THERMOANALYTICAL STUDIES ON LOBSTER SHELL
II. Quantitative analysis

I. Garcia Alonso and *D. Oviedo Vega*

INSTITUTE OF FUNDAMENTAL BRAIN RESEARCH ACADEMY OF SCIENCES OF CUBA, HAVANA, CUBA

The chemical composition of lobster shells was determined by thermal analysis without previous sample preparation. The humidity and ash content were directly calculated from the TG curve. A correlation between the percentage weight loss is a particular temperature interval and the concentration of chitin and protein in the starting material was found, and based on this new method is proposed for the quantitative determination of both polymers. The residual protein after the deproteinization could be estimated with 0.3% error.

The decomposition of fresh lobster shells is characterized by main thermal effects at 100, 340, 520 and 730° associated with the water evolution and with the chitin, protein and carbonate degradation [1, 2]. The DTA, TG and DTG curves showed that the decomposition steps of the organic moiety are too complicated and no better resolution could be achieved in nitrogen atmosphere or under quasi-equilibrium conditions [1]. This study was carried out in order to elucidate the thermal effects due to chitin and protein and to suggest a way for a rapid, easy and simultaneous quantitative lobster shell analysis.

Experimental

Shells from lobsters captured in 1983 in the zone of Batabanó - Gulf, Cuba, were used. They were cleaned from any moss residue, dried at room temperature and ground in a ball mill.

Chitin-protein mixtures were prepared in weight ratios 7:3, 3:2, 1:1, 2:3 and 3:7. The chitin sample was obtained from the Cuban Pharmaceutical

Factory "Mario Muñoz" and the protein was casein, Merck p.a. Analyses were done in duplicate.

The ash and humidity analysis in the shell was performed by classical methods [3]. Nitrogen was determined by Kjeldahl method [4]. Proteinic nitrogen was evaluated by difference between the total nitrogen content and the one left in the solid sample after a treatment with 5% NaOH at 100° during 5 hours. The chitin concentration was estimated by the Hackman gravimetric method [5] as well as from the nitrogen content determined in the solid after protein extraction. The reported values are the average of both analyses.

A procedure previously reported was modified to obtain the protein fractions [6].

Thermal analysis was performed with a Derivatograph (MOM, Hungary). The sample weight was 100 mg. The heating rate was 5 deg/min and the atmosphere was air.

Results and discussion

The thermal curves of chitin-protein mixtures were similar to those of the shells, but in the models the first decomposition step in the DTG curve was
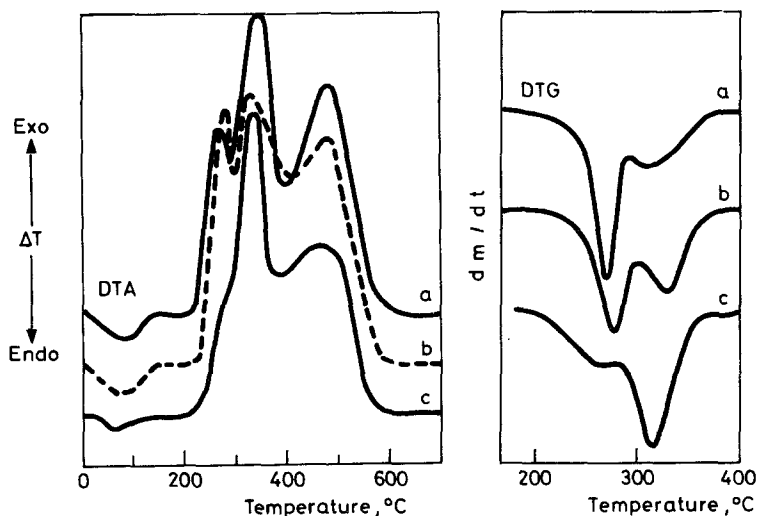

Fig. 1 Chitin: Protein mixtures. DTA and DTG curves. a) 30 % Ch + 70 % P; b) 38 % Ch + 62 % P; c) 62 % Ch + 38 % P

resolved in two overlapping peaks with relative intensities dependent on the protein-chitin ratio (Fig. 1).

Table 1 Weight loss % associated with the main thermal effects of the chitin-protein mixtures

T_p, DTG °C	Δm_0, %	Δm_1, %		Δm_2, %		
	280	320	340	460	480	500
Chitin			62±1			16[2]
Ch 70 % + P 30 %	12		46±2	23±1		
Ch 62 % + P 38 %	16	41±2		25±0.5		
Ch 51 % + P 49 %	20	35±1			28±1	
Ch 42 % + P 58 %	23	30±1			30±0.3	
Ch 38 % + P 62 %	24	28±1			31±0.2	
Ch 30 % + P 70 %	27	23			33±1	
Protein	32		6[3]			40±0.5

1 Δm_1 begins at 220°C

2 Δm evaluated in the complete range 360-600°C is 33±1%

3 Δm reported for the temperature range. There is any DTG effect

Table 2 Experimental and calculated content in the chitin:protein mixtures

Chitin, %	Calc. chitin, %[1]	Protein, %	Calc. prot., %[2]	Calc. prot., %[3]
100[4]	94	0	0.6	0
70	71	30	30	31
62	62	38	38	42
51	51	49	50	52
42	42	58	59	60
38	41	62	62	62
30	30	70	71	70
0	6	100[4]	94	94

1 Ch % = 1.6 Δm_1 − 0.1 prot. %

2 P % = 2.6 Δm_2 - 0.65 Δm_1

3 P % = 2.6 Δm_0

4 Considering samples humidity free

Correlation coefficient between experimental and calculated values:

Ch: 0.997   P[2]: 0.997   P[3]: 0.996

The analysis of the results revealed that the weight loss between 180-280° (Δm_0) fundamentally corresponds to the protein decomposition while that

occurring in the 280-360° range (Δm_1) is due to the chitin degradation with certain contribution from the residual protein decomposition (Table 1). Also the presence of both polymers affect the weight loss detected between 420-560° (Δm_2).

On this basis the following empirical relations were established:

Protein % = 2.6 Δm_0

Protein % = 2.6 Δm_2 − 0.65Δm_1

Chitin % = 1.6 Δm_1 - 0.1 Prot. %

The correlation coefficients between the experimental and calculated values are 0.99 in all cases (Table 2).

Table 3 Chitin and protein content calculated in the lobster shell

Exp.	ΔT_1, °C	Δm_1, %	ΔT_2, °C	Δm_2, %	Ch, %	P, %
1	300-360	7.2	440-540	7.8	9.9	15.6
2	300-360	6	440-560	6	8.4	11.7
3	310-370	7	420-520	6	10.1	11.1
4	320-390	6	440-540	7	8.2	14.3
5	300-360	7	440-540	7	9.6	13.6
Average value	310-370	6.6	440-540	6.8	9.3	13
Stand. deviation	10	0.5	10	0.7	0.9	2

Table 4 Chemical composition of the lobster shell

	Hum, %	Ash, % $T=600°C$	Ash, % $T=900°C$	CO_3^{2-}, %	Ch, %	P, %
Experimental	10±1	55			10±1	12±1
Calc. from TG curve	11±1	54±3	34±2	45	9.3±0.9	13±2

Table 5 Influence of the heating rate over the calculated values

Heat. rate, deg/min	Δm_1, %	Δm_2, %	Protein, %	Chitin, %
2.5	9.8±0.2	7.3±0.3	12.5±0.7	14.5±0.7
5	7.7±0.7	6.2±0.6	11±1	11.3±0.9
10	5.1±0.6	6.4±0.8	16±2	6.5±0.7

The former relationships were applied to the determination of chitin and protein in the original shell (Table 3) and it could be demonstrated that thermal analysis not only allows the qualitative characterization of the lobster shell but also the quantitative evaluation of the humidity, ash, chitin
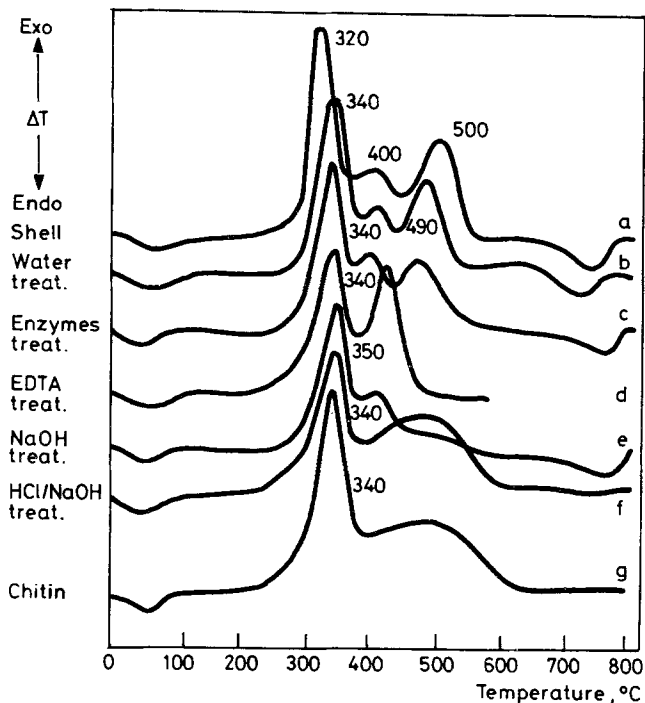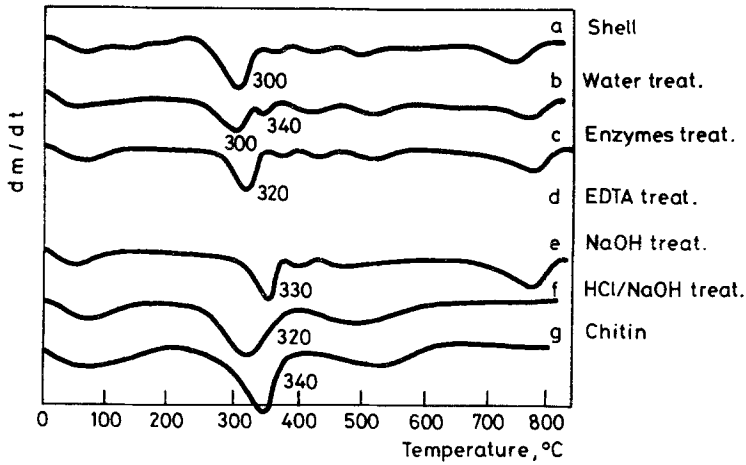
Fig. 2 DTA curves of the lobster shell after deproteinization treatment. a) original shell; b) residue water treatment; c) residue enzymes treatment; d) residue EDTA treatment; e) residue NaOH treatment; f) residue HCl/NaOH treatment; g) chitin

and protein contents in a single sample. There are no significant differences between these results and the ones obtained by classical methods (Table 4).

Nevertheless it should be pointed out that the heating rate affects the Δm associated with the thermal effects and consequently the calculated concentration of the polymers. Nor quicker neither slower heating rate than 5 deg/min result in a good agreement between calculated and experimental chitin concentrations. The influence of this parameter (Table 5) was not detected for the protein determination.

On the other hand, after the different treatments used for the partial deproteinization of shells [6], the temperatures of the thermal effects in the pellets were the same, but the peak intensities at 300° increased and at 500° decreased in proportion to the degree of protein removal (Figs 2 and 3).

Fig. 3 DTG curves and Δm values of the shell and deproteinization residues. a) original shell; b) water treatment; c) enzymes treatment; d) EDTA treatment; e) NaOH treatment; f) HCl/NaOH treatment; g) chitin

Sample	$\Delta m, \%$			
	60–120°	240–360°	360–540°	>540°
a	10	16	15	53
b	11	19	14	48
c	11	21	13	49
d	10	29	24	37
e	4	16	11	69
f	8	53	36	2
g	8	57	34	0

Table 6 Relation between the protein content and the Δm evaluated at 240-340°C temperature range

Treatment	P, % exp. (y)	Δm^1, (x)	P, calc. $(\hat{y})^2$ %	$(y - \hat{y})$
shell	12	44.4	11.5	0.5
water	9.5	47	9.8	0.3
enzymes	6.5	52	6.4	0.1
EDTA	4	55	4.4	0.4
NaOH 2 %	3	57	3.0	0
HCl/NaOH 2 %	1.6	59	1.7	0.1
chitin	0	62	–0.3	0.3

[1] for dry and ashless material

[2] $y = 41.69 - 0.68 \, x \quad r = 0.997$

The weight losses associated with both steps also change, mainly the one appearing between 240-340° (Table 6).
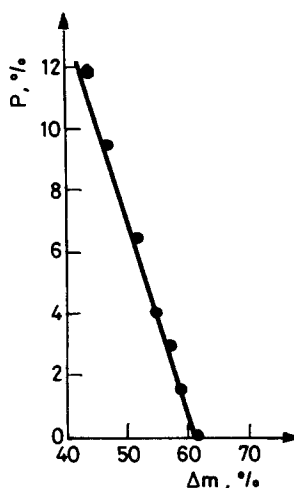
Fig. 4 Correlation between the protein content and the mass loss evaluated in the temperature interval 240-340°C

A linear relationship between the remainder cuticle protein and the weight loss in the former range was established (Fig. 4) and in this way an easy and rapid method was found to evaluate the protein content in chitin with 0.3 % error.

Once more, thermal analysis constitutes a useful method for the characterization of complex materials and without any sample pretreatment it is possible to determine the chemical composition of lobster shells avoiding the inconvenience and errors of the classical methods usually employed for quantitative chitin determination [5].

References

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Zusammenfassung — Mittels Thermoanalyse wurde ohne Probenaufbereitung die chemische Zusammensetzung von Hummerpanzern ermittelt. Feuchtigkeits- und Aschegehalt wurden aus der TG-Kurve direkt berechnet. Es konnte ein Zusammenhang zwischen der

prozentuellen Massenabnahme in einem bestimmten Temperaturintervall und der Konzentration von Chitin und Protein in der Ausgangssubstanz ermittelt und auf dieser Grundlage ein neues Verfahren zur quantitativen Bestimmung beider Polymere vorgeschlagen werden. Das nach der Deproteinierung verbleibende Protein konnte mit einem relativen Fehler von 0.3 % bestimmt werden.