The potential use of near infrared spectroscopy for monitoring mushroom (*Agaricus bisporus*) compost quality during production

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Introduction

Mushroom cultivation is an important horticultural crop in Holland, Belgium, France, the UK, Ireland and Italy. In the past 15 years, the mushroom industry in Europe has changed significantly in terms of the production and growing systems. *Agaricus* is the most widely cultivated mushroom in the world and its popularity is rising with commercial cultivation increasing world-wide. Total European production of Phase II compost was estimated to be over 4 M tonnes per annum in 1998, with a value of £340 M. Total annual production of mushrooms in the EU is near 900 M tonnes with a farm gate value of *c*. £ 450 M per annum.

Mushroom and compost productions are important sectors of the horticultural industry in the UK and are worth more than £150 million annually. The total annual sales turnover is now valued at nearly £50 million[1] and is the success story of the Northern Ireland horticultural sector. This represents more than 50% of the horticultural output of the province and currently more than 2000 people are employed in the different sectors of the industry comprising, compost production. casing (peat and lime mixture) manufacture, growering, marketing companies, spawn supply and their associated industries.

Compared to other sectors of the horticultural industry, the turnover generated by mushroom producers understates the relative importance to the economy in terms of gross margins. With low input costs, the industry contributes significantly to the European economy. The industry also maintains much needed employment in rural areas where the agricultural workforce would otherwise be in decline, due to the reduced viability of the traditional small family farm. In addition, the composting sector is a major user of waste materials, namely straw and poultry litter from other agricultural sectors.

The growing system adopted by industry in Northern Ireland consists of centralised compost production, specialist small-scale growing facilities and large-scale marketing co-operatives and this has undoubtedly accounted for much of the success. Centralised compost production has enabled extensive capital investment, necessary to achieve the higher standards of compost quality and environmental pollution control required, while economies of scale have maintained the price of compost in recent years. Small growing units require relatively low initial capital investment but are run by highly motivated growers, who give the meticulous attention to detail, required to grow the high quality mushrooms demanded in the market place. With supermarket dominance in the UK, the organised marketing structure in Northern Ireland is well placed to meet the demand of specialised production, short supply chains and inherent flexibility.

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Near infrared spectroscopy

Of all the techniques available for rapid assessment of materials, NIR is the most suitable method for process analysis. This technique has been used as a non-destructive analytical tool for assessment of quality and composition of litter,[1] animal feeds,[2] food,[3] brewing,[4] soil[5] and many others.[6] The spectral data from the materials at each stage of production could be used for monitoring important parameters and if possible, control the process during pasteurisation and conditioning of compost according to defined conditions. Currently, not all changes in NIR spectra of compost can be explained by the existing knowledge of the production process and, in addition, the spectrum contains extensive information on the substrate, which has yet to be fully understood. Complex biochemical changes are taking place, which cannot be measured by the existing analytical tools. Recently, Sharma and Kirkpatrick.[7] reported that the NIR spectra of pasteurised compost, after suitable sample preparation, could be used for predicting potential yield of fruiting bodies. The spectrum contains fingerprints of all the microbial, biochemical and physical changes taking place during the preparation of compost and the spectral markers/characteristics shift in correspondence to the transformation within the substrate. The influence of these factors on the spectrum can be described as multidimensional and the data matrices obtained can only be analysed using complex algorithms, such as principal component analysis and partial least squares regression methods.

Methods currently available for quality control during production are pH, total nitrogen, ammonia, conductivity and ash.[8–10] The potential for using NIR, to measure changes in key quality parameters has been investigated by Sharma *et al*.[11] The value of NIR as a rapid, inexpensive environmentally-friendly instrumental method has been widely accepted as an alternative to traditional wet techniques for determining nitrogen and fibre fractions.[12] The development of multi-channel NIR spectroscopy for on-line analysis in chemical, pharmaceutical and animal feed industries has shown that the technology could potentially be applied to compost production as part of a routine quality control system.

Research objectives

The fundamental problem of mushroom production is the extensive variation in the quality of raw materials used, such as wheat straw and chicken litter/horse manure/other animal waste, and the ensuing effect on compost quality and yield of fruiting bodies. The project aims to achieve the following targets:

1. To develop quality standards for commonly used raw materials of mushroom compost
2. To develop NIR calibration equations for assessing both dry and fresh compost so that substrates can be modified during production and investigate the feasibility of using an on-line NIR system
3. Minimisation of the environmental impact of compost production by improving efficiency of the production process

NIR spectroscopy for monitoring compost quality

Assessment of raw materials

Raw materials have a decisive influence on the quality of the substrate. The incoming materials must be checked not only for quality and composition but also for identity. The quality of wheat straw can differ depending on variety, application of fungicides and growth hormones, storage conditions after harvesting, age and variety. Significantly, the NIR spectra are also different for these samples as the cellulose and hemicellulose contents are variable. In addition, the quality of the chicken litter can also vary depending on the bedding materials (i.e. wood chips or straw) and the ingredients of feed used. NIR spectroscopy could be used for the determination of colour, nitrogen, moisture content, fibre fractions, ash and other components of the raw materials used for preparing compost. In some parts of Eu-

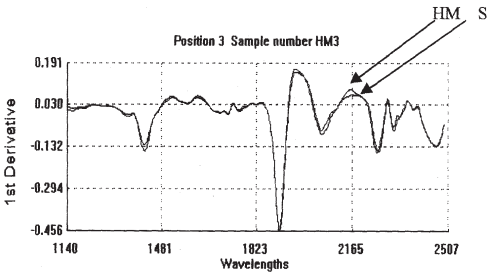

Figure 1. Mean spectra of dried samples of horse manure (HM) and wheat straw (S) after mathematical treatments of the spectral data (SNV/DT-144) where the first number—the order of the derivative function, the second—the segment length in data points over which the derivative was taken and the third—segment length over which the function was smoothed.

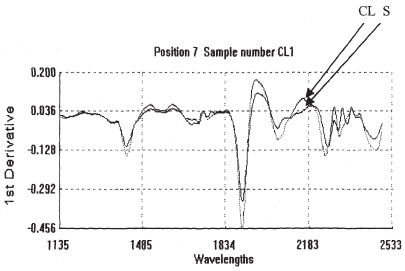

Figure 2. Mean spectra of dried samples of straw (S) and chicken litter (CL) after mathematical treatments of the spectral data (SNV/DT-144) where the first number—the order of the derivative function, the second—the segment length in data points over which the derivative was taken and the third—segment length over which the function was smoothed.

rope, horse manure is still used as raw material for compost production. Comparisons of the NIR spectra of dried and milled samples of straw, chicken litter and horse manure are presented in Figures 1 and 2. The main differences between the spectra of horse manure and straw can be detected at the following wavelengths 1416, 1508, 1620, 1976 and 2046 nm. In contrast the spectral differences between chicken litter and straw are extensive: 1416, 1508, 1620, 1976, 2046, 2166, 2266 and 2466 nm. From the spectral data, it may be possible to identify key spectral combinations as indicators of the raw material quality. The compost production consists of two stages.

Phase I

Compost is prepared either by windrow or bunker systems capable of handling 2–5 k tonnes of material per week. The differences between production methods of the two phase I systems are as follows:

Windrow system

The windrow compost is prepared by mixing wheat straw and chicken litter at a ratio of 1000 kg straw: 450 kg chicken litter. The square bales of straw are broken up and wetted using recycled water, this stage lasting for up to 48 hours. The wet straw is then mixed with a slurry of chicken litter and gypsum, blended at a ratio of 1000 kg chicken litter : 50 kg gypsum. The mixed materials are piled up in an open yard and this stage can last for 5–6 days. During this period, microbial succession takes place starting with mesophiles as primary colonists, followed by thermophilic fungi and actinomycetes. The core temperature may reach 60–70°C. The microbial activity at this stage of fermentation is dependent on the availability of carbohydrates and nitrogen under aerobic conditions.[8,13] Ammonia, released after breaking down urea and protein, softens the straw and makes it accessible to microbial degradation. After which, the materials are transferred to a covered yard and made into windrows or stacks (typical size 100 × 2 × 2 m). In order to control the build-up of high temperature and anaerobic conditions, the windrows are turned every two to three days using a compost turner and this process can last up to six days depending on the weather conditions. During this stage, the fibre fractions, primarily hemicellulose and cellulose, are degraded by the polysaccharide degrading enzymes released by the

thermophiles.[8,13] Minimal breakdown of cellulose is desirable, as too much loss at this stage of production would lead to over-composting. Instrumental probes for measuring oxygen demand, temperature and NIR can be used for monitoring the degree and speed of straw breakdown caused by a succession of micro-organisms.

Bunker system

The formulae for bunker compost is similar to that of the windrow production system, except that a lower proportion of chicken litter is used (1000 kg straw : 400 kg chicken litter). The square bales of straw are wetted using recycled water with the aid of a watering gantry and this stage can last for up to 48 hours after which the bales are broken up by bale-breaker and mixed with a slurry of chicken litter and gypsum blended at a ratio of 1000 kg chicken litter : 50 kg gypsum. The mixed materials are transferred to bunkers (capacity 400 tonnes) for rapid fermentation and the bunkers are enclosed on three sides with concrete walls (8 ×10 × 50 m). The floors of the bunkers (10 × 50 m) are lined with air-inlets (2.5 mm) at a density of 16 units m^{-2} and air is pumped through these ports at a fixed rate of 4 m^3 of air $tonne^{-1}$. In order to control the build up of high temperature and consequent rapid breakdown and depletion of oxygen levels inside the compost pile the air handling system is linked to temperature and oxygen probes.

Within the bunker. compost temperatures can increase to over 60°C within 3–4 hours and this stage can last for four days. The core temperature within the bunker may reach 80°C after two days and ,consequently, microbial activities are reduced although non-biological breakdown (unpublished data) of the substrate can still take place. The straw is softened by ammonia released during breakdown of protein. After four days, the compost is transferred to an adjacent bunker to improve consistency and water (10 l $tonne^{-1}$) is added at this stage to compensate for moisture loss as a result of the airflow through the pile. Minimal breakdown of the fibre fractions, including cellulose, takes place as the environmental conditions are not favourable for thermophilic fungi and bacteria. After four days, the phase I compost is emptied into a concrete yard and left overnight to reduce the temperature to below 45°C. NIR, oxygen and temperature probes could be positioned in the bunker at different depths for monitoring moisture content, fibre breakdown, microbial activity and nitrogen content. NIR spectra of dried phase I samples, prepared by the windrow and bunker systems are compared in Figure 3. The main differences can be detected at the following wavelengths: 1416, 1902, 1958, 1990, 2050, 2266 and 2466 nm. The spectral combinations could be used for predicting key quality parameters of the phase I compost.[11]

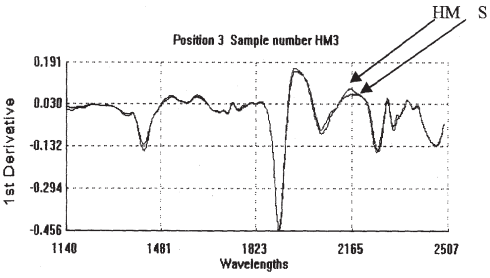

Figure 3. Mean reflectance spectra of dried phase I windrow (W) and bunker (B) composts after mathematical treatments of the spectral data (SNV/DT-144) where the first number—the order of the derivative function, the second—the segment length in data points over which the derivative was taken and the third—segment length over which the function was smoothed.

Phase II stage

The composts prepared by either bunker or windrow systems are pasteurised at 57°C for 24 h in peak-heat tunnels, with a capacity of 150 tonnes each, and the temperature is then gradually reduced to 45°C to complete the conditioning process in six days. The tunnels are equipped with state-of-the-art computer facilities for monitoring and controlling compost and air temperatures. Ammonia levels are checked regularly during this stage, as this is one of the key indicators of compost quality. NIR, ammonia, and oxy-

gen probes could determine the changes, during composting in phase II tunnels. NIR spectra of dried phase II samples, prepared by the two phase I methods, are compared in Figure 4. The main differences can be detected at the following wavelengths 1416, 1508, 1902, 1958, 1990, 2050, 2266, 2320 and 2466 nm. From the spectral data, it is possible to identify key parameters as indicators of the phase II compost quality.[11]

In the final stage of production, pasteurised and conditioned phase II compost is inoculated with *Agaricus bisporus* spawn. During the 14 day spawn run period, the compost is colonised by the *A. bisporus* mycelium. Later, the colonised compost is cased with a mixture of peat and lime and cropped in insulated plastic houses under controlled conditions for four flushes.[14]

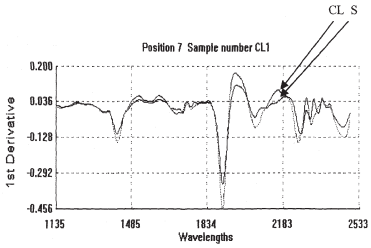

Figure 4. Comparison of NIR spectra of dried phase II compost samples prepared by bunker (B) and windrow (W) phase I production system after mathematical treatments of the spectral data (SNV/DT-144) where the first number—the order of the derivative function, the second—the segment length in data points over which the derivative was taken and the third—segment length over which the function was smoothed.

Calibration development

Calibrations for a range of parameters have been developed using dried and milled composts. The methods employed for assessing microbiological and chemical differences in compost samples have been previously reported by Sharma. *et al.*[14] Protocols for sample selection and development of calibration equations have been reported extensively and the repeatability of the reference method is the most important factor affecting the performance of the NIR regression equation to predict the refer-

Table 1. Calibration statistics of important parameters of compost samples pH, nitrogen dry matter (NDM), thermophilic population, carbon, C : N ratio, neutral detergent fibre (NDF) and acid detergent fibre (ADF).[11]

			Calibration		Validation	
Variable	*n*	Mean	*SEC*	r^2	*SECV*	r^2
PH	184	7.09	0.141	0.946	0.178	0.914
NDM (%)	175	1.98	0.072	0.959	0.079	0.948
Thermophiles	139	7.67	0.333	0.964	0.461	0.931
(log cells g^{-1})						
Carbon %	182	37.72	0.712	0.960	0.824	0.946
C:N	138	15.67	0.878	0.949	0.986	0.935
ADF (%)	137	48.57	1.329	0.956	1.410	0.950
NDF (%)	134	44.86	1.114	0.940	1.182	0.933
Ash (%)	183	23.06	0.753	0.954	0.994	0.920

SEC—standard error of calibration, *SECV*—standard error of cross-validation, r^2— squared for calibration and validation, Mathematical treatments (133)— indicates the transformation applied to the spectral data where the first number—the order of the derivative function, the second—the segment length in data points over which the derivative was taken and the third the segment length overwhich the function was smoothed.

ence values.[15–18] Calibration and validation statistics for pH. conductivity, ash, NDM, thermophiles, CN ratio, NDF (neutral detergent fibre), ADF (acid detergent fibre) and lignin are presented in Table 1. Calibration and validation errors, as a percentage of the mean value, were largest for pH, conductivity and ash. A detailed study on the selection of suitable spectral data, scatter correction and mathematical treatment to apply to these data, was recently investigated by Sharma *et al.*[11] Since the colour of dried composts differs due to variation in raw materials and degree of fermentation, three spectral bands, 400–1100 nm 1100–2200 nm and 2200–2500 nm were assessed for their calibration effect on compost parameters and mushroom yield. Whenever the wavelength range from 400 to 700 nm was used, the cross-validation error became larger. The best prediction equations were obtained by excluding the spectral region below 1100 nm.

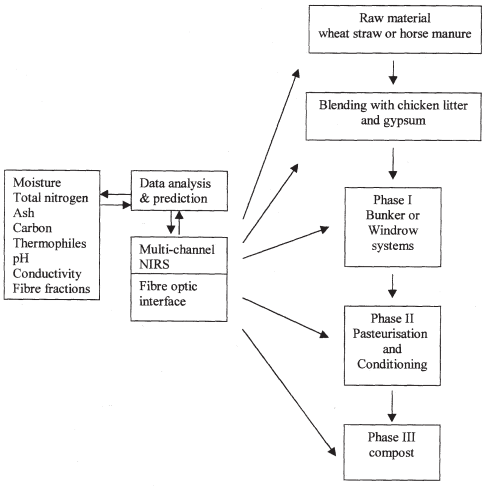

Figure 5. Schematic flow of mushroom production monitored by mult-channel NIR spectrophotometer.

Monitoring system

On-line or at-line monitoring of the production stages, i.e. input raw materials. Phase I (windrow or bunker) and Phase II could be carried out using a multi-channel analyser (Figure 5). For analysis of the spectra from fresh samples, individual calibration equations for each of the key parameters such as NDM, moisture content, ash. fibre fractions and others, have to be developed from the database. After mathematical treatment, the spectral data could be assessed using these calibrations. All components of the test substrates will have their own spectral images for a given period during production. The initial calibrations necessary for measuring key parameters can be developed using a bench top model (such as Foss, 6500 NIR system). The instrument can scan fresh and dried samples taken at different stages of production: raw material, beginning of phase I, filling of the phase II peak-heat tunnel at the end of phase I, and at the end of phase II. Moisture contents of fresh phase I and II samples can range from 70–75% and 64–69%, respectively. The spectra are dominated by two peaks at near 1449 and 1960 nm representing water and the peak in the 400–600 nm region, showing the dark brown colour in the visible band (Figure 6). With high moisture content, the relationships between NIR spectra and key compost parameters may not be linear and selected segments of the spectra may have to be used for the development of robust calibrations.

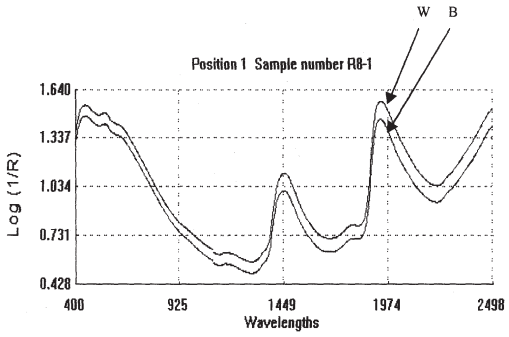

Figure 6. Comparison of visual and NIR spectra of fresh phase II composts prepared by bunker (B) and windrow (W) phase I production systems showing three major peaks representing colour in the visible part, 400–600 nm and water peaks at 1449 nm near 1960 nm in the NIR region.

Conclusion

NIR analysis of mushroom compost needs a convenient method of sample preparation, a precise reference method and robust prediction equations. Development of the best prediction equation for wet samples required the evaluation of all spectral and data processing techniques, monitoring the performance of the equation and recalibration, when necessary. The accuracy of these predictions could be maintained by establishing a library of spectra for composts and expanding the calibration database with selected samples. Real time spectral data acquisitions during on-line assessment will open up new frontiers for interpretation and utilisation of the hardware. The industry needs a rapid turnaround of analytical information on the changes taking place during preparation in order to control and optimise the production process. This would result in improved productivity and quality.

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