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Comparison of graphite furnace and hydride generation atomic absorption spectrometry for the determination of selenium status in chicken meat

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Abstract

Four different procedures for the determination of selenium in chicken meat by atomic absorption spectrometry were investigated. They consisted on conventional ambient pressure acid digestion carried out before and after sample drying, associated or not with fat extraction. For all procedures muscle and skin were analyzed separately. Drying was carried out in a conventional oven at 65 °C for 24 h. For fat extraction different solvents and solvent mixtures were investigated considering both extraction yield and sample adequacy for further AAS measurement. Acid digestions were carried out with mixtures of HNO_3 and $HClO_4$. After digestion, selenium was measured either by Hydride Generation (HGAAS) or by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). For the reduction of Se(VI) prior to the HGAAS determination, 8% (w/v) NaBr, 6 mol/l HCl (both with and without sulfamic acid), as well as UV radiation were investigated. Tests with spiked samples have shown that either UV radiation (pH 8) or NaBr/sulfamic acid presented good recoveries. In this way the HGAAS determination of selenium in tissue was carried out without interference whereas for the fatty fraction the results were satisfactory only if GFAAS was used. The results showed that drying the sample and extracting the fat prior to digestion is advantageous once the amount of acid necessary can be significantly reduced. The precision, expressed as relative standard deviation, was about 6.5% and 0.8% for GFAAS and HGAAS measurements, respectively. The limits of detection for HGAAS and GFAAS, based on three times the standard deviation of the blanks were 1 μg/l and 0.6 μg/l, respectively. The results have shown that in chicken meat 59% of the selenium is found in the muscle tissue while the skin responds for 41%.

Keywords: Selenium status; Chicken meat; Sample digestion; Comparison HGAAS and GFAAS

1. Introduction

Selenium is an essential micronutrient for humans required in very small amounts for the basic functions of life (Sager, 1994). It is a component of an enzyme, glutathione peroxidase, which is responsible for the removal of hydrogen peroxide from cells. Increasing experimental evidence has suggested that selenium possesses antineoplastic properties since studies have demonstrated that dietary selenium supplementation can inhibit chemically-induced tumors in rats (Fishbein, 1991). Most studies employ inorganic forms of selenium (selenite, selenate, or selenium dioxide) added to the diet or drinking water, but there is some disagreement in relation to the effectiveness of the various forms of selenium as cancer-preventive agent: while in one study the inorganic forms were found to be more effective, in another, selenite and selenomethionine were found to be equally efficacious (Fishbein, 1991).

On the other hand, selenium can also be toxic to most organisms at higher concentrations (Sager, 1994). Toxicity

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as well as deficiency syndromes are both known, and the optimal range for beneficial effects seems to be narrow. The adequate daily dietary selenium intake ranges from 50 to 200 μg, with an average value of 55 μg for adult humans (Food & Nutrition Board, 2000).

Food is the main source of selenium for man, but its uptake depends on its chemical form. Various plants growing on selenium-rich soil absorb and accumulate this element (Pickering, Prince, Salt, & George, 2000). It is found in plants as organic compounds, including amino acids. Selenomethionine has been shown to be the predominant form of selenium in wheat, soybeans and selenium-enriched yeast (Lobinski, Edmonds, Suzuki, & Uden, 2000; Stadlober, Sager, & Irgolic, 2001). Selenium concentration in individual food products is influenced by its origin and the way of processing. Dietary intake of selenium occurs mainly via vegetable foods and certain seafood (Fishbein, 1991). Moreover, there is a widespread use of selenium supplementation by enriching commercial foodstuffs for farmed animals with sodium selenite or selenium-enriched yeast. Meat, meat products and dairy products are very important selenium sources in the human diet.

Selenium can be either determined by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) or Hydride Generation Absorption Spectrometry (HGAAS). The choice of these techniques depends on sample volume availability, the matrix composition, and selenium species present in the sample. While HGAAS response is strongly dependent on the selenium form, GFAAS is adequate for the measurements of both organic and inorganic species (Welz, Melcher, & Schlemmer, 1983), providing that adequate conditions such as the use of the proper modifiers and Stabilized Temperature Platform Furnace (STPF) conditions are taken into account. Acids normally used for sample digestion are also a matter of consideration: nitric, sulfuric, and perchloric acids may interfere either in the HGAAS (Cutter, 1983; Guo, Sturgeon, Mester, & Gardner, 2003; Welz & Sperling, 1999) or in the GFAAS (Aller, 1996; Ni, He, & Han, 1994) measurements. There are several reports about interferences in the determination of selenium by both techniques (D'Ullivio, Gianfranceschi, Lampugnani, & Zamboni, 2002; Fernandez & Beaty, 1984; LeBihlan, Cabon, & Elleouet, 1992; Radziuk & Thomasen, 1992; Welz, Bozsai, Sperling, & Radziuk, 1992) but only a few comparing HG with GF regarding AAS selenium determination. Welz and Schubert-Jacobs (1991) compared figures such as characteristic mass and concentration, sample volume, and also the possibility of carrying out the HG in a batch and flow injection (FI) system.

Lambert and Turoczy (2000) compared digestion methods for the determination of selenium in fish tissue. The digestion techniques included wet and dry ashing, oxygen ashing, UV irradiation and high pressure. The authors found out that ashing followed by high-pressure decomposition and HGAAS determination was the only satisfactory combination. They concluded that incomplete destruction of organic matter and selenium losses by volatilization were the causes for the failure of the other procedures.

The HGAAS technique is free from spectral interferences if suitable equipments are used. It is well known, however, that a number of transition metals, mainly those of Groups 8 and 11, can cause several signal depression, and selenium is one of the elements most affected by these interferences (Welz & Melcher, 1984). However, these interferences may be minimized by increasing the acidity of the sample solution or by the addition of masking agents.

The present study aimed at evaluating the adequacy of different sample pre-treatment and detection techniques, namely, hydride generation and graphite furnace atomic absorption spectrometry for the determination of selenium status in chicken meat.

2. Experimental

2.1. Apparatus and AAS measurement conditions

A SpectrAA 200 atomic absorption spectrometer (Varian, Australia), equipped with a VGA 77 system for continuous flow hydride generation and a GTA 100 atomizer for electrothermal atomization were used. A selenium hollow cathode lamp (Varian, Melbourne, Australia) was employed as radiation source. The operating conditions of the spectrometer for the determination of selenium were: wavelength 196.0 nm; bandwidth 1.0 nm; lamp current 10 mA. Hydride generation was conducted carrying reductant, 1% (m/v) sodium tetrahydroborate (III) in 0.1 mol/l NaOH, and acid, 6.0 mol/l HCl, at a flow rate of 1.0 ml/min, and sample at a flow rate of 8.0 ml/min. Atomization temperature 850 °C. Graphite furnace atomization was conducted using 10 μl sample volume and 5 μl chemical modifier volume. The temperature program was: drying 5 s at 85 °C + 30 s at 95 °C + 10 s at 120 °C; pyrolysis 35 s at 1100 °C + 17.6 s at 1100 °C with reduced air flow (0.5 l/min); atomization 2.8 s at 2600 °C; cleaning 2 s at 2600 °C.

An airflow oven (Ehret, Emmendigen, Germany), a 705 UV Digestor with 500 W Hg lamp (Metrohm, Herisau, Austria), a Berghof BSB 939-IR sub-boiling distillation apparatus (Berghof, Eningen, Germany) and a Digimed D-20 pH meter (Digimed, São Paulo, Brazil) were used.

2.2. Reagents

Deionized and further purified Milli-Q high purity water device (Millipore, Bedford, USA) was used throughout. The sodium selenate standard solution containing 1000 mg/l was prepared from the respective salt (Fluka, Buchs, Switzerland). The selenite standard solution was prepared by diluting a Titrisol ampoule (Merck, Darmstadt, Germany) according to the manufacturer's recommendation. Se–methionine standard solution (1 g/l)

(Acrós Organics, Morris Plains, USA) was prepared by dissolving the amino acid in water. Se–cystine standard solution (1 g/l) (Acrós Organics, Morris Plains, USA) was prepared dissolving the amino acid in 0.1 mol/l HCl. Working standard solutions were prepared daily diluting the standards in 6 mol/l HCl for HGAAS and in 0.1 mol/l HNO_3 for the GFAAS measurements, respectively.

Freshly prepared solution of sodium tetrahydroborate (III) (Merck, Darmstadt, Germany) was used as a 1% (w/v) solution in 0.1 mol/l NaOH. HCl (36% (m/m), 1.19 g/ml) and HNO_3 (63% (m/m), 1.14 g/ml) (Merck, Darmstadt, Germany) were further purified by sub-boiling distillation. All other reagents used were of analytical-reagent grade.

A nickel solution containing 2.5 g/l Ni, used as chemical modifier, was prepared by dissolving an appropriated amount of $Ni(NO_3)_2$ in 0.1 mol/l HNO_3.

2.3. Contamination control

To avoid contamination, all laboratory ware, plastic (polyethylene) and glass, was immersed for at least 48 h in a 10 % HNO_3 in ethanol (v/v) mixture and washed with Milli-Q purified water shortly before the use.

To avoid contamination from the air, all steps in the sample and reagents preparation were carried out in a class 100 clean bench.

2.4. Samples

Chicken breast was obtained in the local market. Skin and muscle were separated from each other before sample treatment. The muscle was processed in a food processor and well mixed. One part of the sample was directly submitted to the acid digestion procedure (see below). Portions of the other part were weighed (0.5 g), transferred to a glass container, covered with a piece of muslin (to avoid dust deposition on the sample), and oven dried at 65 °C for 24 h. The skin was cut in small pieces, well mixed, and, like the muscle, weighed and oven dried.

2.5. Digestion procedures

Sample digestion was carried out in glass tubes for sample digestion (Merck, Darmstadt, Germany) with screw caps and 22 ml capacity.

Direct acid digestion: To approximately 0.5 g of meat or skin 5 ml of concentrated nitric acid was added. The mixture was let overnight, heated in a water bath at 100 °C for 3 h; 5 ml of concentrated perchloric acid were then added, and this mixture was heated for four more hours. The volume was made up to 20 ml with water, and selenium was determined in this solution by GFAAS. These samples were further treated for the pre-reduction of Se (VI) for HGAAS measurements as described below.

Acid digestion of the dried samples: Meat and skin samples (0.5 g) was made into small fragments and oven dried as described above. Three milliliters of concentrated nitric acid was added and this mixture was let overnight. Two milliliters of concentrated perchloric acid were then added and after heating in a water bath at 100 °C for 4 h, the volume was made up to 20 ml with water. Selenium was directly determined in this solution by GFAAS and, after pre-reduction by HGAAS.

Acid digestion of the dried samples after fat extraction: Meat or skin (0.5 g) was let to dry as described above. Dried samples were made into small fragments and 4 ml of a 2:3 mixture of methanol/dichloromethane were added. The samples were shaken overnight and the remaining solid residue was withdrawn, let to dry, and processed as described above for acid digestion of dried samples but using only 2 ml nitric acid and 1 ml perchloric acid. The organic fraction was dried and the residue further dissolved with 5 ml of 4:1 methanol/dichloromethane. The selenium content of this fraction was measured by GFAAS using selenium standards prepared in the same methanol/dichloromethane medium.

2.6. Selenium measurement by GFAAS

Nickel, copper, palladium and magnesium (nitrate salts, Merck) in concentration of 1 g/l and 2.5 g/l were tested as chemical modifiers (Gayon, Uria, & Sanz-Medel, 1997) for the determination of selenium either in aqueous or in organic mediums, as well as in the presence of the matrix constituents after sample digestion. Individual standard solutions (20 μg/l) and also samples spiked with the same amount of all selenium species were analyzed by GFAAS using these modifiers, following the conditions described in Section 2.1.

After choosing the best modifier for the samples (2.5 g/l Ni), temperature programs for GFAAS measurements were optimized for inorganic and organic selenium forms using aqueous and methanolic standards. Pyrolysis and atomization temperatures were investigated for samples spiked with each species separately. Temperatures from 900 up to 1500 °C were tested for pyrolysis and from 2400 up to 2700 °C for atomization. All measurements were carried out using a 2.5 g/l Ni solution as modifier.

2.7. Pre-reduction treatments

UV treatment: One milliliter of the digested sample was diluted to 8 ml with water, the pH was adjusted to 8 with 1 mol/l NaOH and the sample was submitted to UV irradiation in time intervals from 1 to 5 h. Every hour 50 μl of 30% (m/v) H_2O_2 were added to the sample. After finishing the reduction step, the volume was made up to 10 ml and the sample was analyzed by HGAAS.

HCl treatment: To 2 ml of the digested sample, 2 ml of concentrated HCl were added. The mixture was heated in a water bath at 100 °C for 15 min, the volume made up to 10 ml and selenium measured by HGAAS.

HCl + sulfamic acid treatment: To 2 ml of the digested sample 2 ml of concentrated HCl and 2 ml of 10% (w/v) sulfamic acid solution were added. The sample was heated in a water bath at 100 °C for 15 min, the volume made up to 10 ml and selenium measured by HGAAS.

NaBr + sulfamic acid treatment: To 2 ml of the digested sample 0.32 g NaBr and 2 ml of 10% (m/v) sulfamic acid solution were added. After heating until the sample becomes colorless, the volume was completed to 10 ml and the analysis carried out by HGAAS.

2.8. Recovery experiments

Spiking was made with a mixture of selenium compounds. A pool containing 10 mg/l of each species, selenite, selenate, selenocystine, and selenomethionine, was prepared, and 100 μl of this solution were added to the samples. Two different recovery experiments were carried out. One consisted of spiking the samples after the digestion procedure and performing the selenium measurement by either HGAAS, to check the pre-reduction step, or by GFAAS to check the instrumental performance. The other one was the addition of the selenium pool to the samples before each treatment to check the whole procedures' performance.

Recovery experiments were also carried out in the presence of copper, iron, chromium and zinc for the HGAAS measurement. A 10 μg/l Se (IV) solution containing Zn and Fe (10 mg/l) as well as Cu and Cr (1 mg/l) was prepared and analyzed by HGAAS at the conditions described above. Another solution containing the same metal concentrations and 10 μg/l of each selenium species was also prepared. To 5 ml of this solution the acids for sample digestion were added, the pre-reduction step with NaBr/sulfamic acid was carried out and the selenium content measured by HGAAS.

2.9. Selenium determination in chicken samples

Three samples of chicken breast from local market were analyzed following the selected procedures. In brief, muscle and skin were treated for fat extraction, and selenium in muscle and skin residues was determined by HGAAS after pre-reduction with NaBr/sulfamic acid treatment and selenium in fat (organic extracts) was measured by GFAAS.

3. Results and discussion

3.1. GFAAS measurements

The conditions for selenium measurement by GFAAS in both aqueous and organic medium were tested, since one goal of this work was to verify whether selenium is accumulated in fat or not.

Nickel, copper, palladium and magnesium were tested as chemical modifiers for the determination of selenium by GFAAS in muscle and skin residues as well as in organic extracts. These preliminary results showed that Ni promoted the best response for both aqueous and organic mediums. However, when the measurements were done in the presence of the acids used for sample digestion (muscle and skin residues), the modifier was not able to reduce interferences.

Pyrolysis and atomization curves are shown in Fig. 1. There is practically no difference in using aqueous or organic mediums (4:1methanol/dichloromethane), since the curves are similar for the same temperature program.

To test the accuracy of the measurement in the fatty fraction, recovery experiments were carried out by spiking the organic extract with organic and inorganic selenium forms separately. The results showed that it is possible to measure both forms by GFAAS since recoveries between 94% and 99% were obtained.

3.2. Sample decomposition and pre-reduction steps

All steps of each digestion procedure are summarized in Fig. 2.

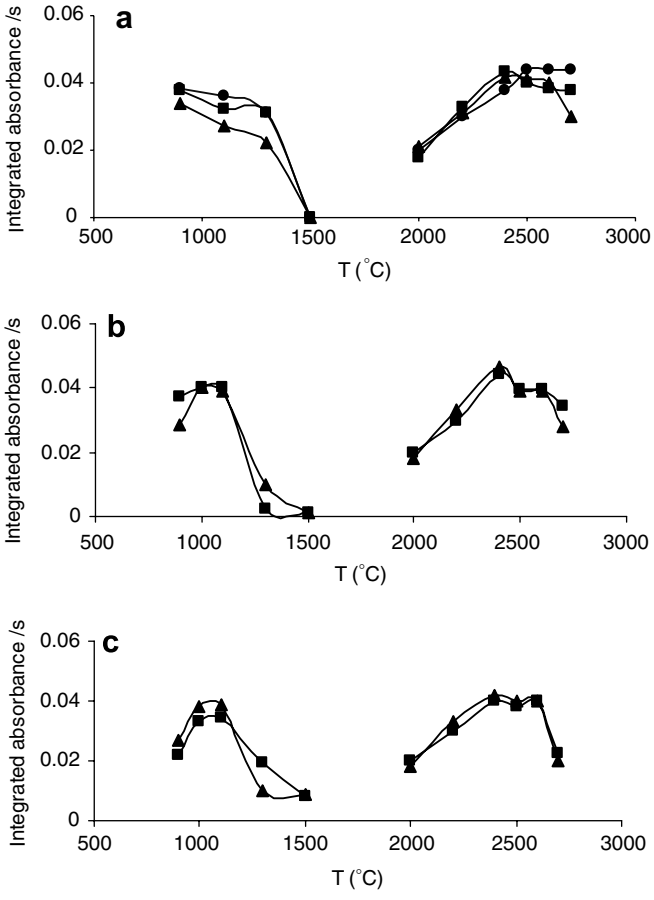

Fig. 1. Pirolysis and atomization curves for Se IV (●), SeM (■) and SeC (▲) in water (a) and in organic extracts from muscle (b) and skin (c). Atomization temperature for pyrolysis curves: 2600 °C; pyrolysis temperature for atomization curves: 1100 °C

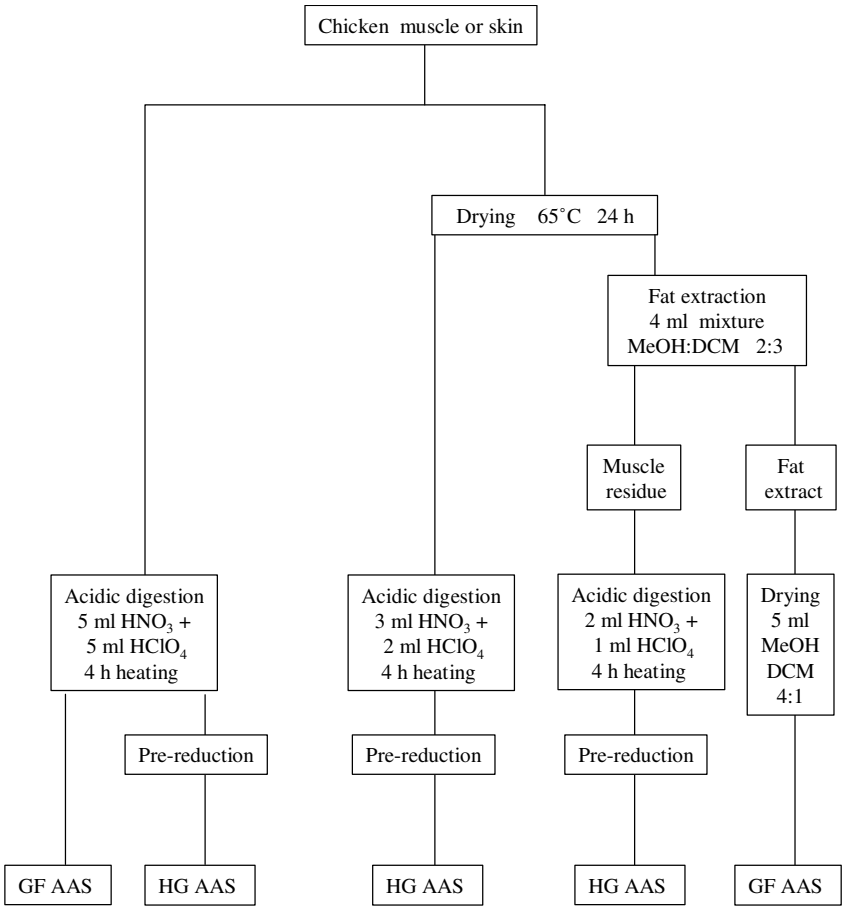

Fig. 2. Summary of all digestion procedures.

As it can be seen in Fig. 2, while 10 ml of acids (HNO_3/$HClO_4$) were necessary for the decomposition of 0.5 g of fresh meat (muscle or skin), 5 ml were necessary for the same sample amount after drying, and only 3 ml after fat extraction. However, even all treatments giving clear solutions, direct selenium GFAAS measurements in these digested samples was not possible due to too high background signals. Concerning the HGAAS determination, Table 1 shows that the best results were obtained with UV irradiation as well as with NaBr in the presence of sulfamic acid (the low recoveries of samples spiked after digestion is commented below). Despite the role of sulfamic acid in this step is much more related to the elimination of nitrogen oxides generated by the decomposition of nitric acid than to the reduction of Se(VI), it seemed to have some influence in the reduction process (Brown et al., 1981). The 64–65% recovery from samples spiked after the digestion procedure corresponds to the inorganic forms only, since the mixture NaBr/sulfamic acid is not able to convert organoselenium compounds into inorganic ones.

Table 1 also shows that the pre-reduction with HCl alone or along with sulfamic acid did not give good results. Poor recoveries on samples spiked after sample digestion might be associated with non-mineralization of organoselenium compounds, and poor recoveries on samples spiked before digestion with incomplete reduction. Spiking samples only with inorganic selenium species (before and after digestion) led to low recoveries as well. These results confirm that for samples spiked before digestion there is an incomplete reduction of selenate to selenite.

UV irradiation also showed good results, however, it was necessary to irradiate the sample for at least 4 h, even

Table 1
Comparison of the different pre-reduction treatments for samples spiked before and after the digestion procedure

Treatment	Tissue	HGAAS	
		Spiking before sample digestion[a] (%)	Spiking after sample digestion (%)
UV irradiation (4 h)	Muscle	96	97
	Skin	92	89
HCl	Muscle	62	35
	Skin	62	39
Sulfamic acid + HCl	Muscle	82	47
	Skin	70	55
Sulfamic acid + NaBr	Muscle	96	64
	Skin	99	65

Digestion procedure carried out after drying and extracting fat from muscle and skin tissues. Measurements carried out by HGAAS.
[a] Corresponds to the sum: Se tissue + Se fat (see Fig. 3b).

in the presence of H_2O_2. Shorter irradiation times (up to 3 h) were not enough to recover all selenium from spiked samples, probably due to the difficulty of reducing the organoselenium species. In Fig. 3a it is possible to see that poor recoveries can be associated with a low conversion rate of the species due to absence of H_2O_2 and insufficient irradiation time. Even the acid treatment itself did not help to shorten the irradiation time.

Incentivated by the good results obtained with UV irradiation and sulfamic acid/NaBr mixture, another experiment was proceeded: the whole procedure was carried out avoiding the use of perchloric acid. The results showed, however, that perchloric acid is a necessary adjuvant for sample decomposition. Poor selenium recovery could be associated not only to incomplete sample digestion but also to the difficulty of mineralizing organoselenium compounds by action of nitric acid alone at ambient pressure (Brindle & Lugowska, 1997; Janghorbani, Ting, Nahapetian, & Young, 1982; Nève, Hanocq, Molle, & Lefebvre, 1982). The treatment with perchloric acid along with sulfamic acid, however, did not help to improve selenium response in the GF measurements, since very high background signals were still observed.

The recovery experiments carried out in the presence of Cu, Zn, Fe, and Cr showed that these metals did not interfere in selenium measurement by HGAAS, at the concentration levels of the experiment. The metal concentration levels were set as described, since they represent those levels expected in poultry meat after sample decomposition.

The idea of extracting fat from the tissues before the measurements had two goals: to verify if selenium is not accumulated in this part of the tissue and, if not, the possibility of carrying out the digestion with smaller volume of acids. Selenium measurement in the organic fraction was carried out by GFAAS without interferences. Since in this fraction mainly organoselenium compound would be present, we did not try to measure selenium in this fraction by HGAAS. As can be seen in Fig. 3b selenium was partially recovered in this fraction. Because selenomethionine and selenocystine are soluble in organic solvents they were partially recovered in this fraction when the samples were spiked before the digestion procedure. Accordingly, for samples spiked only with inorganic selenium no selenium was found in the organic extract.

Table 2 shows the amount of selenium found in each part of three different samples of chicken breast, divided into skin and muscle, and these in turn into fat and solid

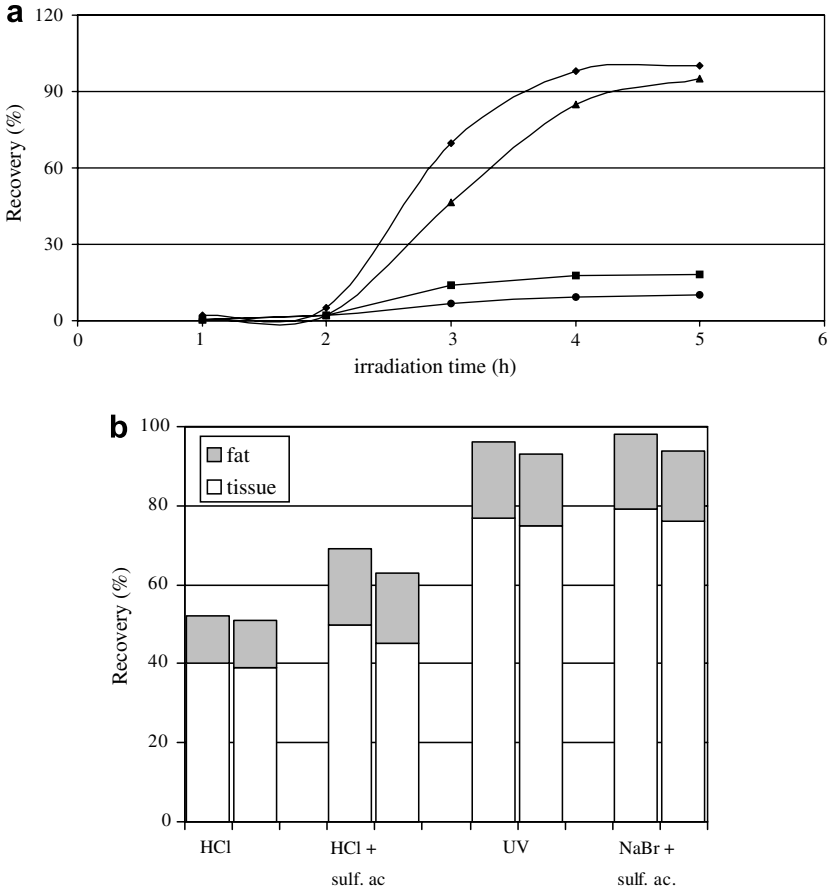

Fig. 3. Selenium recovery. (a) From muscle tissue after UV treatment with and without H_2O_2 addition; (♦) spiking before digestion with H_2O_2; (▲) spiking before digestion with H_2O_2; (■) spiking before digestion; (●) spiking after digestion. (b) From samples spiked before digestion and with fat extraction. Selenium in fat measured by GFAAS and in tissue residues by HGAAS after different pre-reduction treatments: (1) muscle; (2) skin

Table 2
Selenium found in fatty extract and residue of both tissues after fat extraction and digestion acid mixture (selenium pre-reduction with NaBr/sulfamic)

Sample	Tissue	Fat extraction			Residue		
		Mass (g)	μg Se (± RSD)[a]	%	Mass (g)	μg Se(± RSD)[a]	%
1	Muscle	0.0147	0.01 ± 0.5	7.1	0.5431	0.13 ± 8.5	92.9
2	Muscle	0.0154	0.04 ± 0.4	19.1	0.4909	0.17 ± 3.4	80.9
3	Muscle	0.0152	0.05 ± 0.9	13.6	0.5106	0.32 ± 7.6	86.4
1	Skin	0.0899	0.01 ± 1.3	8.8	0.4923	0.10 ± 9.3	91.2
2	Skin	0.0849	0.01 ± 0.8	4.5	0.4565	0.21 ± 6.5	95.5
3	Skin	0.0965	0.02 ± 1.0	14.2	0.4416	0.12 ± 3.3	85.8

[a] $n = 3$.

residues. Selenium was found in all tissues. More than 80% of the selenium is present in the solid residue of a tissue (muscle or skin) and less than 20% in the fatty fraction of these tissues.

Table 3 displays a balance of selenium status in the three different chicken meat samples, considering the relative mass of each part. If skin represents 10% of the total mass of a chicken breast (without bones), a considerable amount of selenium is found in this tissue. Keeping the massic fractions and calculating the relative amount of selenium in each part, approximately 59% of the total selenium was found in the muscle and 41% in the skin.

3.3. Figures of Merit

The linear regression equations and the coefficients of correlation for aqueous and methanolic solutions, under the selected analytical conditions for GFAAS and HGAAS measurements, were $y = 6.00 * 10^{-3}\,x - 8.00 * 10^{-4}$ and $y = 2.03 * 10^{-2}\,x - 7.30 * 10^{-3}$, where y corresponds to concentration in μg/l and x to absorbance, and 0.9922 and 0.9990, respectively.

The detection limits of both measurement techniques were calculated from blank samples, using the criterion of three times the standard deviation. All procedure, fat extraction, digestion with $HNO_3/HClO_4$ and pre-reduction with NaBr/sulfamic acid, was performed and selenium measured in both organic solvent fraction and acidic digested by GFAAS and HGAAS, respectively. Limits of detection were 5 ng absolute in a 5-ml sample volume or 1 μg/l for GFAAS and 6 ng absolute in a 20-ml sample volume or 0.6 μg/l for HGAAS measurements.

Table 3
Selenium distribution between skin and muscle in the three different chicken samples

Sample	Skin			Muscle		
	Mass (g)	μg Se	%	Mass (g)	μg Se	%
1	0.5822	0.11	44.0	0.5571	0.14	56.0
2	0.5414	0.22	51.2	0.5063	0.21	48.8
3	0.5381	0.14	27.5	0.5258	0.37	72.5
		Mean = 40.9			Mean = 59.1	

A mean of six replicates performed with the same standard (20 μg/l Se) gave a variation coefficient of 8.7% (GFAAS). Recovery rates obtained by calculation of blank-corrected data ranged from 89% to 99%. These results show the repeatability of the proposed analysis strategy. The precision, obtained in the analysis of different chicken samples is reflected in the standard deviation of the results in Table 2. The mean relative standard deviation was approximately 6.5% in fat fraction (GFAAS measurement) and 0.8% in residue fraction (HGAAS measurement).

4. Conclusion

Considering the sample digestion procedure used, the assessment of the selenium status in chicken meat was better carried out by HGAAS in comparison to GFAAS. Interferences caused by the presence of sample concomitants, even after sample digestion, or interference caused by reagents or reagents decomposition products could not be overcome in the GFAAS measurement. The chemical modifier (Ni) was not able to eliminate interferences, even permitting a relatively high pyrolysis temperature.

The complete sample treatment included sample decomposition with $HNO_3/HClO_4$ mixture, elimination of nitrogen oxides with sulfamic acid and selenium pre-reduction with NaBr. In spite of also presenting good results, UV irradiation is more time consuming and the need of pH adjusting is an inconvenient additional step.

Spiking the samples before and after the digestion treatment showed that this step not only decomposes organic matter but also promotes the mineralization of organic selenium and, consequently, helps in the further reduction of selenium species to selenite.

The separation of fat from tissues allows the reduction of the amount of all reagents necessary for sample treatment. However, considering that selenium was found in both organic extracts (muscle and skin), the procedure including fat extraction may either be skipped or included in the whole procedure. If included, the selenium level in the extracts must be evaluated and added together to the total, and this determination can be performed by GFAAS.

The measurement of the selenium content in chicken skin allowed concluding that part of the ingested selenium

is stored in this tissue. Whereas 59% of selenium was found in the muscle, 41% was found in the skin. However, since only about 10% of the whole chicken meat is skin, the selenium amount in this tissue is 4 times as big as in the muscle, for portion.

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