EXPRESSION SYSTEMS FOR PRODUCTION OF RECOMBINANT PROTEINS

Yvonne Piotrowski

Department of Chemistry, Faculty of Science and Technology, University of Tromsø, Norway, Tromsø
e-mail: yvonne.piotrowski@uit.no

The discovery of the restriction enzymes and the development of the polymerase chain reaction (PCR) made it possible to amplify a gene of interest and to clone it into an appropriate vector.

Both are common and well established techniques in research labs and the basis for gene expression of recombinant proteins.

For structure-determination methods like X-ray crystallography or for biochemical characterization, e.g. of newly discovered cold-adapted enzymes, a rather elevated amount of pure protein (> 95%) is needed. Unfortunately the production of recombinant proteins in prokaryotic or eukaryotic systems is not always straightforward. Some time has to be spent on optimization to get a sufficient amount of protein, preferably in soluble form to get around the longsome process of protein refolding. In some research labs it is already common practice to generate several constructs per protein and thus to test for better solubility in parallel, e.g. using different fusion tags or host systems.

An overview of available host systems, vectors and fusion tags will be presented. A number of examples will be given showing how each of these can be applied to increase the expression yield and/or solubility of the protein.

SUBSTANTIATION OF THE SHELF LIFE FOR THE FROZEN SOFT SALMON CAVIAR

N.A. Platonova

Russian Federal Research Institute of Fisheries and Oceanography (VNIRO), Russia, Moscow
e-mail: vniro-test@vniro.ru

Salmon caviar, which has high taste and nutrient properties, represents one of the most valuable food substances.

It is known that the caviar quality during its storage depends on the observance of technological conditions during its preparation, sanitary conditions on the factory, type of packaging, storage conditions, and some other factors.

The salting and freezing are the traditional methods of the caviar preservation. However, until now the freezing of salmon caviar was not widely used. Therefore, in our opinion, the technology of freezing of the salmon caviar became relevant.

The purpose of our study was to substantiate the shelf life for the salmon caviar at –18ºC in the absence of any preservatives.

The object of our study was hunchback salmon caviar, prepared according to the State Standard 1629–97 (Soft salmon caviar) without any preservatives.

The sampling, preparation of the average sample, and determination of quality and safety parameters were carried out using the corresponding standard methods of study.

Caviar samples (1000 g each) were packed into polymeric bags, approved for the use with fish products, and stored at minus 18 ºC. The shelf life was determined according to the methodical recommendations of the State Sanitary and Epidemiological Inspection “Sanitary and epidemiological assessment of the substantiation of the shelf life and storage conditions for foodstuffs” (Methodical recommendations 4.2.1847-04).

The dynamics of caviar quality parameters during the storage was characterized by changes in the content of nitrogenous compounds (nonprotein nitrogen, nitrogen from volatile bases (NVB)) and oxyacids and in the values of lipid hydrolysis and lipid oxidation indices.

The organoleptic evaluation was carried out during the whole storage period by at least 2 experts, experienced in the field of the certification of fish, non-fish objects, and the corresponding food substances.

In all samples during the whole storage period we observed the absence of bacteria from the colibacillum group, *Salmonella* genus, *Staphylococcus aureus*, sulphite-reducing clostridia, and also the mould. The microbial contamination of caviar was stable during the whole experiment and was equal to 7.0×10^3 CFU/g to the 13th month of the storage. The yeast number was lower than the normalized value, being equal to 5.0×10^1 CFU/g.

The organoleptic analysis showed that during the storage the eggs remained elastic and had a slightly wet surface; individual eggs could be easily separated from others. The caviar had nice smell and taste, typical for this product. We did not observe any detractive features of the product.

Two weeks after the caviar preparation, the content of nonprotein nitrogen was only 0.16% of the total nitrogen content; to the end of storage (13 months) the value of this parameter gradually increased up to 0.29% (Table 1). The most intensive NVB accumulation was observed during the first 6 months (from 22.06 to 25.13 mg%); during the next 7 months the NVB content slightly increased up to 27.01 mg%.

Table 1. Nonprotein nitrogen, nitrogen from volatile bases (NVB) in caviar

Parameter	Storage time, months					
	1	2	6	8	12	13
Nonprotein nitrogen,%	0.16	0.19	0.23	0.25	0.28	0.29
NVB,%	22.06	23.51	25.13	25.93	26.36	27.01

During the storage time, we observed an increase in the acidity index value from 3.1 to 4.6 mg KOH per 1 g of oil; the oxyacid content increased from 0.3 to 0.6%. The most intensive increase in the number of free fatty acids was observed during the first months of the storage (from 1.3 to 2.6 mg of KOH per 1 g of oil). At the same time, we did not reveal any regularity in the peroxide number changes (Table 2).

Table 2. Acidity index, oxyacid content and peroxide number in caviar

Parameter	Storage time, months					
	1	2	6	8	12	13
Acidity index, mg KOH/1 g of oil	3,1	3,6	3,9	4,2	4,4	4,6
Oxyacid content,%	0,3	0,4	0,4	0,6	0,5	0,6
Peroxide number,% J_2	0,11	0,68	0,23	0,19	0,93	0,48

The results concerning peroxide numbers do not correlate with the observed organoleptic and microbiological changes in the examined samples.

The peroxide number does not reflect the level of oxidative damages, arising during the storage of a product; therefore, this parameter can not be used to characterize such damage of products. This fact is confirmed by publications of other authors and by the data, obtained in the VNIRO-TEST research laboratory during the study of the oxidative damage of fish oil.

The analysis of such parameters as nonprotein nitrogen content, NVB content, oxyacid content, and oil acidity index showed the absence of any explicit hydrolytic and oxidative processes during the storage of salmon caviar; this fact is also confirmed by the organoleptic analysis.

Thus, the results of microbiological, physicochemical, and organoleptic studies confirmed the quality of frozen caviar, stored at minus 18°C in a polymeric packaging, corresponded to the requirements of the State Standard 1629–97 and Sanitary Standard 2.3.2.1078-01 during the whole storage period.

The freezing of soft salmon caviar without any preservatives makes it possible to keep its quality for 12 months in the case of its storage at minus 18 ºC. The results of our studies, conducted jointly with the TINRO-Center state unitary company and devoted to the storage of the frozen salmon caviar, became the basis for the development of the State Standard R 53353–2009 «Frozen soft salmon caviar».