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PROXIMAL AND SENSORIAL EVALUATION OF FETTUCCINE PASTA WITH MUSHROOM FLOUR PARTIAL REPLACEMENT *PLEUROTUS OSTREATUS*

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— Abstract—

Pleurotus ostreatus is a saprophytic fungus that grows mainly on living or dead lignocellulose substrates, poor in nutrients and with low levels of minerals and vitamins. The propagation of this organism requires lignocellulose substrates. This fungus is edible with important functional properties. Because the population of trees located in the gardens of the Universidad de Ciencias y Artes de Chiapas generates a considerable amount of organic waste, these can be used as raw material for the cultivation of fungi. Two crops of the mushrooms were carried out in different substrates, the first from leaves of *benjamina* (*Ficus benjamina*) and the second was a combination of fig leaves (*Ficus carica*) and *capulín* (*Prunus serotina*). The edible mushrooms obtained from the crops were dehydrated by convection of hot air at 50°C for 24 hours, then ground and stored for disposal in the integration of edible paste formulations. This food has become a staple food because of its variety in processing and consumption, however, its protein content can be low and of poor quality; therefore, it is necessary to incorporate other sources of protein of better quality without altering eating habits or organoleptic characteristics. In this work we studied the nutritional composition and consumer acceptance that results with the addition of mushroom flour in the production of fettuccine pasta. Addition levels were 10% and 20% mushroom meal (HS), the chemical composition (raw protein, carbohydrates, fiber, fat and ash) of both formulations was analyzed based on the AOAC (2000). The results indicated a considerable increase in the percentage compositions of fiber and protein when 20% HS was added to the fettuccine pasta formulation with respect to the reported sample without the addition of the mushrooms. In addition, the taste levels perceived by the sensory judges, for the attributes of smell and taste, were also high when 20% HS was incorporated with respect to the fettuccine control paste. This proposal can be considered useful for the preservation of mushrooms, since when dehydrated, despite the low yields, it can become an apt and versatile condiment, because it increases the nutritional components and improves the sensory properties of this type of food, without downplay the sustainable use of waste from plants in university gardens.

Keywords
Mushrooms; Fettuccine; Mycoprotein.

The rustic production of *Pleurotus ostreatus* mushrooms in the state of Chiapas represents for the rural and urban communities an alternative of food production, the generation of employment and an additional economic income (Albores and Álvarez, 2015). In addition to its nutritional and gastronomic characteristics, this fungus has beneficial properties for the organism, among which are the stimulation of the immune system, cholesterol reduction and glucose levels, as well as the decrease in the probability of tumors and cancer (Álvarez *et al*., 2016). From the gastronomic point of view, its flavor, its different tonalities and its versatility as a culinary accessory, make it grow in popularity and be increasingly accepted by consumers.

The state of Chiapas is one of the most biologically diverse areas in the country and in the world, where more than ninety-eight different types of tropical trees grow (Cruells *et al*., 2002). However, these trees generate tons of waste that, far from being used, become an environmental and public health problem (semarnat, 2015; semahn, 2017). Growing mushrooms on garden tree waste can contribute to food security, as it represents an environmentally friendly alternative to add value to waste.

On the other hand, the majority of foods processed from vegetables, such as cereals, possess protein with deficiency of essential amino acids (Elizalde *et al*., 2010), which are essential for a good physical and mental development. Based on the requirements of the human body, the essential amino acids are arginine, phenylalanine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan and valine, which are compounds that must be obtained from the diet since the body does not synthesize them (Quevedo, 2012). So that the protein content of edible fungi reaches 19 to 35% of usable proteins in dry weight, compared to most fruits and vegetables, which have between 7.3 to 13.2%, as other foods for common consumption: rice (7.3%), corn (11.2%), beans (24.2%), avocado (7.1%) and orange (5%), contain protein concentrations similar or lower than fungi (Gaitán, 2017). It is also significant if compared with 13.2% of wheat, 25.2% of milk and 26.04% of red meat (sagarpa, 2016, Rubio *et al*., 2013). In addition, the fungal protein contains all the essential and required amino acids by man, being particularly rich in lysine and leucine remembering that most fibrous cereals contain little or are lacking. The objective of this work was to take advantage of the mushrooms (*Pleurotus ostreatus*) through its incorporation as flour to improve the nutritional and organoleptic quality of the fettuccine pasta.

MATERIALS AND METHODS

Establishment conditions for the cultivation of the mushroom Pleurotus ostreatus

The crop was established in a metal rack covered with black polypropylene bags to perform the first phase of the crop also known as "dark". The cultivation area was cleaned and disinfected with a 200 ppm solution of sodium hypochlorite. For the dark phase the shelf remained closed and without air circulation.

Phases of mushroom (Pleurotus ostreatus) cultivation in tree leaf substrate

The preparation of the substrate for the sowing consisted of the preparation of 30 kilos approx. of *benjamina* (*Ficus benjamina*), fig (*Ficus carica*) and *capulín* (*Pronus serotina*) trees leaves of the gardens of the Universidad de Ciencias y Artes de Chiapas (Tuxtla Campus). Once collected, they were cleaned to remove excess dust and were placed with calcium hydroxide solutions (5%). Subsequently, the substrate for the planting was pasteurized, allowed to cool to room temperature to later carry out the sowing of the inoculum.

The leaves of each tree were drained and treated separately. The substrate was pasteurized for 1 hour in boiling tap water with 1% CaO, and then sieved in a disinfected stainless steel strainer. Later they were accommodated in transparent polypropylene bags with a capacity of 10 kg of substrate. 0.5% inoculum was added to each bag (seeds of sorghum colonized with mycelium of *P. ostreatus*), starting with a first layer of leaf at the bottom of the bag, spreading the inoculum on the edges of the bag and on the layers of leaves. After sowing, the containers were labeled and subsequently incubated on the shelf for the start of the dark phase.

After 72 hours of seeding, holes of 1 cm in length were made aseptically when signs of condensation were noticed in the plastic covering the substrate. After two or three weeks, the incubation conditions with higher aeration and photoperiod of 18 hours of light were changed when colonization of the mycelium on the substrate was observed. In the light phase, holes of approximately 2 cm were again made to favor the transfer of oxygen to the interior of the crop and thus be able to start with the production of mushrooms. The irrigation during the luminous phase of crop was three times a day with an approximate cost of 250 ml of purified water per bag each day. Both stages were maintained at room temperature between 25° to 35°C.

The complete cultivation cycle had a total duration of 4 months from sowing with three harvests. Once the mushrooms were harvested, the length of the fruiting body was recorded, length, width and thickness of the carpophorus and width of the pileus. The color was determined with a Konica Minolta brand colorimeter model CR-400.

Preparation of dehydrated mushroom powder (Pleurotus ostreatus)

The freshly harvested mushrooms were aseptically cut and chopped into pieces of 2-3 cm^3, they were weighed and placed on drying trays at 50°C for 24 h until a dry material (moisture of less than 12%) was obtained in a drying oven FE-291A model brand Felisa® at 50°C. The dehydrated mushrooms were milled with a speed blender, BPST02-B00 model Oster ®, and the material obtained was sieved with a number 30 and 50 TYLER * 5195 ® mesh, and placed in an airtight container.

Fettuccine pasta production

The preparation of the fettuccine pasta consisted of mixing the ingredients in stainless steel utensils according to what is described in table 1. The procedure consisted in pouring the flour into the container making a space in the middle of the mixture to incorporate egg, and start mixing, adding water (approximately 100 mL) and oil (approximately 30 mL) in sufficient quantity to form a homogeneous paste, then wrapped in non-stick paper and refrigerated at 7 ° C for 20 minutes.

Table 1. Elaboration of fettuccine pasta with 2 concentrations of mushroom flour (HS)

Ingredients	Control	Pasta (10% of HS)	Pasta (20% of HS)
Wheat flour (g)	200	180	160
Mushroom flour (g)	0	20	40
Salt (g)	0.002	0.002	0.002
Egg (g)	100 gr	100 gr	100 gr

After removing from the refrigerator, the dough was flattened in an Atlas® Roller 180 pasta machine. Five levels of extrusion were used to form a thin layer, and then passed through the fettuccine cutter, obtaining strips of 25cm in length, which were placed in a stainless steel tray. The paste was dehydrated for 24 hours at room temperature.

Proximate chemical composition of fettuccine pasta

To determine the composition of the pasta, the dried strips of fettuccine pasta with two concentrations of mushroom flour were crushed into pieces. The content of moisture and ash (%) was measured according to the methods of the AOAC, 1990. 934.01 and AOAC, 1990. 942.05. The protein content (%N x 5.7) was analyzed with the micro Kjieldahl method 928.08, the ether extract or fats 942.05 and the raw fiber 978.10; the carbohydrates were determined by difference. All determinations were made in triplicate.

Sensory analysis of fettuccine pasta samples added with mushroom flour

The sensory tests were performed in the S Laboratorio de Evaluación Sensorial of the Universidad de Ciencias y Artes de Chiapas with a panel of 25 semi-trained judges. The judges went to individual cabins under white light at room temperature and received two samples of 30 grams of fettuccine (A and B), an evaluation instrument and purified water for rinsing. The scale used was an unstructured hedonic. The responses of the variables were recorded with a horizontal scale. At one end of the scale was the word "Pleasant" and at the opposite end "Unpleasant". The graphic signal on the horizontal line was measured in centimeters with a scale; this numerical scale can be translated to a 9-point verbal hedonic scale. The judges rated its pleasure level without any information about the product or its nutritional value.

Statistical data analysis

Descriptive statistics were applied to the results of the proximal chemical analyzes with the help of the Microsoft Excel 2013 version, while the Minitab program version 17 was used for the statistical analysis (ANOVA) of the results of the sensory tests.

RESULTS

Cultivation of Pleurotus ostreatus

During the cultivation of the mushrooms, in the treatments where it was used as a substrate of fig leaves and capulin (HHC), there was a smaller amount of mushrooms than in the bags with *benjamina* leaf substrate (HB), which took maximum one week to originate primordium. In the HB substrate, more mushrooms were produced but they were smaller and darker in color than in the HHC substrate, the latter being the support of larger, aromatic and light-colored mushrooms. The moment of the cut is when the mushroom becomes slightly darker in spite of adding water constantly, the harvest is made by cutting from where the trunk of the mushroom starts, to then make the measurements of length, length by width of the hat, width of the stem, thickness of the hat, using a device known as vernier (Table 2).

Table 2. Size and color of produced mushrooms

Quantified parameters	HHC	HB
Fruitful body length (cm)	8	4.8
Length and width of the carpophore (cm)	11.5 x 9.6	4.5 x 4.5
Pileus Width (cm)	1.1	0.6
Carpophore thickness (cm)	0.4	0.3
Color	L* 80.87 A* 1.27 B* 11.92	L* 78.82 A* 1.99 B* 18.64
Mushroom weight (g) x substrate Kg	230	300

Elaboration and proximal evaluation of fettuccine pasta

Fettuccine pasta was made by adding dehydrated fungus. In the control pasta, that is, without the addition of mushroom flour, a light yellow color with a characteristic wheat odor was observed, with a smooth texture (Image 1A). The pastas with 10% mushroom flour had a light brown color and with its characteristic mushroom smell, in terms of its texture it turned out to be softer and smoother with a granular appearance similar to whole-wheat flour (Fig. 1B). The pasta with 20% mushroom flour had a slightly darker color than the pasta with 10%, its smell being a little more pronounced to mushrooms without losing its soft texture and its granular appearance (Image 1C). With these dough fettuccine pastas were made as shown in Image 1 (D: control, E: 10% HS, F: 20% HS). Table 3 shows the results of the proximal analyzes of the three different types of processed pasta.

Image 1. Dough and fettuccine pasta with microprotein added. A and D control; B and E 10% HS; C and F 20% HS

Table 3. Proximal analysis of fettuccine pasta added with 2 concentrations of mushroom flour (10% and 20%)

		Wet base (BH)		Wet base (BS)	
Component		Pasta (10%HS)	Pasta (20%HS)	Pasta (10%HS)	Pasta (20%HS)
Moisture	10.76±0.12	7.77±0.06	8.35±0.06	-----------	-----------
Ashes	0.62±0.02	1.49±0.02	1.69±0.00	1.61±0.04	1.84±0.01
Fiber	0.44±0.08	0.85±0.25	3.04±0.95	0.92±051	3.31±0.74
Fat	2.67±0.01	4.68±0.00	4.49±0.00	5.07±0.01	4.89±0.01
Protein	11.15±0.05	16.53±0.87	17.49±0.03	17.92±0.93	19.08±0.03
Carbohydrates	72.60±0.04	68.67±1.08	64.95±0.86	74.46±1.23	70.86±1.00

Sensory evaluation of fettuccine pasta

Table 4 shows the results obtained from the sensory analysis, in the rows the attribute ratings for the samples of fettuccine control (0% HS), 10% HS and 20% HS are shown.

Table 4. Variance analysis (α = 95%) of the ratings given by the semi-trained judges to the three formulations of fettuccine pasta during the sensory tests

% HS	Appearance	Color	Smell	Taste
0	7.64 ± 2.6	7.91 ± 2.1	6.47 ± 1.9	7.32 ± 2.4
10	6.84 ± 2.5	7.06 ± 2.4	7.45 ± 3.0	6.98 ± 3.1
20	5.22 ± 3.5	6.01 ± 3.4	7.11 ± 3.0	7.70 ± 2.2

DISCUSSION OF FINDINGS

The ability of the *Pleurotus* species to colonize and fructify in almost any agroindustrial waste is mainly due to the fact that it has an enzymatic "arsenal" that allows the degradation of the main components of the cell wall of plants, lignin and cellulose (Xie *et al.*, 2016). Being this ability of the fungus so versatile, in this work, lignocellulose residues of the Universidad de Ciencias y Artes de Chiapas' gardens were used. The results show that *Pleurotus ostreatus* is able to colonize and fructify leaves of *benjamina* (*Ficus benjamina*), fig leaves (*Ficus carica*) and *capulín* (*Prunus serotina*). The contribution of these results are remarkable in two senses, firstly because it is the first report to date of the colonization and fruiting of this fungus on residues of these three tropical trees.

Second, because it constitutes a creative and innovative proposal on the disposal of lignocellulose waste. In this work these residues were from university gardens, however this proposal can be applied to lignocellulose waste from urban and domestic spaces. In the last ten years, the *Pleurotus ostreatus* has become one of the most cultivated mushrooms in the world (Vieira and Andrade, 2016), which shows that the use of waste can be carried out both in cities and in rural areas of Chiapas or any tropical region of the world.

In Chiapas the cultivation of *Pleurotus spp*. constitutes a value chain in several regions such as Altos tsotsil-seltal, Meseta Comiteca and Frailesca (Albores and Álvarez, 2015). There are several reasons why the cultivation of mushrooms has become so popular, among them are the mushroom's ability to grow in a wide range of agroindustrial and forest residues (Ingale and Ramteke, 2010), the low technical requirements to carry technology (Ahmed *et al*., 2016), the organoleptic qualities and the delicate flavor of the fungus (Mohamed *et al*., 2011), the versatility as a fresh ingredient in traditional dishes (Jandaik and Anjana, 2017) and the health benefits that contributes its consumption (Masri *et al*., 2017). In contrast, one of the challenges of mushroom cultivation is post-harvest handling and the short shelf life of fresh mushroom. That is why this work presents a viable alternative to lengthen the shelf life and its incorporation into a very popular food in the cuisine world, as is the fettuccine pasta.

The incorporation of mushroom flours to the pasta required three stages; the first consisted in obtaining the mushroom flour; the second, in the elaboration and proximal analysis of fettuccine pasta and the last one in the sensory analysis of the obtained product to assure its acceptance as safe food.

The yield of the mushroom flour with respect to the fresh mushroom was 10 to 15% of its original weight. The yield in dry matter of mushrooms (*Agaricus bisporus, white strain*) and portobellos (*Agaricus bisporus*, *dark strain*) fresh is very low, around 7% to 10%, and is mainly composed of carbohydrates, proteins, fiber and minerals (Roncero, 2015), while the Shiitake mushroom (*Lentinula edodes*) has a dry matter performance of 11.7%

(Martínez *et al*., 2004). This indicates that the mushroom flour obtained in this work has an acceptable performance compared to that of other mushrooms.

The fettuccine pasta was elaborated replacing the wheat flour of the control formulation with 10 and 20% mushroom flour. From the proximal chemical analyzes performed on the fettuccine pasta, the protein quantified in wet base for the pasta with 10% HS was 16.53±0.87%, while for the pasta with 20% HS was 17.49±0.03%, and for control pasta (without mushroom flour) was 11.15±0.05%. In the protein contribution comparison of the pasta obtained in this work, with the protein contribution reported by Maroto-Sánchez (2016) in fettuccine pasta (12.41 ± 0.04%) and González (2010) clearly indicates that the substitution of mushroom flour increases protein intake by 50 to 70%.

Regarding the fiber content, in the mushroom flour pasta there is a notable increase with respect to the control pastas. The fiber content obtained in this work was between 0.92 (10% HS) and 3.31% (20% HS) on a dry basis and 0.44% for the control, so that the addition of HS doubles and quadruples the contribution of fiber. The addition of non-conventional ingredients helps to increase the nutritional value of the pasta, generating improvements in quantity and protein quality by complementing essential amino acids and increasing the fiber content. Wheat flour is commonly used in bread and pasta products; however, it is deficient in amino acids, specifically in lysine (Torres *et al*., 2014). In contrast, the protein quality of mushrooms is significant. The tendency to develop products with more and better protein content has motivated the development of flours with pea (*Pisum sativum*), chickpea (*Cicer arietinum*), lentils (*Lens culinaris*), (Torres *et al*., 2014), quinoa (*Chenopodium quinoa*) and carrot (*Daucus carota*), (Elizalde, 2010), among others, in combination with wheat flour.

As for the sensory analysis, the paste added with 10% presented good acceptability in color and odor, although the ratings in flavor are shown below the normal pasta, however, it is above the average which means that it can reach to be an accepted product. In this sense, the addition of 20% HS improves the judges' acceptance of flavor compared to control pasta, probably because the mushrooms provide a characteristic flavor (umami), being a protein indicator that most relate to an essential nutrient for survival. This acceptance of flavor by the judges in the pasta added with HS is similar to what happens with the acceptance of dehydrated Shiitake, which contains high concentrations of guanylate, one of the main components of umami (UMAMI Information Center, 2017). In parallel, it is likely that the acceptance of the pasta was related to judges perceiving volatile compounds present, since it is reported that mushrooms have water-soluble volatile components (Zawirska *et al.*, 2009).

Despite the color difference in the control pulp compared with the added pastas, which had a light brown color for 10% HS and coffee for 20% HS, the judges accepted the appearance attribute above the average. This acceptance phenomenon was probably due to the fact that the brown color is associated with the whole food line, which provides fiber and improves protein quality. This may be a preference recently acquired in consumers who choose foods with an appearance that indicates that the product may have ingredients that provide some benefit to health. That is why recently there is a tendency to develop products with more and better protein content adding flour to pasta and bakery products. The addition of pea (*Pisum sativum*), chickpea (*Cicer arietinum*), lentils (*Lens culinaris*) (Torres *et al.*, 2014), quinoa (*Chenopodium quinoa*) and carrot (*Daucus carota*) flours (Elizalde, 2010) has been reported in combination with wheat flour, since the latter is deficient in amino acids, specifically in lysine (Torres *et al.*, 2014).

CONCLUSION

The substitution of 10 and 20% of mushroom flour in the formulation of fettuccine pasta allowed the obtaining of a food with higher protein and fiber levels, besides having a taste and appearance accepted by the potential consumers. This proposal allowed enriching the content of high quality proteins and fungal origin in fettuccine pasta with sensory properties without downplaying the sustainable use of plant residues in university gardens.

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