SCREENING FOR GMO IN FERMENTED SOY SAUCE

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Short communication

Abstract

Soy sauce is worldwide popular condiment of Asian origin. With the advent of GM soybean production, soy sauce drew the interest of food safety control. Samples collected for inspection are generally of industrial grade soy sauce type, which is produced from hydrolyzed soybean and grain. Following the failure to perform RealTime PCR based GMO screening on a number of submitted samples we tested our screening system on soy sauce produced following traditional method based on fermentation. Four batches of soy sauce were produced and DNA extracted. DNA concentration ranged from 32,68 to 65,36 ng/µl. Amplification of taxon specific target was successful with rather high Ct (> 30). Promoter P-35S sequence was not detected, but T-NOS was detected in three samples with values reaching or exceeding LOD of the method. The results show that it is possible to detect transgenic elements in traditionally produced soy sauce while DNA extraction from industrial grade soy sauce is not possible.

Key words: *Soy sauce, DNA extraction, RealTime PCR, GMO*

Introduction

Soy sauce is an Asian condiment that originated in China about 2500 years ago. Its use was later spread to the other parts of Asia while, it was brought to Europe in the 17th century. Its recognition in the Western societies followed the rise in popularity of Asian cuisine. Traditional soy sauce is mature, fermented product, made from soybeans, grains and salt. The production process is slow and does not achieve industrial scale. As current cumulative consumption greatly surpasses the capacities of traditional production, industrial grade soy sauce is produced from hydrolyzed soy protein and grain. While this process results in affordable product of satisfactory sensory qualities, acid hydrolyses renders this type of product inaccessible for DNA based analysis such as those applied in food safety sector. With the increasing presence of GM soybean in food supply, soy sauce became an object of scrutiny from food safety perspective.

According to ISAAA report 83% (92.1 million hectares) of the 111 million hectares of the globally planted soy are biotech (James, 2015). Legal framework on GMO of the European Union and Law on GMO in Bosnia and Herzegovina (2009) require labeling of products

that contain more than 0.9 % GM content per species. Successful implementation of any legal framework depends on the abilities of analytical laboratories to provide correct information. Therefore, official laboratories utilize validated analytical methods and DNA based methods are the methods of choice when it comes to GMO analysis (Anklam et al., 2002). Unfortunately, validation is generally performed on general type of matrix such as grain or flour and does not guarantee comparable performance on different type of matrix. Thus, when DNA extraction from industrial grade soy sauce proved to be challenging, we decided to explore the matrix rather than alter the method. We hypothesized that the DNA extraction method validated for soybean would provide sufficient quantity of PCR grade DNA from soy sauce produced by traditional method.

Materials and methods

We prepared soy sauce by traditional method in order to test the capacity of CTAB DNA extraction procedure validated for soybean to yield sufficient quantity of PCR grade DNA.

The production method was derived by analyzing a number of traditional recipes and extrapolating common ingredients and steps. All ingredients were procured locally. Soybean, being the target ingredient, was obtained from different manufacturers (Tab. 1). Raw soybeans were soaked in water for 15 hours and then boiled for about an hour. Cooked and smashed soybeans were mixed with ca. half the amount of wheat flour to form a homogeneous mixture. The obtained mixture was divided in smaller cakes and left for about 20 days in a warm and humid place and allowed for mold to develop. Following the development of mold, the cakes were dried in a warm place and soaked in salt brine (6 % NaCl solution). Fermentation was allowed to proceed slowly with occasional agitation. The fully fermented mixture was filtered to remove the solids while liquid soy sauce was used for DNA extraction and GMO screening.

Table 1. List of analyzed samples

Sample ID	Country of origin	GMO label
1/16	Abu Dhabi, United Arab Emirates	-
2/16	China	-
3/16	Netherlands	GMO free
4/16	Bulgaria	-

Genomic DNA was extracted from 2 ml of soy sauce using CTAB precipitation method validated and recommended by the European Union Reference Laboratory for GM Food and Feed (EN ISO 21571: 2005. Annex A, par. A3). The quality and quantity of extracted DNA was measured by UV spectrophotometry (UV-VIS Spectrophotometer, Shimadzu, Japan).

To test if the obtained DNA extracts contain amplifiable DNA we used RealTime PCR amplification of lectin ie. taxon specific target for soy (EURL-GMFF & ENGL, 2010). GMO screening was performed using RealTime PCR methods which target most common elements of transgene construct – cauliflower mosaic virus promoter (p-35S) and nopaline synthase gene terminator from Agrobacterium tumefaciens (t-NOS).

These methods are validated by the Italian National Reference Laboratory for GMO (IZSLT, 2011). RealTime PCR reactions were carried out in an ABI 7300 RealTime PCR instrument (*Applied Biosystems*). Certified reference materials by JRC-IRMM were analyzed parallel to samples and used as positive controls. The raw data were analyzed using Sequence Detection Software (Applied Biosystems).

Results and discussion

Following traditional recipe we prepared four batches of soy sauce originating from different sources. Some physical and chemical characteristics of the obtained soy sauces are listed in Tab. 2. While soy sauces made by industrial procedure have liquid consistency, dark color and sour or salty flavor our traditional products have thicker consistency and lighter color.

Table 2. Physical and chemical characteristics of soy sauces produced following the traditional recipe

Sample ID	Color	Consistency	Flavor	pH value
1/16	Light brown	Mash	Sour, salty	4.49
2/16	Light brown	Mash	Sour, salty	3.70
3/16	Light brown	Mash	Sour, salty	3.80
4/16	Dark brown	Mash	Sour, salty	3.90

Genomic DNA was successfully extracted from all samples. Absorbance at 260 nm, 280 nm and 230 nm showed sufficient purity and quantity of DNA extracts for further analysis. However, A260/A230 ratio showed carbohydrates residues which were expected, considering the type of starting material. DNA concentration ranged from 32,68 to 65,36 ng/µl.

Taxon specific target was amplified in all analyzed samples. Even though the amplicons were detected in all samples Ct values were quite high especially compared to CRM Ct value. Despite approximate DNA concentration of 2/16-4/16 samples Ct values varied. Sample 1/16 which showed the lowest DNA concentration (32,68 ng/µl) had lower Ct value compared to 3/16 and 4/16 (40 ng/µl). However, if we consider that the total DNA also included DNA of lactobacilli, the discrepancy in DNA concentration and Ct values ratio is not unexpected. Sample 1/16 also had the highest pH value, indicating less advanced fermentation.

High Ct values for lectine (30.69-34.53) indicated low concentration of target DNA but still within limit of detection (LOD) of the method. All samples tested negative for p-35S, while 3 samples tested positive for t-NOS. High Ct values for t-NOS reactions (34.49-36.30) indicated that concentration of the target DNA approaches LOD of the method (LOD≥4C). Considering LOD of the t-NOS method only sample 1/16 tested positive.

According to high Ct values for lectine we can conclude that concentration of target DNA was low which reflected to Ct values for t-NOS. Additional optimization of DNA extraction protocol such as adjustment of amount of the starting material and additional purification step with chloroform are necessary to increase target DNA yield.

Table 3. Ct values of RealTime PCR reactions

Sample ID	Lectine	p-35S	t-NOS
1/16	32.58	-	34.49
2/16	30.69	-	35.28
3/16	34.53	-	-
4/16	34.50	-	36.30
10 % CRM	18.26	-	20.38

Previous analyses of industrial soy sauces in our laboratory were unsuccessful regardless of used DNA extraction protocol. Industrial soy sauce is

made from acid-hydrolyzed soy protein. It is assumed that unssuccessful DNA extraction is a consequence of absence of target DNA in the substrate because it is also subjected to acid hydrolysis. Amplification of lectin gene in DNA extracted from soy sauce made following traditional recipe once again confirmed that industrial soy sauce is not suitable for GMO screening using PCR methods.

Conclusions

Although DNA was successfully extracted from traditional soy sauce low DNA concentration and high Ct values for lectine (30.69-34.53) indicated the necessity of additional optimization of DNA extraction procedure. All analyzed samples tested negative for p-35S, while t-NOS was detected in three samples. However, high Ct values for t-NOS reactions (34.49-36.30) indicated that concentration of the target DNA approaches LOD of the method which is bellow the labeling threshold and only sample 1/16 can be considered as positive. The results show that it is possible to detect transgenic elements in soy sauce made following traditional recipe while DNA extraction from industrial soy sauce is not possible.

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