Development of a DNA-based quick test for the detection of soy in food

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MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

Introduction

Food allergy is an increasingly serious problem affecting around 1-2% of the population worldwide. In most cases it requires a strict diet, i.e. the elimination of allergenic ingredients from the diet. Soy contains a number of allergenic proteins that reduce the positive health effects of soy and cause allergic symptoms in soy-allergic individuals. Due to the general labelling requirements for allergens, the presence of soy must be indicated on the label of products containing soy and the exemption must be regularly checked. In our research, we aimed to develop a rapid, specific and highly sensitive assay method for the detection of soy content.

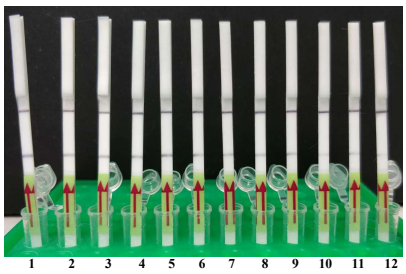
Primer sequences

Forward primer: Biotin-GAGAAGTTTCGAAGAAGGTGTTTTGTTAGA-3'
Reverse primer: 5'- ACCAAAATTGGCAATTTTGGTCAGATAACC -3'
Probe: FAM –CAAGTAGTCTTTGAAAGAAGACATATGATTCG(THF)GGCTTTCTTTTCTATCC –3'

Results

First, we adapted two DNA-based simple PCR methods. We compared the methods (chloroplast AtpA gene-specific and LecI gene-specific) to see which one is more suitable to detect the DNA of a Hungarian soybean variety, Pannonia kincse, at the lowest possible concentration. We found that the AtpA gene-specific primers were more sensitive. Secondly, a lateral flow soybean DNA assay was developed based on the selected chloroplast AtpA primer pair. The assay is based on recombinase polymerase amplification. In our research, we determined the parameters for the optimal operation of the PCR reaction, specifity of used primers and the detection limit. We have successfully demonstrated the proper functioning of the developed soy-specific DNA assay by testing meat products with and without soy component.

Evaluation of Milenia Hybridetect strip
1 positive sample, **2** negative sample
The upper horizontal band is the control band for checking strip reation, and lower band is the signal of positive RPA reaction when the soy-specific sequence is amplified.



Detection limit: For the measuring range analysis, we used 12 samples of fivefold diluted soy DNA. Based on our results the presence of soy in the sample can be also been detected in small copy number, we got weak signal at 1-24 DNA copy number too.

Sample type	Positive results (%)	Remark
Two-component model meat	100	10% soy content+ 90% pork
Raw sausage model-sample	100	10% soy content
Sausage model-sample	66	10% soy content
Turkey cold meat	66	„Soy-free"
Pork cold meat	66	„Soy-free"
Pork sausage	66	„Soy-free"
Turkey sausage	33	„Soy-free"
Oven roasted ham	33	„Soy-free"
Chicken cold meat	100	Contains soy
Pork liver pate	100	Contains soy
Beef salami	66	May contain traces of soy
Veal cold cuts	100	Contains soy
Cooked beef	33	May contain traces of soy

Detection of soy content from different food samples

Conclusion

Detection of soy from foods is very important for allergic individuals to ensure their safety diets. We have successfully adapted two types of DNA-based methods (lectin gene and AtpA gene specific PCR methods) and optimized the polymerase chain reaction method for soybean detection in foods. Comparing the two primers, AtpA primers were found to be more sensitive, so the detection reaction was more effective. The explanation is that the lectin gene has one copy in a plant, while the AtpA gene is originated from mitochondrial, and can be present in the eukaryotic organism in thousands of copies. To verify that "soy-free" products do not contain soy, PCR methods based primarily on the detection of the AtpA gene are highly offered to apply. Based on these primers we developed a rapid DNA-based method - TwistAmp™ nfo kit - to detect soy-content from food samples.
The developed RPA based field test is a sensitive and fast method for detecting soy ingredient in raw foods, food ingredients and food products, even if the sample matrice contain large amounts of fat, spices, which inhibit the conventional test methods. The field test can be used in non-laboratory conditions (shops, industry or restaurant) as the developed method is fast, easy to perform, does not require complicated technical background and the results are available after 30-45 minutes.

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