istituto zooprofilattico sperimentale
del Piemonte, Liguria e Valle d'Aosta

DETECTION OF VERTEBRATE DNA IN VEGAN FOOD BY REAL-TIME PCR METHOD

Tramuta Clara, Morello Sara, Avena Cristina, Bianchi Daniela Manila

Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta - Centro di Referenza Nazionale per la Rilevazione negli Alimenti di Sostanze e Prodotti che Provocano Allergie e Intolleranze – CReNaRiA, Torino, Italy

Introduction

In recent years is increasing the number of consumers who choose vegan or vegetarian diet for ethical, health, nutritional or environmental reasons. A product is vegan if it does not contain any animal ingredients or by-products. To date, the term vegan is not subject to any specific regulation; only the voluntary ISO 23662 standard provides labeling definitions and technical criteria for products and ingredients compatible with dietary choices (1). This does not guarantee the absence of cross-contamination with products of animal origin and consequently may pose a risk to people with allergies and intolerances.

Detection of animal DNA presence in food samples is important to improve the traceability in the food supply chain and to control the cleaning processes in production lines. DNA-based assays, such as PCR methods, are appreciated worldwide because of the minor effect that food processing has on DNA compared with the effect of processing on the expressed protein (2).

Food safety official laboratories rely on validated and accredited methods in order to guarantee high performances, repeatability and reproducibility of analytical results.

Here we present validation of a real-time PCR assay commercially available for the detection of vertebrate DNA in food products.

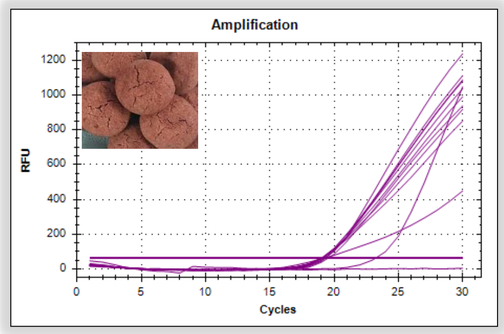
Material and methods

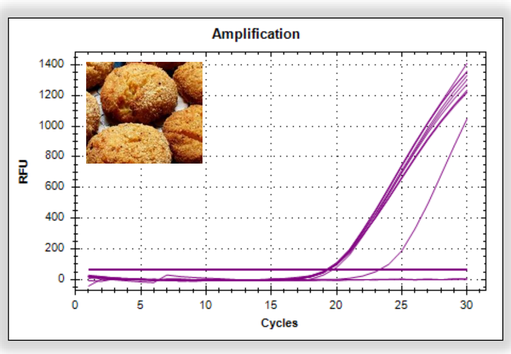
- **Food samples**. The method was tested on three classes of food matrices (blank samples): i) bakery and pastry products (chocolate biscuit), ii) gastronomic preparations (vegan burger), iii) sauces (tomato sauce with onion, carrots, peas and celery) and vegetable drinks (oat and almond drink).
- **Specificity**. The specificity of the primers and probes was compared *in silico* by confronting the sequences in the NCBI BLASTn database of non-target matrices. The specificity of the PCR primers was then tested by amplifying the DNA extracted from blank samples processed in 30 replicates (10 for each of the 3 food matrices). The extracted DNA of vertebrate animals, plant species and non-vertebrate animals was also tested (Table 1).
- **Sensitivity**. DNA extract from a pool of meat samples (*Bos taurus*, *Sus scrofa domesticus* and *Gallus gallus domesticus*) was diluted with DNA extracted from blank samples to a final concentration of 0,01% corresponding to 100 mg/kg (ppm) by Sure Food PREP Advanced kit (r-Biopharm). DNA was then amplified by SureFast Vegan real-time PCR kit (r-Biopharm) on a CFX real-time PCR system (Bio-Rad): vertebrates were detected in the Cy5-channel and plants in the FAM-channel.
- **Robusteness**. To determine method robustness, DNA extracts of the spiked chocolate cookies was amplified on a CFX Opus 96 real-time PCR system (Bio-Rad).

Results

All 30 blank samples were tested negative for the target vertebrate and no amplification signals in Cy5 were noted. The method was rated specific for the detection of vertebrates and no cross-reaction were observed (Table 1). About sensitivity, results demonstrate that the real-time PCR here validated proved to be sensible, showing a LOD of 100 mg/kg (mean Ct 19.3) in all food matrices considered (Figures 1-3). Congruent results for all samples indicated that the method is robust.

Figure 1. Amplification curves of the vegan real-time PCR analysis of DNA extracts from the samples to the LOD concentration corresponding to 0.01% (100 mg/kg)





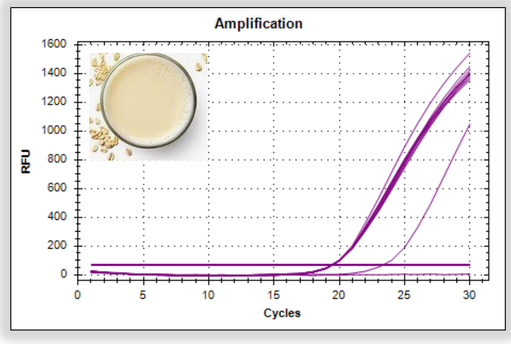


Table 1. List of animal and plant species used in the study and specificity results of real-time PCR

Sample	Vertebrate animals (Cy5)	Real-time PCR cycle threshold (Ct)
Almond (*Prunus dulcis*)	negative	> 30
Barley (*Hordeum vulgare*)	negative	> 30
Beef (*Bos taurus*)	positive	18.7
Cacao (*Theobroma cacao*)	negative	> 30
Carrot (*Daucus carota*)	negative	> 30
Celery (*Apium graveolens*)	negative	> 30
Chicken (*Gallus gallus domesticus*)	positive	19.1
Horse (*Equus caballus*)	positive	20.4
Insect (*Acheta domesticus*)	negative	> 30
Oat (*Avena sativa*)	negative	> 30
Onion (*Allium cepa*)	negative	> 30
Peas (*Pisum sativum*)	negative	> 30
Pig (*Sus domesticus*)	positive	19.3
Plaice (*Pleuronectes platessa*)	positive	26.5
Rice (*Oryza sativa*)	negative	> 30
Scallop (*Pecten jacobaeus*)	negative	> 30
Soy (*Glycine max*)	negative	> 30
Shrimp (*Caridea Dana*)	negative	> 30
Tomato (*Lycopersicon esculentum*)	negative	> 30
Tuna (*Thunnus*)	positive	28.1
Turkey (*Meleagris*)	positive	21.1
Wild boar (*Sus scrofa*)	positive	20.1

Discussion and Conclusion

- Italian Reference Centre for Food Allergens Detection (CReNaRiA) can share validation protocols to the national network of official laboratory to guarantee high performance and reproducibility among official analyses
- Based on our results, the present real-time PCR method proved suitable for vertebrate DNA detection and demonstrated specificity, sensitivity, and robustness.
- Methods that are both specific and sensitive for the detection of vegan foods are needed to ensure the authenticity of vertebrate-free products and to protect allergen-sensitive consumers.
- The methods were found adequate for the needs of a food safety laboratory and can be considered as a potential tool for ensuring avoidance from exposure to allergens for allergic consumers.

In conclusion, the method here proposed provides reliable information to implement handling and cleaning protocols.

References

1) ISO 23662:2021 Definitions and technical criteria for foods and food ingredients suitable for vegetarians or vegans and labeling and claims

2) Madrid R. et al. Survey of Commercial Food Products for Detection of Walnut (Juglans regia) by Two ELISA Methods and Real Time PCR. Foods 2021, 10, 440.

This study was funded by the Italian Ministry of Health IZSPLV 08/23 RC.

EAVLD 2024 - 7th Congress of the European Association of Veterinary Laboratory Diagnosticians, 21st-23rd October 2024