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Nutritional Enhancement of Barley in Solid State Fermentation by *Rhizopus oligosporus* ML-10

Rubina Nelofer[1]*, Muhammad Nadeem[1], Muhammad Irfan[2] and Quratulain Syed[1]

*[1]*Department of Biotechnology and Food Research Center, Pakistan Council of Scientific and Industrial Research (PCSIR), Pakistan*

[2]*Department of Biotechnology, University of Sargodha, Pakistan*

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***Corresponding author:** Dr. Rubina Nelofer, Department of Biotechnology and Food Research Center, Pakistan Council of Scientific and Industrial Research (PCSIR), Laboratories Complex, Pakistan, Email: rubinanelofer@gmail.com

Abstract

The present study was undertaken for nutritional enhancement of whole barley grains by optimizing solid state fermentation conditions with *Rhizopus oligosporus* ML-10 in polythene bags. The influence of various soaking time, boiling time, inoculum size, incubation time and temperature on the levels of proteins, fats and carbohydrates were evaluated during fermentation of barley. 500grams whole barley grains after soaking for 12h in 1L distilled water (pH, 4.5) were allowed to boil for 20min followed by drying at 80 °C for 15min. After this pretreatment, dehulled barley grains were inoculated with 1.5 % (v/w) spore suspension containing 1.0 x10^6 spores per mL of 96h aged *R. oligosporus* ML-10 in 25 x 25 cm2 polythene bags and incubated at 30 °C for 36h. The nutritional value of barley in terms of protein contents increased significantly from 10.25% to 16.85% after solid state fermentation. However, the level of total carbohydrate and fat after fermentation process was found to decrease from 68.6% to 63.55 and 2.13 % to 1.62 % respectively. From the results of present study it was concluded that the solid state fermentation of whole grain barley resulted in increasing the protein contents up to 64.3 % under optimized conditions by *Rhizopus oligosporus* ML-10 in polythene bags.

Keywords: Barley; Solid state fermentation; Protein; Fat; Carbohydrates; *R. oligosprous*; Nutritional value; Protein; Calorie; *Hordeum vulgare*

Introduction

Fermented foods either from plant or animal origin are commonly used in all parts of the world as a low cost protein source which fulfill the protein/calorie deficiency problems especially in developing countries where meat products are in poor supply [1- 4]. Barley is one of the most popular staple foods in Pakistan and many other countries. The barley (*Hordeum vulgare*) is reported to contain (w/w) 6.75 % crude fiber, 11.67% crude protein, 2.31 % crude fat and 2.22 % ash [5] .The consumption of barley and its products is found to assist in decreasing the risk of type 2 diabetes [6-7]. Moreover, it also helps in reducing the total serum lipids and LDL-cholesterol which in turn reduces the risk of cardiovascular diseases [8-9]. Whole grain barley has also been reported as a rich source of phytochemicals [10]. Therefore, increasing consumption of fermented cereals, would lower food cost and promote better health [11].

Microbial fermentation is also considered as one of the oldest and most economical methods for value additions and preservation of food. The fermentation process helps in increasing the digestibility and bioavailability of proteins, carbohydrates, lipids, minerals and vitamin contents in cereals. Moreover, it shortens the cooking time and increases the microbial safety [12].

The present study was undertaken to enhance the nutritional value of barley by optimizing the process parameters such as soaking and boiling time, inoculum size, incubation time and temperature, in solid state fermentation to study their effect on protein, fat and carbohydrates levels.

Materials and Methods

Microorganism maintenance

The fungal strain of *Rhizopus oligosporus* ML-10 used in the present study was obtained from Microbiology Lab, Biotechnology and Food Research Centre, PCSIR Labs Complex, Lahore. The culture was grown on potato dextrose agar (Oxoid) slant at 30 °C for 96h.The culture was then preserved at 4 °C in refrigerator and sub-culturing was done after every 4-6 week for further study.

Preparation of inoculums

Ten ml of sterilized distilled water was transferred to 96h aged potato dextrose agar slant of *Rhizopus oligosporus* ML-10. The spores were dislodged by using a sterile inoculation needle under aseptic conditions. The spore suspension containing approximately 1.0 x10^6 spores per mL was used as inoculum for the fermentation of pretreated barley grains with *Rhizopus oligosporus* ML-10.

Substrate: The whole barley grains used in the present study was purchased from local

Market of Lahore, Pakistan.

Solid-state fermentation

The solid state fermentation of barley was carried out in accordance with the modified method of Berg et al. [11]. 500g of whole barley grains were soaked for 12h in 1L distilled water in plastic beaker at room temperature. After manual dehulling, the soaked barley grains were allowed to boil in tap water for 20 min. Decant off water and kept the grains in oven at 80 °C for 15 min to remove excess of water. Then the spore suspension of *Rhizopus oligosporus* ML-10 at the rate of 1.5 % (V/W) was mixed well with pretreated barley grains at room temperature. Packed the inoculated barley grains in pre- holed 25 x 25cm^2 polythene bags and kept at 30 °C for 36h. All the experiments were conducted in triplicate. The flow sheet of the process is given in Figure 1.

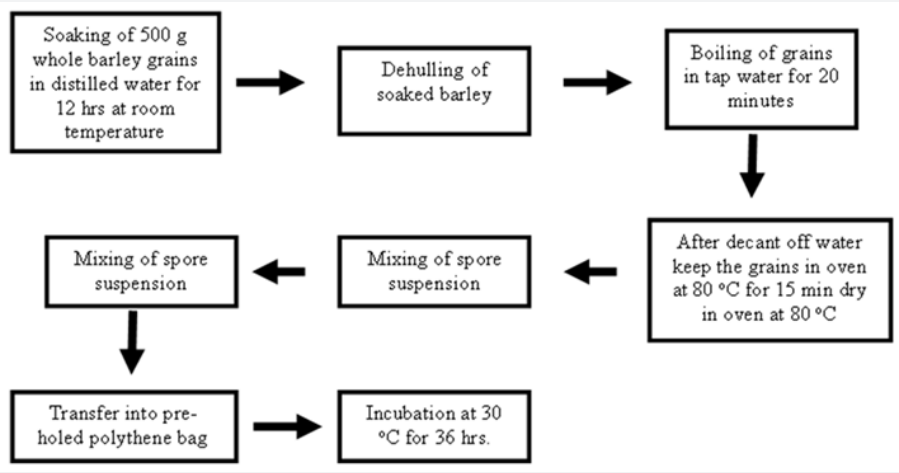

Figure 1: Flow sheet of the process for the fermentation of Barley by *Rhizopous oligosporus* ML-10.

Proximate composition: Moisture, Fat, total carbohydrates and total nitrogen (microkjeldahl) were determined according to AOAC [13].

Result and Discussion

Effect of soaking time

The soaking time on the fermentation of barley is important in solid state fermentation due to its effect on the softening of kernels for smooth mycelial growth of the *Rhizopus oligosporus* ML-10. Therefore, soaking time was varied from 6, 12, 18 and 24h in distilled water at room temperature. The data in Figure 2 revealed that the soaking time of 12h was found to be optimum for the production of nutritionally rich fermented barley with the increase in protein contents from 10.25 % to 16.55 %. Further increase in soaking time resulted in decreasing the protein contents as evident from Figure 2. However, the percentage of carbohydrates and fat in fermented barley was found to decrease slightly from 65 to 63.5 % and from 1.72 % to 1.52 %, respectively, with the increase of soaking time. It has been reported earlier that during soaking of soybean, organic acids are formed which resulted in lowering the pH from 6.0 to 3.9 [14]. Moreover, during soaking, the bacterial fermentation occurred resulting in acidification. Many workers, described the addition of < 0.5% lactic acid or <0.25 % acetic acid during soaking stage which lowered the initial pH, allowing the mould to grow and suppress the bacterial growth [15]. In the present study, the pH was also noted to decrease from 6.5 to 4.3. Our results are in good agreement with earlier investigations that a natural microbial acidification occurred during the soaking process in Indonesia soybeans fermentation [16]. According to other investigators, the changes observed during soaking were due to leaching of soluble components and also due to enzyme activities during sprouting and fermentation [17,18].

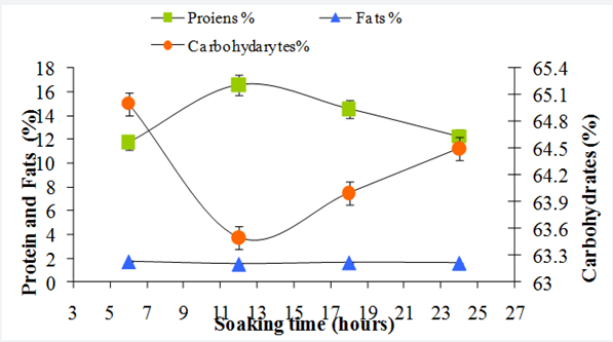

Figure 2: Effect of soaking time on protein, fat and carbohydrate content of barley by *Rhizopus oligosporus* ML-10 at 30 °C for 36h. Bars represents the SD among triplicates.

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Effect of boiling time

Partial cooking or boiling of the cereals was also known to play a vital role in the production of fermented food. Therefore, boiling time ranging from 15 to 30mints was evaluated for the production of nutritionally rich barley in solid state fermentation as given in Figure 3. Boiling of cereals in water has been reported to destroy contamination bacteria and anti nutritional factors and also release of some nutrients which are essential for mould growth [15]. According to Steinkraus et al. [12] a heat stable and water soluble mold inhibitor is leached out during boiling process, which is discarded later. The results in Figure 3 revealed that combination of soaking and boiling was most favorable for the growth of *Rhizopus oligosporus* ML-10. Longer boiling time alone did not give the same elasticity of the seed as did the combination of soaking and boiling. For longer boiling the gelatinization of starch content occurred and the cake formation deteriorated. Therefore, the boiling time of 15min was found to be suitable for the production of fermented whole grain cereal by *Rhizopus oligosporus* ML-10 in solid state fermentation. Mulyowidaro et al. [19] reported that mild boiling (15 min at 95 °C) of soaked and biologically acidified beans results in sufficient survival of a mixed flora of lactic acid bacteria and *Bacillus* spp. A strong proteolytic *Bacillus* spoilage was also observed in beans which had been heated for 60 min at 95 °C or 15 min at 121 °C [14]. Moreover, these workers also hypothesized that the lack of beany flavor in tempeh was due to the result of inactivation of the lipooxygenase associated with the formation of such flavors during the boiling stage. According to earlier investigations, the combination of cooking and fermentation improved the nutrient quality of all tested sorghum seeds and reduced the content of anti nutritional factors to a safe level in comparison with other methods of processing [17].

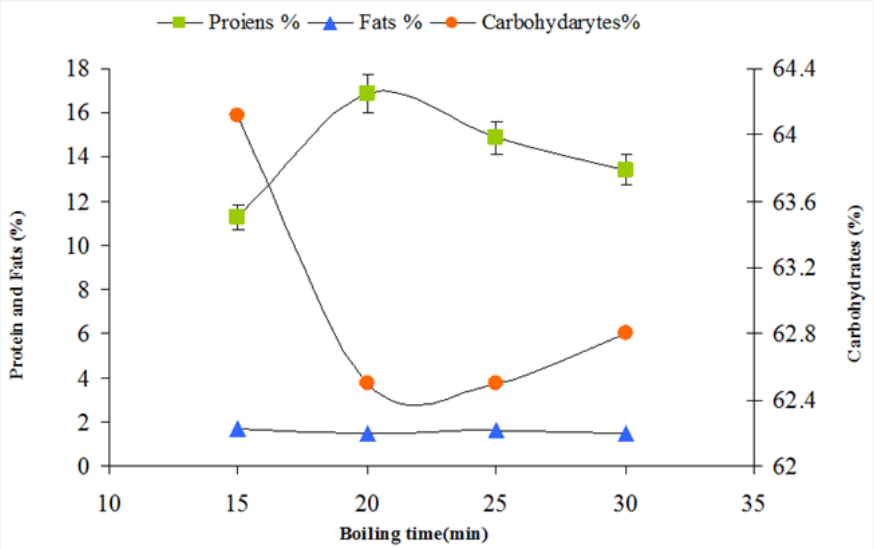

Figure 3: Effect of boiling time on protein, fat and carbohydrate content of barley by *Rhizopus oligosporus* ML-10 at 30 °C for 36 h. Bars represents the SD among triplicates.

Effect of inoculum size

Homogeneous seedling of substrate with adequate spore inoculation along with rapid starting of growth is important to achieve successful fermentation of the cereals for improved nutritional quality. *Rhizopus oligosporus* is the most preferred fungus in tempeh fermentation due to rapid growth rate at high temperature, high proteolytic and lypolytic activities and strong antioxidant properties [20]. Therefore, various inoculum sizes of 96hrs aged *Rhizopus oligosporus* ML-10 containing 10^6 spores/ml was used for nutritional enhancement of barley by fermentation. In Figure 4, the data shows that with low inoculum size 0.5 % (v/w) the fungus grew slowly and barley dense cake formation did not form as also indicated from low protein contents 9.01 %. This slow growth may increase the risk of contamination in barley cereals with microbes. The optimum inoculum size of 1.5 % (v/w) containing 1.0 x10^6 spores of *Rhizopus oligosporus* ML-10 per ml was found to enhance protein contents up to 10.55 % in fermented barley. At high inoculum size (2 %), the fungus grew rapidly and the dense cake formation was uneven resulting in slight decrease in protein level, whereas the carbohydrates level significantly decreased down to 62.5 %.

These findings coincide with the earlier investigations regarding tempeh production by using different substrates [14, 21,22]. Some workers reported that 3.35x10^4cfu/gm is the optimum level of inoculum for quinoa tempeh [23]. Similar findings have also been reported by Xin-Mei Feng [10]. According to them *Rhizopus oligosporus* was inoculated at approximately 10^4 spores/g moist substrate. The data in Figure 4 shows that when *Rhizopus oligosporus* was inoculated at approximately 1.5x10^4spores/g moist barley, the time for obtaining dense mycelial growth was shortened to 36 h. However, the growth was uneven, probably due to oxygen limitation in the center. Similar

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results have also been reported for tempeh fermentation of soy bean by Nout [24]. Earlier investigation showed the widening effect of oxygen on hyphae grown in a gradient of limiting oxygen [25].

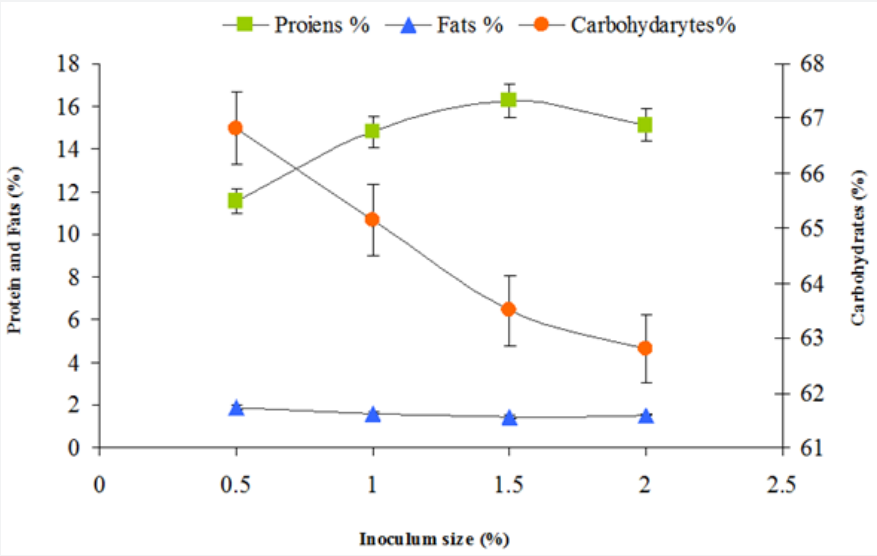

Figure 4: Effect of inoculum size on protein, fat and carbohydrate content of barley by *Rhizopus oligosporus* ML-10 at 30 °C for 36h. Bars represents the SD among triplicates.

Effect of incubation temperature

The incubation temperature was found to be considerable importance for the production of nutritionally rich fermented barley. The best mycelia growth was reported at 30 °C as shown in Figure 5 in which maximum protein contents i.e. 16.75 % were found. These results are in agreement with the work reported by Steinkraus et al. [26]. The workers observed good mycelial growth at 30 °C. Reu et al. [27] also supported that inoculation of beans with *Rhizopus oligosporus* at various temperatures followed by incubation at 30 °C resulted in both increased and decreased periods for the lag phase of fungal growth. According to them maximum difference of 3 h lag phase was found between initial bean temperatures of 25 and 37 °C. However, in another investigation, the optimum growth of *Rhizopus oligosporus* was reported as 35 °C [11]. The data in Figure 5 also revealed that with the increase in temperature the fat and carbohydrates contents did not show significant variation.

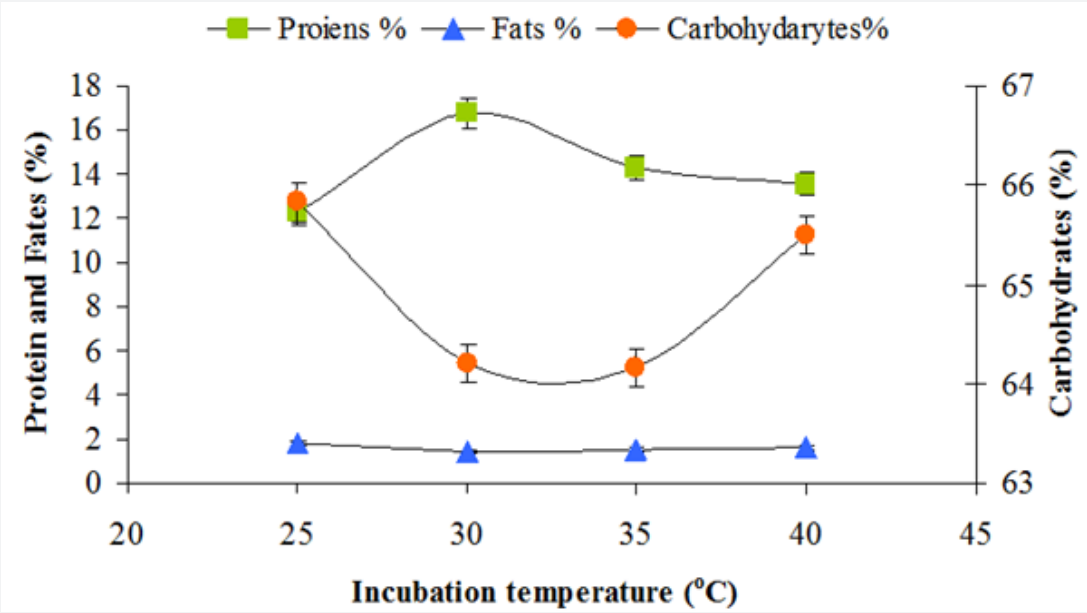

Figure 5: Effect of incubation temperature on protein, fat and carbohydrate content of barley by *Rhizopus oligosporus* ML-10 at 30oC for 36 h. Bars represents the SD among triplicates.

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Effect of incubation time

The results shown in Figure 6 demonstrate that the 36h of incubation time is optimum for the fungal mycelia growth because after this period , the sporulation would be started which had adverse effect on the production of a fermented cereal product in respect of protein contents, and in particular fermented whole grain barley based product of tempe type. It was found that during this time period the fungus growth was very good, and the fungus mycelia evenly distributed in the entire grains. As the time of the incubation was increased the sporulation would be started.

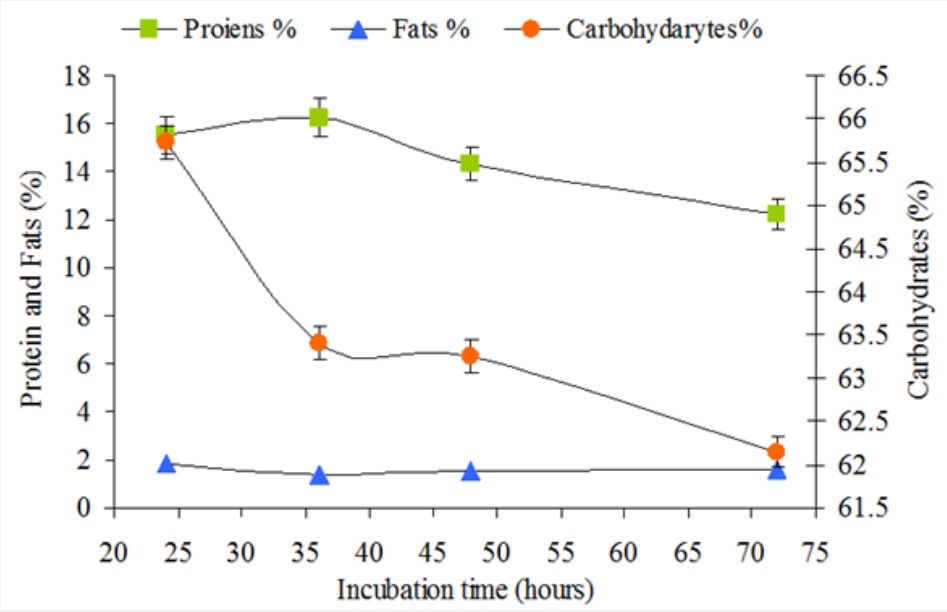

Figure 6: Effect of incubation time on protein, fat and carbohydrate content of barley by *Rhizopus oligosporus* ML-10 at 30 °C for 36h. Bars represents the SD among triplicates.

The fermented barley in solid state fermentation with 1.5 % (v/w) spore suspension of 96h aged *R. oligosporus* ML-10 in 25 x 25cm^2 polythene bags at 30 °C for 36h was analyzed for proximate composition to evaluate the level of protein, fat and carbohydrates as compared with unfermented barley.

The results in Table 1 revealed that protein contents increased significantly whereas the carbohydrates and fats were found to decrease after fermentation with *Rhizopus oligosporus* ML-10 in pretreated barley. It was reported by other workers that the analysis of fermented cereals showed significant decreased in ash contents after fermentation [28]. In fermented soybean, a slight increase was found in crude protein and crude fiber content of Tempe as compared with unfermented soybeans [29]. It was also observed that the growth of fungus reduced the concentration of low molecular carbohydrates and increased the dietary fiber content in fermentation [14]. A decrease in carbohydrate level after fermentation might be due to the partial removal of non starch constituents during solids state fermentation process [30]. It has been reported that during the fermentation process of cereals, proteases, lipases, phytases and a variety of carbohydrases are produced resulting in the degradation of macromolecules into lower molecular weight products thereby improving the nutritional quality of fermented product [24].

Table 1: The proximate analysis of the fermented barley as compared with unfermented barley.

Parameters	Unfermented Barley (%)	Fermented Barley (%)
Moisture	10.12 ± 0.57	14.25 ± 0.68
Proteins	10.25 ± 0.51	16.85 ± 0.55
Crude Fats	2.13 ± 0.07	1.62 ± 0.06
Carbohydrates	68.65 ± 4.53	63.5 ± 3.02

Nutritionally rich fermented barley has earlier been reported by Hesseltine et al. [31]. A patented barley tempe product has been described by fermenting pearled barley kernel with selected strains of *Rhizopus* sp [11]. The barley fermentation process on a new barley genotype has been reported by Eklund-Jhonson et al. [32]. Variations in total fat contents of fermented and unfermented samples were observed under the influence of different parameters as presented in (Figure 2-6). According to Khaterpaul [33] that natural fermentation increases whereas the pure culture fermentation decreased the fat contents in cereals.

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Fatty acids present in glycerides have been reported to decrease during fermentation of soy bean from 30% natural lipid by the action of lipases activity [24]. The protein contents increased initially as a result of fermentation carried out from 6 to 12h. This enhancement in protein level can be attributed to microbial synthesis from metabolic intermediate during the growth [34]. Similar findings were reported regarding the level of protein in the pearl millet after fermentation [35]. It has also been reported that the water soluble protein increased from two to three times after soy fermentation and four to six times after miso fermentation [36,37]. However, in another investigation, it was found that the fermentation process either decreased or did not change the protein contents of pearl millet flour [33].

From the results of present study it was concluded that the solid state fermentation of whole grain barley resulted in increasing the protein contents up to 64.3 % under optimized conditions by *Rhizopus oligosporus* ML-10 in polythene bags.

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