



Research Article

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## On the Optimization of Hydrolysis Conditions for Simultaneous Determination of Amino Acids and Amino Sugars in Marine Sediments

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### Abstract

In the present work different approaches were tested for the optimization of the hydrolysis conditions for the joint determination of amino acids and amino sugars in chitin, an important structural polymer in fungi, invertebrates, algae and the exoskeleton of zooplankton. Ten treatments were evaluated, involving different combinations of hydrolysis temperature and duration. The obtained results indicate that slightly lower temperatures (105°C) than for the classical combined amino acid hydrolysis and longer times (22 hours) than for chitin minimized losses and maximized recoveries of monomeric amino acids and amino sugars. This represents a large advantage in comparison to the usual methods of separate hydrolysis for high-performance liquid chromatography (HPLC) determination, allowing a simultaneous analysis of both compound classes in a single run.

### Keywords

Amino acids; Amino sugars; Sediments; Hydrolysis; Methodological optimization; Temperature treatment

### Introduction

Organic matter (OM) in aquatic ecosystems consists in a mixture of products of synthesis, degradation and re-synthesis originated from biotic and abiotic mechanisms [1]. When OM reaches the bottom, its composition will reflect the combined effect of several processes, reflecting changes in its input rate, transport, degradation and preservation [1]. Thus, marine sediments contain an archive of past climatic and environmental settings influencing primary production and sedimentation dynamics [1,2]. The preservation in the sedimentary column of substances or compositional patterns characteristic for primary producers (pigments, determined amino acids and amino sugars), zooplankton (chitin) or bacterial biomass (muramic acid and D- amino acids), together with more general parameters such as particulate organic C (POC) and N (PON), C:N ratios, or stable C and N isotopes can allow the identification of periods with higher or lower primary production and the corresponding variations in biological activity [3,4].

Amino sugars are important monosaccharides in living organisms [5]. For example, glucosamine is part of the structure

of the polysaccharides chitosan and chitin, which compose the exoskeletons of crustaceans and other arthropods, cell walls in fungi and several higher organisms, being an important indicator of biogeochemical secondary production [4]. Amino acids are one of the most abundant forms of organic nitrogen in living organisms, in which they have essential metabolic and structural functions [6]. The amino acid composition of OM in seawater is relatively similar to that of living organisms, yet with higher proportions of glycine, alanine and others indicative of biodegradation [4]. The relative abundance of the individual amino acids is changed during OM decomposition following well-known patterns, and is used to describe its diagenetic state [7-9]. The amino acids glutamine, alanine, glycine, serine and the amino sugars glucosamine and galactosamine were chosen for this study for representing the major proportion of total amino acids and amino sugars in the marine environment [10,11].

The determination of amino acids and amino sugars in sediments is often carried out by HPLC by monomer pre-column derivatization after hydrolysis of the polymers in OM. A large variation in the conditions for treatment and hydrolysis can be found in the literature, depending on the type of sample material and the specific analytes. Several types of hydrolysis have been proposed for each of these compound classes [6,11-13], with different degrees of recovery. The hydrolysis times for soils and sediments range from 4.5 to 22 hours. Conditions that can be at an optimum for the determination of amino acids in e.g. bacteria (6 N HCl, 140°C, 70 min), are destructive for amino sugars [13]. For soils samples, Zhang and Amelung [11] proposed 6 N HCl at 105°C for 8 hours for amino sugar determination. Other authors [6] used the same conditions for the determination of amino acids and amino sugars (6N HCl, 110°C, 22 hs), which also had been adopted in our laboratory. However, after testing this method we detected losses in amino sugars after hydrolysis of marine sediments.

The situation described above has typically led to separate hydrolysis of replicate sediment samples, with a duplication of the analytical effort in order to obtain the best results for both substance groups. In the present work it was aimed to establish the optimum hydrolysis conditions that could allow a common protocol for both amino acids and amino sugars, allowing a simultaneous determination. For this purpose, ten different combinations of temperature and duration of acid hydrolysis were tested on natural marine sediment samples.

### Materials and Methods

During this work, sediments of significantly different organic matter content were used in order to compare yields at hydrolysis conditions assumed to be most favorable according to literature and preliminary experiments. Samples were obtained from the Argentinean continental shelf in 2009 and from the German Bight in 2010. The organic carbon content (%Corg) was determined by removing inorganic C by acidification with 1 N HCl, in order to calculate the necessary sample amount for the determination of amino acids and amino sugars, taking into account that ca. 0.5 mg of organic C/sample are necessary for a measurement under optimum conditions with the Fisons NA 2100 analyzer. This was carried out by

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**Table 1:** Different hydrolysis treatments for sediment samples from the Argentinean shelf.

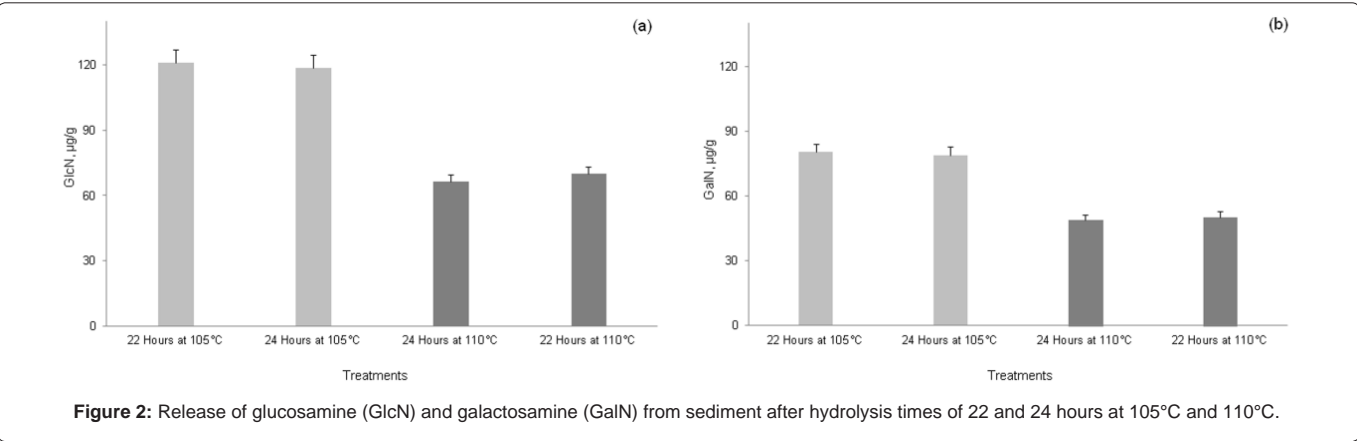
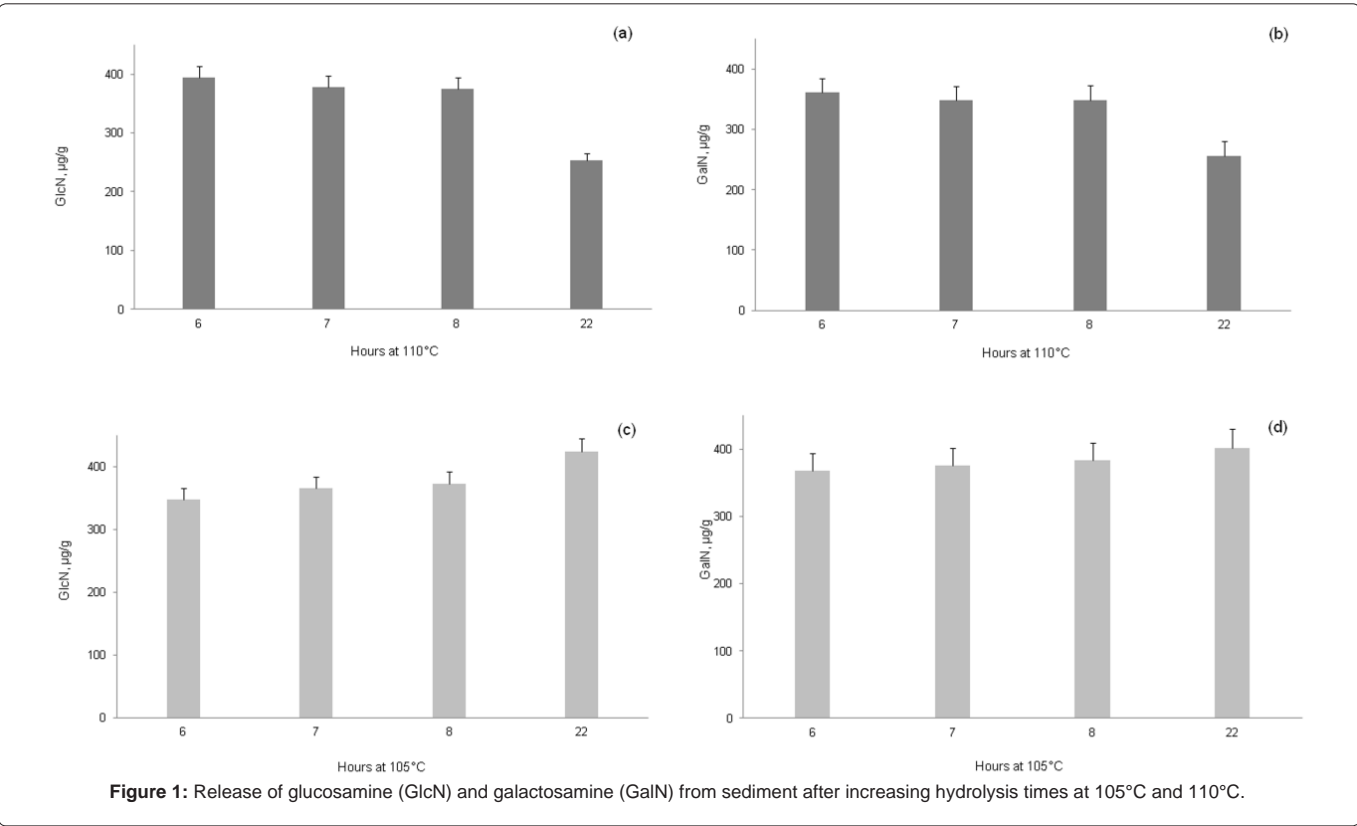
Treatment	Duration (hours)	Temperature (°C)
1	6	105
2	7	105
3	8	105
4	22	105
5	6	110
6	7	110
7	8	110
8	22	110

**Table 2:** Different hydrolysis treatments for sediment samples from the German Bight.

Treatment	Duration (hours)	Temperature (°C)
A	22	105
B	24	105
C	22	110
D	24	110

HPLC with o-phthalaldehyde (OPA) pre-column derivatization and fluorescence detection [13].

In a first experimental setting, sediment from the Argentinean Shelf (% Corg =1.6) was divided in 8 duplicate aliquots of 30.5 mg that were put into 10 mL glass ampoules. To each of them, 4 mL of



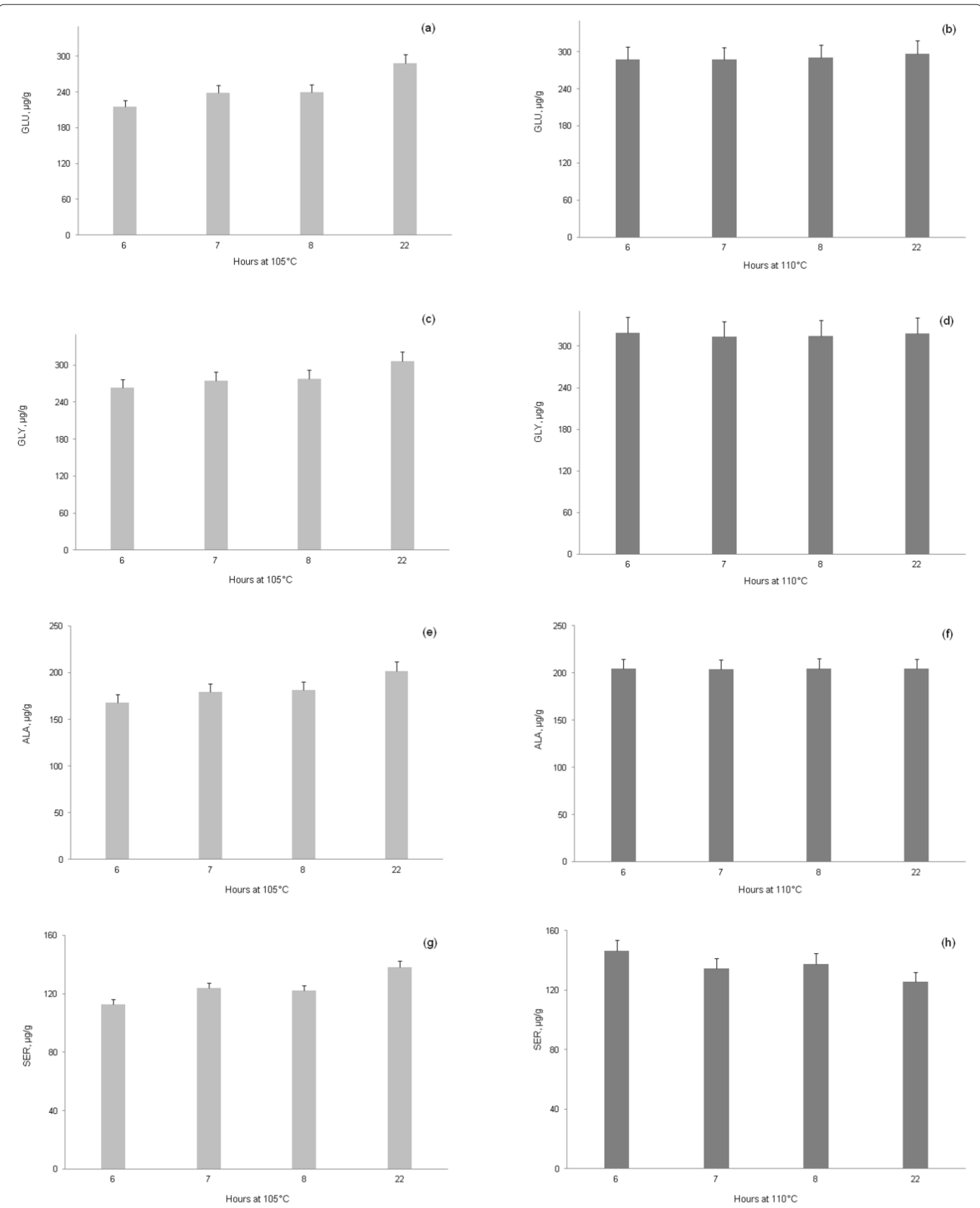


Figure 3: Release of glutamine (GLU), glycine (GLY), alanine (ALA) and serine (SER) from sediment after increasing hydrolysis times at 105°C and 110°C.

6N HCl was added, the dead volume was purged with N<sub>2</sub> and they were immediately flame sealed. Hydrolysis was carried out following 8 treatments (Table 1) combining two temperatures (105° and 110°C) and four durations (6, 7, 8 and 22 hours). In a second setting, a sample from the German Bight (%Corg = 0.5) was divided in four duplicate aliquots of 100.6 mg, and hydrolyzed under 4 different treatments (Table 2, 105° and 110°C during 22 and 24 hours).

After hydrolysis and cooling, 1 mL of the supernatant was withdrawn and evaporated to dryness (~2 hours) under low pressure with a Heidolph Synthesis 1 evaporator. After that, 2 mL of sodium citrate buffer (pH: 2.65) were added and the tube contents vortexed to dissolve and homogenize the hydrolysate. From that solution, two 800 µL aliquot duplicates were pipetted out, transferred to vials and kept at -18°C until analysis.

## Results and Discussion

### Amino sugars

The first set showed clearly different responses in the recovery of glucosamine and galactosamine from sediments (Figure 1). For both amino sugars, at 110°C there was a decrease of around 4% from 6 to 7 hours, keeping practically constant to 8 hours and decreasing ~30% after 22 hours (Figure 1a and 1b). At 105°, from 6 to 22 hours there was a smooth increase of 18% for glucosamine and 9% for galactosamine (Figure 1c and 1d). The lowest amount from both compounds was recovered in treatment 8 (22 hours at 110°C), which is usually used for the hydrolysis of combined amino acids, also in our laboratory, while treatment 4 (22 hours at 105°C) yielded the highest amounts. The difference between treatment 4 and treatment 8 was 40% and 36% for glucosamine and galactosamine, respectively.

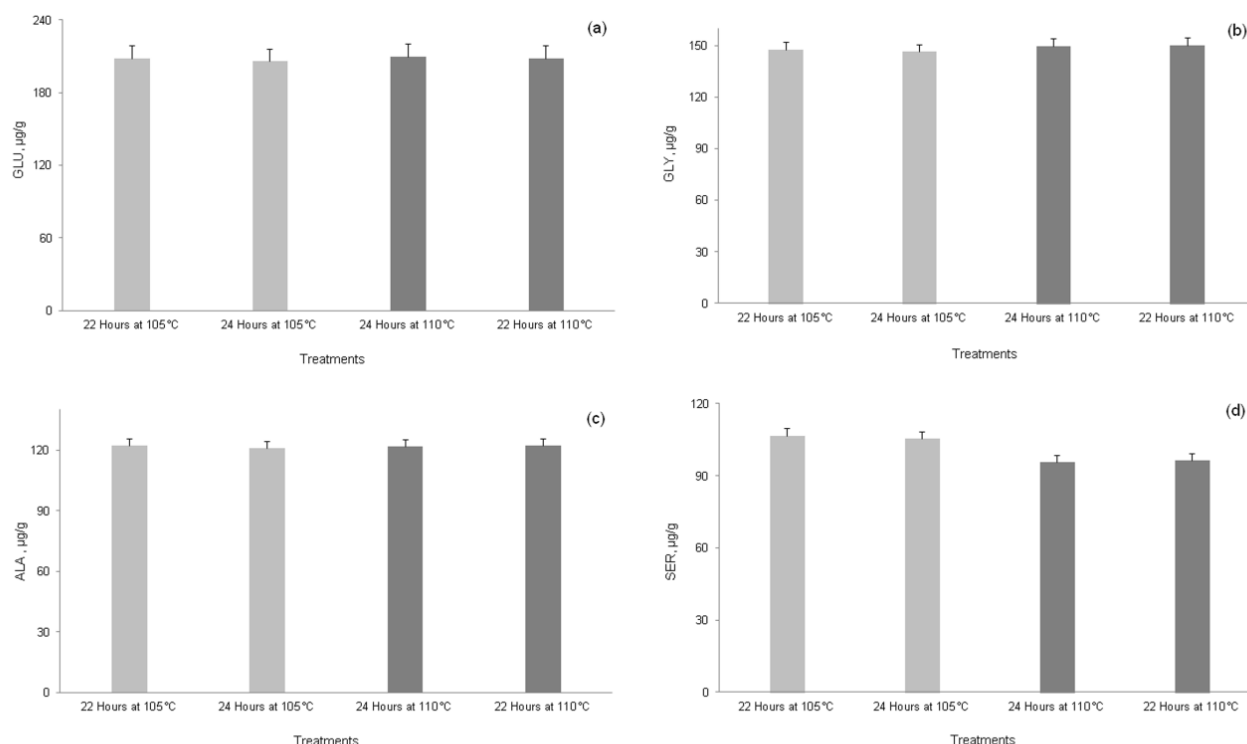
In the second array (Figure 2) the best yields for both amino sugars were also obtained with 22 hours at 105°C, notably higher than those at 110°C, either for 22 or 24 hours. For galactosamine, the yield of treatment with 22 hours at 105°C was 39% higher than with 22 hours at 110°C, while compared to treatment with 24 hours at 105°C the difference was only 2%. At 110°C, the yield was slightly enhanced (3%) for a hydrolysis duration of 22 hours as compared to 24 hours.

### Amino acids

In the hydrolysates of the table 1 array, the amounts of glutamine, glycine, alanine and serine were quantified (Figure 3) in order to evaluate the amino acid yields at the hydrolysis conditions used for amino sugars. These specific compounds were selected for representing a large percentage of the combined amino acids in marine organic matter [14]. Comparing hydrolysis during 22 hours at 105°C and 110°C, the amount of glutamine, glycine and alanine were respectively 3, 4 and 1% higher at 110°C, while serine was 9% lower. At 105°C recoveries increased with time from 6 to 22 hours, while at 110°C the variation was lower, excepting for serine, which decreased 10% from 6 to 22 hours.

The results from the second set (Figure 4) showed practically no differences for glycine and alanine between 22 and 24 hours at 110°C, and these yields were both equal as or only slightly higher (1.5%) than in treatments with 22 and with 24 at 105°C. In contrast, serine was about 10% lower in treatments at 110°C compared to treatments at 105°C.

The obtained yields for glucosamine and galactosamine, indicate that in the range of used temperatures and HCl concentration, a difference of only 5°C could be critical in the process of chitin



**Figure 4:** Release of glutamine (GLU), glycine (GLY), alanine (ALA) and serine (SER) from sediment after hydrolysis times of 22 and 24 hours at 105°C and 110°C.

hydrolysis and decomposition of the produced glucosamine, as well as of other amino sugars present in the sedimentary organic matter. At 105° the acid cleavage of the  $\beta$ -1,4 glycosidic linkage of chitin and the deacetylation of N-acetyl-D-glucosamine produces glucosamine in amounts increasing with time from 6 to 22 hours. In contrast, hydrolysis at 110°C shows a maximum glucosamine yield at 6 hours (with a lower yield than at 105°C, 22 hours) and decreases to a minimum at 22 hours. This behaviour is likely attributable to the deamination of glucosamine, producing glucose, not detectable using the OPA-derivatization method, which reacts with primary amines. Oppositely, at the same hydrolysis conditions (110°C, 22 hours) slightly higher values of the amino acids glutamine, glycine and alanine were obtained than at 105°C.

Zhang and Amelung [12] reported a reduction in the glucosamine yields when hydrolyzing soil samples at 105°C for more than 8 hours and attributed this fact to presence of lignin phenols. Yet, in our case, since marine sediments contain much less lignin compound than terrestrial soils, [4,14], this is unlikely the reason for the decrease. Kaiser and Benner [11] suggested that the optimum hydrolysis conditions for amino acid determination would probably be destructive for amino sugars, what is confirmed by our results.

## Conclusion

Taking into account that (a) the amino acid yields after 22 hours of hydrolysis at 105° did not greatly nor consistently differ from those at 110°C (<4% lower for glutamine, glycine and alanine and ~10% higher for serine), (b) the treatment at 110°C produced a amino sugar yield of ~35% lower than at 105°C, and (c) the hydrolysis during 24 hours did not bring any obvious advantage for neither method; the hydrolysis at 22 h hydrolysis at 105° appears adequate for the processing of large sediment sample series in which both, amino acids and amino sugars must be determined. We believe this choice represents a satisfactory balance between analytic efficiency and quality, costs and time reduction, particularly adequate for regional, synoptic evaluation of marine sediment characteristics.

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