LIÈGE université
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Agro-Bio Tech

Le Corps professoral de

Gembloux Agro-Bio Tech - Université de Liège vous prie

de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

**Monsieur YANG Jinhui,**

**Titulaire d'un *degree of agriculture, master of animal nutrition and feed science*,**

présentera en vue de l'obtention du grade et du diplôme de

**DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,**

le 21 janvier 2019, à 10h00 précises (personne ne sera admis après cette heure),

en l'auditorium ZT1 (Zootechnie, Bât. 1),

Passage des Déportés, 2, à 5030 GEMBLOUX.

Cette dissertation originale a pour titre :

« Milk protein adulteration detection via mass spectra and infrared spectroscopy ».

**Le jury est composé comme suit :**

Présidente : Prof. M.-L. FAUCONNIER, Professeur ordinaire,
Membres : Prof. H. SOYEURT (Promoteur), Prof. J. WANG (Promoteur - CAAS, Chine), Dr V. BAETEN (Copromoteur - CRA-W), Prof. Y. BECKERS, Prof. S. DANTHINE, Prof. G. LOGNAY.

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**Summary**

Milk protein adulteration concerns the addition of cheap foreign substance having high nitrogen content into milk and dairy products, to mask low natural protein in milk. Plant protein is a potential candidate of adulterants; soy, pea, wheat, and rice proteins being the most popular. The consumption of those adulterants could induce serious Human health problems. Therefore, the development of detection methods for protein milk adulteration is of great importance to guarantee food safety. The current thesis aims to test and compare methods such as two-dimensional gel electrophoresis (2-DE), liquid chromatography tandem mass spectrometry, and infrared spectroscopy to detect foreign protein spiked in milk, especially hydrolyzed plant proteins. Here are the major results:

(1) According to the protein spots highlighted on the polyacrylamide gel of adulterated milk, β-conglycinin and glycinin were detected in milk adulterated with soy protein, while legumin, vicilin, and convicilin indicated the addition of pea protein, and β-amylase and serpin marked wheat protein. 2-DE-based protein profile allowed to identify milk spiked with soy and pea protein, with a detection limit of 4% plant protein in the total protein.

(2) Sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE) gels revealed clearly that centrifugation at 20 000 g for 60 min reduced band intensity of casein and albumin in milk. No obvious protein line was observed for hydrolyzed wheat and rice protein. Results of nano-HPLC-MS/MS highlighted the major proteins of soy (β-conglycinin, glycinin), pea (vincilin, convicilin, legumin) and wheat (glutenin and gliadin) in adulterated milks. So, this method allows the detection of hydrolyzed soy and wheat protein at the level above 0.5% in total protein, and pea protein at the level of 2 and 4%. No rice protein was identified in milk samples adulterated with hydrolyzed rice protein. Combined with principal component analysis (PCA), nano-HPLC-MS/MS discriminated all the adulterated samples from authentic milk.

(3) Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-MIR) applied to commercial skimmed milk was also tested to identify protein milk adulteration and to estimate the non-protein nitrogen content (eNPN). Spectral clusters revealed by PCA depended on the level and type of adulterant. The developed partial least square (PLS) regression showed good performance of ATR-FTIR to predict eNPN ($R^2$ = 0.70, RMSE = 0.06 g/100 g of full cross-validation). The addition of adulterants to milk increased the eNPN level for all samples and ranged from 0.04 to 0.37 g/100 g. Based on the difference between the known NPN range in normal milk and the predicted eNPN of adulterated samples, it was possible to detect samples adulterated with hydrolyzed rice (HRP), soya (HSP) or wheat (HWP) with a content higher or equal to 3.8 g/L. This study cannot be generalized as the natural milk composition change was not taken into account. Therefore, a second study was conducted to validate these first observations and to enlarge the use of infrared spectroscopy to near-infrared.

(4) So, 9 raw cow milk samples were adulterated with different levels of HRP (2.5-40 g/L), HWP (1.875-30 g/L), whey (1.875-30 g/L), urea (0.5-8 g/L), and water (3.125-50 g/L). Those 234 samples in total were analyzed using ATR-MIR and near-infrared (NIR) spectrometers. The developed NIR and ATR-MIR PLS - discriminant analysis did not discriminate control milk from adulterated samples. Raw ATR-MIR spectra discriminated better on HRP and HWP adulteration above 6.25%, while first derivative NIR spectra detected whey content above 12.5% in milk. Good prediction performance was observed to quantify the level of HRP and urea in raw milk using both ATR-MIR and first derivative NIR spectra (validation $R^2$p> 0.96). ATR-MIR showed better prediction on HWP level than NIR (validation $R^2$p=0.95 *vs.* 0.88), while NIR had a better accuracy in whey level quantification (validation $R^2$p=0.97 *vs.* 0.40). Moreover, both technics predicted well the total protein adulterant level (without water) in adulterated samples (validation $R^2$=0.87-0.98, RMSE=2.04-4.11 g/L). Consequently, the use of untargeted quantitative analysis is possible to detect the level of protein adulterants (such as hydrolyzed plant protein, urea or whey) in milk using NIR or MIR infrared spectroscopy, although classification of samples between adulterants types gave contrasted performances depending on the adulterant level and the king of spectroscopy used.