

# Indoor composting of vine by-products to produce substrates for mushroom cultivation

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## Abstract

This paper describes a new technique for producing high quality, low cost organic substrates for use in the cultivation of the most widely consumed edible mushroom, *Agaricus bisporus* (Lange) Imbach. With production, economic and environmental concerns in mind, the aim of this work was to provide fresh impetus to the edible fungus industry by introducing alternative materials to those traditionally used in substrate production: in this case waste products from grape growing and wine making (vine shoots, grape stalks and grape pomace). These were composted under controlled conditions. While no more biologically efficient than traditional compost, the agronomic assessment of the alternative indoor substrates (performed using different proportions of grape stalks and vine shoots as base materials and using two varieties of *Agaricus bisporus*) showed them to be economically viable (their use reduces costs) and environmentally advantageous (their use reduces the emission of volatile compounds).

**Additional key words:** *Agaricus bisporus*, alternative raw materials, compost.

## Resumen

### Compostaje indoor de subproductos de la vid en la producción de sustratos para cultivo de champiñón, *Agaricus bisporus* (Lange) Imbach

El presente trabajo se ha llevado a cabo con la finalidad de obtener y definir unas tecnologías de carácter aplicado que permitan elaborar metodológicamente, en condiciones controladas, sustratos orgánicos de cultivo de calidad y bajo coste, para el principal hongo comestible cultivado, *Agaricus bisporus* (Lange) Imbach. Se pretende con ello aportar una mejora productiva, económica y medioambiental al sector profesional de la producción de hongos comestibles y contribuir al relanzamiento de la actividad a partir de la utilización de materiales alternativos a los tradicionales, concretamente materiales residuales de la viticultura y la vinicultura (sarmientos de vid, raspón de uva y orujo de alcohología), así como mediante la obtención de compost en condiciones controladas. La evaluación agronómica de sustratos *indoor* alternativos, utilizando diferentes proporciones de sarmiento y raspón como materiales de base, con dos diferentes variedades de micelio de *Agaricus bisporus*, ha mostrado como, aunque no se superan los valores de eficiencia biológica proporcionados por el compostaje tradicional basado en paja de cereales, sí que supone una notable mejora tanto económica, reduciendo los costes de elaboración, como medioambiental, principalmente por la supresión de emisiones de compuestos volátiles.

**Palabras clave adicionales:** *Agaricus bisporus*, compost, materias primas alternativas.

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## Introduction

The commercial production of mushrooms (*Agaricus* spp.) traditionally involves the use of a substrate obtained by composting cereal (wheat and barley) straw, the fermentation of which is activated by a nitrogen source. This fermentation process has two phases – one that takes place in the open air and another that occurs under

controlled conditions (Gerrits, 1988; Wood and Smith, 1988). The first phase, however, is associated with productive, economic and environmental disadvantages. This led to the introduction of the so-called «indoor method» of controlled fermentation. Performed in controlled environment chambers, the aim of this is to increase the regularity of production while introducing new materials and limiting the environmentally harmful effects of the emissions associated with traditional composting (Gerrits, 1996). The main problems associated with compost production, including the influence

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of the weather on phase I in outdoor conditions, the strong dependence on cereal straw (the price of which is increasing), and the duration of the process which leads to high losses of dry matter (Pardo, 1999), are thus overcome to some extent.

Successful attempts have recently been made to partially replace straw with vine shoots (*Vitis vinifera* L. cv. Bobal) as the base material for composting (Pardo *et al.*, 2006a), with the best results (from a biological efficiency standpoint) obtained with a 2:1 (w/w) straw/vine shoot mixture. The present work describes and agronomically assesses a technique that provides a substrate for cultivating *Agaricus bisporus* (Lange) Imbach. This indoor fermentation process requires no cereal straw; vine shoots and grape stalks are used as the base materials. Further, chicken manure — the normal nitrogen supplement — is substituted by grape pomace. These waste materials are available in large quantities near the main mushroom-producing areas of Spain (La Manchuela in Castilla-La Mancha and La Rioja/Navarra).

## Material and Methods

### Composting processes

The composts examined in this work were made at a pilot indoor plant at the *Centro de Investigación, Experimentación y Servicios del Champiñón*, at Quintanar del Rey in the Province of Cuenca (Central Spain). Traditional compost made under controlled conditions and employing conventional materials (cereal straw, poultry manure and urea as nitrogen supplements, and gypsum as a structure corrector), was used as a control. Three alternative substrates were made in which straw was entirely substituted by different combinations (3:1, 1:1 and 1:3, w/w) of vine shoots and grape stalks. The chicken manure normally used was substituted by grape pomace. Gypsum was maintained as a structure corrector and urea was used to adjust the final nitrogen content; the adjustments made to all four substrates are recorded in Tables 1 and 2.

For each of the substrates, composting was performed in two phases in specially designed chambers that provided circulating air, and into which steam could be injected. During phase 1, which lasted 11 days, a temperature of 45-47°C was maintained until the last 24 h, when it was raised to 70°C. The chamber was then emptied and the contents mixed with 5% commercial

compost before re-filling for phase II. This conventional phase lasted 7 days including an 8-10 h pasteurisation period at 60°C, a 6-8 h period at 53°C, and a gradual lowering of the temperature to 45°C for 4 days. The compost was then rapidly cooled to the spawning temperature (27°C).

### Physical, chemical and biological analyses

The composts and casing soil were characterised by recording the following variables: moisture content (MAPA, 1994), pH and electrical conductivity (Ansorena, 1994), total N content (TECATOR, 1987; MAPA, 1994), organic matter and ash content (Ansorena, 1994; MAPA, 1994), the C:N ratio, the crude fibre and crude fat contents (MSC, 1985), particle real density (MAPA, 1994), bulk density, total porosity, water-holding capacity (Ansorena, 1994), pathogenic nematode content (Nombela and Bello, 1983), mite content (Brady, 1969; Krantz, 1986), and the presence of *Trichoderma* spp. (Tello *et al.*, 1991) (see Tables 3 and 4).

### Spawn used

The substrates were inoculated (10 g kg<sup>-1</sup>) with one of two commercial strains of mushroom mycelium:

— Gurelan 45 (Micelios Gurelan, Huarte-Pamplona, Spain). This strain belongs to the group of large off-white hybrids. Its spawn is particularly recommended for winter and spring cultivation. Its optimum growth conditions include a fructification temperature of 18°C (although it can fructify at 15°C), a relative humidity of 87%, medium ventilation, and 1,000-1,500 ppm of CO<sub>2</sub>. Under such conditions this strain provides large white mushrooms which are normally destined for fresh consumption.

— Portobello (Micelios Fungisem, Autol, La Rioja, Spain). This strain is recommended for winter spawning. Its optimum fructification temperature is 16.5-18°C, and it can withstand up to 2,000 ppm of CO<sub>2</sub>. It provides large brown mushrooms also destined for the fresh market.

### Experimental design

The experimental design used was a 4 × 2 equilibrated factorial plan with 4 repetitions. Factor 1, with

**Table 1.** Experimental mushroom compost formulations

Ingredients	Fresh weight (g)	Moisture (g kg <sup>-1</sup> )	Dry weight (g)	Nitrogen (g kg <sup>-1</sup> )	Total nitrogen (g)	Ash (g kg <sup>-1</sup> )	Organic matter (g kg <sup>-1</sup> )	Total carbon (g)
<i>Formulation 1 (vine shoots-grape stalks 3:1, w/w)<sup>1</sup></i>								
Vine shoots	750.0	94	679.5	6.2	4.2	39.0	961	378.7
Grape stalks	250.0	135	216.3	13.2	2.9	76.4	923.6	115.8
Urea	13.5	0	13.5	460.0	6.2	0.0	1,000.0	2.7
Grape pomace	450.0	108	401.4	23.5	9.4	76.1	923.9	215.1
Gypsum	5.0	200	4.0	0.0	0.0	1,000.0	0.0	0.0
Total	1,468.5		1,314.7		22.7			712.4
<i>Formulation 2 (vine shoots-grape stalks 1:1, w/w)<sup>2</sup></i>								
Vine shoots	500.0	94	453.0	6.2	2.8	39.0	961	252.5
Grape stalks	500.0	135	432.5	13.2	5.7	76.4	923.6	231.7
Urea	12.0	0	12.0	460.0	5.5	0.0	1,000.0	2.4
Grape pomace	450.0	108	401.4	23.5	9.4	76.1	923.9	215.1
Gypsum	5.0	200	4.0	0.0	0.0	1,000.0	0.0	0.0
Total	1,467.0		1,302.9		23.5			701.7
<i>Formulation 3 (vine shoots-grape stalks 1:3, w/w)<sup>3</sup></i>								
Vine shoots	250.0	94	226.5	6.2	1.4	39.0	961	126.2
Grape stalks	750.0	135	648.8	13.2	8.6	76.4	923.6	347.5
Urea	8.5	0	8.5	460.0	3.9	0.0	1,000.0	1.7
Grape pomace	450.0	108	401.4	23.5	9.4	76.1	923.9	215.1
Gypsum	5.0	200	4.0	0.0	0.0	1,000.0	0.0	0.0
Total	1,463.5		1,289.2		23.3			690.6

<sup>1</sup> Adjustment: C/N, 31.4; nitrogen, 17.3 g kg<sup>-1</sup>. <sup>2</sup> Adjustment: C/N, 29.9; nitrogen, 18.0 g kg<sup>-1</sup>. <sup>3</sup> Adjustment: C/N, 29.6; nitrogen, 18.1 g kg<sup>-1</sup>.

four levels, corresponded to the type of substrate and Factor 2, with two levels, corresponded to the strain of mycelium used. Each block was composed of four 20 kg plastic bags filled with spawned compost, overall presenting a total area of 1.2 m<sup>2</sup>. A total of 128 bags of substrate were used.

## Growth cycle management

Growth tests were performed in an experimental growth chamber provided with a humidification system, a heating/cooling system, and internal air circulation/outside ventilation. This allowed for the automatic

**Table 2.** Conventional mushroom compost formulation (straw-chicken manure)

Ingredients	Fresh weight (g)	Moisture (g kg <sup>-1</sup> )	Dry weight (g)	Nitrogen (g kg <sup>-1</sup> )	Total nitrogen (g)	Ash (g kg <sup>-1</sup> )	Organic matter (g kg <sup>-1</sup> )	Total carbon (g)
Wheat straw	666.7	121	586.1	7.6	4.5	69.4	930.6	316.33
Barley straw	333.3	131	289.7	3.4	1.0	59.8	940.2	157.96
Chicken manure	450.0	208	356.4	34.6	12.3	176.8	823.2	170.17
Urea	8.5	0	8.5	460.0	3.9	0.0	1,000.0	1.70
Gypsum	5.0	200	4.0	0.0	0.00	1,000.0	0.0	0.00
TOTAL	1,463.5		1,244.6		21.7			646.15

Adjustment: C/N, 29.8.4; nitrogen, 17.4 g kg<sup>-1</sup>.

**Table 3.** Compost analysis

	VS/GS 3:1 (w/w)	VS/GS 1:1 (w/w)	VS/GS 1:3 (w/w)	Conventional
pH (1:5, p/v)	7.03	7.56	6.82	8.72
Moisture (g kg <sup>-1</sup> )	640	639	559	746
Total nitrogen (g kg <sup>-1</sup> , dm)	19.9	23.2	22.5	18.0
Ash (g kg <sup>-1</sup> , dm)	78.8	107.5	77.1	148.9
Organic matter (g kg <sup>-1</sup> , dm)	921.2	892.5	922.9	851.1
C/N	26.8	22.3	23.8	27.4
Crude fibre (g kg <sup>-1</sup> , dm)	558.3	558.3	502.5	508.8
Crude fat (g kg <sup>-1</sup> , dm)	10.3	11.1	15.2	5.4
Pathogenic nematodes	Absent	Absent	Absent	Absent
Mites	Absent	Absent	Absent	Absent
<i>Trichoderma</i> spp.	Absent	Absent	Absent	Absent

VS/GS: vine shoots-grape stalks formula. dm: dry matter.

control of temperature, relative humidity and carbon dioxide.

The growth cycle conditions were those suggested for the selected strains (CIES, 2005). A spawn run period of 17 days was allowed, and the usual disinfectant (formalin, 18 ml m<sup>-2</sup>), insecticide (diflubenzuron 25%, 3.6 g m<sup>-2</sup>) and fungicide (prochloraz 46%, 0.62 g m<sup>-2</sup>) treatments applied after casing. The casing [a binary mixture of loamy mineral subsoil and black peat (5:1, v/v) (Table 4); thickness 3 cm] was deeply ruffled after 7 days. Ventilation was performed nine days after casing to stimulate the formation of primordia. The casing was moistened to between 60% and 70% of its water-holding capacity by regular and uniform watering with between 0.5 and 1.5 L m<sup>-2</sup> (depending on need) according to the usual cultivation technique (Wuest, 1982). The total growth cycle lasted 67 days; four flushes of mushrooms were harvested.

**Table 4.** Casing soil analysis

Characteristics	Value
Moisture (g kg <sup>-1</sup> )	88
pH (1:6, v/v)	8.37
Electrical conductivity (1:6, v/v) (μS cm <sup>-1</sup> )	450
Bulk density (fresh) (g cm <sup>-3</sup> )	1.245
Bulk density (dry) (g cm <sup>-3</sup> )	1.135
Particle real density (g cm <sup>-3</sup> )	2.67
Total pore space (ml L <sup>-1</sup> )	575
Water holding capacity (kg kg <sup>-1</sup> )	0.37
Organic matter (g kg <sup>-1</sup> )	45.2

### Harvesting, production and commercial quality variables

Mushrooms were harvested every day at their optimal commercial stage of development, corresponding to morphogenetic stages 2, 3 and 4 according to the classification established by Hammond and Nichols (1976).

Weight before stipe trimming and the total number of mushrooms produced were recorded. Yield was expressed with respect to the cultivated area and the quantity of compost used (biological efficiency). Total mushroom production was separated into two groups according to size: large (≥40 mm) and medium (<40 mm).

The unit weight of the mushrooms was obtained from the yield and number of mushrooms picked. A second estimate of size, expressed as cap diameter in mm, was determined from previously established non-linear regression curves based on the diameter and weight of the mushrooms of the three first flushes.

On the day when most mushrooms were picked during each of the first three flushes, the moisture, protein and ash contents of mushrooms of uniform size and at the same stage of development were determined. The weighted means were calculated for the relative yield of each of the first three flushes.

Moisture levels were measured as the loss of weight after oven drying at 105°C for 72 h (Lau, 1982).

The protein content of the mushrooms was calculated by multiplying the total nitrogen content, obtained by the Kjeldahl method (TECATOR, 1987; MAPA, 1994), by a conversion factor of 4.38 (Delmas, 1989).

For the determination of the ash content, mushrooms were ashed at 540°C for at least 6 h (MAPA, 1994).

## Statistical analysis

ANOVA was used to analyse the data and the Tukey-HSD test employed to establish significant differences between means ( $p=0.05$ ). All calculations were performed using Statgraphics Plus v. 4.1 software (Statistical Graphics Corp., Princeton, NJ, USA).

## Results

Among the noteworthy characteristics of the experimental composts at the end of phase II (Table 3) was the organic matter content of 851.1-922.9 g kg<sup>-1</sup>; this is quite high compared to the values obtained with the traditional composting process, and was responsible for the high C/N ratios seen (between 22.3 and 27.4). The high pH of the compost made with conventional materials (8.72), and the low moisture content (559 g kg<sup>-1</sup>) of the 1:3 (w/w) vine shoot/grape stalk compost, are also noteworthy.

Table 5 shows that the greatest number of mushrooms per m<sup>2</sup> (944) was obtained with the combination of conventional substrate and Gurelan 45. This same combination, and the combination of conventional

substrate plus the Portobello strain, gave the greatest yield and biological efficiency values. Of the various shoot/stalk combinations, the best results were obtained with the 1:1 (w/w) proportion and with the Portobello strain.

When the substrates were considered independently of the mushroom variety used, the greatest number of mushrooms was obtained with the conventional indoor substrate and the 1:1 shoot/stalk combination (766 and 796 mushrooms m<sup>-2</sup> respectively). However, the best yield per unit area and the greatest biological efficiency were obtained with the control indoor substrate (18.28 kg m<sup>-2</sup> and 90.0 kg 100kg<sup>-1</sup> compost; this compares well with the values obtained with conventional outdoor compost) because of its greater production of large mushrooms. The worst results were obtained with the 1:3 (w/w) shoot/stalk combination, perhaps due to its low moisture content (559 g kg<sup>-1</sup>), although the more moist (640 g kg<sup>-1</sup>) 3:1 combination also produced poor results. In this last case, the structure of the compost may have been important; a high proportion of shoots ground to < 3 mm might impart a high apparent density and low porosity. This could hinder air circulation in the fermentation chamber. It therefore appears important to optimise the moisture content and the particle size of the materials used.

**Table 5.** Quantitative production variables (means)<sup>1</sup>

	Number of mushrooms per m <sup>-2</sup>	Mushroom weight (g)	Mushroom yield (kg m <sup>-2</sup> )			Biological efficiency (kg 100kg <sup>-1</sup> compost)	Earliness (days since casing)
			Large size (≥40 mm)	Medium size (15-40 mm)	Total mushroom yield		
VS/GS 3:1-Gurelan 45	280 d	24.1 bc	4.71 cd	1.36 b	6.07 c	21.1 c	24.2 bc
VS/GS 3:1-Portobello	341 cd	22.8 bc	6.52 c	1.02 b	7.55 c	26.2 c	22.2 d
VS/GS 1:1-Gurelan 45	871 ab	15.9 c	6.93 c	6.82 a	13.74 b	47.6 b	25.0 ab
VS/GS 1:1-Portobello	721 ab	22.7 bc	11.48 b	4.79 a	16.26 ab	56.3 b	23.0 cd
VS/GS 1:3-Gurelan 45	110 d	17.7 bc	1.36 d	0.60 b	1.95 d	5.5 d	24.3 bc
VS/GS 1:3-Portobello	50 d	26.6 ab	1.25 d	0.10 b	1.35 d	3.8 d	26.0 a
CONTROL-Gurelan 45	944 a	19.3 bc	11.90 b	5.72 a	17.62 a	86.8 a	25.2 ab
CONTROL-Portobello	589 bc	33.3 a	16.90 a	2.05 b	18.95 a	93.2 a	23.3 cd
VS/GS 3:1	311 b	23.5	5.62 c	1.19 b	6.81 c	23.6 c	23.2 b
VS/GS 1:1	796 a	19.3	9.20 b	5.80 a	15.00 b	52.0 b	24.0 ab
VS/GS 1:3	80 c	22.1	1.30 d	0.36 b	1.65 d	4.7 d	25.1 a
CONTROL	766 a	26.3	14.40 a	3.88 a	18.28 a	90.0 a	24.2 ab
Gurelan 45	551	19.3 b	6.22	3.62	9.84	40.2	24.7 a
Portobello	425	26.3 a	9.04	1.99	11.03	44.9	23.6 b
MEAN	488	22.8	7.63	2.80	10.44	42.6	24.1

<sup>1</sup> Values followed by a different letter within a column and factor or combination of factors are significantly different at the 5% level according to the Tukey test. VS/GS 3:1: vine shoot-grape stalk formula (3:1, w/w). VS/GS 1:1: vine shoot-grape stalk formula (1:1, w/w). VS/GS 1:3: vine shoot-grape stalk formula (1:3, w/w).

The only difference observed in the behaviour of the mushroom strains was in the unit weight of their carpophores, which was greater in Portobello, and in the earliness of the latter strain compared to Gurelan 45.

Table 6 shows no differences were found between the different substrates with respect to carpophore diameter, dry weight or protein content. The substrate based on the 1:3 (w/w) shoot/stalk combination, the least productive of all, produced the mushrooms with by far the greatest ash content. The Portobello strain produced larger mushrooms with higher protein and ash contents.

## Discussion

The technique of indoor composting arose from the need to solve the growing cost problems associated with traditional composting, as well as concerns over its related environmental problems. In the new method, phase I is performed in specially designed controlled environment chambers. The washing and/or biofiltration processes involved avoid the emission of volatile acids, sulphur compounds, nitrogen compounds, phenols, ketones and other compounds into the atmosphere; such emissions are regularly detected during conventional phase I fermentations (Duns and Rinker, 2004).

Apart from this environmental advantage, the indoor production of substrates using alternative materials—while not necessarily improving composting in terms of biological efficiency—reduces costs through increasing the number of materials that can be used, its faster processing time, and its smaller losses of dry matter. This technique also requires less production space (Pardo *et al.*, 2006b).

Indoor composting normally involves a low temperature process (Gerrits and Van Griensven, 1990; Gerrits, 1996) and two high temperature processes (with and without a gradient) (Gerrits, 1994, 1996). In the present case it involved two phases. The first phase, adapted from Laborde *et al.* (1986) and Gerrits and van Griensven (1990), consists of two steps: 1) a low temperature treatment (45-47°C) designed to ensure the rapid development of thermophilic microflora (fungi, bacteria and *actinomycetes*) in fundamentally aerobic conditions of high humidity; this flora breaks down the organic matter to release large quantities of sugars and amino acids, and 2) treatment at 70°C; this allows chemical reactions to occur that contribute to the ultimate selectivity of the compost towards the cultivated mushroom (Laborde *et al.*, 1986). Phase II follows the conventional practices of pasteurisation and subsequent thermophilic conditioning. This procedure has been used successfully with wheat and barley

**Table 6.** Qualitative production variables (means)<sup>1</sup>

	Diameter of the sporophore (mm)	Dry matter (g kg <sup>-1</sup> )	Protein (g kg <sup>-1</sup> )	Ash (g kg <sup>-1</sup> )
VS/GS 3:1-Gurelan 45	41.9 abc	88.3 ab	209.8 c	113.0 cd
VS/GS 3:1-Portobello	41.1 bc	85.1 abc	227.3 bc	117.2 bcd
VS/GS 1:1-Gurelan 45	35.5 c	85.1 abc	209.9 c	112.1 cd
VS/GS 1:1-Portobello	41.0 bc	81.2 bc	233.2 bc	122.9 abc
VS/GS 1:3-Gurelan 45	37.1 c	79.3 c	219.6 c	129.5 ab
VS/GS 1:3-Portobello	43.9 ab	88.1 ab	285.4 a	131.9 a
CONTROL-Gurelan 45	38.3 bc	89.1 a	208.4 c	107.3 d
CONTROL-Portobello	48.2 a	80.5 c	254.4 ab	127.6 ab
VS/GS 3:1	41.5	86.7	218.5	115.1 b
VS/GS 1:1	38.3	83.1	221.5	117.5 b
VS/GS 1:3	40.5	83.7	252.5	130.7 a
CONTROL	43.3	84.8	231.4	117.4 b
Gurelan 45	38.2 b	85.4	211.9 b	115.4 b
Portobello	43.6 a	83.7	250.0 a	124.9 a
MEAN	40.9	84.6	231.0	120.2

<sup>1</sup> Values followed by a different letter within a column and factor or combination of factors are significantly different at the 5% level according to the Tukey test. VS/GS 3:1: vine shoot-grape stalk formula (3:1, w/w). VS/GS 1:1: vine shoot-grape stalk formula (1:1, w/w). VS/GS 1:3: vine shoot-grape stalk formula (1:3, w/w).

straw mixed with other materials (Pardo, 2004; Pardo *et al.*, 2006a).

The type of processing and materials used condition the characteristics of the resulting compost. It is therefore not surprising that indoor composting produces composts that differ from the traditional product in technical/economic, physiological and microbiological terms. The shorter processing time and consequently smaller loss of dry matter condition the physical and chemical characteristics of the compost obtained.

In general, chicken manure is the nitrogen supplement most widely used in the production of composts for mushrooms. In the present experiment, the smaller organic matter content of the chicken manure compared to the grape pomace led to the conventional compost having a high ash content (148.9 g kg<sup>-1</sup>) compared to the experimental composts (77.7-107.5 g kg<sup>-1</sup>). The high pH of the conventional compost (8.72) suggests the presence of competitor moulds normally associated with insufficiently composted substrates (*Scopulariopsis fimicola*, *Coprinus cinereus*, *Doratomyces stemonitis*, *Chaetomium globosum*, *Oedocephalum* spp., *Papulaspora byssina* etc.). However, the spawn run step reflected no problems of this type.

Among the materials left over from wine making, grape stalks and grape pomace were the first to be used in mushroom cultivation. Pardo and Sánchez (1989), Pardo and Pardo (1991), Pardo (1994, 1995) and Pardo *et al.* (1995) described the use of these materials (which are widely available in Castilla-La Mancha and La Rioja/Navarra) for conventional composting. More recently, Pardo (2004) described the indoor composting of formulations in which 1:1 (w/w) mixtures of cereal straw and, among other materials, grape stalks, vine shoots and grape pomace, were used as base materials. Biological efficiency values of 70-78 kg of mushrooms per 100 kg of compost (dry weight) were obtained. Subsequently, experiments were performed using different proportions of straw and vine shoots, including grape stalks and chicken manure as a nitrogen supplement (Pardo *et al.*, 2006a). The best results were obtained with a 2:1 (w/w) mixture of straw and vine shoots, the biological efficiency reaching 86.7 kg of mushrooms per 100 kg compost (dry weight). These results compare favourably with those obtained commercially with traditional compost, and better than those obtained in the present study [56.3 kg of mushrooms per 100 kg compost (dry weight)]. *Pleurotus ostreatus* (Jacq, ex. Fr.) Kummer has also been successfully grown using substrates containing, among others,

grape pomace and vine shoots (Pardo *et al.*, 2005a,b).

The most noteworthy aspect of the present work was the production of specific substrates for the cultivation of *Agaricus* spp. without the need for cereal straw or chicken manure: the only materials required were waste products from grape growing and wine making.

In summary, the proposed indoor composting method provides mushroom yields comparable to those obtained when using traditional compost. The 1:1 (w/w) shoot/stalk combination, while producing somewhat more modest results, opens up the possibility of using these abundantly available materials in different proportions, with different grain sizes, and in combination with other materials. The indoor production of substrates from alternative materials could lower production costs by eliminating over-dependence on one particular base material, accelerate composting, reduce losses of dry matter, and reduce the space required for compost production. In addition, the proposed method offers environmental advantages by avoiding the emission of volatile sulphurous and ammoniacal compounds etc. into the atmosphere.

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