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Pushing HSI to the Limit for the Quantification of Peanut Traces in Bulk Powder Foods

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REVIEW

CEI Moncloa as the Origin of this Interdisciplinary Research

The CEI_Moncloa was a proposal coordinated by the Complutense University of Madrid (UCM) and the Technical University of Madrid (UPM), approved in 2011, in which 13 institutions have been integrated. It houses around 10,000 researchers, 10% of the national scientific production and approximately 80,000 students (75% of the UCM and 25% of the UPM), including the Agri-Food and Health Cluster. It is situated in the so-called Agroalimentary Corridor, which groups together the activities that all these groups carry out in the production of agricultural and livestock products, and their subsequent processing for the creation of safe, healthy and nutritious food and feed. In this context we have been committed to finding synergistic and stable relations among research groups and as a consequence of a think tank set up to do this, there has been a steady scientific production since the first published works in 2014 **(Figure 1).**

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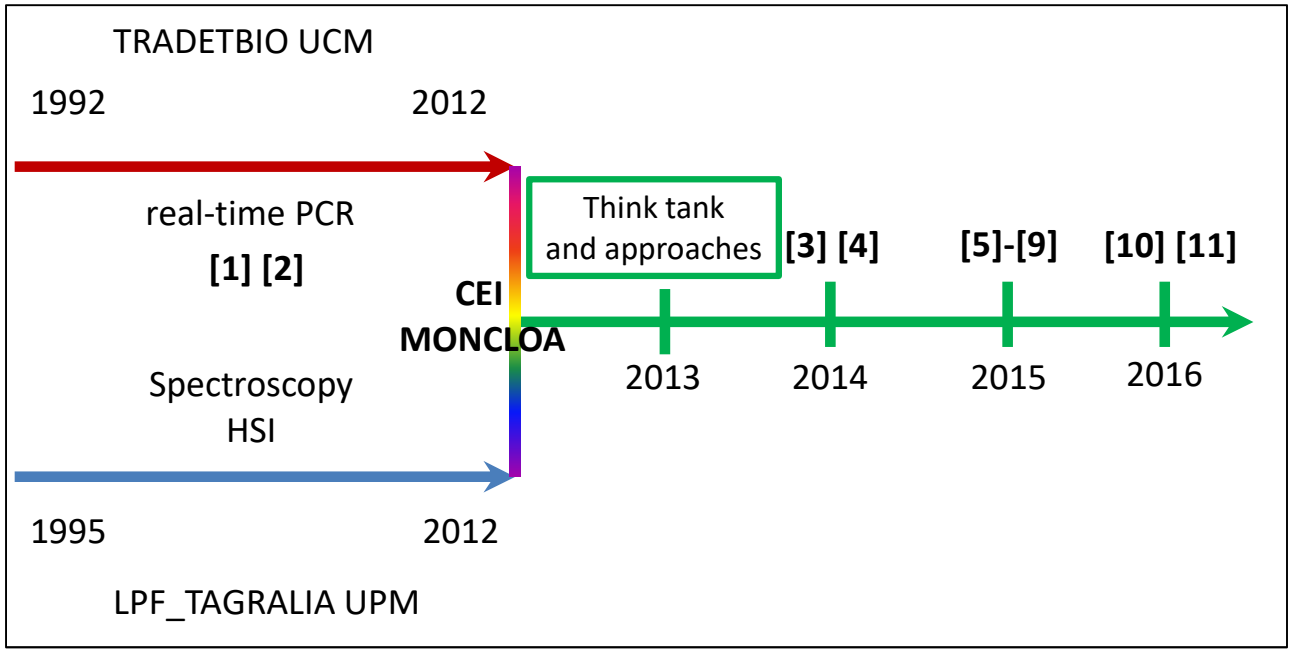

Figure 1. Time line of the research at CEI_Moncloa(UCM_UPM), Agri-Food and Health Cluster.

Detection Limit of the Reference Measurement

The main outcome of the think tank was the realisation that there was a need (both in biomedicine and the Agrofood industry) for low-cost real-time non-destructive methods for detecting peanut traces in powdered food ingredients such as flour, milk and cocoa. According to IUPAC[1], "trace" means any element having an average concentration of less than about 100 parts per million atoms (ppm) or less than 100 ug g-1. One of the relevant issues for the development of a new procedure is the need of a reliable reference procedure which should be at least one order of magnitude more sensitive than the proposed one. In this research the reference procedure was developed (prior to CEI_Moncloa) by the UCM research group (TRADETBIO) and consisted of both PCR and real-time PCR [1,2] for the quantification of peanut traces with 0.1 ppm resolution.

On the other hand, the spatial resolution of the Hyper Spectral images (HSI) images proposed by UPM (LPF_Tagralia) was identified as the cornerstone for the detection of peanut traces, therefore at the very initial steps the optimal configuration of HIS was set as to characterize particles of 70um size.

Selection of Wavelength Range

VIS and NIR spectroscopy and HIS have been evaluated in consecutive trials [3-9] using IRMM[i]-481e originating from Jumbo Runners, USA to guarantee the highest variability among peanut origins and treatments. The main conclusion being that VIS is sensitive to peanut variability, and does not provide sufficient specificity regarding milk and flour, based on results validated with commercial peanut samples (**Figure 2**). On the other hand, NIR spectroscopic imaging provides characteristic bands for peanut detection: 950 nm arising from O-H bond, 1212 nm arising from 2nd overtone of the C-H stretch of CH2group and 1450 nm arising from the 1st overtone of the O-H stretch, while HIS measurements at 70 µm resolution has shown the feasibility to detect up to 100 ppm (0.01 %) of peanut traces in any of the proposed powder foods (**Figure 3).**

SPECIFICITY ANALYSIS

Detecting peanut traces is relevant when it is sufficiently specific. Therefore, it is mandatory to avoid both false positives and false negatives. To this end, 49 commercial samples of cereals, legumes, oilseeds and nuts were obtained from local market of Madrid, Spain. All samples were ground with precautions to avoid cross-contamination. After grinding, all powdered food samples were sifted by passing through a sieve of 212 um, since diffuse reflectance properties are dependent on the size of particles, non-uniformity in size can cause scatter effects in the spectra. In this case the wavelength range proposed included all NIR data, except for the bands with highest sensitivity to peanuts. The result (see procedure in **Figure 5a**), attained for calibration and external validation [10,11] showed that the greatest challenge was in isolating peanuts from pine nuts. **Figure 4b** provides an indication or the most relevant wavelength bands for specificity.

[1] IUPAC, International Union of Pure and Applied Chemistry
IRMM International Reference Material
https://ec.europa.eu/jrc/en/reference-materials

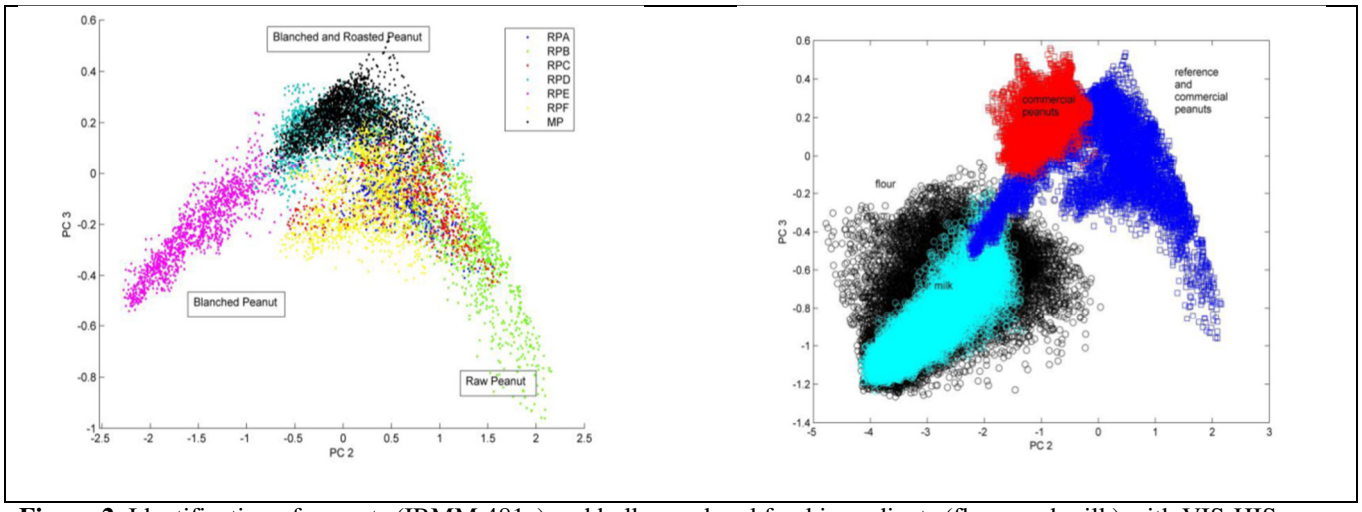

Figure 2. Identification of peanuts (IRMM 481e) and bulk powdered food ingredients (flour, and milk) with VIS-HIS.

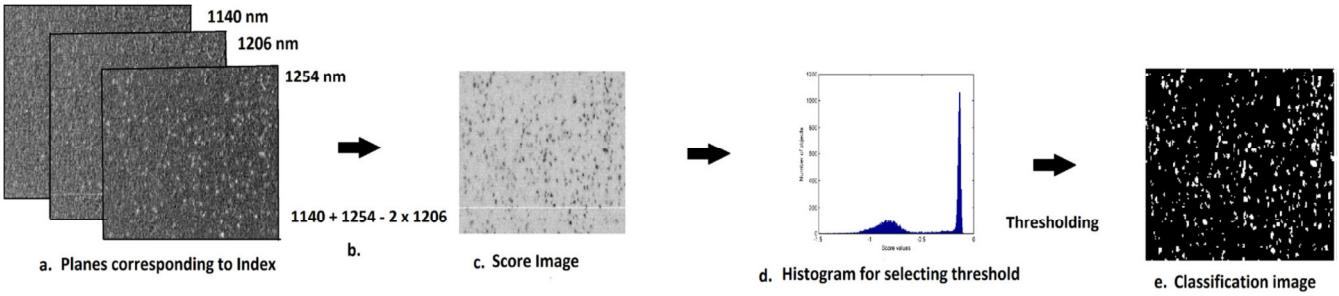

Figure 3. Process in the identification of peanut traces by NIR HSI.

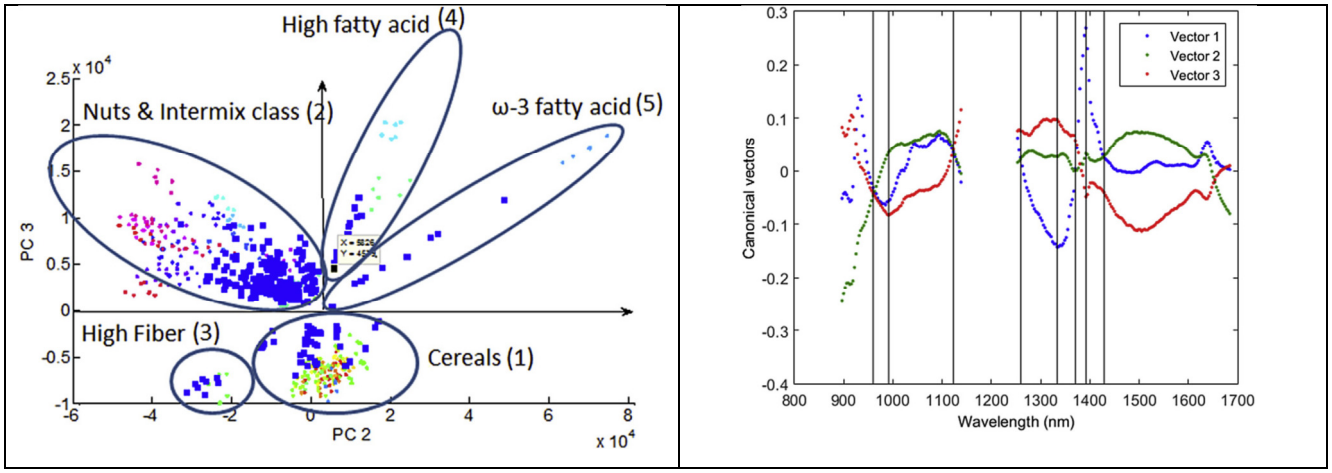

Figure 4. Segregation of bulk food materials from nuts (left), and wavelength used for specificity analysis (right)

Calibration Transfer, Transcontinental Approach

Finally, it was necessary to determine whether our results were reproducible irrespective of the instrumental platform, a hypothesis which was dismissed after initial trials at the transcontinental level [12]. Therefore the development of calibration transfer techniques is required, and this step is still ongoing. **Figure 5b** summarizes some pros and cons of NIR spectroscopic techniques for the specific identification and quantification of peanut traces in powdered foods.

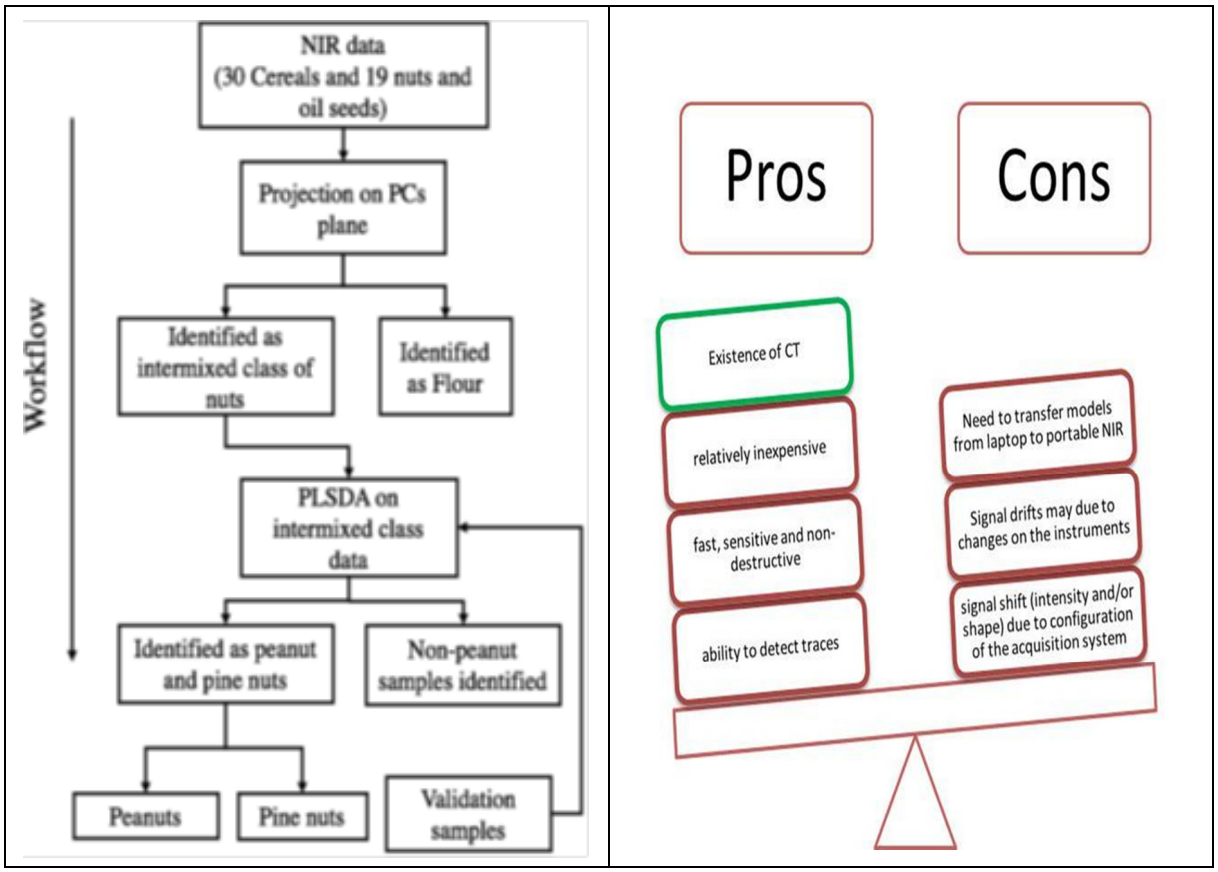

Figure 5. Summary of Chemometric procedure for the specific identification of peanut traces (left), and the pros and cons of NIR Spectroscopy for the identification of peanut traces in bulk food materials.

Prospective of Use for the Agro-Food Industry

The main conclusion of nearly a decade of research is that low-cost non-destructive NIR multispectral equipment associated with corresponding chemometric approaches should be commercially available in the near future, as derived from our AECOC[2] award in 2016. This technology can be complemented with real-time PCR when required with a highly significant reduction in cost, since it will only be used for contamination levels below 100ppm (0.01 %).

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[2] AECOC Asociación Española de Codificación Comercial

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