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A preliminary study to evaluate the physicochemical and microbial population dynamics of a blend of pearl millet flour and buttermilk during spontaneous fermentation

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ABSTRACT

Objectives: The present study assessed the microbial population dynamics and physicochemical test during the spontaneous fermentation of pearl millet flour and buttermilk blend.
Materials and Methods: Pearl millet flour samples were taken from the local market of Rewari and Mahendergarh district of Haryana, whereas buttermilk samples were taken from nearby villages of Mahendergarh, Haryana. pH and total titratable acidity were estimated for physicochemical analysis of the blend sample. Microbiological analysis has been carried out to estimate the microbiological population dynamics of samples using different media i.e., 1) Potato Dextrose Agar; 2) Plate Count Agar; 3) *Lactobacillus* MRS agar; 4) Eosin Methylene Blue Agar.
Results: The subject study mainly deals with the physicochemical test and microbiological analysis of pearl millet, i.e., pH ranges from 3.0 to 6.84 where it was lowest in buttermilk sample at 72 h, and highest is 6.84 in flour sample at 0 h, temperature ranges from 13°C to 31.5°C at different conditions (shaking, refrigeration, room temperature). Total titratable acidity as gm/lactic acid was recorded in the range of 0.4 to 0.96, lowest in the blend sample at 0 h, and highest in the blend at room temperature (72 h). In the samples of the blend the total aerobic count, Lactic acid bacteria count, *E. coli* count, were up to 7.65 (log cfu/ml), up to 6.8 (log cfu/ml), and 3.48 (log cfu/ml), respectively after fermentation.
Conclusion: A preliminary study results suggest that the best storage condition is refrigeration for all the samples. However, on 72 h of fermentation LAB counts were increased. This is only a preliminary study, a study with a large sample and much-organized methods need to be investigated for the outcome of these different conditions of microbial dynamics.

Key words: Pearl millets, Fermentation, Buttermilk, Lactic acid bacteria, Microbiological analysis

Introduction

The word "millet" refers to the whole group or class of small-sized food grains, which form a different group of varied compositions; maize and sorghum are also considered under the millet category. Some important millets are *bajra* or pearl millet (*Pennisetum glaucum*), *samai* or little millet, *ragi* or finger millet, *haraka* or kodo millet, *navane* or foxtail millet, *banti* or barnyard millet, etc., which are widely produced in African and Asian countries. Nutrition apart, millets provide health benefits in diet, consequently, help in overcoming some disorders such as diabetes, obesity, mellitus, and hyperlipidemia.[1] They are rich in micronutrients; specifically, minerals and vitamin B; hence are

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very advantageous for health. Millet is the main food source in many African and Asian countries. In millet-producing areas, it is also used in making different traditional food and beverages such as bread, infant and snack foods, *idli, dosa, chakli, papad,* porridges.[2] Food fermentation is known to be the oldest biotechnological method. Fermentation adds many qualities to food, such as flavor, texture, shelf-life, and nutritional benefits. Fermentation is a process by which consumable food products are prepared using microorganisms. It is also stated that protein digestibility, as well as starch digestibility of food grains, is also improved by fermentation.[3] There is a wide range of fermented foods worldwide such as beer, bread, cheese, pickles, and sausages. Fermentation has ample scope for microbial and enzymatic processes that could be applied to food ingredients to achieve the desired qualities such as attractive flavor, improved safety, prolonged shelf life, enrichment of nutritional value, and promoting health benefits.[4] A recent study reported the microbial dynamics of traditional fermented foods (grawa, tej, and borde) of Ethiopia. Results showed that yeast counts were highest in tej (9.41 Log CFU/ml) and LAB was highest in borde (7.33 Log CFU/ml) samples, at the end of fermentation. Furthermore, during fermentation, pH of samples was dropped (grawa: 4.18–3.62), (borde: 4.58–4.22) but increased for tej (5.26–5.50) during first 24 h, after that decreased later down to 3.81 at 144 h.[5] Foods prepared by natural fermentation are nutrient-rich foods as a result of microbial metabolic activity. Curd is one of such frequently used popular fermented foods which helps cure many diseases. Such widely consumed foods in India are *idlis, dosas, wadas, dhoklas,* and *kadhi*. In a study, the microbial dynamics of traditional fermented food "*Korefe,*" microbial counts were assessed. Moreover results showed that lactococci and lactobacilli were increased from 4.0 to 9.7 log CFU/ml during fermentation. Furthermore, the study concluded that pH decreased from 5.18 to 4.0 during the fermentation processes.[6] The fermented food helps in increasing the absorption of essential minerals, thereby preventing mineral shortage. In India, pearl millet is grown in Rajasthan, Gujarat, Maharashtra, U.P, and Haryana. It is rich in minerals and fiber and contains beneficial micronutrients such as calcium, iron, potassium, magnesium. A large amount of nutrients is present in pearl millet which makes it suitable to produce a large variety of food items example, snack foods, baby foods, and dietary food items. In addition to its dietary merit, it consists of a considerable amount of antinutrients such as phytic acid or phytates. It also possesses tannins and polyphenols, which affect the bioavailability of minerals and restrict their utilization as food or feed. Therefore, improvement needs arise to curtail the antinutritional effects. Many traditional methods such as fermentation soaking reduce the antinutrients content of plant-based crops.[7]

This study was carried out to examine the physicochemical analysis and microbial dynamics of spontaneously fermented pearl millet (*P. glaucum*) flour and buttermilk-based product. This research is only a preliminary trial to standardize the quality of buttermilk-based products.

MATERIAL AND METHODS

Collection of samples

Pearl millet flour samples were taken from the local market of Rewari and Mahendergarh district of Haryana, whereas buttermilk samples were taken from nearby villages of Mahendergarh, Haryana. Aseptic sterile containers were used for the collection of the samples. Samples were then stored at 4°C for microbiological analysis and physiochemical test. The flow chart of our study plan is shown in [Figure 1].

Physicochemical analysis

pH

The pH of different samples and the blend of pearl millet and buttermilk were measured by a digital pH meter.

Total titratable acidity (TTA)

TTA was described in terms of % lactic acid using the standard method.[8]

Fermentation process

Blend (flour + buttermilk) samples were stored at different storage conditions (i.e., shaking, refrigeration, and room temperature) and spontaneously fermented for up to 72 h. Fermented samples were taken on 24 h (0 h, 24 h, 48 h, and 72 h) of intervals for the evaluation of microbial dynamics.

Microbiological analysis

The examination of microbiological population dynamics and risk was carried out with the help of the pour plate technique using the following media:

- Potato dextrose agar (PDA), plate count agar, *Lactobacillus* Man, Rogosa, and Sharpe (MRS) agar, and Eosin methylene blue agar

Isolation and enumeration of microbes

Dilutions of different samples were carried out according to the well-defined serial dilution method. For the isolation of microbes, 1 ml of homogenized sample was pipetted out aseptically from each serially diluted sample and was plated on different Petri plates labeled as different dilutions with different general purpose/selective media using the pour plate method. After the incubation duration, colonies of bacteria, yeast and mold, and *E. coli* grow in different media.

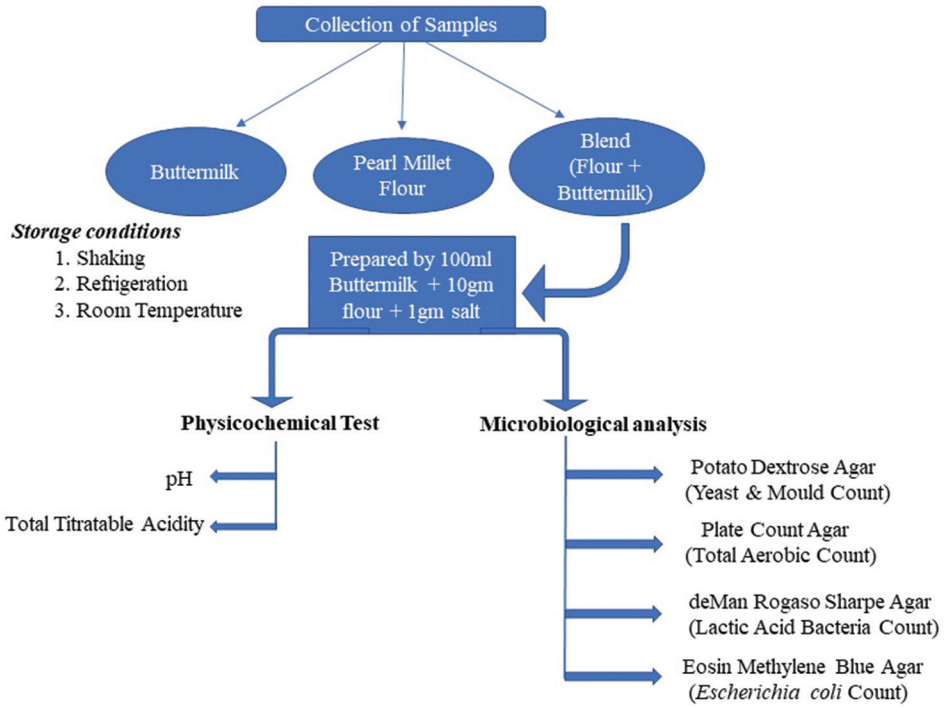

Figure 1: A flow chart for studying physicochemical and microbial population dynamics of the blend.

RESULTS AND DISCUSSION

Physiochemical analysis

pH

The pH values of samples in triplicates were recorded using pH meter for three different samples, that is, blend of flour and buttermilk, pearl millet flour, and buttermilk. pH reading was in the range of 3.9–6.84. The pH after 4 days of storage at different temperatures was found in the range from 3.0 to 5.96. The pH concentration decreased from 5.2 to 3.6 (0 h to 72 h.) in the blend of flour and buttermilk, and the natural decline in pH concentration from 6.84 to 5.34 (0 h to 72 h) was observed in pearl millet flour. At the last sample of buttermilk, pH decreased from 5.6 to 3 (0 h to 72 h). The significant decrease in pH might be due to increased acid production by lactic acid bacteria (LAB) in the different samples. Lactic acid fermentation of buttermilk and other samples leads to producing a large quantity of vitamins (Group B and K), the amino acid lysine, folate, and micronutrients in the fermented products.[9,10]

pH of the blends at different conditions, that is, room temperature, refrigeration, and shaking at different fermentation duration (0 h, 24 h, 48 h, and 72 h). As the fermentation duration increases, the pH decreases from 4.1 to 3.42 at room temperature. On the refrigeration condition, pH increases from 4.1 to 4.37. Shaking pH decreases from 4.1 to 3.4 as the hours of fermentation increase. The decline in the pH during spontaneous fermentation of pearl millet flour and buttermilk is mainly due to the degradation of complex organic substances (mucilage) into simpler sugars in the fermenting blends, which produces the acid components by the action of microbes.

TTA

The TTA as gm/lactic acid was recorded in 0.4–0.96 in various samples. The blend sample titratable acidity naturally increased from 0.4 to 0.9 as fermentation hours increased. The flour sample titratable acidity smoothly increases from 0.43 to 0.81, and at last, in the buttermilk sample, the acidity from 0.53 to 0.83 from duration 0h to 72h, slightly increases after fermentation. Relevant information about the titratable acidity of blends samples at different conditions shows a significant rise from 0.41 to 0.96 during the period, while the shaking and refrigeration showed a slight increase from 0.41 to 0.82 and 0.41 to 0.43, respectively. As the days of fermentation were increased, titratable acidity samples were also found to be increased significantly. Lactic acid is the dominant metabolite of LAB fermentation, although acidification occurs due to acetic acid.

Microbiological dynamics

The microbiological dynamics of blends of pearl millet flour and buttermilk were examined using aerobic plate count, yeast and mold count, *E. coli* count, and LAB count method. The PDA often noted is a common microbial growth medium made from an infusion of potato and dextrose. It is used widely for growing fungi and yeasts. PDA can be supplemented with different antibiotics to i€nhibit bacterial growth that may interfere with yeasts and mold. PDA is generally recommended

for the isolation and microbial enumeration of yeasts and molds in dairy, cosmetics, and clinical samples.[11] Similarly, an aerobic plate count for bacteria indicates the level of bacteria in a product and can sometimes be used to indicate the quantity and spoilage level of the product.[12] de MRS agar is developed primarily for culturing lactobacilli from various sources. This media shows good productivity for nearly all LAB. MRS agar gives excellent colony counts and a characteristic colony size and morphology for lactobacilli and other LAB.[13] The Eosin Methylene Blue agar is a differential microbiological medium, which slightly inhibits the growth of Gram-positive bacteria and provides a color indication that differentiates between microorganisms that ferment the lactose (e.g., *E. coli*) and those that do not (e.g., *Salmonella* and *Shigella*).[14] The different samples were examined for microorganism dynamics based on the type of agar medium.

Spontaneous fermentation

Here, the total aerobic count (log cfu/ml) of a blend (flour and buttermilk) at different conditions (room temperature, shaking, and refrigeration) after different fermentation durations (0 h, 24 h, 48 h, and 72 h) are discussed. Refrigeration condition indicates the control sample in which growth remains almost constant because at particular refrigeration temperature, the growth of microorganisms was controlled, but in room temperature and shaking condition count starts from 6.2 (log cfu/ml) at 0 h and increases smoothly to 7.65 (log cfu/ml) after fermentation of 72 h but shaking indicates slightly decrease after 72 h from 7.44 to 7.20 (log cfu/ml).

A microbial succession of fermented blends showed a quantitative and qualitative increase in the initial condition, which later stabilized with a slight decrease. The yeast and mold count of the blend at the different conditions and different fermentation temperatures were recorded. The room temperature and shaking in which the growth rate suddenly increases from 0 h to 24 h fermentation duration and after it was constant, when estimated on 48 h, it slightly decreased. In control (refrigeration) shows the constant growth rate throughout the fermentation. Due to the accessibility of sugary mucilage with optimum conditions, the growth of yeast showed a qualitative increase initially.

Results found that the *E. coli* count (log cfu/ml) in different blend samples in room temperature and shaking condition were increased after fermentation of 24 h, that is, from 3.05 to 3.28 and 3.05 to 3.48 (log cfu/ml), respectively. A previous recent study also found the *Enterobacteriaceae* counts, that is, 4.87, 4.48, 4.84, and 4.99 log cfu/g in flour, porridge, Mkarango, and Busaa. These are selected maize-based fermented products from Western Kenya.[15] *Enterobacteriaceae* are the pathogens (such as *E. coli* etc.,) that may be responsible for foodborne ailments in individuals who frequently consume contaminated foods.[16] Health Protection Agency set, the upper *Enterobacteriaceae* counts limit in ready-to-eat products, that is, 10^4 cfu/g (4.0 log cfu/g).[17]

The relevant information about the microbial population of LAB count (log cfu/ml) of the spontaneously fermented blend at different conditions is discussed. The bacterial count increased with increasing fermentation days. The bacterial count of room temperature and shaking condition samples ranged from 5.61 to 6.4 and 5.61 to 6.17 (log cfu/ml). There is a slight increase in the first 24 h after constant growth due to increased acidity. The bacterial count of shaking condition was highest at 72 h of fermentation. The growth pattern of associated microorganisms followed a normal bacterial growth curve which might be due to the activity of microbes in the new environment of fermentation. Pre-treatment of the samples (fermentation) leads to a higher population of microbial counts.

Here, the finding suggested the count of total aerobic bacteria in given different samples, that is, buttermilk, flour, mix, and their control. The control samples (buttermilk, flour, and mix) were kept at 4°C temperature, whereas the same samples of buttermilk, flour, and mix were kept at room temperature 28°C. The bacterial growth increases with increase in temperature, and their growth seize at 4°C; therefore, it was observed that the samples which are kept at room temperature have more viable cell count hence leading to the increase in the total number of aerobic counts as compared to the samples kept at 4°C.

The amount of LAB dominated all samples and increased throughout the fermentation. Results show that the growth of LAB in control samples was not increasing as the growth seizes due to room temperature. Still, at room temperature, the amount of LAB increased in ranges from 6.07 to 6.90 and 6.2 to 6.8 (log cfu/ml) in buttermilk and mix samples, but in pearl millet flour LAB colonies were not found.

Finding stated relevant information about *E. coli* count for the taken samples (buttermilk, flour, and mix) and control samples. The growth rate of *E. coli* in the buttermilk sample at room temperature ranges from 3.91 to 3.46 (log cfu/ml). It first slightly increased after 48h and declined after 72h of fermentation. In the mixed sample, there is a slight increase in *E. coli* count after 48 h and then dropped after 72 h, but in the flour sample, it was slightly increased from 3.75 to 4.32 (log cfu/ml) as fermentation hours increased. Hence, the highest growth occurred in the flour sample, which was kept at room temperature as compared to all control samples, which were kept at 4°C.

The yeast and mold count in the different samples in which the growth of yeast and mold was first increased at 24 h and a slight decline was observed at 48 h fermentation duration in their growth and then increases after 72 h fermentation as compared to control samples which were kept at 4°C growth of control samples was almost constant throughout the fermentation duration.

CONCLUSION

The important sources contributing to the functional nutrients are mainly pearl millet and dairy products processed under fermentation. The flour obtained from pearl millet contributes to important sources of advantageous microbes, especially LAB and yeast. As the days of fermentation increase, the properties such as titratable acidity and temperature of fermented blend also increase while pH decreases proportionally. The fermented blend has two contradictory properties (pearl millet flour and buttermilk). One is the existence of LAB and yeast, which helps improve the nutritional qualities of fermented products and hence benefits the consumer.

On the other hand, bacteria like coliforms put forth some risks to product quality and consumer health. The process of handling, storage, and processing by the producers does not pay much attention to the hygienic of the product. As a result, local producers must be provided with special training to improve the hygienic properties of fermented foods. Further desirable functional properties can be achieved by doing more studies on the characterization of microbial isolates by bringing into practice molecular techniques and screening isolated cultures. Preliminary study results suggest that best storage condition is refrigeration for all the samples. However, on 72 h of fermentation, LAB count was increased. This is only a preliminary study, a study with a large sample and much-organized methods need to be investigated for the outcome of these different conditions of microbial dynamics.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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