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CHARACTERIZATION OF THE MAIN SOURCES OF CHITIN IN CUBA

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The chemical composition and thermal behaviour of crab, shrimp and lobster shells were studied. The lobster cephalothorax and its main parts also constitute important sources of both polymers. Their chitin and protein content are 20 and 40 %, respectively (dry base) while in these cases the proteins are less associated to the matrix than in the carapace. The chitin level in the chitinous concentrates isolated from different sources is over 80 % in all cases but the polymer characteristics change in dependence on the raw material.

Chitin is not a simple polymer, but a family of products narrowly related and derived from natural chitin-protein complexes [1-3] which join properties that make them particularly attractive in their study and useful in almost all industrial branches.

The solubility, molecular weight, acetylation degree and the specific rotation of the chitin samples, depend on the isolation methods and the species used as source [3, 4].

The production and trade of chitin depend, among other factors, on the composition and characteristics of the raw materials. This situation justifies the investigations that have been carried out on the cuticles of differents species of crab and shrimp, taking into account that these shells have constituted up to now, the raw material for the industrial production of chitin and its derivates [5, 6].

In Cuba the high volume of residual crustaceans is obtained from the prosecution of the lobster. The entire waste and their integral parts constitute the principal potential source of both chitin and protein, but shrimp and crab shells may also be used.

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The objective of this work is the physico-chemical characterization of all these materials.

Experimental

The residues of lobsters, crabs and shrimps were obtained in the zone of the Batabano Gulf (Southwest Cuba) during the first months of 1983. The samples of lobster were divided in two aleatorie lots. In the first the integral cephalothórax was processed and from the other one the shell, legs, antennae, internal tegument of the shells and the rest of the cephalothorax were analyzed separately.

The crab, the shrimp, the branchial lobster and the pleon lobster shells were cleaned of all mass residues, dried under laboratory conditions and finally ground. The other materials were triturated and kept frozen until use.

The humidity and ash contents were evaluated according to AOAC methods [8]. The values were compared with the results obtained from the thermogravimetric curves (TG).

The nitrogen was determined according to the Kjeldahl method [9]. The N content determined in the residue after an extraction with 5 % NaOH during 4 hours at 100° was considered as the chitinous one.

The chitin and protein contents were also calculated by the empirical equation, derived on the basis of the TG curves [10].

The thermal analysis was performed in a Derivatograph (MOM, Hungary). Samples of 100 mg size were analyzed in air at a heating rate of 10 deg/min.

The % of acetyl groups was calculated also on the basis of the TG curves [11].

The free protein was removed from all the materials by extraction with water at room temperature during 4 hours. For the extraction of the associated protein double and successive treatments during 30 min at 80° with 0.5 and 2 % NaOH solution were realized. The chitinous concentrate was obtained with acid/basic treatments according to [12] reported before.

Results and discussion

The whole dry shell of the lobster represents 16.5 % of the fresh animal weight. 3.7 % corresponds to the pleon shell and 12.8 % to the branchial

Table 1 Chemical composition of the cuban crutacean shells

Shell	Hum. %	Ash %	Ch %	P %	N %
Branch. cephalothorax	10±1	37±1	10±1	12±1	2.5±0.2
Pleon	10±1	26±1	25±3	19±2	4.8±0.1
Pink shrimp	9±2	28±1	29±2	18±3	4.9±0.1
Blue crab	11±1	31±1	14±2	19±3	4.0±0.1

Table 2 Protein fractionation of the shells

Shell	P_t %	Free P %	Associated P		Residual
			OH^- 0.5%	OH^- 2%	P %
Branc. cephalothorax	12	2.5±0.2	2.3±0.4	5.8±0.4	1.6±0.1
Pleon	19	2.2±0.4	9.7±1.1	5±1	0.2-1.4
Pink shrimp	18	3.1±0.4	8.5±0.7	5.4±1	0-1
Blue crab	19	11.9	5.3	0.9	0-1

Table 3 Chemical composition of the lobster cephalothorax and its main parts

Source	Ave, (1) Weight	Hum.%		Ash %			Ch %		Prot. %		N
		A	B	A	B	B	A	B	A	B	%
Entire cephal.	-	64±4	59	19±2	17	11	8±2	6	14±1	11	2.7±0.3
Branch. shell	18	10±1	10	54±3	54	37	10±1	10	12±1	13	2.5±0.2
Antennae	14	60±2	59	17±2	17	11	8±2	10	15±1	14	3.1±0.2
Legs	23	59±2	57	20±4	19	13	6±1	6	12±2	12	2.4±0.2
Cehphal. rest	43	64±4	67	10±3	8	5	8±3	8	16±2	17	3.1±0.3
Non calcified int. tegument	-	7±1	-	3.5±0.4	-	-	27±4	-	50±4	-	10.3±0.5

A traditional methods; B thermal analysis; (1) relative to the cephalothorax

Table 4 Protein fractionation of the cephalothorax

Source	P_t	P remov.	P residual	Free P	Associated P	
	%	%	%	%	OH^- 0.5%	OH^- 2%
Entire cephal.	14	13±0.8	N.D.	4.6	6	2.3
Branch. shell	12	10±0.6	1.6±0.1	2.5	2.3	5.8
Antennae	15	13.5±0.6	N.D.	5.7	5.4	2.3
Legs	12	11.8±0.2	N.D.	5.1	3.4	2.6
Cephal. rest	16	16.0±0.4	N.D.	7.3	7.2	1.5

cephalothorax one. Both cuticles differ in chemical composition as shown by data in Table 1. The pleon shell is a less calcified material with a higher chitin content.

The thermal analysis as a qualitative method of characterization allowed us to assess the differences and similarities between the lobster shells and crab and shrimp shells (Fig. 1).

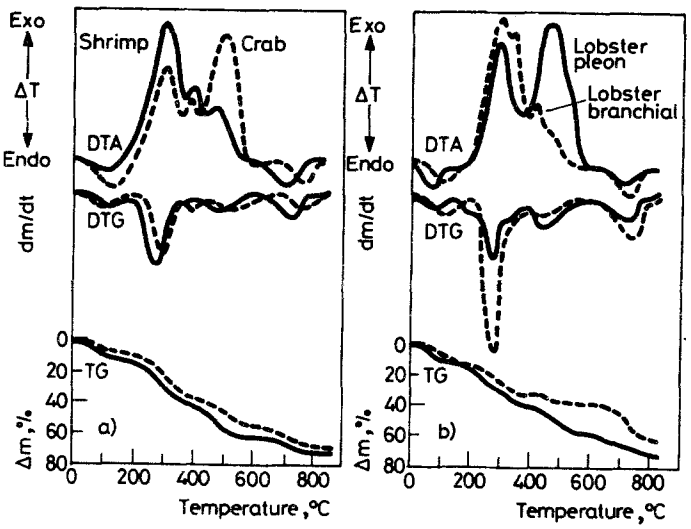

Fig. 1 DTA, DTG and TG curves of the shrimp, crab (a) and lobster shells (b)

For all the cuticles the typical thermal behaviour of the constituents of chitin, protein and carbonates were detected but with particularities for each source. So the effect at 720-740° owing to the decomposition of the carbonates [13] not only confirmed their presence in all the shells studied, but also that the branchial looster shell contained the highest inorganic material content.

For all the cuticles there appeared an effect at 300° characteristic of the decomposition of glycosamineglycan with very similar DTA peak intensity weight loss associated, though the last one is slightly greater for the shrimp shell. This behaviour is in correspondence with the higher chitin content detected in the shrimp cuticle. On the other hand in the DTG curve pleon of two shoulders at 200 and 320° were detected which constitute a difference from the other shells studied.

Nevertheless the fundamental differences among the species was found around 450-500°, in the zone of the second step of the protein degradation [13, 14]. In spite of the fact that the protein content is similar for the pleon of the lobster, the shrimp and the crab shells, the intensity of this peak is lower for the shrimp as well as for the branchial lobster shell. In addition, the peak temperaturs of this effect seems to constitute an index of the predominant presence of glycoproteins in these cuticles, except for the crab carapace where the protein seems to be not associated to the chitinous matrix.

The % free protein was not so high in accordance with the former results, and only in the case of the blue crab 60 % of the protein was easily removed with water (Table 2). About 80 % of the entire protein content in the lobster and shrimp shells seems to be retained by the chitin. Still after a drastic basic treatment remain 1.6 % of protein with high gly content in the lobster branchial shell [15].

Nevertheless the exclusive use of the lobster shell does not resolve satisfactorily the problem concerning the elimination and utilization of the crustacean wastes in Cuba. The cephalothorax itself constitutes 65 % of the fresh lobster weight and only 18 % of it belong to the branchial shell (Table 3).

The chemical composition of the cephalothorax and its parts can be assessed from the table where it is observed how the humidity, ashes, chitin and protein contents calculated from the TG curves coincide with the results obtained by means of the traditional methods.

The thermal behaviour of the cephalothorax and its integral parts indicate that they are essentially constituted by chitin, protein and carbonates (Fig. 2).

Significant differences among the appendix and the integral cephalothorax were not found and in all the cases the peak at 300-320° characteristic of the principal degradation of chitin as well as the exothermic effect in the region of 500° owing to the second etap of protein decomposition were observed in the DTG and DTA curves. The associated weight loss evaluated in dry materials was not different either. The endothermic effect characteristic of the thermal decomposition of the carbonates was detected at 740°. It should be mentioned that for the cephalothorax rest the peaks are, in general, wider as a consequence of the major heterogeneity of the material.

The protein fractionation proved that in spite of the high % of associated protein a treatment with 0.5 % aqueous NaOH is sufficient to remove practically 80 % of the protein present in the cephalothorax and its parts except

in the shell. 90 % of the cephalothorax rest protein is removed in this procedure (Table 4).

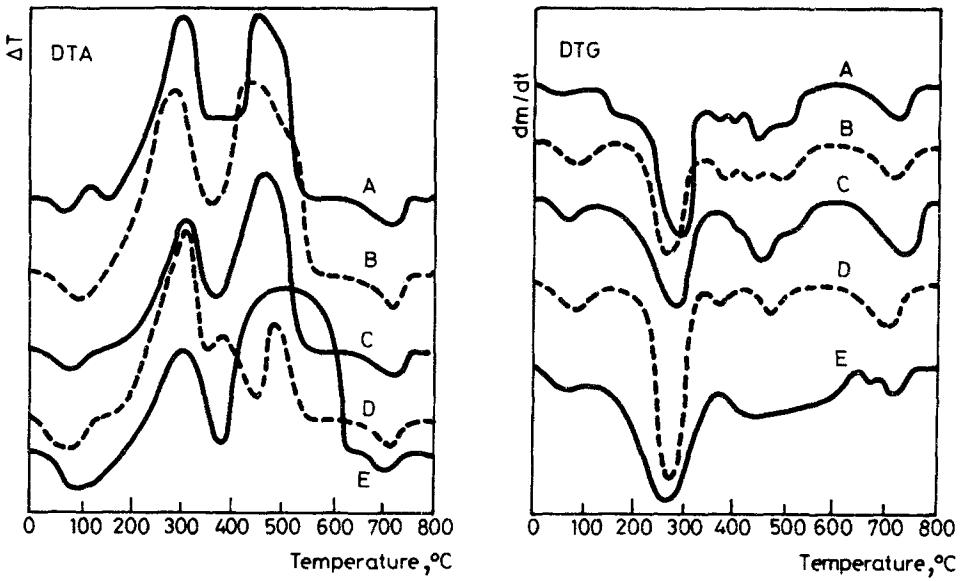

Fig. 2 DTA and DTG curves of the lobster cephalothorax and its main component parts. A lobster cephalothorax; B antennae; C legs; D inner tegument; E residual cephalothorax

Table 5 Chemical composition of the isolated chitins

Source	Hum.,%	% Ash, % 660°C	% P cont.	% Ch	Ac
Shrimp shell	8±2	0.5±0.2	0.4–3	90±1	16
Crab shell	5	0.5	0.3	94±1	17
Pleon lobst. shell	9±1	0.1	3	87±2	16–17
Branch. lobst. shell	8±2	0.2–1.5	2–3	90	16
Antennae	6	3	5–6	84	16
Legs	6	3	5	87	15
Cephal. rest	6	14	9	73	15–16
Entire cephal	6	2–7	6	84±1	15–16
Int. tegument	9.6±0.5	0	11	81	15

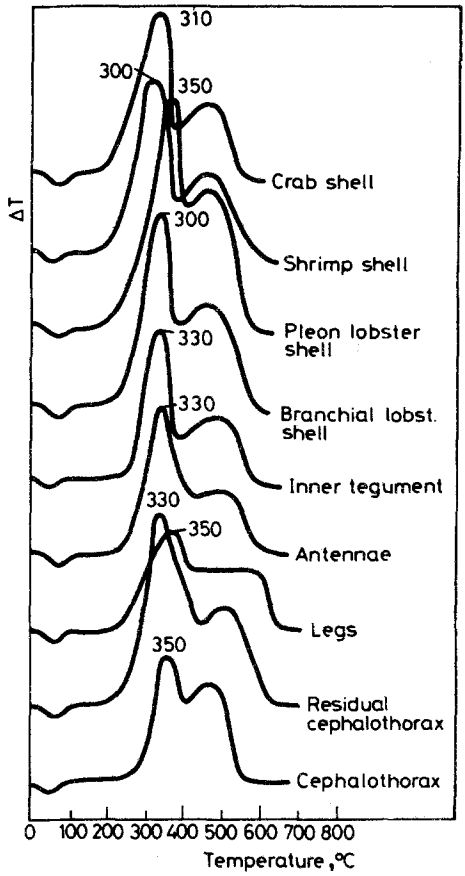

Fig. 3 Chitins DTA curves

The not calcified intern tegument of the shell does not constitute an appreciable part of the cephalothorax weight but the results are interesting as a consequence of its much lower ash content. The acid treatment was then unnecessary and only the extraction with NaOH is performed in order to preserve the original chitin characteristics as much as possible.

The composition of the chitinous concentrates obtained from the different sources is shown in Table 5. In all the cases the chitin contents exceed the average reported for the cephalothorax chitin [7] while the residual protein and inorganic concentration as well as the acetylation degree of the polysaccharide and the DTA peak temperature (Fig. 3) change according to the source. In general the chitin isolated from the shells is purer.

The differences among the chitins can be observed mainly in their physico-chemical characteristics and biological properties.

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Zusammenfassung – Es wurde die chemische Zusammensetzung und das thermische Verhalten von Krabben-, Garnelen- und Hummerpanzern untersucht. Der Hummer cephalothorax und auch seine Hauptteile bilden wichtige Quellen für beide Polymere. Der Chitin- und Proteingehalt beträgt 20 bzw. 40 % (bezogen auf Trockensubstanz), während in diesem Falle die Proteine weniger an der Matrix assoziiert sind als im Panzer. Der Chitingehalt in aus verschiedenen Quellen gewonnenen Chitinauszügen liegt in allen Fällen über 80 %, die Eigenschaften der Polymere schwanken jedoch in Abhängigkeit vom Ausgangsmaterial.