Mushroom compost production, sampling and sample selection for development of NIR calibrations

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Introduction

Mushroom (Agaricus bisporus) production in Europe has increased dramatically in the past decade, although growth in the industry has slowed down in the past 2-3 years due to consolidation of production and growing technologies. However, future growth in mushroom consumption is still expected due to increased consumer awareness of food safety issues and environmental concerns. Small and medium enterprises dominate the industry, comprising of raw material suppliers, compost producers, spawn producers, supplement manufacturers, growers and casing producers with a labour requirement of 120,000 employees. Total European production of compost was ca 4.5 Million tonnes per annum with a value of 400 Million Euro in 2002. Total annual production of mushrooms in the EU is more than 1,000,000 tonnes with a farm gate value of ca 3000 Million Euro per year.[1,2]

Production systems

Traditionally mushroom compost is prepared by composting wheat straw/horse manure, chicken litter, gypsum and supplements, during two stages of production known as phase I and II lasting 18 - 21 days. The phase II substrate promotes the growth of mushroom mycelium to the exclusion of competing microorganisms. This is achieved by manipulating the natural succession of microorganisms present in raw materials.[3,4,5] Production of mushroom compost in the British Isles is largely different from the process employed by composters in the Netherlands and Belgium.[6] The producers in N. Ireland and Ireland have adopted outdoor bunker phase I composting followed by phase II pasteurisation in indoor tunnels for nearly 18 hrs (Figure 1).

This is followed by conditioning in the tunnels at 45^0C for 4-5 days before inoculating with *A. bisporus* spawn in plastic bags. After an incubation period of 15-18 days in environmentally controlled plastic houses, the substrate bags are layered with a mixture consisting of sphagnum peat and ground limestone (known as casing). The lime is used for neutralising the acidity of peat. In contrast, other European composters have adopted an indoor (Figure 1) composting (a combined phase I, II & III stages in enclosed chambers) process, due to strict environmental regulation on pollution and to shorten production time.[7,8,9,10,11,12] After 15-20 days the compost is inoculated with spawn in an enclosed chamber and after an incubation period of 15-18 days the spawn run substrate (phase III compost) is transferred to metal trays and cased with casing to harvest three to four flushes of mushrooms during a 4-week period. Average mushroom yield of phase II compost after a

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spawn run period of 15-18 days is 250 kg/tonne and for spawn-run phase III compost the yield is higher at 315 kg/tonne.

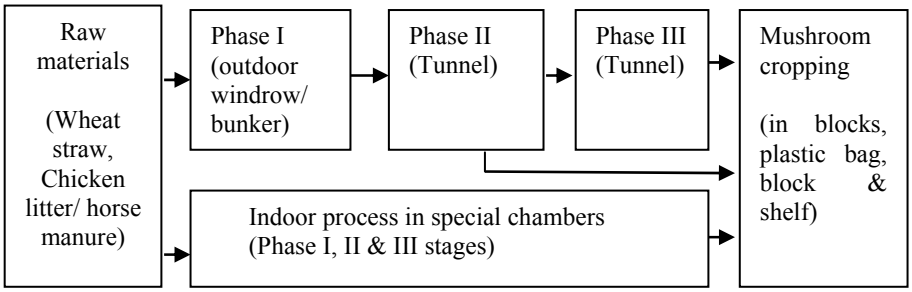

Figure 1. A graphical representation of the different mushroom compost production systems showing the production steps from raw material to the final cropping stage.

Near infrared spectroscopy

Recent improvements in instrumentation, computer hardware and software have enhanced the robustness of this technique. Near infrared spectroscopy (NIRS) is a powerful tool for rapid analysis of a range of agricultural and horticultural materials.[13] In the past 8 years, application of NIRS for assessing compost quality has been investigated in Northern Ireland and early research focused on the development of calibrations for key parameters, using milled samples due to lack of facilities on site. The parameters are pH, nitrogen, thermophilic population, carbon, ash, fibre fractions, dry matter content and potential yield.[6,14,15,16] However research during the past 4 years has concentrated on the development of calibrations using fresh materials in order to reduce sample preparation time and this approach could reduce accuracy of the calibrations due to high moisture content of phase I and II samples. Recently, a review of the work carried out in Belfast and elsewhere in Europe has been reported by Sharma.[17,18]

The inherent problems of compost production are the variability of the raw materials used and volume of substrate produced in order to sustain profitability. A moderate size compost yard producing 250,000 tonnes of phase II compost may require 250,000 tonnes of straw and 100,000 tonnes of chicken litter per year. The volume of materials going through the phase I and II stages pose difficulties during sampling and monitoring of quality. The materials need to be analysed by both NIR and reference wet chemistry methods to develop calibrations and unless the samples are representative of the production batch, the integrity of the tests is compromised. Therefore sampling is one of the factors that determine robustness of NIR calibrations. This paper highlights the protocols developed for handling fresh compost.

Sampling

Primary objective of sampling is to collect a truly representative material of the whole sample. However, a number of factors including, area of sampling, size and type of materials, physical nature, size, blending, frequency of sub-sampling and foreign materials, such as stone and wood chips, will determine sampling error, accuracy of the wet chemical analysis and robustness of the NIR calibrations. Therefore materials must be sampled carefully in order to obtain a composite sample of the batch. However, variance within the population needs to be assessed by analysing all

sub-samples and this will determine the total number of sub-samples needed to be taken from the batch. For example a reliable guide is to collect 50 sub-samples (5 kg for each sub-sample) from a bunker containing 500 tonnes of phase I compost.

Raw materials

Metal probes that consist of steel bars of various lengths can sample bales of straw and the ends of the steel bars carry several barbs. When the probe is pushed into the bale a sample can be obtained by twisting and withdrawing the probe. However, in practice it is more convenient to sample straw after opening the bale with a mechanical aid, such as a bale breaker. The main components of straw are cellulose, hemicellulose, lignin and ash and the composition can differ between wheat varieties.[19] The fractions are degraded during outdoor storage due to microbial action, sun, wind and rain. Since the bales are stored on top of each other in ca 5 meter high rows, exposed bales on the top are usually broken down rapidly compared to bales underneath. The degraded bales are usually discarded and not used for compost production. Although the bales can be stored indoor, composters generally store the bales outdoor to comply with health and safety-regulations.

The nitrogen content of chicken litter can vary significantly between batches, due to various factors including feed quality and hygiene regulations of the broiler industry.[6] Unless a recipe for blending of straw, chicken litter and gypsum is adjusted based on nitrogen content of the chicken litter, the microbial activity during the production process will be affected. Sampling of chicken litter is relatively easy compared to straw, but the protocols must comply with current health and safety regulations, as pathogenic microorganisms can be present in poultry litter.

Phase I, II and III

During sampling of materials from compost yards, three main sources of error can be associated with the sample and these are as follows: source of the material, methods employed to sample and composition of the sample. Substrate analysis is beset with difficulties, as materials have to be taken from different depths of the windrow, bunker and tunnel to determine variance within the production batch and the degree of variation will determine frequency of sub-sampling.

Locating suitable sub-sampling positions in a bunker/windrow/tunnel is not a random process, as the materials have to be taken in a planned manner. Where access to a particular area is dangerous from a health and safety point of view, mechanical aids should be used to minimise risks to personnel. In compost yards, sampling is routinely carried out at the beginning and end of phase I, II & III (Figure 1). For example, when the blended-raw materials are being transferred into a bunker, samples can be taken at regular intervals manually from the conveyor belt and similar procedures can be followed at each stage during filling or emptying of a tunnel. It is not feasible to install automatic sampling arms, as used by the grain industry in the US and Canada.

However, sampling from an indoor production system has to be organised even more carefully as the production batch can be contaminated with saprophytes, weed mould fungi and pathogens. Therefore sampling protocols have to follow strict hygiene similar to the standards followed by the food industry.

Sample handling and preparation

After sampling, the materials must be labelled, packaged safely and transported from the production site to a laboratory for further sample preparation. Although storage of the samples is not recommended, it may be unavoidable when sampling a bunker with a 500 tonne capacity. After transportation of the sub-samples (50 bags), the sample bags can be stored in a cool-room (-4^{o}C)

overnight before sample preparation and analyses could start the next day. Sub-samples of chicken litter can be blended using a mechanical aid to reduce variation but this method is not practical for straw or phase I and II samples and the blender causes more problems than actually solving it. Therefore sub-sampling of the materials is usually carried out manually by ensuring that the correct proportion of old and new straw is maintained and the efficiency of this process should be monitored during the process. Care should be taken to minimise loss of moisture and materials during this stage of preparation.

Where possible, prepared materials should be analysed after stabilising the temperature of the samples in a controlled environment. Key parameters, such as moisture content, microbial population, pH and conductivity can change significantly after storage and it is advisable that the four parameters should be measured on the day of sampling before storage.

Prior to wet chemical analyses of samples, drying is necessary and should be dried at below 85^{o}C overnight on trays lined with aluminium foil in small 1-2 kg batches. Care should be taken to avoid contamination of the samples and introduction of physico-chemical artefacts. Changes in moisture content after drying can be minimised by storing in sealed bags before milling. Measurement of volatile compounds present in compost can be carried out by drying at 35^{o}C to a low enough moisture content to enable milling, after which it can be analysed. In contrast freeze drying of samples provides a better preparation step which minimises loss of volatiles.

Milling of samples

Reference wet chemical analyses for nitrogen, ash, fibre fractions and total carbon, can be carried out using dry ground samples and milling of the dried samples must be carried out carefully to avoid degradation of the material. There are five types of grinders: burr, cutting action, hammer, impeller and centrifugal. For mushroom compost, Cyclotec mill (model no 1093) employing fan type impeller with cyclone action manufactured by Foss Tecator is ideal and similar products from other manufacturers are also available. Performance of a grinding mill is influenced by many factors including, type of material, sample composition, physical texture, operational speed of the grinder and grinding chamber. Mills must be serviced and cleaned regularly to avoid the affects of reduction in grinder performance on particle size distribution, incidence of high temperature during grinding and rate of throughput.

Sample presentation

Although a range of NIR sample cells can be used for fresh mushroom compost, a natural product cell is the best due to its large sample window area for maximising reflectance. The cell can also be used for milled samples and convenient to fill a 3-4 mm layer without stratification, which causes inaccuracy and variance during scanning. The sample must be thoroughly re-mixed between scans. For fresh samples, it is advisable to scan a minimum of 4-5 different sub-samples for each sample. Filling the cell with a known weight is also important for maintaining precision of spectral reflectance and this can be achieved by starting with a known weight of the sub-samples before scanning. Over-filling and under-filling the cell will change the degree of sample compaction and under certain circumstances the sample could force the backing plate to open in the transport chamber causing damage leading to breakage of the quartz glass window of the cell.

The moisture content of materials from different stages of mushroom production can range between 10-80%. Compost samples with high moisture content can cause difficulties during sub-sampling and packing. Handling of odorous samples including chicken litter and phase I compost, need to comply with health and safety protocols. Use of non-PVC cling film or bags to house the material can overcome safety regulations by subtracting the spectral signature of the bag from the sample spectrum to eliminate spurious absorbance arising from the plastic. This can be achieved by

using WINISI or other chemometric tools. The cell need to be cleaned in between samples and care should be taken to avoid breaking the quartz glass window due to stones or hard materials in the sample. Accuracy of NIR analysis is dependent on sample characteristics, such as moisture content, composition, fineness or coarseness of the materials, temperature and humidity in the laboratory,

Sample selection for calibration development

In order to develop a robust calibration, a database of samples with visible and NIR spectra representing full range of target reference parameters is needed and the calibration must be validated using blind samples. This can be achieved by either generating enough samples with database to develop and validate the calibrations, or selection of samples strictly on the basis of spectral characteristics followed by reference analysis of only those samples that display comprehensive variance in the spectral data. The two approaches are referred to as conventional and spectral methods of sample selection.[20]

Conventional

This approach requires identification of all possible sources of variance likely to be encountered including seasonal factors, different production systems, phase I and II production parameters, range of the target parameters and physical characteristics including colour. It is better to select samples with uniform distribution with respect to the range of constituents to be determined.[23] Recommended sample size for calibration development is ca. 100 for a target composition range of 10% variation, for example a moisture content range of 62 - 72% will require approximately 10 samples for an increment of 1% in the moisture range (62-63%). Since key target parameters for compost are moisture content, microbial population, pH, nitrogen, ammonia, carbon, ash and fibre fractions, even greater number of samples representing a full range of each parameter will be necessary to develop robust NIR calibrations for all parameters.

Spectral sample selection

This procedure is based on selection of samples on the basis of their spectral characteristics. Although this method can reduce the number of samples to be analysed by the reference methods, a large number of samples must be collected and scanned to provide spectra displaying maximum variance. A detailed description of each sample will be necessary to explain variance within the set and different samples from the set may be used for a particular parameter, due to variation in their spectra. Spectral variation due to differences in the particle size of the materials must also be taken in to account during the sample selection process, as this could be the reason for differences between the samples rather than the chemical constituent. However, effects of this factor can be minimised by transformation of the raw spectral data using a number of mathematical tools. Principal component analysis can also be used for sample selection using all wavelengths. Spectral sample selection tool offered by WINISI software is convenient for removing spectrally similar samples.

Conclusion

Since materials from compost yards are usually high in moisture content, it is best to scan the samples first and analyse prior to calibration development for the key parameters. Spectral interactions of the major and minor components in varying degrees of composition are complicated and the differences in spectral intensities between samples cannot be easily interpreted from an overlay.[19] In practice sample selection for calibration development can only be carried out

effectively on the basis of the key parameters. The development of a robust NIR calibration is dependent on four main factors: sampling technique, minimising change in the sample during preparation, sample presentation to the instrument and accuracy of the reference method.

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