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The effect of processing factors on detection of genetically modified soy in flour by ELISA assay

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ABSTRACT

Genetic modification (GM) techniques have been an important research area of food and feed industry since the 19th century. There is a strong consumer concern over genetically modified organisms (GMOs) because of their potential risks on health and environment. For this purpose, various countries including Turkey have released labelling regulations for products derived from GMOs. These legal enforcements brought the necessity for reliable detection methods. The aim of our study was to evaluate the effect of processing factors on the detection possibility of GMOs by using a commercial Enzyme Linked Immunosorbent (ELISA) assay. For this, flour mixtures containing 0.5%, 1%, 5%, 10%, 100% were prepared by mixing the appropriate amount of RUR-GM and non GM standard soy flour and main processing techniques most used in the food industry (baking, autoclaving and freezing) were applied. According to our results, the detection of GMOs was possible at all concentrations of autoclaved and frozen samples. In dry heated samples, GMOs could not be detected containing below 5% GMOs. ELISA method cannot be recommended as a reference method for evaluation of the compliance with the regulations, but it can serve as a practical alternative to be used as an online monitoring tool in production lines for raw and mildly processed foods.

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Introduction

Genetic modification (GM) techniques have been an important research area of food and feed industry since the 19th century. The most significant product of this field is Genetically Modified Organisms (GMOs). “GMOs are organisms that genetic materials (DNA) have been altered in a way that does not happen in normal ways” as defined by World Health Organization (WHO). In this context, it is intended to get edible vaccines or medicines, functional foods, enhanced shelf life and nutritional composition, also growing adaptable and strong plants such as herbicide tolerant or insect resistant in various environmental conditions via using GM technology. Improving the quality of certain crops is believed to be the most significant advantage of GMOs (Arun Ozgen et al., 2015).

Since they were first approved, GMOs have received a worldwide demand and GM crops have been planted on a very large scale. The plantation area of GM crops have increased 110-fold from 1996 to 2016 and finally reached 2.1 billion hectares (ISAAA, 2016). Soybean, maize, cotton and canola are the most cultivated GM crops in 26 countries which grows GM crops. Most of these crops have been approved to be used in food/feed in several countries and thus enters the food chain (Anonymous, 2018).

Despite this huge market share, there is a strong

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consumer concern over GMOs because of their potential risks on health and environment. For this purpose, various countries including Turkey have released labelling regulations for products derived from genetically modified organisms (GMOs). The main policy of such regulations are to leave the decision making to the consumer (Anonymous, 2003; Anonymous, 2009; Anonymous, 2010). These regulations lay emphasise on that; GM food or feed must not have any adverse effect on the environment or human/animal health. Also, labelling must not misguide the consumer (Varzakas et al., 2007).

These legal enforcements brought the necessity for reliable detection methods. For this purpose, numerous analytical methods, supporting the regulations have been carried out to monitor and verify the presence of GMOs in food or feed samples (Anklam et al., 2002). These methods mainly based on detection of novel DNA or protein present in the product. DNA-based methods widely used both qualitatively and quantitatively for the detection of GMOs product in transgenic raw or unprocessed soy products (Meyer et al., 1996; Lipp et al., 2000; Lipp et al., 2001; Taverniers et al., 2001). Qualitative testing is used to identify GM or non GM material or to distinguish certified or noncertified material. Quantitative testing, on the other hand is used for confirming the official thresholds (Quist and Chapela, 2001; Windels et al., 2001 and 2003; Chowdhury et al., 2003a and 2003b ; Hernandez et al., 2004; Collonnier et al., 2005; Nielsen et al., 2005; Ortiz-Garcia et al., 2005; Saji et al., 2005; Taverniers et al., 2005; Aono et al., 2006; Messean et al., 2007). DNA based methods are proved to be the most reliable methods for detection of GMOs in food and feed. However, PCR methods need an expensive laboratory infrastructure and experienced staff. In such cases, protein based methods may be used as an alternative cheap and easy testing especially for quality control laboratories performing routine process monitoring. ELISA is the most widely used protein based method for detection of the proteins expressed by GMOs especially in raw food (Vollenhofer et al., 1999; Ahmed, 2002; Arun Ozgen and Garrett, 2009; Suchitra and Ali, 2013). The method can be used for qualitative and quantitative purposes.

Food processing causes serious degradation of proteins in food. In many studies, it was reported that heat processing such as cooking, baking, drying, sterilizing or freezing causes a severe denaturation on food proteins (Asensio et al., 2008).

Taking these into consideration the aim of our study was to evaluate the effect of processing on the detection possibility of GMOs by using a commercial ELISA assay.

Materials and methods

Raw Material Preparation : Flour mixtures containing 0.5%, 1%, 5%, 10%, 100% were prepared by mixing the appropriate amount of Roundup Ready® (RUR) GM and non GM standard soy flour (SDI diagnostics, USA) and main processing techniques most used in the food industry were applied to the mixtures. Utmost care was taken to avoid contamination between samples and different steps.

Dry Heat Treatment (Baking): For baking 0.5 g of each flour mixture containing RUR GM soy were mixed with 1000 µl milli Q water and cooked at 100°C for 20 min in a sterilizer (Murray, 2007).

Wet Heat Treatment (Autoclaving): For autoclaving process; 0.5 g of each flour samples were mixed with 3750 µl milli Q water and autoclaved at 121°C, 15 Ibs pressure for 20 min (Hirayama, HV-50L, Japan) (Murray, 2007).

Freezing: For freezing; 1 g of each flour mixture were mixed with 1000 µl milli Q water and stored at –18°C for three days (Murray, 2007).

All of these preparations were performed in duplicate and analysed with ELISA method.

GMO detection with ELISA method: For detection and quantification of GMOs in the treated and raw samples were performed with Romer Labs Agraquant Toasted Meal Plate Kit (No: 7099999). The ELISA kit is designed to detect the CP4 EPSPS protein in RUR soybeans in toasted meals. According to the manufacture© instructions 100 mg of each standard (0%, 0.3%, 1.25%, and 2.5% RUR soy flour) and samples were mixed with 16 and 13 ml extraction buffer, respectively and vortexed for 1 min. The wells of the plate were filled with 100 µl of these extracts in duplicate and processed incompliance with the manufacturer's instructions. The absorbance of the developed colour was read at 450 nm using a plate reader (ELISA Plate Reader ELX 800, Biotek-Inst, ABD).

Results and Discussion

The main objective of this study was to evaluate the detection and quantification capability of a commercial ELISA based GMO detection assay on heat treated samples. For this; five different concentrations of RUR soya (0.5, 1, 5, 10 and 100%) flour samples were heated in two different conditions (baking, autoclaving) and also they were frozen to simulate the common processes in the industry. Detection and

quantification of GMOs in these samples were performed with Romer Labs Agraquant Toasted Meal Plate ELISA assay. The results of this study are summarised in Table 1 and 2.

Table1. Detection results of ELISA assay

Samples	100 °C Dry Heat Treatment	121 °C Wet Heat Treatment	-18 °C Freezing
0.5% GMO	Not detected	Detected	Detected
1% GMO	Not detected	Detected	Detected
5% GMO	Detected	Detected	Detected
10% GMO	Detected	Detected	Detected
100% GMO	Detected	Detected	Detected

GMO = Genetically modified organism

According to these results, the detection of GMOs was possible at all concentrations of autoclaved and frozen samples. In dry heated samples, GMOs could not be detected in samples containing GMOs below 5%.

The quantification results of frozen samples were significantly closer to the true values while the results of autoclaved samples were still close although slightly deviated compare to frozen samples. However, there was a significant bias between true values and results of dry heated samples as in detection.

Table 2. Quantification results of ELISA assay

Samples	100 °C Dry Heat Treatment	121 °C Wet Heat Treatment	-18 °C Freezing
0.5% GMO	<0.00%	0.44%	0.49%
1% GMO	<0.00%	0.74%	1.17%
5% GMO	0.26%	>2.50%	>2.50%
10% GMO	0.70%	>2.50%	>2.50%
100% GMO	>2.50%	>2.50%	>2.50%

GMO = Genetically modified organism

Our previous studies performed with PCR also showed that baking (dry heating) process has a significant effect on detection of GM DNA in food samples (Arun Ozgen et al., 2016). Similarly, several other researchers indicated the degradation/ denaturation effect of heat on DNA and proteins (Asensio et al., 2008, Arun Ozgen and Garrett, 2009). Although autoclaving is performed at 121°C detection and quantification was more successful at these samples. Several other studies also proved that the degradation effect of dry heat is stronger than wet heat (Corbisier et al., 2005; Vijayakumar et al., 2009; Bergerová et al., 2010; Ballari and Martin, 2013). This was incompliance with our study on novel protein.

Conclusion

Despite the detection and quantification is effected from dry heating and detection limit of the method is significantly higher compared to PCR based methods, it can still produce reliable results on wet heated (autoclaved) and frozen samples. ELISA method for sure cannot be recommended as a reference method for evaluation of the compliance with the regulations. However, it should be taken into consideration that, ELISA does not require a sophisticated laboratory infrastructure and expertise and thus it can serve as a practical alternative to be used as an online monitoring tool in production lines for raw and mildly processed foods.

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