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Determination of Hydration Properties and Thermal Behavior of *Paecilomyces variotii* by Differential Scanning Calorimetry[1]

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Abstract—Due to the structure and the composition of *Paecilomyces variotii*, the mycelia of this fungus could have potential applications as ingredients in wettable foods. For this use, drying could be employed, justifying the study of thermal behavior of *P. variotii*. The objectives of this work were to perform a study of thermal behavior of *P. variotii* isolates, to evaluate the hydration properties of these mycelia and to analyze the effect of different technological parameters on the latter properties. Wet cultures exhibited a wide endothermic transition, with mean values of peak temperature of 61°C and denaturation enthalpy of 4 J/g dry matter. Initial (50°C) and final (80°C) temperatures of the endothermic transition were used to dry the mycelia. Freeze-drying was also assayed. For all dried mycelia, a decrease in denaturation enthalpy between 40 and 50% was observed for drying at 50°C and freeze-drying, and a drastic decrease of almost 100% for drying at 80°C. According to the hydration properties, wet mycelia exhibited water holding capacity (WHC) value of 45 g water/g dry matter. Significant differences among dried mycelia, resulting WHC values in order: 50°C > freeze-dried > 80°C ($p < 0.05$) were revealed for each *P. variotii* strain. Fungi obtained by drying at 50 C and by freeze-drying, showed a rapid water absorption ($t_{1/2} < 0.1$ min). Ionic strength, pH and particle size of dried mycelia influenced the hydration properties.

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The genus *Paecilomyces* was split from *Penicillium* by Bainier in 1907 [1] on the basis of their differences in phialide shape and conidial color. *Paecilomyces* species are important as soil fungi and insect pathogens. Only two species are commonly isolated from foods: *Paecilomyces variotii* and *Paecilomyces lilacinus* [2]. *P. variotii* has not been reported as a mycotoxin producer [2], and is used in the food industry as a producer of enzymes. This fungus produces a thermostable glucoamylase [3]. α-amylases have the widest range of industrial applications [4, 5] including brewing, baking, textile and detergent production [6, 7]. *P. variotii* also produces tannase [8], an enzyme that cleaves ester linkages in hydrolyzable tannins.

Single cell protein (SCP) is a term coined in the 1960's to embrace microbial biomass products of fermentation [9]. *P. variotii* has been used for the production of microbial protein because of its excellent ability to grow in a variety of highly-polluting industrial effluents, such as molasses, hydrolyzed wood, spent sulfite liquor and vinasse [10–13]. *P. variotii* was the first fungus used in an industrial process for the production of microbial protein for animal feed, known as "Pekilo" process [10].

The successful adoption and use of microbial biomass depend on the properties of the fungus, which determine its usefulness as a functional food ingredient. Functional properties are defined as the physicochemical properties that contribute with the rheological parameters and stability and sensory features of a food product. These properties can be classified as hydration, structuring, surface and organoleptic properties [14]. The water holding capacity (WHC) and the water absorption capacity (WAC) expresses the hydration properties. WHC refers to the ability of a hydrated material to retain water against the action of an external force of centrifugal gravity or compression, whereas WAC refers to the ability of a material to absorb water in its structure spontaneously when it comes in contact through a surface that remains wet. Both WHC and WAC are relevant for each food system [15].

The possible inclusion of Pekilo biomass has been studied in different food systems, such as sausages and meat balls [16]. *P. variotii* has been found to increase the hardness of sausages up to 30% substitution level, probably owing to its good water imbibing and gelling properties reported earlier [16]. Pekilo has also been studied in doughs, particularly in white wheat bread and soft and hard rye bread. This biomass has also been observed to increase the water binding of the dough from about 62% to approximately 72% when the flour substitution level is increased from 0 to 8%. The mild taste of Pekilo powder is distinctly found in bread, even at the lowest substitution levels [17].

[1] The article is published in the original.

In our previous work [18], we isolated and identified filamentous fungi from several sources. Non-toxic fungal strains, characterized by the *Artemia salina* bioassay [19], were used to study their composition (protein, total dietary fiber and β-glucan) and hydration properties (WAC and WHC). We found that these mycelia give the following composition: protein content ranged from 30 to 47% of dry weight, total dietary fiber content changed from 16 to 53% of dry weight and β-glucans content varied from 1 to 26% of dry weight. The highest values of these parameters were found in *P. variotii*. According to hydration properties, this fungus showed the highest values for WHC and WAC, with a rapid water absorption rate (mycelia dried at 50°C) [18]. These results suggest the potential application of this fungus as ingredient in wettable foods. For this use, *P. variotii* may require drying, which justifies the study of its thermal behavior.

Then, the objectives of this work were to perform a study of thermal behavior of *P. variotii* isolates, to evaluate the hydration properties of these mycelia and to analyze the effect of different technological parameters on the latter properties.

MATERIALS AND METHODS

Fungal strains and culture conditions. Four strains of *P. variotii* isolated from pepper were used. The isolates were identified according to Pitt and Hocking [2]. The cultures were inoculated by 1 1×10^5 conidia/ml and grown in liquid media containing (g/L): yeast extract—20 and sucrose—40 by shaking at 135 rpm and 25°C for 7 days. Mycelia samples were collected by filtration through Whatman N° 1 filter paper (USA) under vacuum and washed twice with distilled water.

Drying process. Wet mycelia of *P. variotii* were fractionated to be subjected to different drying conditions: a—drying in stove with air circulation at 50°C and 80°C until constant weight (moisture content < 0.5%), and b—freeze-drying. For this latter process, samples were frozen at −80C for 5 days. Freeze-drying was performed using HETO FD4 (Denmark) coupled to a Rotary Vane Vacuum Pump RZ5, for 30 h.

Protein, total dietary fiber and β-glucans determination. Dry mycelia were analyzed to determine protein [20], total dietary fiber (TDF) [21, 22] and β-glucan. The latter was analyzed by the Megazyme TM commercial kit instruction (Ireland).

Milling and fractionation. Dried mycelia were ground in an FW100 Model Chincan miller (China) and fractionated to obtain different particle sizes (>850 μm, 500–850 μm and < 500 μm) using IRAM 1501/76 ASTM–E–11–81 sieves No. 35 and No. 20 (Argentina).

Thermal behavior. The thermal behavior of wet and dried mycelia of *P. variotii* was studied by modulated differential scanning calorimetry (MDSC) using a Q200 calorimeter (TA Instrument USA, amplitude: +/− 1°C, frequency: 60 sec). The samples were brought to 80% of water either by partial dehydration in the case of wet mycelia by Whatman No. 1 paper or by addition of water in the case of dry mycelia. Samples (12–15 mg) were hermetically sealed in standard aluminum pans and heated from 10 to 90°C at a heating rate of 5°C/min. An empty pan was used as reference. Enthalpies and peak temperatures (Tp) of endotherms were obtained from thermograms. Enthalpies (H) were expressed as J/g dry matter by perforating the pans and heating overnight at 105°C [23]. All assays were performed in duplicate.

Hydration properties. WAC was determined on dried mycelia. Water absorption kinetics was followed using the Baumann equipment [24], which consists of a funnel connected to a horizontal capillary. A 50 mg sample was dusted on a wetted filter paper which was fastened to a glass filter placed on top of the funnel filled with water. The apparatus was kept at 20°C. The amount of water uptaken by the sample at equilibrium was read in the graduated capillary. The maximum amount of water absorbed and the time to reach WAC were determined [25]. The half time ($t_{1/2}$) was defined as the time of the samples to absorb half of the maximum amount of water.

WHC was determined on both wet and dried mycelia of *P. variotii*. For WHC determination on wet mycelia, a full pellet was dispersed in distilled water and then shaken (for 2 h at 25°C and 100 rpm) and centrifuged (for 20 min at 15°C and 15000 × g. The pellet was dried in a stove with air circulation at 50°C until constant weight and finally weighed. For WHC determination of dry mycelia, 100 mg of sample was stirred with 9 ml of distilled water in an orbital shaker at 100 rpm for 2 h and then centrifuged for 30 min at 800 × g. The supernatant decanted and the pellet finally weighed. WHC and WAC were expressed as g of water/g of dry matter. Both determinations were performed using distilled water or 0.01 M phosphate buffer (pH 5.0 or 7.0) with different concentrations of NaCl (0, 1, 2 and 3%). Besides, hot water (80°C) was used to determine WHC. To this end, 100 mg of sample was stirred with 1 ml of distilled water at room temperature, in an orbital shaker for 1 min and then 8 mL of hot distilled water (80°C) was added. In the second assay, 100 mg of sample was stirred with 9 mL of hot distilled water (80°C). Both experiments were performed as previously described to determine WHC.

Viability of *P. variotii* dried at 50°C. A suspension (1% m/V) was obtained from dried fungi at 50°C (*P. variotii* 101 and *P. variotii* 440) in distilled water. Malt extract agar plates were inoculated at central point with 5 μL of suspension. Then, these plates were incubated at 5°C or 25°C. The diameter of colonies developed was measured daily (in duplicate) from the first to the seventh day of cultivation and was expressed

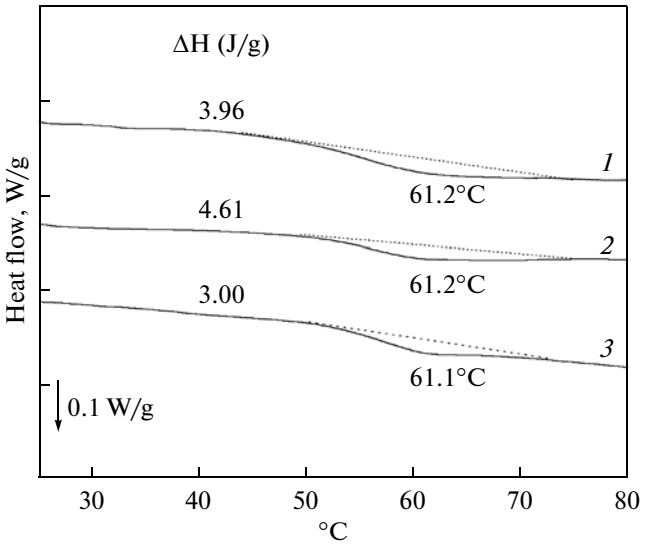

Fig. 1. Differential scanning calorimetric thermograms of wet mycelia of different *P. variotii* strains: *1*—*P. variotii* 101, *2*—*P. variotii* 102 and *3*—*P. variotii* 439.

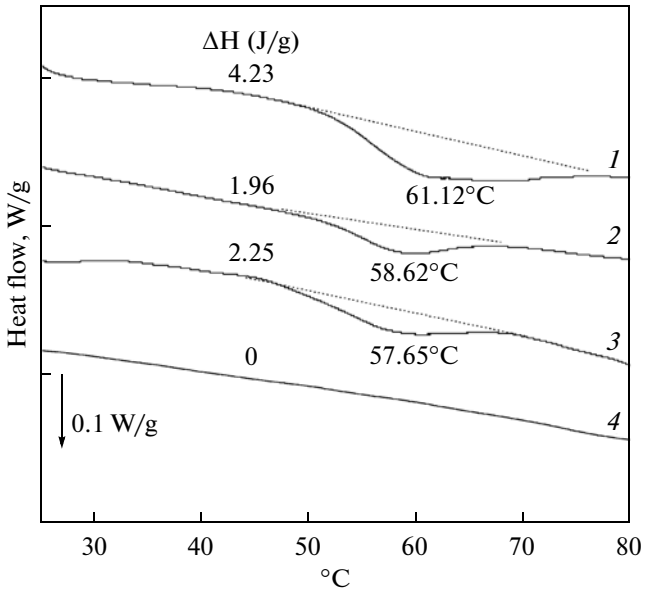

Fig. 2. Effect of drying conditions on differential scanning calorimetric thermograms of *P. variotii* 102: *1*—wet mycelium, *2*—freeze-dried mycelium, *3*—mycelium dried at 50°C and *4*—mycelium dried at 80°C.

in millimeters. From the growth curves obtained, the growth rate of mycelia (K = mm/day) was calculated by regression analysis.

Statistical analysis. The statistical analysis was performed by analysis of variance and tested of the least significant difference ($P < 0.05$) using the statistical program Statgraphics Plus 5.1.

RESULTS AND DISCUSSION

Protein, total dietary fiber and β-glucans determination. Dry mycelia of *P. variotii* 101, 102, 440 and 439 exhibited a protein content of 26, 28, 26, 29% of dry weight a TDF content of 51, 50, 58, 48% of dry weight and a β-glucans content of 26, 22, 22 and 26% of dry weight respectively.

Thermal behavior. Differential scanning calorimetric (DSC) thermograms of wet cultures for all the *P. variotii* strains exhibited a wide endothermic transition in the range between 45–50°C and 75–80°C, which could be attributed to protein denaturation (Fig. 1). An endothermic peak was observed with a temperature peak (Tp: 61°C) with the enthalpy mean value of 4 J/g of dry matter. Figure 1 shows the thermograms corresponding to *P. variotii* strains 101, 102 and 439. As mentioned before, the mycelia analyzed in this work have about 30% of protein/dry weight and the enthalpy values are in the order of 13 J/g of protein. This is in accordance with the results of Otero et al. [26], who obtained ΔH values of approximate 14–17 J/g of protein for *Saccharomyces cerevisiae* and *Kluveromyces fragilis*, respectively, working with intact cells.

The drying temperature used in this work was obtained from the DSC thermograms: 50°C and 80°C corresponding to the beginning and the end of the endothermic transition, respectively. Freeze-drying was an additional condition. Figure 2 demonstrates the thermograms obtained for *P. variotii* 102 as an example of the general behavior of all the strains studied. In comparison to wet control mycelium, for all dried mycelia (particle size <500 μm), a decrease in denaturation enthalpy between 40 and 50% was observed for drying at 50°C and freeze-drying, and a drastic decrease of almost 100% occurred for drying at 80°C.

Hydration properties. *Wet mycelia*. All samples analyzed showed good hydration properties. Wet mycelia of the *P. variotii* strains showed high WHC, with mean values of 45 g of water/g of dry matter.

Effect of the drying conditions of mycelia. The dried mycelia used in this assay were sieved in <500 μm particle size, and distilled water was used to determine their hydration properties. Figure 3 shows the WHC values for the different strains of *P. variotii* dried under different conditions: 50°C > freeze-dried > 80°C ($p < 0.05$). Mean values of WHC were 8.8 g of water/g of dry matter at 50°C, 7.4 g of water/g of dry matter at freeze-drying and 3.5 g of water/g of dry matter at 80°C. These values correspond to losses in WHC of 80, 83 and 92%, respectively, compared with wet mycelia.

During study of the effect of the different drying conditions on WAC a similar pattern was revealed in all *P. variotii* strains. As an example, Fig. 4 shows the

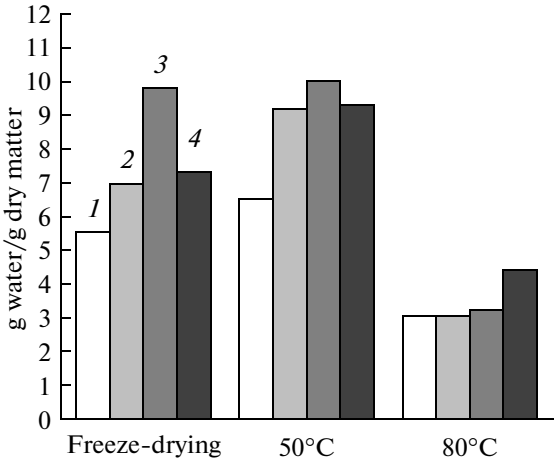

Fig. 3. The WHC of the *P. variotii* strains dried under different conditions. *1*—*P. variotii* 101; *2*—*P. variotii* 102; *3*—*P. variotii* 439; *4*—*P. variotii* 440.

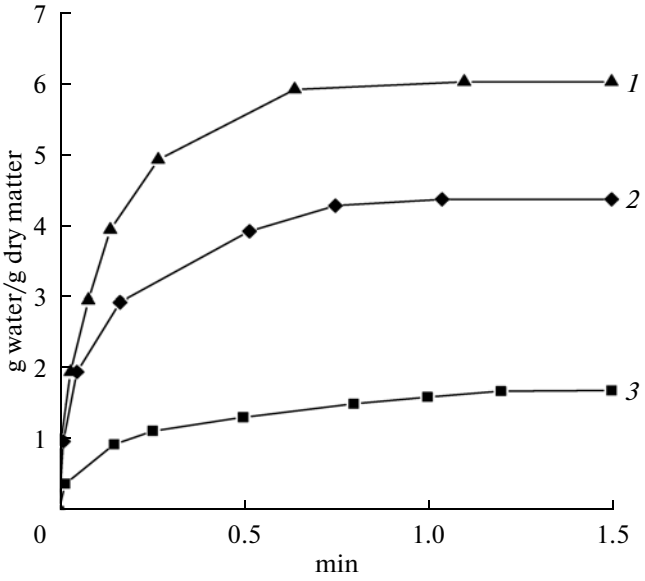

Fig. 4. Water absorption kinetics for *P. variotii* strain 101 dried under different conditions: *1*—freeze-drying, *2*—50°C and *3*—80°C.

water absorption kinetics for *P. variotii* 101. The samples dried at 50°C and freeze-drying showed rapid water absorption, with $t_{1/2}$ values shorter than 0.1 min. Freeze-dried mycelia exhibited the highest values of WAC (mean values: 6.4 g of water/g of dry matter). These values correspond to losses in WAC of 17% at 50°C and 75% at 80°C, respect to freeze-dried mycelia.

As a consequence of drying to 80°C, a pronounced loss of both hydration properties analyzed was observed. This effect could be related to the total protein denaturation of mycelia at this temperature.

Effect of particle size. Mycelia dried at 50°C were used for this assay. The larger particles of mycelia tended to improve WHC, except for *P. variotii* 101, where no significant differences ($p < 0.05$) for the different particle sizes were observed (Table 1). For WAC determination, the 500–850 μm particles showed the highest values for all strains (Fig. 5), except for *P. variotii* 440. The kinetic values obtained for the larger particles were not reproducible among the repetitions. This was probably due to the lack of uniformity in the particle population.

Table 1. WHC (g of water/g of dry matter) of mycelial particles from different strains of *P. variotii* dried at 50°C*

Fungal strain	Particle size, μm		
	<500	500–850	>850
P. variotii 102	8.8 ± 0.1^{a}	10.8 ± 0.9^{b}	10.3 ± 0.9^{b}
P. variotii 439	9.2 ± 0.7^{a}	10.4 ± 0.8^{ab}	12.6 ± 1.2^{b}
P. variotii 440	6.8 ± 0.3^{a}	7.5 ± 0.1^{a}	8.6 ± 0.5^{b}
P. variotii 101	7.8 ± 0.4^{a}	7.4 ± 0.3^{a}	7.7 ± 0.4^{a}

* All results are mean values and S.D. of 3 replicates. Values followed by the different letter (a, b) within the row are significantly different ($p < 0.05$) by the LSD test.

Effect of the environmental medium. At pH 5.0 and 7.0 in the presence of 0 and 1% of NaCl mycelia samples exhibited the highest values of WAC at shorter times. As the concentration of NaCl increased, the WAC decreased, which is in agreement with that found in previous works on yeast protein [27] and isolated soy protein [23]. The reduced WAC can be attributed mainly to the shielding effect of NaCl ion pairs, which prevents water molecules from interacting with protein molecules [28].

At pH 7.0 0.1 M phosphate buffer increased the holding capacity of the mycelia compared to distilled water.

Table 2 demonstrates a trend towards a decrease in WHC at pH 7.0 as the NaCl concentration increased, reaching an average maximum decrease of 20% (3% NaCl) compared to control (0%), being *P. variotii* 102 the strain that suffered the sharpest decrease (26%).

At pH 5.0, the trend was similar but with lower absolute values. The mean maximum decrease was 14% (3% NaCl) compared to control, being *P. variotii* 439 the strain that suffered the largest decrease (16%).

Because we found no data on hydration properties of whole organisms, we compared our results (mean value of 11.9 g of water/g of dry mycelia) with works performed on proteins isolated from different sources.

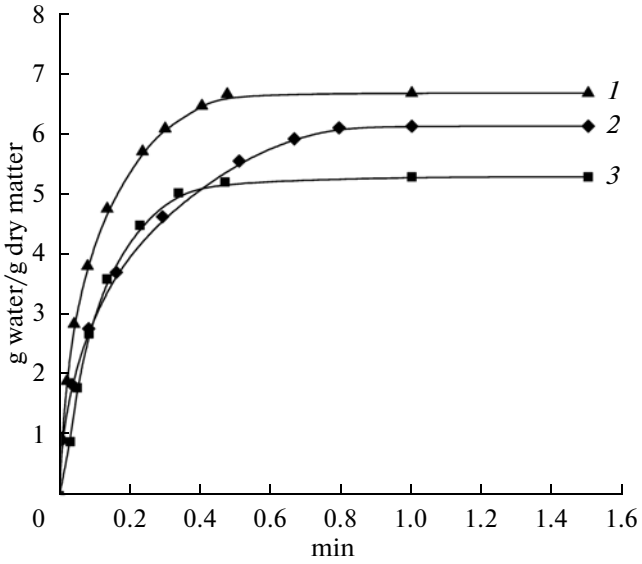

Fig. 5. Influence of particle size of mycelia on water absorption kinetics for strain *P. variotii* 439 dried at 50°C: *1*—500–850 μm, *2*—<500 μm, and *3*—>850 μm.

Thus, protein from plant sources, such as soybean, cottonseed and sunflower, exhibited WHC values ranging from 2.5 to 6.0 g of water/g of protein [27]. Protein isolates from yeast (*S. cerevisiae* and *K. fragilis*) treated under different conditions of incubation and precipitation exhibited WHC values from 3.58 to 6.94 g of water/g of protein [26]. These values of WHC are lower than those obtained in this work for *P. variotii* with 0% NaCl at pH 7.0 dried at 50°C. These results revealed the important contribution provided by the fibers, in addition to the protein, in the WHC of the whole organisms.

Effect of hot water on WHC. The WHC values were not negatively modified when hot water was used instead of room temperature water (Table 3). After these assays, DSC determinations were performed on the pellets obtained. A complete protein denaturation was observed for the pellets treated with hot water (data not shown). These results suggest that rehydration in hot water, although producing total protein denaturation, does not affect WHC values.

Viability of *P. variotii* dried at 50°C. The growth rate of *P. variotii* dried at 50°C was 19.9 mm/day and 18.1 mm/day at 25°C for *P. variotii* 101 and *variotii* 440, respectively. There was no germination of these fungi when suspensions were incubated at 5°C for 30 days.

Wet mycelia of *P. variotii* exhibit good water holding capacity. The drying process required for the use of mycelia as a food ingredient affected both the WHC and WAC of mycelia. Drying at 50°C, at which protein denaturation was only partial, the WHC values were higher than those obtained with freeze-drying. Although the rehydration of fungal strains denatured the proteins completely in hot water at 80°C, it did not influence the WHC. On the other hand, drying at 80°C significantly reduced the hydration properties. This result might be due to a simultaneous process of protein denaturation and aggregation.

Environmental conditions, such as pH and ionic strength of the growth medium, also influence the water absorption and holding capacities, although not more than 20%. This behavior could be attributed mainly to the effect of the mentioned parameters on mycelial proteins and polysaccharides. These properties are also dependent on the particle size of dried mycelia.

The results of this study demonstrate the potential use of *P. variotii* in food, marketed dry to be then reconstituted in both cold and hot aqueous media, and be consumed at the time or after a short cooling time. However, this product should be subjected to rigorous toxicity studies imposed by the Food and Drug Administration (USA) for new ingredients and/or additives in food.

Table 2. WHC (g of water/g of dry matter) of different strains of *P. variotii* dried at 50°C and pH 7 for particles of 500–850 μm*

Fungal strain	NaCl, %			
	0	1	2	3
P. variotii 102	12.6 ± 0.7^{b}	12.9 ± 0.1^{b}	9.8 ± 0.3^{a}	9.1 ± 0.7^{a}
P. variotii 439	15.7 ± 0.9^{b}	13.2 ± 0.5^{a}	12.9 ± 0.9^{a}	12.5 ± 0.2^{a}
P. variotii 440	10.9 ± 0.7^{b}	10.3 ± 0.4^{b}	8.4 ± 0.2^{a}	9.1 ± 0.3^{a}
P. variotii 101	8.5 ± 0.1^{bc}	9.1 ± 0.2^{c}	8.1 ± 0.5^{ab}	7.7 ± 0.2^{a}

* All results are mean values and S.D. of 3 replicates. Values followed by the different letter (a, b, c) within the row are significantly different ($p < 0.05$) by the LSD test.

Table 3. WHC (g of water/g of dry matter) of *P. variotii* 101 and *P. variotii* 440 dried at 50°C under different measurement conditions*

Strain	Conditions	WHC
P. variotii 101	A	6.54 ± 0.35
	B	6.57 ± 0.21
	C	6.16 ± 0.08
P. variotii 440	A	6.47 ± 0.21
	B	6.67 ± 0.14
	C	7.65 ± 0.21

* A—100 mg of sample + 9 ml of distilled water at room temperature. B—100 mg of sample + 1 ml of distilled water at room temperature and 8 ml of hot distilled water (80°C). C—100 mg of sample + 9 ml of hot distilled water (80°C).

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