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β-Glucan Content and Hydration Properties of Filamentous Fungi

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Abstract—The aim of this work was to isolate and identify filamentous fungi from several sources to study the dietary fiber and β-glucan content. The fungal hydration properties such as water absorption and water holding capacities were also evaluated. Total dietary fiber of isolates exhibited a noticeable variability from 16 to 53% and the highest values were obtained for the genera *Paecilomyces* and *Penicillium*, a fact consistent with a higher content of β-glucans (24 and 17%, respectively), higher than previously reported for Basidiomycetes and yeast. We observed a large decrease (75%) in the water holding capacity when the mycelia were dried. Isolates of filamentous fungi with greater water holding capacity also exhibited greater absorption capacity. *Paecilomyces variotii* and *Penicillium nalgiovense* had the best hydration properties. Our results contribute to the search for new unconventional ingredients providing a high protein and β-glucans content. The addition of these dried mycelia could change the hydration properties in the food system.

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Fungi have been part of the human diet for thousands of years either as a food item itself, especially mushrooms, or as part of fermented foods like yeasts used in bread and beverages. Other well-known fermented products using filamentous fungi are blue and white cheeses, sausages, tempe and miso [1, 2].

Since the sources of animal protein are becoming insufficient, several studies have focused on the search for suitable microbial sources as substitutes of conventional food proteins [3]. Microbial proteins compete successfully with animal protein due to their nutrient profile. In this sense, there are numerous works on the use of unicellular fungi as a diet supplement, but not so many filamentous fungi have been used for this purpose so far [4]. These fungi are ideal as nutritional supplement because they have a fairly high content of protein, which contains all essential amino acids and are virtually free of cholesterol [5]. However, since vegetable protein sources are abundant in the market at competitive prices, microbial food products of the 21-st century can not base their market success solely on their protein content. They should offer additional features, such as probiotic effects, as well as technological functionality, such as rheological properties. An example of this is mycoprotein Quorn extracted from *Fusarium venenatum*. This product is the only source of mycoprotein for human consumption, where the filamentous nature of the organism provides the final product a resemblance to animal or fish meat, thus being favorably compared to soybean protein and often considered "a meat substitute". Nowadays, its sale is growing in Europe and spreading also to the USA [6, 7]. On the other hand, edible mushrooms are a potential source of dietary fiber: fungal cell walls contain chitin, other hemicelluloses, mannans, and P-glucans. The latter have attracted attention because of their bioactive and medicinal properties such as their immune-stimulating, anti-inflammatory, antimicrobial, anti-infective, anti-viral, anti-tumoral, cholesterol lowering, radioprotective and wound-healing properties [8, 9]. Manzi [10] studied different mushroom species and observed a large variability in their β-glucan content (0.22 to 0.53 g/100 g on dry basis). However, this component has not yet been studied in filamentous fungi.

Due to the structure of filamentous fungi with high levels of proteins and polysaccharides [7, 11], they could have food applications as ingredients in wettable food with suitable functional properties as water absorption and holding capacities.

Water absorption capacity (WAC) indicates the ability of a material to absorb water in its structure spontaneously when it comes in contact through a surface that remains wet. Water holding capacity (WHC) means the ability of a hydrated material to retain water against the action of an external, force of centrifugal gravity or compression. Both WAC and WHC are relevant according to the food system [12],

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Principal components of different genera of fungi

Fungi	Composition, % w/w			
	protein	TDF *	β-glucans	RNA
Absidia corymbifera [3]	36.0 ± 7.0	23.9 ± 3.7	0.7 ± 0.6	4.8 ± 0.1
Mucor hiemalis [3]				
Mucor circinelloides [2]	40.6 ± 3.1	24.5 ± 4.3	1.9 ± 1.8	6.1 ± 0.7
Mucor racemosus [1]				
Rhizopus oryzae [3]	47.5 ± 8.2	16.3 ±5.3	0.9 ± 0.3	3.7 ± 0.2
Fusarium gramineraum [1] *Fusarium* sp. [7]	37.7 ± 8.3	34.0 ± 11.4	6.0 ± 1.3	4.6 ± 1.5
Aspergillus candidus [3]	36.0 ± 2.4	35.9 ± 0.1	3.4 ± 1.2	4.5 ± 0.2
Trichoderma harzianum [3]	44.5 ± 2.1	27.2 ± 6.8	6.2 ± 0.8	6.2 ± 0.2
Paecilomyces lilacinus [1] *Paceilomyces variotii* [4]	29.9 ± 5.7	51.7 ± 5.5	23.8 ± 2.4	3.6 ± 0.5
Penicillium nalgiovense [7]	31.0 ± 3.8	53.3 ± 5.2	17.0 ± 3.5	3.8 ± 0.6

* TDF–total dietary fiber.

dietary fiber and β-glucan content. Hydration properties, such as WAC and WHC were also measured.

MATERIALS AND METHODS

Thirty-seven non-toxic hyaline fungi strains isolated from cereals and vegetables were identified according to Samson et al. [13] and Pitt and Hocking [14]. Toxicity of fungi was characterized by the *Artemia saline* bioassay [15]. The positive control of toxicity was *Fusarium graminearum* NRRL 22198, while *F. graminearum* A 3/4 NRRL 26139 (QuornTM) used as the negative control. These control strains were kindly provided by Kerry O'Donnell (United States Department of Agriculture, USA).

Isolated fungal strains belonged to the genera *Paecilomyces* [5], *Fusarium* [8], *Mucor* [6], *Absidia* [3], *Rhizopus* [3], *Trichoderma* [3], *Aspergillus* [3] and *Penicillium* [7], After inoculation of 1×10^5 conidia/ ml the cultures were grown in liquid YES medium containing (g/L): yeast extract—20 and sucrose—40, at 25°C and 135 rpm for 7 days.

Samples of mycelium were collected by filtration through Whatman JVel filter paper (England) under vaccum, washed twice with distilled water, dried at 50°C in stove with air circulation until constant weight, ground and sieved (0.5 mm mesh). Dry mycelia were analyzed to determine protein [16], total dietary fiber (TDF) [17, 18], RNA [19] and β-glucan content.

TDF content was determined according to the AOAC (http://www.aoac.org/) method using Megazyme TM commercial kit (Ireland). The AOAC method is an enzymatic-gravimetric procedure. In brief, aliquots of dried filamentous fungi (1.0 g of dry matter) were treated with 2 amylases, a heat stable α-amylases (Megazyme cat. N° E-BLAAM, 10.000 U/mL on soluble starch) for 15 min in a boiling water, then with an amyloglucosidase (Megazyme cat. N° E-AMGDF, 3300 U/ mL on soluble starch) for 30 min at 60°C to remove glycogen, and a purified protease (Megazyme cat. N° E-BSPRT-350 tyrosine units/mL) for 30 min at 60°C to solubilise protein. After an ethanol precipitation, the ethanol-insoluble residue recovered by filtration was dried and weighed.

For the β-glucan assay, the Megazyme TM commercial kit (Ireland) was used. Briefly, the dried mycelia were solubilized in 1 ON hydrochloric acid and then extensively hydrolyzed by 1.3 N HC1 at 100°C for 2 h. After that, a hydrolysis incubation at 40°C for 60 min with 0.1 mL of a mixture of highly purified exo–1,3-β-glucanase (20.0 U/mL) plus β-glucosidase (4.0 U/ml) was performed. D-glucose formed was measured at 510 nm [20].

WHC and WAC, as hydration properties, were also determined. WAC was determined on dried mycelia, while WHC was determined on both wet (before drying)

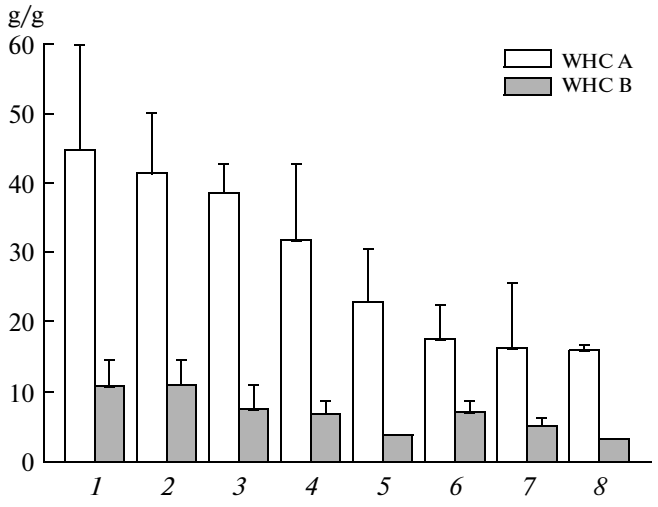

Fig. 1. Water holding capacities of wet (WHC A) and dried (WHC B) mycelia by different species of filamentous fungi. *1—Penicillium nalgiovense, 2—Paecilomyces lilacinus* and *P. variotii*, *3—Aspergillus candidus*, *4—Fusarium graminearum* and *Fusarium* sp., *5—Trichoderma harzianum*, *6—Mucor hiemalis*, *M. racemosus* and *M. circinelloides*, *7—Rhizopus oryzae*, *8—Absidia corymbifera*.

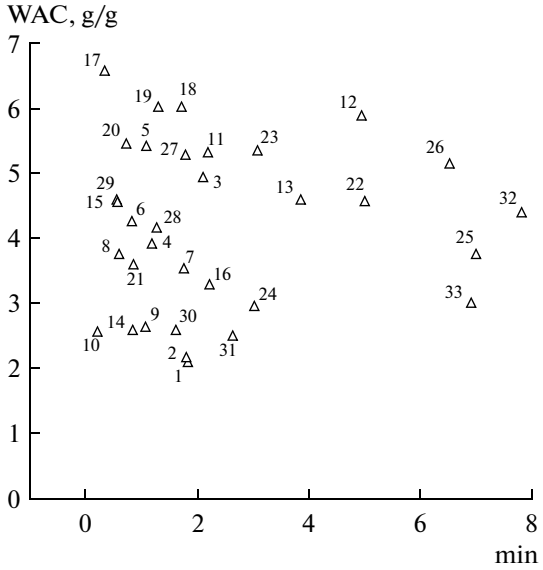

Fig. 2. Relationship between WAC (g of weight/g of dry sample) and absorption time (min) of different strains of filamentous fungi. *1—Absidia corymbifera* T62, *2—A. corymbifera* T61, *3—Fusarium graminearum* NRRL 26139, *4—Fusarium* sp. 201, *5—Fusarium* sp. 213, *6—Fusarium* sp. 214, *7*—Fusarium sp. 235, *8—Fusarium* sp. 237, *9*—Fusarium sp. 239, *10—Fusarium* sp. 229, *11—Mucor circinelloides* G31, *12—M. circinelloides* G32, *13—M. hiemalis* A11, *14—M. racemosus* 250, *15—M. hiemalis* 307, *16—Paecilomyces lilacinus*, *17—P. variotii* 101, *18—P. variotii* 102, *19—P. variotii* 440, *20—P. variotii* 439, *21—Penicillium nalgiovense* 262, *22—P. nalgiovense* S1-2, *23—P. nalgiovense* S14–4, *24—P. nalgiovense* S15–1, *25—P. nalgiovense* S15–3, *26—P. nalgiovense* S16-2, *27—P. nalgiovense* S35–1, *28—Aspergillus candidus curso*, *29—A. candidus* 508, *30—Trichoderma harzianum* 464, *31—T. harzianum* 465, *32—Rhizopus oryzae* 503, *33—P. oryzae* 514.

and dried mycelia. For WHC determination, 100 mg of sample was stirred with 9 mL of water in an orbital shaker (for 2 h at 100 rpm), then centrifuged (for 30 min at 800 g) and finally, the pellet was weighed. Water absorption kinetics was followed using the Baumann equipment for 50 mg of sample [21]. Maximum amount of water absorbed (WAC) and time to reach WAC were measured. WHC and WAC were expressed as g of water/g of dry matter. In this assay, we also analyzed dried samples of lean beef, *Agaricus bisporus* (mushroom) and commercial soy isolate (dried at 50°C to constant weight) as controls. Samples were dried as previously described.

RESULTS AND DISCUSSION

Table shows the principal components of filamentous fungi studied. The protein content was in the range of 30–47%, being the highest value for the genus *Rhizopus*. On the other hand, great intra-species variability was observed in all genera. RNA content was 3.6–6.2%, in agreement with the values (2.5–6.0%) obtained for mushrooms [4].

Total dietary fiber exhibited a noticeable variability 16.3–53.3%, which agrees with that previously reported for edible fungi and yeast [22–25]. The highest values were obtained for the genera *Paecilomyces* and *Penicillium*, a fact consistent with a higher content of β-glucans (23.8 and 17.0%, respectively). These values are at least three times higher than the β-glucan content in the rest of the genera studied. Also they are higher than those previously reported for Basidiomycetes and yeast [10, 26–28]. In our study, β-glucans represent 46 and 32% of their total dietary fiber for *Paecilomyces* and *Penicillium*, respectively. In 2004 Manzi [29] has reported that for commercial mushroom (*Boletus group, Agrocybe aegerita* and *Pleurotus eryngii*), β-glucans constitute between 2 and 13% of their total dietary fiber. On the other hand, Williams [26] found that in yeast this value was 22%. Thus, filamentous fungi *Paecilomyces* and *Penicillium* could be used as a source of this polysaccharide.

With respect to hydration properties, Fig. 1 shows that the lowest values for WHC in wet mycelia were those of Zygomycetes genera (*Absidia, Mucor* and *Rhizopus*), being in average 16.5 g of water/g of dry sample, according with mean values of β-glucans lower than 2.0% (Table). The highest values of WHC corresponded to the genera Paecilomyces and Penicillium, with an average of 43.2 of water/g of dry sample, according with the higher values of β-glucans. *P. lilacinus* show the lower WHC values inside the genus (data not showed). We observed a large decrease

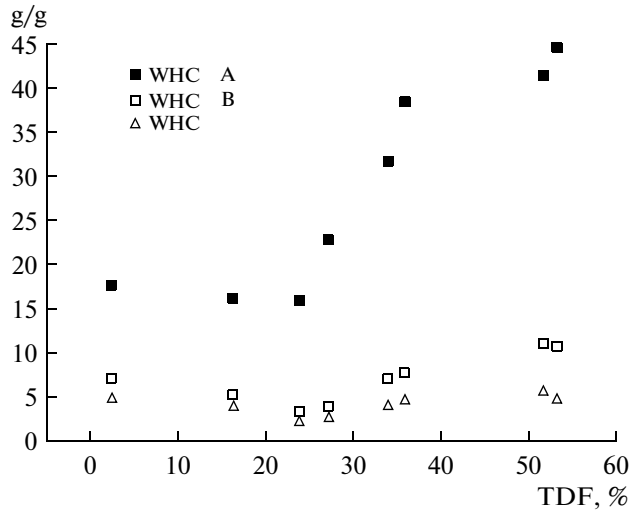

Fig. 3. Relationship between hydration properties and total dietary fiber in different genera of filamentous fungi. WHC A—water holding capacity of wet mycelia; WHC B—water holding capacity of dried mycelia; TDF—total dietary fiber.

(75%) in the WHC, when the mycelia were dried, except for isolates of the genus *Mucor*, which showed a decrease lower than 60%. The latter genus together with *Paecilomyces, Penicillium* and *Aspergillus* had the highest water retention for mycelium in dried state (Fig. 1).

We observed a rapid water absorption velocity, which reached the maximum value in less than 2 min for 60 % of the isolates of filamentous fungi (Fig. 2). It is important to highlight that the isolates belonging to the species *Paecilomyces variotii* presented the highest values of WAC, with an average of 6 g water/g dry sample. These values were higher than those found for dried lean beef (2.93 g water/g dry sample) and the dried *Agaricus bisporus* mycelia (2.19 g water/g dry sample). The WAC value found for the commercial soy isolate was 8.5 g water/g dry sample, which is consistent with that found in previous reports on soy protein isolates [30, 31]. Although this value exceeds by 30% those achieved for *P. variotii* strains, the latter reached maximum WAC twice faster. Taking into account this fact, the results of *P. variotii* are promising.

The highlights of this work are: (1) the isolates of filamentous fungi with greater water retention also exhibit greater absorption capacity in the most cases; (2) *P. variotii* and *Penicillium nalgiovense* show the highest content of dietary fiber and are the species with the best hydration properties; (3) a relationship between hydration properties and total dietary fiber of different genera of filamentous fungi was found (Fig. 3); (4) the species belonging to *P. variotii* and *P. nalgiovense* have a β-glucan content much higher than that reported for Basidiomycetes and yeast, which is a very important finding that had not been reported so far.

Our results contribute to the search for new unconventional ingredients providing a high protein and β-glucans content. The addition of these dried mycelia could contribute the hydration properties in the food system. Further studies are needed for the search of a versatile food matrix for the incorporation of these ingredients and the influence of technological parameters on the functionality of the matrix.

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REFERENCES

1. Wainwright, M., *Introducción a la Biotecnologia de los Hongos*, Zaragoza: Acribia S.A, 1995.
2. Thrane, U., *Food Mycology. A Multifaceted Approach to Fungi and Food*, Dijksterhuis, J. and Samson, R., Eds., Utrecht: CRC Press, 2007, pp. 353–360.
3. Snyder, H.E., *Adv. Food Res.,* 1970, vol. 18, pp. 85–140.
4. Villalobos, A., *Agronomía Mesoamericana*, 2004, vol. 15, no. 1, pp. 93–106.
5. Moore, D. and Chiu, S.W., *Bio-Exploitation of Filamentous Fungi*, Pointing S.B. and Hyde, K.D., Eds., Hong Kong: Fungal Diversity Press, 2001, pp. 223–251.
6. Edelman, J., *J. Chem. Technol. Biotechnol.,* 1988, vol. 43, pp. 279–283.
7. Weibe, M.G., *Appl. Microbiol. Biotechnol.*, 2002, vol. 58, pp. 421–427.
8. Cheung, P., *J. Nutr.*, 1998, vol. 128, no. 9, pp. 1512–1516.
9. Freimund, S., Sauter, M., Kappeli, O., and Dutler, H., *Carbohydr. Polym.*, 2003, vol. 54, no. 2, pp. 159–171.
10. Manzi, P. and Pizzoferrato, L., *Food Chem.*, 2000, vol. 68, no. 3, pp. 315–318.
11. Rodger, G., *Food Technol.*, 2001, vol. 55, no. 7, pp. 36–41.
12. Fennema, O., *Quimica de los Alimentos,* 2nd ed., Zaragoza: Acribia, 2000.
13. Samsom, R.A., Hoekstra, E.S., Frisvad, J.C. and Filtenborg, O., *Introduction to Food- and Airborne Fungi*, 6th ed., Baarn, Netherlands: Centraalbureau voor Schimmelcultures, 2000.
14. Pitt, J.I. and Hocking, A.D., *Fungi and Food Spoilage*, 3rd ed., Dordrecht: Springer, 2009.
15. Harwing, J. and Scott, P.M., *Appl. Microbiol.*, 1971, vol. 21, no. 6, pp. 1011–1016.
16. *Official Methods of Analysis*, 16th ed., Washington: Association of Official Analytical Chemists, 1996.
17. Prosky, L., Asp, N.-G., Schweizer, T. F., DeVries, J. W. and Furda, I., *J. Assoc. Off. Anal. Chem.*, 1988, vol. 71, pp. 1017–1023.

18. Lee, S.C., Prosky, L. and DeVries, J.W., *J. Assoc. Off. Anal. Chem.*, 1992, vol. 75, pp. 131–133.
19. Rut, M., *Kvasny Prumysl*, 1973, vol. 19, pp. 131–133.
20. Mixed-Linkage beta-Glucan Assay Procedure, K-BGLU 07/11 (McCleary Method), Megazyme, 1985.
21. Torgensen, H. and Toledo, R.T., *J. Food Sci.*, 1977, vol. 42, no. 6, pp. 1615–1618.
22. Regula, J. and Siwulski, M., *Acta Scient. Polon. Technol. Aliment.*, 2007, vol. 6, no. 4, pp. 135–142.
23. Díez, V.A. and Alvarez, A., *Food Chem.*, 2001, vol. 75, no. 4, pp. 417–422.
24. Cheung, P.C.-K., *J. Sci. Food Agric.*, 1997, vol.73, no. 2, pp. 225–260.
25. Kalac, P., *Food Chem.*, 2009, vol. 113, no. 1, pp. 9–16.
26. Williams, D.L., McNamee, R.B., Jones, E.L., Pretus, H.A., Ensley, H.E., Browder, I.W., and DiLuzio, N.R., *Carbohydr. Res.*, 1991, vol. 219, pp. 203–213.
27. Y.-T., Ko and Y.-L., Lin, *J. Agric. Food Chem.*, 2004, vol. 52, no. 11, pp. 3313–3318.
28. Kim, K.S., Chang, J.E., and Yun, H.S., *Enzyme Microb. Technol.*, 2004, vol. 35, no.6–7, pp. 672–677.
29. Manzi, P., Marconi, S., Aguzzi, A., and Pizzoferrato, L., *Food Chem.*, 2004, vol. 84, no. 2, pp. 201–206.
30. Wagner, J. and Añón, M., *J. Food Sci.*, 1990, vol. 55, no. 3, pp. 765–770.
31. Añón, M., Sorgentini, D., and Wagner, J., *J. Agric. Food Chem.*, 2001, vol. 49, no. 10, pp. 4852–4858.