity similar to that of propyl gallate (Fig. 1): raw *Lentinus* (98% inhibition on the 30th day of storage) > raw *Cantharellus* (98%) > frozen *Lentinus* (97%) ≡ canned *Lentinus* (97%) > raw *Agrocybe* (96%) ≡ BHT (96%) > raw *Lepista* (95%) > propyl gallate (95%) > frozen *Agrocybe* (91%) > frozen *Cantharellus* (89%) > canned *Cantharellus* (87%) ≡ raw *Hydnum* (87%) ≡ frozen *Hydnum* (87%) ≡ frozen *Lepista* (87%) > canned *Hydnum* (86%) > raw *Picoa* (85%) > BHA (84%) > raw *Terfezia* (77%). These substances showed very high levels of antioxidant activity during the 30 days of storage.

A second group of substances (Fig. 1) included canned *Agrocybe,* frozen *Terfezia,* α-tocopherol, and frozen *Picoa,* which exhibited medium levels of antioxidant activity after 30 days of storage, with inhibition percentages of 59, 50, 27, and 23%, respectively. However, canned *Agrocybe,* α-tocopherol, and frozen *Terfezia* showed strong antioxidant activity until day 21 of storage, at which time their inhibition percentages were 77, 63, and 62%, respectively.

The third group (Fig. 1) included substances with no antioxidant activity (canned *Terfezia,* canned *Picoa,* and canned *Lepista*); the absorbances of these samples were similar to that of the control at the end of the assay. Therefore, these results show that antioxidant activity is lost in two truffles and in *Lepista* after canning. In the long term (30 days), increased losses of antioxidant activity were measured for the samples with poor free radical–scavenging capacity.

TEAC assay. TEAC values can be assigned to all compounds able to scavenge ABTS by comparing the scavenging capacities of these compounds with that of Trolox,

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/65/10/1614/1672364/0362-028x-65_10_1614.pdf by University of Murcia user on 02 December 2021

TABLE 5. *Scavenging of ABTS radical anions by raw and industrially processed (frozen and canned) truffles and mushrooms compared with the activity of common food antioxidants*[a]

Substance added to reaction mixtures	Processing	TEAC[b]
None (control)		—
Trolox (0.05 mM)		1.00 ± 0.0 A
Trolox (0.5 mM)		10.00 ± 0.0 F
Truffles		
Terfezia	Raw	4.77 ± 0.1 C
	Frozen	3.57 ± 0.1 B
	Canned	2.52 ± 0.1 AB
Picoa	Raw	3.91 ± 0.1 BC
	Frozen	2.57 ± 0.1 AB
	Canned	0.56 ± 0.1 A
Mushrooms		
Lepista	Raw	3.19 ± 0.1 B
	Frozen	2.36 ± 0.1 AB
	Canned	2.01 ± 0.1 AB
Lentinus	Raw	9.05 ± 0.1 E
	Frozen	7.59 ± 0.1 DE
	Canned	6.93 ± 0.1 D
Agrocybe	Raw	10.39 ± 0.1 EF
	Frozen	9.99 ± 0.1 EF
	Canned	3.04 ± 0.1 B
Cantharellus	Raw	11.41 ± 0.1 F
	Frozen	10.92 ± 0.1 F
	Canned	4.88 ± 0.1 C
Hydnum	Raw	1.56 ± 0.1 A
	Frozen	1.25 ± 0.1 A
	Canned	0.46 ± 0.1 A
α-Tocopherol		1.16 ± 0.1 A
BHA		0.44 ± 0.1 A
BHT		0.26 ± 0.1 A
Propyl gallate[c]		3.47 ± 0.1 B

[a] Statistical differences were analyzed by ANOVA ($P < 0.05$). Values with the same letter are not significantly different.

[b] TEAC is the millimolar concentration of a Trolox solution having an antioxidant capacity equivalent to that of the dilution of the substance under investigation.

[c] Propyl gallate dilution was selected to reduce the measurement within the appropriate part of the Trolox standard curve.

a water-soluble vitamin E analog. The quantitative evaluation of antioxidant capacity based on TEAC can be used to provide a ranking order of antioxidants *(48)*.

Table 5 shows the TEACs of different raw truffles and mushrooms, of samples subjected to industrial processing, and of common food antioxidants. The sample with the highest TEAC value was that of raw *Cantharellus*; the order of the remaining raw samples with regard to antioxidant capacity was as follows: *Agrocybe, Lentinus, Terfezia, Picoa, Lepista,* and *Hydnum. Cantharellus* and *Agrocybe* lost 4% of their antioxidant activity in the freezing process. Antioxidant activity losses of 16 to 34% were detected for the rest of the frozen samples. Canned samples showed considerable losses of total antioxidant capacity, with losses ranging from 24 to 86% for *Lentinus* < *Lepista* < *Terfezia* < *Cantharellus* < *Agrocybe* ≡ *Hydnum* < *Picoa*. Antioxidant activity loss percentages were calculated on the basis of the data obtained for raw samples.

Of the common food antioxidants analyzed, propyl gallate exhibited the highest TEAC value. α-Tocopherol, BHA, and BHT exhibited lower TEAC values than did raw and frozen truffle and mushroom samples.

DISCUSSION

Mushrooms represent biological systems in which antioxidant compounds are very powerful *(16)*. The protective properties of mushrooms are attributable to several compounds, such as water-soluble polysaccharides, which are effective in protection against hydroxyl and superoxide radical–scavenging activities *(25)*. In fact, α- and β-glucan polysaccharides isolated from *L. edodes* exhibit free radical–scavenging activity and antiviral, hepatoprotective, antifibrotic, antiinflammatory, antidiabetes, and hypochloesterolemic activities *(41)*.

Other structures, including vitamins *(9, 10),* pigments (carotenoids) *(35),* and phenolic compounds, may also be related to the capacity to reduce and chelate the ferric iron that catalyzes lipid peroxidation *(16)*. The molecular structures of these compounds include an aromatic ring with hydroxyl groups containing mobile hydrogens *(4)* that lies within the phytin and phytin-P antinutritional constituents of the mushrooms. The ability of mushrooms to chelate certain mineral elements, especially Ca, Mg, Fe, and Zn, has also been described *(2)*. Other compounds isolated from different types of mushrooms, such as inoscavin A, curtisians A through D (*p*-terphenyl compounds) *(50),* hydrazine *(51)* and the indole derivatives isolated from *Agrocybe,* are also associated with antioxidant capacity and the inhibition of lipid peroxidation *(22)*.

Polyphenoloxidase, a constituent of mushrooms, is a widely distributed copper-containing protein that catalyzes two different reactions to *o*-quinones that generally decrease the quality and the antioxidant capacity of food, either directly through the oxidation of reducing substrates or indirectly through the oxidation of ascorbic acid by the quinones and the oxidized products generated in the enzymatic oxidation of their substrates *(42)*.

Although freezing did not affect the folate vitamins *(47)* or the dietary fiber and unsaturated and saturated fatty acid contents of truffles *(37),* processing and storage can profoundly alter the antioxidant composition of fruits and vegetables *(18)*. A reduction in free radical–scavenging capacity has been observed for several foods heated at different temperatures (grapes *(23),* tuna *(34)*), because polyphenols and flavonoids are associated with the antioxidant capacity of the samples and are usually oxidized at high temperatures *(23)*. Also, with heating, lipids increase in extractability and proteins are denatured, while vitamins such as ascorbic acid *(3),* vitamin E *(38),* vitamins B_1, B_2, and B_6 *(40),* and carotene *(27)* suffer degradation and losses of antioxidant activity.

Taking into account that several compounds are responsible for antioxidant activity and some of them can be reduced after processing, the loss of total antioxidant activity in mushroom tissues was evident after the application

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/65/10/1614/1672364/0362-028x-65_10_1614.pdf by University of Murcia user on 02 December 2021

of different blanching treatments *(12).* However, while short heat treatments were found to decrease antioxidant potential, prolonged heating times were found to lead to a recovery of antioxidant properties, suggesting that the initial reduction in the overall antioxidant activity could be attributable not only to the thermal degradation of naturally occurring antioxidants but also to the formation of early Maillard reaction products with oxidant properties *(3).*

CONCLUSIONS

The results of this study indicate that canned truffles and mushrooms exhibit significant losses ($P < 0.05$) of antioxidant activity, while the frozen products exhibit less extensive losses. Raw truffles and mushrooms exhibit strong antioxidant activity as scavengers of several oxygen species. This finding supports the replacement of synthetic antioxidants with natural truffle and mushroom extracts.

Although they are eaten for their flavor *(26),* truffles and mushrooms may also be a valuable protein supplement. They have high dietary fiber and unsaturated fatty acid contents *(37)* and may compare favorably with other vegetables with regard to antioxidant activity *(33).* Thus, raw and frozen truffles and mushrooms can be considered promising candidates for industrial processing, thus permitting their year-round consumption, and they can also be considered functional foods *(32).*

ACKNOWLEDGMENT

This work was funded by a FEDER grant (1FD97-1746) from Ministerio de Educación y Cultura, Spain.

REFERENCES

1. Aeschbach, R., R. Bächler, P. Rossi, L. Sandoz, and H. J. Wille. 1994. Mechanical extraction of plant antioxidants by means of oils. *Fat Sci. Technol.* 11:441–443.
2. Aletor, V. A. 1995. Compositional studies on edible tropical species of mushrooms. *Food Chem.* 54:265–268.
3. Anese, M., M. C. Nicoli, R. Massini, and C. R. Lerici. 1999. Effects of drying processing on the Maillard reaction in pasta. *Food Res. Int.* 32:193–199.
4. Aruoma, O. I. 1996. Assessment of potential prooxidant and antioxidant actions. *J. Am. Oil Chem. Soc.* 73:1617–1625.
5. Aruoma, O. I., A. Murcia, J. Butler, and B. Halliwell. 1993. Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives. *J. Agric. Food Chem.* 41:1880–1885.
6. Aruoma, O. I., J. P. E. Spencer, R. Rossi, R. Aeschbach, A. Khan, H. Mahmood, A. Muñoz, A. Murcia, J. Butler, and B. Halliwell. 1996. An evaluation of the antioxidant and antiviral action of extracts of rosemary and provençal herbs. *Food Chem. Toxicol.* 34: 449–456.
7. Bokhary, H. A., and P. Sarwat. 1993. Chemical composition of desert truffles *Terfezia claveryi*. *J. Food Comp. Anal.* 6:285–293.
8. Borchers, A. T., J. S. Stern, R. M. Hackman, and C. L. Keen. 1999. Mushrooms, tumours and immunity. *Proc. Soc. Exp. Biol. Med.* 221: 281–293.
9. Breene, W. M. 1990. Nutritional and medicinal value of speciality mushrooms. *J. Food Prot.* 53:883–894.
10. Coskuner, Y., and Y. Özdemir. 1997. Effects of canning processes on the elements content of cultivated mushrooms (*Agaricus bisporus*). *Food Chem.* 60:559–562.
11. Danell, E., and D. Eaker. 1992. Amino acid and total protein content of the edible mushroom *Cantharellus cibarius*. *J. Sci. Food Agric.* 60:333–337.
12. Devece, C., J. N. Rodríguez-López, L. G. Fenoll, J. Tudela, J. M. Catalá, E. De los Reyes, and F. García-Cánovas. 1999. Enzyme inactivation analysis for industrial blanching applications: comparison of microwave, conventional, and combination heat treatments on mushroom polyphenoloxidase activity. *J. Agric. Food Chem.* 47: 4506–4511.
13. Feofilova, E. P., I. B. Gornova, A. S. Memorskaia, and L. V. Garibova. 1998. Lipid composition of fruiting bodies and submerged mycelium from *Lentinus edodes* (Berk.) Sing (*Lentinula edodes* (Berk.) Peglar). *Mikrobiologiia* 67:655–659.
14. Food and Agriculture Organization, World Health Organization. 1999. Codex alimentarius. Food and Agriculture Organization, World Health Organization, Rome.
15. Frankel, E. N. 1993. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends Food Sci. Technol.* 4:220–225.
16. Gazzani, G., A. Papetti, M. Daglia, F. Bertè, and C. Gregotti. 1998. Protective activity of water soluble components of some common diet vegetables on rat liver microsome and the effect of thermal treatment. *J. Agric. Food Chem.* 46:4123–4127.
17. Gazzani, G., A. Papetti, G. Massolini, and M. Daglia. 1998. Anti- and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *J. Agric. Food Chem.* 46:4118–4122.
18. Halliwell, B. 1995. Antioxidants: elixirs of life or tonics for tired sheep? *Biochemist* February/March:3–6.
19. Halliwell, B., M. A. Murcia, S. Chirico, and O. I. Aruoma. 1995. Free radicals and antioxidants in food and *in vivo*: what they do how they work? *Crit. Rev. Food Sci. Nutr.* 35:7–20.
20. Hokama, Y., and J. L. R. Y. Hokama. 1981. In vitro inhibition of platelet aggregation with low dalton compounds from aqueous dialysates of edible fungi. *Res. Commun. Chem. Pathol. Pharmacol.* 31:177–180.
21. Honrubia, M., A. Cano, and C. Molina-Niñirola. 1992. Hypogeous fungi from southern Spanish semiarid lands. *Persoonia* 14:647–653.
22. Kim, W. G., I. K. Lee, J. P. Kim, I. J. Ryoo, H. Koshino, and I. D. Yoo. 1997. New indole derivatives with free radical scavenging activity from *Agrocybe cylindracea*. *J. Nat. Prod.* 60:721–723.
23. Larrauri, J. A., C. Sánchez-Moreno, and F. Saura-Calixto. 1998. Effect of temperature on the free radical scavenging capacity of extracts from red and white grape pomade peels. *J. Agric. Food Chem.* 46:2694–2697.
24. Lin, S., P. Zhu, and H. Liao. 1998. Blocking effect of 4 edible mushroom beverages on the carbon tetrachloride induced rat liver lipid peroxidation. *Zhongguo Gonggong Weisheng Xuebao* 17:15.
25. Liu, F., V. E. Ooi, and S. T. Chang. 1997. Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sci.* 60:763–771.
26. Longvah, T., and Y. G. Deosthale. 1998. Compositional and nutritional studies on edible wild mushroom from northeast India. *Food Chem.* 63:331–334.
27. Lu, G., Z. Shi, and X. Gao. 1998. Influence of different processing treatments on nutrients of sweet potato. *Zhongguo Liangyou Xuebao* 13:32–35.
28. Manzi, P., L. Gambelli, S. Marconi, V. Vivanti, and L. Pizzoferrato. 1999. Nutrients in edible mushrooms: an inter-species comparative study. *Food Chem.* 65:477–482.
29. Manzi, P., and L. Pizzoferrato. 2000. Beta-glucans in edible mushrooms. *Food Chem.* 68:315–318.
30. Martínez-Tomé, M., F. García-Carmona, and M. A. Murcia. 2001. Comparison of antioxidant and pro-oxidant properties of broccoli amino acids with those of common food additives. *J. Sci. Food Agric.* 81:1019–1026.
31. Martínez-Tomé, M., A. M. Jiménez, S. Ruggieri, N. Frega, R. Strabbioli, and M. A. Murcia. 2001. Antioxidant properties of Mediterranean spices compared with common food additives. *J. Food Prot.* 64:1412–1419.
32. Mattila, P., K. Suonpa, and V. Piironen. 2000. Functional properties of edible mushrooms. *Nutrition* 16:694–696.
33. Mau, J. L., K. T. Wu, Y. H. Wu, and Y. P. Lin. 1998. Non-volatile

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/65/10/1614/1672364/0362-028x-65_10_1614.pdf by University of Murcia user on 02 December 2021

taste components of ear mushrooms. *J. Agric. Food Chem.* 46:4583–4586.
34. Medina, I., M. T. Satue-Gracia, J. B. German, and E. N. Frankel. 1999. Comparison of natural polyphenol antioxidants from extra virgin olive oil with synthetic antioxidants in tuna lipids during thermal oxidation. *J. Agric. Food Chem.* 47:4873–4879.
35. Mui, D., T. Feibelman, and J. W. Bennett. 1998. A preliminary study of the carotenoids of some North American species of *Cantharellus. Int. J. Plant Sci.* 159:244–248.
36. Murcia, M. A., A. M. Jiménez, and M. Martínez-Tomé. 2001. Evaluation of the antioxidant properties of Mediterranean and tropical fruits compared with common food additives. *J. Food Prot.* 64: 2037–2046.
37. Murcia, M. A., M. Martínez-Tomé, A. M. Vera, A. Morte, A. Gutiérrez, M. Honrubia, and A. M. Jiménez. Effect of industrial processing on desert truffles (*Terfezia claveryi* Chatin and *Picoa juniperi* Vittadini): proximate composition and fatty acids. *J. Sci. Food Agric.,* in press.
38. Murcia, M. A., A. Vera, and F. García-Carmona. 1992. Determination by HPLC of changes in tocopherol levels in spinach after industrial processing. *J. Sci. Food Agric.* 60:81–84.
39. Nöel-Suberville, C., C. Cruz, J. Guinberteau, and M. Montury. 1996. Correlation between fatty acid content and aromatic compound release in fresh blewit (*Lepista nuda*). *J. Agric. Food Chem.* 44:1180–1183.
40. Olleta, F. J., E. Llanos, R. Barcos, M. C. Ancin, and O. Martin. 1993. Determination of vitamins B_1, B_2, B_6 and C in mushroom and lentils by HPLC. Effect of transformation in canning and storage time. *Quim. Anal.* 12:155–158.
41. Ooi, V. E. C., and F. Liu. 2000. Immunomodulation anti-cancer activity of polysaccharide-protein complexes. *Curr. Med. Chem.* 7: 715–729.
42. Rodríguez-López, J. N., L. G. Fenoll, J. Tudela, C. Devece, D. Sánchez-Hernández, E. De los Reyes, and F. García-Cánovas. 1999. Thermal inactivation of mushroom polyphenoloxidase employing 2450 MHz Microwave radiation. *J. Agric. Food Chem.* 47:3028–3035.
43. Rosas-Romero, A. J., B. Rojano, C. A. Hernández, C. Martínez Manchado, J. Silva, and J. C. Herrera. 1999. A novel approach to quantitative structure-property relationships in antioxidants. *Ciencia* 7: 78–87.
44. Schwarz, K., and H. Ernst. 1996. Evaluation of antioxidative constituents from thyme. *J. Sci. Food Agric.* 70:217–223.
45. Sensidoni, A., G. Bortolussi, C. Orlando, G. Lognay, P. Fantozzi, and M. Paquot. 1995. Composition and oxidative stability of borage (*Borago officinalis* L.) and borage-virgin olive oil blends. *Lebensm.-Wiss. Technol.* 28:343–346.
46. Sprong, R. C., A. Winkelhuyzen-Jansen, C. Aarsman, J. van Oirschot, T. Van der Bruggen, and B. Van Asbeck. 1998. Low-dose N-acetylcysteine protects rats against endotoxin-mediated oxidative stress, but high dose increases mortality. *Am. J. Crit. Care Med.* 157: 1283–1293.
47. Vahteristo, L. T., K. E. Lehikoinen, V. Ollilainen, P. E. Koivistoinen, and P. Varo. 1998. Oven-baking and frozen storage affect folate vitamer retention. *Lebensm.-Wiss. Technol.* 31:329–333.
48. van den Berg, R., G. R. M. M. Haenen, H. Van den Berg, and A. Bast. 1999. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* 66:511–517.
49. Yen, G.-C., S.-C. Wu, and P.-D. Duh. 1996. Extraction and identification of antioxidant components from the leaves of mulberry (*Morus alba* L.). *J. Agric. Food Chem.* 44:1687–1690.
50. Yun, B.-S., I.-K. Lee, J.-P. Kim, and I.-D. Yoo. 2000. Curtisians A~D, new free radical scavengers from the mushroom *Paxillus curtisii. J. Antibiot.* 53:114–122.
51. Zhou, S., L. C. Dickinson, L. Yang, and E. A Decker. 1998. Identification of hydrazine in commercial preparations of carnosine and its influence on carnosine's antioxidative properties. *Anal. Biochem.* 261:79–86.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/65/10/1614/1672364/0362-028x-65_10_1614.pdf by University of Murcia user on 02 December 2021