Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v40i1.39341

CROP PRODUCTION

Recycling of the biomass waste defatted almond meal as a novel nutritional supplementation for cultivated edible mushrooms

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ABSTRACT. Defatted almond meal (DAM) is an useless biomass waste obtained after oil extraction. The substrate designed for mushroom cultivation is achieved through a controlled composting process from agricultural by-products (chicken manure and wheat straw). This work shows the potential of DAM as efficient compost supplement for culturing the species *Agaricus bisporus* (J.E. Lange) Imbach and *Pleurotus ostreatus* (Jacq.) P. Kumm. Supplementation during *A. bisporus* cultivation results in larger mushrooms with a firmer texture and higher dry matter and protein contents in comparing with the non-supplemented substrate. In *P. ostreatus,* supplementation at a dosage of 15 g kg^{-1} provided a yield improvement up to 31.8%, compared to the control without supplement. The supplementation with DAM supposed equivalent or better yield than the commercial supplements. Therefore, the technique developed assessed good agronomic potential for application of DAM at the commercial scale in *P. ostreatus* cultures, adding value to a worthless organic by-product.

Keywords: defatted almond meal; yield, quality; *Agaricus bisporus*; *Pleurotus ostreatus*; recycled waste.

Reciclagem de farinha de amêndoa desengordurada com resíduo de biomassa para uma nova suplementação nutricional em cogumelos comestíveis cultivados

RESUMO. A farinha de amêndoa desengordurada (DAM) é um resíduo de biomassa inútil obtido após extração de óleo. O substrato designado para o cultivo de cogumelos é formulado através de um processo de compostagem controlado utilizando subprodutos agrícolas (cama de frango e palha de trigo). Este trabalho demosntra o potencial da DAM como um suplemento de composto eficiente para o cultivo das espécies *Agaricus bisporus* (J.E. Lange) Imbach e *Pleurotus ostreatus* (Jacq.) P. Kumm. A suplementação durante o cultivo de *A. bisporus* resulta em cogumelos maiores com textura firme e elevado teor de matéria seca e proteína, em comparação com o substrato não suplementado. Em *P. ostreatus*, a suplementação utilizando a dosagem de 15 g kg^{-1} proporcionou um aumentono rendimento para 31,8%, em comparação com o substrato nãosuplementado. A suplementação com DAM proporcionou rendimento equivalente ou melhor do que os suplementos comerciais. Portanto, a técnica desenvolvida avaliou o bom potencial agronômico para aplicação de DAM na escala comercial em culturas de *P. ostreatus*, agregando valor a um subproduto orgânico sem valor.

Palavras-chave: farinha de amendoa desengordurada; rendimento; qualidade; *Agaricus bisporus*; *Pleurotus ostreatus*; resíduos reciclados.

Introduction

Several alternative methods can be applied to extract the oil contained inside the almond (Marzouki, Piras, & Rosa, 2008; Mezzomo, Martínez, & Ferreira, 2009). Among them, extraction using pressure is the best option because it yields high quality oils at an affordable price (Da, Hongzhi, Li, Hui, & Qiang, 2014; Sena-Moreno et al., 2015). The by-product obtained from this process is a DAM, which has high protein content, minerals, dietary fibre and substances with antioxidant capacity (Martínez, Marín, Gili, Penci, & Ribotta, 2017), although it does not have an extensive application.

The lipid, protein, carbohydrate and mineral fractions are the main components in the almond seed. Additionally, a large group of compounds called phytochemicals are present; these compounds are found in low quantities but play a significant role in the almond quality. The percentages of these fractions differ based on the variety, type of culture and geographic origin (Yada, Lapsley, & Huang, 2011; Yada, Huang, & Lapsley, 2013; Kodad, Estopañán, Juan, Socias, & Company, 2014a; Kodad et al., 2014b). Yada et al. (2011) reported that the total protein

(considering a nitrogen conversion factor of 5.18) values ranged between 14 and 61 g for 100 g of raw almonds, whereas the soluble sugar ranges were between 1.8 and 7.6 g for 100 g and between 2.5 and 12 g for 100 g of raw nuts.

The almond is the most important nut cultured in the world in terms of its commercial production. Most of the world's production is concentrated in three regions, which include California, the Mediterranean and Central Asia/Middle East (Food and Agriculture Organization of the United Nations [FAO], 2017). The almond tree is a very significant crop primarily due to its high adaptability to extremely dry conditions, such as the conditions that occur in the Mediterranean basin. In this region, the maintenance of the almond as a crop and the genetic reserve of the species is important since the vegetal material presents a very high variability (Homet-Gutierrez, Schupp, & Gómez, 2015).

The mushrooms use nutritional supplements directly to increase the yield without influencing the quality. The yield increase can range between 10 and 20% and occasionally may be higher. The types of nutrients, the best moment for the application, the cost of the supplement and the economic benefits should be analysed in advance (Naraian et al., 2009; Royse & Chalupa, 2009; Royse, 2010; Sánchez, 2010).

Cereal grains and oilseeds, which are widely used as supplements for mushroom composting, contain variable proportions of the three basic nutritional requirements for mushrooms (carbohydrates, proteins and lipids) (Sánchez, 2010; Arce-Cervantes et al., 2015). Most commercialised supplements use raw materials based on vegetables rich in protein, among them soybean meal is the main ingredient (Zied et al., 2011; Arce-Cervantes et al., 2015). Zervakis et al. (1996) also studied the effect of olive mill residues on the production of two *Pleurotus* species. Recently, the viability of the use of defatted pistachio meal for the supplementation of substrates for the cultivation of *A. bisporus* and *Pleurotus ostreatus* was also successfully validated (Pardo-Giménez et al., 2016).

Worthy of note is that the technology based on supplementation is highly attractive since its application stimulates yield while improving the quality, shelf life and preventing disease occurrence (Wheeler & Wach, 2006). The supplementation equilibrates the nitrogen content and the C/N ratio, increasing the production yields and even provides benefits to the nutritional content of the harvested basidiomes (Zied et al., 2011; Pardo-Giménez, Zied, Álvarez-Ortí, Rubio, & Pardo, 2012). Since both the cereal straw and sawdust, agricultural by-product that are commonly used as base materials for the production of mushroom substrates, are poor nitrogen sources; nitrogen depletion in the substrate during fruiting (when most of the nitrogen is used for mycelial growth) is unsuitable and limits the yields. An immediate consequence of this situation is a drastic drop in production after the first flush, which may be caused by nutrient depletion, among other factors. The supplementation technique shows high potential to correct this issue.

Agaricus and *Pleurotus* are the two most cultivated genera worldwide, and Europe is the second largest producer after China (Royse, 2014), in a global market valued over 4,700 $ billion only considering the species *Agaricus bisporus* (Sonnenberg et al., 2011). Mushrooms are recognised as a very healthy source of nutrients and its consumption has significantly increased within the last years (Roncero-Ramos, Mendiola-Lanao, Pérez-Clavijo, & Delgado-Andrade, 2017).

The aim of the present study was to investigate the viability to recycle an interesting by-product, DAM, as a novel nutritional substrate supplement for the mushroom industry of the species *Agaricus bisporus* and *Pleurotus ostreatus*.

Material and method

Characterization of substrates and supplements employed

A commercial *A. bisporus* Phase III compost based on wheat straw and chicken manure inoculated with the Amycel XXX® (Amycel, Watsonville, CA, USA) mycelium (thick white hybrid) and provided by Champinter S.C.L. (Villamalea, Albacete, Spain) was used as substrate for the *A. bisporus* production. Euroveen® (Euroveen BV, Grubbenvorst, The Netherlands), which is a commercial mixture of Dutch origin, was used as the casing layer. The thickness of the casing was 3.5 cm.

A commercial *P. ostreatus* substrate based on wheat straw inoculated with the Sylvan Spoppo mycelium (Sylvan Inc., Kittanning, PA, USA) (25 g kg^{-1}) provided by Champinter S.C.L. (Villamalea, Albacete, Spain) was used for the production.

The variety Marcona was used in this study, which is the most cultivated in Spain in upland farming. The almonds were acquired from the Manchega Almond Cooperative (Cooperativa Manchega de la Almendra) of Villamalea. Shelled

almonds were placed on trays and the DAM was obtained following the procedure described by Pardo-Giménez et al. (2016). Untreated substrates and commercial supplements designed for use in *A. bisporus* (Promycel 600, Amycel, Watsonville, CA, USA) and *P. ostreatus* (Promycel Pleurotus, Amycel, Watsonville, CA, USA) cultures were used as negative and positive controls respectively.

The following parameters were determined for the physical, chemical and biological characterisation of the substrate, the casing materials and the supplements: humidity, pH, electrical conductivity, total nitrogen content, raw material and ashes, C:N ratio, crude fibre, crude fat, nitrogen-free extract, cellulose, hemicellulose, lignin and neutral detergent-soluble fibres, real density, apparent density, total porosity and water retention capacity, and the presence of nematodes, mites and competitor moulds, as described by Pardo-Giménez et al. (2012) and Pardo-Giménez et al. (2016).

Research layout

Two trials in crop were performed (the first for *A. bisporus* and the second for *P. ostreatus*) using a random block design with six replicates. For *A. bisporus*, boxes containing 10 kg of compost with 70 kg m^{-2} of load density were used. A total of 30 boxes were evaluated, corresponding to the six replicates per treatment of the three doses (5, 10, and 15 g kg^{-1}), the non-supplemented controls and the controls using the commercial *A. bisporus* supplement (10 g kg^{-1}) assayed. For *P. ostreatus*, transparent pierced polyethylene bags with a 6 kg substrate capacity were used; four orifices (25 mm in diameter) were uniformly distributed on the lateral surface of each bag. Similarly, a total of 30 bags were used corresponding to six replicates per treatment of the three doses used (5, 10, and 15 g kg^{-1}), the non-supplemented controls and the commercial *P. ostreatus* supplement (8 g kg^{-1}) assayed.

Crop cycle

The mushroom crop cycle was conducted in a climate-controlled growing room at the Mushroom Research, Experimentation and Service Centre (CIES, Quintanar del Rey, Spain). The room is supplied with automatic systems for humidification, heating/cooling and recirculation/external ventilation to control temperature, relative humidity and the carbonic anhidric ratio as recommended by the spawn supplier. Peat-based casing was applied the same day as the filling; then, the routine insecticide (diflubenzuron 25%, 3.8 g m^{-2}) and antifungal (prochloraz-Mn 46%, 0.47 g m^{-2}) treatments were applied for *A. bisporus* production. The casing was ruffed 8 days after application, and fruiting was induced 24h later. Three flushes were harvested along a crop cycle of 37 days.

An air-conditioned greenhouse tunnel that was similarly equipped with humidification, heating/cooling, recirculation/external ventilation and lighting systems was employed for growing *P. ostreatus*. The culture cycle was performed under the temperature, relative humidity, carbonic anhidric ratio and lighting conditions recommended by the spawn manufacturer. The substrate was incubated for 17 days with neither external ventilation nor lighting; then, fruiting was induced using ventilation, lighting and decreasing temperature and relative humidity, during a crop cycle of 70 days.

Mushroom harvest and quality parameters

For *A. bisporus*, harvesting was performed daily at the optimal commercial development stages. Mushroom picked were daily weighed and counted. Additionally, the production was separated into two groups based on their sizes as follows: thick (≥ 40 mm) and medium (15-40 mm) for *A. bisporus* crop. The production was determined from the yield by surface unit, considering the load density of the compost and its humidity content. The unit weight of the mushrooms was determined from the obtained yield and the number of harvested mushrooms. A second estimation of the size (expressed as the diameter of the cap in mm) was determined using the non-linear regression curves previously determined from the measurement of the diameter and the weight of the mushrooms corresponding to the three flushes of the chosen variety of commercial mycelium. The earliness was expressed as the time from the application of the casing until the harvesting of the first flush considering the daily relative production of the harvesting (Pardo-Giménez, Pardo-González, & Zied, 2011).

On the day of the maximum harvest of each flush, mushrooms of uniform size and maturity were chosen for the assessment of other quality parameters. The colour, dry matter content and texture of the mushrooms were determined as described by Pardo-Giménez et al. (2016). For *P. ostreatus*, mushroom harvest was performed daily at the optimal commercial development stages. The number of harvested mushrooms was determined by counting during the whole crop cycle. The amount of mushrooms produced daily was weighed per each bag to calculate the yield. The yield provided per unit area was assessed by taking into account the load density of the substrate in the bags and the humidity content. The others quantitative (earliness) and qualitative (dry matter, colour and firmness) were analysed using the same methods.

Mycochemical characterisation

In order to analyse the composition of both *Agaricus* and *Pleurotus* mushrooms, the content of water (Lau, 1982), carpophore protein (Miles & Chang, 1997), ash (Lau, 1982), crude fat (Ankom XT10, Ankom, 2009), crude fibre (Ankom 220 Fiber Analyzer, Ankom, 2008), total carbohydrate (Sullivan, 1993), available carbohydrates (nitrogen-free extractives) (Lau, 1982) and energy value (Lau, 1982) was determined from uniform sized and matured basidiomes.

Statististics

The data obtained were processed by one-way analysis of variance (ANOVA). The Fisher's least significant difference (LSD) test, at 5% probability, was employed to establish significant differences between the mean values. The analysis was performed using the software package Statgraphics Plus software, v. 4.1 (Statistical Graphics Corp., Princeton, NJ, USA).

Result

Characterization of composts and supplements employed

Table 1 shows the determined composition of the different composts and supplements used in the crop trials.

Table 1. Physical-chemical characteristics of the various substrates and supplements used.

Parameter	*Agaricus bisporus* compost	*Pleurotus ostreatus* substrate	Deffated almond meal	Promycel 600	Promycel Pleurotus
pH (1:5, w/v)	6.09	8.04	6.19	5.90	6.00
Moisture (g kg^{-1})	634	741	70	120	126
Total nitrogen (g kg^{-1})	24.3	6.15	67.2	81.7	66.5
Protein (g kg^{-1})	151.9	38.4	420.0	510.4	415.6
Ash (g kg^{-1})	277.8	72.3	58.0	64.3	67.9
Organic matter (g kg^{-1})	722.3	927.7	942.0	935.7	932.1
C/N	17.2	87.5	8.2	6.6	8.1
Crude fibre (g kg^{-1})	260.0	433.8	200.4	87.9	128.2
Crude fat (g kg^{-1})	5.3	5.6	248.3	31.4	38.1
N-free extract (g kg^{-1})	305.1	449.9	73.4	306.0	350.2
Total carbohydrates (g kg^{-1})	565.1	883.7	273.8	393.9	478.4
Hemicellulose (g kg^{-1})	179.0	261.9	278.8	188.7	215.5
Cellulose (g kg^{-1})	256.3	452.0	88.5	50.0	87.7
Lignin (g kg^{-1})	160.5	95.7	201.7	132.3	80.0
Neutral-det. sol. (g kg^{-1})	126.5	118.1	373.1	564.6	548.9

Among the supplements used for *Agaricus*, it is remarkable the lower protein content of the almond meal (420.0 g kg^{-1}) compared to the control Promycel 600 (510.4 g kg^{-1}). Higher crude fibre (200.4 *vs* 87.9 g kg^{-1}) and much higher crude fat (248.3 *vs* 31.4 g kg^{-1}) contents have been detected. Additionally, we highlight a lower total carbohydrate content (273.8 *vs* 393.9 g kg^{-1}). The higher hemicellulose, cellulose and lignin contents represent a lower neutral detergent-soluble fibre content (373.1 *vs* 564.6 g kg^{-1}).

The supplement of DAM shows a remarkable higher crude fat content (248.3 g kg^{-1}) compared to the reference Promycel Pleurotus (38.1 g kg^{-1}) used for *Pleurotus* (Table 1). Notably, the lower nitrogen-free extractive (73.4 *vs* 350.2 g kg^{-1}), total carbohydrate (273.8 *vs* 478.4 g kg^{-1}) and neutral detergent-soluble fibre contents (373.1 *vs* 548.9 g kg^{-1}) should be highlighted; the latter effect was due to the higher hemicellulose content and, particularly, higher lignin content.

Agaricus bisporus yield and quality

Although no significant differences were found for most of the quantitative production parameters, some findings should be highlighted (Figure 1, Table 2). The non-supplemented substrates provided lower unit weights of the mushrooms (18.7 g) than the supplemented substrates, although the difference was not significant. However, larger unit weights were obtained for the mushrooms produced after the addition of almond meal regardless of the dose. Variations caused by the dose applied did not allow us to obtain conclusions.

Regarding the yields, a larger production (28.12 kg m^{-2}) was obtained with the substrate supplemented with almond meal at a dose of 10 g kg^{-1}, although the differences were neither significant (Figure. 1).

Table 2. Results obtained for the main qualitative production parameters assessed in *Agaricus bisporus* mushrooms from the various treatments.

Supplement	Commercial class (kg m^{-2})		Cap diameter mm)	Colour			Mush. Unitary wt (g)	Firmness	
	Large sized ≥40 mm	Medium sized <40 mm		L⋆	b⋆	ΔE⋆		Puncture force (N)	Compression energy (mJ)
NS	15.57	11.29	37.9	93.8	8.2	7.3	18.7	19.01	132.7
PRO600-D10	17.17	10.32	38.6	93.9	8.2	9.0	19.6	19.95	157.3
DAM-D5	18.22	9.37	39.6	94.3	7.8	8.4	20.7	19.50	144.6
DAM-D10	17.85	10.26	39.3	94.2	8.0	8.7	20.4	19.92	154.9
DAM-D15	18.56	8.65	40.0	93.7	8.3	9.1	21.3	20.71	164.7

Values followed by different superscript letters within a column are significantly different at $p \leq 0.05$ (LSD Fisher's test); NS: non-supplemented control, PRO600: Promycel 600 control, DAM: deffated almond meal, D5: supplement amount 5 g kg^{-1}, D10: supplement amount 10 g kg^{-1}, D15: supplement amount 15 g kg^{-1}.

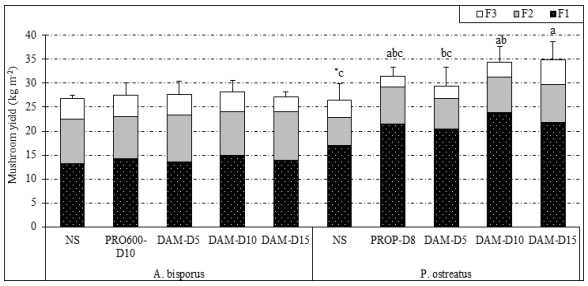

Figure 1. Mushroom yield (kg m^{-2}) obtained in the trials with *Agaricus biporus* and *Pleurotus ostreatus* supplemented crops. F1: first flush; F2: second flush; F3: third flush.
*Letters over bars in each trial mean statistical significant differences at $p \leq 0.05$ (LSD Fisher's test) among treatments. NS: non-supplemented control, PRO600: Promycel 600 control (*Agaricus*), PROP: Promycel Pleurotus control, DAM: defatted almond meal, D5: supplement amount 5 g kg^{-1}, D10: supplement amount 10 g kg^{-1}, D15: supplement amount 15 g kg^{-1}.

All the supplemented substrates registered yield increases in the first flush, and even more significant for almond meal at a dose of 10 g kg^{-1}, which was the only parameter that showed significant differences. However, the behaviour found in the subsequent flushes did not follow a pattern associated with the applied supplement dose.

The distribution of the production by sample size increased the yield of thick mushrooms with the supplemented substrates, albeit not significantly. This result is related to the finding that the diameter of the mushrooms is the largest for the supplemented substrates with similar unit weight, resulting in larger diameter mushrooms produced with the substrate supplemented with almond meal at a 15 g kg^{-1} dose (40.0 mm), although the difference was not significant.

The only significant differences found regarding the qualitative parameters concern the dry matter content of button mushrooms. The non-supplemented substrate produced mushrooms with a low dry matter content (71.3 g kg^{-1}), which was significantly below the content of the mushrooms produced with substrate supplemented with the highest dose of almond meal (75.6 g kg^{-1}) (Table 3).

In fact, considering the relation between the dry matter content and the texture, a lower puncture force and compression energy have also been obtained with mushrooms using a non-supplemented substrate (19.01 N and 132.7 mJ, respectively) (Table 2). Similarly, higher breaking strength and compression energy were obtained for mushrooms produced with the substrate supplemented with the highest dose of almond meal (20.71 N and 164.7 mJ, respectively), although the differences were not significant. Neither significant differences were found regarding the colour of the carpophores (Table 2).

Regarding the mushroom mycochemical characteristics (Table 3), no significant differences were found in any of the analysed parameters. Despite the lack of significant differences, supplementation increased the protein content of the carpophores with the increase in the almond meal dose, which could be explained by the additional contribution of the supplementation with nitrogen-rich materials. For the remaining parameters, supplementation resulted in an increase in the crude fibre content and decreases in the total carbohydrate, nitrogen-free extractive and ash contents, although the differences were not outstanding.

Pleurotus ostreatus yield and quality

Regarding productivity, all supplemented substrates showed important increases compared to the non-supplemented substrate (26.47 kg m^{-2}), with a significant increase up to 34.90 kg m^{-2} observed with the substrate supplemented with DAM at the 15 g kg^{-1} dose, achieving a maximum increment of 31.8% (Figure 1). All recorded values were considered outstanding compared with the usual values obtained with commercial culture. Regarding the behaviour of the production by flush, all supplemented substrates provided higher productivity values than the non-supplemented substrates for the first and second flushes. Larger increases were found for the first flush, where the differences were significant compared to the control except for the 5 g kg^{-1} dose. For the second flush, only the differences obtained with the highest dose were significant. In addition to leading the greatest significant increase recorded, the maximum dose resulted in a more uniform temporal production distribution considering the higher yields reached during the second and third flushes. Taking into account the days elapsed until the last harvesting, the difference was not significant even though the supplemented substrates provided larger production value rates than the non-supplemented substrate (between 1.93 and 1.96 kg dt^{-1} day^{-1} *vs* 1.63 kg dt^{-1} day^{-1}). No significant differences were found regarding the unit weight and earliness.

The qualitative parameters defining the colour and texture (firmness) did not show significant differences, although the dry matter content of the carpophores differed significatly (Tables 3 and 4). The lowest records corresponded to the non-supplemented substrate (75.9 g kg^{-1}) and were significantly lower than the results for the substrates supplemented with the 10 and 15 g kg^{-1} doses (83.2 and 86.0 g kg^{-1}, respectively). Thus, the dose increases translated to 9.6 and 13.3% respectively, in the dry matter content of the harvested mushrooms (Table 3).

Table 3. Mycochemical characterization of *Agaricus bisporus* and *Pleurotus ostreatus* harvested from the various treatments.

	Supplement	Dry matter (g kg^{-1})	Crude protein (Nx4.38, g kg^{-1} d.m.)	Crude fat (g kg^{-1} d.m.)	Total carbohydrates (g kg^{-1} d.m.)	N-free extract (g kg^{-1} d.m.)	Crude fiber (g kg^{-1} d.m.)	Ash (g kg^{-1} d.m.)	Energy value (kcal 100 g^{-1} d.m.)
A.bisporus	NS	71.3^{b}	194.1	17.8	686.4	613.3	73.1	101.7	345
	PRO600-D10	74.5^{ab}	209.5	18.4	672.6	591.6	81	99.6	342
	DAM-D5	74.2^{ab}	201.9	16.2	683.3	602	81.3	98.7	342
	DAM-D10	74.6^{ab}	202.9	16.2	680.9	604.7	76.2	100	343
	DAM-D15	75.6^{a}	207.5	19.8	674.7	599.8	74.9	98.1	346
P. ostreatus	NS	75.9^{c}	143.5^{b}	18.4^{ab}	778.9	706.9	72.1	59.1	366
	PROP-D8	78.0^{c}	154.9^{ab}	17.6^{abc}	769.7	689.3	80.4	57.9	362
	DAM-D5	79.0^{bc}	153.2^{b}	19.0^{a}	768.8	683.7	85.1	58.9	360
	DAM-D10	83.2^{ab}	165.1^{ab}	14.5^{c}	766	678.4	87.6	54.5	359
	DAM-D15	86.0^{a}	176.2^{a}	15.0^{bc}	752.6	679.5	73.1	56.2	364

Values followed by different superscript letters within a column in each trial are significantly different at $p \leq 0.05$ (LSD Fisher's test). NS: non-supplemented control, PRO600: Promycel 600 control (Agaricus), PROP: Promycel Pleurotus control, DAM: defatted almond meal, D5: supplement amount 5 g kg^{-1}, D10: supplement amount 10 g kg^{-1}, D15: supplement amount 15 g kg^{-1}, d.m.: dry matter.

Table 4. Results obtained for the main qualitative production parameters assessed in oyster mushrooms from the various treatments.

Supplement	Oyster mushroom unitary wt (g)	Colour			Firmness	
		L★	a★	b★	Puncture force (N)	Compression energy (mJ)
NS	17.8	64.91	3.538	14.136	215.4	731.2
PROP-D8	16.6	64.37	3.718	13.880	215.6	802.9
DAM-D5	19.0	65.06	3.467	13.818	240.9	874.0
DAM-D10	17.7	64.62	4.115	15.377	238.7	739.5
DAM-D15	18.0	64.70	4.063	15.106	198.4	701.4

Values followed by different superscript letters within a column are significantly different at $p \leq 0.05$ (LSD Fisher's test); NS: non-supplemented control, PROP: Promycel Pleurotus control, DAM: deffated almond meal, D8: supplement amount 8 g kg^{-1}, D5: supplement amount 5 g kg^{-1}, D10: supplement amount 10 g kg^{-1}, D15: supplement amount 15 g kg^{-1}.

The supplementation tended to produce significant decreases in the carpophore water content similar to the observations with the *A. bisporus*. It also induced an increase in the protein content in all cases. This is even larger when the applied almond meal dose rises, reaching a maximum of 176.2 g kg^{-1} for the supplementation with 15 g kg^{-1}, which was significantly greater than the non-supplemented control. On the contrary, the addition of supplements decreases the fat values significantly. For the remaining parameters, supplementation improved the crude fibre content and reduced the total carbohydrate, nitrogen-free extractive and ash content, similar to the results obtained for *A. bisporus*, although the differences observed were not significant (Table 3). Compared to *A. bisporus*, mushrooms from the genus *Pleurotus* showed lower protein and mineral (ash) content and a higher carbohydrate content and energy value.

Discussion

There is controversy regarding the nutrient content within supplements used for mushroom growing. In mushroom compost, wheat straw in the major carbon source while chicken litter is the major nitrogen source, but frequently the colonized compost (Phase III compost used for *A. bisporus* cultivation) is supplemented with sterile protein rich nutrients (Vos et al., 2017). According to Arce-Cervantes et al. (2015), *A. bisporus* needs a simultaneous source of oil and protein in order to increase yields, and that with a source of polysaccharides it is possible to formulate a supplement equivalent to the traditional one based on crushed soybeans (25% protein and 18% oil). Dahlberg (2004) suggested that carbon metabolism is also important in the mushroom growth process. Ingredients containing cellulose and hemicellulose are preferred as supplements because formulations based on simple sugars or starch are subjected to the attack of microorganisms or pathogenic moulds such as *Trichoderma*. Moreover, base ingredients with high carbohydrate contents are cheap and easily available which is an added value.

The supplements used in this research showed high fibre, fat, hemicellulose and lignin content and low carbohydrate, and resulted in equal and even higher yield than commercial supplements consecrated in the market. Therefore, our results contradict the broadly accepted theory that supplements used in the production of mushrooms must necessarily present both high protein and high carbohydrate content.

Larger sizes are usually preferred by farmers due to the fresh market demand and the reduction of labour costs in harvesting; however, medium sizes may be preferable by facilities prepared for mechanical harvesting and canned industry. Typically, high yields are associated with high fruiting rates. Thus, when the number of harvested mushrooms increases, the mushroom size decreases (Pardo-Giménez et al., 2012). This statement is consistent with our results. Besides, the mushroom dry matter content is a parameter of special interest because it directly reflects the quality and

commercial lifetime. Mushrooms with a high dry matter content, most likely the button mushroom cultivated with DAM supplementation, show a firmer texture, a higher nutritional value and a lower sensitivity to microbial spoilage (Diamantopoulou & Philippoussis, 2015).

Considering the cost of the substrate and the supplement, in addition to the increase in production registered, a substantial improvement in the benefits can be achieved by incorporating the by-product defatted almond meal as a nutritional supplementation for enhancing mushroom composition and yield.

Conclusion

The by-product defatted almond meal can be successfully recycled for enhancing mushroom production and quality on compost application. The species *P. ostreatus* had a higher response than *A. bisporus* in terms of yield. The quality of mushrooms improved after supplementation. Doses of DAM between 10 and 15 g kg^{-1} of fresh substrate show good potential for application at the commercial scale in *P. ostreatus* primarily due to the expected production increases, with direct incidence on the benefits and cost-effectiveness of the mushroom industry. Complementary the recycling of an useless by-product contributes to the sustainability of the agriculture and to optimize the biomass generated from a relevant agronomical activity base on almonds trees.

Supplementary material (Table S1) collects the main quantitative production parameters along the crop cycle.

Acknowledgements

We would like to thank the 'Diputación Provincial de Cuenca', the 'Consejería de Agricultura, Medio Ambiente y Desarrollo Rural de Castilla-La Mancha' and the University of Castilla-La Mancha (Spain) for the financial support.

References

Ankom. (2008). *Crude fiber analysis in feeds by filtering bags technique. Ankom Technology Method 7, AOCS Approved procedure Ba6a-*. Macedon, NY: Ankom Technology.

Ankom. (2009). *Rapid determination of oil/fat utilizing high temperature solvent extraction. Ankom Technology Method 2, AOCS Official Procedure Am 5-04*. Macedon, NY: Ankom Yechnology.

Arce-Cervantes, O., Saucedo-García, M., Lara, H. L., Ramírez-Carrillo, R., Cruz-Sosa, F., & Loera, O. (2015). Alternative supplements for *Agaricus bisporus* production and the response on lignocellulolytic enzymes. *Scientia Horticulturae*, *192*(50), 375-380. doi: 10.1016/j.scienta.2015.06.030

Da, C., Hongzhi, L., Li, L., Hui, H., & Qiang, W. (2014). Quality comparison of almond oil extracted with different processes and correlation analysis of quality indexes. *Journal of the Chinese Cereals and Oils Association*, *2*, 47-52.

Dahlberg, K. R. (2004). Carbohydrate-based mushroom supplements. *Mushroom News*, *52*(7), 6-11.

Diamantopoulou, P., & Philippoussis, A. (2015). Cultivated mushrooms: preservation and processing. In Y. H. Hui, & E. Özgül Evranuz (Ed.), *Handbook of vegetable preservation and processing* (p. 495-525). Boca Raton, FL: CRC Press.

Food and Agriculture Organization of the United Nations [FAO]. (2017). *FAOSTAT, production of almonds by region average 1994-2014.* Retrieved on June 15, 2017 from http://www.fao.org/faostat/en/#data/QC

Homet-Gutierrez, P., Schupp, E. W., & Gómez, J. M. (2015). Naturalization of almond trees (*Prunus dulcis*) in semi-arid regions of the Western Mediterranean. *Journal of Arid Environments*, *113*(1), 108-113. doi: 10.1016/j.jaridenv.2014.10.005

Kodad, O., Estopañán, G., Juan, T., Socias, I., & Company, R. (2014a). Tocopherol concentration in almond oil from Moroccan seedlings: Geographical origin and post-harvest implications. *Journal of Food Composition and Analysis*, *33*(2), 161-165. doi: 10.1016/j.jfca.2013.12.010

Kodad, O., Estopañán, G., Juan, T., Alonso, J.M., Espiau, M.T., Socias, I., & Company, R. (2014b). Oil content, fatty acid composition and tocopherol concetration in the spanish almond genebank collection. *Scientia Horticulturae*, *177*(2), 99-107. doi: 10.1016/j.scienta.2014.07.045

Lau, O. (1982). Methods of chemical analysis of mushrooms. In S. T. Chang, & T. H. Quimio (Ed.), *Tropical mushrooms. Biological nature and cultivation methods.* (p. 87-116). Hong Kong, CH: The Chinese University Press.

Martínez, M. L., Marín, M. A., Gili, R. D., Penci, M. C., & Ribotta, P. D. (2017). Effect of defatted almond flour on cooking, chemical and sensorial properties of gluten-free fresh pasta. *International Journal of Food Science & Technology*, *52*(10), 2148–2155. doi: 10.1111/ijfs.13493

Marzouki, H., Piras, A. M., & Rosa, A. D. (2008). Extraction and separation of volatile and fixed oils from berries of *Laurus nobilis* L. by supercritical CO_2. *Molecules*, *13*(8), 1702-1711. doi: 10.3390/molecules13081702

Mezzomo, N., Martínez, J., & Ferreira, S. R. (2009). Supercritical fluid extraction of peach (*Prunus persica*) almond oil: kinetics, mathematical modelling and scale-up. *The Journal of Supercritical Fluids*, *51*(1), 10-16. doi: 10.1016/j.supflu.2009.07.008

Miles, P. G., & Chang, S. T. (1997): The chemical composition of fungal cells. Useful generalizations. In P. G. Miles, & S. T. Chang (Ed.), *Mushroom biology.*

Concise basics and current developments (p. 33-35). Singapore: World Scientific Publishing Co. Pte. Ltd.

Naraian, R., Sahu, R. K., Kumar, S., Garg, S. K., Singh, C. S., & Kanaujia, R. S. (2009). Influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on corn cob substrate. *The Environmentalist*, *29*(1), 1-7. doi: 10.1007/s10669-008-9174-4

Pardo-Giménez, A., Pardo-González, J. E., & Zied, D. C. (2011). Evaluation of harvested mushroom and viability of *Agaricus bisporus* growth using casing materials made from spent mushroom substrate. *International Journal of Food Science & Technology*, *46*(4), 787-792. doi: 10.1111/j.1365-2621.2011.02551.x

Pardo-Giménez, A., Zied, D. C., Álvarez-Ortí, M., Rubio, M., & Pardo, J. E. (2012). Effect of supplementing compost with grapeseed meal on *Agaricus bisporus* production. *Journal of the Science of Food and Agriculture*, *92*(8), 1665-1671.

Pardo-Giménez, A., Catalán, L., Carrasco, J., Álvarez-Ortí, M., Zied, D. C., & Pardo, J. E. (2016). Effect of supplementing crop substrate with defatted pistachio meal on *Agaricus bisporus* and *Pleurotus ostreatus* production. *Journal of the Science of Food and Agriculture*, *96*(11), 3838-3845. doi: 10.1002/jsfa.7579

Roncero-Ramos, I., Mendiola-Lanao, M., Pérez-Clavijo, M., & Delgado-Andrade, C. (2017). Effect of different cooking methods on nutritional value and antioxidant activity of cultivated mushrooms. *International Journal of Food Science & Technology*, *68*(3), 287-297. doi: 10.1080/09637486.2016.1244662

Royse, D. J., & Chalupa, W. (2009). Effects of spawn, supplement, and phase II compost additions and time of re-casing second break compost on mushroom (*Agaricus bisporus*) yield and biological efficiency. *Bioresource Technology*, *100*(21), 5277-5282. doi: 10.1016/j.biortech.2009.02.074

Royse, D. J. (2010). Effects of fragmentation, supplementation and the addition of phase II compost to 2nd break compost on mushroom (*Agaricus bisporus*) yield. *Bioresource Technology*, *101*(1), 188-192. doi: 10.1016/j.biortech.2009.07.073

Royse, D. J. (2014). A global perspective on the high five: *Agaricus, Pleurotus, Lentinula, Auricularia* & *Flammulina*. In M. Singh (Ed.), *Proceedings of ICMBMP8* (p. 1-6). New Delhi, IN: ICAR-Directorate of Mushroom Research.

Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology*, *85*(5), 1321-1337. doi: 10.1007/s00253-009-2343-7

Sena-Moreno, E., Pardo, J. E., Catalán, L., Gómez, R., Pardo-Giménez, A., & Álvarez-Ortí, A. (2015). Drying temperature and extraction method influence physicochemical and sensory characteristics of pistachio oil. *European Journal of Lipid Science and Technology*, *117*(5), 684-691. doi: 10.1002/ejlt.201400366

Sonnenberg, A. S. M., Baars, J. J. P., Hendrickx, P. M., Lavrijssen, B., Gao, W., Weijn, A., & Mes, J. J. (2011). Breeding and strain protection in the button mushroom *Agaricus bisporus*. In J. M. Savoie, M. Foulogne-Oriol, M. Largeteau, & G. Barroso (Ed.), *Proceedings of ICMBMP7* (p. 7-15). Arcachon, FR: INRA.

Sullivan, D. M. (1993). Proximate and mineral analysis. In D. M. Sullivan, & D. E. Carpenter (Ed.), *Methods of analysis for nutrition labeling* (p. 105-109). Arlington, VA: AOAC International.

Vos, A. M., Heijboer, A., Boschker, H. T., Bonnet, B., Lugones, L. G., & Wösten, H. A. (2017). Microbial biomass in compost during colonization of *Agaricus bisporus*. *AMB Express, 2017*(7). doi: 10.1186/s13568-016-0304-y

Wheeler, D., & Wach, M.P. (2006). Re-evaluating supplementation. *Mushroom News*, *54*(7), 4-11.

Yada, S., Lapsley, P., & Huang, G. (2011). A review of composition studies of cultivated almonds: Macronutrients and micronutrients. *Journal of Food Composition and Analysis*, *24*(4-5), 469-480. doi: 10.1016/j.jfca.2011.01.007

Yada, S., Huang, G., & Lapsley, K. (2013). Natural variability in the nutrient composition of California-grown almonds. *Journal of Food Composition and Analysis*, *30*(2), 80-85. doi: 10.1016/j.jfca.2013.01.008

Zervakis, G., Yiatras, P., & Balis, C. (1996). Edible mushrooms from olive oil mill wastes. *International Biodeterioration & Biodegradation*, *38*(3), 237-243. doi: 10.1016/S0964-8305(96)00056-X

Zied, D. C., Savoie, J. M., & Pardo-Giménez, A. (2011). Soybean the main nitrogen source in cultivation substrates of edible and medicinal mushrooms. In H. A. El-Shemy (Ed.), *Soybean and nutrition* (p. 433-452). Rijeka, CR: In Tech Open Access Publisher.

Received on September 6, 2017.
Accepted on January 12, 2018.

Supplementary material

Table S1. Results obtained for the main quantitative production parameters assessed in *Agaricus bisporus* and *Pleurotus ostreatus* mushrooms originating from the various treatments.

	Supplement	Number of mushrooms (mushrooms m^{-2})	Mushroom yield (kg m^{-2})				Biological efficiency (kg dt^{-1} compost)	Production rate (kg dt^{-1} d^{-1})	Mushroom unitary wt (g)	Earliness (days from casing)
			First flush	Second flush	Third flush	Total yield				
A.bisporus	NS	1445	13.21^{b}	9.37	4.27	26.86	107.3	2.9	18.7	21.5
	PRO600-D10	1416	14.23^{ab}	8.86	4.4	27.49	107.2	2.91	19.6	21.4
	DAM-D5	1348	13.63^{ab}	9.66	4.3	27.59	108.8	2.95	20.7	21.4
	DAM-D10	1388	14.98^{a}	9.13	4.01	28.12	109.5	2.96	20.4	21.4
	DAM-D15	1296	13.90^{ab}	10.17	3.14	27.21	104.7	2.83	21.3	21.4
P. ostreatus	NS	-	16.94^{b}	5.95^{b}	3.58	26.47^{c}	100.3^{b}	1.63	17.8	25.4
	PROP-D8	-	21.50^{a}	7.63^{ab}	2.34	31.47^{abc}	117.0^{ab}	1.96	16.6	25.9
	DAM-D5	-	20.37^{ab}	6.45^{b}	2.53	29.34^{bc}	109.8^{ab}	1.94	19	24.6
	DAM-D10	-	23.92^{a}	7.33^{ab}	3.06	34.31^{ab}	126.9^{a}	1.94	17.7	25.7
	DAM-D15	-	21.89^{a}	7.86^{a}	5.15	34.90^{a}	127.6^{a}	1.93	18	26.2

Values followed by different superscript letters within a column in each trial are significantly different at $P \leq 0.05$ (LSD Fisher's test) ; NS: non-supplemented control, PRO600: Promycel 600 control (*Agaricus*), PROP: Promycel Pleurotus control, DAM: defatted almond meal, D5: supplement amount 5 g kg^{-1}, D10: supplement amount 10 g kg^{-1}, D15: supplement amount 15 g kg^{-1}.

The most accurate way to express mushroom productivity is the parameter known as "biological efficiency". The biological efficiency is expressed as kg dt^{-1} compost (dt= 100 kg of compost in dry matter) and was determined from the yield by surface unit when taking into account the load density of the compost and its humidity content. However, the production rate as kg of mushroom per unit area, is more intuitive, for this reason that was the parameter used to build the Figure 1 included in the article. The production rate (mean daily biological efficiency) was also calculated and determined from the total biological efficiency and the time from the filling of the culture room until the last harvesting day. The unit weight of the mushrooms (expressed as grams) was determined from the obtained yield and the number of harvested mushrooms. The earliness was expressed as the time from the application of the casing until the harvesting of the first flush considering the daily relative production of the harvesting (Pardo-Giménez et al., 2011).

As discussed in the article, the table submitted as supplementary material provides the information concerning production rate in each flush, including statistically significant differences detected among treatment during each flush.