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Alkalization and heat treatments of substrates for cultivation of edible mushrooms in pupunha and cocoa residues

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**Alkalization and heat treatments of substrates for cultivation of edible mushrooms in pupunha and cocoa residues**

**Abstract**

In search of a healthier diet, the consumption of edible mushrooms has been expanding, as well as the use of agro-industrial residues for cultivation and the use of less costly techniques. In this work residues of pupunha palm (Bactris gasipaes Kunth.) and cocoa (Theobroma cacao) were tested as a substrate to produce Pleurotus pulmonarius CCB19, as well as two forms of treatment: wet heat (autoclaving) and alkalization in calcium hydroxide solution. Substrate compositions based on the pupunha palm residue (P) mixed with the cocoa testa (T) were tested in three different proportions (100% P; 90% P + 10% T; and 80% P + 20% T). The production time, the biological efficiency (BE), the production rate (PR) and the diameter of the cap of the mushrooms were analyzed. These parameters did not show any difference when substrates formulations were compared, nor with the disinfestation methods. The results show that P and T have the potential to be used in mushroom cultivation and that the alkalization technique is as effective as autoclaving for the tested substrates. Alkalization technique is a good alternative for the production of edible mushrooms by the small rural producer, since it is less expensive and easier to handle.

**Keywords:** Bactris gasipaes Kunth, Theobroma cacao, Pleurotus pulmonarius, Biological Efficiency, Agro-industrial residues.

**Introduction**

Within the agricultural scenario, the cultivation of edible mushrooms becomes an interesting income option for rural producers, especially when agricultural waste can be used as substrates for the cultivation of the mushrooms. Edible mushrooms are foods which provide benefits to human health by having high protein content, essential amino acids, carbohydrates (mainly fibers) as well as low content of lipids (Bach et al., 2017; Okwulehie et al., 2014; Ragunathan & Swaminathan, 2003). They may also be a source of minerals such as calcium, potassium, iron and zinc (Mallikarjuna et al., 2013) and vitamins such as riboflavin, niacin and folates (Mattila et al., 2001). Also, they have bioactive substances that give them medicinal properties, making them functional foods (Roncero-Ramos & Delgado-Andrade, 2017). They were initially cultivated in China, around the sixth century (Chang & Miles, 2004). In 2015, mushroom production was recorded in more than 100 countries (Singh, 2015). The species most produced in the
world are *Lentinula edodes* and species of the genera *Pleurotus, Auricularia, Agaricus* e *Flammulina* (Royse et al., 2017). *Pleurotus* mushrooms are fast and easy to grow and have the ability to colonize many agricultural residues (E.S. Dias, 2010). In the production of *Pleurotus*, substrates such as coffee shells, cocoa shells, coconut fibers, sawdust from coconut husks supplemented with rice bran, cupuaçu exocarp, corn cobs and sugarcane bagasse have been tested (Bermúdez et al., 2001; Fonseca et al., 2015; Hoa & Wang, 2015; Marino et al., 2008). In order to decrease the initial microbial load of the substrate, different forms of disinfestation have also been studied, including autoclaving, considered a standard method on a large scale for mushroom production, and alternative methods, such as alkalinization (Contreras et al., 2004; León-Monzón et al., 2004; Mateus D Nunes et al., 2017). Due to the cost, energy consumption and necessary training, autoclaving substrates is a methodology that is difficult to access for small and medium rural producers. Considering the costs, alkalinization can be an alternative for disinfesting substrates. This technique consists in raising the pH of the substrate to alkaline levels, inhibiting the growth of contaminating fungi and bacteria without substantially affecting the development of *Pleurotus* sp. (Bernabé-González & Cayetano-Catarino, 2009; Contreras et al., 2004; León-Monzón et al., 2004; Mateus D Nunes et al., 2017).

Another way to reduce the cost of production is the use of local agroindustrial residues as substrate for mushroom cultivation. In the southern of Bahia, the production activities of peach palm (*Bactris gasipaes* Kunth.) for obtaining industrialized palm hearts, and cacao (*Theobroma cacao*) for obtaining cocoa beans, in 2019, was 5,200 tons and 41,637 tons, respectively (IBGE, 2019). In these productive chains, a large amount of waste is produced. Approximately 13 kg of waste is generated to obtain 400 g of palm heart (Fermino et al., 2010) and to obtain one ton of cocoa almond, 80-120 kg of cocoa seed shell is generated after processing (Silva et al., 2015). So, considering the availability of these agro-industrial residues, the objective of this study was to evaluate the use of them for the production of *Pleurotus pulmonarius* CCB19 and the effectiveness of alkalinization in calcium hydroxide solution as a disinfestation method.
Material and methods

Microorganism

*Pleurotus pulmonarius* CCB19 was obtained from the culture collection of the State University of Maringá, Paraná, Brazil, and maintained at 4°C in potato dextrose agar (PDA). Reactivation was performed in Petri dishes containing PDA medium and incubated at 25 ± 2 °C in the absence of light until the PDA medium was completely covered by the fungal mycelium (Oliveira et al., 2007).

Preparation of the inoculum or seed

The inoculum was prepared using wheat grains in accordance to (Bononi et al., 1999) with some modifications. The grains were previously cooked in boiling water for 15 min. After, the excess water was drained and calcium carbonate (CaCO$_3$) and plaster (1:4, w:w) were added in the proportion of 3% of the dry mass of the grain. Portions of 300 g were placed in polypropylene bags and sterilized for 1 h at 121 °C. After cooling the grains, plugs of the *P. pulmonarius* mycelium grown in PDA (of one third of the cultivated mycelium) were inoculated on the surface of the autoclaved wheat grains. The inoculated bags were kept at 25 °C, in the absence of light, until complete colonization of the grains by the mycelium of *P. pulmonarius* CCB19.

Residues, substrate preparation and mycelium inoculation

The pupunha leaf sheath residue discarded in the processing of palm heart was tested. This residue was subjected to drying in a forced air greenhouse at 50 °C for 2 days and then crushed in a knife mill, using a 12 mm sieve. The dried and crushed cocoa testa (cocoa shell) was also tested with the pupunha residues. For the preparation of the substrates, residues were mixed in three different proportions: 100:0; 90:10 and
80:20 of pupunha leaf sheath and cocoa testa, respectively. After, they were subjected to two different
types of disinfection, heat treatment (autoclaving) and immersion in alkaline solution (CaOH₂),
composing 6 different types of tested substrates (S1 to S6), as shown in Table 1.

The autoclaving and alkalinization treatments were developed based on the studies of (Mateus D
Nunes et al., 2017). The water holding capacity was adjusted between 66 to 67% and 500 g of substrates
(S1-S3) were distributed in polypropylene bags and autoclaved at 121 °C for 2 h. For the alkalinization
treatment, the residues were subjected to immersion in a 2% CaOH₂ for 18 h and centrifuged at 363 g for
6 min to remove the liquid excess. After that, 500 g of each substrate (S4-S6) were packed in plastic bags.

For pH analysis, 5 g of sample were diluted in 50 mL of distilled water and stirred for 30 min. For
moisture content analysis, 5 g of the previously treated substrate sample was taken to the greenhouse (50
°C) until a constant mass was obtained. The residues were also submitted to analysis of carbon and
nitrogen content in accordance with the Brazilian Ministry of Agriculture (MAPA, 2006). For the analysis of the
carbon content, 5 g of the substrate previously dried in a greenhouse was submitted to a muffle
incineration at 550 °C for 1 h to determine the organic matter content and then, the carbon content was
calculated. The nitrogen analysis was performed by the Kjeldahl method by distillation (Kjeldahl, 1883).

Cultivation of P. pulmonarius CCB19

After autoclaving or CaOH₂ solution immersion, the substrates were inoculated with 10% of the inoculum
(seed) and then incubated at 25 °C, moisture of 60 - 70% (monitored by a hygrometer) and absence of
light until the fungal mycelium occupies all the substrate (Oliveira et al., 2007). The time (days) required
for colonization of the substrate by the fungus (mycelial run) was monitored. After complete colonization,
the mycelium was subjected to thermal shock at 4° C for 24 h. For fruiting, the colonized substrates were
transferred to a room with a temperature of 20 °C, moisture of 80 - 90% and the presence of light until the
initial formation of the mushrooms (Mateus Dias Nunes et al., 2012). Then, the bags were opened to
expose the substrate surface to air until the basidiocarps harvesting. After the first harvest (1st flow), the substrates were retransferred to the incubation room (25 °C, moisture of 60 - 70%, absence of light), to allow new mycelial growth aiming at the second harvest (2nd flow) of basidiocarp production.

**Determination of Biological Efficiency (BE), productivity rate (PR) and morphological analysis (cap size)**

From each cultivation bag, the mass of the harvested mushrooms was determined and with the dry mass of the substrate, the biological efficiency (BE) was determined using the formula:

\[
BE (\%) = \frac{\text{fresh mushroom mass}}{\text{initial dry mass of the substrate}} \times 100.
\]

In addition, the productivity rate (PR) was calculated by dividing the BE by the time in days required for harvesting (Oliveira et al., 2007). After harvesting, the size of each mushroom's cap was measured with a millimeter ruler (Liasu et al., 2015).

**Statistics**

The experimental design was completely randomized in a 1 x 3 x 2 x 4 factorial scheme, corresponding to 1 fungal strain, 3 substrate formulations, 2 substrate disinfestation modes and 4 repetitions for each treatment, totaling 24 portions represented by each culture bag. The data were submitted to analysis of variance and compared by Tukey's test at 5% probability.

**RESULTADOS**

*Physical-chemical evaluation of the cultivation substrate and cultivation time of P. pulmonarius CCB19*

The values of the carbon and nitrogen ratio (C / N), moisture, pH, mycelial colonization time (TMC), and mushroom harvest time (MH) of the two fruiting cycles are presented in table 2. The pH values of the
initial substrates were 4.6 in the autoclaved substrates (S1, S2 and S3) and between 10 and 11 in the alkalized substrates (S4, S5 and S6). Moisture values ranged from 66 to 67% for autoclaved substrates, and from 71 to 73% for alkalized substrates. The carbon-nitrogen ratio ranged from 33.6 to 37.7 for autoclaved substrates and 41.7 to 44.1 for alkalized substrates. The time (days) of colonization and harvest were recorded during the first and second flows. In the first flow, the colonization time of the alkalized substrates (26 days) was significantly shorter (p<0.05) than the colonization time of the autoclaved substrates (37.3 to 41.3 days). In this same first flow, the harvest time of alkalized substrates was also significantly shorter (30.5 to 31.3 days) than for autoclaved substrates (43.3 to 46.8 days). In the second flow, the colonization time was significantly longer for S1 when compared to the alkalized substrates, and the harvest time was significantly longer also for S1, but only when compared to two of the alkalized substrates (S4 and S6). In general, only the first flow showed a significant difference in colonization time and harvest time between alkalized and autoclaved substrates. Considering the same treatment of disinfestation, autoclaving (S1, S2, S3) or alkalinization (S4, S5, S6), in these two flows, different proportions of pupunha leaf sheath e cocoa testa substrates did not show significant difference for the mycelial colonization time and harvest time.

Biological efficiency (BE), productivity rate (PR) and morphological analysis (cap size)

Table 3 shows the biological efficiency results for each flow and total. In the first flow, the BE values between the different substrates (autoclaved or alkalized, with or without cocoa testa) did not show significant difference, except for the value of S1 which was higher than S4 and S5. In the second flow, in general, a difference in BE was observed between autoclaved and alkalized treatments (p < 0.05), and the alkalized substrates presenting a higher EB value. The results of total BE (first flow + second flow) varied from 49 to 71.2%, and PR was between 0.7 and 1.2. BE and PR showed no significant difference between treatments.
The results of the cap size of the mushrooms collected during the two flows are presented in table 4. In flow 1, the cap size varied from 2.0 to 3.2 cm and only the mushrooms collected in substrate S1 had a significantly smaller cap size when compared to the other mushrooms. In flow 2, the cap size varied from 2.6 to 3.6 cm and S1 continued to have the smallest cap, but it differed significantly only from S4 and S6.

**Discussion**

*Physical-chemical evaluation of the cultivation substrate and cultivation time of P. pulmonarius CCB19*

Values of pH, moisture and the carbon and nitrogen ratio (C/N) are variables that impact on the time of substrate colonization by the fungal mycelium and, consequently, the basidiocarp harvest time (Bellettini et al., 2016). The difference between the pH values between autoclaved and sterilized substrates was due to the solution composed of CaOH₂ used only in the alkalinization methodology. According to (Chang & Miles, 2004) the pH range for mycelial growth of *Pleurotus* spp. is about 5,5 to 6,5. However, some *Pleurotus* spp. have good growth over a wider pH range, from 5,5 to 7,5 (Yadav, 2001). Some studies have shown that the pH of the substrate lower than is recommended can reduce the mycelial growth rate (Odero, 2009; Sardar et al., 2015). In this work, the initial pH of the autoclaved substrates (S1, S2, S3) was 4,6, lower than the range recommended in the literature. This may have negatively influenced the mycelial growth rate of *P. pulmonarius*, increasing the mycelial colonization time, which was from 37,3 to 41,3 days, and consequently, a longest harvest time in the first flow (43,3 to 46,8 days). In alkaline substrates (S4, S5, S6), where the pH was higher (10 to 11), mycelial colonization time (26 days) and harvest time (30,5 to 31,3 days) were shorter. In general, in the second flow there was no difference between autoclaved and alkalized substrates for the times of mycelial colonization and harvest time. Similar results were found by (Mateus D Nunes et al., 2017), when testing the feasibility of alternative methods for producing *Pleurotus ostreatus* in coffee husks. These authors report that the method of immersion in alkaline solution for 4 h produced mushroom was faster (31,5 days) than the
autoclaved treatments (46.6 days) in the first flow. However, in the second flow, no mushrooms were harvested from the autoclaved substrates (Mateus D Nunes et al., 2017). Another factor to consider when growing mushrooms is the substrate moisture content (Eustáquio Souza Dias et al., 2003; Rajarathnam & Bano, 1988). On the other hand, the lower concentration of water reduces mycelial growth, since water is essential for the assimilation and transport of nutrients (Staments & Chilton, 1983). The recommended substrate moisture content for basidiomycete growth is between 50% and 75% (Chang & Miles, 2004). However, Oliveira et al. (2007), managed to grow *P. pulmonarius* on a substrate formed by a mixture of wheat bran, corn straw, peanut husks with 80% of humidity. In this work, the alkalized substrate had a higher moisture content (from 71 to 73%) than the autoclaved substrates (66 to 67%). This difference was probably due to the different wetting processes between the two disinfection treatments (autoclaving and alkalinization). The autoclaved substrates were moistened with the controlled addition of water. In alkalinization, the substrate was immersed in an aqueous solution for 18 h, a difference that probably did not negatively affect the development of the mycelium.

Different C/N ratios have been used for cultivation of *Pleurotus*, with values from 32.6 to 47.99, providing good production of mushrooms (Bernardi et al., 2013; Cueva et al., 2017; Kurt & Buyukalaca, 2010). In this work, the C/N ratio was 33.6 to 37.7 for the autoclaved substrates (S1, S2 and S3) and 41.7 to 44.1 for the alkaline substrates (S4, S5 and S6), following what has already been described in the literature (Bernardi et al., 2013; Cueva et al., 2017; Kurt & Buyukalaca, 2010).

**Biological efficiency (BE), productivity rate (PR) and morphological analysis (cap size)**

From the point of view of the producer, the biological efficiency, productivity and morphology of mushrooms show how much a given technique is viable. In this study, the alkalinization technique had results that did not significantly differ from the sterilization technique, showing that the alkalinization of the substrates tested is as viable as autoclaving in the cultivation of *P. pulmonarius*. In addition, alkalinization has the advantages of greater electricity savings and easier handling.
So far, few scientific works have studied the potential of peach palm residue as a substrate for the
cultivation of edible mushrooms. In addition, there is no work in the literature so far that reports on this
residue being treated with alkalinization. Sales-Campos et al. (2010) used sterilized substrate prepared
with peach palm stem for the cultivation of *P. ostreatus*, and obtained BE values ranging from 123.13 to
128.66%.

Regarding the cultivation of *P. pulmonarius* in several agro-industrial residues, the literature shows
different values of BE. The evaluation the production of seeds with different substrates and using the
same lineage of *P. pulmonarius* CCB19 of this study, BE ranging from 25 to 43% as was reported
( Oliveira et al., 2007). When another lineage of *P. pulmonarius* was grown in different types of wood
(Milicia excelsa, Gmelina arborea, Afzelia africana and Khaya senegalensis), BE ranging from 25.56 (A.
africana) to 36.13% (G. arborea) was observed (Adewoyin & Ayandele, 2018) and when grown in
brachiaria straw treated with different volumes of CaCO₃ solution, BE ranged from 69.87 to 135.50%
(Iossi et al., 2018). Another experiment using dried banana leaves or dried leaves of Chrysalidocarpus
lutescens, a palm tree, treated with immersion in alkaline solution or in hot water at 80 °C for 1 h. The
highest BE was 120.1% for mushrooms grown on banana leaves immersed in alkaline solution, followed
by BE of 81.24% for the same substrate treated in hot water. On the other hand, the BE of dry palm leaves
were 41.4 and 44.9% in the treatments with alkaline solution and hot water, respectively (Bernabé-
González & Cayetano-Catarino, 2009). When using corn stalk supplemented with different concentrations
of wheat bran or corn flour, an BE of 113% and 132% was found, respectively (Mkhize et al., 2016).
These authors indicated that the addition of supplements had a positive influence on the production of *P.
pulmonarius*, thus showing the importance of supplementation for some residues. In this study, the cocoa
seed testa was used as a potential supplement for the pupunha residue. The different concentrations of the
studied substrates (0, 10 and 20% of cocoa seed testa) did not result in a difference (p> 0.05) regarding
BE and PR with the tested mushroom (Table 3).
Therefore, it turns out that the pupunha residue with or without cocoa seed testa is a substrate with potential to produce *P. pulmonarius* CCB19 mushrooms. This is also reinforced by PR between disinfestation treatments, since there was no statistical difference between them (Table 3).

Regarding the morphological analysis, mushrooms grown on different substrates, in general, did not show difference in the size of the cap between the different disinfestation methods (Table 4). Cap size varied from 2.0 to 3.2 cm in the first flow, and from 2.6 to 3.6 cm in the second flow. It could be an advantage for the producer, in terms of cap size, in continuing the production of the mushroom in the second flow. Oliveira et al. (2007), working with the same isolate from this study (*P. pulmonarius* CCB19), obtained mushrooms with a cap size between 5 to 10 cm, when the inoculum was prepared with corncob residue. Cap sizes similar to that found in this work (less than 5 cm) were obtained when the inoculum was prepared with rice husk. Other studies cultivating *P. pulmonarius* on different substrates, found cap sizes ranging from 3.20 to 5.50 cm (Mkhize et al., 2016) and from 2.9 to 13.5 cm (Liasu et al., 2015). The size of the cap of the mushrooms is affected by environmental conditions, such as aeration, characteristics of each species and by a specific response of each species to environmental conditions. In addition, substrate conditions, such as type, the presence or absence of supplementation, and the technique for treating the substrate can influence the size of the cap (Chang & Miles, 2004; Ng’etich et al., 2013; Yang et al., 2013). In this work, the use of different disinfestation methods (autoclaving and alkanization), different concentrations of pupunha and cocoa testa residues did not demonstrate an effect on the cap diameter size of the *P. pulmonarius* CCB19.

**Conclusions**

Pupunha residues and pupunha residues plus cocoa are equally efficient to produce *P. pulmonarius* CCB19 mushrooms. The use of the calcium hydroxide solution as a chemical agent for disinfesting the substrate has biological efficiency similar to the cultivation in autoclaved substrates. Considering the
production of mushrooms by small farmers, the method of treating the substrate by alkalisation is a better alternative, considering the BE, the PR of the mushrooms, as well as the ease of access to the technique, low cost and easy handling.

Acknowledgements

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Table 1 - Proportions of residues of pupunha leaf sheath and cocoa testa, and disinfestation treatments (autoclaving and alkalization) of the substrate for P. pulmonarius CCB19 cultivation
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Substrate Formulation (%)</th>
<th>Disinfestation treatments</th>
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<tr>
<td></td>
<td>Pupunha</td>
<td>Cocoa</td>
</tr>
<tr>
<td>S1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>S2</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>S3</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>S4</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>S5</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>S6</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 2 – Physical-chemical evaluation (pH, moisture and C/N), Time of mycelial colonization (TMC) and mushroom harvest (MH), in two production flows of *P. pulmonarius* CCB19 in pupunha leaf sheath and cocoa testa residues in different proportions and submitted to autoclaving or alkaline treatment.
S1: 100% pupunha residue (autoclaved); S2: 90% pupunha residue + 10% cocoa testa (autoclaved); S3: 80% pupunha residue + 20% cocoa testa (autoclaved); S4: 100% pupunha residue (alkalinized); S5: 90% pupunha residue + 10% cocoa testa (alkalinized); S6: 80% pupunha residue + 20% cocoa testa (alkalinized). Means followed by different letters in the same column show a significant difference (p<0,05) according to the Tukey test.

Table 3 – Results of biological efficiency (BE) of the two flows, total BE and productivity rate (PR) in the different substrates for the cultivation of *P. pulmonarius* CCB19 in residues with different proportions of pupunha leaf sheath and cocoa testa residues submitted to autoclaving and alkaline treatment

<table>
<thead>
<tr>
<th>Substrates</th>
<th>BE (%)</th>
<th>BE Total (%)</th>
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<tr>
<td></td>
<td>Flow 1</td>
<td>Flow 2</td>
<td>(Flows 1 +2)</td>
</tr>
<tr>
<td>S1</td>
<td>55,4 a</td>
<td>11,1 b</td>
<td>66,5 a</td>
</tr>
<tr>
<td>S2</td>
<td>42,0 ab</td>
<td>13,4 b</td>
<td>55,3 a</td>
</tr>
<tr>
<td>S3</td>
<td>32,6 ab</td>
<td>16,4 ab</td>
<td>49,0 a</td>
</tr>
<tr>
<td>S4</td>
<td>26,9 b</td>
<td>37,0 a</td>
<td>63,9 a</td>
</tr>
<tr>
<td>S5</td>
<td>31,5 b</td>
<td>22,6 ab</td>
<td>54,1 a</td>
</tr>
<tr>
<td>Substrates</td>
<td>Cap Diameter (cm)</td>
<td>Flow 1</td>
<td>Flow 2</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>S1</td>
<td></td>
<td>2,0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2,6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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<td>S2</td>
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<td>3,1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,0&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td></td>
<td>3,2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,8&lt;sup&gt;ab&lt;/sup&gt;</td>
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BE: biological efficiency; PR: productivity rate; S1: 100% pupunha residue (autoclaved); S2: 90% pupunha residue + 10% cocoa testa (autoclaved); S3: 80% pupunha residue + 20% cocoa testa (autoclaved); S4: 100% pupunha residue (alkalinized); S5: 90% pupunha residue + 10% cocoa testa (alkalinized); S6: 80% pupunha residue + 20% cocoa testa (alkalinized). The different
<table>
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<tr>
<th>Substrate</th>
<th>Parameter 1</th>
<th>Parameter 2</th>
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<tr>
<td>S4</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>S5</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>S6</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

S1: mushrooms grown in 100% pupunha (autoclaved); S2: mushrooms grown in 90% of pupunha residue + 10% cocoa testa (autoclaved); S3: mushrooms grown in 80% of pupunha + 20% cocoa testa (autoclaved); S4: mushrooms grown in 100% pupunha residue (alkalinized); S5: mushrooms grown in 90% of pupunha residue + 10% cocoa testa (alkalinized); S6: mushrooms grown in 80% of pupunha residue + 20% cocoa testa (alkalinized). The different letters in the same column show a significant difference (P<0.05) according to the Tukey test for an average of four repetitions per substrate.