Food Safety Monitoring: The Use of Specific Animal DNA Detection Methods to Ensure Vegan Authenticity

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ABSTRACT

More and more consumers are choosing a vegan or vegetarian lifestyle, whether for ethical, environmental, health or religious reasons.

In the food industry, binding legal definitions of the terms 'vegan' and 'vegetarian' have not been adopted yet with regulators globally. As a result, there are not a standardised criteria for a reliable labelling of vegan and vegetarian products and can lead to incorrect labelling by the producers themselves. This legal gap causes uncertainty between consumers and producers.

In addition, animal-DNA testing is crucial in the animal feed production industry to ensure produce authenticity and avoid animal cannibalism which has proven association with potential pathologies, such as spongiform encephalopathy.

INTRODUCTION

Detection of animal DNA presence in food samples via molecular techniques is crucial to improve the traceability and control in the food supply chain, as well as a necessary quality control for handling and cleaning processes in production lines. Furthermore, DNA analysis is more sensitive and more specific than similar strategies, such as protein-based analysis. Thermo Scientific™ RapidFinder™ Vegan ID kit (Thermo Fisher Scientific) has been used to analyze a total of 83 samples including food, animal feed, vegetable derivates, wine etc. using a highly sensitive analytical method (limit of detection >0.01%).

MATERIALS AND METHODS

A total of 83 samples submitted between March 2016 and March 2021 by multiple manufacturers (food and feed industry, consultancy and quality control laboratories) were included in the study.

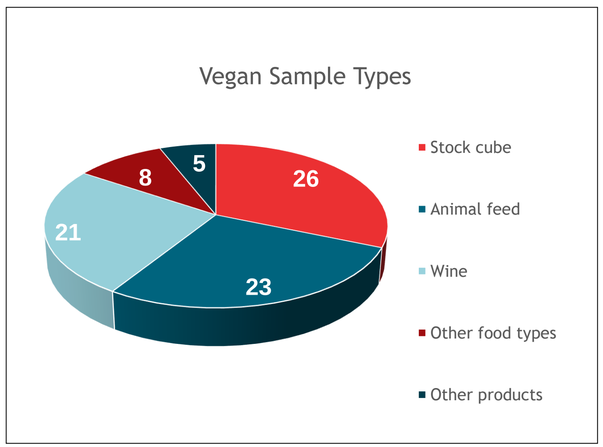
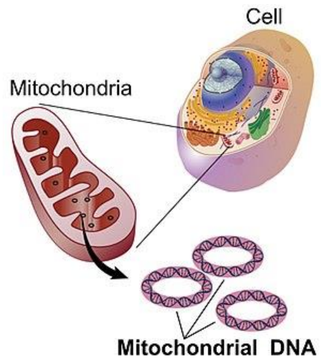


Figure 1. Sample types analyzed

For genetic analysis, DNA was extracted from each sample using a commercial **GMO Extraction Kit** (Thermo Fisher Scientific). To ensure optimal representativeness of the sample, maximum amount of each of them were homogenized. Once homogenized each sample, 10 g of feed, food and others, 10 mL of wine or 5 g of Bouillon tables (of which a smaller quantity is started because they are matrices very rich in salts and larger quantities could inhibit PCR) were incubated in lysis buffer, proteinase K and RNase reagent according to the manufacturer's instructions.

The quality and quantity of the purified DNA sample was studied by spectrophotometry. The analytical monitoring was performed by real-time PCR using the Applied Biosystems™ 7500 FAST Real-Time PCR System and RapidFinder Vegan ID kit.For this, a total amount of 50 ng of total DNA were used to set up the PCR reaction.



Animal DNA was detected from conserved *16S* gene, a highly specific mitochondrial DNA region, which confers great specificity and analytical sensitivity with a limit of detection established at 0.01%.

RESULTS

Our results indicated that all DNA samples were optimal for the genetic analysis. The **GMO Extraction kit** is optimal for low-target detection in assays that require great sensitivity as it processes over 20-times more sample than most commercial kits. In addition, it yields high DNA concentration and purity suitable for any PCR-based downstream assays.

The **RapidFinder Vegan ID kit** is a highly sensitive system capable to detect less than 100 ppm of animal DNA. This sensitivity allows food and feed manufacturer to reliably label and trace the authenticity of the sample. Diagnostic detection of animal DNA using Real-Time PCR is interpreted based on a cycle threshold (C_t) value[1]. The cut-off value is stablished on 0.01% above which a C_t value is deemed negative.

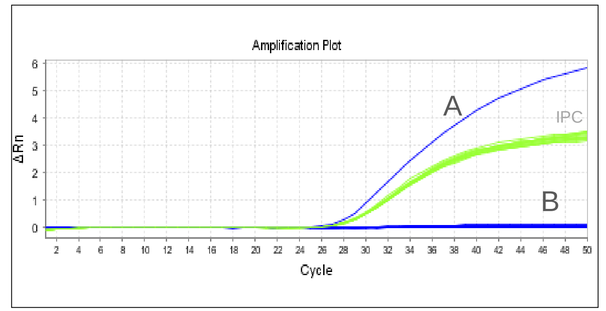


Figure 2. Real-Time PCR amplification plot.
A) Detection results obtained from a DNA sample containing animal DNA. Animal DNA is labelled with FAM, and the internal positive control (IPC) with VIC. The IPC consists of a synthetic DNA region directed to function as a PCR control to confirm the correct set up and functioning of the PCR;
B) Animal-free sample suitable for Vegan labelling

Analytical surveillance confirmed that 100% of the expected animal-free products were free of animal DNA.

Overall, only 3 of the samples contained animal DNA. These animal containing samples consisted of poultry feathers and viscera flour intended for animal feed production.

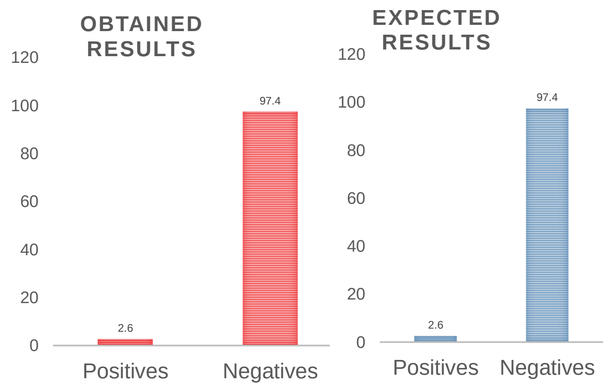


Figure 3. Graphic representation of the 83 samples analyzed. The red bars, represent the results obtained with the RapidFinder Vegan ID; whereas blue bars, represent the expected results obtained with the RapidFinder Vegan ID.

The results enabled the users to implement additional control measures when needed to ensure the animal DNA-free composition.

Follow-up monitoring informs food and feed manufacturers of a breakage in the cleaning and decontamination process in the production chain enabling them to seek solution to mitigate cross-contamination with animal-containing products.

Regulators are currently investigating. The objective is now to set up a consensual and globally accepted definition of the vegan claim. For instance, the EU Commission released a recommendation indicating follow-up controls shall be performed[2,3] and the removal of debris and product changes must be ensured to comply the acceptability limit of <1% accidental contamination.

CONCLUSIONS

The presented study indicated that a highly sensitive analytical tool is capable of detecting animal DNA to ensure the authenticity of vegan products directed to human consumption, and the safety of animal by-products intended for animal feed production in absence of cannibalism. Until there is a standardized criteria, this traceability control test provides the food and feed manufacturer reliable information to implement handling and cleaning protocols.

In conclusion, the present study highlights the importance of implementing specific and sensitive analytical surveillance methods to ensure the authenticity of animal-free products.

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TRADEMARKS/LICENSING

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