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Traceability of genetically modified Roundup Ready soybean: A case study on sampling and analytical uncertainty along processing chain

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ABSTRACT

A study on the fate of Genetically Modified (GM) Roundup Ready soybean (RRS) was undertaken on the following products: flour, protein flour, lecithin, crude and refined oil, broken grain, hull and expander of an industrial soybean manufacturing plant, with the aim to evaluate the possible effects of processing on the reliability of control plans. A sampling control plan was applied to all the products of the industrial manufactory plant. The best sampling point was identified based on the lowest impact of the analytical and sampling uncertainty.

The best "fit for purpose" sampling point for the accurate evaluation of the Genetically Modified Organism (GMO) concentration measurement was identified in the processed products, e.g. flour and protein flour, thanks to the homogeneity on RRS in the batch and the better yield and quality of the extracted DNA.

This study presents a practical approach to assess the two main factors that affect the reliability of the control plans: analytical and sampling uncertainty. The work was undertaken on GM soybean derived products, nevertheless the conclusions we reached could be also applied to verify compliance with GMO labelling threshold.

1. Introduction

The EU General Food Law Regulation EC No 178/2002 defines traceability as "the ability to trace and follow food, feed, food producing animals and other substances intended to, or expected to be incorporated into food or feed, through all stages of production, processing and distribution". Furthermore, the current legislation on GMO traceability, Regulation EC No 1830/2003, was set up to enable controls and verifications of labelling claims and to guarantee freedom of choice for consumers and farmers. All food and feed products that use GMOs along the production chain need to be labelled, even if no GM content is detectable in the final product. In fact the "analytical" traceability may be inapplicable to highly processed products, e.g. starch, highly refined oil or lecithin, due to the DNA degradation during processing, therefore only a documentary traceability system guarantees the GM origin of these products. In order to successfully apply the legislation on labelling, an adequate implementation of both sampling and detection methodologies is required: the more stringent the labelling requirements, the more expensive and complicated the strategies that meet these requirements will be.

For what concerns the performances of GMO detection methods there are several factors that are responsible for the DNA degradation along the food manufacturing chain: enzyme-catalyzed chemical reactions, or simple mechanical procedures, such as milling (Moreano, Busch & Engel, 2005). Several authors carried out studies on GMO detection in processed food trying to verify if the quantified GMO percentage reflected the original amount of the GM raw material used for their preparation (Berdal & Holst-Jensen, 2001; Hupfer, Hotzel, Sachse & Engel, 1998; Lipp et al., 2001; Matsuoka et al., 1999; Rizzi, Panebianco, Giaccu, Sorlini & Daffonchio, 2003). Berdal & Holst-Jensen (2001) recovered higher percentages of GM soybean in processed food compared to percentages obtained from raw material. Yoshimura et al. (2005) showed how common food production processes can lead to significant GMO percentage variation (ranging from 13% to 81%). In contrast, according to Debode, Janssen & Berben (2007), when both species specific and transgenic targets sequences have similar size, a physical degradation of DNA does not affect the relative quantification of GMO content.

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With reference to sampling, it represents the starting and the most crucial step of the whole analytical chain, especially when analytes are heterogeneously distributed in the lot, as for the case of GMOs and Mycotoxins (Miraglia et al., 2004). The overall objective of a good sampling is to provide representative samples for the analysis, this is particularly relevant for GMO analysis considering that a wrong sampling plan may affect the reliability of the measured levels resulting in legal disputes and trade barriers.

General guidelines on sampling are described in the Codex Alimentarius document CAC/GL 50-2004 (Codex 2004) but they are specific for homogeneously distributed analytes and should not be used for heterogeneously distributed ones. A first specific sampling procedure for GMO was described by Brera et al. (2005), who performed a pragmatic study on a sampling procedure for RRS. After that, the KeLDA (Kernel Lot Distribution Assessment) project, the most important study on sampling for GMOs funded by the 5th framework EU program, pointed out the issue of heterogeneity of GMO kernel distribution and the variability of distribution patterns among the analysed soybean shipments lots. It was highlighted the need to develop sampling protocols based on statistical models free of distribution assumption, capable of considering the specific variability of spatial patterns that KeLDA project has demonstrated (Paoletti et al., 2006).

At present, the only legislative sampling guideline for GMOs is the Recommendation EU No 787/2004, that was specifically developed for GMO inspections and controls, based on KeLDA results. Among the general principles, the Recommendation claims that it is the Member States' responsibility to select the points in the supply chain where the official control is to be undertaken. The Recommendation envisages the procedure for bulk agricultural commodities and it states that alternative sampling procedures may be applied. However, it doesn't take into consideration the needs that may arise at the productive stage, where "fit for purpose" sampling strategies may be useful.

In the present study a soybean processing chain was chosen in consideration of the significant presence of soybean both in food and feed products. The experimental sampling plan applied aimed at identifying the potential effects of processing on the reliability of the determination of RRS concentration as well as on traceability systems throughout all the products. The quality and quantity of the extracted DNA of all sampled products were evaluated. The analytical and sampling contributions to the total uncertainty were investigated through the evaluation of RRS percentage in different soybean products obtained from the industrial plant.

This study proposes a "fit for purpose" approach for the optimization of control strategies based on the evaluation of between-increment variation (sampling uncertainty) and the effect of analytical uncertainty, at different sampling points of the soybean processing plant.

2. Materials and methods

2.1. Samples

The samples were collected from an Italian industrial plant (Bunge - Italy) that operates oilseed crushing and refining facilities. The main steps of processing are shown in Fig. 1.

The first step is the preparation of soybean grain for oil extraction. This stage comprises drying, tempering, cleaning, cracking, dehulling, conditioning and flaking. We collected during this step the following by-products: broken grain, hull and expander.

The second step is the solvent extraction of oil by means of non-polar solvents and the removing and recovering of the solvent from the micella and from the meal. We collected during this step two types of soy flour which are made grinding finely the defatted meal: flour passing at 97% through a 100-mesh screen and at 95% through a 200-mesh screen. Flour passing through the highest rated screen, hereafter referred to as protein flour, has an higher protein content (49% crude protein), that the other one (44% crude protein).

Soybean oil (crude and refined) and lecithin obtained from soybean oil by a degumming process were also collected during processing.

2.2. Sampling and sub sampling procedures

The collection of samples throughout the milling process were performed according to Brera et al. (2005). The systematic and dynamic sampling procedures were carried out during the processing of 50 tons of soybean grain. The applied sampling procedure is illustrated in Fig. 2. The sampling plan was optimized according to the processing steps in order to maintain the coherence between the starting materials (grain) and its derived products. Ten samples of 2.4 kg each were collected at regular intervals, 7 min, for grain, broken grain, hull, expander, flour, protein flour and 5 samples of 100 mL each were collected for fluid lecithin, crude and refined soybean oil. The samples were split into two portions of 1.2 kg each: one to be used as an increment to produce the bulk sample (12 kg), the second (incremental sample, IS) to be analyzed individually. The same procedure was applied to the samples of lecithin and crude and refined soybean oil, the only difference being the size of samples (bulk samples of 250 mL and IS of 50 mL).

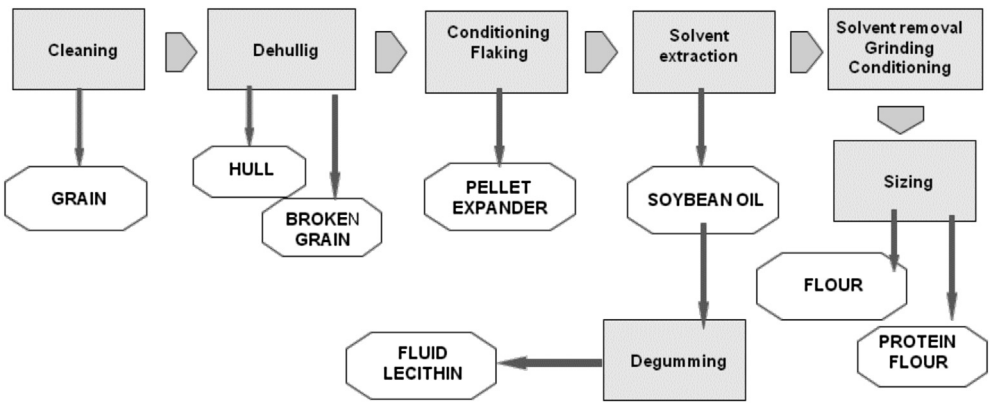

Fig. 1. Schematic description of soybean processing steps.