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# Research Article
# Probiotic-fermented Milk Supplemented with Rice Bran Oil

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## Abstract

**Objective:** Fermented milks are widely consumed for their benefits and refreshing effects. An attempt to prepared healthy yoghurt sample fortified with probiotic bacteria as well as Rice Bran Oil (RBO) was conducted. **Materials and Methods:** Yoghurt was prepared from cow's milk by using starter culture and *B. bifidum* as well as fortified by RBO. Yoghurt milk was standard to 3% fat and used for preparing control. Skim milk was incorporated with 1, 2 and 3% commercial rice bran oil, mixed and homogenized to serve three treatment ($T_1$, $T_2$, $T_3$), respectively. All samples were used to prepare yoghurt and then storage at 5±1°C for 10 days .Total solids , pH value, acidity, acetaldehyde and diacetyl as well as viscosity were determined through storage period. The sensorial properties were evaluated and cell viability of the fermented products were also estimated. **Results:** Obtained data elucidated that the count of bacteria was increased from 0 time tell the 5th day followed by a decreasing rate again. All treated samples had the recommended levels ($10^6$-$10^7$ CFU $g^{-1}$) of bacteria till the 10 days. The viscosity of the obtained yoghurt samples was significantly increased as RBO percent increased either fresh or at all the storage period up to the 7th day of storage and after that a slightly decreased was observed in the end of the storage. Treatment $T_3$ had excellent sensorial characteristics which were almost very close to the control one. **Conclusion:** Data revealed that it is possible to prepare yoghurt product fortified with *Bifidobacterium* and 3% rice bran oil, which is similar to the control sample and which had satisfied properties with preferable viscosity beside the healthy benefit of rice bran oil and *Bifidobacterium* strain.

Key words: Probiotic-yoghurt, rice bran oil, omega oils, viscosity, *Bifidobacterium* strain

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Yoghurt is generally consumed for its healthy and refreshing action. It could be saying that its popularity attributed to its flavors and milder cultures[1]. It have a positive nutritive image[2,3], which can be further increased by using probiotic bacteria that have therapeutic properties[4]. The most of probiotics that are emerging in functional food are lactic acid strains and *Bifidobacterium*[5].

On other hand, rice bran (*Oryza sativa*) is a by-product of milling factories despite having nutritious components which normally used as animal feed and poultry[6]. Using of RB or its fractions in food industry is a recent trend[7,8]. Rice bran contents about 10% of rice grain and contains oil around 18-22 %. Its oil is appeared to be yellow, limpid (at 20°C) has no odor and its acid index was <0.50. The value of its density (at 20°C) was around 0.920-0.930. The refractive index ranged from 1.471-1.475 (at 20°C). For the smoke point is >200°C and it has pleasant lightly sweet flavor. The oleic acid was 38.4%, while linoleic acid was 34.4% and linolenic acid was 2.2% as unsaturated fractions. Palmitic acid content was about 21.5% and stearic acid was 2.9% as saturated fractions[9]. Oil contains also a rich un-saponifiable materials as sterols (43%) and (28%) as triterpene alcohols and less polar components (19%)[9]. The most important ingredient of oil is γ-oryzanol, (2% ) as an antioxidant, it is a single compound when fresh while it is a mixture of steryl and triterpenylesters of ferulic acids when storage[10]. Average of saturated fats was 23% which distributed as myristic (0.7%), palmitic (20.1%) and stearic (2.2%). The total unsaturated fats was 75% and monounsaturated was 38%[10]. Chemical composition of Rice bran oil is similar to peanut oil, for the mono-unsaturated, poly-unsaturated and saturated fatty acids. Rice bran oil is also rich in omega 3 (more than olive oil) and omega 6. The ratio of omega 6/omega 3 is so higher than olive oil. A high omega 6/omega 3 ratio is a factor in enhancing the inflammation[10,11].

One of health aspect, various studies have been conducted on the influence of the oil in rice bran and its gamma-oryzanol effect on fat metabolism and oxidation in rats[12], rabbits[13], hamsters[14], monkeys[15] and humans[16]. The active constituents in rice bran oil generally improve blood cholesterol and increasing the HDL cholesterol proportion[17]. Minhajuddin *et al.*[18] mentioned that a decrease in total cholesterol recorded 42% accompanying with a 62% drop in LDL cholesterol, when the researchers supplied test subjects' meals (for animal) with vitamin E isolated from rice bran oil. Rice bran oil help lower cholesterol in the blood, because the oil has substances that might decrease absorption of cholesterol as well as might increase elimination of cholesterol. On other hand; rice bran might decrease calcium absorption which might help reduce the formation of certain types of kidney stones.

The present study was aimed to evaluate the physicochemical, microbiological and sensorial properties of fermented product (set yoghurt) prepared with *Bifidobacterium* strain and rice bran oil.

## MATERIALS AND METHODS

Fresh cow's milk had 12.61% total solids, 3.3% fat, 3.2% total protein, 4.85% lactose and 0.65% Ash was used for this study, it was obtained from the Farm of Faculty of Agriculture, Cairo University, Egypt.

Strains belonging to *L. delbrueckii* subsp., *bulgaricus* and *S. salivarius* subsp., *thermophilus* and *B. bifidum* were obtained from Chr., Hansens Laboratory, Denmark A/S.

**Rice Bran Oil (RBO):** It was obtained from Thai edible oil Co., Ltd., Product of Thailand. Its average composition is serving size 1 tablespoon (15 mL), total fat 14 g (22%), saturated fat (18%) trans-fat (0%), mono-saturated fat 6 g, poly-saturated fat 4.5 g, cholesterol 0%. Sodium was 0%, total carbohydrate, fiber, sugars and protein were 0%, while vitamin E was 4%.

**Experimental:** Fresh cow's milk was divided into 2 parts. The first part was standard to 3% fat and used as a control. The second part was separated. Skim milk was mixed and homogenized with 1, 2 and 3% Rice Bran Oil (RBO) to serve three treatments $T_1$, $T_2$ and $T_3$, respectively. The milk of the four treatments was heated at 90°C/5 min, then cooled at 35°C. The *L. delbrueckii* subsp., *bulgaricus* and *S. salivarius* subsp., *thermophilus* and *B. bifidum* (1:1:1) were added at the rate of 3% (v/v) served as mixed starter culture into the milk. Each of different yoghurt treatments was distributed into individual 120 mL plastic cups and incubated at 42°C till firm curd formed. The prepared yoghurt was kept in refrigerator (5±1°C) for 10 days. All samples of yoghurt were analyzed when fresh and after 3, 7 and 10 days of storage. Three replicates of each treatment were conducted.

**Method of analysis:** The total solids and titratable acidity of yoghurt samples were estimated according to the methods described by AOAC[19]. The pH value was measured using a laboratory pH meter-type HANNA instrument (8417). Acetaldehyde content was determined as described by Lee and Jago[20], diacetyl content was estimated as described

by Lee and Jago[21]. Viscosity was evaluated using RVDV-ii Brookfield viscometer-adapter at 20 rpm and sample treatments was maintained at 25°C and expressed in cent poise (cP).

**Microbiological examination:** Viable cell count of *S. thermophilus* were enumerated on M17 agar (oxoid) after incubation at 30°C 48 h and *L. bulgaricus* count was determined as mention by Gilliland and Walker[22]. The count of *B. bifidum* was numerated according to Blanchette *et al.*[23]. Coliforms were also accounted according to Harrigan and McCance[24], while molds and yeasts were also estimated according to the standard procedures[25].

**Sensory evaluation:** Eleven panelists (from the members of Dairy Science Department, National Research Centre in Egypt) were selected for evaluated the organoleptic activity. Yoghurt samples of all treatments were evaluated for flavor scores (45 points), viscosity (30 points), appearance (15 points) and acidity (10 points). All acceptability was 100 points.

**Statistical analysis:** The GLM procedure with software was applied for analysis the obtained data[26]. Duncan's multiple procedure was conducted to compare the means. A probability to $p \leq 0.05$ was used to recognize the statistical significance.

## RESULTS AND DISCUSSION

**Total solids contents:** Total Solids (TS) content of the yoghurt samples with rice bran oil were logical slightly increased as the storage period increased. This may be attributed to the development of acidity and also, to the natural evaporation. Replacement of milk fat with RBO did not significantly affect the TS values (Table 1). These results are in agreement with those reported by Abbas *et al.*[8], Ibrahim *et al.*[27] and Salem *et al.*[28].

Table 1: Changes of total solids percent of the yoghurt samples with rice bran oil during the storage periods at 5±1°C

| | Storage period (days) | | | | |
|---|---|---|---|---|---|
| Treatments | Fresh | 3 | 5 | 7 | 10 |
| Control (3% milk fat) | 12.60$^{a}$ | 12.60$^{a}$ | 12.65$^{a}$ | 12.70$^{a}$ | 12.75$^{a}$ |
| $T_1$ | 11.40$^{c}$ | 11.40$^{c}$ | 11.44$^{c}$ | 11.45$^{c}$ | 11.49$^{c}$ |
| $T_2$ | 11.63$^{b}$ | 11.68$^{b}$ | 11.75$^{b}$ | 11.78$^{b}$ | 11.84$^{b}$ |
| $T_3$ | 12.25$^{a}$ | 12.28$^{a}$ | 12.31$^{a}$ | 12.35$^{a}$ | 12.38$^{a}$ |

Same capital letters between columns or rows are not significantly at $p \leq 0.05$, $T_1$: 1% rice bran oil, $T_2$: 2% rice bran oil, $T_3$: 3% rice bran oil

**Values of pH and titratable acidity:** In Table 2 and 3, no significant differences were also noticed between all treatments of yoghurt and control in the pH values or acidity percent. Meanwhile, results reveal a slight decreased in pH during storage period of all treatments.

**Viscosity:** Table 4 reveled the viscosity of yoghurt samples. It elucidated that the viscosity of resultant yoghurt was significantly increased as Rice Bran oil percent increased either fresh or at all the storage period up to the 7th day of storage and then slightly decreased up to the end of the storage period. Similar results were also noticed by Tamime and Robinson[29] and Alroubaiya[30].

**Acetaldehyde content:** Results in Fig. 1a, indicated that the acetaldehyde content significantly developed as the storage period was advanced. Also, yoghurt of 3% rice bran oil was very close to the control one. Similar results were reported by

Table 2: Changes of pH value of yoghurt samples with rice bran oil during the storage periods at 5±1°C

| | Storage period (days) | | | | |
|---|---|---|---|---|---|
| Treatments | Fresh | 3 | 5 | 7 | 10 |
| Control (3% milk fat) | 4.81$^{a}$ | 4.77$^{a}$ | 4.66$^{a}$ | 4.51$^{a}$ | 4.49$^{a}$ |
| $T_1$ | 4.76$^{ab}$ | 4.59$^{b}$ | 4.51$^{ab}$ | 4.46$^{a}$ | 4.74$^{b}$ |
| $T_2$ | 4.74$^{b}$ | 4.66$^{b}$ | 4.55$^{b}$ | 4.53$^{a}$ | 4.46$^{a}$ |
| $T_3$ | 4.73$^{ab}$ | 4.65$^{b}$ | 4.52$^{b}$ | 4.49$^{a}$ | 4.47$^{a}$ |

Same capital letters between columns or rows are not significantly at $p \leq 0.05$, $T_1$: 1% rice bran oil, $T_2$: 2% Rice bran oil, $T_3$: 3% Rice bran oil

Table 3: Changes of titratable acidity (%) of the yoghurt with rice bran oil during the storage periods at 5±1°C

| | Storage period (days) | | | | |
|---|---|---|---|---|---|
| Treatments | Fresh | 3 | 5 | 7 | 10 |
| Control (3% milk fat) | 0.77$^{a}$ | 0.78$^{b}$ | 0.83$^{a}$ | 0.88$^{a}$ | 0.92$^{a}$ |
| $T_1$ | 0.79$^{a}$ | 0.84$^{a}$ | 0.87$^{a}$ | 0.89$^{a}$ | 0.93$^{a}$ |
| $T_2$ | 0.83$^{ab}$ | 0.83$^{a}$ | 0.87$^{a}$ | 0.92$^{a}$ | 0.95$^{a}$ |
| $T_3$ | 0.79$^{ab}$ | 0.82$^{a}$ | 0.85$^{a}$ | 0.91$^{a}$ | 0.95$^{a}$ |

Same capital letters between columns or rows are not significantly at $p \leq 0.05$, $T_1$: 1% rice bran oil, $T_2$: 2% rice bran oil, $T_3$: 3% rice bran oil

Table 4: Changes in viscosity (cP) of yoghurt with rice bran oil during the storage periods at 5±1°C

| | Storage period (days) | | | | |
|---|---|---|---|---|---|
| Treatments | Fresh | 3 | 5 | 7 | 10 |
| Control (3% milk fat) | 84$^{a}$ | 90$^{a}$ | 96$^{a}$ | 102$^{a}$ | 101$^{a}$ |
| $T_1$ | 51$^{c}$ | 52$^{c}$ | 53$^{c}$ | 55$^{c}$ | 48$^{c}$ |
| $T_2$ | 66$^{b}$ | 69$^{b}$ | 70$^{b}$ | 72$^{b}$ | 68$^{b}$ |
| $T_3$ | 86$^{a}$ | 87$^{a}$ | 94$^{a}$ | 99$^{a}$ | 105$^{a}$ |

Same capital letters between columns or rows are not significantly at $p \leq 0.05$, $T_1$: 1% Rice bran oil, $T_2$: 2% rice bran oil, $T_3$: 3% rice bran oil

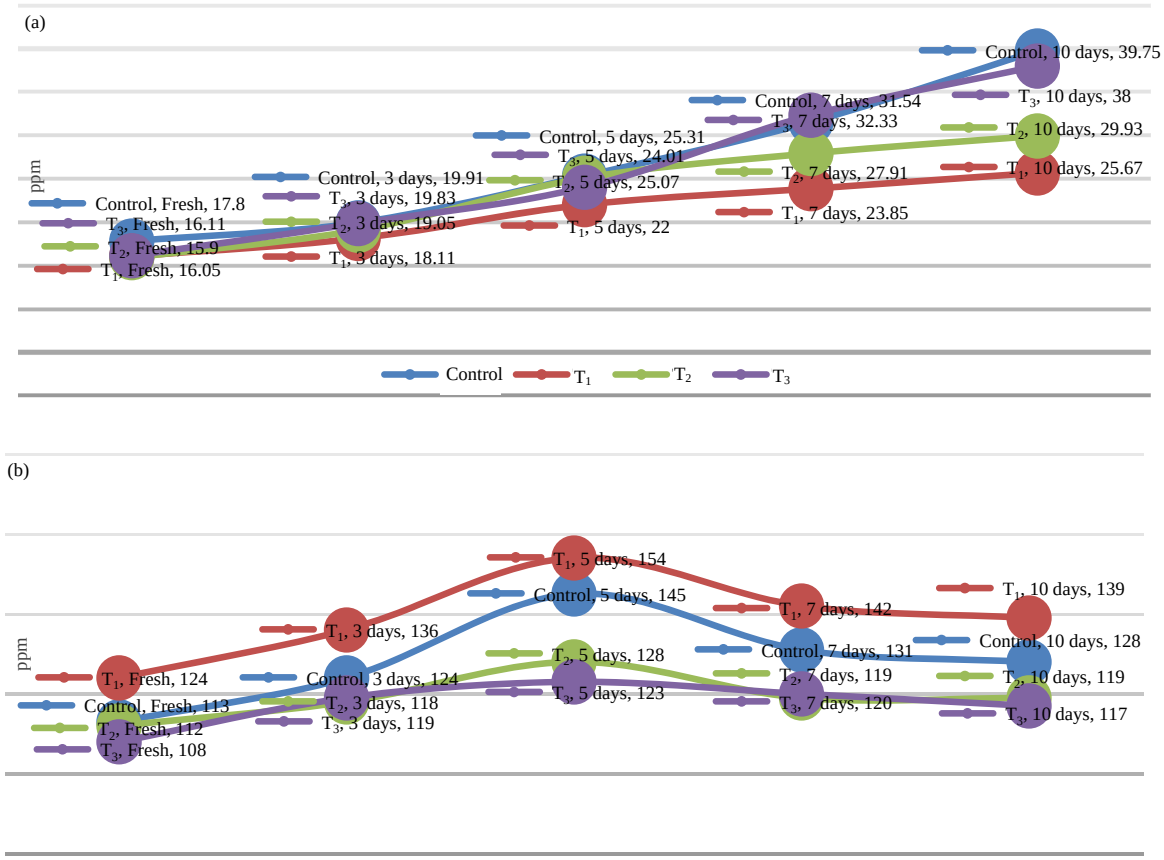


Fig. 1(a-b): Changes in (a) Acetaldehyde content and (b) Diacetyl content yoghurt with rice bran oil during the storage periods at 5±1°C

Fatma[31], Zedan *et al.*[32] and Tamime and Robinson[33]. Replacement of milk fat with RBO did not significantly affected acetaldehyde content and so had no effect on flavor components.

**Diacetyl content:** Diacetyl content of the resultant yoghurt with rice bran oil in Fig. 1b gradually increased as the storage period prolonged up to the 5th day, then strongly dropped at 10th day. Such decreased in diacetyl content could be due to transferring the diacetyl to acetaldehyde. As previously reported by Tamime and Robinson[33]. Highest diacetyl content was for the control is close to the prepared of yoghurt with 3% rice bran oil ($T_3$).

**Microbiological analysis:** The effect of rice bran oil on the viability of probiotic bacteria is presented in Fig. 2. Their maximum count increased at the 5th day of storage, then the trend was decrease gradually till the end of storage. Whereas the control sample reached the maximum level of viability in the 3th day then decreased gradually to the end of storage. The reduction in number of probiotic strains may be due to the sensitively of these bacteria to the acid produced during the storage period. Numbers of all probiotic bacteria remained more than $10^6$ CFU $mL^{-1}$ in all treatments of yoghurt, until the end of storage period. The Japanese fermented milk and lactic acid bacteria beverages association, a minimum of $10^6$-$10^7$ viable microorganisms per gram or military should be present in food product in order to meet the requirements of a probiotic food[34].

**Organoleptic properties:** Table 5 showed that $T_3$-yoghurt had good flavor and very close to the control either fresh or during storage period. No appreciable differences were obtained for the acidity of the yoghurt with rice bran oil which more likely close to the control. Similar results were recorded by Fatma[31]. Prepared yoghurt with rice bran oil had high viscosity which was noticed in $T_2$ and $T_3$ which were almost similar to the control treatment. Also, viscosity of the yoghurt with rice bran oil and the control were slightly decreased as the storage period increased. The appearance score of the resultant yoghurt with rice bran oil was similar for the $T_2$ or $T_3$, which are more or less similar to the control.

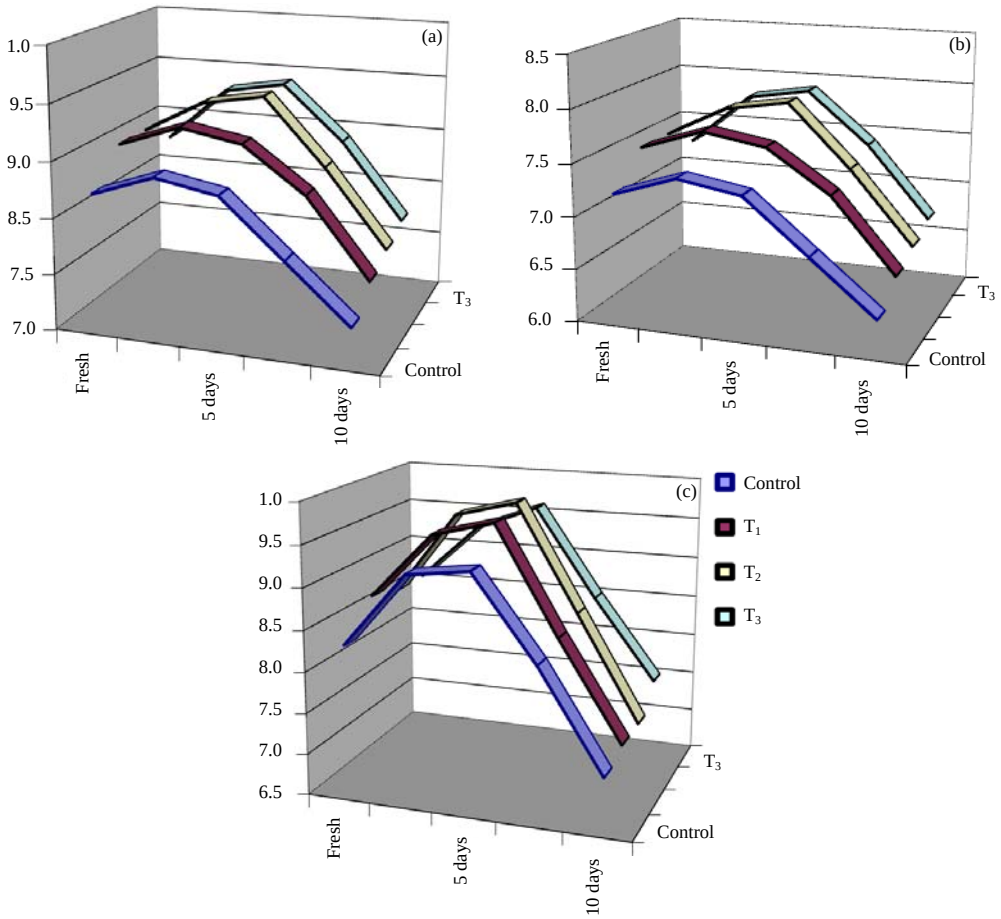


Fig. 2(a-c): Effect of rice bran oil on (a) *S. thermophilus*, (b) *L. bulgaricus* and (c) *B. bifidum* during the storage periods of yoghurt at 5±1°C

Table 5: Organoleptic properties of yoghurt with rice bran oil during the storage periods at 5±1°C

| | | Organoleptic properties | | | | |
|---|---|---|---|---|---|---|
| Storage period (days) | Treatments | Flavor (45 points) | Viscosity (30 points) | Acidity (10 points) | Appearance (15 points) | Total acceptability (100 points) |
| Fresh | Control | 40[a] | 26[a] | 8[a] | 14[a] | 88[a] |
| 1 | $T_1$ | 34[a] | 24[a] | 7[a] | 12[a] | 77[a] |
| | $T_2$ | 39[a] | 25[a] | 6[a] | 13[a] | 83[a] |
| | $T_3$ | 39[a] | 25[a] | 7[a] | 13[a] | 84[a] |
| 3 | Control | 42[a] | 24[a] | 8[a] | 13[a] | 87[a] |
| | $T_1$ | 33[b] | 22[b] | 7[a] | 13[a] | 75[a] |
| | $T_2$ | 41[a] | 25[a] | 6[a] | 13[a] | 85[a] |
| | $T_3$ | 40[a] | 25[a] | 8[a] | 13[a] | 86[a] |
| 5 | Control | 40[a] | 23[a] | 8[a] | 12[a] | 83[a] |
| | $T_1$ | 33[b] | 21[a] | 6[b] | 13[a] | 73[a] |
| | $T_2$ | 38[a] | 23[ab] | 6[b] | 12[a] | 79[a] |
| | $T_3$ | 40[a] | 23[a] | 8[a] | 12[a] | 83[a] |
| 7 | Control | 40[a] | 21[a] | 8[a] | 12[a] | 81[a] |
| | $T_1$ | 32[b] | 21[a] | 5[b] | 13[a] | 71[a] |
| | $T_2$ | 37[a] | 23[a] | 6[ab] | 12[a] | 78[a] |
| | $T_3$ | 39[a] | 22[a] | 8[a] | 12[a] | 81[a] |
| 10 | Control | 39[a] | 22[a] | 7[b] | 12[a] | 80[a] |
| | $T_1$ | 30[b] | 20[a] | 5[ab] | 12[a] | 67[a] |
| | $T_2$ | 36[a] | 22[a] | 6[ab] | 12[a] | 76[a] |
| | $T_3$ | 39[a] | 22[a] | 7[a] | 12[a] | 80[a] |

Same capital letters between columns or rows are not significantly at $p \leq 0.05$

## CONCLUSION

In general the highest total score of the organoleptic properties resultant yoghurt with rice bran oil was for the control followed by 3, 2 and 1% fat yoghurt with rice bran oil when fresh and at 3 days of storage. However, the yoghurt with rice bran oil of $T_3$ was of as score as the control one up to the end of storage period.

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