

## Lactic Fermentation of Cereal Flour: Feasibility Tests on Rice, Oat and Wheat

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### Abstract

**Background and objective:** Consumers show increasing interests in probiotic foods and lactic acid fermentations. Cereal flour, can be a good fermentable substrate due to its prebiotic nature; from which, synbiotic products can be prepared. The aim of the current study was to investigate if three various cereal flours rice, oat and wheat would be good potentially functional foods.

**Material and methods:** Fermentation tests were carried out on rice, oat and wheat flours, using *Lactobacillus paracasei* CBA L74 and 1.5-L fermenter with 1-L working volume. After 24 h, microbial growth, pH value, lactic acid production and starch consumption were assessed.

**Results and conclusion:** In all three flours, pH reduction was seen; particularly in rice flour. The highest *Lactobacillus* growth and lactic acid production were achieved at the end of rice fermentation. The greatest starch consumption was reported at the end of rice fermentation. In conclusion, lactic fermentation of cereals as potentially functional foods was possible for the three flours. However, the best result belonged to rice flour.

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## 1. Introduction

In industrial countries, roles of foods have progressively been changed. In the past, the term of nutrition referred to disciplines that simply aimed at ensuring adequate calorie intakes. Nowadays, nutrition can be considered as a tool to prevent various diseases. Further attentions have been paid to foods and food ingredients, resulting in a significant development of the so-called functional foods. A functional food can be described as a food with additional functions, often linked to health promotion or disease prevention. Particular attentions are now paid to probiotics, the live microorganisms with health benefit on hosts when supplied in adequate quantities [1]. The most studied and used bacteria within the probiotics include lactic acid bacteria (LAB) and bifidobacteria [2]. Based on the

literatures, the minimum quantity to achieve a temporary colonization of the intestine by a lactic fermenting strain includes at least  $10^7$  CFU  $g^{-1}$  [3]. Currently, the most well-known and marketed functional foods are dairy products [4]. However, non-dairy products, especially cereals, are becoming preferred vehicles for probiotics [5-7]. Cereal-based functional foods represent a new frontier due to their vast resources and ease of storage, compared to other products from various sources [8]. Experimental evidence prove good growth of LAB in cereal matrices, suggesting that the incorporation of probiotic strains into cereal substrates results in production of fermented foods with characteristics that can contribute to the development of consumers' well-being [9-12]. Cereal flour is a good

example of fermentable substrates because of its composition. Indeed, cereal flour is a rich source of nutrients such as resistant starches, water soluble and insoluble  $\beta$ -glucans and arabinoxylans as well as indigestible oligosaccharides that make cereal flours good prebiotics. A prebiotic is described as an indigestible food ingredient that beneficially affects the host by selectively stimulating growth and/or activity of one or more resident bacterial species in colon and thus improves host health [13]. Fermented cereal flour can be considered as a symbiotic; in which, probiotic and the prebiotic effects are present. Therefore, lab-scale tests were carried out on submerged fermentations of cereal flours using *Lactobacillus (L.) paracasei* CBA L74. The aim of the current study was to investigate feasibility of the fermentation, especially on cereal flours and direct use of these food products as functional foods. Another aim was to prepare a semi-finished product to introduce in production process of the functional foods. Various cereal flours of rice, oat and wheat were used to investigate effects of cereal characteristics such as starch chemical-physical properties, protein and mineral concentration and nutritional values on fermentation. The differences are verified for the *Lactobacillus* growth, pH values and lactic acid production (the main fermentation product) as the strain is homo-fermentative.

## 2. Materials and methods

### 2.1 Strain

The strain used in starter culture included *L. paracasei* CBA L74, (Heinz, Italia SpA) with the International Depository Accession Number of LMG P-24778. That strain was a Gram-positive homo-fermentative, facultative anaerobic bacteria. The bacterial strain was stored in freeze-dried form at  $-20^{\circ}\text{C}$  and recovered at  $37^{\circ}\text{C}$  in 0.9% sodium chloride solution, 10 min before fermentation tests. The inoculum bacterial density included  $10^8$  CFU  $\text{ml}^{-1}$ .

### 2.2 Rice, oat and wheat flours

Rice, oat and wheat flours were provided by Heinz, Italia SpA. The starch content of the flours included 67.0, 60.0 and 46.7% for rice, wheat and oat, respectively. Before each fermentation process, flour was subjected to heat treatment ( $120^{\circ}\text{C}$  for 90 min) to decrease possible microbial loads.

### 2.3 Lab-scale fermentation bioreactors

The experimental lab-scale bioreactors for fermentations included four components of a vessel and system of mixing, thermal conditioning and pH and temperature measurements. The vessel, with a maximum capacity of 1.5 l, was a cylindrical Pyrex (20 cm high, 10 cm ID) equipped with an external jacket that allowed circulation of a service fluid necessary to preserve a

constant temperature in the entire apparatus. The fluid was thermostatically regulated through a thermo-controlled water bath (Haake, USA) set at  $37^{\circ}\text{C}$ . The mixing system consisted of a stainless steel impeller, having an inclined blade and a Rushton impellers connected to a motor (a three-phase asynchronous electric motor of 0.25 Hp; 0.18 kW; 1310 rpm with a speed reducer of 170-880 rpm) that allowed adjustment of the stirring speed and hence axial and radial mixings. Therefore, efficient mixing and good homogeneity of the substrates were achieved, using mixing speed of 180 rpm and food dye; as demonstrated by preliminary mixing tests (not shown). Briefly, the best mixing speed (180 rpm) was considered as a speed that allowed achieving a perfect distribution of the food dye in the suspension; mostly within 10 min. The vessel, the mixing system and the entire mechanism used for the lab-scale experiments are shown in Figs. 1A, B and C, respectively. A Mettler Toledo device equipped with an autoclavable In Pro 3100 Probe (Mettler Toledo, USA) was used for the continuous measurement of pH and temperature.



**Figure 1.** The vessel, mixing system and bioreactor. A) The vessel was built using Pyrex and equipped with an external jacket that allowed the circulation of service fluid necessary to maintain the entire apparatus at a constant temperature. B) The stainless steel impeller with two various types of turbines: a blade turbine to reach an axial flow and a Rushton turbine to reach a radial flow. C) The complete equipment was used for the lab-scale experiments.

### 2.4 Lab-scale fermentation protocols

Submerged fermentation was carried out using 1.5-L fermenter with 1-L working volume. The first step included sterilization of the fermenter and mixing system

at 121°C for 20 min, using autoclave. Then, 150 g of each flour (rice, oat and wheat flours), previously sanitized by dry heat, were mixed in 0.850 l of sterilized distilled water under laminar flow hood to avoid contamination. The mixture was tyndallized using two consecutive cycles of heating (90°C) and cooling (37°C) to decrease the microbial load. The fermentation temperature was controlled at 37°C under aerobic conditions and the fermentation run was stopped after 24 h. This protocol was used for the three flours.

### 2.5 Analytical methods

To characterize the fermentation process, a small quantity of the substrate was aseptically collected from the bioreactor at specific sampling times ( $t_0$ ,  $t_4$ ,  $t_{14}$ ,  $t_{20}$ ,  $t_{24}$ ; intended as hours from the inoculum phase). These samples were used for microbiological and chemical analyses. After serial diluting, substrate was spread on Petri dishes of MRS agar (Oxoid, UK) and incubated at 37°C for 48 h under anaerobiosis, using Anaerogen Compact anaerobic kits (Oxoid, UK). To investigate the presence of contaminants, samples were spread on Petri dishes prepared with PCA bacteriological agar, yeast extract agar, peptose peptone, glucose, McConkey agar and gelatine peptone agar (Oxoid, UK).

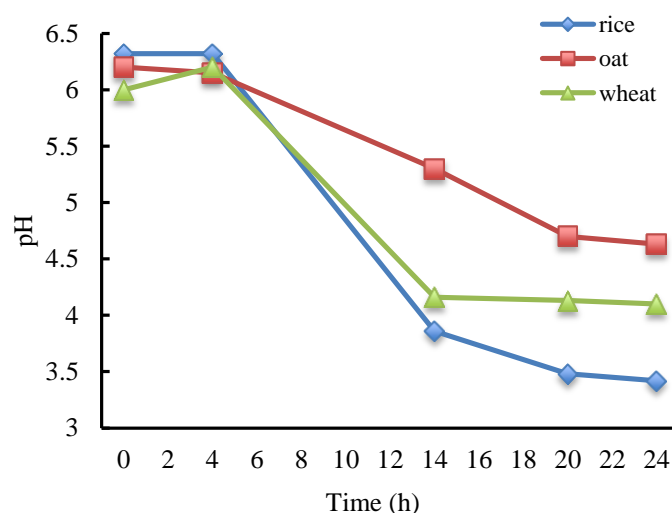
The quantity of lactic acid produced during fermentation was assessed using a high performance liquid chromatography (Agilent Technologies 1100, USA), equipped with a C18 column (Agilent Zorbax, 4.6 × 150 mm, pore size of 80 Å) and a UV detector. The eluent included 0.1 M of  $\text{NH}_4\text{H}_2\text{PO}_4$  with a flow rate of 0.8 ml  $\text{min}^{-1}$ . The mobile phase included ammonium phosphate with a pH of 2.7 and detection at 218 nm. The temperature of analysis included 30°C. Secondary acid products such as butyric and acetic acids were assessed using gas chromatography (Agilent Technologies 6890, USA) equipped with a Capillary Poraplot Q column (25 m × 0.32 mm). The flow rate included 200 ml  $\text{min}^{-1}$  and the mobile phase included helium gas. The starch content in raw materials and fermented samples was assessed using Total Starch Assay Kit (AA/AMG) (Megazyme, Ireland) through spectrophotometric analysis at 510 nm.

### 2.6 Statistical analysis

Each test was carried out in triplicate. Statistical analysis was carried out using GraphPad Prism 7.0a (San Diego, CA, USA). Mean and standard deviation of the experiments were calculated and their significance was evaluated using student's t-test. The significant was reported when values  $P \leq 0.05$ .

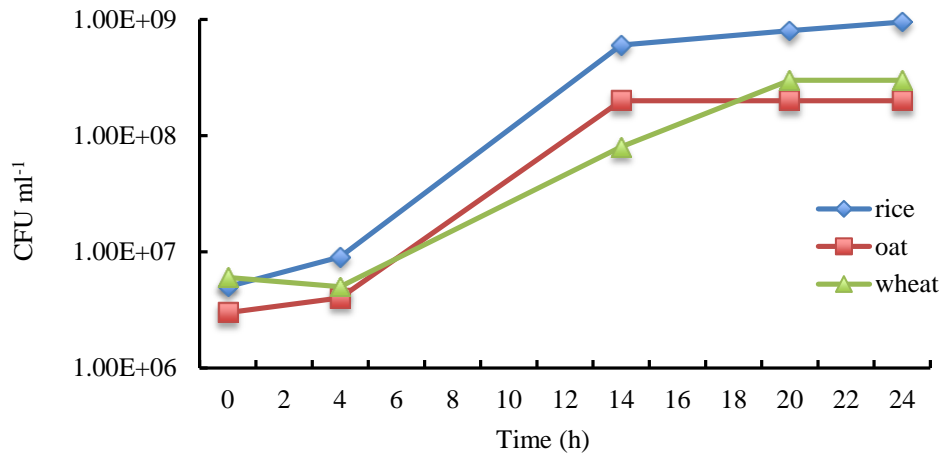
## 3. Results and discussion

Although dairy foods are the most common substrates used for the probiotic production, cereal based foods offer a valid alternative. In this study, three flours of rice, oat and wheat were fermented. The *L. paracasei* CBA L74 was used as fermenting culture. This was a homo-fermentative bacterial strain with lactic acid final product. To test the flour fermentation, the lactic acid concentration, pH values (for increased production of the lactic acid, lower pH levels were expected), *L. paracasei* growth and starch consumption were assessed. The aim of the present work was to investigate feasibility of lactic acid fermentation of cereal flours as potentially functional foods. Based on the literatures, a minimum level of  $10^6$  viable probiotic bacteria per milliliter or gram of the food product was considered sufficient [14]. The pH results from the fermentation of the three flours (rice, oat and wheat) are shown in Fig. 2.



**Figure 2.** The pH values for the three flours tested at various sampling times ( $t_0$ ,  $t_4$ ,  $t_{14}$ ,  $t_{20}$ ,  $t_{24}$ ). Blue, red and green lines represented results for rice, oat and wheat, respectively. Each result was the mean value of a triplicate analysis using student t-test ( $P < 0.001$ ).

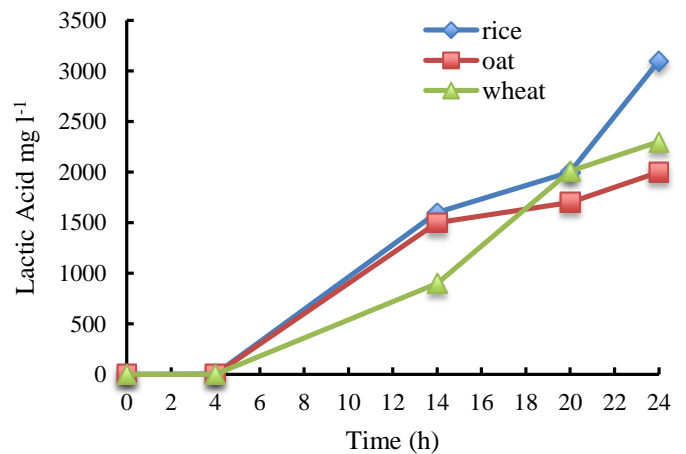
The  $t_0$  value of pH was near 6 in the three flours. After a lag time of nearly 4 h; in which, the pH value remained almost constant for the three flours, pH progressively decreased during the fermentation time reaching at  $t_{24}$ , values of  $3.42 \pm 0.055$ ,  $4.63 \pm 0.050$  and  $4.1 \pm 0.060$  for rice, oat and wheat, respectively. Each result was the mean value of triplicate fermentations. The lactobacilli growth on the three flours is shown in Fig. 3. For all the flours, a lag phase of about 4 h could be assessed with a limited growth of the *Lactobacillus*.



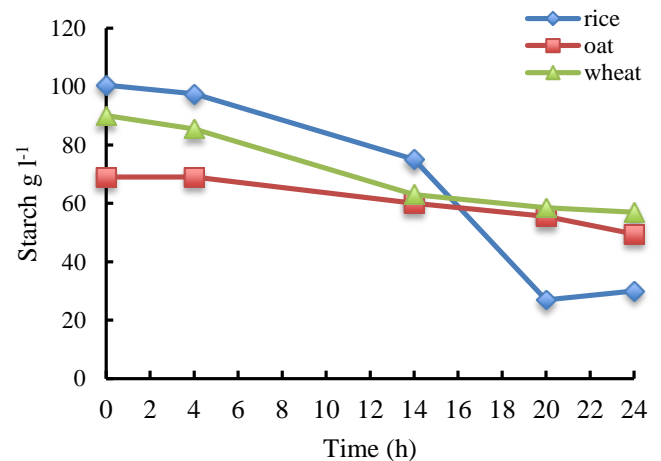
**Figure 3.** Growth rates (CFU ml<sup>-1</sup>) for the three flours. Blue, red and green lines represented results for rice, oat and wheat, respectively. Each result was the mean value of a triplicate analysis using student t-test ( $P < 0.001$ ).

Various growth rates could be calculated for these cereals. The exponential phase for rice shifted from  $t_4$  to  $t_{14}$  with a viable LAB concentration of  $6 \times 10^8 \pm 1.5 \times 10^8$  CFU ml<sup>-1</sup>; after which, a stationary phase could be observed up to the end of fermentation process ( $t_{24}$ ) with  $9.5 \times 10^8 \pm 1.3 \times 10^8$  CFU ml<sup>-1</sup>. The exponential phase for oat shifted from  $t_4$  to  $t_{14}$  with  $2 \times 10^8 \pm 7 \times 10^7$  CFU ml<sup>-1</sup>. This remained almost constant until the end of fermentation. The period of lag phase for wheat was similar other cereals, while the exponential phase was longer (nearly 6 h). This was continued to  $t_{20}$  with a LAB concentration of  $3 \times 10^8 \pm 1.7 \times 10^8$ . Concentration of viable *Lactobacillus* remained constant until  $t_{24}$ . The lactic acid quantity is shown in Fig. 4. No lactic acid was detected at the beginning of fermentation. An almost linear production rate of lactic acid was detected after lag phase until the end of fermentation. Values of  $3100 \pm 380$ ,  $2000 \pm 130$  and  $2300 \pm 143$  mg l<sup>-1</sup> were measured for rice, oat and wheat, respectively. No secondary metabolites (butyric and acetic acids) were detected using gas chromatography analysis, proving absence of the contaminants.

Starch content of the substrates during the fermentations is shown in Fig. 5. Starting with an initial starch concentration of  $100.5 \pm 2.5$ ,  $69 \pm 4$  and  $90 \pm 4$  g l<sup>-1</sup> for rice, oat and wheat flours respectively, a progressive consumption was found over the time. For oat and wheat flours, decreases in starch content were almost linear. For rice flour, a significant decrease was seen until  $t_{20}$ ; after which, the concentration was almost constant. The final concentrations of starch included  $30 \pm 4$ ,  $50 \pm 4.3$  and  $57 \pm 5$  g l<sup>-1</sup> for rice, oat and wheat flours respectively, corresponding to consumptions of 70, 28 and 37% of the initial starch contents. Results for the rice, oat and wheat fermentations are summarized in Table 1.



**Figure 4.** Lactic acid production (mg l<sup>-1</sup>) during the fermentation of the three flours. Samples included no lactic acids at  $t_0$  min. Concentrations after 24 h included  $3100$  mg l<sup>-1</sup> for rice,  $2000$  mg l<sup>-1</sup> for oat and  $2300$  mg l<sup>-1</sup> for wheat. Blue, red and green lines represented results for rice, oat and wheat, respectively. Each result was the mean value of a triplicate analysis using student t-test ( $P < 0.001$ ).



**Figure 5.** Starch concentrations (g l<sup>-1</sup>) for the three flours. Blue, red and green lines represented results for rice, oat and wheat, respectively. Each result was the mean value of a triplicate analysis using student t-test ( $P < 0.001$ ).

**Table 1.** Summary of the results for rice, oat and wheat at  $t_0$  and  $t_{24}$  fermentations

	Time [h]	Rice	Oat	Wheat
pH	0	6.32 ± 0.13	6.24 ± 0.04	6.00 ± 0.25
	24	3.42 ± 0.05	4.63 ± 0.05	4.10 ± 0.06
Bacterial density [CFU ml <sup>-1</sup> ]	0	5.00E+06 ± 5.29E+05	3.00E06 ± 8.19E05	6.00E+06 ± 8.19+05
	24	9.50E+08 ± 1.30E+08	2.07E+08 ± 6.81E+07	3.00E+08 ± 6.08E+07
Lactic acid [mg l <sup>-1</sup> ]	0	0	0	0
	24	3100 ± 380	2000 ± 130	2300 ± 143
Starch [g l <sup>-1</sup> ]	0	100.50 ± 2.50	69 ± 4	90 ± 4
	24	30 ± 4	50 ± 4.30	57 ± 5

Concentrations of the starch following fermentation could be still high in a food product for human consumption. Since the major aim of the current study was to test the feasibility of cereal fermentation to obtain a semi-finished product for using in other foods, the highlighted concentrations of starch might not be considered high. The greatest *L. paracasei* growth and starch consumption were seen at the end of rice fermentation. This could be due to several factors. First, rice starch granules included the smallest size [15] and small granules were hydrolyzed more rapidly than larger ones using  $\alpha$ -amylase; as suggested by Vasanthan and Bhatta [16]. This could make rice starch more accessible to hydrolysis than oat and wheat starches. Second, high amylose starches were particularly resistant to hydrolysis [17]. It seemed that rice, containing amylose of 21-25% w w<sup>-1</sup> on total starch dry basis [18], included smaller amylose contents than oat (amylose content of 27.5-29.8% w w<sup>-1</sup> on total starch dry basis) [19] and wheat (amylose content of 25.6% w w<sup>-1</sup> on total starch dry basis) [18]. These data of granule size and amylose content suggested an easier hydrolysis of the rice starch, compared to that of oat and wheat starches. Regarding lactic acid, the highest concentration was found for the rice flour (3100 mg l<sup>-1</sup>). Significantly lower levels were reported for the oat (2000 mg l<sup>-1</sup>) and wheat (2300 mg l<sup>-1</sup>) flours. This could be linked to the growth of *Lactobacillus*. Several studies were carried out on cereal fermentation. In most of these studies, a spontaneous fermentation was studied under uncontrolled conditions [20-22]. In other studies, simultaneous saccharification and fermentation were carried out using hydrolytic enzymes [23,24]. Marko et al. [25] studied static fermentations of various cereal suspensions such as wheat flour in water using *L. plantarum* as the fermenting strain. A lag phase longer than that of the present study (8 instead of 4 h) was seen. Starch concentration was lower and starch consumption and lactic acid production were smaller than those found in the current study. In contrast, a similar  $\Delta$

growth of nearly three logs was reported by Marko et al. [25] and Charalampopoulos et al. [8]. Rice and wheat flours were used as substrates in another study [26]. Fermentation was carried out under static condition, anaerobiosis and pH control. The substrate for fermentation was composed of a simple broth added with various concentrations of pure starch or cereal flour. Results showed greater starch consumption and lactic acid production for rice and wheat flours, compared to that results of the present study did in significantly longer time (6 days instead of 24 h).

#### 4. Conclusion

Based on the current findings, rice seemed the best cereal for the fermentation because of a greater growth, lactic acid production and starch consumption by lactobacilli; possibly due to the granule characteristics of the rice starch. Although oat and wheat seemed to be good substrates for the fermentation, their fermentation process needed optimization. For example, this optimization could be carried out by adding a further available source of carbon such as glucose. In all the fermented substrates, a sufficient bacterial density to consider the products as potential functional foods was achieved. However, In vitro and In vivo experiments are necessary to demonstrate functionality. Fermented flours can be used as semi-finished products in various foods (e.g. baby foods) to further develop their properties such as immunomodulatory activity or digestibility. A future perspective could be process optimization of oat and wheat fermentations using glucose. Furthermore, the process could be improved by controlling pH value during the fermentation and/or by modifying the atmosphere to decrease the O<sub>2</sub> concentration. Study on various flour mixes could be scheduled to verify if each flour alone could affect other flours in terms of microbial growth and lactic acid production.

## Contributions

Roberto Nigro and Marianna Gallo designed the research; Federica Nigro, Francesca Passannanti, Dana Salameh and Paola Schiattarella carried out the research; Andrea Budelli provided the raw materials and *L. paracasei* CBA L74; and Marianna Gallo and Francesca Passannanti drafted the manuscript. Marianna Gallo has the primary responsibility for the final content. All authors read and approved the final manuscript.

## 5. Acknowledgements

Dr Andrea Budelli is currently employed by Heinz BV, Netherlands. He provided the raw materials (rice, oat and wheat flours) and *L. paracasei* CBA L74 and participated in design of the study. He did not have any additional roles in collection and analysis of data, decision of publishing or preparation of the manuscript.

Heinz BV did not provide any financial support to the authors for the experimental activity and did not have any additional roles in the study design, data collection and analysis, publishing decision or manuscript preparation.

## 6. Conflict of interest

The authors declare no conflict of interest.

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## تخمیر لاکتیکی آرد غلات: آزمون‌های امکان‌سنجی بر روی برنج، جو دوسر و گندم

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۴- مرکز نوآوری کرافت هینز، نیو دوکنبرگسوتر ۱۹، ۶۵۳۴، نیجمن، هلند.

### چکیده

**سابقه و هدف:** مصرف کنندگان تمایل رو به افزایشی را برای غذاهای زیست‌بار<sup>۱</sup> و تخمیر لاکتیکی نشان می‌دهند. آرد غلات، به دلیل ماهیت کمک زیست‌یاری<sup>۲</sup> می‌تواند ماده اولیه مناسبی برای تخمیر باشد که از آن می‌توان فرآورده‌های فرزیست‌بار<sup>۳</sup> را تولید کرد. هدف مطالعه حاضر این است که آیا سه نوع آرد غلات شامل برنج، جو دوسر و گندم می‌توانند مواد غذایی فراسودمند بالقوه‌ای باشند یا خیر.

**مواد و روش‌ها:** آزمون‌های تخمیر آرد برنج، جو دوسر و گندم با استفاده از لاکتوباسیلوس پاراکازئی CBA L74 و فرمانتور ۱/۵ لیتری با حجم کاری یک لیتر انجام شد. پس از ۲۴ ساعت، رشد میکروبی، میزان pH، تولید اسید لاکتیک و مصرف نشاسته تعیین شد.

**یافته‌ها و نتیجه‌گیری:** در هر سه نوع آرد به‌ویژه آرد برنج کاهش pH مشاهده شد. بالاترین رشد لاکتوباسیلوس و تولید اسید لاکتیک در پایان تخمیر برنج به‌دست آمد. بیشترین میزان مصرف نشاسته در پایان تخمیر برنج گزارش شد. در نتیجه، تخمیر لاکتیکی غلات، به‌عنوان مواد غذایی بالقوه فراسودمند برای این سه نوع آرد امکان‌پذیر است. اگرچه، بهترین نتیجه به آرد برنج تعلق داشت.

**تعارض منافع:** نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافی مرتبط با انتشار این مقاله ندارند.

### تاریخچه مقاله

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### واژگان کلیدی

▪ آرد غلات

▪ تخمیر لاکتیکی

▪ لاکتوباسیلوس پاراکازئی CBA

L74

▪ زیست‌بار

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- Probiotic<sup>۱</sup> غذاها یا مکمل‌هایی که به دلیل داشتن ریزاندامگان‌ها قادر به بازسازی یا تغییر گیاهان میکروبی روده هستند و در حفظ سلامت انسان مؤثرند.

- Prebiotic<sup>۲</sup> غذاهای غیرقابل هضمی که باعث تحریک رشد ریزاندامگان‌های پس‌روده/کولون می‌شوند.

- Symbiotic<sup>۳</sup> مکمل غذایی حاوی مواد زیست‌بار و کمک‌زیست‌بار.