The development of the system for quantitative assessment of soya bean content in meat products by real time PCR

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Abstract - **The object of this research was to develop the system for quantitative assessment of soya bean content in meat products (regardless of the type of their technological processing) by real time PCR. The search of the DNA template was conducted on the Internet using the open access genetic database of the US National Center for Biotechnological Information. CLC Sequence Viewer and Primer Express 2 were used for primer design. Specificity of the selected primers was studied theoretically using on-line the interactive system BLAST. DNA was extracted by the method based on DNA extraction and purification with ion detergent cetyltrimetylammonium bromide (CTAB) followed by sorption on a carrier. PCR was carried out on the ABI PRISM 7000 device with the detection of the amplification products in real time. In total, 8 sausage samples with a-priori known soya bean content were analysed. The quantitative PCR assay showed that the soya bean content in these samples corresponded to the amount of soya bean incorporated into the product according to the formulation ± 27% in dry or gel form. The obtained results confirm the possibility to use the system of soya bean quantitative assessment by real-time PCR to detect the possible falsification of the product or to confirm product authenticity in accordance with the regulations or the information on the label and in the accompanying documents.**

Keywords: **real time PCR, meat products, soya bean**

I. INTRODUCTION

Plant protein additives used in meat products manufacture allow to increase volume and decrease costs of production of finished products. Among protein additives of plant origin the most prevalent are soya bean proteins. The specific attention to them is associated primarily with the availability of raw material (the worldwide soya bean sown area occupies more than 70 mln ha; the total volume of soya bean seeds production is 160 mln tones; it can be obtained up to 731 kg of protein from 1 ha) and secondarily, with the unique chemical composition of soya bean seeds, which contain 40% of protein and 20% of lipids, and thus, have a high biological and nutritive value. Furthermore, soya bean proteins are the most frequently used proteins in meat products manufacture. Soya bean protein preparations have a wide range of functional properties at less cost comparing to the alternative additives of animal origin such as dried milk, casein, egg yolk, egg albumen or gelatine.

Soya bean proteins are incorporated into majority of functional and multifunctional mixtures, emulsifying agents, and emulsifying and stabilizing agents used in meat industry. Moreover, soya bean proteins, specifically soya bean lecithin, are used as a carrier for flavour components.

In case of unauthorized addition of soya bean proteins to a product formulation without placing the information about this addition on a label of a product, manufacturers not only deliberately falsify their produce, but also seriously endanger a particular group of population, for which soya bean proteins are allergens even in small doses. These people need to clearly control their nutrition and avoid the presence of allergenic proteins in their diet. It is well known that allergens can cause both a small reaction and a very strong one including anaphylactic shock. With this in mind, manufacturers are obliged to place the information about the presence of soya bean (both transgenic and grown without genetic modification) on a label of a product manufactured with the use of preparations containing soya bean.

It follows from the above arguments that identification of plant ingredients incorporated into meat products (in this case, soya bean) occupies a special place in their quality assessment.

II. MATERIALS AND METHODS

The search of the DNA template was conducted on the Internet using the open access genetic database of the US National Center for Biotechnological Information. CLC Sequence Viewer and Primer Express 2 were used for primer design. Specificity of the selected primers was studied theoretically using on-line the interactive system BLAST. DNA was extracted by the method based on DNA extraction and purification with ion detergent cetyltrimetyl-

ammonium bromide (CTAB) followed by sorption on a carrier. PCR was carried out on the ABI PRISM 7000 device with the detection of the amplification products in real time. In total, 8 sausage samples with a-priori known soya bean content were analysed.

III. RESULTS AND DISCUSSION

The test system for soya bean DNA identification in meat products was developed in the laboratory 'Production hygiene and microbiology' of VNIIMP named after V.M. Gorbatov. The basis of this test system is a method of real time PCR. Its principle lies in multiple copying *in vitro* the particular species-specific DNA site, namely, the site of the lectin gene nuclear DNA of soya bean (*Glycine max*) and DNA site specific to all species of slaughter animals and poultry used as templates. Modification in real time allows to synchronize amplification and detection of amplification products, which can be observed on the computer monitor in the form of logarithmic fluorescence curves.

Thus, if soya bean is a part of a meat product, it will be identified by the presence of its DNA. It is known that DNA is the most resistant to external exposure biopolymer. It is impossible to completely destroy soya bean DNA during technological processing; thus, PCR method is highly effective. Furthermore, the method is highly sensitive and allows to reliably detect the presence of soya bean in a product in the quantity from 0.001% and higher. However, when restricting to qualitative analysis, it is impossible to quantify added soya bean preparations, which is also important in the product quality assessment. Consequently, the necessity arises to develop the quantitative method.

To this end, a test system for the quantitative assessment of the percent content of soya bean raw material relative to meat raw material was also constructed. Similar to the qualitative method, the quantitative method of assessment is based on real time PCR. It is well known that real time PCR gives an opportunity to evaluate process kinetics, which depends on the initial amount of testing material. If we compare the reaction kinetics in test and standard specimens, we can make a conclusion on the initial DNA concentration. Taking into account this peculiarity, we developed the test system, in which the quantitative assessment of the target DNA (i.e., soya bean) was based on the simultaneous analysis of the test specimens and the series of the calibration (standard) specimens with the calculations made from the calibration graphs.

Calibration specimens were the decimal dilutions of the genetic engineering construction carrying species-specific insertions of sites of Lec1 gene ($1x10^6$ copies per µl) and Myo2 gene ($1x10^6$ copies per µl) (table 1, fig.1).

Table 1 The dilutions of the calibrators Lec1 and Myo2

$1x10^6$ copies per µl Lec1/ Myo2	$1x10^5$ copies per µl	$1x10^4$ copies per µl	$1x10^3$ copies per µl	$1x10^2$ copies per µl	1x10 copies per µl	1 copy per µl
	10 µl $1x10^6$ + 90 µl H_2O	10 µl $1x10^5$ + 90 µl H_2O	10 µl $1x10^4$ + 90 µl H_2O	10 µl $1x10^3$ + 90 µl H_2O	10 µl $1x10^2$ + 90 µl H_2O	10 µl $1x10^1$ + 90 µl H_2O
100%	10%	1.0%	0.1%	0.01%	0.001%	-

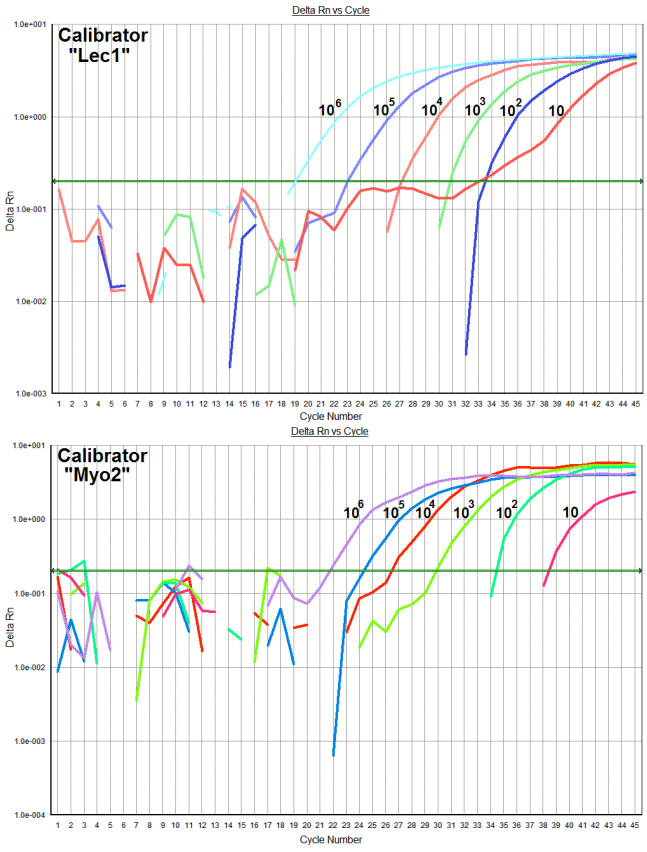

Fig.1. Fluorescence curves of the real time PCR accumulated products from the calibration specimens Lec1 and Myo2 with known target gene copy number ($1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$, $1x10^2$, $1x10^1$). $R^2 = 0.9874$

Figure 1 presents the fluorescence curves of the PCR accumulated products. These curves express the dependence of fluorescence on the number of

conducted PCR amplification cycles. In the process, the apparatus automatically detects the point of the beginning of the log phase, expressed in the number of executed cycles. This point characterizes the initial amount of the DNA template in the reaction mixture. The difference in the threshold cycle values of two reactions, carried out simultaneously (the calibrators Lec1 and Myo2), is used for the construction of the calibration line for the calculation of the soya bean content in the analyzed samples. Data processing was done using MS Excel software.

The control samples of the meat products manufactured in the different meat processing plants and provided for the analysis with the quantitative data on the soya bean preparations content in their formulation (2% to 8%) were used for the evaluation of the quantitative test system. DNA was extracted from all samples and amplified simultaneously with the calibrators. The results of the analysis corresponded to the amount of soya bean incorporated according to the recipe with the error ±27% (table 2).

Table 2 Quantitative assessment of soya bean by PCR test system

No	Test sample	Amount of soya bean according to the recipe, %	Results of PCR assay, %
1	Sausage 'Molochnaya'	2	3.39±0.6
2	Pâté	2	1.58±0.3
3	Frankfurters	2	3.7±0.7
4	Sausage 'Doctorskaya'	7	7.24±1.4
5	Sausage 'Govyagja narodnaya'	5	2.58±0.5
6	Sausage 'Lubimaya'	8	6.43±1.3
7	Sausage 'Doctorskaya bistro'	4	2.9±0.6
8	Sausage 'Russkaya bistro'	7	6.5±1.3

IV. CONCLUSION

The obtained results show the possibility to use PCR test systems for the qualitative and quantitative assessment of soya bean content in food products in order to confirm the authenticity of an individual product according to the requirements of regulations or the information on the label and in the accompanying documents.