The assessment of the mycological quality of dry food ingredients using near infrared spectroscopy

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Introduction

It has become increasingly important in both agricultural and food research to determine mould contamination of horticultural crops in field and during processing. Under the applied conditions of processing technology or decontamination treatments partial inactivation of viable moulds is evident. However, toxic metabolites produced by moulds (many mould species are able to produce mycotoxins) may not be inactivated and may eventually present in the processed foods. Therefore, detection of viable and not viable mould content of food is important because of the fungal spoilage and the potential public health hazard, indicating whether food products have been prepared from uncontaminated, high-quality materials or not. Methods able to recognise moulds in foods under all circumstances would be useful for evaluating the possible risks related to fungal contamination.

As traditional methods different procedures are available for detecting moulds in food. They include cultural methods,[1] electrical measurements using conductance and other changes in electrical properties of culture media,[2] detection of heat-stable mould components like chitin[3] microscopic methods for detecting mycelium,[4] application of the direct epifluorescent filter technique[5] etc. Among these methods the mould colony count and Howard Mould Count (HMC) are used in many countries as a standard method for quality control purposes. Most of the current methods have certain disadvantages.

With traditional culturing methods heat-damaged or inactivated moulds escape detection and they are time consuming (time requirement is five to eight days). Microscopical detection of mycelium lacks precision. Using for example the ATP assay, has not widely been applied due to the problems of separating mycelium from food materials. Chemical methods based on detection of fungal chitin are not totally acceptable because the chitin content varies among species of mould.

Other alternative techniques have been developed to replace HMC. One of such methods is the quantification of ergosterol as an indicator of fungal contamination [6–8]. Determination of ergosterol content has been further developed using flourodensitometry for measuring fungal contamination in compound feeds.[9] Some immunological methods were described for detection moulds in foods.[10–13] They developed serological methods using enzyme-linked immunosorbent assay (ELISA) for detection of extra-cellular polysaccharides (EPS) produced by various moulds. A sensitive technique of latex agglutination was described.[14,15] Different volatile fungal metabolites are also used as indicators of fungal growth.[16–18] Jonsson and co-workers used an electronic nose (a sensor array with different type of sensors) for predicting the odour classes of good, mouldy, weak and

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strong musty oats.[19] A new fluorescent lectin test was developed for quantifying mould in raw tomato juice.[20]

Asher achieved good results in determining spores of yellow and brown rust of barley as well as brown rust of wheat for each fungal species, using near infrared (NIR) spectroscopy.[21] Preliminary studies on screening tomato puree for excessive mould content by NIR were reported by Davies.[22] Roberts and co-workers reported results about chemical and spectral quantification of mould in hay[23] and in contaminated barley[24] by NIR. Aramaki estimated mycelial weight in rice koji by NIR.[25]

Materials and methods

Wheat bran samples were collected by selecting the wheat carefully from practically mould-free crops. Practically mould-free samples can be defined as samples without any visible mould contamination. Samples were collected in 1998, 1999 and 2000. Three varieties were used for the experiments from each year: “Régia”, “Mlynské otruby” and “Ilona”. They were irradiated with 30 kGy irradiation dose, which made possible to get sterile samples. Paprika powder samples were obtained from retail store, black pepper samples from Kotanyi GmbH, Vienna (Austria).

The following mould strains were made available for the experiments: *Aspergillus flavus* (non-aflatoxigenic), *Penicillium verrucosum* (non-ochratoxigenic) and *Fusarium graminearum* (non-fumonisinogenic). At Szent István University (formerly University of Horticulture and Food Industry, Budapest), these strains were grown on surface of Czapek–Dox agar (Merck 1. 05460.0500) for one week at 25°C. Dilution fluid (containing 0.1% pepton and 0.85% NaCl) was pipetted onto the surface of Czapek–Dox agar containing mould cultures. Mycelia were rubbed off from the agar surface by using a small sterile stainless steel stick into the sterile dilution water and homogenised by vortexing. This suspension was used for inoculation. In the case of *Fusarium graminearum* suspension mycelia were homogenised by applying small sterile glass pearls in the glass tube during vortexing.

For determining ergosterol content of different samples a modified method of Seitz and Paukstelis[26] was used. Quantitative determination of ergosterol was performed by high–performance liquid chromatography (HPLC).[27] This HPLC method was developed originally for carotenoid analysis, but it is also suitable for separation and determination of ergosterol content. The accuracy (repeatability) of this method is $\pm 1 \times 10^{-3}$ mg ergosterol/g at low ergosterol content (around 0,04 mg ergosterol/g) and 100×10^{-3} mg ergosterol/g at high ergosterol content (around 0,6 mg ergosterol/g).

Samples were examined by NIR measurements using Spectralyzer (PMC type10-25, Switzerland) scanning NIR spectrometer. Log(1/*R*) spectra were recorded in the 1000–2500 nm wavelength region in 2 nm steps. Qualitative evaluations were performed according to Kaffka and Seregély[28] using the Polar Qualification System (PQS) program. "Quality points" of the samples were determined in the "quality plane". The quality point is defined as the centre of the spectrum (of the spectral points) represented in polar coordinate system.

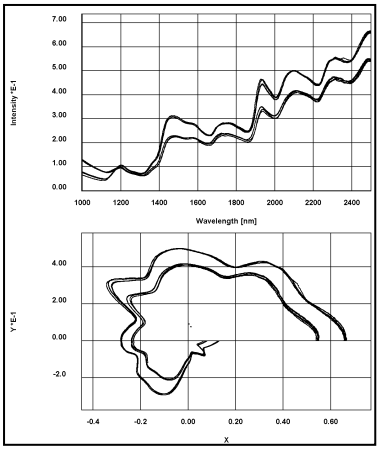


Figure 1.The log(1/*R*) spectra of bran samples in the rectangulat (top) and in the polar (bottom) coordinata system, red: control, blue: double ergosterol content

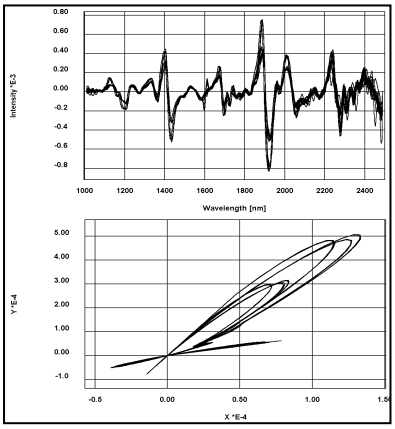


Figure 2. The 2nd derivative spectra of bran samples in the rectangular (top) and in the polar (bottom) coordinate system. The optimal wavelength range is 1418–1590 nm, red: control, blue: double ergosterol content

Figure 1 shows the log(1/*R*) spectra of the bran samples—having the highest and the lowest mould contamination—in the rectangular (top) and in the polar (bottom) coordinate system The polar spectrum might be unusual but the two spectra are equivalent. Since the absorption peaks of the same components can be situated 180° to each other on the quality plane balancing their shifting effect to the location of the quality point, the algorithm of the so called "wavelength range optimisation" was developed to solve this problem. The goal of the wavelength range optimisation is to determine that wavelength range (that part) of the spectrum which gives the best distinction of two samples according to one selected chemical or physical property using their quality points. The criterion of the "best distinction" must be of course defined. Three formulas were introduced with the help of which the best distinction could be determined[28]. The maximum of the "absolute distance" or the "normalised distance" or the "sensitivity" are the possible criterions of the optimum. The maximum value of normalised distance is 1, while the value of the sensitivity shows how much is the distance between the centres of the clusters higher than their standard deviations. By calculating these terms the effectiveness of the classification can be expressed numerically making it possible to compare the results obtained by using different classification models.

Results and discussion

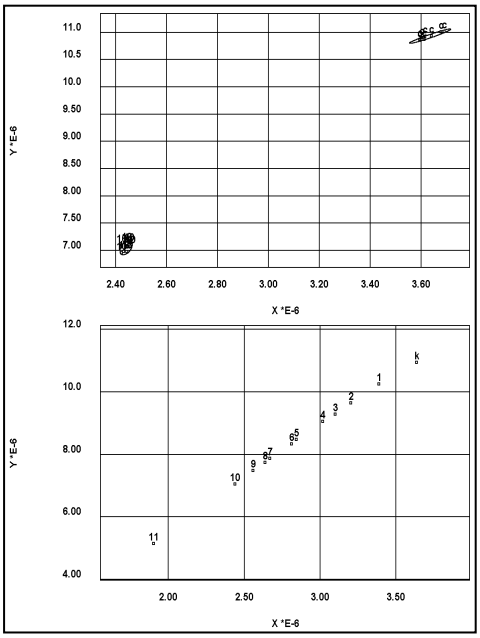
Before qualifying the samples with the above method several pre transformation procedure commonly used in the field of NIR spectroscopy (smoothing, derivative, MSC, etc) were applied to eliminate the effect of noise, particle size distribution etc. The effect of these procedure to the discrimination power of PQS in this special application were investigated by comparing the values of the maximum sensitivities. The results (optimal wavelength ranges and the corresponding sensitivities between the outermost contamination levels) of the wavelength range optimisations following the above pre-treatments are summarised in Table 1.

Table 1. The results of the optimisation of the wavelength ranges of the BRAN samples, produced from ILONA, REGIA and MLYNSKE wheat varieties infected with *ASPERGILLUS flavus* 301, *PENICILLIUM verrucosum* 499 and *FUSARIUM graminearum* 457 mould strains, comparing the effect of the applied pre-treatment to the log(1/R) spectra (top: optimal wavelength range, bottom: sensitivity in each cells of the table)

Sample	log(1/*R*)		2nd. Der		Norris		MSC1		MSC2		MSC3 Number of Points:45	
	Set to Zero	Omit	Set to Zero	Omit	Set to Zero	Omit	Set to Zero	Omit	Set to Zero	Omit	Set to Zero	Omit
REGIA												
Regia Aspergillus	1472–1586	1118–1228	1302–1418	1152–1374	1148–1268	1418–2002	2000–2216	1156–1198	1008–1018	1156–1198	1302–1418	1152–1374
	737,44	1158,56	1843,30	1692,03	559,47	261,08	1379,68	824,54	1022,44	379,49	1843,30	1692,03
Regia Penicillium	1552–1562	1122–1206	1144–1214	1052–1264	1272–1334	1122–1272	1986–1996	1136–1208	1002–1052	1122–1206	1144–1214	1052–1264
	1355,41	3422,00	2624,28	1831,58	974,42	497,59	2589,93	1468,19	5525,54	1060,60	2624,28	1831,58
Regia Fususarium	1542–1554	1122–1262	1152–1210	1156–1374	1182–1570	1148–2058	2224–2248	1210–1292	1002–1034	1122–1332	1152–1210	1156–1374
	427,25	2424,57	1648,47	1325,16	369,32	174,92	731,40	930,54	1292,64	557,29	1648,47	1325,16
MLYNSKE												
Mlynske Aspergillus	1092–1102	1000–1928	1184–1224	1134–1382	1320–1330	1096–1328	2048–2058	1000–1930	2042–2056	1050–1306	1184–1224	1134–1382
	454,38	1622,21	1469,64	757,46	501,70	141,37	5740,06	729,80	11139,68	1554,40	1469,64	757,46
Mlynske Penicillium	1098–1108	1048–1250	1426–1440	1426–1788	1344–1368	1078–1328	1866–1876	1054–1220	1032–1042	1012–1246	1426–1440	1426–1788
	379,37	1277,17	1572,71	365,72	382,65	117,06	1223,34	604,33	920,54	710,54	1572,71	365,72
Mlynske Fusarium	2168–2200	1004–1268	1282–1350	1154–1386	1154–1544	1038–1398	2204–2288	1014–1250	2240–2276	1006–1254	1282–1350	1154–1386
	476,29	2008,11	1067,41	616,00	397,87	242,14	2023,91	783,35	3227,50	1498,98	1067,41	616,00
ILONA												
Ilona Aspergillus	2060–2084	1118–1192	1152–1282	1092–1382	1154–1170	1052–1192	2006–2366	1030–1926	1288–1348	1120–1192	1152–1282	1092–1382
	2812,39	1884,84	1855,47	1430,72	353,20	166,33	3009,44	1545,60	15242,84	1477,75	1855,47	1430,72
Ilona Penicillium	1570–1624	1026–1730	1430–1500	1224–1650	1082–1138	1182–1982	1502–1516	1456–1880	1294–1368	1112–1444	1430–1500	1224–1650
	4392,73	2569,56	2209,42	1220,70	593,55	361,43	1636,83	1235,21	4093,33	1762,72	2209,42	1220,70
Ilona Fusarium	1114-1218	1000-1946	1142-1262	1114-1344	1082-1144	1016-2166	1366-1846	1052-2368	1998-2182	1128-1260	1142-1262	1114-1344
	2998,13	1014,17	1214,78	569,38	423,75	131,08	1959,71	1082,07	1487,34	860,58	1214,78	569,38

Figure 2 represents the repeatedly measured 2nd derivative spectra of the bran samples having the highest and the lowest mould contamination in the rectangular (top) and in the polar (bottom)

coordinate system. The calculated optimal wavelength range is 1418–1590 nm. This range is bordered by dotted lines on the top figure while the polar diagram shows this selected range only. The centres (quality points) of these spectra are shown in the top part of Figure 3. As it can be seen the distance between the sample groups is high compared to their standard deviations. In the bottom part of Figure 3 the location of the quality points of the samples can be seen having mould content in between the outermost contamination levels on the same quality plane using the same wavelength range. The increasing marking numbers denote increasing contamination levels (from 0–50% in 5% steps, c: 0%, 1: 5%,...10: 50 %, 11: 100% relative mould content). As it is shown the points of the outermost samples locate in corresponding sequence between the samples taking part in the optimisation.



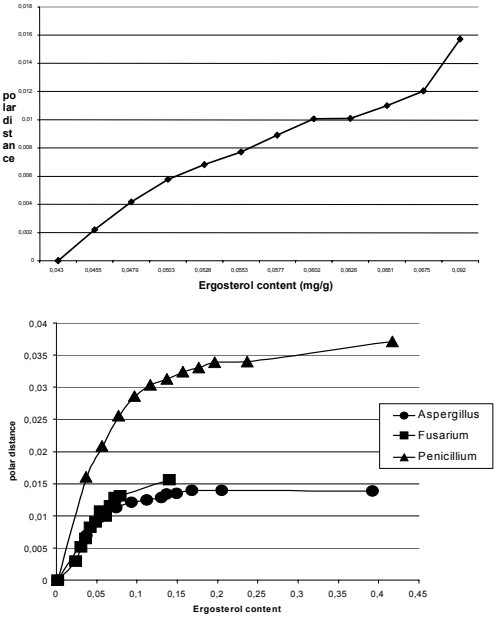


Figure 3 The quality points of bran samples having the highest and the lowest mould contamination calculated from the 2nd derivative spectra using the optimal wavelength range (top). The quality points of the samples having mould content in between the outermost contamination levels (bottom). The increasing marking numbers denote increasing contamination levels

Figure 4. The polar distances between the quality points of the samples contaminated differently with *Fusarium* and control sample, harvested in year 2000 versus the ergosterol content (top). The polar distances between the quality points of the samples contaminated with *Fusarium, Aspergillus*, *Pennicillium* and their control samples, harvested in year 1999 versus the ergosterol content (bottom).

The top part of Figure 4. demonstrates the polar distances between the quality points of the samples contaminated differently with *Fusarium* and the control sample, harvested in year 2000 versus the ergosterol content. The bottom part shows the polar distances between the quality points of samples contaminated with *Fusarium*, *Aspergillus Penicillium* and their control samples, harvested in year 1999 versus the ergosterol content. With the help of Figure 4 the tendency of the

changes caused by mould development can be observed and compared among the studied mould strains. As it is shown the growing of *Penicillium* has the most sensitively detectable effect resulted in the location and shift of the centres of the polar spectra.

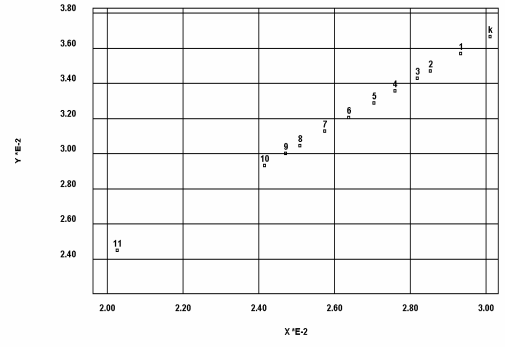


Figure 5. The quality points of red pepper samples contaminated with *Pennicillium* having mould content in between the outermost contamination levels. The increasing marking numbers denote increasing contamination levels. The optimal wavelength range is 2330–2340 nm

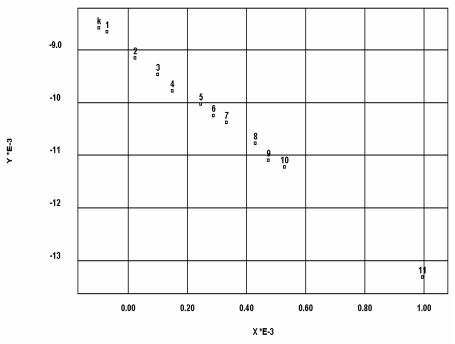


Figure 6. The quality points of black pepper samples contaminated with *Pennicillium* having mould content in between the outermost contamination levels. The increasing marking numbers denote increasing contamination levels. The optimal wavelength range is 1040–1332 nm

In the Figures 5 and 6 you can see the quality points of red and black pepper samples contaminated with *Pennicillium* in different levels in their optimal wavelength range using PQS.

Conclusion

The contamination with moulds is one of the most important microbiological quality parameter of bran products. Moulds can produce various toxic metabolites, which are not eliminated or inactivated by the processing technology, while viable moulds are drastically reduced. Therefore, it is important to detect both viable and non-viable mould biomasses together. The available microscopical, immunological and chemical methods are slow, expensive or inaccurate. NIR spectroscopy would provide non-destructive, reagentless method for this purpose opening new perspectives in application of PQS offering a rapid, accurate, cheap and simple method for qualifying or identifying products, using their near infrared spectra.

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