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An in situ pre-concentration method for fluorine determination based on successive digestions by microwave-induced combustion

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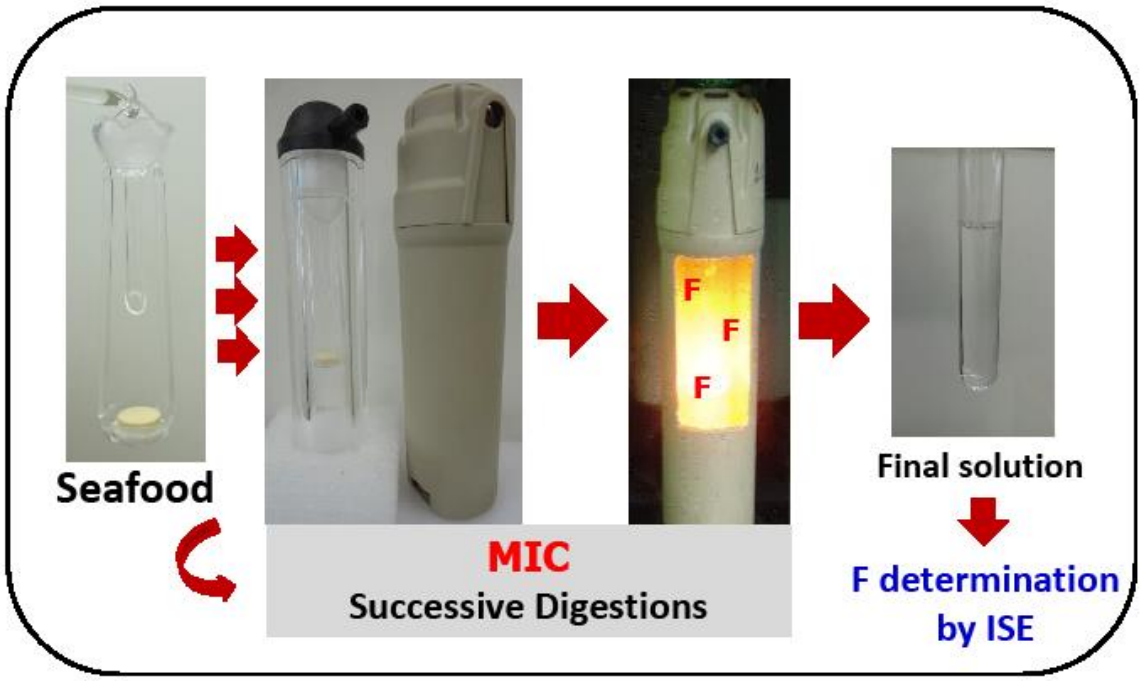
ABSTRACT

A new strategy based on successive digestion by microwave-induced combustion (MIC) in the same reaction vessel with a single absorbing solution was proposed. As a proof of concept, F was determined in seafood digests by ion-selective electrode (ISE). Samples were pressed as pellets (up to 0.7 g) and combusted in closed quartz vessels pressurized with oxygen. Sequential digestions were each performed up to 4 combustion cycles in the same vessel and using the same absorbing solution. In each cycle, a new filter paper,

igniter and sample pellet (0.7 g of sample) were used. Ammonium hydroxide solutions (10 to 100 mmol L^{-1}) were evaluated for F absorption. Accuracy of the proposed method was evaluated using certified reference material of oyster tissue (NIST 1566a) and also by comparison of results obtained after pyrohydrolysis method. Up to 3 digestion cycles (0.7 g each cycle, total mass of 2.1 g) could be used with 50 mmol L^{-1} NH_4OH as an absorbing solution. Results were in agreement with those obtained using pyrohydrolysis and also to certified reference values; the coefficient of variation after 3 cycles was below 5%, and it was considered as suitable for F determination even at low concentration. The residual carbon in digests was lower than 25 mg L^{-1}, allowing F determination by ISE virtually free of interferences due to dissolved organic matter. The limit of detection (LOD) for F was 1.3 µg g^{-1} (using 2.1 g of seafood), which is almost 4 times lower than the LOD obtained using the reference method (pyrohydrolysis). Contrary to the reference method, this relatively low LOD allowed the determination of F in all the seafood samples analysed in this work. Taking into account that only 6 mL of diluted NH_4OH solution (50 mmol L^{-1}) were used and the suitable LOD, the proposed sequential digestion MIC method can be recommended for further F determination in trace levels in seafood, even using a low-cost technique such as ISE, instead of other, more powerful techniques, such as ion chromatography.

Graphical Abstract:



Keywords:

Fluorine determination; Seafood analysis; Microwave-Induced Combustion; Ion-selective electrode; Sample preparation; Pyrohydrolysis.

1. Introduction

Fluorine is considered an important element for human growth and development. In low levels it can prevent dental caries, but at high concentrations it can cause disease, such as fluorosis and osteoporosis [1-3]. Due to these aspects, F concentration has been monitored in food in order to avoid excessive intake [4,5]. In this sense, it is important to establish suitable methods for F determination in food, even in very low concentrations. Despite the use of ion chromatography [6,7] and atomic

spectrometry [8,9] for F determination, potentiometry with ion-selective electrode (ISE) is the most used technique for this purpose due to its simplicity, relatively low cost and availability for most laboratories [10-12].

However, depending on the concentration of F in food and the dilution extent of digests, the use of ISE has been limited due to its poor sensitivity. For organic matrices, the digestion step is generally unavoidable [13,14] in order to assure the complete sample decomposition necessary to obtain F in the solution and also to reduce the risk of interferences during F determination by ISE [15]. Concentrated acids could be used for microwave-assisted wet digestion [16,17], but this medium can not be suitable for F due to the risk of losses by volatilization [18,19]. In addition, to avoid systematic errors due to the formation of insoluble fluorides in the digests, such as CaF_2 and MgF_2, separation procedures are always mandatory prior to the determination. On the other hand, pyrohydrolysis and combustion methods are particularly suitable for the digestion of organic matrices for further F determination due to the possibility of using diluted alkaline solutions, which are suitable for F absorption [20].

The microwave-induced combustion (MIC) method involves the combustion of organic samples in closed quartz vessels pressurized with oxygen [21,22]. This system has been successfully applied for the digestion of several kinds of organic matrices, such as carbon nanotubes [23], crude oil [24], coal [25], elastomers [26], food [27-29] and tobacco [30], among others, for further halogens determination. However, for solid samples with high protein content (such as seafood), MIC has been usually applied only for sample masses up to 0.7 g due to the limited amount of oxygen inside the vessel required to assure safety (20 bar of O_2 is typically used) [28].

Based on the relatively few options for sample preparation and F determination in food at low levels, this work proposed MIC for the first time as a method of high

efficiency sample digestion, and also for fluoride separation and concentration for further F determination by ISE. The use of sequential cycles of digestion was performed to decompose relatively high sample masses and consequently improve the LOD obtained by ISE. In order to demonstrate the feasibility of the proposed method, seafood was used as an example of application. The same vessel and absorbing solution were used to perform the decomposition of up to four portions from the same test sample. Sample mass and composition of absorbing solution were investigated as well as the digestion efficiency, which was evaluated by carbon determination in digests. The accuracy was evaluated using certified reference materials (CRM) with similar matrix compositions and also by comparison of results with those obtained using pyrohydrolysis [11], and further F determination by ISE and IC.

2. Experimental

2.1. Instrumentation

A microwave sample preparation system (Multiwave 3000, Anton Paar, Austria) equipped with eight high-pressure quartz vessels was used for the proposed MIC method. The internal volume of vessels was 80 mL and the maximum pressure and temperature were 80 bar and 280 °C, respectively. A commercial quartz holder (Anton Paar, Cat. no. 16427) was used to insert the samples inside the quartz vessels.

Fluoride determination in digests was performed using a potentiometer (781 pH/Ion meter, Metrohm, Switzerland) equipped with an ion-selective electrode for F (part number 6.0502.150, Metrohm) and a reference electrode (Ag/AgCl, part number 6.0726.100, Metrohm). For F measurements, samples and reference solutions were diluted 1+1 using a total ionic strength adjustment buffer (TISAB) with pH 5.5 [11]. Operational conditions were selected according to a previous work [31]. For

comparison of results, F in digests was also determined by ion chromatography (Metrohm Ion Analysis, Herisau, Switzerland) with an anion-exchange column (Metrosep A Supp 5, polyvinylalcohol with quaternary ammonium groups, 150 x 4 mm i.d.), a guard column (Metrosep A Supp 4/5 Guard), a chemical suppressor module and a conductivity detector. A sample loop of 100 µL was used. The mobile phase was 3.2 mmol L^{-1} Na_2CO_3 and 1.0 mmol L^{-1} $NaHCO_3$ flowing at 0.7 mL min^{-1}. Operational conditions were selected according to a previous work [20]. An inductively coupled plasma optical emission spectrometer (Optima 4300 DV, PerkinElmer, USA) with axial view configuration was used for carbon determination in digests. A concentric nebulizer and a cyclonic spray chamber were also used. Operational conditions were set according to a previous work [32]. Argon 99.996% (White Martins, Brazil) was used for plasma generation, nebulization and as auxiliary gas.

Seafood samples used in this work were dried in an oven (400/2ND, Nova Ética, Brazil) and further ground in a cryogenic mill (6750, Spex Certiprep, Freezer Mill, EUA). For milling, samples were initially frozen in liquid argon for 2 min and further ground for 2 more min For MIC, pellets of seafood samples were prepared using a hydraulic press at 1 ton for 1 min (Specac, UK). All weighings were performed using an analytical balance (AY 220, Shimadzu, Japan).

For comparison of results, seafood samples were digested using a pyrohydrolysis system as previously described [33]. The system was composed of an electrothermal furnace, a quartz tube and a quartz sample holder. A peristaltic pump (Minipuls, Gilson, USA) equipped with a Tygon® tube was used for water transport to a ceramic capillary placed at the inlet of the quartz tube and connected with a silicone stopper. The outlet of the quartz reactor was connected to a glass coil immersed into an ice bath in order to condense the gaseous products from pyrohydrolysis reaction. The condensed solution

was collected in a polypropylene vessel containing absorbing solution for subsequent F determination by ISE.

2.2. Reagents, reference solutions and samples

Ultrapure water (resistivity of 18.2 MΩ cm) obtained from a Milli-Q system (Millipore Corp., USA) was used for preparing reagents and analytical solutions. All the reagents used in this work were of analytical grade (Merck, Germany). Absorbing solutions used to retain the analytes were prepared from 25% NH_4OH solution. Ammonium nitrate solution (6 mol L^{-1}) was used as igniter for MIC added to a small disc of filter paper (15 mm of diameter, 12 mg) with low ash content (Black Ribbon Ashless, Schleicher and Schuell GmbH, Germany). The filter paper was previously cleaned with ethanol for 20 min in an ultrasonic bath and further washed with water and dried in a class-100 laminar flow bench (CSLH-12, Veco, Brazil).

Fluoride reference solution was prepared by the dissolution of sodium fluoride salt (Merck) in water. Analytical standards for F determination by ISE (250 to 2500 µg L^{-1}) and IC (10 to 100 µg L^{-1}) were prepared by sequential dilution of a 1000 mg L^{-1} stock reference solution in water. A TISAB solution, used in all samples and standard solution, was prepared by dissolving 58.4 g of NaCl, 4.5 g of 1,2-cyclohexilenediaminetetraacetic acid and 57.5 mL of acetic acid in 500 mL of water [11]. After dissolution, the pH was adjusted at 5.5 with a 20% (m/v) sodium hydroxide solution, and the final volume was adjusted up to 1000 mL with water.

For carbon determination, standards (25 to 500 mg L^{-1}) were prepared using a C reference solution (1000 mg L^{-1}, Spex CertiPrep, Metuchen, USA) and yttrium (1000 mg L^{-1}, Spex CertiPrep) was used as internal standard (1 mg L^{-1}) for standards and samples.

2.3. Samples

Corrupt goldfish (*Carassius auratus*), mussel (*Limnoperna fortunei*), octopus (*Octopus vulgaris*), salmon (*Salmo salar*), shrimp (*Litopenaeus vannamei*), squid (*Loligo vulgaris*) and wolf fish (*Anarhichas lupus*) samples were purchased in a local market. Samples were dried in an oven at 60 °C for 24 h and afterwards ground in a cryogenic mill (particle size lower than 100 µm). Shrimp was arbitrarily selected for development of the proposed MIC method. A CRM of oyster tissue (NIST 1566a) from the National Institute of Standards and Technology (NIST) was used for evaluation of the accuracy of the proposed MIC method.

2.4. Proposed MIC Method for Sequential Digestion

For the proposed MIC method, pellets of shrimp (0.7 g) were weighed and placed on the quartz holder with the filter paper. Ammonium nitrate solution (50 µL of 6 mol L^{-1}) was added to the paper, and the holder containing the sample was placed inside the quartz vessel, which was previously charged with 6 mL of absorbing solution. Ammonium hydroxide solutions ranging from 10 to 100 mmol L^{-1} were evaluated as absorbing solutions. After closing the vessels and capping the rotor, vessels were pressurized with 20 bar of oxygen. The rotor was placed inside the oven and the heating program was applied as follows: 1400 W for 5 min and 0 W for 20 min (cooling step). After finishing the heating program (including cooling step), each vessel was carefully opened to release the pressure. Quartz holders were washed using a minimum volume of water (about 2 mL) and then another quartz holder containing a second pellet of 0.7 g of sample was introduced into the vessel. The absorbing solution was the same used in the first digestion, and the digestion was performed using the same procedure

previously described. This procedure was applied up to four times, reaching the digestion of up to 2.8 g of sample. Afterwards, the final solution was transferred to volumetric flask and volume adjusted to 25 mL. It is important to explain that, in order to obtain the maximum number of cycles that could be performed using the proposed method, the determination of F was also performed after each cycle of digestion, and results were compared with reference values obtained after pyrohydrolysis digestion and determination by ISE.

Cleaning of vessels and holders was carried out with 6 mL of concentrated HNO_3 using a microwave heating program with 1400 W for 10 min and 0 W for 20 min for cooling. The vessels and holders were then rinsed with ultrapure water.

2.5. Pyrohydrolysis digestion

Results of F concentration in seafood were also compared with those obtained after pyrohydrolysis. The procedure was adapted from previous works [11,34]. In this procedure, seafood samples (0.5 g) were mixed with V_2O_5 (1.5 g) in a quartz holder that was introduced in a quartz tube heated to 1,150 °C with an electrical furnace. Water was pumped through a heated ceramic capillary flowing at 0.5 mL min^{-1} for water vapour generation. The air flow rate, used as carrier gas, was set at 200 mL min^{-1}. The gaseous products of pyrohydrolysis were collected during 10 min in a vessel containing 10 mL of absorbing solution, and the final solution was diluted to 25 mL. Fluorine determination was performed by ISE, as previously described [35].

2.6. Method validation

Method validation was carried out according to the Eurachem Guide [36]. The accuracy of the proposed method was evaluated using a CRM of oyster tissue in three

concentration levels. The limits of quantification (LOQs) were calculated as ten times the standard deviation of 10 consecutive measurements of blanks, taking into account the sample mass and the final volume of digests. Linearity was expressed as the related coefficients (*R*) of analytical curves, and the minimum acceptable value of *R* was 0.990. Robustness was evaluated by the reliability of analysis taking into account variations of the following method parameters: sample mass, number of digests cycles, concentration of absorbing solution and some ISE parameters. Repeatability (intra-day precision) and intermediate precision (inter-day precision) were estimated based on the coefficients of variation (CV) by analysing each matrix on three different days and with three replicated analyses per day. All statistical calculations were performed using GraphPad InStat (GraphPad InStat Software Inc., Version 3.00, 1997) software and a confidence level of 95%.

3. Results and discussion

The development and optimization of MIC conditions for seafood digestion and pre-concentration for subsequent F determination was performed using a shrimp sample. The reference value of F for this sample was 39.1 ± 2.9 μg g^{-1}, which was determined by ISE after pyrohydrolysis, as previously described [11]. Fluorine determination was also performed by IC, and the results were in agreement with those obtained by ISE.

3.1. Seafood digestion by MIC

In general, around 0.5 g of food sample [28,37] can be digested by MIC using 20 bar of oxygen [38,39]. For combustion of higher masses, the oxygen pressure should usually be increased, but this approach is not commonly used in order to avoid

excessive pressure during digestion [40]. On the other hand, the use of higher sample masses, keeping the same dilution factor, could improve the LOD for F determination. One possibility still not explored in MIC is the use of sequential combustion cycles without adding or changing the absorbing solution.

This work was performed in order to evaluate the feasibility of such an approach. Initially, a study was performed using 0.7 g of shrimp, oxygen pressure of 20 bar and 6 mL of 100 mmol L^{-1} NH_4OH as absorbing solution. After finishing the heating program and cooling step, each vessel was carefully opened to release the pressure. Then another quartz holder containing a second pellet of 0.7 g of sample was introduced into the vessel. This procedure was applied up to four times, reaching digestion of up to 2.8 g of sample. After this, the final solution was transferred to volumetric flask and volume made up to 25 mL. In the first experiment, 0.7 g of shrimp sample were digested by MIC. After, two cycles of digestion were carried out and 1.4 g of sample was digested (0.7 + 0.7 g of sample). Then, 3 and 4 cycles of digestion were performed, totalizing the digestion at 2.1 and 2.8 g of sample, respectively. After each digestion cycle, F determination was performed by ISE in order to obtain the maximum number of cycles that could be performed using the proposed method. Results for F determination in shrimp by ISE after proposed MIC method are shown in Fig. 1. Results report agreement with the reference values obtained by ISE after digestion by pyrohydrolysis.

Using four digestion cycles (reaching 2.8 g of sample), the agreement of results obtained by MIC with reference value obtained by pyrohydrolysis was about 52%. On the other hand, using 1, 2 or 3 cycles of digestion the agreement ranged from 96 to 100%. The pH of the absorbing solution after each digestion of the first 3 cycles were

5.0, 4.5 and 3.9. Nevertheless, after the fourth digestion cycle, the pH was lower than 1.0, which can be unsuitable for F absorption and could lead to the formation of volatile fluorine species (e.g., HF) with consequent analyte losses. Subsequently, the concentration of absorbing solution was increased in order to improve the recovery of F, but even with 200 mmol L^{-1} NH_4OH, the results were lower than 75% of reference value. Based on these results, subsequent experiments were performed using 3 digestion cycles, reaching 2.1 g of seafood sample. It is important to mention that the use of sequential digestions increased the precision of results obtained with coefficients of variation (CV) of 8%, 6% and 5% for 1, 2 and 3 digestion cycles, respectively. This behavior could be explained by the higher concentration of F in digests, which allows ISE determination at a lower uncertainty range.

3.2. Evaluation of the kind of absorbing solution

The suitability of the absorbing solution for F absorption is an important aspect for MIC digestion, since the type and concentration of solution generally are not necessarily the same for different analytes. Although F could be absorbed using many solutions, even water [11], alkaline solutions are generally preferred in order to absorb and stabilize halogens in the solution [34]. Therefore, experiments were carried out to evaluate the suitability of ammonium hydroxide solution (ranging from 10 to 100 mmol L^{-1}). Experiments were performed using 3 sequential cycles of digestion with 0.7 g of shrimp sample per cycle; the results are shown in Fig. 2.

For all NH_4OH solutions, the agreement with reference values ranged from 98 to 103% after seafood digestion using three sequential cycles. However, when 10 or 25 mmol L^{-1} NH_4OH solution was used, the CV was slightly higher (around 10%). On the

other hand, from 50 to 100 mol L^{-1} NH_4OH, the CV was below 5%. It is important to mention that blank values obtained after MIC with 10 to 100 mmol L^{-1} NH_4OH were always negligible. Although 100 mmol L^{-1} NH_4OH was suitable for F absorption, a solution of 50 mmol L^{-1} NH_4OH was chosen for subsequent experiments in order to avoid the use of an excessive amount of reagents, reducing blanks values and reagents consumption.

3.3. Determination of F in seafood samples

Seafood samples were analysed using either one digestion cycle (conventional MIC method) with successive digestions (3 cycles, proposed MIC method) or pyrohydrolysis methods. As can be seen in Table 1, results obtained by using the proposed method presented no statistical difference (ANOVA, confidence level of 95%) when compared with those obtained after MIC (1 cycle) and pyrohydrolysis methods.

Seafood samples showed F concentration ranging from 3.37 to 39.8 μg g^{-1}. Samples of shrimp and corrupt goldfish showed higher concentration of F than other samples. It is important to mention that the World Health Organization (WHO) recommends the optimal concentration of F as between 200 to 250 μg g^{-1} in food [41]. In this way, by taking into account the highest F content found in the shrimp sample (approximately 40 μg g^{-1} F, Table 1), the consumption of approximately 5 and 6 g of shrimp per adult per day can be suggested as a source of fluoride intake. On the other hand, one must be aware that excessive intake of F may cause health problems [41,42]. Moreover, with sequential cycles (sample mass of 2.1 g), it was possible to determine F in all test samples, including wolf fish and mussel, which were not detected by using ISE after pyrohydrolysis or MIC applied in the conventional way.

Whilst there are some published works for F determination presenting LOQs lower than those obtained using the proposed method, it is important to mention that the use of acid solution for food digestion [43] for subsequent F determination by ISE can cause interference during the determination step. Moreover, fusion method using open vessels allows the use of high sample masses, decreasing the LOQs' values [19]. However, losses by volatilization during fusion procedure and interferences during F determination can be observed due to the presence of excessive amounts of reagents used during sample preparation.

3.4. Analytical figures of merit

Accuracy and precision of the proposed method (MIC using 3 cycles) were evaluated by using a CRM of seafood (NIST 1566a, oyster tissue). Experiments were carried out using 2.1 g of sample, 6 mL of 50 mmol L^{-1} NH_4OH as absorbing solution and a reflux time of 5 min. The comparison with certified values (Table 1) showed no statistical difference (*t*-test, 95% of confidence level) between certified and found values using the proposed method. The analytical precision expressed by CV was lower than 5%. Moreover, according to the results shown in Table 1, by using sequential cycles of MIC, better LODs were achieved when compared with those obtained by conventional MIC (one cycle). The LODs obtained by ISE after MIC using 1 and 3 cycles were 5.4 and 1.3 μg g^{-1} F, respectively. Moreover, it is important to mention the improvement of precision using the proposed method. Although suitable CVs have been obtained after pyrohydrolysis and conventional MIC methods (about 8% for both methods), with the proposed method, CVs were always lower than 5%. Moreover, using the proposed MIC method, up to eight samples could be simultaneously processed;

using pyrohydrolysis, only one sample can be processed for time, which is an important consideration for routine analysis that aims for a high sample throughput.

4. Conclusions

The feasibility of successive digestions by MIC in the same digestion vessel with a single absorbing solution aiming at the determination of F by ISE was successfully demonstrated for the analysis of seafood samples. By digesting the same test sample in sequential cycles of MIC, it was possible to efficiently decompose a relatively high sample mass (2.1 g). The final digests presented relatively low residual carbon (< 25 mg L^{-1}), avoiding possible interference from the presence of high concentrations of dissolved organic compounds. An additional advantage was that blank values were negligible when compared to the reference methods (MIC and pyrohydrolysis). Consequently, the LOD was improved about 4 times related to one run digestion procedure, allowing, contrary to pyrohydrolysis method, the determination of F in very low concentrations in all seafood test samples. With the proposed MIC, up to 8 samples can be simultaneously digested in the same run, a better performance compared to pyrohydrolysis method, which allows only one sample digestion per run. A relevant aspect is that as F concentration in digests grow higher and the digests are basically well diluted alkaline solutions, even a relatively simple and inexpensive technique, such as ISE, can still be used to assure suitable LODs. It is an important aspect of routine analysis that there is no need of a more powerful and inexpensive technique as ISE. In summary, it is important to point out that the proposed method for seafood digestion combines safety, low reagent consumption, high efficiency and better sample throughput, which are relevant aspects aiming at routine trace analysis.

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Table 1. Results for F determination by ISE after digestion of CRM and seafood by MIC and pyrohydrolysis. Results in μg g^{-1} (mean and standard deviation, n = 3).

Samples	Pyrohydrolysis	MIC	
		1 cycle**	3 cycles***
Corrupt	34.8 ± 2.8	36.8 ± 2.5	35.3 ± 1.4
Wolf fish	< 5.0	< 5.4	4.68 ± 0.15
Mussel	< 5.0	< 5.4	3.37 ± 0.13
Octopus	6.01 ± 0.49	6.21 ± 0.51	6.18 ± 0.18
Shrimp	38.7 ± 2.8	39.1 ± 2.9	39.8 ± 0.9
Squid	6.29 ± 0.51	6.69 ± 0.56	6.45 ± 0.27
NIST 1566a*	230 ± 17	234 ± 19	237 ± 10

*Informed value (F: 240 μg g^{-1})
**using 1 cycle of digestion (0.7 g, LOD 5.4 μg g^{-1} F)
***using 3 cycles of digestion (2.1 g, LOD 1.3 μg g^{-1} F)

Fig. 1. Influence of MIC seafood digestion cycles (0.7 g of shrimp in each digestion cycle and 100 mmol L^{-1} NH_4OH as absorbing solution). Error bars represent the mean and standard deviation, n = 3. Reference value was obtained by pyrohydrolysis and determination by ISE.

Fig. 2. Influence of the concentration of NH_4OH absorbing solution for F determination in seafood using three sequential digestion cycles. Determination by ISE and mean results in µg g^{-1} (error bars represent the standard deviation, n = 3).

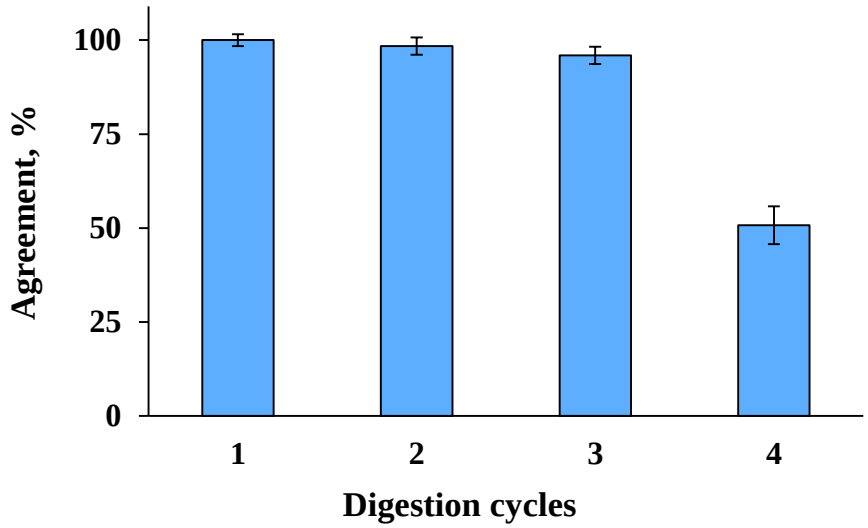

Fig. 1

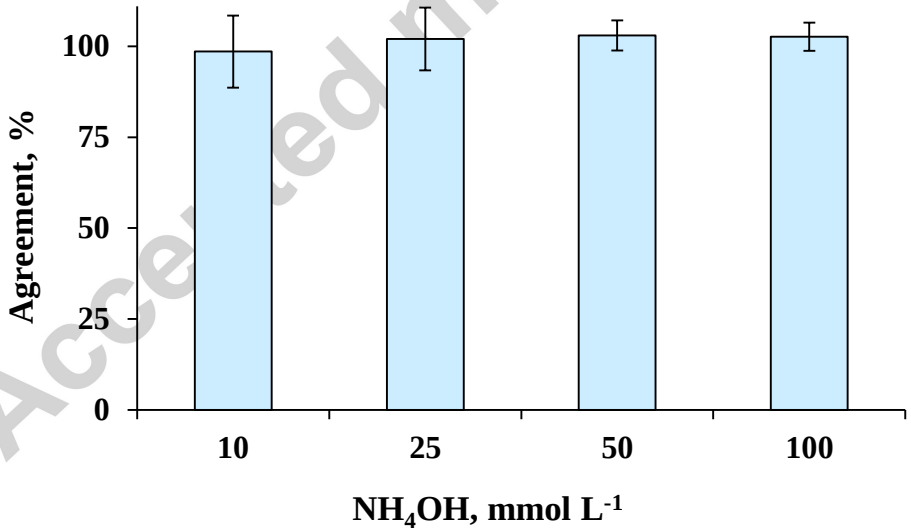

Fig. 2

Highlights

- For the first time a method using successive cycles of MIC for further F determination by ISE was proposed.
- In the proposed work the determination of F was performed in seafood samples using up to 2.1 g.
- Only diluted alkaline solution was used as absorbing solutions, minimizing reagents consumption.
- The throughput was considerable suitable to routine analysis (8 samples can be digested in each run).

- proposed method is according to the recommendations of green chemistry.