Quantification with reference material – is this reliable?

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Sensitive, fast, and inexpensive analytical techniques are necessary to supervise the composition of processed food. Additionally, it is important to differentiate between contamination and intentional addition. Therefore, quantitative methods are mandatory.

One such method is quantitative real-time polymerase chain reaction (qPCR). By means of amplification of species specific DNA sequences, even traces of DNA can be detected. And as DNA is relatively heat stable[1], this method can be applied for processed food. When probes with different fluorochromes are used, several DNA sources can be simultaneously detected (multiplex qPCR).

The main problem is quantification. An easy and widely used solution is to apply a serial dilution of the target DNA. However, the results are given in copies of DNA and therefore, no direct conclusion about the actual added amount of target material is possible. Another approach is to produce reference material containing the target material in different concentrations within the detection range. The thereof isolated DNA samples are then used as a straight calibration line. In this way the results are presented in g of added target per kg food product and the direct conclusion of how much target was added is possible.

The aim of the presented study was to validate the reliability of quantification via reference material. For this purpose, emulsified type sausages were chosen as a model for processed food and the six main cereal species as target DNAs. And to be fast and inexpensive, two triplex qPCR systems (barley, oat, and rye and maize, rice, and wheat) were developed. Emulsified type sausages were produced and spiked with 0.0005-0.1% plant protein per species and produced at different conditions. As production parameters, post-processing (none vs. grilling vs. storing), packaging (artificial casings vs. cans), and production temperature (75°C vs. 121°C) were investigated. For the quantification analysis, emulsified type sausages produced at 117°C in cans and without any post-processing were used. In the last step, the production of reference material was validated at low and medium temperatures for three concentrations.

While the post-processing and packaging parameters had no influence on the detectability of plant DNA in meat products, differences were seen for the production temperature. The recovery rate varied between 161±64% - 260±71% at low and between 62±11% - 95±22% at high production temperature in comparison to the reference sausages. Therefore, the production temperature had to be checked only for the validation of the production of reference material. At low production temperature, the values for the coefficient of variation (CV) varied between 19.5-39.0% (0.001% plant protein), 11.0-28.1% (0.01% plant protein), and 7.8-18.1% (0.1% plant protein). At medium production temperature, the CV values were 18.1-40.3% (0.001% plant protein), 29.9-44.7% (0.01% plant protein), and 17.3-28.9% (0.1% plant protein). Hence, the accuracy was satisfactory at 0.01% and 0.1% plant protein at low temperature and at 0.1% plant protein at medium temperature (CV ≤ 30%). At all other conditions, the accuracy was not given (CV ≥ 30%)[2].

All in all, the quantification with reference material is possible. However, as the measurement uncertainty is quite high and dependent on the production temperature, it is neither reliable nor simple. Therefore, either the reference material has to be produced at the same production temperature as the sample or other analytical methods for quantification have to be developed.

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References:

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