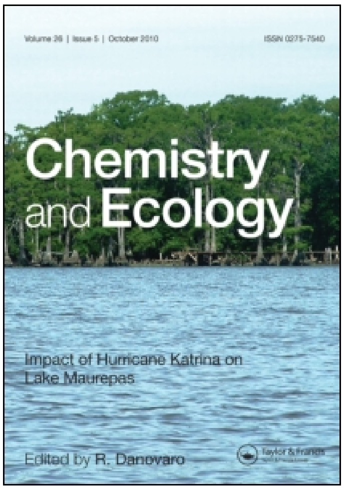

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A FUNCTIONAL APPROACH TO THE ASSESSMENT OF THE NUTRITIONAL VALUE OF PARTICULATE ORGANIC MATTER

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Organic matter lability can be determined with different approaches, based on the quantitative and qualitative estimate of its nutritional value. The C/N ratio and the particulate organic matter (as the sum of protein, carbohydrate and lipid bulks) vs total suspended matter (POM/TSM) deal with a quantitative assessment of the organic matter bulk, while other indices such as proteins/carbohydrates ratio (PRT/CHO) may give also qualitative information. Gordon (1970) proposed a functional approach, based on organic carbon decrease after enzymatic digestion. Coupling Gordon's approach with the evaluation of the main biochemical components (proteins, carbohydrates and lipids) contained in the particulate organic matter, a simple method to assess lability and nutritional value of organic substrates is proposed.

Water samples were collected at the surface and close to the bottom in a shallow coastal station (10 m depth) in the Marconi Gulf (Ligurian Sea, NW Mediterranean). The difference between "total labile" and "hydrolyzed labile" organic matter (respectively before and after enzymatic digestion) suggests a relevant presence of resuspended refractory materials vs about 60% of labile hydrolyzed organic matter, thus confirming the importance of a more qualitative approach to organic matter studies.

Keywords: Hydrolytic enzymes; particulate organic matter; nutritional value

INTRODUCTION

The flux of particulate matter from surface productive layers of oceans is the main food source for planktonic and bottom organisms

(Fukami *et al.*, 1981; Smith *et al.*, 1992). The organic content and the nature of particles may affect the trophic pathways and the biochemical processes of the ecosystems (Amy *et al.*, 1987). Physical, chemical and biological processes modify sinking particles, enhancing or inhibiting their aggregation, influencing the solubility of organic matter and determining the settlement of chemical components (Biddanda, 1988; Biddanda and Pomeroy, 1988; Azam *et al.*, 1992). Grazing and degradation processes are the major factors responsible for depletion of energetic resources sinking to the bottom (Hobson, 1967; Cawet, 1978).

The changes in total vs labile organic matter ratio can be used as an effective nutritional value index as well as to measure detritus "aging" (Nival *et al.*, 1972; Fabiano *et al.*, 1992). Organic carbon and nitrogen assessment can offer a good nutritional value index for the organic matter, although the available fraction could be overestimated. In fact, a relevant fraction of these elements is linked to refractory compounds, like phenols or some kind of carbohydrates (Tenore and Rice, 1980; Rice, 1982). The labile fraction of organic matter may be identified with the sum of proteins, carbohydrates and lipids, measured with analytical procedures commonly employed in biochemical studies (Fabiano *et al.*, 1984; Tanoue, 1985; Fichez, 1991). Sometimes the labile fraction can be assessed based on its energetic properties (Fabiano *et al.*, 1993).

However, organic matter lability is determined by the ability of organisms to ingest and assimilate it. Assimilation processes are carried out by enzymatic activities and depend, initially, on enzyme affinity for substrates. A simple oligomeric compound is likely to resist assimilation by organisms due to poor affinity of their hydrolytic enzymes. Furthermore, a specialized organism can get energy from highly refractory substrates like vegetal debris (Stockton and DeLaca, 1982). Thus, the definition of labile substances has to take into account the hydrolysis performed by enzymes on the organic matter. Following this assumption, Gordon (1970) proposed to quantify the hydrolytic activity of some classes of enzymes (proteinases and amilases) on natural samples in order to evaluate the labile fraction in terms of carbon decrease, after digestion of the organic matter bulk.

This paper proposes a method which, matching Gordon's functional approach with the study of the changes of protein, carbohydrate and lipid bulks, can directly measure the organic matter available to consumers. The sum of proteins, carbohydrates and lipids makes up the "total labile" organic matter, while the same components after enzymatic digestion constitute the "hydrolyzed labile" organic matter.

MATERIALS AND METHODS

Sampling

Water samples were collected at the surface and close to the bottom at a shallow coastal station (10 m depth) of the Marconi Gulf (Ligurian Sea, NW Mediterranean) with a 5 l Van Doorn bottle from November 1993 to February 1995. The samples were prefiltered through a 200 μm mesh to avoid sampling of the mesoplanktonic fraction. Subsamples (0.5–1 litres, depending on water transparency) were then filtered through Whatman GF/F glass fibre filters (nominal pore diameter 0.45 μm), previously calcinated in a muffle furnace (450°C, 4h).

Biochemical methods

Protein analysis: The reaction suggested by Hartree (1972) for protein determination can be distinguished in two phases: copper and proteins react in a few minutes. One copper atom links up with one amino acidic residue out of four. In the second part, the Folin-Ciocalteu reactive added to the sample has a short and pH-specific reaction, because of which the sample becomes blue. Spectrophotometric analysis is undertaken as soon as possible, with 650 nm absorption light. Bovine serum albumin was used as standard. Variation coefficient: 6.6%.

Carbohydrate analysis: Carbohydrate analysis was carried out according to Dubois *et al.* (1956). The reaction between glucides and phenol in a strong acid medium (concentrated sulphuric acid) gives a colour increase proportional to glucide concentration, detectable in a spectrophotometer with 490 nm absorbance light. Standards of D(+)-glucose were utilized. The analysis coefficient of variation was 6.0%.

Lipid analysis: Lipid extraction was performed according to Bligh and Dyer (1959) with chloroform-methyl alcohol solutions. The Marsh and Weinstein's technique (1966), based on the reaction between lipids and sulphuric acid at high temperature (200°C), was applied. The colour increase is proportional to lipid concentration. A 375 nm absorbance light was employed for spectrophotometric analysis. Tripalmitine, dissolved in chloroform, was used as standard. Variation coefficient: 7.8%.

Carbon conversion factors: Carbon equivalents were obtained with the following conversion factors: 0.75 for lipids, 0.40 for carbohydrates and 0.49 for proteins.

Enzymatic methods

Because of the overwhelming bulk of natural hydrolytic enzymes, it is quite difficult to select three enzymatic systems capable of sustaining widespread diffusion between marine organisms and at the same time ensuring efficiency on the substrates. However, based on literature experience and recent scientific trends, trypsin (E. C. 3.4.21.4), β-glucosidase (E. C. 3.2.1.21) and lipase (E. C. 3.1.1.3), purchased from Sigma Chemicals Co. were chosen. These enzymes, since no commercial purified enzymes from marine organisms are available, are extracted from plants, bacteria and vertebrates. They have hydrolytic activities quite similar to natural marine organisms, and they are quite widespread among autotrophes and heterotropes (Dall and Moriarty, 1983; Morton, 1983). Enzyme solutions are prepared in TRIS (hydroxymethyl aminomethane) buffer at 0.05 M. To avoid undissolved enzyme crystals, solutions were filtered through Nuclepore policarbonate filters (0.2 μm pore diameter). Optimal pH and temperature conditions for purified enzymes are shown in Table I. From experiments carried out to determine the best analysis conditions, proper enzyme concentration was found at 80 mg in 1000 ml TRIS. Complete hydrolysis of digestible substrates can be performed with this solution (Fig. 1). The increase in enzyme concentrations had no effect on hydrolytic processes and damaged the replicability of samples, thus increasing variation coefficients. The most effective incubation times are shown in Figure 2 (a, b and c).

Analysis procedure: Analyses were conducted with paired and one blank sample filters in their plastic petri dishes. A sample of 5 ml of

TABLE I Optimal pH, temperature and time lag of hydrolysis for trypsin, β-glucosidase and lipase

Enzyme	*pH*	*Temp. (°C)*	*Time (min)*
Trypsin	7.6	25	15
β-glucosidase	5.0	37	120
Lipase	7.4	37	30

enzyme solution was pipetted on to one sample filter and on to the blank one, and 5 ml of TRIS on to the other sample filter (reference sample). After hydrolysis, each filter was carefully removed from its dish, placed in a filter-holder and rinsed with the solution remaining in the dish, to return any particles that may have floated off the filter, as well as with 5 ml of deionized water. Control sample results give the initial (total) concentration of proteins, carbohydrates and lipids. Blank sample results are relatively high for proteinase assay and quite negligible for β-glucosidase and lipase. Sample results, corrected with

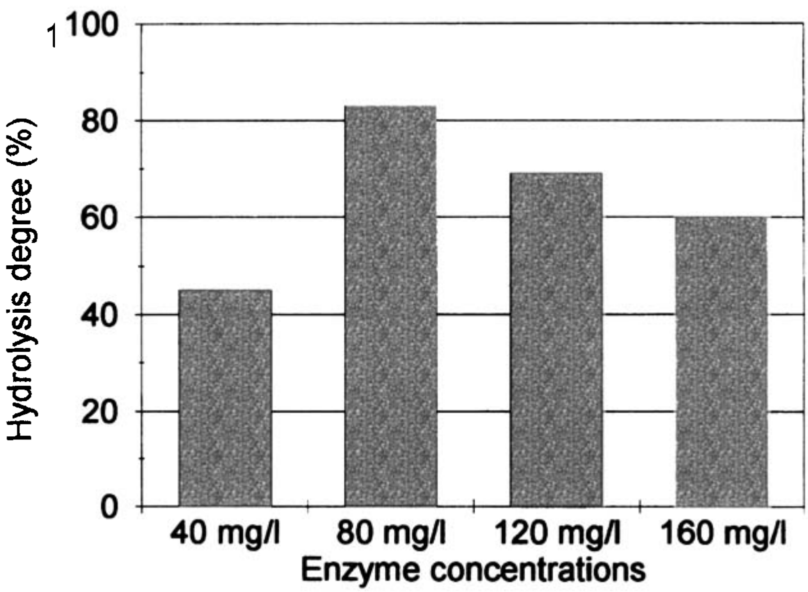

FIGURE 1 Hydrolysis (percentage) response to different trypsin concentrations in natural samples

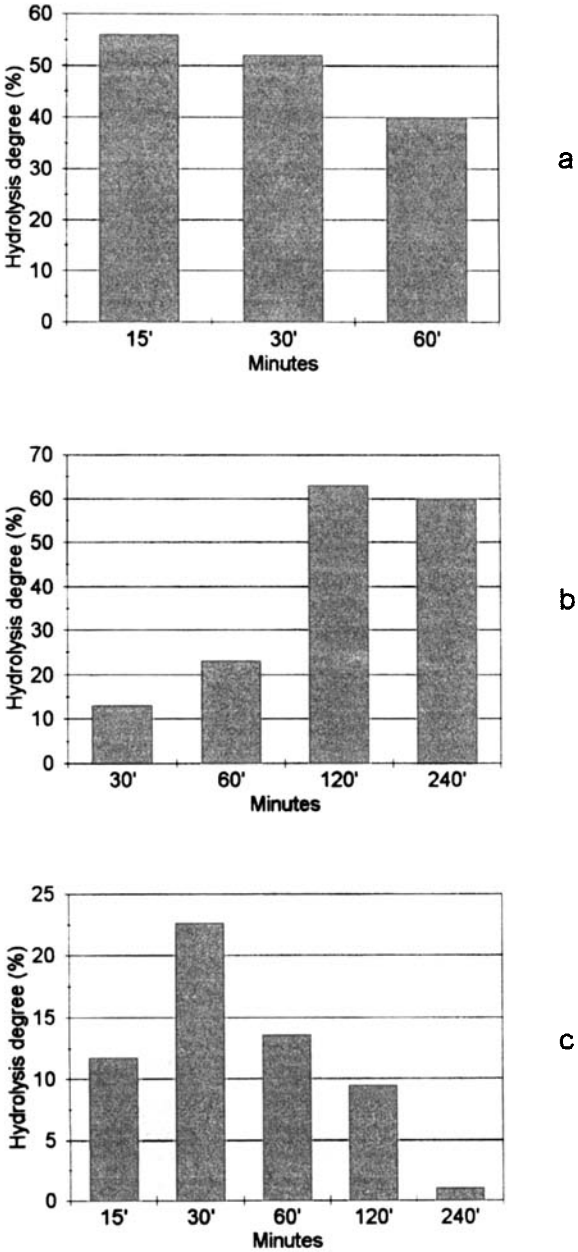

FIGURE 2 Hydrolysis response (percentage) to different incubation periods of the enzymes in natural samples. a: trypsin, b:β-glucosidase, c: lipase.

the blank results, give the concentration of refractory compounds, because of the cleavage of labile substances by enzymatic activity. The degree of hydrolysis is given by the percentage of labile vs the total compounds:

$$100 - [(SMP - BLK)/(REF - blk)]*100$$

where:
SMP = sample concentration;
BLK = blank concentration after enzyme solution treatment
REF = control sample concentration
blk = blank concentration before enzyme solution treatment.

CONSIDERATIONS

Among the different procedures used in the literature to assess the organic matter nutritional value, the total C/N ratio has often been used for suspended particulate matter as well as for sediment organic matter (Bodungen *et al.*, 1986; Nelson and Smith, 1986; Treguer *et al.*, 1988; Fabiano *et al.*, 1993). Russell-Hunter (1970) stated that high quality organic matter is characterized by a C/N ratio of less than 17, a value based on tissue C and N contents. Thus, this could be considered as an index of appetizing food for consumers when applied to living organisms. However, it does not seem to be as effective on detrital organic matter, originating from qualitatively and quantitatively different consumption and decomposition processes.

In the Marconi Gulf waters, the C/N ratio amounts to 6.0 average in surface waters and 10.5 in the waters close to the bottom. (Tab. III). With this index, a clear distinction is made between surface and bottom waters. The significantly higher value of the examined material at the surface may relate to concern nutritional values typical of microorganisms such as bacteria (Fukami *et al.*, 1985). However, by just measuring carbon and nitrogen content, no assessment can be made on the type of molecule in which these elements are included nor can this be established if the material is labile or refractory. Therefore, the C/N ratio can be used for quantitative estimates and only marginally to understand the nutritional value of the organic matter.

TABLE II Hydrolysis percentage for proteins (PRT), carbohydrates (CHO), lipids (LIP) and particulate organic matter (POM) in superficial and bottom water

Date	*Surface*				*Bottom*			
	PRT	CHO	LIP	POM	PRT	CHO	LIP	POM
04.11.93	100.0	38.1	52.0	74.7	81.5	70.9	35.2	66.4
30.11.93	99.6	27.5	15.9	69.1	76.0	0.0	16.4	49.9
08.02.94	81.1	29.0	0.0	56.1	69.0	12.5	28.0	50.6
02.03.94	98.9	46.7	32.5	68.5	53.5	32.1	0.0	24.2
07.04.94	96.3	30.5	47.0	70.4	82.1	11.2	30.4	47.3
27.04.94	78.3	17.0	32.1	57.2	94.3	18.5	17.8	65.7
25.05.94	81.7	8.4	57.5	63.7	100.0	7.5	11.9	63.1
07.06.94	100.0	0.0	0.0	62.2	100.0	27.6	40.2	71.7
25.07.94	100.0	48.6	45.8	69.8	100.0	15.6	35.4	67.8
07.09.94	100.0	30.6	63.6	73.9	69.7	0.0	54.9	53.0
28.09.94	92.7	50.5	52.4	79.6	100.0	2.3	55.3	71.3
12.10.94	100.0	45.1	54.2	81.6	79.8	0.0	63.6	62.9
16.11.94	100.0	3.1	60.0	65.0	71.1	0.0	63.3	50.4
14.12.94	100.0	67.8	57.0	80.4	71.1	9.4	71.6	58.4
22.02.95	58.6	56.6	55.4	57.4	60.9	56.2	49.1	56.6
AVG	92.5	33.3	41.7	68.7	80.6	17.6	38.2	57.3

Similarly, the POM/TSM ratio, where POM is the sum of protein, carbohydrate and lipid bulks and TSM the total suspended matter, can be considered as a quantitative food index (Fabiano *et al.*, 1993; Navarro *et al.*, 1993). This ratio has been used successfully also on detrital organic matter. However, as far as our data are concerned, this index fails to identify any significant difference between surface and bottom water.

Referring to the POM (total)/TSM ratio (Table III), average values range between 14.5 and 16.1 for surface and bottom waters. Conversely, when considering the POM hydrolyzable portion only, these figures are

TABLE III Comparison between different food indices applied to Marconi Gulf data

Food index	*Surface*	*Bottom*
C/N	6.0	10.5
POM tot/TSM	14.5	16.1
POM hyd/TSM	9.9	9.0
PRT tot/CHO tot	2.6	3.2
PRT hyd/CHO hyd	12.5	30.3

much lower (9.9 and 9.0). This ratio, although able to give us an idea on the availability of suspended particulate matter, provides scarce information on its actual nutritional value. In fact, organic matter is used with different strategies depending on nutritional needs, and biochemical components have different features with different nutritional values for consumers.

The POM value has been used to estimate the labile fraction of particulate organic matter (Fabiano *et al.*, 1984; Tanoue, 1985; Fichez, 1991). However, although biochemical components are supposed to be digestible by organisms, the efficiency of hydrolyzing tools was not taken into account. With the hydrolytic approach, these limitations are overcome and a suitable estimate of the labile fraction as well as a good index of potential utilization of the organic matter can be obtained.

Hydrolyzed labile POM in the Marconi Gulf is about 60% of total labile matter, which provides evidence of the oligotrophic conditions in the Ligurian Sea (Table II). Hydrolysis percentage can be quite different among the three biochemical components. The protein pool is known to be more sensitive to hydrolytic attack than the other fractions, and it is likely to be utilized and modified before other more refractory products like carbohydrates, which may contain aromatic compounds (Williams and Carlucci, 1976; Hollibaugh and Azam, 1983; Newell and Field, 1983; Bodungen *et al.*, 1986; Muller *et al.*, 1986; Fabiano *et al.*, 1992). These assumptions are in agreement with the experiments carried out on enzyme hydrolytic efficiency, showing that proteinases, like trypsin, are more effective than β-glucosidase and other carbohydrate hydrolases (Antranikian, 1992). Moreover, in our experience, protein hydrolysis often reaches 100 percent (Table II).

During algae growth, the bulk of phytoplankton biomass shows a carbohydrate fraction predominance, and the newly produced proteins are quite difficult to be digested by hydrolytic enzymes because of the strong cell wall of living phytoplankton. Similarly, resuspension from the sediment of substrates linked with phenols or other compounds, which increase their refractory features, is responsible of hydrolysis degree variations. Other processes such as grazing and decomposition, modify the quantity and the chemical composition of seston particles (Fabiano *et al.*, 1992). Damaged phytoplanktonic cells, zooplanktonic faecal pellets and the bulk of excretive substances, which characterize the post-productive periods, are a good substrate for bacterial colonization and growth (Dowgiallo, 1970; Rice and Tenore, 1981; Krog *et al.*, 1986). Moreover, hydrolysis, expecially for lipids, may be related to zooplanktonic activities and to their life cycle and physiological status (Fabiano *et al.*, 1992).

The proteins/carbohydrates ratio (PRT/CHO) seems to be suitable for a qualitative study of organic matter (Fabiano *et al.*, 1993). Low values of PRT/CHO ratio, generally, indicated a mainly phytoplanktonic seston and/or the presence of an aged detritus, leading to a particulate organic matter of low nutritional quality for consumers (Nival *et al.*, 1976; Roy *et al.*, 1991; Pusceddu and Fabiano, 1996). On the other hand, the colonization of fresh algae-derived detritus by microorganisms increases the detritus nutritional value, with a higher PRT/CHO ratio (Fukami *et al.*, 1981).

The difference between total PRT/CHO ratio of surface waters in the Marconi Gulf (2.6 on average) and of bottom waters (3.2 on average) does not seem to be significant (Table III), thus leading to the same conclusions offered by a quantitative food index as POM/TSM. The hydrolyzed PRT/CHO ratio is more realistic, since it is based on the capacity of organisms to consume the organic matter and not rather its quantity. It is also a more sensitive parameter: it can detect a rather significant difference between the environment of surface (12.5) and bottom water (30.3), while stressing the importance of the area close to sediments in organic matter change processes. Despite the constant resuspension of refractory materials in this area, decomposition processes of the solid organic structures into easily assimilable materials are promoted by organic matter build-up.

CONCLUSIONS

Following the analyses carried out on samples from the Marconi Gulf, some conclusions can be drawn:

— The enzymatic method overcomes the evaluation of the labile organic matter intended as sum of proteins, lipids and carbohydrates, while it focuses on the lability and nutritional value of the main biochemical fractions of suspended matter.

— The hydrolyzed labile organic matter seems to be remarkably less abundant than labile organic matter (indicated as the sum of lipids, proteins and carbohydrates).

— The hydrolyzed PRT/CHO ratio seems to be the most suitable food index. It can give a more sensitive and realistic qualitative estimate of particulate organic matter nutritional value. This result further supports the observations made on total PRT/CHO ratio and supplies additional information to quantitative indices like POM/TSM, which failed to identify any differences between the two layers, and C/N, which gave an indication on the total amount of nitrogen containing organic material. The latter, although more abundant in surface waters, was not necessarily more appetizing.

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