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Analysis of Protein Oxidation Markers α-Aminoadipic and γ-Glutamic Semialdehydes in Food Proteins Using Liquid Chromatography (LC)−Electrospray Ionization (ESI)−Multistage Tandem Mass Spectrometry (MS)

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To elucidate the formation of protein oxidation biomarkers α-aminoadipic semialdehyde (AAS) and γ-glutamic semialdehyde (GGS) in food proteins was the main purpose of the present study. Food proteins, namely, myofibrillar proteins, α-lactalbumin, and soy proteins, as well as bovine serum albumin (BSA), were suspended in a piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) buffer and oxidized by Fe^{3+} and H_2O_2 while kept in an oven for 14 days at 37 °C. For the analysis of semialdehydes, a derivatization procedure with *p*-aminobenzoic acid (ABA) and $NaCNBH_3$ followed by liquid chromatography (LC)−electrospray ionization (ESI)−multistage tandem mass spectrometry (MS) was performed. For comparative purposes, the dinitrophenylhydrazine (DNPH) method was also employed as a routine method to assess carbonyl gain. Both semialdehydes were specifically and accurately detected by LC−MS in all oxidized proteins proving that GGS and AAS are formed as a consequence of the oxidation of lysine, proline, and arginine amino acid residues from BSA and other food proteins. Proteins from an animal source and, particularly, BSA were more susceptible to undergo oxidative reactions than soy proteins. The results from the present paper highlight the significance of using both semialdehydes as protein oxidation indicators in meat and dairy products. The analysis of GGS and AAS in real food systems would contribute to the understanding of the precise mechanisms involved in food protein oxidation and shed light on the fate of oxidizing amino acids during food processing and storage.

KEYWORDS: Oxidation; myofibrillar proteins; α-lactalbumin; soy protein; BSA; semialdehydes; DNPH; LC−ESI−MS

INTRODUCTION

The oxidation of edible oils and food lipids has been profoundly studied for decades, whereas oxidized proteins have remained in the background. Nowadays, protein oxidation is a hot topic of increasing interest among food researchers because of the relevant and scientifically sound results derived from recent studies. These studies have shown the complex mechanisms implicated in protein oxidation (*1*−*3*) and the large variety of oxidation products derived from food proteins, including cross-links (disulfide bonds and dityrosines), amino acid-oxidized derivatives, and protein carbonyls (*1*, *4*−*6*). Protein oxidation is known to affect protein functionality and food quality (*7*−*10*), and certain plant phenolics have been reported to be effective inhibitors of protein oxidation in food systems (*6*, *11*). Nevertheless, the fate of oxidizing proteins and amino acids during food processing and storage is still poorly understood. Advanced methodologies are required to deepen the knowledge of protein oxidation and fulfill the upcoming challenges.

Thus far, the quantification of protein carbonyls through the dinitrophenylhydrazine (DNPH) method (*12*) has been the most widespread routine method for assessing protein oxidation in food systems (cf. review by Estévez et al. *13*,). This method has been described as robust and accurate, although the DNPH does not react specifically with protein carbonyls because it might bind lipid carbonyls, leading to an overestimation. Lately, fluorescent spectroscopy has also been introduced for measuring protein oxidation products in bovine serum albumin (BSA) and dairy and myofibrillar proteins (*3*, *11*). These methods are limited to measure total protein carbonyls formed by diverse and unspecific pathways, and thus far, the chemical structure and the oxidation mechanisms of carbonyls derived from food proteins remain unknown.

Two carbonyls, α-aminoadipic and γ-glutamic semialdehydes (AAS and GGS, respectively), have been highlighted as biomarkers of oxidative damage to proteins (*14*). AAS is an oxidative deamination product of lysine, whereas GGS

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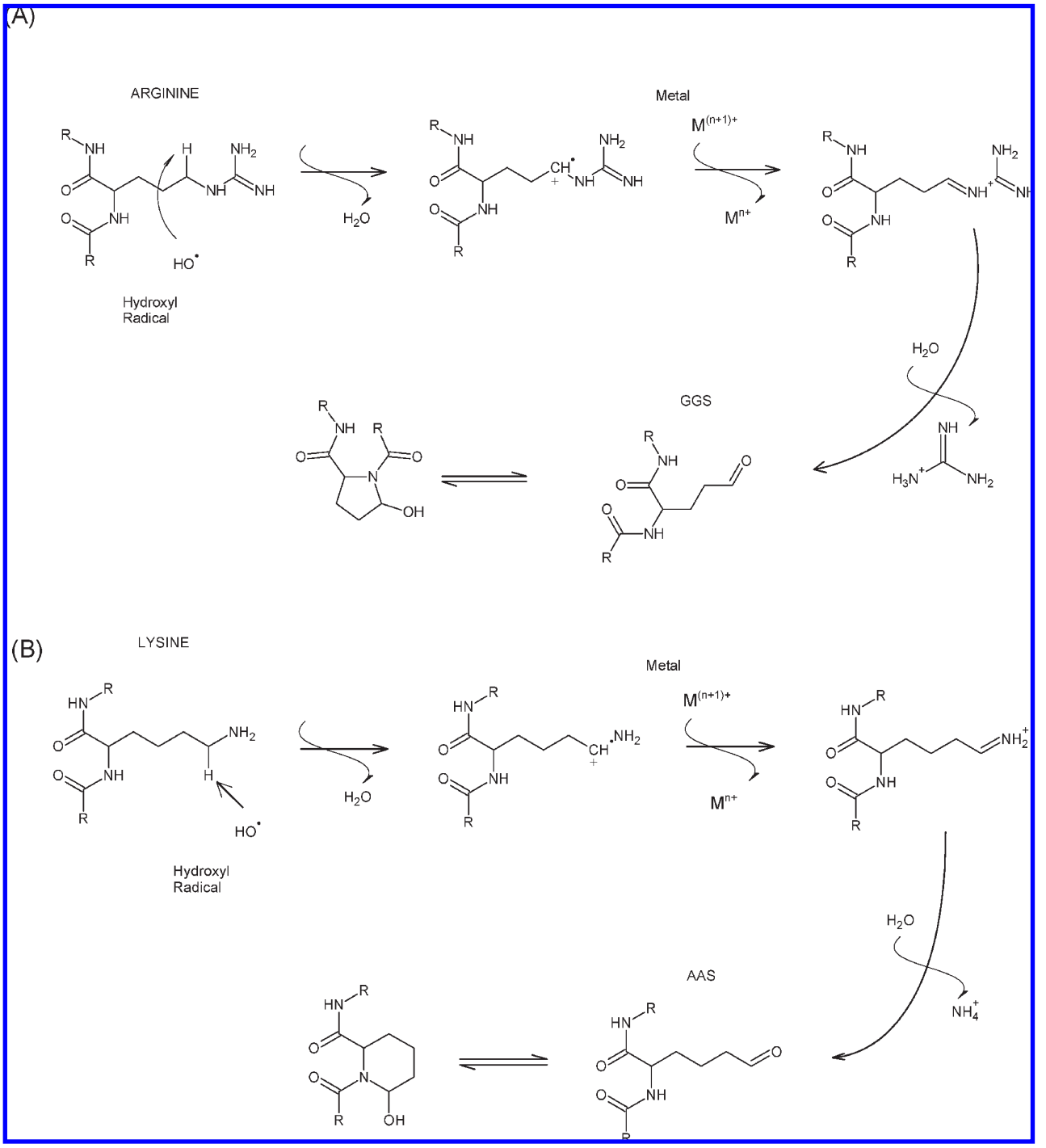

Figure 1. Formation of (A) GGS and (B) AAS as a result of the oxidative degradation of protein-bound arginine and lysine, respectively.

originates from arginine and proline residues (**Figure 1**). Both compounds have been reported as major carbonyl products of metal-catalyzed oxidation of plasma and liver proteins (*14*, *15*). In medical research, these semialdehydes have been used as biomarkers of oxidative stress and indicators of serious age-related disorders, such as Alzheimer's disease (*16*). Several procedures have been described for the analysis of AAS and GGS in biological samples. They involve a preparative derivatization step using either $NaBH_4$ or fluoresceinamine ($FINH_2$) followed by gas chromatography−mass spectrometry (GC−MS) or high-performance liquid chromatography−diode array detector (HPLC−DAD) analysis, respectively (*14*, *15*). More recently, Akagawa et al. (*17*) developed a novel derivatization procedure using *p*-aminobenzoic acid (ABA) coupled to HPLC coupled to fluorescence detection (FLD).

As far as we know, no previous studies have been carried out to detect specific protein carbonyls in food systems and the formation of AAS and GGS in oxidized food proteins remains unknown. Therefore, our objective was to analyze BSA and several food proteins, namely, myofibrillar proteins, α-lactalbumin, and soy protein, for the presence of AAS and GGS and evaluate the suitability of using these biomarkers as protein oxidation indicators in food systems. For this purpose, both compounds were innovatively analyzed using the ABA-derivatization procedure coupled to liquid chromatography−electrospray ionization−mass spectrometry (LC−ESI−MS).

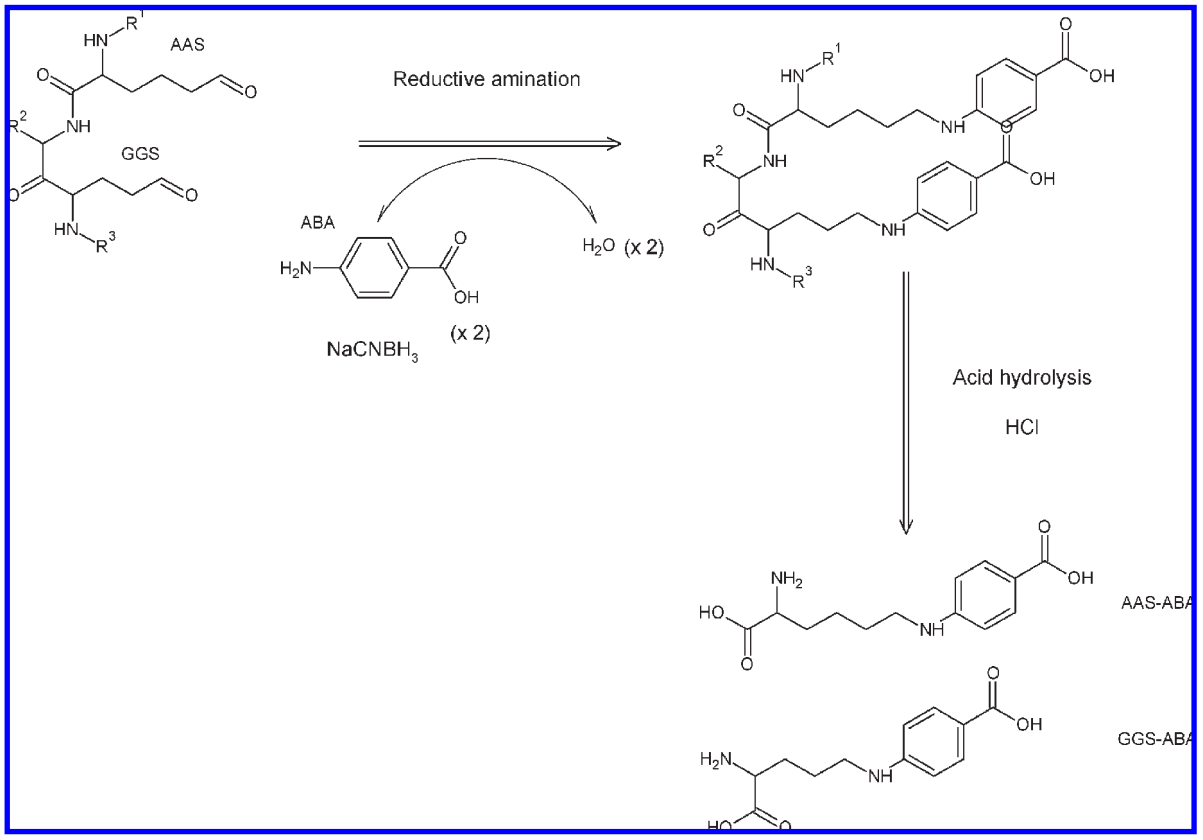

Figure 2. Preparation of semialdehydes for LC−FLD−MS analysis: derivatization of AAS and GGS with ABA and subsequent hydrolysis in the presence of HCl.

MATERIALS AND METHODS

Materials. All chemicals were supplied by J. T. Baker (Deventer, Holland), Riedel dehaen (Seelze, Germany), and Sigma Aldrich (Steinheim, Germany). All chemical were of analytical grade, except HPLC-grade methanol. BSA and α-lactalbumin were obtained from Sigma Aldrich (Steinheim, Germany), while purified soy protein isolate and porcine longissimus dorsi muscle were purchased in a local supermarket in Helsinki, Finland. Myofibrillar proteins were isolated and purified from porcine muscles according to a method described elsewhere (*11*). Water used was purified by passage through a Milli-Q system (Millipore Corp., Bedford, MA). An egg shell membrane was isolated from fresh white leghorn hen eggs, thoroughly washed with distilled water, cut into small pieces (5 × 5 mm), and finally dried with filter paper before use.

Preparation and *in Vitro* Oxidation of Protein Suspensions. Myofibrillar proteins, α-lactalbumin, soy protein, and BSA, were suspended (20 mg/mL) in 15 mM piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) buffer (pH 6) containing 0.6 M sodium chloride. Protein suspensions (30 mL) were dispensed in sealed vials and oxidized (10 μM $FeCl_3$, 0.1 mM ascorbic acid, and 1 mM H_2O_2) while constantly stirred and kept in a oven at 37 °C for 14 days. Sampling was carried out at days 1, 4, 7, 10, and 14 for analyses.

Determination of Total Carbonyls by the DNPH Method. Total protein carbonyls were quantified in protein suspensions at sampling times according to the method described by Butterfield and Stadtman (*18*), with minor modifications. An aliquot (100 μL) of protein suspensions was dispensed in 2 mL eppendorf tubes. Proteins were precipitated by cold 10% TCA (1 mL) and subsequent centrifugation for 5 min at 5000 rpm. One pellet was treated with 1 mL of 2 M HCl (protein concentration measurement), and the other pellet was treated with an equal volume of 0.2% (w/v) DNPH in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterward, samples were precipitated by 10% TCA (0.8 mL) and washed twice with 1 mL ethanol/ethyl acetate (1:1, v/v) to remove excess of DNPH. The pellets were then dissolved in 2 mL of 6 M guanidine HCl in 20 mM sodium phosphate buffer at pH 6.5, stirred, and centrifuged for 2 min at 5000 rpm to remove insoluble fragments. The protein concentration was calculated from absorption at 280 nm using BSA as a standard. The amount of carbonyls was expressed as nanomoles of carbonyl per milligram of protein using an absorption coefficient of 21.0 nM^{-1} cm^{-1} at 370 nm for protein hydrazones.

Synthesis of AAS−ABA and GGS−ABA. *N*-Acetyl-L-AAS and *N*-acetyl-L-GGS were synthesized from *N*α-acetyl-L-lysine and *N*α-acetyl-L-ornithine using lysyl oxidase activity from egg shell membrane following the procedure described by Akagawa et al. (*17*). Briefly, 10 mM *N*α-acetyl-L-lysine and 10 mM *N*α-acetyl-L-ornithine were independently incubated with constant stirring with 5 g of egg shell membrane in 50 mL of 20 mM sodium phosphate buffer at pH 9.0 and 37 °C for 24 h. The egg shell membrane was then removed by centrifugation, and the pH of the solution was adjusted to 6.0 using 1 M HCl. The resulting aldehydes were reductively aminated with 3 mmol of ABA in the presence of 4.5 mmol of sodium cyanoborohydride ($NaCNBH_3$) at 37 °C for 2 h with stirring. Then, ABA derivatives were hydrolyzed by 50 mL of 12 M HCl at 110 °C for 10 h. The hydrolysates were evaporated at 40 °C *in vacuo* to dryness. The resulting AAS−ABA and GGS−ABA were purified using silica gel column chromatography and ethyl acetate/acetic acid/water (20:2:1, v/v/v) as elution solvent.

Derivatisation and Acid Hydrolysis of Oxidized Proteins. Oxidized proteins were prepared for LC analysis according to the procedure described by Akagawa et al. (*17*, *19*), with minor modifications, in accordance with a previous study (*20*) (**Figure 2**). At sampling times, an aliquot (200 μL) of protein suspension was dispensed in 2 mL eppendorf tubes. Proteins were precipitated with 2 mL of cold 10% TCA and subsequent centrifugation at 2000 rpm for 30 min. The resulting pellets were treated again with 2 mL of cold 5% TCA, and proteins were precipitated after centrifugation at 5000 rpm for 5 min. Pellets were then treated with 0.5 mL of 250 mM 2-(*N*-morpholino)

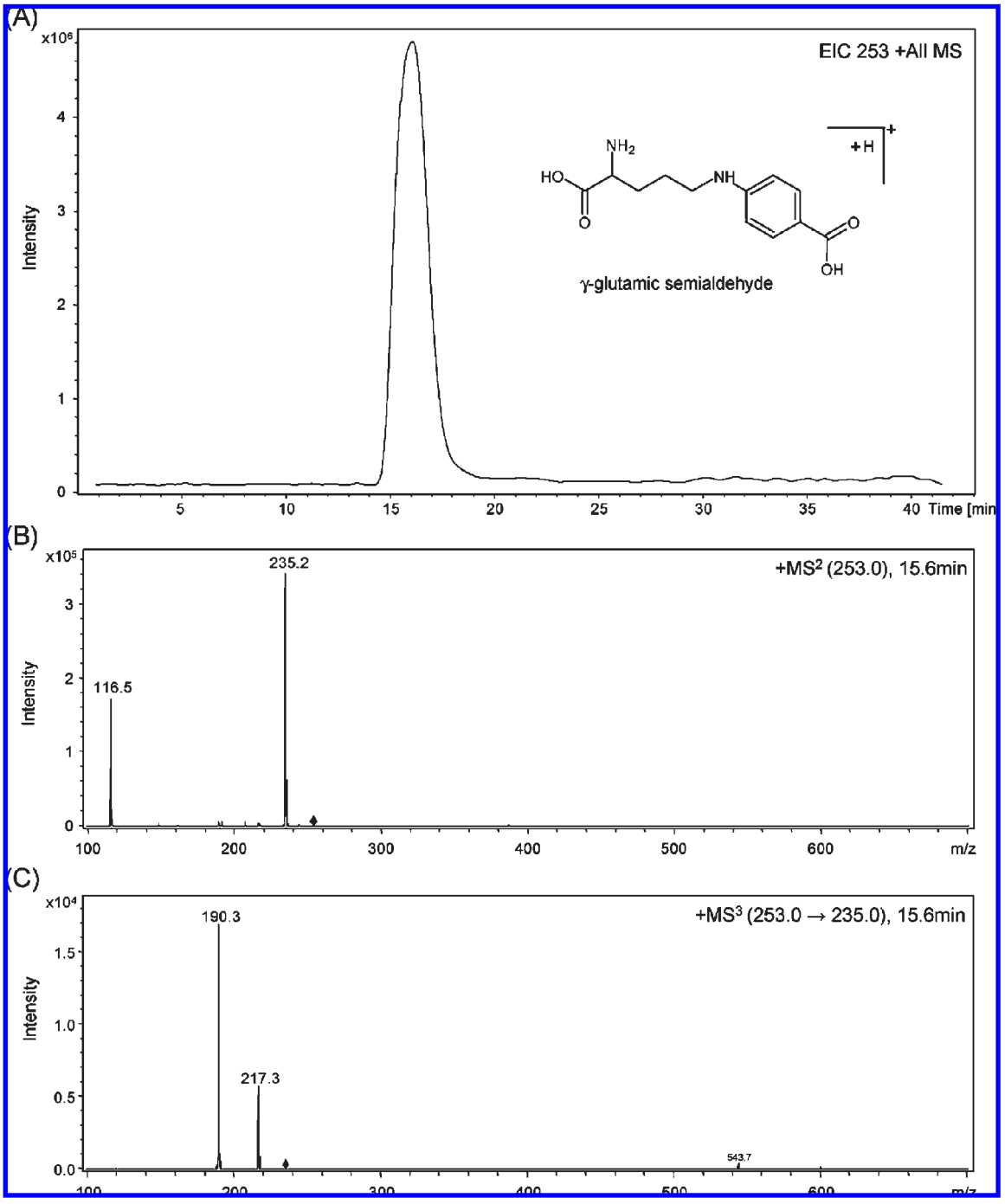

Figure 3. (A) Extracted ion chromatogram (EIC) of *m/z* 253 γ-glutamic semialdehyde and mass spectra after subsequent (B) MS^2 and (C) MS^3 fragmentations.

ethanesulfonic acid (MES) buffer at pH 6.0 containing 1% sodium dodecyl sulfate (SDS) and 1 mM diethylenetriaminepentaacetic acid (DTPA), 0.5 mL of 50 mM ABA in 250 mM MES buffer at pH 6.0, and 0.25 mL of 100 mM $NaCNBH_3$ in 250 mM MES buffer at pH 6.0. The derivatization was completed by allowing the mixture to react for 90 min while tubes were immersed in a water bath at 37 °C and stirred regularly. All solutions employed for the derivatization procedure were freshly made at sampling days. The derivatization reaction was stopped by adding 0.5 mL of cold 50% TCA, followed by a centrifugation at 5000 rpm for 5 min. Pellets were then washed twice with 1 mL of 10% TCA and 1 mL of ethanol/diethyl ether (1:1, v/v). Centrifugations at 5000 rpm for 5 min were performed after each washing step. Protein hydrolysis was performed at 110 °C for 18 h in the presence of 6 M HCl. Hydrolysates were finally dried *in vacuo* at 40 °C using a rotaevaporator. Hydrolysates were finally reconstituted with 200 μL of Milli-Q water and filtered through hydrophilic polypropylene GH Polypro (GHP) syringe filters (0.45 μm pore size, Pall Corporation, East Hills, NY) for LC analysis.

Detection of AAS−ABA and GGS−ABA by LC−ESI−MS. Samples (2 μL) were injected into an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA) equipped with a Luna reversed-phase (RP) column (5 μm C_{18} II column, 150 × 1.00 mm inner diameter, Phenomenex Torrance, CA) eluted at a flow rate of 50 μL/min with isocratic water/2.5% acetic acid (solvent A; 95%) and methanol/2.5% acetic acid (solvent B; 5%). The column was operated at a constant temperature of 30 °C. The fluorescence detector attached to the HPLC was set at excitation and emission wavelengths of 283 and 350 nm, respectively. Standards (0.2 μL) were run and analyzed under the same conditions.

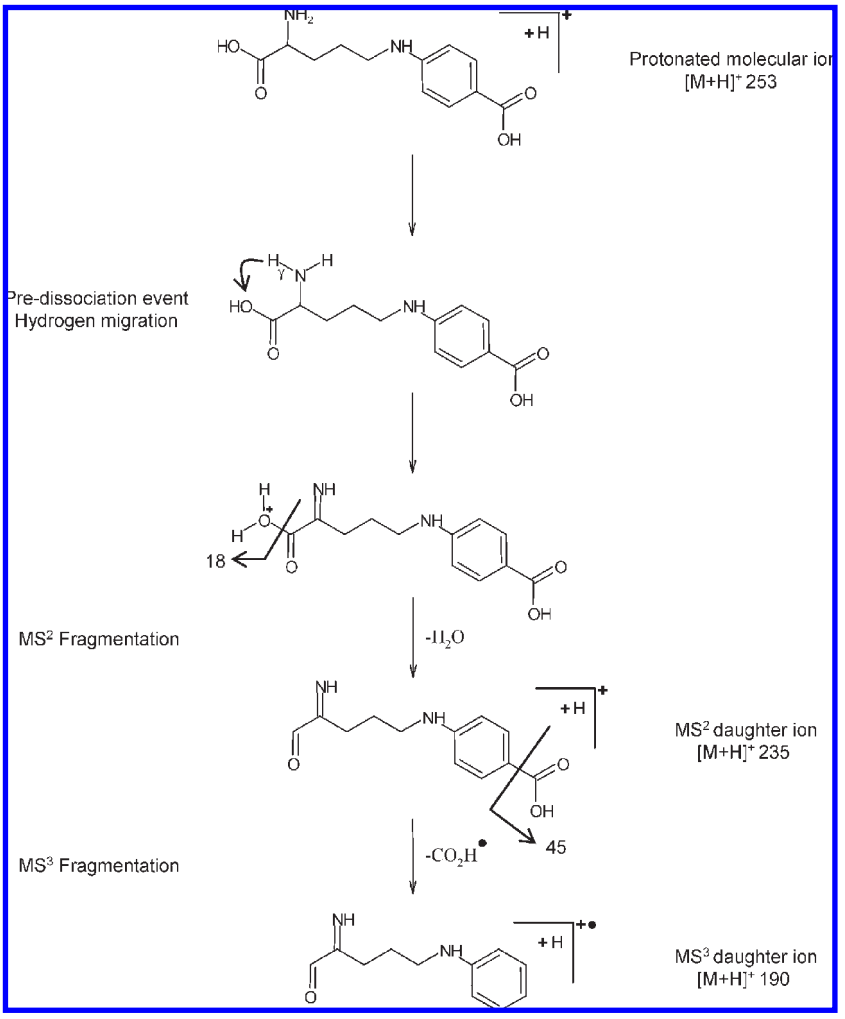

Figure 4. Fragmentation pattern proposed for GGS.

Mass spectrometric analysis was carried out on an Esquire-LC quadrupole ion trap mass spectrometer equipped with an ESI interface (Bruker Daltonics, Bremen, Germany) and LC−MSD Trap software, version 5.2 (Bruker Daltonics). MS^n methods were carried out for identification purposes and optimized for AAS−ABA and GGS−ABA. Capillary voltage was 3500 V; capillary exit offset was 25 V; skimmer potential was 15 V; and the trap drive value was 36. Conventional ESI−MS data were recorded using a scan range of m/z 100−700. Nebulizer (nitrogen) pressure was 50 psi; dry gas (nitrogen) flow was 8 L/min; and dry temperature was 300 °C. MS^2 and MS^3 were subsequently carried out for molecules of interest and recorded using helium (99.996%) as the collision gas. The peaks corresponding to the protonated AAS−ABA and GGS−ABA were manually integrated from extracted ion chromatograms (EIC), and the resulting areas were used as arbitrary indicators of the abundance of both semialdehydes. Results are expressed as arbitrary area units (AU).

Data Analysis. All types of suspensions were made in triplicate in three independent experiments, and all analyses were made in duplicate ($n = 6$). Data obtained from instrumental analysis were used as variables and computed in an analysis of variance using SPSS for Windows version 6.1 to detect differences between protein suspensions for the formation of protein hydrazones and protein semialdehydes. Student t tests were performed to compare means derived from suspensions with a different protein source. Pearson correlations were also calculated to establish relationships between parameters. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Detection and Fragmentation Patterns of Derivatized Protein Semialdehydes. Synthesized standards, GGS−ABA and AAS−ABA, appeared in FLD chromatograms as two main peaks at retention times of 15.5 and 29.7 min, respectively. The suitability of using ABA as a derivatization agent for the detection of semialdehydes by LC−FLD was reported in a previous paper (*19*) and confirmed in the present study. Other derivatization procedures have been described as a preliminary step for the chromatographic analysis of AAS and GGS (*14*, *15*). The procedure employed in the present study provides some advantages, as reported by Akagawa et al (*17*). The great stability of ABA-derivatized semialdehydes against acidic hydrolysis and cold storage was observed in a preliminary study (*20*) and is also in agreement with previous reports (*14*, *21*). The identification of both compounds was confirmed by LC−MS. Protonated molecules $[M + H]^+$ for GGS−ABA (m/z 253) (**Figure 3**) and AAS−ABA (m/z 267) (Figure **5**), formed as a result of the electrospray ionization, were found in MS chromatograms at the aforementioned retention times (**Figures 3** and **5**). Protonated molecules of interest were selected and fragmented at multiple stages (MS^n). Fragmentation of $[GGS-ABA + H]^+$ at MS^2 (**Figure 3B**) led to the formation of a main daughter molecule with m/z 235 caused by a neutral loss of H_2O. A minor daughter molecule with m/z 116 was also formed, which involved the loss of a fragment with a mass of 137 that might correspond to the derivatization agent

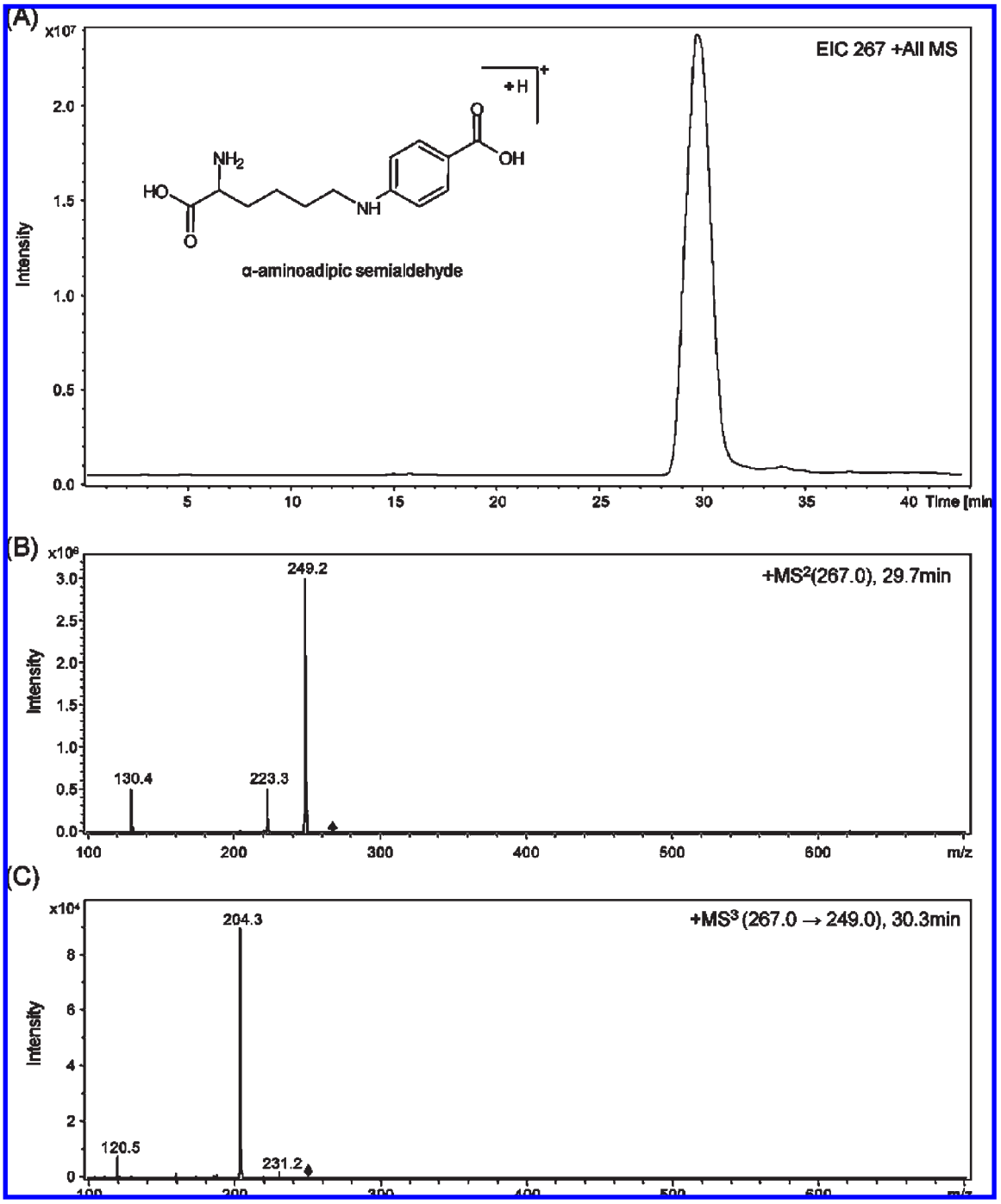

Figure 5. (A) EIC of *m/z* 267 α-aminoadipic semialdehyde and mass spectra after subsequent (B) MS^2 and (C) MS^3 fragmentations.

(ABA). The main daughter molecule from MS^2 $[GGS-ABA + H - H_2O]^+$ was further fragmented at MS^3 (**Figure 3C**) mainly into $[GGS-ABA + H - H_2O - 45]^+$ with *m/z* 190. Fragmentation of AAS−ABA (**Figure 5A**) followed a similar pattern. MS^2 of AAS−ABA (**Figure 5B**) also involved the dehydratation of the protonated molecule to form a main daughter molecule $[AAS-ABA + H - H_2O]^+$ with *m/z* of 249. In this case, other minor daughter molecules derived from the cleavages of ABA (137 mass units) and CO_2 (44 mass units) were also found. A neutral loss of 45 was also the main fragmentation route for $[AAS-ABA + H - H_2O]^+$ at MS^3, yielding a main molecule $[AAS-ABA + H - H_2O - 45]^+$ with *m/z* of 204. The similar chemical structures and the presence of ABA attached to the carbonyl group explain that both semialdehydes showed equivalent fragmentation patterns. The fragmentation patterns of $[GGS-ABA + H]^+$ and $[AAS-ABA + H]^+$ and proposals for cleavages and molecular structures are shown in **Figures 4** and **6**, respectively. Multistage tandem MS has never been applied before for the analysis of ABA-derivatized GGS and AAS; therefore, no parallel comparison to previous results is applicable. According to our proposal, the loss of H_2O from the protonated molecule in MS^2 involves the migration of a γ-hydrogen from the amino group to the carboxylic oxygen group, which triggers the subsequent fragmentation. McLafferty-type rearrangement reactions are common under soft ionization conditions, such as electrospray, leading to the abstraction of a γ-hydrogen by a group with high proton affinity, such as −OH, finally yielding H_2O from alcohols, carbonyls, and carboxylic acids (*22*, *23*). In fact,

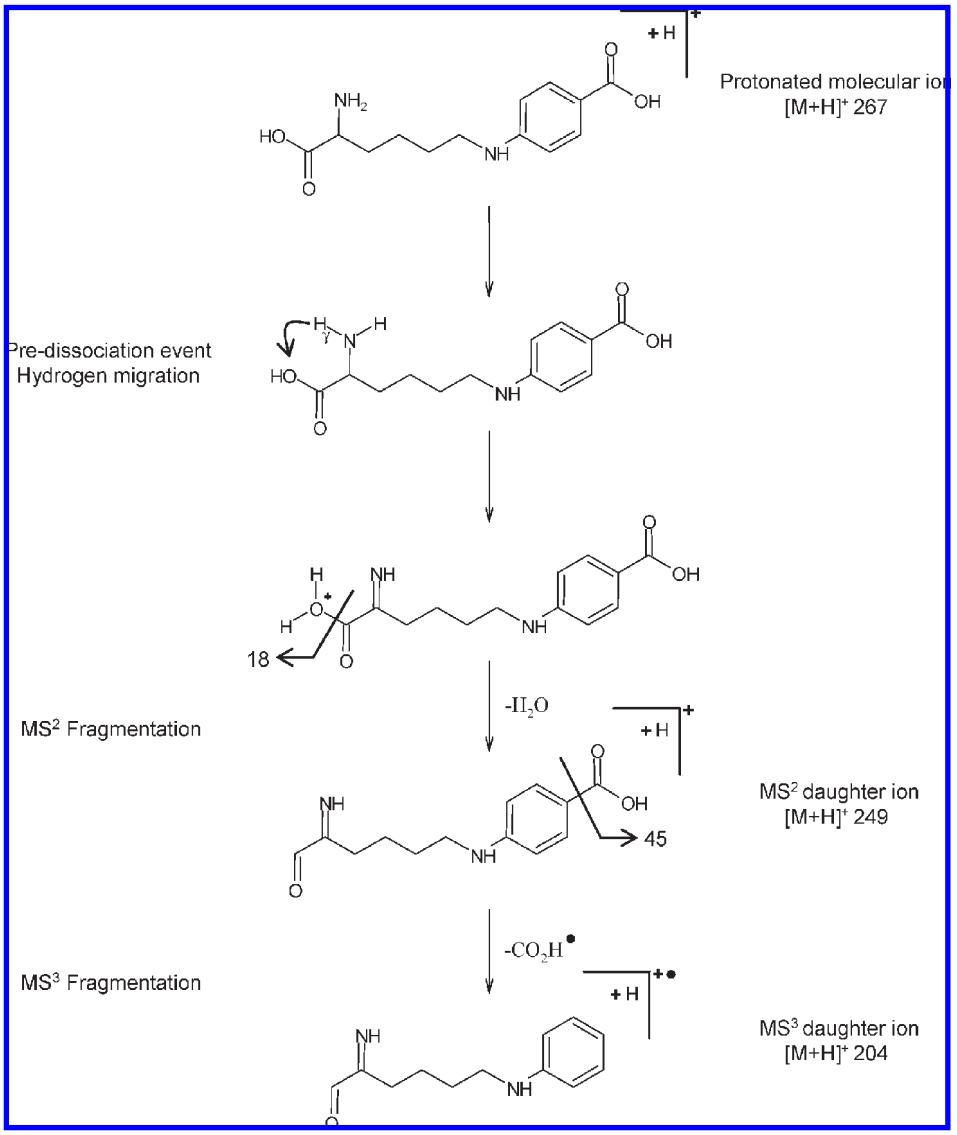

Figure 6. Fragmentation pattern proposed for AAS.

rearrangement events have been previously described for amino acids and peptides subjected to MS^n analyses (*24*, *25*). At MS^3, both compounds were preferentially fragmented to yield a loss of 45 and form a protonated molecule with even mass (*m*/*z* 190 for GGS and *m*/*z* 204 for AAS). Considering that the original protonated molecules contained an even number of N and, consequently, uneven masses, the appearance at MS^3 of a daughter molecule with even mass necessarily involves either the loss of a neutral loss containing one atom of N or the formation of a radical cation. Liu et al. (*26*) recently reported that certain dinitrophenylated (DNP) amino acids are fragmented to yield $[M + H - 45]^+$ molecules. Although the loss of a $COOH^\bullet$ radical was originally considered, these authors concluded that the loss of $(CH_3)_2{-}NH$ (45 Da) was more likely. In the present study, however, the loss of that neutral fragment is not compatible with the structure of the mother molecules because both $-NH_2$ groups are located within the hydrocarbon structure. Neither a simple cleavage nor a rearrangement would reasonably explain the loss of such a neutral fragment. Therefore, the loss of 45 Da may correspond to the cleavage of the aromatic carboxylic acid. The unpaired electron in the resulting radical cation could be stabilized by delocalization mechanisms in the aromatic ring. Although it is highly endothermic and, thus, generally improbable, the production of a radical cation from an even-electron molecule containing aromatic carboxylic acids has been described (*27*). In fact, Dhananjeyan et al. (*28*) have recently reported the formation of intermediate radical cations during the fragmentation of ABA and ABA-derivatized molecules using LC−multistage tandem MS.

Fragmentation studies carried out on amino acids and other related compounds showed that neutral losses of 18 (H_2O) and 45 ($COOH^\bullet$) are not particularly common (*29*, *30*). Simple cleavages leading to losses of NH_3 and CO_2 from amino and carboxylic groups, respectively, are the most likely fragmentation routes for nonmodified and nonderivatized amino acids. Therefore, the oxidative modification of the amino acids and the following derivatization process with ABA considerably alters the fragmentation pattern of the original amino acids, leading to distinctive patterns that could be employed as fingerprints for GGS and AAS when analyzed under the conditions of the present study.

Semialdehydes in Oxidized Food Proteins. GGS−ABA and AAS−ABA were found in FLD and MS chromatograms corresponding to samples from oxidized food proteins. Identification of both semialdehydes was confirmed because compounds from protein samples had identical

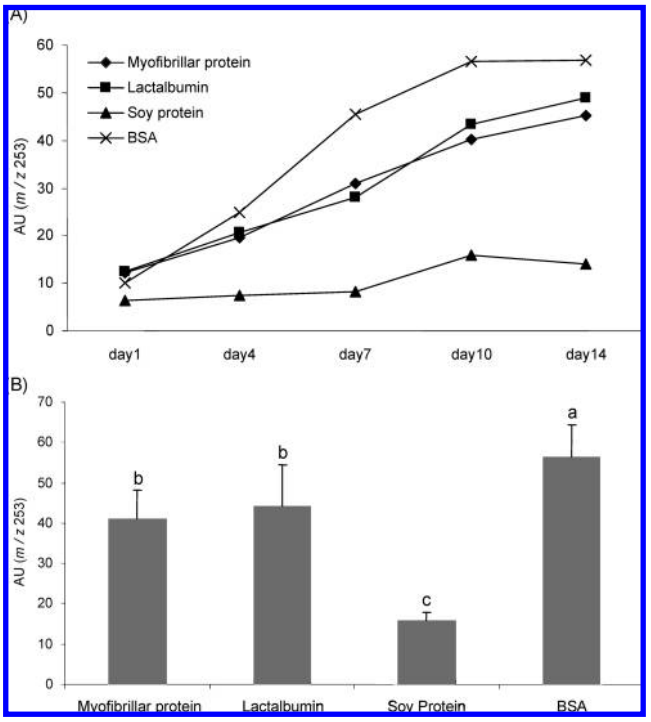

Figure 7. (A) Evolution of GGS−ABA during *in vitro* oxidation of proteins for 14 days and (B) comparison between groups at day 10 (means ± standard deviation).

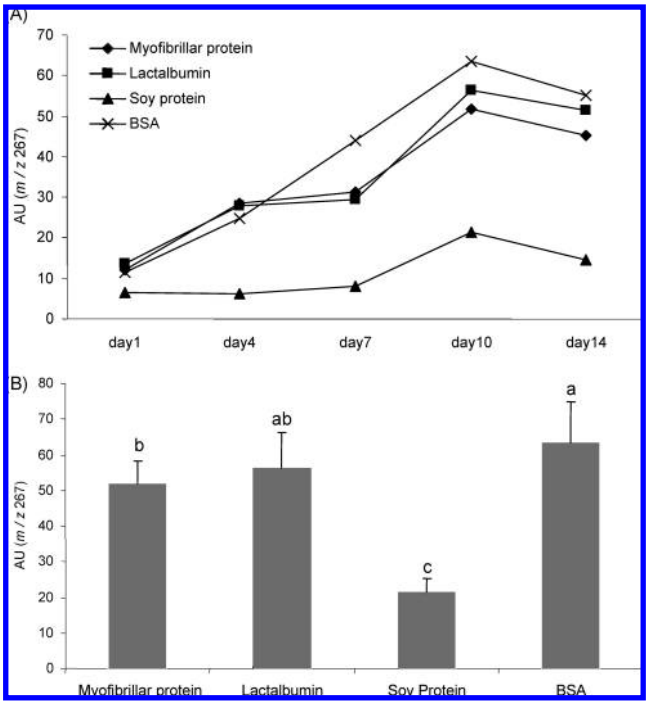

Figure 8. (A) Evolution of AAS−ABA during *in vitro* oxidation of proteins for 14 days and (B) comparison between groups at day 10 (means ± standard deviation).

retention times, mass spectra, and fragmentation patterns as standard compounds. The MS signal for both semialdehydes increased in all native proteins analyzed, namely, BSA, myofibrillar proteins, α-lactalbumin, and soy protein, throughout the oxidation assay (**Figures 7** and **8**), indicating that an accumulation of semialdehydes occurred in suspensions as a result of the oxidative reactions. The present results highlight that lysine, proline, and/or arginine from BSA and

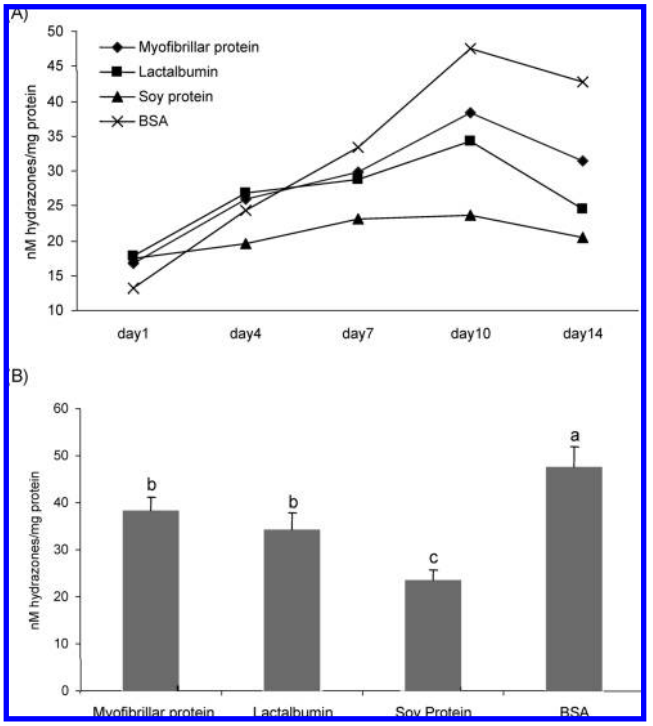

Figure 9. (A) Evolution of the total amount of protein carbonyls during *in vitro* oxidation of proteins for 14 days and (B) comparison between groups at day 10 (means ± standard deviation).

diverse food proteins are oxidized in the presence of Fe and H_2O_2 to yield GGS and AAS. In the present experiments, the reaction might be initiated by $OOH^{\bullet}$ radicals derived from the Fenton reaction between Fe^{3+} and H_2O_2. The oxidative deamination from the intermediate radical molecule occurs in the presence of Fe^{3+}, yielding the semialdehyde (**Figure 1**). The resulting Fe^{2+} could propagate the oxidative degradation to new amino acid residues. This metal would be involved in additional Fenton reactions with H_2O_2 for the formation of hydroxyl radicals that initiate further reactions. The oxidation of BSA and food proteins during the assay was confirmed by the results obtained from the DNPH method. The evolution of the total amount of protein carbonyls in protein suspensions under the pro-oxidant conditions (**Figure 9**) is consistent with the trends observed for GGS and AAS. Both semialdehydes are known to be the main protein carbonyls in biological samples and comprise around 90% of total carbonyl compounds in BSA subjected to metal catalyzed *in vitro* oxidation (*15*). The results obtained in this study, including the significant correlations found between DNPH measurements and GGS (0.79; $p < 0.01$) and AAS (0.73; $p < 0.01$), contribute to support the fact that both semialdehydes could be highly representative of the total amount of carbonyl compounds formed during metal-catalyzed oxidation of food proteins. Another portion of the protein carbonyls in these samples might be made by carbonyls derived from the oxidation of the side chains of amino acids other than arginine, proline, and lysine.

In general, GGS increased constantly over time to reach the highest levels at day 14 (**Figure 7**), whereas the amount AAS increased until day 10, showing by the end of the storage a slight decrease (**Figure 8**). This observation was also found for the evolution of total carbonyl compounds (**Figure 9**). This result suggests that AAS might not be a final oxidation product because this semialdehyde could be involved in further reactions. In fact, Requena et al. (*15*)

reported that protein semialdehydes might react with nonmodified amino acid residues to form cross-links and Schiff bases. Whereas in previous studies carried out on BSA and other plasma and liver proteins, GGS and AAS were formed in oxidized proteins to similar extents (*15*, *31*); in the present study, AAS formation was slightly more intense than the formation of GGS. The differences in the yield of both oxidation products might respond to the relative amount of the particular amino acids from which they are formed in the proteins and the susceptibility of these amino acids to be oxidized. The slight differences reported in this study could reflect the amino acid composition of proteins because lysine (AAS source) is more abundant in most analyzed proteins than proline and arginine together (GGS sources) (*32*−*35*).

Proteins analyzed in the present study suffered the oxidative reactions to significantly different extents. At day 10, when the highest oxidation rates were recorded, BSA had the highest amount of GGS and AAS, followed by myofibrillar proteins and α-lactalbumin, while soy proteins contained significantly lower amounts of both semialdehydes. The overall susceptibility of food proteins to oxidative reactions as assessed by the DNPH method is in good agreement with GGS and AAS results. Requena et al. (*15*) reported differences in the intrinsic susceptibility of proteins (BSA, lysozyme, and other pancreas and liver enzymes) to undergo metal-catalyzed oxidation and yield GGS and AAS. Measuring oxidation in whey proteins by means of tryptophan loss, Viljanen et al. (*3*) also reported differences in susceptibility to oxidation between BSA and α-lactalbumin. Using fluorescence spectroscopy, we (*36*) previously reported that myofibrillar proteins are more resistant to oxidative reactions than BSA, which is in good agreement with the present results. The stability of food proteins against oxidative reactions should be attributed to a variety of exogenous and endogenous factors, including, among the latter, the tertiary structure of the proteins, their size, their amino acid composition and sequence, and the distribution of amino acids on the protein structure (*1*, *34*, *36*). The native structure of proteins was found to play a major role in the susceptibility of myofibrillar proteins and BSA to oxidation (*36*). In solution, myofibrillar proteins form tightly packed structures that are less accessible than small globular proteins to oxygen and other pro-oxidants, such as metals and radicals, hindering initiation of oxidation. Moreover, amino acids with reactive side chains (−SH, −OH, and $-NH_2$) are particularly sensible to undergo oxidative reactions, and therefore, proteins enriched in those amino acids may show a higher overall susceptibility to oxidation. Among all essential amino acids, cysteine has been highlighted as the most sensible amino acid residue and the first in being oxidized (*1*). It is generally known that proteins from animal sources (myofibrillar and whey proteins) have higher amounts of the GGS and AAS sources (proline, arginine, and lysine) as well as sulfur-containing amino acids (cysteine and methionine) compared to proteins from vegetable sources, such as soy proteins (*32*, *33*, *35*). Among proteins from animal sources, BSA contains considerably higher levels of cysteine (∼6/100 g) than myofibrillar proteins (∼1.2/100 g) and α-lactalbumin (<1/100 g). The early oxidation of cysteine and other sensible amino acids in animal food proteins and, particularly, in BSA could have increased the overall instability of these proteins, enhancing the yield of the semialdehydes and other protein oxidation products. Some other factors, such as the exposure of sensible amino acid side chains to the water surface of food proteins as well as the presence of traces of phenolic compounds in the soy protein isolate, could help to explain the results obtained in the present study.

In summary, the present results show that GGS and AAS are carbonyls derived from the oxidation of food proteins and can be accurately detected using LC−ESI−MS. The results obtained in the present study emphasize the interest of using both semialdehydes as protein oxidation indicators in meat and dairy products. In comparison to procedures commonly used as routine methods for protein oxidation assessment (DNPH method), the analysis of GGS and AAS in food systems provides precise information about chemical structures and oxidation pathways. The methodology proposed in the present study could be greatly useful to understand the precise mechanisms involved in food protein oxidation and shed light on the fate of oxidizing amino acids during food processing and storage.

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