ภาคผนวก

Influences of Calcium and Silicon Supplementation into *Pleurotus ostreatus* Substrates on Qualities of Fresh and Canned Mushrooms

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Abstract
Gypsum (calcium source), pumice (silicon source), and pumice sulfate (silicon and calcium source) were supplemented into substrates for oyster mushrooms (*Pleurotus ostreatus*). The addition of pumice up to 30% had no effect on total yield, size distribution and caps diameter. The supplementation of gypsum at 10% decreased total yield and although gypsum at 5% did not affect total yield, the treatment increased the proportion of large-sized caps. Too much content (> 10%) of pumice sulfate resulted in the lower yield. Calcium and silicon contents in the fruit bodies were not influenced by supplementations. The centrifugal drip loss values and solid content of fresh mushrooms and percentage of weight gained and firmness of canned mushrooms cultivated in substrates supplemented with gypsum, pumice sulfate and pumice were significantly ($p<0.05$) higher than those of the control. Scanning electron micrograph revealed the more compacted hyphae of mushroom stalks supplemented with silicon and/or calcium after heat treatment comparing to the control.

Key words: *Pleurotus ostreatus,* silicon, calcium, texture

Introduction

Processed vegetables that maintain firm and crunchy textures are highly desirable. The appearance of a soft or limp product may cause a lack of acceptance even prior to consumption (Ni et al. 2005). The operations utilized in the production of canned or frozen vegetables frequently result in a significant loss of textural integrity. The demand for 'fresh like' processed plant based foods has prompted progressive research in ways of improving the texture of thermally processed products. Various approaches have been employed including low temperature blanching (<70 °C) prior to sterilization or freezing. Preheating at mild temperatures (50-70 °C) typically for ≥30 min prior to high temperature processing (≥100 °C) has been used to improve the texture of thermally processed plant based foods (Sila *et al.* 2008). This process results in better texture preservation. However, the limitations of this method are the availability of endogenous divalent ions and pectin methylesterase (PME) (Ng and Waldron, 1997), hence a combination of preheating with calcium impregnation (calcium soaking and vacuum or pressure infusion) is preferable.

Recently, solutions containing PME and calcium have been vacuum-infiltrated. (Degraeve, et al. 2003; Duvetter et al., 2005; Guillemin et al, 2006; Guillemin et al 2008, Javeri et al. 1991). This resulted in the preservation of firmness in processed fruits and vegetables especially in apples (Guillemin et al, 2006) and strawberries (Degraeve, et al. 2003; Duvetter et al., 2005), which contain low amounts of PME. However, enzyme infusion to alter textural features is not successful in all applications. A limitation to successful enzyme infusion is contact with the substrate. Because the substrates for many reactions are within the cell, their enzymatic modification would require penetration of the cell by the enzyme. Moreover, an impermeable skin or lack of interior voids may minimize the effects of infusion as an example in the infusion of PME and calcium into blueberries (Baker and Wicker 1996). Therefore, there is still a need for alternative methods to help improve plant-based product textural qualities.

Silicon is the second most abundant element on the surface of the earth (Epstein, 1994). Although silicon has not been classified as an essential element, it has been shown to be beneficial for plant growth (Epstein, 1999; Liang et al., 1994). In Thailand silicon supplementation for the cultivation of vegetables such as lettuce, cabbage, Chinese cabbage, Chinese broccoli, green/string bean, celery as well as mushrooms increased resistance to diseases and pests and reduced pesticide and fungicide usage. It also has also been reported to help improve crispy texture, prolong self-life during storage and distribution (Chaiwongkeit 1998). These beneficial effects could contribute from the modifications of the plants cell wall architecture (Chérif et al. 1992; Hossain et al. 2007).

Calcium is known to stimulate fruiting in *A. bisporus*. Recently, irrigation water supplemented with calcium both in the form of calcium sulfate (gypsum) and calcium chloride has been shown to improve postharvest storage by reducing the surface bacterial population (Simons and Beelman, 1996). Calcium chloride addition improved yield and increased the calcium contents of fruit bodies (Chiu et al. 1998).

These studies suggested that it is possible to apply some minerals during cultivation. If those minerals, such as calcium and silicon, are important to textural integrity, supplying those minerals during cultivation may help improving the texture of processed products. A crucial benefit of this method is that minerals supplied are more

evenly distributed in plant tissue unlike other techniques such as soaking or vacuum infusion.

To our knowledge, there is currently no information available about the possible beneficial effects of silicon and calcium supplementation during cultivation on the textural integrity of vegetables that will later undergo thermal processing. In this study we investigated the effects of silicon and/or calcium supplemented into substrates for *Pleurotus ostreatus* production on productivity, accumulation of silicon and calcium in mycelium and fruiting bodies as well as quality attributes of fresh and processed mushrooms. Although mushrooms are actually fungi, they are also classified as edible fresh produce and possess cell walls similar to plant cells. Mushrooms are commercial produced with a short harvesting time. Silicon and silicon-calcium fertilizer are likely to help improve the qualities of mushrooms and increase shelf-life similar to adding calcium into irrigated water previously reported. Results from this study will provide an alternative method for providing a better quality of fresh oyster mushroom as well as improving qualities of mushrooms that later undergoing thermal processing. This technique may be applicable for other types of mushrooms and other processed vegetables.

Materials and Methods

Source of silicon, calcium and silicon-calcium

Pumice, volcanic rock high in silica, was used as a silicon source. It is powdered and light-colored. Gypsum ($CaSO_4$) was used as a calcium source and Pumice sulfate was used as a silicon-calcium source. They were agricultural grade and produced in Thailand.

Pumice contained 68.7% silicon, 13.6% Aluminum oxide (Al_2O_3), 2.18% Sodium oxide (Na_2O), 0.46% Magnesium oxide (MgO), 4.69% Potassium oxide (K_2O) and 1.02% Calcium oxide (CaO).

Gypsum ($CaSO_4$) for agricultural use commercially produced in Thailand (GreenCal 97) was 97% purity and contained 23.3% calcium and 17.8% sulfur (SO_4).

Pumice sulfate contained pumice and calcium sulfate as two major compositions. It composed of 50.0% pumice, 7.0% sulfate, 12.0% calcium, 0.20% phosphoric acid (H_3PO_4), 0.01% magnesium, 0.01% iron and 0.0005% zinc.

Other chemicals used in this study were analytical grade.

Substrates and preparation of *Pleurotus ostreatus* production

Pleurotus ostreatus (Gray oyster mushrooms originally from Bhutan) strains were available from the Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. Mycelia were first grown in the solid medium (Potato dextrose agar). After inoculation, the cultures were incubated in Petri dishes at 28 °C for three weeks. A small piece (1 cm^2) of mycelium from the solid medium was then transferred to several bottles of sterilized cooked sorghum grains and incubated at 28 °C for three weeks before growing on substrate mix.

The substrate standard formulation for *P. ostreatus* production (60% moisture) included 60.0% of sawdust from rubber trees, 6.0% of rice bran, 0.12% $MgSO_4$ (industrial grade), 0.6% of $CaCO_3$ (industrial grade). All basic ingredients were mixed with various levels of gypsum ($CaSO_4$): 5 and 10%, pumice (source of silicon): 10, 20, 30% and pumice sulfate (source of silicon and calcium): 10, 20 and 30%. Amounts of

these three supplement materials were calculated as a percentage of sawdust weight. Substrate formulations were summarized in Table 1.

Following mixing the substrate ingredients, substrates were filled into 25x35cm sized plastic bags on a 0.9 kg wet weight basis and then the bags were closed, tied up and sterilized at 80 °C for 8 h and spawned with mushroom mycelia grown on sorghum grains, on the dry weight basis of substrate. The bags were subsequently placed into a spawn running room at room temperature. After completion of spawn running, the bags were placed into a production room set environmentally at 25-30 °C and 80–90% RH. Bags were unfolded at the upper parts. Water was sprayed to maintain moisture up to the desired level in the form of fine mist with the help of a nozzle.

At least 25 bags were used for each growing trial. Biological efficiency (BE) was calculated using the following equation:

$$BE\ \% = \frac{\text{Weight of fresh mushroom harvested}}{\text{Weight of dry substrate used}} \times 100$$

Determination of silicon content in the fruit bodies: Mushrooms from each treatment (at least 1 kg) from each harvesting period were separated into caps and stalks, dried in an oven at 60 °C for 12 h, ground by a blender and sieved though a 20 mesh sieve. Ground dried mushroom caps and stalks were wet-digested by 10 mL of HNO_3-H_2O_2-distilled water (4:2:4). The amount of silicon was determined by colorimetric method according to Samadi-Maybodi and Atashbozorg (2006) using a UV-vis spectrophotometer (HACH DR/4000, 48000, Loveland, Colorado) at 815 nm at ambient temperature. Standard silicon was prepared from silicon atomic absorption standard solution (100 ppm of silicon in 1.9% NaOH, Sigma). At least three replicates were analyzed for each test.

Determination of calcium content in the fruit bodies: After the ground dried mushroom caps and stalks were wet digested by HNO_3 and $HClO_4$, calcium content was determined with an atomic absorption spectrophotometry (GBC model Avanta PM, Australia). At least three replicates were analyzed for each test.

Texture measurement: Fresh and processed mushroom caps with diameter of 7 cm and mushroom stalks with diameter of 1 cm were used to determine texture. Mushroom caps were cut into 1 cm x 2 cm pieces. Each piece was cut from the same location on the caps. Mushroom stalks were cut to 2 cm long. Texture (expressed as shear values) of mushroom caps and stalks were measured using a Universal Testing Machine (4411, Instron Laboratory Inc., USA) equipped with a Warner-Bratzler shear cell. The maximal force (top value) needed to break through the mushroom caps and stalks was used to quantify the instrumental firmness of the mushrooms. At least 10 replicates were used for each treatment over three flushes and the results averaged.

Solids content: Fresh mushrooms with a cap diameter of 7cm and a stalk diameter of 1 cm were randomly sampled for solids content determination from each treatment. Mushroom caps and stalks were cut into small pieces. Samples (100g) and were placed in moisture cans and oven dried at 105°C for >12h (ShelLab, 1375FX, Cornelis, Oregon). Dried mushrooms were placed in a desiccator on a laboratory bench for 2 h to cool before weighing. Ten replicates per treatment were used and the solids contents were recorded as a percentage of dry mushroom weight (Mamiro and Royse 2008).

Centrifugal drip loss of fresh mushroom: To characterize the amount of free water in fresh mushroom stalks and caps, drip loss was assessed by measuring the water loss after centrifugation according to (Redmond and Gormley, 2004). Drip loss was assessed by measuring the water loss after centrifugation. Fresh mushrooms with a cap diameter of 7 cm and a stalk diameter of 1 cm were randomly sampled from each treatment. Approximately 3 g of diced mushroom caps and stalks were accurately weighed into a paper filter thimble and placed in a centrifuge tube containing a layer of glass beads 20 mm deep. Samples were centrifuged at 223×*g* for 10 min at 10 °C using a centrifuge (Sorvall, Biofuge Stratus, Germany). After centrifugation the samples were removed and re-weighed. Percentage drip loss was expressed as follows:

$$\text{Drip loss (\%)} = \frac{(wi - wf)}{wi} \times 100$$

where *wi* is the initial weight, and *wf* is the final weight of the sample. Measurements were made in triplicate and the results averaged.

Effect of silicon and calcium in a liquid medium on mycelial growth

WPDY (wheat grain–potato–dextrose–yeast extract) was used as the liquid growth medium (Serafin Mun˜ oz et al. 2006). Liquid growth medium WPDY was obtained as follows: 400g of wheat grain and 300g of potato slices were suspended in 600mL of deionized water and boiled (100°C, 1h), and the mixture was filtered through Whatman no. 1 paper. The filtrate was recovered and supplemented with 20g of dextrose, 2g of yeast extract, 0.5g of $MgSO_4$, 0.5g of KH_2PO_4, and 1.0g of NaCl. Finally, the volume was brought to 1,000mL with deionized water (pH 5.0 ± 0.5). Sterilization was carried out in autoclave (121°C, 15min). The solutions of sodium metasilicate ($Na_2Si_3 \bullet 5H_2O$, Ajax Finechem) and calcium sulfate ($CaSO_4 \bullet 2H_2O$, Ajax Finechem) salts were sterilized by filtration (0.45mm) and added directly to the growth medium to obtain the following final concentrations: 5, 10mg/l silicon and 5, 10 mg/l calcium and combination of 5mg/l silicon + 5mg/l calcium.

P. ostreatus strains were available from the Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. Mycelia were first grown in the Potato dextrose agar (PDA) solid growth medium. After inoculation, the cultures were incubated in Petri dishes at 28 °C for three weeks. A small piece ($1cm^2$) of mycelium from the solid medium was then transferred to the liquid medium (several 50 ml flasks) containing silicon, calcium and combination of silicon and calcium. The cultures were incubated at 25 °C for three weeks. Control mycelia with no metal were also obtained.

The mycelia were filtered through Whatman no. 1 paper, washed with de-ionized water and dried. The homogenization of cells was performed by mortar grinding, yielding a fine powder. Ground dried mycelia were wet-digested by 10 mL of HNO_3-H_2O_2-distilled water solution (4:2:4). The amount of silicon and calcium was determined by Inductively coupled plasma atomic emission spectroscopy (Perkin Elmer model PLASMA-1000).

Canning process

Fresh mushrooms were randomly sampled from each treatment from Crop I and II. Mushrooms from each treatment were graded according to their cap diameters. Only mushrooms with cap diameter of 7.30 ± 2.26cm were chosen. Mushroom stalks were cut to obtain 3 cm long stalks. The mushrooms were blanched in boiling water for 30 sec and filled into 12 ounces jars. Portions (approximately 200g) of mushrooms were packed into

glass jars and a 2% NaCl and 0.05% citric acid solution (brine) was added. Closed jars were sterilized at 121°C for 15 min. After sterilization, the samples were stored at 25°C until firmness evaluation, drain weight and grade determination.

Determination of weight and grade of canned mushrooms

Weight was determined, according to current guidelines, by weighing the product after draining the can content for two min in a perforated stainless steel tray. The determination of grade was done by measuring the diameter of all the caps in each can with a vernier caliper.

Both parameters were determined in fresh and canned mushrooms. The weight gained and grade losses during the process of sterilization were calculated and expressed as a percentage of weight gained or loss grade.

Weight gained (%) = $\frac{(w_d - w_i)}{w_i} \times 100$

where w_i is the initial weight of fresh mushrooms before canning, and w_d is the weight after draining of canned mushrooms. Measurements were made in triplicate and the results averaged.

Size loss (%) = $\frac{(cd_i - cd_d)}{cd_i} \times 100$

where cd_i is the average initial cap diameter of fresh mushrooms before canning, and cd_d is the average cap diameter after the draining of canned mushrooms. Measurements were made in triplicate and the results were averaged.

The data obtained from the experiments was evaluated by SPSS statistics 17.0 program and include ANOVA (analysis of variance) and Duncan's new multiple range test applied at 95% confidence level.

Preparation and observation of scanning electron microscope (SEM) specimens

Structural analysis was performed by Scanning Electron Microscope (Leo1455VP). The preparation of SEM specimens was based on Ko et al. (2007) and Zivanovic et al. (2000) with modification. Samples of fresh and canned oyster mushroom (with stalks and caps in cross-sections) were fixed in a modified Karnovsky's fixative, consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.05M Cacodylate buffer at pH 7.0 for 24 h at room temperature. Samples were then removed from the fixative and rinsed three times with the same buffer each time for 5 min. Samples were then dehydrated in a series of ethanol at 30%, 50%, 70%, 90% with 5 min in each concentration, followed by 2 dehydrations in absolute ethanol, 5 min each. Samples were then placed in metal basket for critical point drying in a critical point dryer. Dehydrated samples were then placed on aluminum stubs and sputter-coated with gold. Prepared samples were then observed and photographed with the SEM.

Results and Discussions

I. Supplement compositions

Minerals such as potassium, calcium, iron, copper, manganese, zinc and, often molybdenum, are required by fungi for growth (Özçelik and Pekşen, 2007; Royse and Sanchez-Vazquez, 2003; Yildiz et al. 1998). Beside major compositions, calcium and silicon, pumice contained trace amount of other minerals required for fungi growth (0.46% magnesium, 4.69% potassium). Pumice sulfate also contained 7.0% sulfur and trace amounts of magnesium (0.01%), iron (0.01%) and zinc (0.0005%). Gypsum

($CaSO_4$) for agricultural use commercially produced in Thailand contained approximately 17.0% sulfur.

Mg, K, Fe and Zn in pumice and pumice sulfate would not have any effect on oyster mushroom production since they are in trace amounts. There is no report on the relationship between K content and yield of *Pleurotus* spp. In the case of shiitake mushrooms, K content in substrates showed no relationship with the yield or BE (Özçelik and Pekşen, 2007). Potassium was the most abundant mineral element in the cultivated *P. ostreatus* var. salignus. The preponderance of potassium in the sporophore tissue may be due to absorption accumulation of this element from the substrate. However, there was no report on the effect of K on yield improvement.

Although sulfur was in significant amounts in pumice sulfate and gypsum, studies found that sulfur showed no effect on oyster mushrooms cultivation. Yildiz et al. (1998) found that sulfur was not detected in the sporophores of *P. ostreatus* var. salignus, which is only found in structures of poisonous mushrooms.

Therefore, it was implied that any effect of pumice, pumice sulfate and gypsum was solely due to the influences of silicon and/or calcium.

II. Spawn run time, time periods of fruit body formation, yield and BE

Supplementation of gypsum, pumice and pumice sulfate in *P. ostreatus* production substrate showed no effect on time periods of spawn running (mycelia development) probably due to the C:N ratio of substrates in each formulation was not different (Table 2). Spawn run time varied depending on mushroom genotype and the C:N ratio of substrates. Plant materials with low C:N ratios decayed more rapidly than those with high C:N ratios, indicating that mycelium extension rate is related to the bioavailability of nitrogen (Özçelik and Pekşen, 2007). Time periods of fruit body formation decreased slightly, about 4-5 days as a result of supplementation. For each treatment, *P. ostreatus* took about 3 weeks for fruit body formation after spawn running (Table 2). Our results were in agreement with findings of Tan (1981) which reported that *P. ostreatus* and other species on cotton waste took 2–3 weeks for fruit body formation after spawn running. The faster fruit body formation for substrates treated with gypsum, pumice sulfate and pumice indicated more concentrated mycelium, available from increased spawn levels, which would provide faster substrate colonization (Royse et al. 2004).

For each treatment, mushroom yield in the first flush was the highest and yields decreased in the second and the third flush. The reduction in yield in the second flush of *P. ostreatus* was reported by Wang et al. (2001), of *P. sajor-caju* as reported by Zhang et al. (2002) and of oyster mushrooms reported by Murugesan et al. (1995). Total yield decreased significantly ($p<0.05$) as gypsum and pumice sulfate content added to the substrate increased to 10% and 20%, respectively. Unlike gypsum and pumice sulfate, addition of pumice to the substrate at 20% and 30% gave a total mushroom yield similar to the control.

BEs calculated from the three flushes followed the same trend as total yield. Substrate supplemented with 20% pumice exhibited the highest BE and its BE was slightly higher than other treatments (BEs of 55-56%) (Table 2). Negative yield effects caused by gypsum were reported in *A. bisporus*. Mau *et al.* (1993) reported that the addition of gypsum (calcium sulfate) to irrigation water decreased yield of *A. bisporus* as concentration increased. The yield decreases were significant and were as high as 20% at

a gypsum concentration of 0.3%. Negative yield effects caused by pumice sulfate could contribute from having calcium sulfate or gypsum as a major composition as well.

Gypsum, pumice sulfate and pumice added to substrates did not inhibit oyster mushrooms cultivations though yield decreased in some conditions.

III. Distribution pattern of individual mushrooms weight and size

Individual mushroom caps (basidiomata) weighed between 0.64g and 62.0g, and had a skewed distribution pattern of individual mushroom weights and frequencies (Figure 1). A sewed distribution pattern of individual mushroom weights was also reported for *P. eryngii* (Rodriguez Estrada and Royse, 2006) although weights were in a different range. In order to obtain ranges for mushroom size, the 33.3% and 66.6% quantiles were used. Mushroom size ranges, as determined by the specified quantiles, were as follows: small, <6.70 g; medium, 6.70-12.38 g; and large, >12.38 g. Distribution of mushrooms within size classes (small, medium and large) were influenced by supplements added to the substrates. More than 40% of the small-sized mushrooms were produced by substrates supplemented with pumice sulfate at 20% and pumice at 10% corresponded to their decrease in total yield. Substrates supplemented with gypsum at 5% produced more than 47% large-sized caps highest over other treatments. The proportion of small- , medium- and large-sized caps was similar (approximately 30% each size) for the control and substrates supplemented with pumice sulfate at 30% and pumice at 20% and 30% (Table 3).

The distribution of mushrooms within size classes were highly influenced by strain and supplements added to substrates. Influence of supplementation of *P. eryngii* substrate with Mn, Cu, and ground soybean on mushroom size was reported by Rodriguez Estrada and Royse (2006).

It has been reported that the average mushroom weight could be positively or negatively affected by calcium chloride, depending on the strain or dose used (Barden et al. 1990 and Solomon et al. 1991). Diamantopoulou and Philippoussis (2001) evaluated calcium chloride irrigation effect on different size categories of *A. bisporus* and found that size of calcium chloride-treated mushrooms was increased in the range applied. There was a pronounced increase of the size of 'large' mushrooms for calcium chloride treatment at 0.10 and 0.15%, while the positive effect of the rest of the treatments was correlated with the size increase of small mushrooms. In our study, substrates supplemented with gypsum at 5% produced more large-sized caps, highest over other treatments. The increase in gypsum content resulted in the increase in the small and medium-sized. However, supplementation of precipitated calcium carbonate ($CaCO_3$) to shiitake (*Lentinula edodes*) cultivation substrate reduced mushroom size (weight) from non-supplemented substrate (16.8 g) compared to substrate supplemented with 0.6% $CaCO_3$ (15.1g) (Royse and Sanchez-Vazquez 2003).

Individual mushroom cap (basidiomata) diameter ranged between 2.0cm and 16.0cm, and had a normal distribution pattern of individual mushroom diameters and frequencies with average diameter of 7.3±2.26cm (Figure 1). Average cap diameters of mushrooms cultivated in substrate supplemented with pumice and pumice sulfate were not significantly different from the control. Highest cap diameters were mushrooms supplemented with gypsum at 5% whereas supplementation with gypsum at 10% resulted in similar cap diameter to the control despite the decrease in total yield.

Table 4 shows that the hardness of the *P. ostreatus* stalks was not influenced by the kind of cultivation substrates. Substrates supplemented with gypsum, pumice sulfate and pumice tended to increase firmness of the mushrooms cap although they were not significantly different. Treatment of *Agaricus bisporus* with calcium chloride showed a variation in texture improvement. Diamantopoulou and Philippoussis (2001) found that there was a texture improvement when low calcium chloride concentrations were used, i.e. 0.05 and 0.10% but in higher dosages (above 0.15% calcium chloride) mushrooms presented a significant softening.

In summary, addition of pumice to substrate up to 30% had no effect on total yield, size distribution and cap diameter. Supplementing gypsum at 10% decreased total yield and although gypsum at 5% did not affect total yield, the treatment increased the proportion of large-sized caps which may not be appropriate for the canning process. Too much content of pumice sulfate added to *P. ostreatus* resulted in a lower yield.

IV. Calcium and silicon content in fresh mushrooms

Table 4 illustrates the calcium content in stalks and caps of oyster mushrooms supplemented with gypsum, pumice sulfate and pumice at two harvesting periods. Calcium content was 0.070-0.137mg/g dry weight for the stalks and 0.050-0.115mg/g dry weight of the caps. Wang et al. (2001) found that *P. ostreatus* cultivated on spent beer grain containing 3.9mg/100g dry matter of calcium content in the fruit bodies which was lower than our results. Calcium content in wild-grown *P. eryngii* and *P. ostreatus* was found to be 0.84 and 1.26mg/g dry weight, respectively (Gençcelep *et al.* 2009). The fruit bodies of *P. sajor-caju* contained calcium, in the range of 0.189-0.362mg/g dry weight of the fruit bodies was reported by Chang *et al.*, (1981). Akindahunsi and Oyetayo (2006) reported calcium content in *P. tuber-regium* caps and stalks to be 2.9 and 1.2mg/g dry matter, respectively. Ragunathan et al. (1996) showed that the fruit bodies of *Pleurotus* spp. (*P. sajor-caju, P. platypus* and *P. citrinopileatus)* contained 0.75-2.45mg/g of calcium. The variation of mineral content depended largely on the species of *Pleurotus* under study and the amount of the mineral in the substrates.

In all cases, calcium content in both the stalks and caps declined significantly in the second flush. Calcium content in mushroom stalks harvested in the 1st flush was not influenced by the kind of cultivation substrates. A slight increase in calcium content was observed in the stalks of mushrooms cultivated in substrates supplemented with gypsum and pumice sulfate. The calcium content in the caps were lower than those in the stalks. Likewise, calcium content in mushroom caps harvested in both flushes was not influenced by supplementations. Unlike our results, for bottom mushroom *Agaricus bisporus*, the addition of calcium both in the form of calcium sulfate (gypsum) and calcium chloride into irrigated water significantly ($p<0.05$) increased the calcium content of mushroom as compared to the control (Simons and Beelman, 1996). The differences were likely to be due to both application method and the kind of mushrooms. Calcium applied to irrigation water might not absorb into the tissue but was likely to accumulate on the surface and therefore was readily detected.

For all treatments, lime ($CaCO_3$) was added to each formulation in order to obtain the desired pH values (5.5±6.5). $CaCO_3$ has a profound effect on pH, while $CaSO_4$ has no or only minimal effect on substrate pH (Yildiz et al. 1998; Royse and Sanchez-Vazquez, 2003). Sawdust from rubber trees and rice bran, two major ingredients in the substrate, could also be a source of calcium beside $CaCO_3$. *P. ostreatus* did not appear to

accumulate more calcium when its substrate was supplemented with high amounts of calcium, therefore it was likely that there was a saturation level with regards to levels of calcium in the substrate. Supplementation with calcium therefore showed no effect on calcium accumulated in the mushrooms. It seemed that the sawdust based cultivation substrate was itself able to provide the required minerals.

Chiu et al. (1998) used soluble $CaCl_2$ in the cultivation of *P. pulmonarius* and found both the stipe and the pileus showed increased contents of calcium unlike insoluble calcium carbonate (up to 5%) which did not have any effect. The author suggested that insoluble calcium salts become available only after mobilization by fungal metabolism such as by secreting organic acids and represents a slow release form of Ca^{2+} ions. Thus a higher amount of insoluble calcium salts was needed to show a stimulatory effect. In our study, gypsum ($CaSO_4$) of agricultural grade rather than soluble $CaCl_2$ was used because it is commonly used in mushroom cultivation in Thailand.

Table 4 illustrates the silicon content in stalks and caps of oyster mushrooms supplemented with gypsum, pumice sulfate and pumice. Silicon accumulated in caps more than in stalks of the mushrooms. The silicon content in mushroom caps was approximately two times higher than that of mushroom stalks for all treatments. The level of silicon in mushroom caps and stalks was quite low compared to silicon in rice flour samples which contained 0.3-0.4mg/g dry sample (Samadi-Maybodi and Atashbozorg, 2006). Rice can accumulate silicon in the tops to levels up to 10% of shoot dry weight (Ma et al. 2002). Whereas, the roots of the spinach plant contains about 4mg/g dry weight of silicon concentrate with about 1mg/g dry wt for the shoot tissue (Gunes et al. 2007). The differences were probably due to mushrooms which cannot accumulate silicon in their tissues unlike those two plant species. The silicon content of plant tops greatly varies with species, ranging from 0.1% to 10.0% in dry weight. Based on the silicon content of the plant tops and the silicon to calcium ratio, plants are classified into silicon accumulator, intermediate type, and silicon excluder species. Rice (*Oryza sativa*) is the most effective Si-accumulating plant known (Ma et al. 2002).

As a result, the silicon content of the *P. ostreatus* stalks and caps was not influenced by the kind of cultivation substrate. Yet, silicon of the caps of mushrooms supplemented with pumice sulfate and pumice increased as their concentration increased, though not significantly. Silicon concentration in mushroom stalks showed no significant different values in all treatments. Unlike our results, the supply of silicon ($Na_2Si_3O_7$) to the soil increased Si concentration in both the roots and shoots of spinach plants significantly (Gunes et al. 2007). Silicon-treated tomato plants prepared by using a pure form of silicon dioxide at the rate 1g/l substrate with a nutrient solution amended with monosilicic acid contained a higher concentration of Si in root compared to –Si plants but the Si contents in stems showed no significant difference (Diogo and Wydra, 2007).

A study on the cultivation of the oyster mushroom, *P. sajor-caju*, on rice and wheat straw showed that the silica content in the substrates increased with time whereas the contents of other components remained the same or decreased. If the content of a component remained the same or decreased, the implication is that *P. sajor-caju* utilized the component (Zhang et al. 2002). This study suggested that silicon was not utilized during mushroom cultivation which was likely to be the case in our study as well.

V. Effect of silicon and calcium in liquid medium on mycelial growth

Table 5 illustrates the calcium content (mg/g dry wt.) and silicon content (mg/g dry wt.) in mycelia of *P. ostreatus* grown on WPDY medium. Liquid media without calcium and silicon, at two concentrations of $CaSO_4$ (5, 10mg/l), to concentration of Na_2Si_3 (5, 10mg/l) and a combination of 5mg/l Na_2Si_3 and 5mg/l calcium were examined. Grain-potato-dextrose-yeast extract (WPDY) medium was chosen because mycelia of *P. ostreatus* grown on this medium produced the highest biomass (Serafin Mun˜oz et al. 2006) as compared to potato-dextrose (PD) and potato-dextose-yeast extract (PDY). The mycelia grown in WPDY medium in the absence and in the presence of calcium and silicon were dried, homogenized, acid-digested and the determination of calcium and silicon was carried out by ICP. Calcium added to the growth medium was incorporated to the biomass more than 1,000 times compared to the incorporation of silicon. Silicon content in mycelium was at an undetectable level due to very low content of silicon incorporated to the mycelium. Apparently, calcium distribution was not affected by the concentration of calcium chloride in medium. At every level of calcium and silicon used, the growth of mycelium was not inhibited. Calcium content in the control mycelium seemed to be lower than those supplemented with calcium and silicon, though not significant in some cases. There was no significant increase of calcium level in mycelia grown in 5mg/l as compared to 10mg/l. These results confirmed previous experiments that *P. ostreatus* cannot accumulate silicon in the tissue. *P. ostreatus* mycelium did not appear to accumulate more calcium when its substrate was supplemented with high amounts of calcium; therefore it was likely that there was a saturation level with regard to levels of calcium. Liquid medium could probably provide enough calcium for the mycelium. A lack of calcium would not be possible due to its requirement for mycelial growth.

VI. Drip loss values and solids content

Although supplementation of gypsum, pumice sulfate and pumice showed no influences on accumulated calcium and silicon contents in mushroom fruit bodies these treatments affected centrifugal drip loss values and solids content as shown in figure 2. The drip loss of mushroom caps and stalks supplemented with gypsum, pumice sulfate and pumice was significantly higher than those of the control mushrooms. The drip loss values increased as supplement concentration increased both for the caps and stalks. The values of centrifugal drip loss increased with increasing concentration of calcium and silicon in the substrates as can be seen in cases of G5 and G10, the available calcium increased from 9.4 to 18.9g/kg substrate and in P10 to P30, the available silicon varied from 48.6 to 145.9g/kg substrate (with calcium <2.2 g/kg substrate). In PS10 to P30 the available silicon varied from 24.3 to 72.9g/kg substrate and available calcium varied from 8.5 to 25.5g/kg substrate. In the cases of G10 (18.9g/kg available calcium) and PS10 containing approximately half of the available calcium of G10 but containing silicon (8.5g/kg available calcium and 24.3g/kg available silicon), their drip loss values were similar, indicating that both silicon and calcium influenced the drip loss value.

When a frozen food is thawed, the moisture is readily separated from the matrix and it causes drip loss and often deterioration of texture quality. Theoretically, for some fresh produces pre-treatment of calcium before processing (freezing or canning) helps strengthen plant cell wall which reduces cell damage during thawing that in turn lowers moisture release and drip loss. A study on the effects of the pre-treatments and the long and short-term frozen storage on the quality of raspberries showed that calcium pre-

treatments before freezing may be used to reduce the induced loss of firmness caused by the frozen storage, as well as the drip loss (Sousa et al., 2005). In case of *P. ostreatus*, calcium incorporated into the mushrooms tissues influenced tissues differently from other produces. In the case of our studies, calcium content in control sample did not differ from supplemented samples, yet centrifugal drip loss values and solids content were higher in supplemented samples. This indicated that the increase in the centrifugal drip loss value might not be due to the strengthening effects of calcium on the hyphal wall but rather indicated the ability to hold larger amounts of free water of the tissues of supplementation samples compared to control samples.

The centrifugal drip loss values corresponded well with moisture the content which in turn related to solids content, as clearly seen in mushroom stalks containing higher solids content and lower centrifugal drip loss than mushroom caps. The solids content of mushroom caps and stalks supplemented with gypsum, pumice sulfate and pumice was significantly higher than those of the control mushrooms. The solids content increased as pumice sulfate concentration increased both for the caps and stalks. The increase in gypsum content in the substrate seemed to increase the solids content in the fruit bodies, though not significant differences. The increase in pumice content in the substrate did not significantly increase solids content of both caps and stalks. It seemed that the amount of calcium available in the substrate was necessary for the increase in the solids content in the fruit bodies. Solids contents of the mushroom caps and stalks supplemented with gypsum and pumice were in the same range.

Moisture content of in *P. ostreatus* cultivated in wheat straw supplemented with sugar beet was 90.7% (solids content of 9.3%) (Bonatti et al. 2004). The values were similar to the solids content of the mushroom caps in our study. The effect of addition of calcium on the solids content varied depending on each study since the study conditions were different. Simons and Beelman (1996) found that an addition of 0.3% calcium chloride to irrigation water significantly increased the solids content of *A. bisporus* samples compared to that of the control treatment. Whereas, irrigation of *A. bisporus* with 0.3% calcium chloride had no effect on solids content of fresh mushrooms as reported by Chikthimmah et al. (2005). The solids content of *Pleurotus spp.* was influenced by the kind of cultivation substrate. *P. sajor-caju* CCB 019 fruiting bodies presented higher moisture when cultivated in rice straw (Bonatti et al. 2004). The additions of micronutrients to the compost may reduce solids content of harvested mushrooms. Significant decreases in solids occurred as a result of adding 276mg/kg manganese (crop 3010) and 92mg/kg manganese (crop 3011) to the compost of *A. bisporus* at spawning. It was speculated that cation imbalances in the compost or casing may have produced this effect (Weil et al, 2006).

In this study, although silicon and calcium did not accumulate into mushroom tissues as a result of supplementation, it influenced the physiological state of the tissue as indicated in its positive effect on increasing drip loss values and solids content. The increase in centrifugal drip loss for supplemented samples indicated an ability to hold larger amounts of free water in the tissues of supplementation samples compared to control samples. Besides, the higher solids content for supplemented samples could also be a good indication of the more compacted mushroom hyphae, basic structural components of mushroom tissues, compared to the control.

VII. Quality attributes of canned mushrooms

According to the high drip loss values and solids content, mushrooms cultivated in substrates containing gypsum at 10%, pumice sulfate at 30% and pumice at 30% were processed into canned mushrooms and qualities were evaluated. The percentage of weight gained after draining, the percentage of size reduction and firmness of canned mushrooms in brine is shown in Table 6. The weight gained was determined by weighting mushrooms after draining the can contents for two min in a perforated stainless steel tray and calculating a percentage as compared to the original weight before canning. The percentage of weight gained of mushrooms cultivated in substrates supplemented with gypsum, pumice sulfate and pumice were significantly ($p<0.05$) higher than that of the control treatment. This result indicated that if the original weights of mushrooms before canning are equal, canning mushrooms supplemented with calcium and/or silicon will produce canned mushrooms with significantly higher drain weight of approximately 20% compared to canned control mushrooms. The results clearly showed the beneficial effect of supplementation of calcium and/or silicon in the mushroom production substrates for drain weight improvement of canned mushrooms.

In term of size loss, the cap diameter of canned mushrooms reduced significantly approximately 10% as compared to fresh mushrooms independent of treatments. The size loss after canning is expected because fresh produces shrink when exposed to thermal treatment. In the case of canned bottom mushrooms, the loss of grade was detected at about 40% for unblanched samples but the value reduced to about 20% for blanched samples (Vivar-Quintana et al. 1999).

Correspondingly to the weight gained values, the firmness of caps and stalks of canned mushrooms with calcium and/or silicon treatments were significantly higher than that of the control treatment. The highest value of firmness was found in stalks of mushrooms supplemented with silicon.

The increase in drain weight and firmness of canned mushrooms supplemented with calcium and silicon was the result of both the improvement in the ability to hold water and the increase in solids content or mushroom hyphae of mushrooms which have undergone calcium and/or silicon treatment compared to non-supplemented mushrooms.

VII. Scanning electron microscope (SEM)

Figure 3 shows the microstructures of mushroom stalks and caps after canning. Under SEM observation, the hyphae of the stipes and pores could be found in the center of each hyphae. After heat treatment by sterilization at 121 °C for 15 min, the release of mucilage from the hyphal wall was observed both for stalks and caps. This was speculated to be in the form of soluble polysaccharides. The polysaccharides run off tissue pores, which cause no increase in springiness. In addition, the collapse and deformation of softened hyphal walls in the section were observed when the mushrooms were heated. The collapsed and deformation of hyphal walls associated with loss of cell components were reported for heated *A. bisporus* (Zivanovic et al. 2000) and winter mushrooms (*Flammulina velutipes*) (Ko et al. 2007). The hyphae of mushroom stalks were more tightly compacted than those of the caps, which corresponded well with higher firmness values of the stalks. This indicated that the more closely compacted the hyphae are, the firmer the texture of the tissue. Zivanovic et al. (2000) reported that softening of heated *A. bisporus* corresponded with the increase in free space between the cells. Hyphae in mushrooms supplemented with silicon and/or calcium seemed tightly compacted and most hyphae remained intact unlike those of the control where its hyphae

was loosely compacted and some hyphae were collapsed. The effect of supplementation of silicon and/or calcium on the compactness of hyphae was clearly shown in microstructures of mushroom stalks after canning. After heat treatment, hyphae walls adhered to adjacent tissues. The aggregation of cells was also found in heated *A. bisporus* (Zivanovic et al. 2000). The more compacted hyphae of mushroom stalks supplemented with silicon and/or calcium was clearly shown after heat treatment compared to that of the control. This contributed to less firmness of the control mushroom samples. These results corresponded well with their firmness values in Table 6. However, this effect was not clearly seen for mushroom caps. This might be because the hyphae of mushroom caps were loosely packed whereas those of the stalks were more compacted. Tissues of mushroom caps were less firm than mushroom stalks, therefore, their hyphae collapsed more easily than those of the stalks.

Conclusion

The addition of pumice to substrate for *P. ostreatus* cultivation up to 30% had no effect on total yield, size distribution and caps diameter. Supplementation of gypsum at 10% decreased total yield and although gypsum at 5% did not affect total yield, the treatment increased the proportion of large-sized caps which may not be appropriate for the canning process. Too much content (> 10%) of pumice sulfate resulted in the lower yield.

The calcium and silicon contents in mushroom caps harvested in both flushes were not influenced by supplementations. *P. ostreatus* did not accumulate silicon in the tissue.

Although silicon and calcium did not influence silicon and calcium in mushroom tissues, they influenced the physiological state of the tissue as indicated in its positive effect on increasing drip loss values and solids content. The increase in centrifugal drip loss for supplemented samples indicated the ability to hold larger amounts of free water in the tissues of supplementation samples compared to the control samples. Besides, the higher solids content for supplementation samples could also be a good indication of the more compacted mushroom hyphae, basic structural components of mushroom tissues, compared to the control.

The percentage of weight gained in canned mushrooms cultivated in substrates supplemented with gypsum, pumice sulfate and pumice was significantly ($p<0.05$) higher than that of the control treatment. This indicated that the canning of mushrooms supplemented with calcium and/or silicon will produce canned mushrooms with a significantly higher drain weight of approximately 20% compared to canning non-supplemented mushrooms. The more compacted hyphae of mushroom stipes supplemented with silicon and/or calcium was clearly shown after heat treatment compared to that of the control by using SEM. This contributed to less firmness of the control mushroom samples. These results corresponded well with their firmness values.

Supplementation of *P. ostreatus* substrates with 20% pumice provided an alternative method for improving the qualities of canned mushrooms since this practice improved drain weight and textural integrity of canned mushrooms. The improvement of drain weight and firmness of canned mushroom was probably because pumice induced strains to produce more hyphae compacted in the tissues as a result; the tissues were able

to hold more water and possessed tougher texture. Using gypsum and pumice sulfate was less desirable due to the decrease in yield and higher cost.

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Table 1. Summary of substrate formulations

Treatment code	**C**	**G5**	**G10**	**PS10**	**PS20**	**PS30**	**P10**	**P20**	**P30**
Basic Ingredients	sawdust (60 kg) + rice bran (6 kg) + MgSO4 (0.12 kg) + CaCO3 (0.6 kg) + water to 60% moisture content								
Gypsum	-	3 kg (5%)	6 kg (10%)	-	-	-	-	-	-
Pumice sulfate	-	-	-	6 kg (10%)	12 kg (20%)	18 kg (30%)	-	-	-
Pumice	-	-	-	-	-	-	6 kg (10%)	12 kg (20%)	18 kg (30%)

(C = control, G = gypsum, PS = pumice sulfate, P = pumice)

Table 2. The time period of different phases of oyster mushroom cultivation, yield and percentage BE of *Pleurotus ostreatus* grown on substrates non-supplemented and supplemented with gypsum, pumice sulfate and pumice.

Supplement		Treatment code	Spawn running (days)	Fruit body formation (days)	Yield per pack				BE# (%)
					1st flush	2nd flush	3rd flush	Total	
Gypsum	0%	C	25.5±1.5	39.4±5.8^{b}	86.05±21.6	74.21±19.0	42.93±15.7	203.19±30.0^{b}	56.44
	5%	G5	24.9±1.8	39.9±4.6^{b}	70.70±29.6	73.85±27.5	55.81±18.6	200.36±35.1^{b}	55.66
	10%	G10	25.2±1.1	34.3±4.8^{a}	57.50±18.0	46.33±10.8	37.78±14.7	141.62±19.1^{a}	37.27
Pumice sulfate	0%	C	25.5±1.5	39.4±5.8^{b}	86.05±21.6	74.21±19.0	42.93±15.7	203.19±30.0^{b}	56.44
	10%	PS10	24.7±1.3	39.4±5.3^{b}	80.53±21.4	71.79±24.9	50.66±22.2	202.97±30.8^{b}	56.38
	20%	PS20	25.2±1.9	33.5±4.0^{a}	74.08±11.4	53.53±13.9	36.55±19.4	163.07±20.6^{a}	45.30
	30%	PS30	24.3±1.2	36.6±5.7^{ab}	66.83±23.3	59.80±22.6	37.05±17.1	163.69±26.9^{a}	45.47
Pumice	0%	C	25.5±1.5	39.4±5.8^{b}	86.05±21.6	74.21±19.0	42.93±15.7	203.19±30.0^{b}	56.44
	10%	P10	27.3±2.1	36.2±4.7^{ab}	62.91±24.0	47.81±17.1	28.28±9.2	138.99±25.8^{a}	38.61
	20%	P20	25.7±2.6	35.2±5.0^{a}	85.14±18.6	72.19±28.6	56.37±24.2	216.33±28.8^{b}	60.09
	30%	P30	26.3±2.0	35.0±4.2^{a}	79.38±27.9	66.76±20.8	58.88±22.6	203.24±34.2^{b}	56.46

Means followed by the same letter in the same column in the same set of supplement are not significantly different according to the Duncan test ($P \geq 0.05$)

Biological efficiency (BE) is the ratio of fresh mushroom harvested per unit of dry substrate and expressed as a percentage. Values were from 3 flushes.

Table 3. Size classes (small, medium, large) expressed as a percentage of the total mushroom production per treatment. Quantiles 33.3% (6.70 g) and 66.6% (12.28 g) were used to delimit mushroom size classes (small, medium, large), mushroom cap diameter (cm) and firmness (kgf) of *Pleurotus ostreatus* grown on substrates non-supplemented and supplemented with gypsum, pumice sulfate and pumice.

Treatment code	Size classes (%)			Cap diameter (cm)	Hardness (kgf)	
	Small	Medium	Large		Stalk	Cap
C	35.03	33.27	31.70	7.3±2.5[a]	3.432±0.139	0.730±0.060[a]
G5	20.49	31.78	47.73	8.1±2.3[b]	3.654±0.505	1.050±0.006[b]
G10	37.32	37.88	24.80	7.2±2.2[a]	3.392±0.471	1.146±0.096[b]
C	35.03	33.27	31.70	7.3±2.5	3.432 ±0.139	0.730±0.060
PS10	27.62	34.78	37.60	7.4±2.2	4.074±0.419	0.990±0.072
PS20	41.70	33.55	24.75	7.0±2.2	3.588±0.658	1.226±0.127
PS30	31.05	33.72	35.23	7.4±2.2	3.260±0.230	1.042±0.120
C	35.03	33.27	31.70	7.3±2.5	3.432±0.139	0.730±0.060[a]
P10	42.69	35.85	21.46	6.9±2.0	3.432±0.130	1.248±0.123[b]
P20	35.05	33.27	31.70	7.1±2.2	3.510±0.283	1.208±0.116[ab]
P30	31.50	32.20	36.30	7.2±2.2	3.342±0.130	0.952±0.108[ab]

* Number of individual mushroom samples taken for size class and cap diameter measurement was 332 for C, 291 for G5, 231 for G10, 346 for PS10, 355 for PS20, 296 for PS30, 290 for P10, 424 for P20 and 284 for P30.
Means followed by the same letter in the same column in the same set of supplement are not significantly different according to the Duncan test ($P \geq 0.05$)

Table 4. The calcium content (mg/g dry wt.) and silicon content (mg/g dry wt.) of *Pleurotus ostreatus* grown on substrates non-supplemented and supplemented with gypsum, pumice sulfate and pumice.

Treatment code	Calcium content (mg/g dry wt.)				Silicon content (mg/ 100 g dry wt.)	
	Stalk		Cap		Stalk	Cap
	1st flush	2nd flush	1st flush	2nd flush		
C	0.117±0.037	0.061±0.005[a]	0.073±0.011	0.033±0.003	0.059±0.011	0.076±0.013
G5	0.103±0.018	0.092±0.003[ab]	0.060±0.019	0.040±0.001	0.040±0.004	0.097±0.038
G10	0.104±0.023	0.110±0.026[b]	0.079±0.012	0.048±0.011	0.058±0.010	0.119±0.048
C	0.117±0.037[ab]	0.061±0.005[a]	0.073±0.011[ab]	0.033±0.003	0.059±0.011	0.076±0.013
PS10	0.085±0.036[a]	0.050±0.025[a]	0.090±0.022[b]	0.052±0.008	0.045±0.013	0.093±0.032
PS20	0.137±0.012[b]	0.115±0.040[b]	0.077±0.021[ab]	0.051±0.015	0.059±0.025	0.098±0.032
PS30	0.088±0.013[a]	0.084±0.017[ab]	0.046±0.005[a]	0.036±0.030	0.068±0.013	0.121±0.029
C	0.117±0.037[b]	0.061±0.005	0.073±0.011[ab]	0.033±0.003	0.059±0.011	0.076±0.013
P10	0.070±0.007[a]	0.035±0.005	0.051±0.002[a]	0.047±0.008	0.050±0.008	0.087±0.003
P20	0.117±0.011[b]	0.066±0.015	0.092±0.032[b]	0.043±0.008	0.048±0.017	0.084±0.020
P30	0.081±0.006[ab]	0.053±0.018	0.042±0.004[a]	0.030±0.002	0.044±0.009	0.101±0.016

Means followed by the same letter in the same column in the same set of supplement are not significantly different according to the Duncan test ($P \geq 0.05$)

Table 5. The calcium content (mg/g dry wt.) in mycelia of *Pleurotus ostreatus* grown on WPDY medium

Treatment	Calcium content **(mg/g dry mycelium)**
Ca_0Si_0 (control)	1.161 ± 0.148^{a}
Si_{Lo}	1.453 ± 0.003^{bc}
Si_{Hi}	1.567 ± 0.082^{c}
Ca_{Lo}	1.504 ± 0.096^{c}
Ca_{Hi}	1.423 ± 0.179^{abc}
$Ca_{Lo}Si_{Lo}$	1.220 ± 0.192^{ab}

Means followed by the same letter in the same column are not significantly different according to the Duncan test ($P \geq 0.05$)

Table 6 Percentage of weight gained and size loss and firmness (kgf) of canned mushrooms cultivated in substrates supplemented with gypsum, pumice sulfate and pumice.

Treatment	Wt. gained (%)	Size loss(%)	Firmness (kgf)	
			Stalks	Caps
Control	5.96±3.75^{a}	11.70±0.77	3.395±0.345^{a}	1.540±0.135^{a}
G10	18.15±2.59^{b}	10.60±0.85	3.977±0.534^{b}	1.802±0.200^{bc}
PS30	19.40±5.84^{b}	10.86±0.24	4.415±0.783^{c}	1.867±0.170^{c}
P30	21.20±4.75^{b}	12.08±0.99	4.491±0.668^{c}	1.714±0.149^{b}

Means followed by the same letter in the same column are not significantly different according to the Duncan test ($P \geq 0.05$)

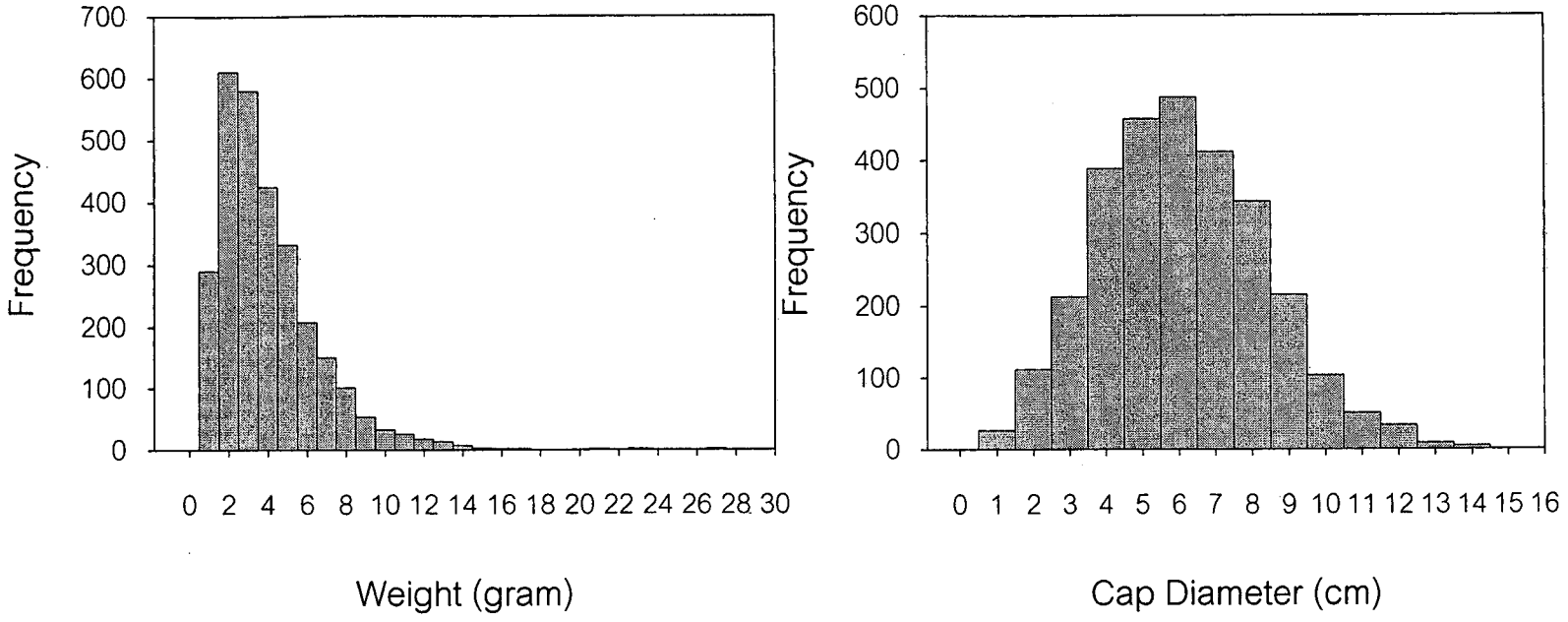

Figure 1 a.) A histogram showing the distribution of individual (2,848) mushroom weights. Quantiles 33.3% (6.70 g) and 66.6% (12.28 g) were used to delimit mushroom size classes (small, medium, large) b.) A histogram showing the distribution of individual (2,848) mushroom cap diameter showing normal distribution curve with mean of 7.3 cm and standard deviation of 2.26 cm. The caps with diameter of 7.3 ± 2.26 cm were used to produce canned mushrooms.

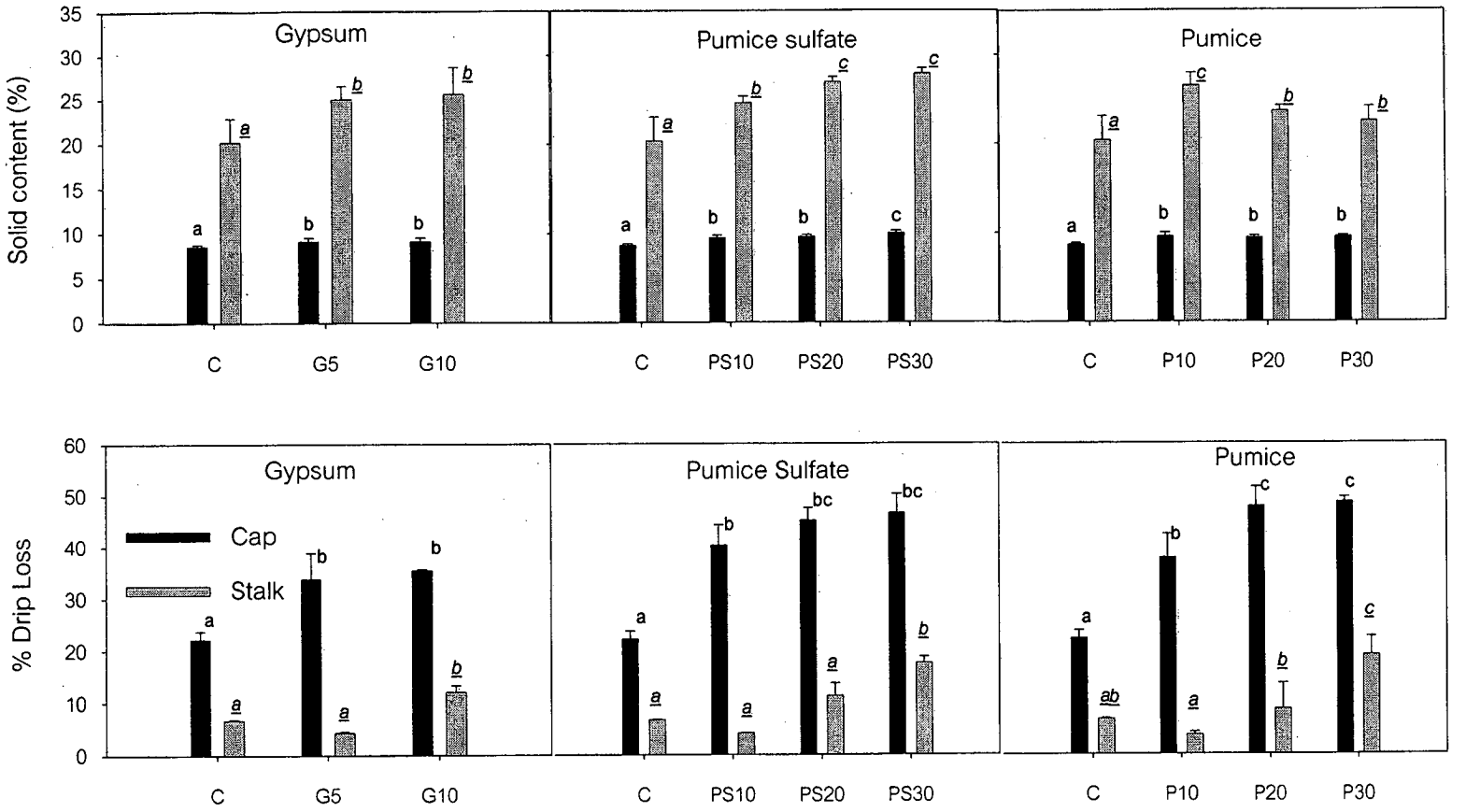

Figure 2. The percentage drip loss and solid content of the caps and stalks of oyster mushrooms grown on substrates non-supplemented and supplemented with gypsum, pumice sulfate and pumice. Means followed by the same letter in the same column in the same set of supplement are not significantly different according to the Duncan test (P≥0.05)

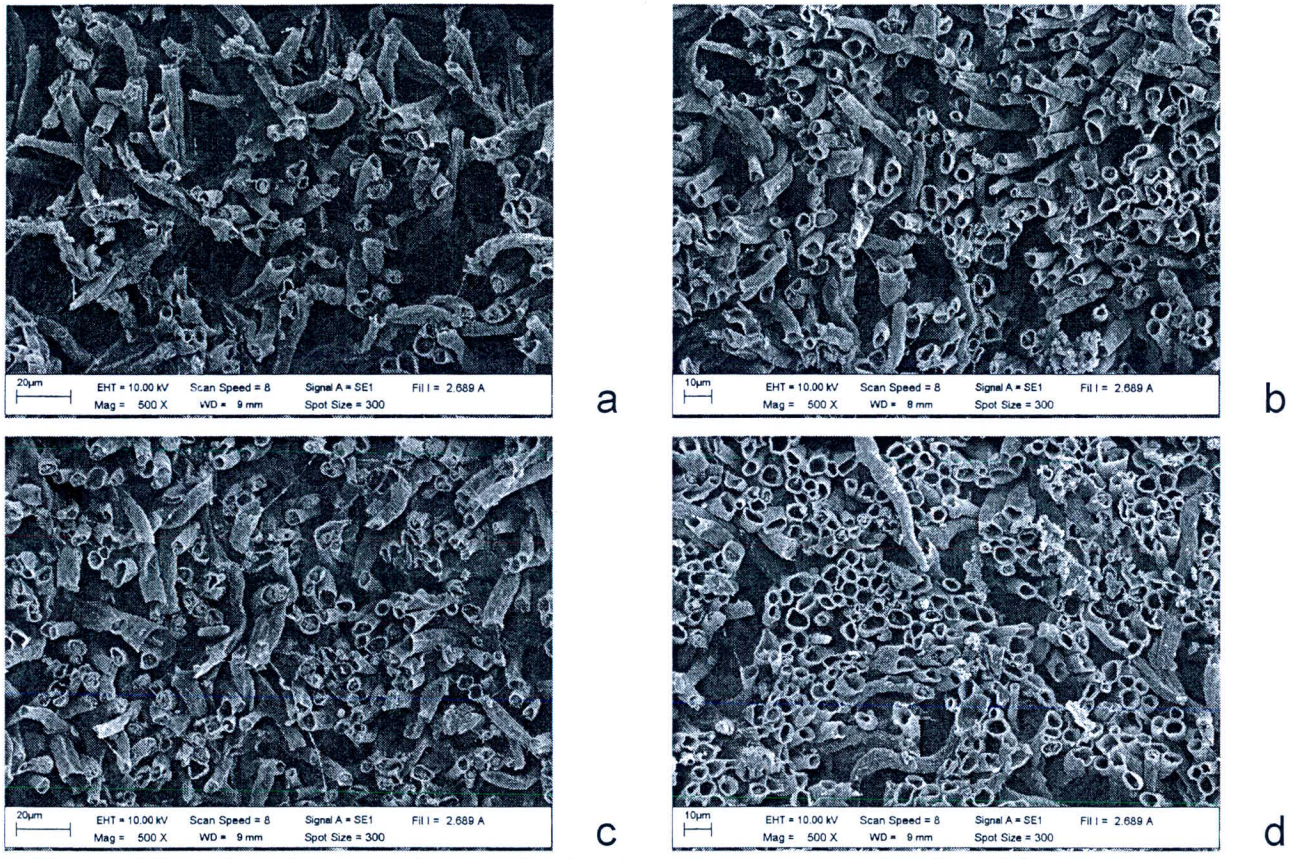

Figure 3 Scanning electron micrographs showing a cross section of stalks of *P. ostreatus* grown on substrates non-supplemented (a) supplemented with gypsum (b) pumice sulfate (c) and pumice (d) after thermal treatment. For each sample, the image was the representative image from 3 different samples randomly collected from at least 10 canned mushrooms. Only mushroom caps with diameter of 7 cm and mushroom stalks with 1 cm thickness were chosen.