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EFFECT OF LOW TEMPERATURE ON THE LIPID COMPONENT OF CHITIN AND CHITOSAN STRUCTURES

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Phospholipids and fatty acids in chitin and chitosan samples undergo qualitative and quantitative changes upon cryogenic treatment.

Key words: natural chitin, chitosan, composition, phospholipids, fatty acids, cryogenic treatment.

The structures of chitin and chitosan have not yet been finally established. The structure of natural chitin is thought to be the homopolymer β-(1→4)-N-acetyl-D-glucosamine associated with proteins, glucans, lipids, and carotenoids [1, 2]. However, natural chitin has been shown to contain glucosamine in addition to N-acetylglucosamine units [1]. The former are potentially highly reactive and form bonds from chitin to other components. Chitin—protein complexes have been thoroughly studied [1-5]. Little information is available on polymer—lipid association [1, 2] although it has been proposed that lipids play an important role in chitin biosynthesis [1]. We observed previously in chitin and chitosan phospholipids (PL) and fatty acids (FA) [6], which can contribute to the pharmacological activity if they enter the structural component of the polymer [7-12]. Furthermore, chitin and chitosan structures may be partially similar to that of lipid A if lipid components are present [13, 14]. Similar pharmacological properties of chitin and lipid A have been reported, in particular, immunomodulatory ones [7, 13, 15].

We present results of single and identical investigations of samples obtained from a single source.

The total lipid content of the studied samples was:

Sample*	Lipids, % (per dry weight)
1	0.65
2	0.50
3	0.20
4	0.20
5	0.17
6	0.19

*Sample numbers are the same as in Experimental.

Cryogenic treatment of samples 1, 3, and 5 was used to improve the taste quality of feed chitosan [16].

Crab-shell samples (1, 2) contained an array of three PL: phosphatidylcholines (PC), phosphatidylethanolamines (PE), and phosphatidylserines (PS). The main one was PC judging from the intensity of the spots on the chromatograms. In chitin subjected to cryogenic treatment (4), a lipid assigned by us as diphosphatidylglycerine (DPG) was found in addition to PC. In chitin (5), only DPG was found; in cryogenically treated chitosan (6), an additional unidentified PL.

Cryogenic treatment of the samples produced both quantitative and qualitative changes in the FA composition (Table 1).

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TABLE 1. Qualitative and Quantitative Compositions of Fatty Acids (% GLC)

Fatty acid	Fatty acid content relative to MEFA* (% of mass)					
	shell**		chitin**		chitosan**	
	1	2	3	4	5	6
12:0	1.2	1.4		4.2	5.9	1.6
13:0					4.2	1.6
14:0	6.2		12.4	4.2	7.7	2.1
14:0-iso				3.2		
14:1	3.8	3.1		3.2		2.6
14:1-cis		3.0				
15:0	3.2	1.6			5.4	2.1
15:0-anteiso					4.2	
16:0	10.7	14.9	22.7	27.6	16.5	18.9
16:0-neo			2.7	2.1		
16:0-iso	1.1					
16:1(6)	8.9	11.1	12.1	11.2		
16:2	3.4	8.2		3.3	6.7	5.1
16:3 (7, 10, 13)				3.3		3.7
17:0		5.0				
18:0	2.0				3.3	8.4
18:0-iso	4.7			2.6		
18:1(9)	22.6	18.1	19.1	4.4	6.2	26.1
18:1-cis				11.6		
18:2(9, 12)	10.1			7.0	9.0	10.9
18:2(6, 9)		4.2	5.4			3.8
18:3(9, 12, 15)			3.4	2.0		1.9
18:4			5.8			
19:0					18.3	
19:0-anteiso		11.0				
19:yn	4.1			2.3	2.8	
20:0	2.2				1.9	
20:1		7.3				9.0
20:yn	5.1	7.1	1.5	4.1	6.1	Tr.
20:3(8, 11, 14)	1.9	1.8			Tr.	
20:4(5, 8, 11, 14)			3.0	Tr.	0.3	
21:0	3.0			1.2	1.7	2.3
22:5(7, 10, 13, 16, 19)	1.6	2.2				
Unidentified	4.4		9.3	0.4		

*MEFA = methyl esters of fatty acids. Results are averages of 3-5 analyses. **Sample numbers are the same as in Experimental.

The content of 16:0 and 16:2 acids increased markedly, new components appeared, and certain acids disappeared in the shell (2).

The 16:0 acid predominates among the FA of chitin (samples 3 and 4, 22.7 and 27.6%, respectively). Cryogenic treatment produced about 10 new components with increased fractions of 18:1 and 18:2 (9, 12) acids.

The most significant change of chitosan (5, 6) FA composition is an increase of 18:1 (9) content from 6.2 to 26.1% and the disappearance after treatment with liquid N_2 of 20: yn and 19:0, which make up a significant fraction of the total FA mass of sample 5.

Our data indicate that PL are probably packed into natural chitin. They become available for extraction after treatment with liquid N_2. We think that sufficiently harsh conditions for isolating chitin and chitosan make it possible to gradually separate PL from the polymer. Whereas crab shell contains three types of PL, chitin has only one, PC. After subjecting the

polymer to the harsh conditions required to obtain chitosan, PC also disappear and DPG becomes available for extraction. Additional cryogenic treatment of the polymers probably loosens further the structure. As a result, additional DPG are found in chitin; unidentified PL, in chitosan.

Thus, the initial data indicate the presence of several PL and FA in the chitin and chitosan structures that are observed after cryogenic treatment of the polymers.

EXPERIMENTAL

We used the following samples:

1) shell of boiled kamchatka crab *Paralitodes camtschatica*;
2) shell of boiled crab held in liquid N_2 for 20 min;
3) chitin isolated from shell (sample 1);
4) chitin isolated from shell (sample 1) with subsequent treatment with liquid N_2 for 15 min;
5) chitosan obtained from chitin (sample 3);
6) chitosan obtained from chitin (sample 3) and treated with liquid N_2 for 15 min.

The fraction of particle size 2 mm was selected for the study after grinding and sizing.

Preparation of Chitin and Chitosan. Chitin from crab shell was isolated by two demineralizations (1 N HCl for 3 h at 23-25°C) alternating with two deproteinizations (1 N aqueous NaOH for 1 h) [16, 17].

Chitosan was prepared by deacetylation of chitin with 50% aqueous NaOH for 0.5 h at 100°C [16].

Lipid Extract. Lipids were extracted by a combined method [18, 19]: a sample (25 g) was treated with $CHCl_3$—CH_3OH (75 mL, 1:2 v/v) and stored under Ar for 2 d in a refrigerator. The supernatant was decanted. The solid was re-extracted with $CHCl_3$—CH_3OH (75 mL, 2:1) and left under Ar for 1 d. The combined extract was treated with water (20%). After the phases separated, the $CHCl_3$ layer was concentrated using a rotary evaporator.

PL Analysis. Qualitative and quantitative analyses of PL were performed using two-dimensional micro-TLC [20]. Two solvent systems were used: $CHCl_3$—CH_3OH—NH_4OH (aq., 25%), 65:35:5 (I); $CHCl_3$—CH_3OH—$(CH_3)_2CO$—CH_3CO_2H—H_2O, 50:10:20:10:5 (II). PL were visualized using H_2SO_4 in CH_3OH, phosphomolybdic acid, ninhydrin, and Dragendorff's solution. Photometry was performed at 825 nm on an SF-26 spectrophotometer.

FA Analysis. Methyl esters of FA were prepared by the literature method [21]. Chromatograms were recorded on a Tsvet-100 GLC with a flame-ionization detector, a glass column (3 m × 3 mm), 6.5% diethyleneglycoladipate on Chromaton N-AW, carrier-gas (Ar) flow rate 35 mL/min, and palmitic acid internal standard.

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