ESA WHITE PAPER ON PLANT METABARCODING NEXT GENERATION SEQUENCING (NGS) ANALYSIS APPLIED TO CULINARY HERBS AND SPICES

MAIN POINTS

- ✓ **NGS should not be used as a direct tool to authenticate herbs and spices nor to detect or quantify them as ingredients in mixtures.**
- ✓ **Reference methods like classical microscopy or validated non-targeted chemical/physical methods (using NMR, NIR/MIR spectroscopy, mass spectroscopy,…) or a combination of them should be used as primary analyses to prove herb and spice authenticity. See this link to the Food Integrity Handbook :** https://secure.fera.defra.gov.uk/foodintegrity/index.cfm?sectionid=83
- ✓ **NGS can be a secondary tool to confirm and identify only additions of exogenous plant matters containing DNA when detected by reference methods.**
- ✓ **NGS is not able to detect any adulterations with economically-motivated additions of endogenous material.**
- ✓ **NGS is not able to detect any adulterations with economically-motivated additions of materials not containing DNA.**
- ✓ **NGS methods using short DNA fragments should be chosen to mitigate the effect on analysis results of processes applied to herbs and spices (milling, steam-treatment,…). Any internal herb and spice databases should be stated and the use of reliable reference materials should be demonstrated.**

TECHNICAL INPUT

1) Endogenous materials

A spice is a well-defined part of the plant it belongs to. It can be either the fruit, the bark, the stigma of the flower, the bud, the seed, the root, the rhizome, the leaf, the kernel or the bulb. And any economically motivated additions of other parts of the same plant are considered as a fraud.

NGS is not capable of distinguishing between parts of the same plant so unable to authenticate spices versus fraudulent endogenous matter additions.

Example: 100% of ground pepper leaves or 100% of ground black peppercorn or a mixture of them will all be identified as 100% pure *Piper nigrum.*

For that reason, only the plant name (Latin and vernacular names) should be reported on NGS plant certificates of analysis not the spice name.

2) Exogenous materials

Component percentages given by NGS analysis are DNA sequence read percentages which tightly depend on :

- the quantity of DNA the plant contains,
- the DNA recovery rate of the extraction step for the considered plant.

It occurs that spices are difficult matrices sometimes containing low levels of DNA what is more, difficult to extract (cinnamon/cassia, black pepper,…). Consequently, the ratio of other natural foreign plant matters, if any, can be increased and thus artificially overestimated. The DNA amount does not correlate with the weight amount of a plant in a mixture and thus the DNA sequence read percentages reported do not give the weight percentages. Such distorted results may mislead the customer.

Consequently, NGS quantitation of herbs and spices may create unfortunate confusion between adulteration and natural presence of foreign matters.

3) Other materials

In herbs and spices **NGS methods are not able to detect fraudulent additions of exogenous materials that contain no DNA** or highly fragmented DNA.

Examples: artificial chemical dyes, extracted/defatted materials, minerals (salt, talc, sand, chalk, brick,…), highly processed materials (starch, maltodextrin, sugars, oil,…),…

4) Composition of mixtures containing herbs and spices

NGS cannot be used to verify the herb and spice composition of a mixture them as ingredients for the following reasons.

- As described in 2), the DNA read percentages are misleading because they cannot be related to actual weight percentages especially for spices. So, no possibility to check the real weight percentages of an ingredient list.
- As described in 2), some spice ingredients can be missed because of their low DNA level and the difficulty to be extracted. Extraction procedures also vary between labs, there is still no norm on the subject for standardised extraction.
- Some spice ingredients can be missed because of the process they have been subjected to (process such as grinding/milling, colouring, bleaching, fumigating, steam treatment or drying may have an influence on the DNA quality jeopardising successful analyses). The shorter the DNA fragments used are, the less sensitive to process the method is.

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