Eggs as a source of nutraceuticals and biomedical substances in the prevention of civilization diseases

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Recent years studies have shown unequivocally that hen's egg due to the content of large quantities of biologically active compounds may serve as a valuable material for biomedical applications. At present it is generally accepted that eggs besides being a source of everyday diet, they can also be used as raw material for special food, nutraceutical, pharmaceutical and cosmetic production. The biological activity of the majority of substances presented in egg matter is related to their anti-microbial and anti-cancer properties and their immunogenic features as well. Due to the use of new generation of feed additives and techniques of immunisation of hens, the chemical composition and biological features of eggs may be modified and enriched with desired components, like n-3 fatty acids (ALA, DHA), vitamins, microelements. Egg material may also be used for the production of new biopreparations for the prophylaxis of various civilisation diseases, such as heart and cardiovascular diseases, cancers, neurogenerative disorders, metabolic bone diseases etc. Current progress in egg science allowed to discover new bioactive substances, like monomeric and dimeric cystatin and yolkin. Nowadays, it is desirable to develop efficient methods of isolation of biologically active substances contained in eggs, which would make it possible to produce new generation of diet supplements and biomedical products. Consortium of Medical University and the Wroclaw University of Life Sciences carries out interdisciplinary in a large-scale study of bio-components of the eggs (Project OVOCURA). The results of this study included the newly discovered substance - yolkin, and further cystatin, phospholipids, products of enzymatic hydrolysis are the subject of this publication.

Keywords: bioactive substances; biopeptydes; cystatin; phospholipids; yolkin.

Introduction

As a result of strong competition on the global markets, which makes pro-innovation system, novel and refined technologies are being implemented. New methods of isolation of bioactive components from eggs, as well as their enzymatic modification or biotransformation using techniques of molecular biology, are the subject of great interest.

Nowadays we observe growing consumer interest in maintaining good health and slowing the aging process therefore the awareness of people about the role of food in the prevention of diseases and the related tendency to self-medicate with "natural" products seems to be conceivable. The development of functional food market is also stimulated by nutritionists and doctors, through the media promoting a healthy lifestyle and encouraging prevention.

There is no any other natural food product which is as perfect as an egg. When a normally laid and fertilized egg is provided with energy in the form of heat (39ºC) for certain period of time, it turns into a living organism (chick) which continues growing and developing. Thus, all substances indispensible

for its existance are present in the egg. This fact confirms the excellence of eggs in terms of their biological value.

The biological activity of the majority of substances present in egg matter is related to their anti-microbial and anti-cancer properties and their immunogenic features as well. Due to the use of new generation of feed additives and techniques of immunisation of hens, the chemical composition and biological features of eggs may be modified and enriched with desired components, like n-3 fatty acids (ALA, DHA phospholipids), vitamins, microelements. Egg material may also be used for the production of new biopreparations for the prophylaxis of various civilization diseases, such as heart and cardiovascular diseases, cancers, neurogenerative disorders, metabolic bone diseases etc. Current progress in egg science allowed to discover new bioactive substances, like monomeric and dimeric cystatin and yolkin. It is desirable, however, to develop efficient methods of isolation of biologically active substances contained in eggs, which would make it possible to create a new generation of nutraceutical, biomedical and biocosmetical preparations.

Selected biocomponents of egg matter as results of OVOCURA Projects

An egg contains thousands of biologically active polymer structures. Although several hundred of them have already been identified and described, nevertheless, only some of them can be isolated in technological processes. This shows, on the one hand, how complex and little known are eggs as material for the production of nutraceuticals and biomedical preparations and, on the other hand, how many possibilities and challenges there are for scientific research and advanced technologies.

For 4 years past we realized Project “Innovative technologies of production of biopreparation based on new generation eggs” co-financed by EU, Innovative Economy Operational Program (2007-2013).

Among the many scientific and technological achievements of the Project, special attention deserve a newly discovered complex of polypeptides called yolkin, thoroughly studied cystatin, new peptide preparations obtained by limited proteolysis of egg protein and still valid phospholipids.

Yolkin

The peptides which are characterized by immunomodulatory properties against such neurodegenerative diseases as e.g. Alzheimer’s disease (AD) are especially important from the therapeutic point of view. Bioactive peptides may exist in a free state, complexed with other substances, or may be a structural element of larger proteins from which they are removed during proteolysis.

Based on it we have shown that hen egg yolk IgY preparation, the main immunoglobulin class of the avian immune system, indeed, is capable of stimulating whole blood cells to release TNF-α and IL-1β cytokines (Polanowski et al., 2012). Alongside IgY, a fraction of low molecular weight proteins was obtained. In cytokine induction experiments it was shown that this protein fraction, named yolkin exhibited much higher inducing activity then a sample of additionally co-purified IgY. The preparation resembles Colostrinine, a prolin rich polypeptide complex separated from colostrum, in terms of immunoregulatory properties and alike it appeared to be a strong inductor of secretion of cytokines so important for the immunological system (Iglot et al.,1996). Yolkin, is a protein mixture consisting of several peptides of MW ranging from over 1 to 35 kDa which were found to be efficient inducers of IL-1β, IL-6 and IL-10 secretion. N-terminal amino acid sequences of eight of the electrophoretically purified yolkin constituents revealed that all of them are homological to some fragments of the C-terminal domain of vitellogenin II and they may be considered as a set of peptides resulting from proteolytic cleavage of the YGP40 mainly at positions 1571S – 1572A and 1731R – 1732M. (Polanowski et al., 2013). This finding reveals a new role for vitellogenin as a reservoir of polypeptides which may play an important role in the innate immune system of the developing embryo.

It is worth noting that all yolkin components separated by means of reverse-phase HPLC, displayed cytokine-inducing activity and, at least partially, are responsible for such properties of separated IgY preparation.

Cystatin

Cystatins are strong reversable inhibitors of cysteine proteases, including cystein cathepsins and legumain, which catalyse the cleavage of protein molecule, leading either to their degradation or, in case of pro-hormones and pro-enzymes, to the creation of their active forms. They are also engaged in the processes of presentation of class II antigens MHC, change of the bone tissue, differentiation of keratinocytes, reproduction and apoptosis. Disorder in the balance between the activity of cysteine proteases and the amount of their inhibitors may result in numerous pathological conditions, including development of carcinomas, muscular atrophy, Alzheimer's diseases, osteoporosis, sclerosis multiplex, chronic inflammations and disorders in the development of epidermis (Vasiljeva et al., 2007). It has been observed that the inhibitor extracted from egg white is active against various bacteria, viruses and fungi and that it prevents the development of numerous diseases (Węsierska et al., 2002, Mine, 2007, Kołaczkowska et al., 2010).

As hen cystatin is, in its structure, similar to human cystatin C, it is often used in model medical research. (Gołąb et al., 2008). The study of melanoma cell line showed a correlation between the low level of this cystatin, high proteolytic activity of legumain and the development and metastases of some kind of cancer (Briggs et al., 2010) The research on the use of cystatin isolated from hen egg proteins in treating cancer and Alzheimer's disease is advanced (Gołąb et al., 2008). However, the use of cystatin in medicine requires development of concentrated preparations of this protein characterized by high activity and stability. Studies showed that cystatin prevents oligomerisation and formation of beta-amyloid fibrils. Beta-amyloid reacts only with the dimeric form of cystatin C. Moreover, it is toxic for hippocampus neurons and cortex as it induces the process of oxidation of proteins and lipids and leads to oxidation stress. Cystatin, however, without any protection, polymerizes and loses its anti-papain activity. The problem of stabilization of cystatin as well as a method of obtaining a concentrated form of monomeric cystatin has been solved by Wroclaw scientists and described in own patent application (Gołąb et al., 2011, 2012). It was observed that cystatin may be stabilized by a properly composed buffering solution and an addition of stabilizers other than glycerol. Solutions of bovine albumin and selected saccharides at the final concentration between 1% and 5 % may be used as stabilizer.

Ovophospholipids

Among non-protein substances contained in egg yolk, the most important – both for pharmacology and industry – are ovophospholipids, esp. ovolecithin. Egg yolk is a rich source of phospholipids which constitute over 22% of its dry mass. The main phospholipids contained in the yolk are phosphocholine (80.5%) and phosphatidylethanolamine (19.1%). Lecithin (phosphocholine) is an important component of all cells and plays an important role in physiology of organisms. It is also widely believed that lecithin and choline increase the functioning of brain, improve the process of memorising and the ability to concentrate. Moreover, they help in digestion of fats and inhibit their build-up in the liver, prevent fatty liver, increases the effect of antioxidative vitamins, prevent tumours and improve the physical ability of human body (Zeisel 2000, Miller 2003, Sosnowska et al., 2011).

Although, since the 30's of the previous century, soya and rape seeds have been the main source of lecithin, egg yolk lecithin is, thanks to its unique chemical composition, an important alternative source of this substance. Egg yolk phosphocholine differs from the lecithin of plant origin in the content of unsaturated fatty acids important for human body: arachidonic acid (AA) and docosahexaenoic acid (DHA). Arachidonic acid takes part in the transmission of intercellular signals and is responsible for the condition of the cell membrane. Docosahexaenoic acid is necessary for the proper development of brain, nervous system and the processes of seeing. From the pharmacological

point of view, soy lecithin, which does not contain these acids, cannot replace egg yolk phosphocholine. Moreover, the content of AA and DHA may be increased thanks to proper feeding programme of the layer hens. Research programmes have been conducted for many years in order to enrich eggs with omega-3 fatty acids and to increase the content of long-chain fatty acids (L-PUFA) in the yolk. Such enriched eggs are referred to as designed eggs and phospholipids extracted from them are unique as they have docosahexaenoic acid (DHA) built in in the sn-2 position. Thus, they may be used for the production of lecithin rich in DHA. Scientists from the Wroclaw University of Environmental and Life Sciences conduct a research programme in that field and both hen and quail eggs are used as material (Kovacs-Nolan, 2005, Sosnowska et al., 2011).

Products of enzymatic hydrolysis

Modification of food proteins by means of enzymatic hydrolysis has been used for many years to improve functional properties and biological activity of protein by-products (Liu and Chiang 2008).

The objective of our study was valorization of egg proteins preparation generated as by-product in industrial process of lecithin and yolkin isolation from egg yolk (YP) (Polanowski et al., 2013) and lysosyme and cystatin from egg white (WP) (Sokołowska et al. (2007). The hydrolysis was perform using a commercial preparation of Neutrase and an uncommercial proteinase of fig-leaf gourd fruit (Cucurbita ficifolia). The antihypertensive and antioxidative properties of YP and WP hydrolysates were evaluated based on their angiotensinconverting enzyme (ACE)-inhibitory activity and radical scavenging (DPPH) capacity, ferric reducing power, and chelating of iron activity. YP hydrolysate obtained with Neutrase showed high levels of antioxidant activity. Our study indicated that YP and WP Neutrase hydrolysates could be used in foods as natural antioxidants and functionality enhancers (Pokora et al., 2013).

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