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ARTICLE

Impact of Thermal Processing on the Activity of Gallotannins and Condensed Tannins from *Hamamelis virginiana* Used as Functional Ingredients in Seafood

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Phenolic extracts from witch hazel, *Hamamelis virginiana*, are efficient antioxidants against fish lipid peroxidation. The impact of fish thermal processes on the hydrolyzable polyphenols from this source was studied. *H. virginiana* polyphenols included 80% of hydrolyzable tannins, characterized by a mixture of glucose gallates containing from 5 to 10 units of gallic acid, hamamelitannin, and 20% of proanthocyanidins. Structural modifications of the polyphenols during thermal processes were determined by HPLC-MS. Changes in their reducing and free radical scavenging capacities as a result of high temperatures were also determined. Thermal processes triggered a significant breakdown of hydrolyzable tannins with 6−10 galloyl units to give pentagalloyl glucose (PGG). The release of high concentrations of free gallic acid especially in long-term thermally processed samples leads to an increase of the antioxidant ability of heated *H. virginiana* extracts. Such an increase was evidenced by an increment in the reducing and radical scavenging capacities as well as an improvement in the antioxidant effectiveness for inhibiting lipid oxidation of processed fatty fish muscle.

KEYWORDS: *Hamamelis virginiana*; hydrolyzable tannins; antioxidants; thermal processes; fish lipid oxidation

INTRODUCTION

Modern trends toward convenience foods have resulted in increased production of precooked and restructured seafood products. However, the high content of polyunsaturated fatty acids of such products leads to a high susceptibility to lipid oxidation and the occurrence of off-flavors (*1*). Rancidity and off-flavors caused by lipid oxidation in preprocessed products could change customers' behavior and cause great challenges to the fish industry. To protect foods against oxidative deterioration, synthetic antioxidants are being replaced by natural antioxidants. Natural polyphenols represent a biologically relevant group of natural compounds, which have generated growing interest from consumers, food manufacturers, and pharmaceutical industries. Their antioxidant abilities and biological in vivo effects have driven their incorporation as functional ingredients in several foodstuffs or their use as nutraceutical supplements (*2*). Natural polyphenols from different vegetable and forest sources have been employed for supplementing fish and meat muscle based products. In recent years, procyanidins from grapes have shown to be effective as inhibitors of fish muscle oxidation. Their properties to inhibit strongly lipid oxidation in fish muscle together with their ability to induce selective apoptosis in colon cancer cells have been demonstrated and employed to promote their use as functional antioxidant ingredients (*3*, *4*).

The antioxidant activity of the different phenols in seafood products seems to be related to their molecular structure, which determines important properties such as the number of donated electrons or their chelating ability. Galloylation, the number of galloyl esters, seems to be an important factor in the antioxidant activities of procyanidins. Torres et al. (*5*) have demonstrated the relationship of galloyl residues to antioxidant activity. Procyanidins showing a moderate galloylation degree are the most effective antioxidants for preserving fish lipid from oxidation. Recently a natural source of galloylated poyphenols, witch hazel (*Hamamelis virginiana*), has been suggested as an antioxidant with antitumoral and anticarcinogenic properties (*6*, *7*). Elevated peroxynitrite and superoxide scavenging activities and total antioxidant potential have been related to the high number of hydrogen-donating antioxidants present in *Hamamelis* extracts (*8*−*10*). Witch hazel has long been used as a therapeutic agent in herbal medicine because of its ability to reduce capillary permeability, to exhibit antihemorrhagic (*11*) and anti-inflammatory properties (*12*), and to act against skin disease injuries (*13*). Furthermore, extracts of witch hazel bark, twigs, and leaves are widely used as components of skin care products and in dermatological treatment of sun burn, irritated skin, and atopic eczema and to promote wound healing via anti-inflammatory effects (*14*). The presence of high galloyl residues in witch hazel extracts suggests a possible utilization as food antioxidants, particularly in foodstuffs susceptible to rancidity such as fish muscle or fish oil based products.

H. virginiana is an important source of hydrolyzable and condensed tannins (*15*, *16*). Tannins, secondary metabolites of higher plants, are oligomeric compounds with multiple structure units with free phenolic groups and molecular weight ranging from 500 to > 20000. The main groups of tannins are hydrolyzable

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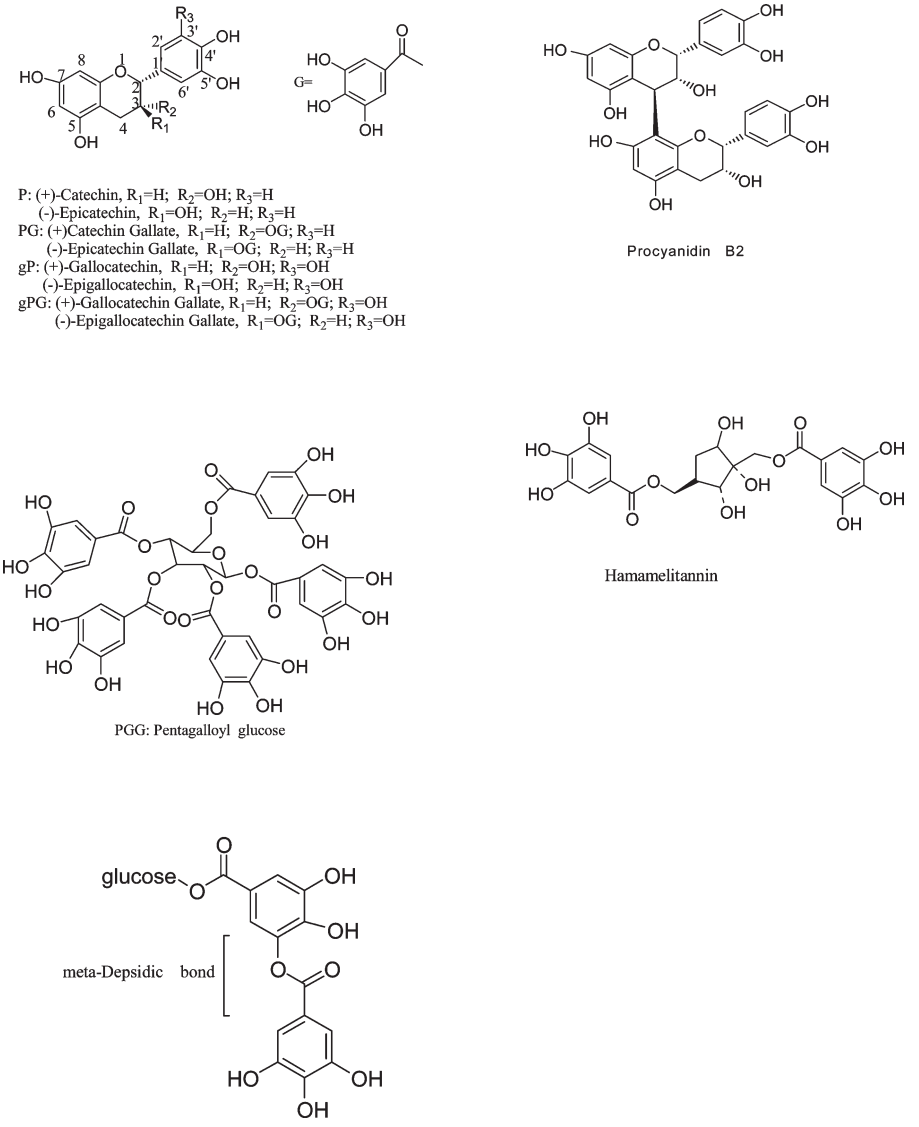

Figure 1. Structures of tannins.

tannins and condensed tannins (*17*) (**Figure 1**). Hydrolyzable tannins are esters of phenolic acids such as gallic acid (gallotannins) or ellagic acid (ellagitannins) with a sugar (generally glucose) or a polyalcohol (quinic acid) (*18*). Condensed tannins or proanthocyanidins consist of coupled flavan-3-ol units that can appear as isolated dimers and compounds with high polymerization grade (*19*).

Despite the possibilities of all these polyphenols to be employed as bioactive antioxidants in foods, one question that remains unclear is the stability of their antioxidant ability after the technological treatments usually employed in industry. As for seafood products, the most important processing methods involve cooking, frying, or sterilization. Heating affects single and polymeric phenols in different manners. Previous works report the epimerization of catechins with temperature (*20*, *21*) or the changes produced in procyanidins after thermal processing (*22*, *23*).

This work was aimed at studying the impact of thermal processes employed in the seafood industry as cooking and sterilization on hydrolyzable tannins present in extracts from *H. virginiana* and proposed as antioxidants for fish products. The study comprises the influence of short- and long-term thermal processes accomplished at high temperatures (100 °C) on the molecular structure of tannins and, as a consequence, on their antioxidant capacity measured as the ability to donate electrons and scavenge radicals. Because sterilization is one of the most common processes in the fish industry, involving high temperatures and prolonged times, the ability of the processed *Hamamelis* extracts to inhibit lipid oxidation of fish muscle was then determined.

MATERIALS AND METHODS

Solvents and Reagents. All solvents and reagents were of analytical or chromatographic grade. Acetonitrile was supplied by Panreac (Castellar del Vallès, Barcelona, Spain). Acetic acid, HCl 37%, ethanol 95%, sodium acetate trihydrate, and sodium carbonate were supplied by Prolabo (VWR Prolabo, Fontenay Sous Bois, France). Tannic acid, Folin−Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) (95%), iron(III) chloride hexahydrate, iron(II) chloride tetrahydrate, iron(II) sulfate heptahydrate, streptomycin sulfate, trichloroacetic acid, 2-thiobarbituric acid, ammonium thiocyanate, 1,1,3,3-tetraethoxypropane and 2,4,6-Tris (2 pyridyl)-s-triazine (TPTZ) were supplied by Sigma-Aldrich (Steinheim, Germany). Methanol, and *n*-hexane were supplied by Merck (Darmstadt, Germany).

Standards. Gallic acid, (+)-catechin, (−)-epicatechin, (−)-gallocatechin, (−)-gallocatechin gallate, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate were supplied by Sigma-Aldrich, and procyanidin B2 and hamamelitannin (HT) were supplied by Fluka (Sigma-Aldrich, Steinheim, Germany).

Samples. *Raw Material*. The starting material was chopped stems of witch hazel (*H. virginiana*) from Martin Bauer GmbH (Alveslohe, Germany).

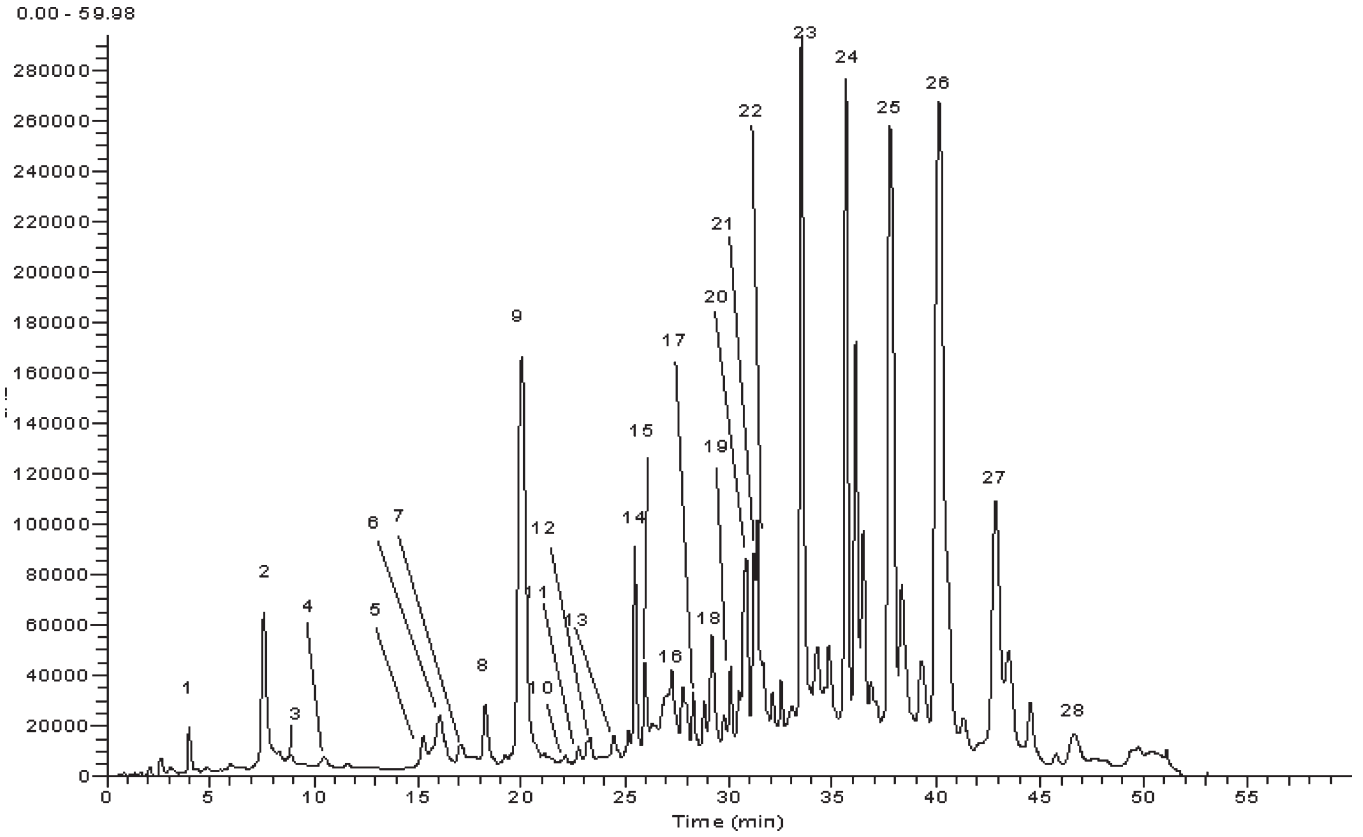

Figure 2. Total scan chromatogram of the intact tannin fraction from *H. virginiana*.

A polyphenolic fraction was obtained as described by Torres (*5*). It resulted from the crude extract soluble in both ethyl acetate and water by fractionation on Toyopearl HW-40F. The fraction selected was the eluate with water/acetone (1:1) after washing with methanol. For thermal studies, 1 mL of water solution of 0.5 mg/mL of the fraction was heated in glass tubes to be subjected to the following treatments: as cooked products, 100 °C during 6 min; and as sterilized products, 100 °C during 120 min. After that, samples were diluted to 1 mL with water.

The phenolic content was determined according to the method of Singleton and Rossi (*24*). Briefly, 1 mL of 0.2 N Folin−Ciocalteu and 0.8 mL of 0.8% Na_2CO_3 were added to 0.2 mL of phenolic solution. After stirring and incubation at 45 °C during 15 min, absorbance at 765 nm was measured. The results were expressed as equivalents of catechin (range of the standard curve from 10 to 200 ppm, $R^2 = 0.9977$).

Liquid Chromatography−Electrospray Mass Spectrometry (HPLC-DAD-MS). HPLC separation was performed on a Waters C18 Symmetry column, 3.5 μm, 2.1 × 150 mm. HPLC-DAD-MS was equipped with a quaternary pump (Spectra System P4000) and a diode array detector (UV 6000LP) coupled to an ion trap mass spectrometer (LCQ DECA XP Plus, Thermo Fisher Scientific) with an electrospray source (ESI) in negative mode.

The mobile phase was a binary solvent gradient system consisting of (A) 0.05% HCOOH and (B) CH_3CN and a flow rate of 0.2 mL/min. The elution profile was as follows: gradient 0−77% B in 30 min, isocratic conditions 77% B for 10 min, and gradient 77−100% B in 12 min at 25 °C. MS conditions were as follows: capillary temperature, 300 °C; sheath gas, 80 units; source voltage, 5 kV; and capillary voltage, 15 V. MS-MS spectra of daughter ions were obtained with a normalized collision energy of 27%. Peaks were identified by comparison of their retention times with those of standards and by interpretation of their mass spectra.

Gallic acid (range standard curve = 2.5−100 ppm, $R^2 = 0.9992$) and HT (range standard curve = 10−200 ppm, $R^2 = 0.9949$) were quantified using commercial standards. Hydrolyzable tannins were quantified using pentagalloyl glucose (PGG) purified in the laboratory (purity = 99%) as standard (range standard curve = 105−2100 ppm, $R^2 = 0.9980$). Calibration curves were built using eight data points. Percentage of procyanidins was obtained by difference between total extract and the percent of hydrolyzable tannins.

Reducing Power of the Phenolic Compounds. The ferric reducing/antioxidant power (FRAP) method was used by adaptation of the procedure of Benzie and Strain (*25*). The FRAP reagent was prepared daily by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM ferric chloride, in the ratio 10:1:1, respectively. TPTZ solution was prepared in 40 mM HCl. Three milliliters of FRAP reagent was added to 100 μL of phenolic solution (0.1 mg/L), and the absorbance was measured at 593 nm after 6 min. The standard curve was built with ferrous chloride. All analyses were performed in duplicate.

DPPH Radical Scavenging Assay. The DPPH radical scavenging capacity was measured according to the method of Von Gadow et al. (*26*). Three milliliters of a 6×10^{-5} M methanolic solution of DPPH was added to 75 μL of the antioxidant solution. The decrease in absorbance at 515 nm was recorded for 16 min. The inhibition percentage (IP) was defined as the percentage of reduction in absorbance measured after 16 min, using a selected concentration of extract. EC_{50} was calculated as the amount of extracts causing a 50% inhibition of the DPPH radical. All analyses were performed in duplicate.

Processed Mackerel Muscle. Fresh Atlantic mackerel (*Scombrus scombrus*) was supplied by a local market. Fish was deboned and eviscerated, and the white muscle was separated and minced. Three different fish muscle systems were prepared:

Control: Minced fish muscle was processed at 110 °C fir 120 min, and then 200 ppm of streptomycine sulfate was added.

System I: Minced fish muscle was processed at 110 °C for 120 min, and then 30 ppm of the *Hamamelis* fraction and 200 ppm (w/w) of streptomycine sulfate were added.

System II: Minced fish muscle was supplemented with 30 ppm of the *Hamamelis* fraction, processed at 110 °C for 120 min, and then 200 ppm (w/w) of streptomycin sulfate was added.

The addition of 30 ppm of *Hamamelis* extract to minced fish muscle did not provoke any effect on the sensory properties of the final fish product. For oxidation experiments, portions of 5 g of fish muscle corresponding to the three systems were placed into 50 mL Erlenmeyer flasks and stored at 4 °C during 18 days. Samples were

Table 1. Identification of Phenolics from the *Hamamelis* Extract[a]

peak	compound	UV (nm)	$(M-H)^-$	$(2M-H)^-$	$(M-H)^{2-}$
1	gallic acid	218, 269, 326	169 (100)		
2	gP	224, 268	305.48 (43.85)	611.23 (100)	
3	P−gP (dimer)	216, 272	593.15 (100)		
4	P−gP (dimer)	216, 272	593.15 (100)		
5	P−P (dimer)	217, 277−278	577.45 (100)		
6	P−P (dimer)	217, 277−278	577.45 (100)		
7	PG−gP (dimer)	217, 275	745.32 (100)		
8	catechin	220, 278	289 (84.49)	579.33 (100)	
9	hamamelitannin	224, 273	483 (100)	967 (84.84)	
10	P−gP (dimer)	216, 272	593.15 (100)		
11	PG−gP (dimer)	217, 275	745.32 (100)		
12	P−gP (dimer)	216, 272	593.15 (100)		
13	procyanidin B2	218, 277−278	577.45 (100)		
14	trigalloyl glucose	224, 276	635.34 (100)	1270.87 (1.56)	
15	P−PG (dimer)	221, 277	729.34 (100)	1458.96 (0.35)	
16	trigalloyl glucose	224, 276	635.34 (100)		
17	composed by P−P and P−PG and PG−gP (all dimers)	218, 277−278	577.45 (100)		
		221, 277	729.34 (27)		
		217, 275	745.32 (85.40)		
18	unknown	221, 270	473.20 (100)	947.06 (15.15)	
19	tetragalloyl glucose	223, 276	787.50 (100)		393.55 (36.40)
20	tetragalloyl glucose	223, 276	787.50 (100)		393.55 (6.25)
21	tetragalloyl glucose	223, 276	787.50 (100)		393.5 (8.85)
22	catechin gallate	223, 276	441 (100)	883.25 (47.02)	
23	pentagalloyl glucose	224−276	939.27 (100)		469.45 (21.18)
24	hexagalloyl glucose	224, 278	1091.22 (100)		545.2 (52.35)
25	heptagalloyl glucose	225, 274	1243.08 (65.04)		621.45 (100)
26	octagalloyl glucose	225, 264	1395.03 (29.06)		697.43 (100)
27	nanogalloyl glucose	222, 274	1546.9 (12.27)		773.48 (100)
28	decagalloyl glucose	222, 273	1699 (0.64)		849.27 (100)

[a] Intensities are indicated in parentheses. Abbreviations are described in **Figure 1**.

prepared in triplicate. Oxidation was measured on different sampling days.

Lipid Extraction. Lipids were extracted from fish muscle according to method of Bligh and Dyer (*27*) and quantified gravimetrically. Analyses were performed in duplicate.

Peroxide Value (PV). The PV of fish muscle was determined by using the ferric thiocyanate method (*28*) and was expressed as milliequivalents of oxygen molecule per kilogram of lipid (mequiv of O_2/kg of lipid). Analyses were performed in duplicate.

Thiobarbituric Acid Reactive Substances (TBARS). TBARS, expressed as milligrams of malonaldaldehyde per kilogram of muscle (mg of MDA/kg of muscle), were determined according to the method of Vyncke (*29*). Analyses were made in duplicate.

Inhibition of formation of peroxides and TBARS was calculated during the propagation period of controls according to the method of Frankel (*1*) as

$$\% \text{ inhibition} = [(C-S)/C]100$$

where C = oxidation product formed and S = oxidation product formed in sample.

Statistical Analysis. All experiments were replicated, and analyses were performed in duplicate. The data were compared by one-way analysis of variance, and the means were compared by a least-squares difference method (*30*). Significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Changes in *Hamamelis* Extract after Thermal Treatment. The first part of this work was aimed at identifying the different components present in the *H. virginiana* fraction. The chromatogram of raw material is shown in **Figure 2**, and **Table 1** shows the identifications of the different peaks. The chromatogram exhibited a complex profile with a great variety of compounds including proanthocyanidins and hydrolyzable tannins.

The proanthocyanidin fraction was mainly composed by monomers of catechin (P) and gallocatechin (gP) and several dimers of procyanidins (catechin or epicatechin) with a galloyl moiety at position 3 of the C-ring (3-*O*-galloyl substituents, PG) and a hydroxyl group at position 3′ of the B-ring (gP). The identification of these compounds was mainly made on the basis of their highly stable $[M-H]^-$ ions. **Figure 3B** represents the mass spectra of the PG−gP in which its corresponding $[M-H]^-$ ion is clearly visible, m/z 745. All dimers identified were B-type procyanidins, in which the linkage pattern was C4→C8 or C4→C6 as can be seen by their molecular weights. The A-type procyanidins, with an unusual second ether linkage between an A-ring hydroxyl group of the bottom unit to position 2 of the C-ring of the T-unit, show molecular weights 2 units lower than those described above.

A high number of hydrolyzable tannins were detected and identified. The first one, peak 9, was hamamelitannin (HT) (**Figure 1**), a furanose ring with two units of gallic acid that was highly concentrated and unequivocally identified by comparison to a commercial standard (**Figure 3A**). The $[M-H]^-$ ion was clearly visible at m/z 483. Also, the $[2M-H]^-$ ion corresponding to the m/z 967.0 ion and attributed to dimerization in the mass spectrometer and the m/z 241 ion corresponding to $[M-2H]^{2-}$ were evident. The presence of this compound has been previously reported in *H. virginiana* (*16*). However, the most abundant hydrolyzable tannins in the *H. virginiana* extract were more complex molecules with a different molecular structure, mainly differing in the sugar moiety (peaks 14, 16, and 19−28). The careful study of their mass spectra demonstrated that they were glucose esters composed by a basic moiety of glucose core esterified with a variable number of galloyl moieties, from 3 to 10 units. As consequence, these compounds showed specific

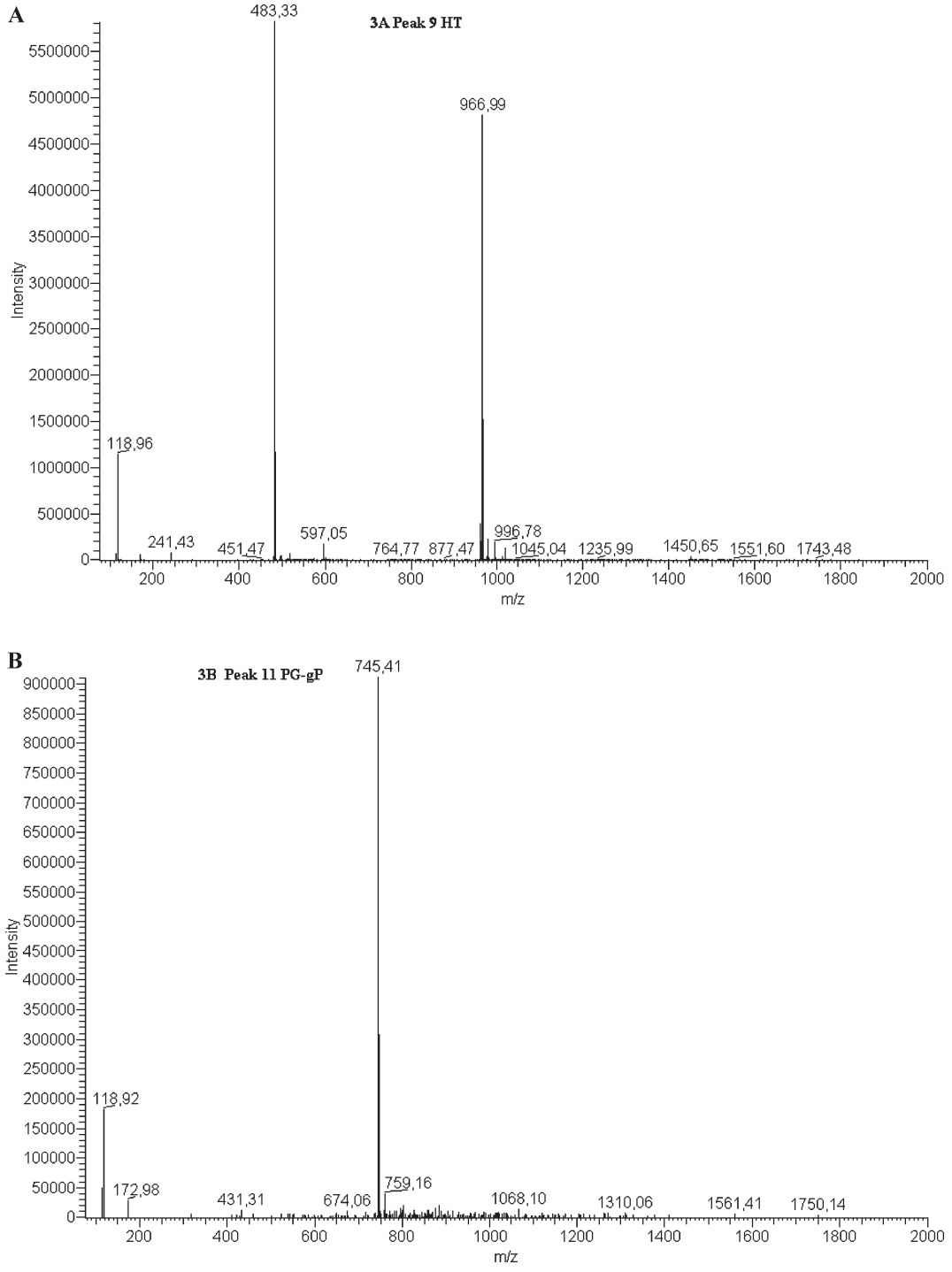

Figure 3. Mass spectra of peaks 11 (gP−GP) and 9 (hamamelitannin).

mass spectra differing in a gap of *m*/*z* 152 corresponding to the galloyl moiety (**Figure 4**). These spectra show as characteristic ions $[M - H]^-$ and $[M - 2H]^{2-}$. A characteristic of the ESI-MS of these compounds is the influence of the number of galloyl units in the ionization pattern that affects significantly the formation of the doubly charged ions. The ratio of intensities of $[M - H]^-/[M - 2H]^{2-}$ ions is >1 for pentagalloyl glucose (PGG) through hexagalloyl glucose (6G) (**Figure 4**). An increment of the number of galloyl moieties higher than 6 resulted in an inversion of the ratio of intensities. Then, for hepta (7G), octo (8G), nano (9G), and decagalloyl glucose (10G) the ratio of the intensities was <1. Therefore, the stability of $[M-H]^-$ ions decreases with the increment of galloyl moieties. This fragmentation pattern had been previously described in hydrolyzable tannins present in *Eucalyptus nitens* wood (*31*) and in other plant extracts (*32*, *33*).

In this way, hexagalloyl glucose is formed by attachment of additional galloyl units to the PGG core via so-called meta- or para-depsidic bond (**Figure 1**). These compounds were identified by the detection of $[M - H]^-$ ions and the examination of MS-MS daughter ions from the selected parent ions. **Figure 5** shows the mass−mass spectrum of hamamelitannin and pentagalloyl glucose. Losses of 18 (water) and 152 units (galloyl moiety) from the $[M - H]^-$ ion are characteristic of C-glucosidic hydrolyzable tannins. Finally, the identification was also possible by comparison of the retention times and mass spectra with those obtained

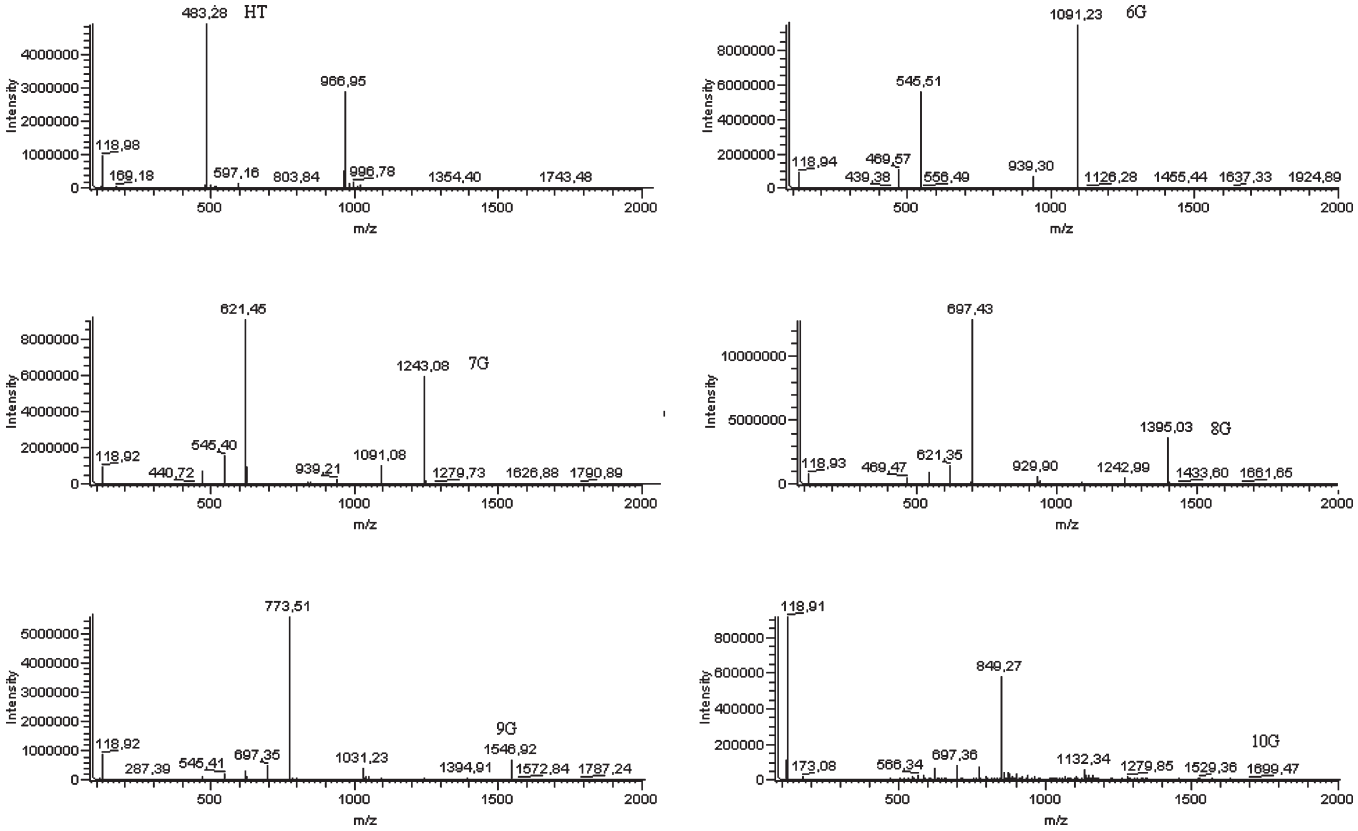

Figure 4. Mass spectra of different hydrolyzable tannins. HT: $[M - H]^-$ = 483.31 and $[M - 2H]^{2-}$ = 241.40. 6G: $[M - H]^-$ = 1091.33 and $[M - 2H]^{2-}$ = 545.38. 7G: $[M - H]^-$ = 1243.04 and $[M - 2H]^{2-}$ = 621.40. 8G: $[M - H]^-$ = 1395.07 and $[M - 2H]^{2-}$ = 697.35. 9G: $[M - H]^-$ = 1546.92 and $[M - 2H]^{2-}$ = 773.59. 10G: [M − H] = 1699.85 and $[M - 2H]^{2-}$ = 849.30.

by the injection of a commercial extract of tannic acid, which consisted of a mixture of different glucose gallates.

This is the first time that hydrolyzable tannins with a high number of galloyl moieties are being described in *H. virginiana*. Haberland and Kolodziej, in a previous work (*15*), reported the presence of a galloylhamamelose with three galloyl units. The analyses of UV maxima (around 224 and 273 nm, **Table 1**) of peak 14 corresponding to m/z = 635.4 and of peaks 19−21 corresponding to m/z 787 suggest that these compounds could be derivatives of hamamelose with three (3G) and four (4G) galloyl units. The mass spectral pattern of these compounds was similar to that of HT and to glucose derivatives with five and six units of gallic acid. The ratio of intensities of $[M - H]^-/[M - 2H]^{2-}$ ions was > 1.

Thermal Treatments. The chromatographic profiles of the *H. virginiana* extract after two thermal treatments differing significantly in the duration time are shown in **Figure 6**. As a result of treatments, significant changes in the chromatographic profile were observed. Variations in the concentration resulting are shown in **Table 2**. Thermal treatment at 100 °C during 6 min triggered significant modifications in the peak profiles. A decrease in glucose derivatives with a number of galloyl units higher than six was observed. As consequence, an important increment in free gallic acid was observed, 275%. Simultaneously pentagalloyl glucose content decreased around 12%. The most important changes were seen as a result of long-term heating, 100 °C during 120 min. In this treatment, the effect on the individual compounds was more severe, leading to an extensive hydrolysis of gallotannins with 6−10 galloyl units to tetra- and pentagalloyl glucose. As a result of this hydrolysis, increments of gallic acid and pentagalloyl glucose concentrations (3706 and 88%, respectively) and a total loss of the gallotannins with 6−10 galloyl units were detected. Therefore, structural modifications of hydrolyzable tannins showed clear time dependence during the thermal processes. In relation to proanthocyanidins, most of the dimers were not detected after any thermal treatments.

Changes in Polyphenolic Content and in Vitro Antioxidant Capacity after Thermal Treatments. Thermal processing altered the total phenolic content determined by the Folin−Cioalteu reactive and the capacities to donate electrons and scavenge free radicals (**Table 3**). The total phenolic content was slightly reduced after heating at 100 °C during 120 min. Treatment at 100 °C during 6 min produced a reduction of 20% of the initial phenolic content. These results are consistent with previous studies in vegetables, which confirm that thermal processes significantly alter the composition and also the functionality in legume sprouts and seedlings (*34*, *35*).

Thermal processing provoked a reduction of scavenging and reducing capacities as can be inferred from data corresponding to heating at 100 °C during 6 min. Such reduction was higher in the DPPH assay. However, improved values of DPPH and FRAP were obtained for the long thermal treatment (100 °C during 120 min). This treatment showed DPPH values similar to those of the raw extract and a significant increase of FRAP values. The increase in accessible reactive groups due to thermal hydrolysis could explain these findings. Gallic acid is able to donate five electrons per mole. The FRAP values of the nonprocessed *H. virginiana* extract indicated 3.6 electrons transferred per mole of extract. Therefore, free gallic acid showed higher reducing capacity than the nonprocessed fraction, rich in hydrolyzable tannins. The significant increase in the amount of free gallic acid as a result of heating in thermally processed samples probably led to an increment in the reducing capacity per mole of heated samples. Such effect probably affected the scavenging ability as well.

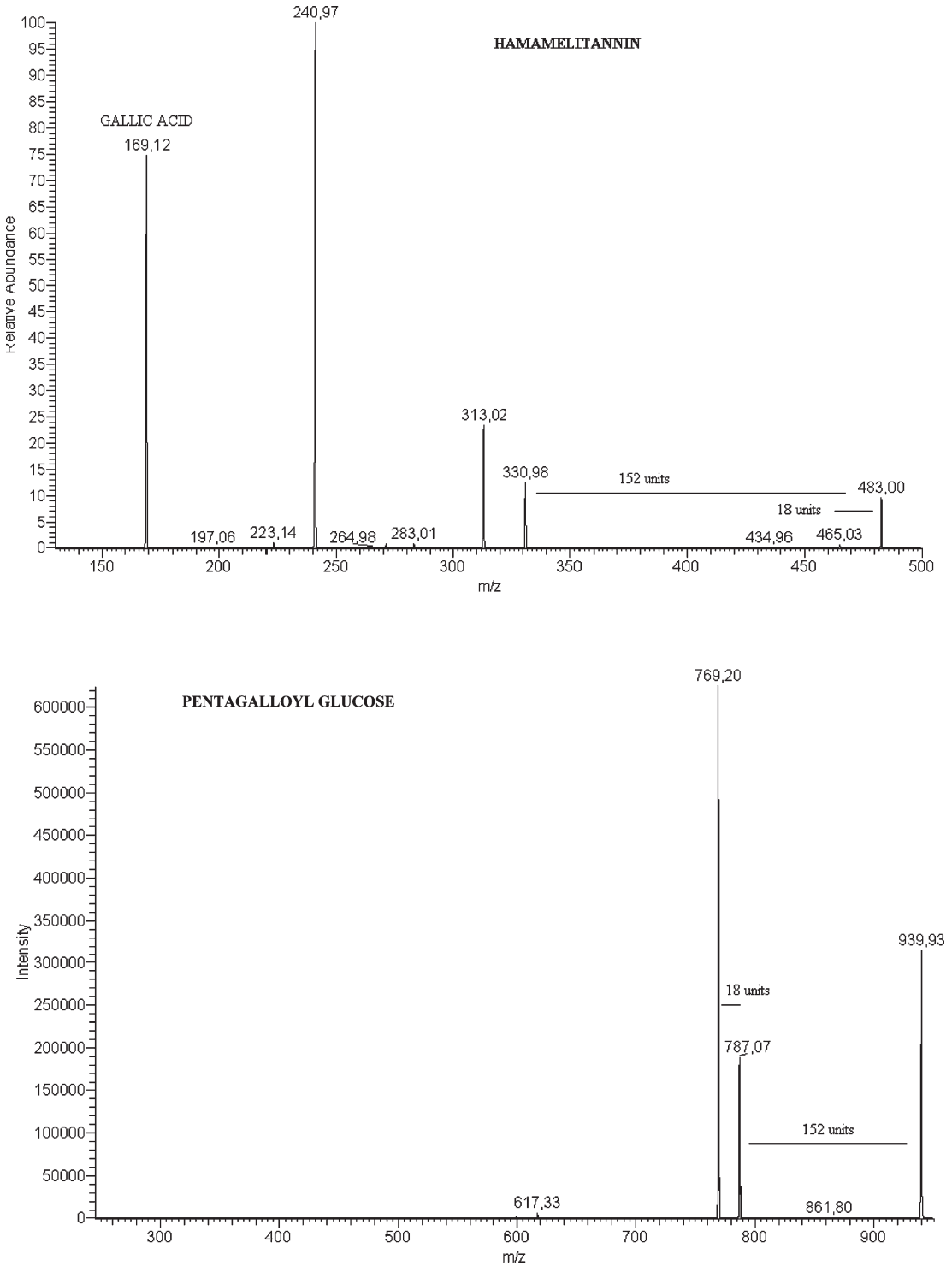

Figure 5. MS-MS of daughter ions from hamamelitannin and pentagalloyl glucose; losses of 18 units (water) and 152 units (galloyl group).

The concentration selected for these studies allowed a careful identification of the changes in both molecular structure and antioxidant capacity. Such changes were consistent with those observed at lower concentrations of the *Hamamelis* extract as the concentration employed in the fish muscle experiments (0.03 mg/mL, equivalent to 30 ppm in aqueous solution).

Lipid Oxidation Study in Processed Mackerel. To study the influence of heating on the activity of *Hamamelis* extract on muscle foods, oxidations of processed mackerel muscle containing 30 ppm of the antioxidant extract added after (system I) of before (system II) heating were compared with controls. Minced mackerel muscle used for the experiment showed a high fat content (4.32 ± 0.27% expressed in wet weight). After thermal treatments, the water content of fish muscle was reduced from 74.22 ± 0.32 to 68.54 ± 1.41%, leading to an increase in the muscle lipid content up to 5.98 ± 0.53%. The monitoring of the formation of peroxides and TBARS during chilled storage of minced mackerel muscle is shown in **Figure 7**. Formation of peroxide products was significantly inhibited in both samples supplemented with the witch hazel tannins. However, significant differences were detected for both systems. Fish samples heated before the addition of the tannins developed significant formation of peroxides more rapidly and in higher concentration than fish samples already containing the witch hazel phenolics when

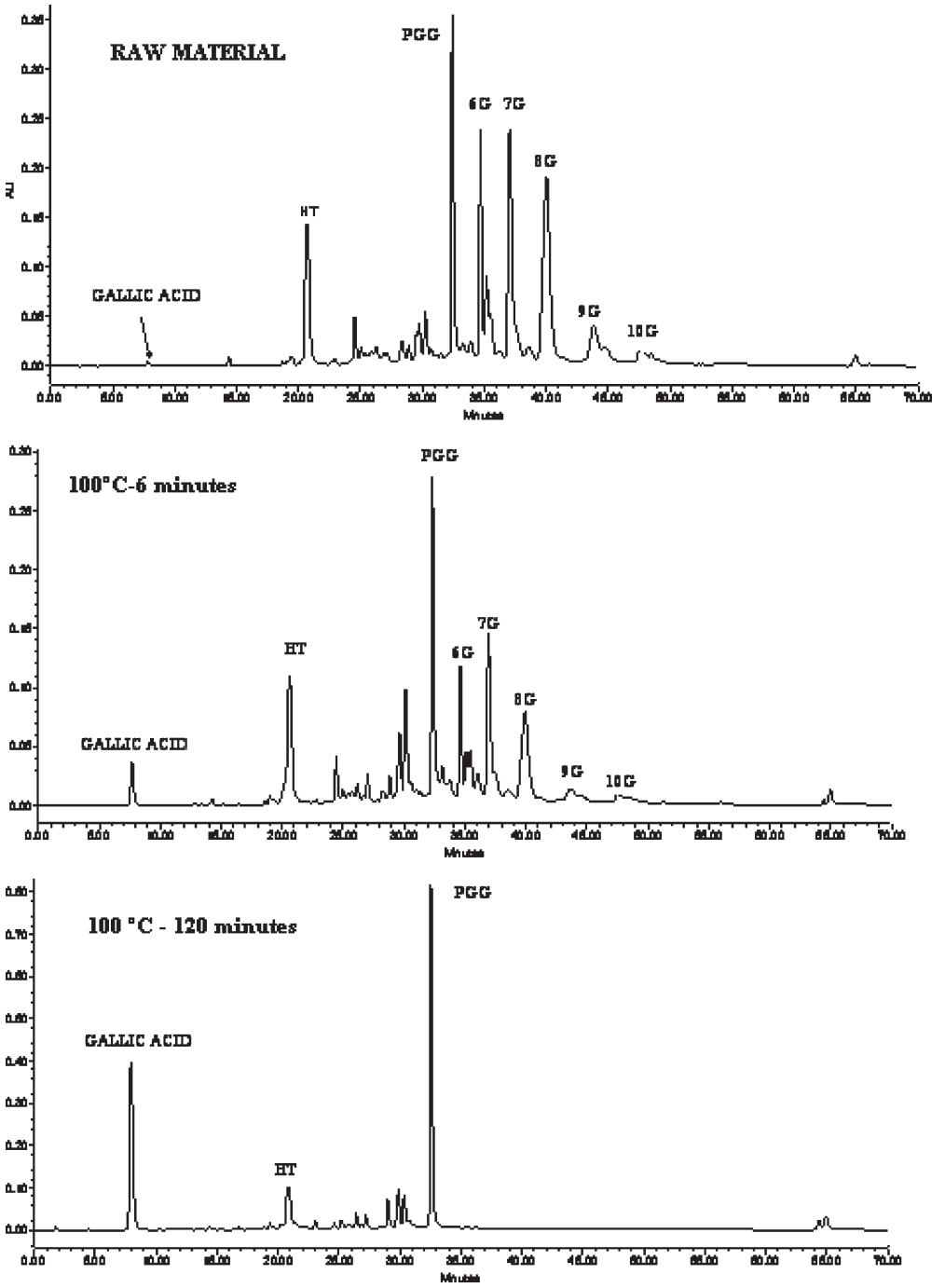

Figure 6. Comparison of chromatographic profiles of the intact tannin fraction from *H. virginiana* and different thermal treatments. PDA detector: λ = 280 nm.

heated. With regard to TBARS formation, differences between both types of samples were more evident. Fish samples corresponding to system I showed scarce inhibition of TBARS formation compared with controls. In agreement with peroxide formation, samples corresponding to system II did not show evidence of oxidation during the whole experiment. Neither were rancid off-flavors detected in these samples. The percentages of inhibition of peroxide formation calculated by the 11th day were $44.92 \pm 2.59\%$ for system I and $83.56 \pm 0.87\%$ for system II. The percentages of inhibition of TBARS formation during the 11th day were $5.31 \pm 1.45\%$ for system I and $98.7 \pm 2.34\%$ for system II. Therefore, chilled experiments evidenced that extracts after thermal treatment were more effective in inhibiting fish lipid oxidation than those without thermal treatment. This finding could be attributed to a possible reduction of radical formation during thermal processing when the antioxidants are added. Such reduction should decrease the level of hydroperoxides, the first oxidation products, during the first steps of the experiments. However, the concentrations of hydroperoxides in controls and samples corresponding to systems I and II, respectively, were quite similar and close to zero during the first three days of chilled storage. Therefore, it seems that the high antioxidant effect observed in processed fish samples (system II) is essentially attributed to the effect of the thermal process itself on hydrolyzable tannins. As described previously, thermal treatments at 100 °C provoked a significant breakdown of the high molecular weight hydrolyzable tannins, leading to a major concentration of free gallic acid and pentagalloyl glucose. This effect was significantly higher when the duration of the thermal processes was 120 min. Data of antioxidant capacity in solution indicated that the

Table 2. Degradation of Polyphenols during Thermal Treatments[a]

	ppm			% of variation[b] related to control	
compound	raw	100 °C, 6 min	100 °C, 120 mim	100 °C, 6 min	100 °C, 120 min
gallic acid	1.89 ± 0.03	7.09 ± 0.79	71.97 ± 6.71	−275.21	−3706.70
hamamelitannin	44.50 ± 4.15	39.33 ± 1.25	37.77 ± 2.13	11.62	15.14
trigalloyl glucose	26.06 ± 2.09	26.35 ± 2.30	10.33 ± 1.55	−1.12	60.35
tetragalloyl glucose	15.66 ± 1.88	16.84 ± 1.58	30.70 ± 2.86	−7.54	−96.05
pentaalloyl glucose	60.99 ± 4.56	53.64 ± 3.27	115.11 ± 9.45	12.05	−88.74
hexagalloyl glucose	61.43 ± 6.99	22.48 ± 1.78	0.00	63.41	100.00
heptagalloyl glucose	66.84 ± 6.43	41.43 ± 3.99	0.00	38.01	100.00
octagalloyl glucose	78.17 ± 3.45	37.00 ± 2.56	0.00	52.67	100.00
nanogalloyl glucose	30.96 ± 2.02	12.12 ± 1.54	0.00	60.86	100.00
decagalloyl glucose	18.20 ± 2.56	14.60 ± 2.56	0.00	19.77	100.00

[a] Gallic acid and hamamelitannin were quantified using the corresponding standards. Hydrolyzable tannins were quantified as pentagalloyl glucose equivalents. [b] Calculated for each compound as follows: $(\text{area}_{raw} - \text{area}_{processed}) \times 100/\text{area}_{raw}$. Negative values indicate compounds for which concentration increases during thermal processing, and positive values indicate compounds for which concentration decreases during thermal processing.

Table 3. Total Phenolic Content and FRAP and DPPH Values of Raw and Heated *Hamamelis virginiana* Extract[a]

Hamamelis extract	total phenolic content expressed as equivalents of catechin	FRAP (mequiv of Fe^{2+}/mg of sample)	DPPH (EC_{50}, ppm)
raw	109.308 ± 0.007 c	$6.925 \times 10^{-3} \pm 0.00002$ b	98.348 ± 0.109 a
100 °C, 6 min	88.457 ± 0.003 a	$6.529 \times 10^{-3} \pm 0.00004$ a	115.658 ± 0.13 c
100 °C, 120 min	101.217 ± 0.003 b	$8.211 \times 10^{-3} \pm 0.00007$ c	100.003 ± 0.000 b

[a] Values in each column with the same letter were not significantly different ($p < 0.01$).

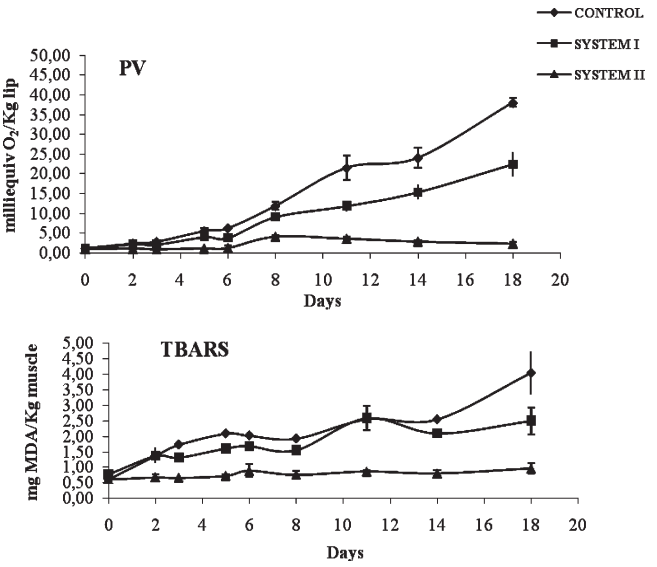

Figure 7. Peroxide and TBARS formation in fish muscle controls, systems I and II, stored at 4 °C.

resultant high amounts of free gallic acid enhanced the reducing power of the heated natural extract. The reducing capacity or amount of donated electrons per molecule of phenolics has been suggested as a major factor involved in the antioxidant protection on chilled fish (*36*). Therefore, the release of free gallic acid and pentagalloyl glucose in the heated natural extract probably provoked a better protection against rancidity in fish processed products. Thermal processing of tomatoes has also enhanced the content of bioaccessible lycopene and total antioxidant activity (*37*). Such increase has been attributed to the release of phytochemicals from the cell matrix to make them more accessible. Also, the formation of other novel compounds such as Maillard products during heat treatment or thermal processing has been associated with the increment of antioxidant activity of thermal food processes (*38*). Studies performed on tomato and coffee have shown that prolonged heat treatment enhanced the antioxidant activity of these foodstuffs, and such increase has been related to the formation of Maillard products (*39*). Maillard reaction products exhibit well-known chain-breaking and oxygen-scavenging activities (*40*). Apart from releasing gallic acid, thermal processing of witch hazel gallotannins added to fish muscle may trigger the formation of novel compounds having also higher antioxidant activity than the original compounds.

Finally, loss of available hydrolyzable tannins due to protein binding in muscle foods should be also considered. It has long been known that relative affinities of tannins for different proteins can vary as much as 10000-fold (*41*). Tannins are thought to act as multidentate ligands to facilitate protein cross-linking and consequent precipitation. The formation of soluble complexes produced through different mechanisms, among which ionic bonding was predominant, has been also described. In the case of fish muscle, protein precipitation would lead to a loss of protein solubility, which could affect the texture of the final fish product. Such change in protein solubility was not observed in fish samples supplemented with *Hamamelis* extract. Alterations in the texture of the fish product were not detected either. Additionally, it has been described that higher molecular weight tannins, such as pentagalloyl glucose highly concentrated in *Hamamelis* extracts, interacted less strongly with proteins than the lower molecular weight tetragalloyl glucose. Considering these aspects, it seems that there was not a significant formation of complexes due to protein binding.

Conclusion. Chemical composition and antioxidant activity of natural extracts of condensed and hydrolyzable tannins are affected by thermal treatment. The effects are more severe in long-term processes. In the case of hydrolyzable tannins based on glucose gallates, their antioxidant activity is increased, especially their ferric reducing/antioxidant power, probably due to the presence of gallic acid from hydrolysis of these compounds. The increase of this ability leads to a major antioxidant effectiveness in processed muscle foods. These results stress the importance of the knowledge of the effect of industrial processes on food ingredients to establish the real capacity of antioxidants.

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