Activity of lysozyme and cystatin of hen’s egg white after concentrated microwave field (CMF) treatment

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Concentrated microwave field (CMF) method is one of the unconventional ways of food preservation where the most important are non thermal aspects. Inactivation of microorganisms is mainly achieved by non thermal influence of CMF what was confirmed in our previous studies. The aim of study was determination of the activity of lysozyme and cystatin of hen’s egg white where different parameters of CMF were used. The study was carried out on eggs collected from 42 week old Tetra SL laying hens kept in battery cages. CMF treatments were carried out in modified “Microwave Reactor RM2001” where power of microwaves was 325 [W] and 160 [W] for 60 or 120 seconds. Control was hen’s egg white without CMF treatment. It was concluded that CMF significantly decreased activity of lysozyme and cystatin in hen’s egg white what can be treated as an undesirable effect. The maximum decrease of both lysozyme and cystatin activity was found for 325 [W] and 120 [s] of CMF treatment: 22.2% and 45.2%, respectively. Nevertheless CMF method can be used in combine preservation systems together with other methods, but microbial inactivation as well as physicochemical, functional and biological aspects like activity of lysozyme and cystatin should be considered.

Keywords: lysozyme, cystatin, concentrated microwave field, hen’s egg white

Introduction

Food industry investigate more and more the replacement of traditional food preservation techniques like intense heat treatments, salting, acidification, drying and chemical preservation by new preservation techniques due to the increased consumer demand for tasty, nutritious, natural and easy-to-handle food products (Devlieghere et al., 2004). The most commonly used method of food preservation today is thermal processing. In spite of achieving good inactivation of microorganisms thermal treatment leads to unwanted reactions in food, involving the loss of flavour and nutrients (Aronsson et al., 2004). In the past years such preservation methods like aseptic processing, ionizing energy, modified atmospheres, oscillating fields, pulsed electric fields, microwave energy were observed to become more and more popular (Cardello, 2003). Many studies indicate microbiological effectiveness of these methods at good level of sensory characteristic of products (Knorr, 1998). Although the most studies are focused on high hydrostatic pressure and pulsed electric fields it must be stated that another methods are also investigated. One of them is concentrated microwave field (CMF) method where thermal effect is not the most important factor to inactive microorganisms. There is microbial reductions after CMF treatment what proved in our previous studies, but combined way of common treatment with another unconventional methods should lead to better reduction of microorganism. Nevertheless another aspects of CMF should be also investigated to compare that method with others. One of that aspect is activity determination of lysozyme and cystatin of hen’s egg white. That biologically active substances of egg white could be potentially sensitive on such factors like CMF treatment, so the main purpose of our study was investigation of activity of lysozyme and cystatin after CMF treatment.
Materials and methods

The studies were carried out on the eggs collected from 42 weeks old laying hens (Tetra SL) fed with fodder enriched with flax seed and fish meal where omega-3 polyunsaturated fatty acids level was higher than in standard fodder. Activities of biologically active components of egg white i.e. cystatin and lysozyme were analysed on 20 eggs from each experimental groups.

Activity of cystatin against papain was analysed according method reported by Siewiński (1991). Antipapain test is based on colorimetric method, in which amount of BANA (hydrochloride Na-benzoyl-DL-arginyl-B-naftylamid) hydrolysis products is measured as a result of cysteine proteinase (papain) activity. One unit of inhibitory activity correspond with one unit of enzymatic activity of papain, that is the quantity of enzyme which is able to hydrolyse 1.0 mM of substrate per minute in standard conditions (37°C).

Lysozyme was analysed by spectrophotometrical method. The principle of the method lies in dynamics of turbidity changes in the suspension made with bacteria Micrococcus lysodeiticus and lysozyme. The measurement were taken at wavelength $\lambda = 450$ nm at stable temperature of 25°C in every 60 seconds during 6 minutes test.

Concentrated microwave field treatments were carried out in modified “Microwave Reactor RM2001” where power of microwaves was 325 [W] and 160 [W] focused in central space of chamber. Treatment time was 60 and 120 seconds, so 5 different groups were investigated (together with control without CMF treatment). All the results were then statistically (n=3) analysed using Statistica 6.0 programme with one-way analysis of variance at the significant level of p=0.05.

Results and discussion

Egg white obtained from the hens was the object of the research. Collected eggs were analysed for the activity of biologically active components of egg white, i.e. lysozyme and cystatin, before and after CMF treatment.

The results showed that the CMF treatment used in the experiment significantly influenced the activity of cystatin (Table 1). The highest activity both of lysozyme and cystatine was found in egg white without CMF treatment (115 566 units and 33.40 units, respectively).

Table 1 Activity of lysozyme and cystatin in egg white before and after CMF treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Activity of lysozyme [unit]</th>
<th>Activity of cystatin [unit]</th>
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<tbody>
<tr>
<td>A) Before CMF treatment - control</td>
<td>115 566 a</td>
<td>33,40 a</td>
</tr>
<tr>
<td>B) After CMF treatment 160 [W] / 60 [s]</td>
<td>112 808 ab</td>
<td>25,99 b</td>
</tr>
<tr>
<td>C) After CMF treatment 160 [W] / 120 [s]</td>
<td>109 183 b</td>
<td>24,30 bc</td>
</tr>
<tr>
<td>D) After CMF treatment 325 [W] / 60 [s]</td>
<td>103 500 c</td>
<td>23,83 c</td>
</tr>
<tr>
<td>E) After CMF treatment 325 [W] / 120 [s]</td>
<td>89 916 d</td>
<td>18,31 d</td>
</tr>
</tbody>
</table>

The same letters at means indicate no significant differences at $p = 0.05$ for activity of lysozyme and cystatin separately

The maximum decrease of lysozyme activity was 22.2%, i.e. from 115 566 units for control to 89 916 units after 325 [W] and 120 [s]. Power of microwave seems to effect more for lowering of lysozyme activity comparing to treatment time. That relation is not so clear for cystatin where both factor (power of microwave and treatment time) seems to lower its activity in similar way. Influence of CMF is lower for lysozyme than to cystatin activity, where the maximum decreasing of 45.2% was found (variant 325 [W] and 120 [s] of CMF treatment). In the all variants microwave energy lowered activities both of lysozyme and cystatin what can be treated as an undesirable effect of CMF treatment. It is important to use the best CMF parameters to obtain acceptable reduction of microorganisms at minimal lowering of activities of such biologically active substances like lysozyme and cystatin in egg white.
It must be stressed that success of novel preservation technology is connected not only with technical aspects but depends also on consumer factors including these social, cultural, contextual and attitudinal ones. Consumers are afraid of some new technologies more than others. Within this context, the application of novel food processing technologies to commercial foods rises high concern among consumers. The lowest level of consumers’ fear of food preservation concerns old technologies like heat pasteurisation, cold preservation, thermal energy but also some new technologies like radio-frequency heating, microwave radiation, pulsed electric fields, ultrasounds and oscillating magnetic fields (Cardello, 2003). In that context concentrated microwave field treatment has chance to be eventually accepted by consumers if that technology would be applied into plant scale.

CMF method can be used in combine preservation systems together with other methods (“hurdle theory”) where the aspects of total microbial inactivation should be the most important (San-Martin et al., 2003; Farkas, 2004). Nevertheless the other parameters like psychochemical, functional or biological aspects like activity of lysozyme and cystatin of investigated liquid food should be also considered in novel preservation methods.

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References


