1. Introduction

The red king crab is one of the most valuable species of industrial cancroid. By its exceptional gustatory properties, a high food value and huge sizes this crab by right deserves to be called “king crab” [1]. Besides, a shell of cancroid is a raw material for the production of such valuable natural polymer as chitin. The complex application of the cancroid shell allows the solution of an urgent ecological problem, namely, the decrease of contamination of cancroid catching regions with waste of their processing [2]. In connection with the foregoing, it is extremely important from point of scientific and applied view to study physicochemical properties of chitin prepared from the shell of red king crab at various stages of its molting cycle.

2. Materials and method

As a method of research works, the differential thermal analysis (DTA) was used [3 - 5]. An experiment in the range of –190 to 400 °C was conducted in helium atmosphere with using a device developed in the laboratory of thermochemistry at the Research Institute of Chemistry Nizhni Novgorod State University [6]. Quartz served a standard. The mass
of a sample and the standard was \( \sim 0.25 - 0.35 \) g. The temperature of the sample and a
difference in temperatures of the sample and the standard were measured with a chromel-
copel thermocouple within to 0.5\(^\circ\)C. The rate of heating in experiments was 50/\(\text{min}\). A
deviation from linearity did not exceed 1\%. To check the DTA device operation the melting
temperature (\(t_m\)) of standard n-heptane and the glass transition temperature (\(t_g\)) of purified
glycerin were determined. The results obtained by us coincided with the corresponding
reliable literature data for n-heptane [7] with an uncertainty of 0.2 °C and glycerin [8] 1 °C.

Seven samples of chitin-protein complex prepared at Russian Federal Research Institute of
Fisheries and Oceanography (VNIRO) (Moscow) were examined. Sample No1 was formed
from exuvium that was shed during the shell molting. An undershell film did not peel. Sample
No 4 was prepared from the shell in about one day after the molting (of an exoskeleton). It
is a stage of postmolting. In this case, the shell is soft and flexible. As to sample No 2, it was
produced from the shell in ~ 4 - 5 weeks after the molting occurred (stage of postmolting).
Sample No 3 was produced from the shell in about 8 weeks after the molting. The shell is
hard, the muscle – filling is weak. It is a stage of intermolting. Sample No 5 was prepared
from the shell at an early stage of intermolting. The shell is hard and filled. Sample No 6
is a product of the shell at a late stage of intermolting. Sample No 7 was formed from the
shell at an early stage of premolting. Those were demineralized. A protein component of the
shell was not separated. Pigments – carotenoids (astoxanthine, xanthoxanthine) -remained
in them too. Our experiments showed that sorbed water contained in air-dried samples in
the amount of 6.6 to 10.5 mass.% (Table 1). Its evaporation was shown on thermograms as
an endothermal peak at \(t_{\text{vap}}(\text{H}_2\text{O}) = 121 – 128 \) °C (Figure 1).

A thermal chamber with the sample and standard was cooled with liquid nitrogen till
\(-190 \) °C at a rate of \(~ 20 \) °/\(\text{min}\). Then it was heated at a rate of 5 °/\(\text{min}\) up to \(~ 140 \) °C (the
first warming-up) to record the endothermal peak of evaporation of sorbed water (Figure 1,
curve 1). The heating was ended, the sample was cooled at a rate of 5 °/\(\text{min}\) down to the
room temperature and simultaneously the evaporated sorbed water was pumped out in
situ in the thermal chamber. Further a crucible with the sample was taken out from the
thermal chamber, weighed on an analytical balance and the quantity of water containing
in an air-dried sample was determined. Afterwards the sample was placed in an excicator
over CaCl\(_2\). The next day the dried sample was located in the thermal chamber and the
experiment was repeated with heating the sample up to \(~ 160 \) °C (the second warming-up).
The third warming-up was performed in a similar way. In course of the last 4\(^{\text{th}}\) warming-up
the samples were heated up to 350 °C (Figure 2) and the loss of mass relative to the sample
subjected to desiccation was determined.

3. Results and discussion

In Figures 1 and 2 thermograms of sample No 7 are demonstrated as an example. The
averaged values and some physicochemical characteristics of the samples studied are given
in Table 1.

As seen from the experimental results, the anhydrous samples of the chitin-protein complex
have relaxation transitions (\(\beta\)-type, 1\(^{\text{st}}\) and 2\(^{\text{nd}}\) glass transition) similar to those of samples of
chitin separated from a wide variety of sources that were studied earlier [9 - 12]. Temperature
Table 1. Physicochemical properties and mean temperatures of physical transitions in the chitin-protein complex isolated from red king crab shell at different stages of molting cycle.

<table>
<thead>
<tr>
<th>Sample</th>
<th>No 4</th>
<th>No 2</th>
<th>No 3</th>
<th>No 5</th>
<th>No 6</th>
<th>No 7</th>
<th>No 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stages of molting</td>
<td>Postmolting</td>
<td>Intermolting</td>
<td>Pre-molting</td>
<td>Molting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{\text{vap}}(\text{H}_2\text{O}), ^\circ\text{C}$</td>
<td>121</td>
<td>125</td>
<td>121</td>
<td>125</td>
<td>128</td>
<td>126</td>
<td>123</td>
</tr>
<tr>
<td>Content of H$_2$O, mass%</td>
<td>8.5</td>
<td>9.8</td>
<td>10.5</td>
<td>7.3</td>
<td>6.7</td>
<td>6.6</td>
<td>9.5</td>
</tr>
<tr>
<td>$t_{\beta}^\circ\text{C}$</td>
<td>61</td>
<td>85</td>
<td>66</td>
<td>64</td>
<td>62</td>
<td>50</td>
<td>81</td>
</tr>
<tr>
<td>$t_{\gamma 1}^\circ\text{C}$</td>
<td>113</td>
<td>118.5</td>
<td>111</td>
<td>117</td>
<td>117</td>
<td>123</td>
<td>113.5</td>
</tr>
<tr>
<td>$t_{\gamma 2}^\circ\text{C}$</td>
<td>144.5</td>
<td>143</td>
<td>144</td>
<td>137</td>
<td>136.5</td>
<td>145</td>
<td>137.5</td>
</tr>
<tr>
<td>$t_{\text{destr}1}^\circ\text{C}$</td>
<td>227</td>
<td>214</td>
<td>215</td>
<td>215.5</td>
<td>214.5</td>
<td>224.5</td>
<td>224.5</td>
</tr>
<tr>
<td>$t_{\text{destr}2}^\circ\text{C}$</td>
<td>291.5</td>
<td>245</td>
<td>274</td>
<td>300</td>
<td>299</td>
<td>295</td>
<td>266</td>
</tr>
<tr>
<td>Loss of anhydrous sample mass, mass%</td>
<td>16.1</td>
<td>22.2</td>
<td>25.4</td>
<td>30.6</td>
<td>38.3</td>
<td>23.9</td>
<td>13.6</td>
</tr>
<tr>
<td>Appearance of sample after destruction</td>
<td>Not resini-</td>
<td>Resini-</td>
<td>Resini-</td>
<td>Not resini-</td>
<td>Not resini-</td>
<td>Not resini-</td>
<td>Resini-</td>
</tr>
</tbody>
</table>

Figure 1. Thermograms of sample No 7 of chitin-protein complex: 1 – 1st warming-up; 2 – 2nd warming-up; 3 – 3rd warming-up.
of β-transition ($t_\beta$) connected with the libration of pyranose rings around glucoside links changes non-monotonously (Table 1, Figure 3). It approaches the maximum 81 - 85 °C for samples No 1 and 2 and the minimum (50 °C) in the case of sample No 7. As to other samples, the temperature is approximately identical (61 - 66 °C).
For some air-dried samples during the first warming-up the relaxation transition of endo-
thermal character at 46 - 48 °C can be seen in front of the endothermal peak of evaporation
of the sorbed water. It is likely to be classified as β-transition. Early studies [9 - 12] of the
effect of water on the physical transitions of chitin showed that it does not exert a subst-
stantial influence on \( t_\beta \).

The samples under consideration have two temperature intervals of devitrification (Table 1).
Such behavior of chitin is associated with the availability of microregions of different
degree of ordering (highly- and weakly ordered) in it [9 - 12]. Other polysaccharides studied
by us behave themselves similarly [13 - 17]. The temperature of the first glass transition
(\( t_{g1} \)) belonging to the process in amorphous microregions does not vary strongly. Sample
No 7 has the highest \( t_{g1} \). The change in the temperature of the second glass transition (\( t_{g2} \))
which is explained with the presence of ordered microregions in the polymer is illustrated in
**Figure 4**. As seen in **Figure 4**, the samples after molting (No 4 and No 2) and sample
No 3 of an intermolting period have the same temperature of the second glass transition
\( t_{g2} = 144 °C \). Then it decreases for samples No 5 and 6 till 137 °C, increases in the case
of sample No 7 up to 154 °C and it again becomes lower (137 °C) in the molting period
(sample No 1).

During the last fourth warming up the samples were heated up to 350 °C. At the same time,
their destruction was observed. Like earlier examined samples of chitin [10 - 12] the chitin-
protein complex decomposed in two stages that was accompanied by energy absorption
(**Figure 2**, curve 4'; **Table 1**). Histograms of destruction temperatures (\( t_{destr1}, t_{destr2} \)) of the
samples are shown in **Figures 5** and **6** (see page 22).

After the decomposition the samples were weighed and the loss of their mass as compared
to the anhydrous samples was estimated (**Table 1**). Besides, a normal change in the sample

![Figure 4. Temperature of the second glass transition of samples of chitin-protein complex
isolated from crab shell at various stages of molting cycle. Number of sample: 1 – No 4;
2 – No 2; 3 – No 3; 4 – No 5; 5 – No 6; 6 – No 7; 7 – No 1.](image-url)
mass was observed (Figure 7). Moreover, the samples looked in different ways. Some of them (No 2, 3 and 1) resinified and others (No 4, 5, 6 and 7) were non-resinified (Table 1).

It should be noted that the relaxation transition appeared for some samples at temperatures below 0 °C ($t_{r1}$) (see Table 1, Figure 1). It can be related to a protein component that is available in the samples of the chitin-protein complex. However, this fact requires additional studies.
Physicochemical properties of chitin-protein complex from shell of red king crab...

4. Conclusions

In conclusion it is worth of note that the studies have shown the substantial differences in the physicochemical characteristics of the chitin-protein complex isolated from the crab shell at various stages of the molting cycle. Perhaps, in the future the results of studies can be taken for the fair assessment of the corresponding step in the crab development.

Results investigations at present in this paper to report in XIII Seminar and Workshop on “New Aspects of the Chemistry and Applications of Chitin and its Derivatives” (17 – 19 September 2007, Wroclaw, Poland). The participation in the Seminar was supported with the Russian Foundation for Basic Research (grant No 07-03-08449).

5. REFERENCES

2. Nemtsev S.V.: Scientific grounds of complex technology of chitin, chitosan from shell of industrial cancroid and products based on them. Author’s abstract for a doctor’s degree, Moscow, 2006.

Figure 7. Mass loss of anhydrous sample of chitin-protein complex after destruction. Number of sample: 1 – No 4; 2 – No 2; 3 – No 3; 4 – No 5; 5 – No 6; 6 – No 7; 7 – No 1.