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# Effects of mixed strains of rhizopus oryzae and lactobacillus on corn meal fermentation

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**Abstract**

As long as they are provided in appropriate proportions, probiotics can be beneficial to the host. These bacteria are increasingly used in food to balance intestinal microbiota and relieve gastrointestinal disorders. However after traveling through the gastrointestinal (GI) tract, surviving probiotic bacteria comprise 10 to 30 % of this population. It is a probiotic bacterium found in many probiotic foods. As a result of its inability to hydrolyze proteins and macromolecule carbs, L. acidophilus grows poorly in cereal products. The goal of the present investigation was a synbiotic beverage made from corn mash and Rhizopus oryzae-fermented corn mash. Starting culture concentration is one such element. Milk powder and Corn mash that had been fermented with Rhizopus oryzae were both researched in depth. Fermented cornflour with R. oryzae had just enough nutrients to support L. acidophilus' survival, but not its development. The proliferation of Lactobacillus acidophilus was not improved by adding sugar (1 or 2 %, w/v). However, once milk powder (1 % or 2 %, w/v) was put in, L. acidophilus developed rapidly. After 10 hours of fermentation using 5.5 % Rhizopus oryzae -fermented corn mash and 2 % Cell counts for skim milk powder were about. 9.0 log CFU/mL. During fermentation, the content of -glucans (approximately 781 mg/L) did not change considerably.

**Keywords:** synbiotic beverage; Rhizopus oryzae; Lactobacillus acidophilus; fermented corn.

**Practical Application:** Practical Application: The goal of the present investigation was a synbiotic beverage made from corn mash and Rhizopus oryzae-fermented corn mash. Fermented cornflour with R. oryzae had just enough nutrients to support L. acidophilus' survival, but not its development. The proliferation of Lactobacillus acidophilus was not improved by adding sugar (1 or 2 %, w/v). However, once milk powder (1 % or 2 %, w/v) was put in, L. acidophilus developed rapidly.

## 1 Introduction

Soluble fiber is abundant in corn. Corn is also a natural source of folic acid, which is a B vitamin. Corn's natural folic acid concentration can be increased by fermentation with lactic acid bacteria (Wang et al., 2019; Zhang et al., 2019). Human nutrition in many places depends on maize and the fermented products generated from it. As a result of fermentation, maize's nutritional value and shelf life are enhanced (Ruan et al., 2019; Tan et al., 2020). Some of the final product properties can be affected by other microbes and microbial succession and the synthesis of antimicrobial substances and vitamins, which boosts the amount of nutrients (Kim et al., 2019; Park & Kim, 2019). Also being investigated are other safety-enhancing actions, such as the phytate detoxification and mycotoxin reduction in maize or corn are believed to have originated in North America. As a result of its extraordinary adaptation to different geographical environments today, Worldwide, it is considered as one of the essential cereals (Alkay et al., 2020; Chen, 2021). It was first brought to Europe in the sixteenth century and subsequently expanded to Africa and East Asia. Although maize has a low protein level, it is regarded as a staple meal in many nations.

In addition to boosting nutritional value and digestibility, fermentation destroys unwanted components as well as inhibits harmful bacteria. Toxic compounds of microbial origin can be found in fermented goods (Verni et al., 2020). So it is essential to understand how these diverse microorganisms interact with one other to enhance the end product's quality and food safety (Muhialdin et al., 2021). On the other hand, the sensory qualities of spontaneously fermented items are influenced by different species or microbiological groupings with different metabolic rates. In reality, microbial interactions in fermented products occur via numerous pathways, and their impact on strain fitness might be favorable, neutral, or negative (Peng et al., 2018). The influence of the various species on taste, rheology, shelf-life, and functional/nutritional properties has been studied (Cagno et al., 2016; Katina, 2005; Scarnato et al., 2017; Taglieri et al., 2021). During maize fermentation, with special

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attention on those activities that determine microbial succession and fermentation growth, such as increased sugar availability via starch breakdown, and synthesis of exopolysaccharides and vitamins, as well as antimicrobial chemicals. Microorganisms also detoxify mycotoxins.

Processed and fermented maize grains are produced all over the world, following diverse traditions. A fermented maize product's production process usually begins with washing and then progressively soaking and grinding the grains in water until they are soft. Fermented maize products are classified into four groups by Ramírez-Vega et al. (2020), based on the techniques by which they are prepared: As follows: 1) dried kernel; 2) soaking ears of corn; 3) millet; and 4). Maize products can also be divided into liquid and solid categories based on their textures. Traditionally, maize grains were soaked in an alkali (usually lime) before being boiled, dried, and crushed into flour; they were also germinated and chewed. The grains go through physical and chemical changes as a result of this process, which functions as the selection of microbiota-directing agents for this substrate's fermentation (Ørskov & Greenhalgh, 1977). When fermented maize is exposed to microorganisms, they consume several nutrients in the corns. They regulate the diversity and activity of microbes through their metabolism. (Sakai et al., 2004). Members of the maize microbiota are defined by environmental influences, for example, pH and temperature, inoculum amount, and fermentation duration. Chicha, a Colombian fermented beverage made from chewed maize, has a greater variety of organisms, including Candida spp.

On the other hand, following the buildup of hazardous end-products or other inhibiting factors, a certain microbe or group of them begins the growth and establishes itself for a specific length of time. Other species that are less susceptible to these inhibitory effects benefit from the microbes' presence. According to culture-independent methods, maize fermented product microbiomes are vastly underestimated (Bik et al., 2018; Ray et al., 2017). LAB (lactic acid bacteria) and yeast coexistence are unavoidable in fermented products made from corn Fungi, AAB, and Bacillus species are also common in various goods (Vieira-Dalodé et al., 2007). Few investigations have documented the presence of E. coli and E. aerogenes, which are pathogenic bacteria that can cause illness. Fermented maize products commonly include LAB, which frequently generates enzymes that may degrade high molecular weight polysaccharides, organic acids, and chemicals that can kill or decrease the microbial population, such as hydrogen peroxide and bacteriocins (Schnürer & Magnusson, 2005). Through the breakdown of antinutritional substances, they can enhance the amount of B group vitamins and free amino acids in a product and improve the availability of zinc, calcium, and iron (Mugula et al., 2003).

LAB and other bacteria may thrive in the presence of free sugars in mature maize kernels, which are produced by endogenous grain amylases. Just several bacteria can utilize starch during the corn fermentation's initial stage. As a result, there is less microbiological diversity as opposed to the later stages. The activity of microbial amylases makes alternative sources of carbon, particularly nonamylolytic strains, more available to a wider range of organisms. Yeasts and other microbes can use the organic compounds produced during fermentation as carbon sources. Due to its ability to increase the energy sources available to nonamylolytic bacteria, amylase activity is crucial during maize fermentation. It also has an impact on pH decrease (Soomro et al., 2002).

Amylolytic activity in LAB species is rare; only a few species convert starch directly into lactic acid in one step. The enzyme amyA, which expresses an external -amylase that is only generated transiently, plays a key role in this bioconversion. Additionally, this is strain-dependent in its amylolytic effect and can be hindered by the pH decrease caused to lactobacilli development as a result of certain pre-fermentation procedures, bacteria with strong amylolytic activity might be selected (Sills & Stewart, 1982). A product's rheology can be affected by bacteria and yeasts' amylolytic activity, which is advantageous for microbiota development as well as rheology. Amorphous areas of starch granules are easily attacked by acid and amylase enzymes. A possible explanation for this is that, during maize fermentation, enzymes generated by LAB break down the glycosidic linkages in the starch granules. This allows the granules to absorb water quicker, resulting in a reduction in viscosity and the cohesiveness of the doughs. Although bulk and starchy weaning gruel viscosity has dropped, its nutritional density has risen, allowing it to retain an appropriate thickness for young infants (Gallant et al., 1992; Robyt et al., 1996).

Several species and species are required for the fermentation of maize products, as has already been reported (Adebiyi et al., 2019; Chaves-López et al., 2020; Decimo et al., 2017). During a particular period of time, one or more species will begin to flourish and settle down in numbers. Consequentially, species that are less susceptible to hazardous end products or other inhibitory factors would experience a decline in growth or perhaps stop growing. During maize fermentation, bacteria and fungus may be inhibited by a variety of chemicals produced by the microorganisms involved. Many microorganisms cannot grow in an environment with a pH that is too low. When they discover extra oxygen, acetic acid bacteria (AAB) create it (Gao et al., 2020; Lynch et al., 2019).

As a result of their acknowledged safety status and probiotic action, lactic acid bacteria (LAB) have gained a lot of interest in recent years. It is unusual for LAB to be employed in solid-state fermentation alone because of their strict moisture and nutrient requirements. In addition to degrading polymeric materials, Rhizopus oryzae can offer nutrients for the development of LAB cultures. By fermenting maize with R. oryzae and LAB, barley would be endowed with a sweet-sour flavor and become a carrier for probiotic bacteria (Barnharst et al., 2021; Ma et al., 2020; Yang et al., 2017).

Acid-loving milk-bacillus Lactobacillus acidophilus is a species of gram-positive bacteria in the Lactobacillus genera. Microaerobically active, L. acidophilus ferments carbohydrates into lactic acid. It grows well at low pH values (pH 5.0 or below) and at temperatures around 37 °C (99 °F) for optimal development. L. acidophilus may be present in humans and other animals' gastrointestinal systems and mouths. L. acidophilus strains with probiotic properties may exist. Streptococcus thermophilus and

Lactobacillus delbrueckii subsp are widely utilized in various dairy products.

It was discovered that pigs fed L. acidophilus had reduced blood cholesterol and more cholesterol in feces (Zeng et al., 2018). Porks were fed the same quantity of food, but one group was given a saline solution containing L. acidophilus while the other group was given saline solution without acidophilus. Comparatively to the control group, the saline-bacteria group exhibited reduced serum cholesterol levels. It was decided to utilize pigs since their digestive systems are comparable to human ones (Beitel et al., 2020).

A number of health advantages have been claimed for Lactobacillus acidophilus when eaten, typically through increasing or repairing gut flora. Bacteria-host interactions and undesirable side effects are rare; however, probiotics are usually regarded to be safe for consumption.

Heterothallic microfungus Rhizopus oryzae is found in soil, animal waste, and decaying plants. Despite its close resemblance to Rhizopus stolonifer, this species is differentiated by its smaller sporangia and sporangiospores that are disseminated in the air. Columellae and sporangiospores are bigger than in R. oligosporus and R. microsporus. Economically, R. oryzae is employed in the manufacture of glucoamylase and lipase, in the synthesis of organic acids, and in the creation of fermented foods, among other things (Guleri et al., 2018).

Anaerobic breakdown of molecules, such as glucose, through the chemical process known as fermentation. Beer and wine are produced by the foamy process known as fermentation, which has been around for more than 10,000 years. Fish, Meat, dairy, soybeans, vegetables, fruits, and grains have all been fermented traditionally (Rupp, 2020). As a result of the microorganisms, nutritional components, and climatic factors that influence the fermenting process, hundreds of distinct fermented food varieties have been developed throughout the years.

Fermentation may be accomplished in two ways. There are a number of ways that foods may be fermented, including spontaneously, which is referred to as wild fermentation or spontaneous fermentation. For example, sauerkraut and some fermented soy products can be fermented naturally. Culture-dependent ferments, like kombucha, can be used to ferment foods. For example, sourdough bread can be fermented by "backslopping." Natural or commercial starters can be employed to commence fermentation and standardize the end product's organoleptic qualities.

Most cultures throughout the world use fermented foods. During the past several years, fermented foods have become more popular in the West due to their alleged health advantages and the growing interest in digestive health. Fermented foods may positively impact health and illness through a variety of methods, and this is due to the presence of lactic acid bacteria, a probiotic microbe. There are at least 106 microorganisms per gram in most fermented goods. Concentrations vary based on the product's area and age when tested or ingested (Tamang et al., 2020). By its protective impact against gastrointestinal conditions, probiotic strains appear to be able to survive in the presence of food matrix (e.g., bile acids). Although bacteria from fermented foods can enter the gastrointestinal tract, their presence in the gut appears to be transitory, according to several research investigations on the topic. Due to their ability to compete with pathogenic bacteria and produce immune-regulatory and neurogenic fermentation by-products, these microorganisms may still have the capacity to exert a physiological effect on the gut. Metabolites that are produced during fermentation might be beneficial to the body's health. A good example of this is lactic acid bacteria (found in dairy and non-dairy fermented foods). Thirdly, fermentation may transform some chemicals into physiologically active metabolites. Flavonoids can be converted by lactic acid bacteria into physiologically active metabolites, for instance. The health advantages of fermented foods include prebiotics and vitamins. In addition to toxins and anti-nutrients, fermentation can also reduce the content of fermentable carbohydrates, which may increase the tolerance of these products in patients with functional bowel disorders. In addition to toxins, fermentation can also reduce the content of anti-nutrients.

β-glucan, cellulose, protein, lipids (unsaturated fatty acids), vitamins, antioxidants, have all been identified as significant dietary fiber types in corns. This physiological impact is considered to be due to the -glucan content of corns 4. - blood glucose, insulin levels, and weight management are all regulated by beta-glucan. (Bulmer et al., 2021). Many corn-based functional foods have been produced as a result of these physiological activities. Probiotic goods are one type of corn-based food that is popular. According to previous research, probiotic bacteria can grow on corns, and -glucans might be utilized as prebiotics. As long as they are provided in appropriate proportions, probiotics can positively affect the host's wellbeing. To balance intestinal microbiota and relieve human gastrointestinal tract dysfunction, microorganisms are being used in food. Lactobacillus Plantarum, Lactobacillus casei, and Lactobacillus bulgaricus were among the lactobacilli utilized in commercial probiotic products (Mohammadian et al., 2017). In order to exhibit their positive effects, probiotic bacteria must be able to live in the upper GI environment. Acid and bile tolerance determines whether probiotic bacteria survive in the GI tract. Following their passage via the Intestinal environment, only 10 to 30 % of such probiotic bacteria endure. A high survival rate of probiotic microorganisms is therefore essential.

Researchers have found that probiotic strains of Lactobacillus species boost immunity, increase resistance to infections, and lower blood cholesterol levels (Nagashima et al., 2013; Pivetta et al., 2020). According to research, Lactobacillus acidophilus can live at high bile and low pH concentrations. According to Curto et al. (2011) study using an in vitro gastric model of digestion, Lactobacillus acidophilus had the best %age of remaining alive in the intestinal tract when compared to the other two species of L. casei.

Due to its low capacity to hydrolyze proteins and macromolecular carbohydrates, L. acidophilus' development is hindered because of a shortage of accessible sugars and amino acids essential for growth. Leuconostoc acidophilus' growth can be boosted by adding protein hydrolysis products and other nutrients to the substrate. By hydrolyzing macromolecular substrates in situ, we hope to promote the growth of L. acidophilus.

Rhizopus oryzae is widely considered as safe in Asia, where it is often employed in fermented dishes. As well as -amylase and proteases, it is recognized for its ability to hydrolyze polymers. Additional secondary metabolites, such as malic acid, are produced by R. oryzae (Cantabrana et al., 2015).

Our synbiotic product was developed by combining the fermentation of L. acidophilus and Rhizopus oryzae and making use of the characteristics of both strains. R. oryzae's saccharification and liquefaction are likely to be to blame for the fast increase in reducing sugar content. Many people are aware that Rhizopus produces a large amount of glucoamylase, which might contribute to the saccharification and liquefaction of cereals and the final breakdown of starch into simple sugars. When it comes to fermenting cereals, Lactobacillus acidophilus may convert glucose into organic acid, which could help the fragrance production and make it somewhat acidic.

## 2 Materials and methods

This was done using a high-speed electric grinder to prepare the substrate for fermentation (Pointner et al., 2014). This was followed by 15 minutes of 121 °C sterilization of the mash. A 500 mL flask filled with 100 grams of corn mash was inoculated with the R. oryzae spore suspension (106 spores/gram of grain) (Bai et al., 2004). This was followed by three days of incubation at 25 °C under static circumstances, followed by 24 and 48 hours of stirring., the high-speed grinder was used to grind fermented corn again after it had fermented for 72 hours. In the next step of our study, we employed fermented corns at varying concentrations (4, 5, and 7 % in sterilized water) as substrates for L. acidophilus. Prior to that, we looked at five alternative therapy options: A fermented corn mash made with R. oryzae, B fermented corn mash made with R. oryzae and 1 % sucrose, C fermented corn mash made with R. oryzae and 2 % sucrose, D fermented corn mash made with R. oryzae and 1 % skim milk powder, fermented corn mash made with R. or After being heated at 90 °C for 10 minutes, each sample's slurry was cooled to 37 °C. This was followed by L. acidophilus inoculations of 2, 5, and 10% (v/v) (7.44 log CFU/mL). For 12 hours, A 37 °C temperature was used for all fermentations.

## 3 Results and discussion

Cells of Lactobacillus acidophilus that are viable in the various sample (Bernet et al., 1994). As shown in Table 1, Rhizopus oryzae fermented cornmeal mashing to change its reducing sugar and free amino acid composition. Degrading sugars and free amino acids also increased dramatically as a result of the fermentation process. In Figure 1, Various cellular groups of L. acidophilus have been identified within the many samples that we have examined. L. acidophilus viable cells did not grow in treatment A. As a result, L. acidophilus was able to survive but not thrive in corn mash fermented with R. oryzae. There was no difference in the number of viable cells of L. acidophilus between treatments B (sucrose,1 % ) and C (sucrose,2 % )., as indicated in Figure 1. For L. acidophilus to thrive, sugar levels must be appropriate. Perhaps other nutrients were lacking, preventing L. acidophilus from growing. As a result, L. acidophilus developed fast in samples D and E (p0.05). After fermentation, There were 8.25 and 8.79 log CFU/mL of viable cells. Viable L. acidophilus cells in samples E and D fulfill the probiotic product viability requirements. According to all of these studies, L. acidophilus can proliferate as a result of certain nutritional components in skim milk powder.

Starter culture concentration: To the R. oryzae-fermented corn mash, starting cultures of L. acidophilus (7.5 logs CFU/mL) were introduced in concentrations of 2, 5, and 10 %. During 10 hours, all samples were fermented at 37 °C. In Figure 2, we show the results of various fermentations. After fermentation with three different inoculum concentrations of L. acidophilus, cell counts were 8, 8.4, and 9 logs CFU/mL, respectively. All of these values were higher than the minimum requirements for probiotic supplements, according to the study. 3.5, 3.81, and 4.72 H+ mmol/100 L, respectively, were the TA values with 2.0, 5.0, and 10% inoculum. Three inoculum samples had pH values between 3.83 and 4.41 after 10 hours of fermentation. All things considered, it may be more appropriate to utilize a starting culture concentration of 10.0 %, which can yield a greater number of Lactobacillus acidophilus.

- Fermented corn mash concentration: After 6 hours, 7.0 % malted barley had a little higher Tmax than 5.5 % malted barley ($p > 0.05$) but was substantially higher than 4.0 % malted barley ($p<0.05$) (Figure 3B). Different concentrations of corn mash can explain this. When R. oryzae is allowed to work its magic on the mash of corns, it produces lactic and malic acids. Lactic acid generated by the lactobacilli may be responsible for the disappearance of the difference in the Titratable acidity( TA) between 5.6 % and 7.2 % of the mash through subsequent fermentation by L. acidophilus. During phase 2 of fermentation, L. acidophilus

**Table 1.** Rhizopus oryzae fermented corn mash, resulting in changes in decreasing sugar and free amino acids.

| | Fermented corn | Unfermented corn |
|---|---|---|
| Reducing sugar (g/kg ) | 249.4 | 24 |
| Free amino acids (mg/ kg) | 2176 | 389.4 |

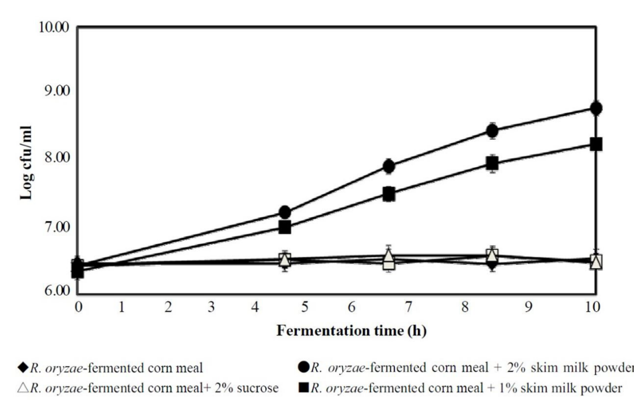


**Figure 1.** Number of L. acidophilus cells that are viable in five separate samples.

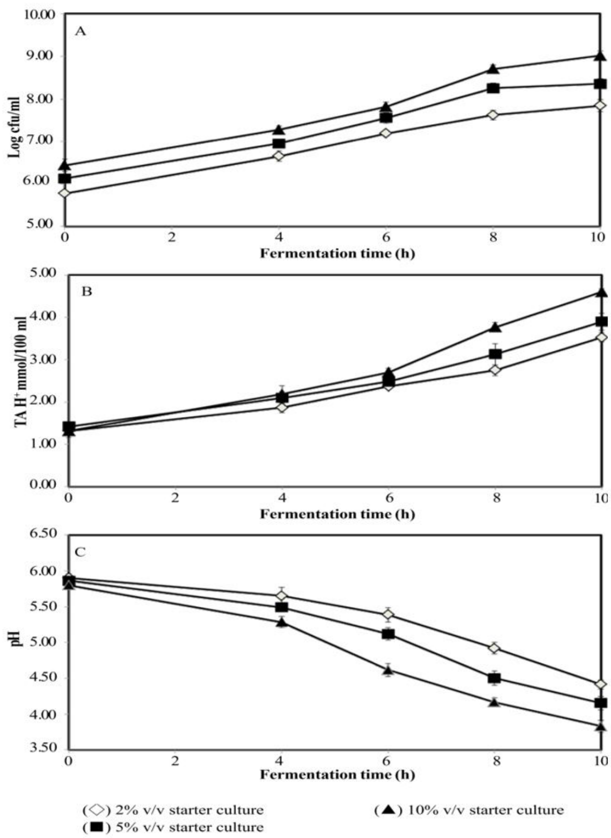


**Figure 2.** R. oryzae-fermented cornmeal prepared with the milk powder, the effect of starting culture concentration was studied. Calculating viable cell numbers(A), TA (B), and pH (C).

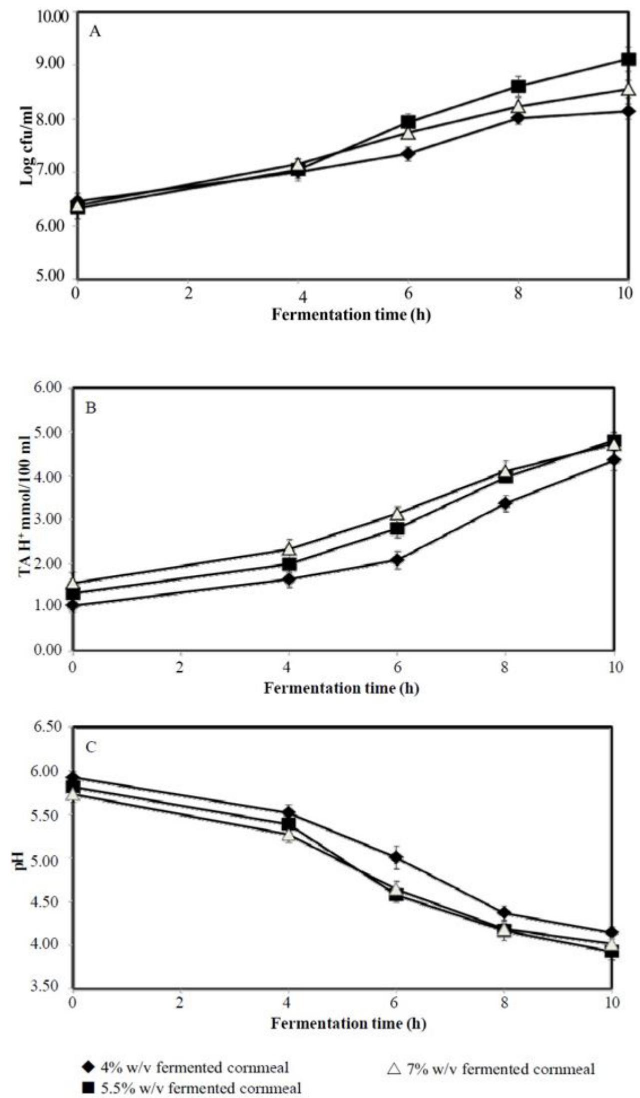


**Figure 3.** Cornmeal fermented with R. oryzae affects L. acidophilus fermentation. Counts of viable cells (A), acidity that can be titrated (B), and pH (C).

fermented faster with 5.5 % mash than it did with 7 % mash. (Figure 3A). Lactic acid would be generated in greater quantities in the 5.5 % and 7.0 % mashings, which would progressively offset the difference in TA induced by the various amounts of corn mash at the outset. Due to R. oryzae fermentation and L. acidophilus viability, the treatment comprising 4.0 % mash received the lowest score of total acidity (TA) during the whole fermentation period (see Figure 2 and Figure 3B).

As shown in Figure 3C, the initial pH values for the various treatments ranged from 5.73 to 5.92 depending on the concentration of R. oryzae-fermented corn mash. Figure 3A shows that these minor pH adjustments had no influence on the growth of L. acidophilus during the first phase. L. acidophilus also developed rapidly in these samples throughout the 10-hour fermentation. As a result of these findings, L. acidophilus is resistant to pH levels below 7.4. In previous studies, L. acidophilus has also been found to grow at pH levels below five and to conform to adverse environmental circumstances.

Milk powder concentratation: R. oryzae-fermented corn mash has enough nutrients to allow L. acidophilus survival, but not growth, as demonstrated in Figure 1. It was found that L. acidophilus grew fast when 1 or 2 % milk powder was introduced to the R. oryzae-fermented maize. In addition to fermentable carbohydrates, L. acidophilus requires a variety of amino acids, vitamins, and other growth factors. L. acidophilus may require certain nutrients to develop, in which case milk powder may be able to give those nutrients. R. oryzae-fermented corn mash was treated with milk powder at concentrations of 1, 2, and 3% (w/v), as indicated in Figure 4. Lachnospira acidophilus viable cell counts in skim milk powder at the conclusion of 10 h fermentation ranged from 8.40 log CFU/mL for 1.0 %, 2.0 %, and 3.0%. (Figure 4A). One-percent skim milk treatment had the lowest TA value of all therapy (Figure 4B).

About 6 hours of fermentation with 2% or 3% additional milk powder resulted in a pH of 4.5, whereas 10 hours of fermentation with 1.0 % skim milk powder resulted in the same pH. These results demonstrate that a 2% concentration of skim milk powder best influences the development of L. acidophilus.

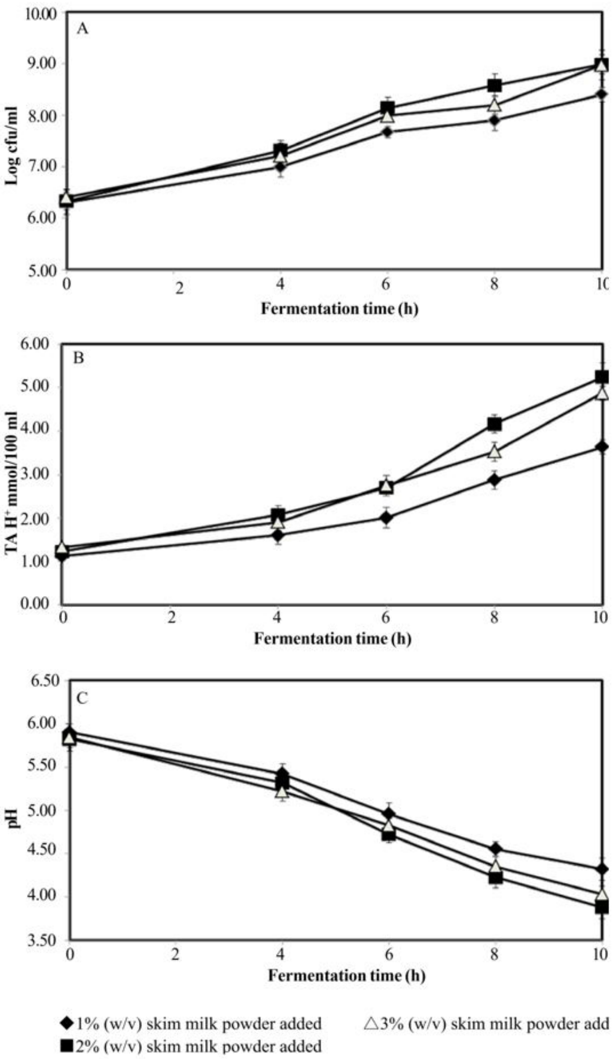


**Figure 4.** Milk powder effect on the fermentation of Lactobacillus acidophilus (LAB). Counts of viable cells (A), acidity that can be titrated (B), and pH (C).

## 4 Conclusions

Fructification affects soluble protein concentration in an important way. Solid-state fermentation is generally considered to be dependent on the protease produced by fungal organisms. According to experts, the Rhizopus produced protease Aspartic Protease has the best activity and stability in acidic environments. As the fermentation continued, soluble protein content increased significantly along with the pH drop and the development of R. oryzae. In addition, a continual decrease in pH levels may have increased the endogenous proteolytic activity in grain. In order to create a synbiotic beverage based on corns, In this study, L. acidophilus was grown on rhizopus oryzae-fermented maize mashes as fermentation substrate. L. acidophilus can survive on R. oryzae-fermented corn mash, although its development is limited. However, adding skim milk powder enabled the fast development of L. acidophilus. As much as 781 mg/L of -glucan remained in the beverage after 10 hours of fermentation. 5.5 % fermented corn supplemented with 2 % As a substrate for L. acidophilus fermentation, milk powder can be used as well.

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