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Prevention of Bread Spoilage and to Enhance the Quality of Bread by using Lactic Acid Bacteria

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Abstract: Baked foods are the staple foods that are consumed by the people all over the world, since from the age old time. The production of bread is easy and can be practiced in all environmental conditions. Bread is highly perishable under humid condition, which paves way for fungi to grow over it and causes spoilage. Generally in baking industries, propionic acid a chemical preservative is added to prevent the spoilage of bread by moulds, which is not preferred by the consumers. Several attempts were made in the recent past for the addition of organic preservatives to control the bread spoilage. Contamination by mould in bread can be inhibited by several microorganism, Lactic Acid Bacteria (LAB) has no degrading effect over the nature, taste and texture of bread, makes it suitable for the bio preservation of bread. In this study, LAB, yeast and mould were added to the dough. Parameters such as presence of LAB, antifungal activity and physical nature were studied after baking.
Keywords: Lactic Acid Bacteria (LAB), antifungal activity, bio preservation.

Introduction:

The history of bread goes back at least 30,000 years. The first bread produced was probably cooked versions of a grain-paste, made from roasted and ground cereal grains and water, and may have been developed by accidental cooking or deliberate experimentation with water and grain flour.Descendants of these early flatbreads are still commonly made from various grains in many parts of the world. In general bakery products should be dry and fresh for its usage. But in wet conditions it paves way for several agents to precede spoilage over them. Spoilage of bakery products is mainly due to fungal growth.The major species involved are *Aspergillus, Fusarium* and penicillium. The most common spoilage fungi of cereal-based products belong to the genera *Penicillium(P),Aspergillus(A.),Fusarium(F.)*(Keshri et al.,2002). Fungi were grown in Potato Dextrose Agar (pH 5.6) (PDA, Oxoid Laboratories) at 25C for 24-72 h. Wheat flour hydrolyzes (WFH) was prepared as described previously (Gobbetti et al., 1994) and used for the determination of conidia germination.

In addition to the great economic losses derived from the presence of moulds,another concern is the potential mycotoxin production that may cause public health problems.Mouldsproduce mycotoxins such as Ochratoxin A and Aflatoxin. Flour contains (approximately) 8000 mouldspores/ 1g. Bread can be easily affected by moisture. Microbiological spoilage by bacteria, yeast and molds is the concern in high moisture products (i.e.) products with a water activity (aw>0.85). NaCl is common inhibitory action to mould growth

Contamination by moulds can be prevented by irradiating the goods with infrared rays or microwaves, by using modified atmospheres during packaging or by adding chemical preservatives such as propionic acid. The addition of chemical preservatives is not mostly preferable.

In recent years, bio preservation (the use of microorganisms and/or their metabolites to prevent spoilage and to extend the shelf life of foods) (stiles 1996) has gained increasing interest due to consumer's demand. Preservatives are commonly used in bread because economic losses from bread spoilage caused by bacteria or by moulds are substantial.

Shelf life is defined as "the life time of the bakery products". Moisture content may reduce the shelf life of bread. Normal bread shelf Life is 2 days. If Calcium propionate (preservative) added, shelf life increases 3-4 days.

The *Lactobacillus* is isolated from cow and goat milk ha sa high antifungal activity [Delavenne, Mounier, Denial, Barbier, and Le Blay(2012)]. LAB, total aerobicmesophilic bacteria (TAMB), Lo¨nner, Welander,Malin,and Dostalek,1986). PH value and dry matter percentages were determined by a method described by (Elgu¨n, Ertugay, Certel, and Kotancılar,1999).As an alternative, the antimicrobial activity displayed by LAB strains may help to combat microbial contamination (Holzapfel, Geisen, &Schillinger,1995; Lu¨cke, 2000).

Numerous studies have described the isolation and characterization of antifungal components from LAB cultures (Corsetti et al., 1998; Lavermicocca et al., 2003) but limited applications of the antifungal strains in baking have been reported (Dal Bello et al., 2007; Lavermicocca et al., 2000).

The selection of the most suitable starter culture to be used in baking applications (Ehrmann and Vogel, 2005). Rapid acid production by lactic acid bacteria is a desired property for selection of starter cultures used for sourdough processes (Clarke, Schober, & Arendt, 2002; Gianotti et al., 1997; Gobbetti et al., 1995).

Wheat FlourYeast and other ingredients

Wheat flour is the key ingredient in most braked food products. Flour quality is particularly important in bread making as the quality of the flour will have a significant impact on the finished product. The elastic framework of gluten holds the gas produced by the fermentation action of yeast.Yeast requires moisture, food and warmth for growth. When these requirements are satisfied, the yeast grows rapidly to produce carbon dioxide gas to enable the dough to rise even at the high temperature. Expand the dough's cellular network to form bread crumb and to give bread its characteristic flavor and aromabyLodder(1970). Ascorbic acid and acetic acid were added to increase the doughness and freshness of the product.

Preparation of culture medium:

The*Lactobacillus plantarum* (MTCC No.1407) strain obtained from Chandīgarh was used throughout the study as a bio preservative agent and plated in the MRS media with the composition of 1% peptone, 0.8% egg extract, 0.4% yeast extract, 2.0% glucose 0.5% sodium acetate trihydrate and other essential chemicals with pH of 6.2 adjusted by phosphate buffer and temperature 25^{o}C.

Mould isolation

Bread acts as a rich source for the mould to grow. The mould can be easily isolated from the spoiled bread which is clearly visible. Piece of contaminated bread sample was serially diluted and plated in potato dextrose agar medium which was sterilized initially and incubated for 27^{o}C for 4 - 5 days.

Anti fungal assay test

Rapid, reliable and sensitive methods for the detection of the antifungal activity of LAB become convenient replacements for chemical preservatives with potential industrial application. The serially diluted mould plates were observed for the number of colonies formed in it. The disc loaded with the LAB organism was placed with different concentration of serially diluted plates ($10^{-1}$to $10^{-8)}$and incubated at room temperature. The zone of inhibition exhibited by the Lab organism was observed.

Viability test

Thus the viability test can be done by several methods like flow cytometry, colony count etc. Generally, counting the number of colonies on an agar plate is the standard method for determining the number

of viable bacterial cells in samples. However, colony formations require one to several days. *Lactobacillus plantarum*, yeast and moulds are mixed completely for the bread preparation in required proportion. The mixture was undisturbed as doughing process is carried out for the bubbles to create. When the sample were baked at 110^{o}C the microorganism was killed and hence to confirm that viability test were carried out.

Quality control tests

Water is an inexpensive ingredient, and manufacturers often try to incorporate as much water as possible, without exceeding the legal maximum requirement. A simple moisture test on incoming raw ingredients and the final product can able to prevent the easy spoilage.The moisture content is the loss in weight of a sample when heated under specified conditions. The percentage of moisture present in the sample is determined using the formula

$$\text{Moisture (\%)} = (A - B) / (A - C) \times 100$$

The ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific inorganic components present within a food, such as Ca, Na, K and Cl. Total ash is the inorganic residual remaining on incineration in a muffle furnace. This reflects the quantity of mineral matter present in the flour. Acid insoluble ash reflects added mineral matter in milled products such as dirt, sand, etc.

The ash content of the sample is determined by

$$\text{Ash} = (W3 - W1) / (W2 - W1) \times 100$$

Yeast is a biological material, and thus its activity is affected by many factors such as storage temperature, relative humidity and moisture content, etc. Such conditions affect the number of viable cells per unit mass and hence the dough raising capacity. In order to produce good quality fermented product it is important to add the optimum quantity of yeast. Therefore, it becomes necessary to check the dough raising capacity of each batch and also periodically for satisfactory gas production during fermentation of the product.

The dough raising capacity can be calculated as

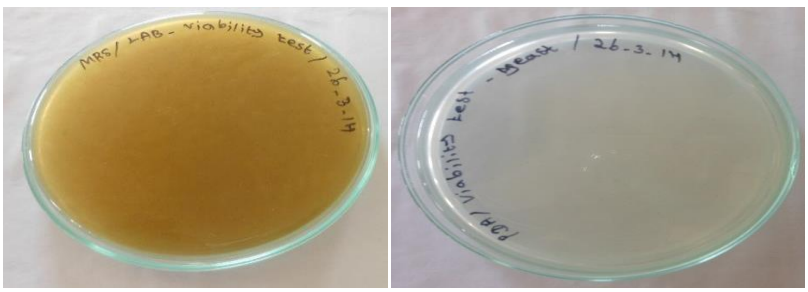
$$\text{Dough raising capacity} = (B - A) / A \times 100$$

Antifungal Assay test

In the current study, we optimized a commercially available discs (9 mm diameter) preloaded with LAB microbes, to determine the susceptibility to various yeast. Molds were sub cultured on potato dextrose agar (PDA) at 30°C for 4 to 15 days. Plates were streaked evenly with a swab dipped into the standardized inoculum and the lids of the jar was opened for 3 min in a laminar flow cabinet to allow for any excess surface moisture to be absorbed into the agar before the drug-impregnated disks were applied. Disks containing the LAB Microbes were placed to the surfaces of the plates. Plates were inverted and incubated at 30°C for 4 to 7 days to allow for fungal growth. An inhibition zone diameter (IZD) was found to be 14 cm when measured in millimeters.

Viability Test after Baking

Figure 1: Viability Test after Baking in lab and Yeast



Viability test– LAB Viability test- yeast

Viable cell detections are very important for analyzing microbial contamination in food or evaluating the cleanliness of facilities in order to protect us from food poisoning and infections. The sample piece from

bread were collected and placed in the agar plates and sealed with adhesive tape at the circular edges of the plates to prevent the contamination. After incubation period it was checked for the presence of viable organism and found no viable or visible growth of LAB was found on the agar plate. The same test was carried out simultaneously for the yeast and no immediate colonies were found after incubation. Thus after baking it was found that no microbial growth (LAB and Yeast) were present, which confirms the food product is completely safe and shown in (Figure-1).

Moisture and ash content test:

Baking is a process which uses heated air to alter the eating quality of foods. A secondary purpose is preservation, by destroying microorganisms and reducing the moisture content at the surface of the food. The texture, taste, appearance, stability and also the shelf life of your final product depend on the amount of water it contains. Knowing the appropriate moisture content will allow the baked product to be successfully manufactured and sold in the market. The moisture content of all samples is determined by measuring the mass of the material before and after the water is removed by evaporation:

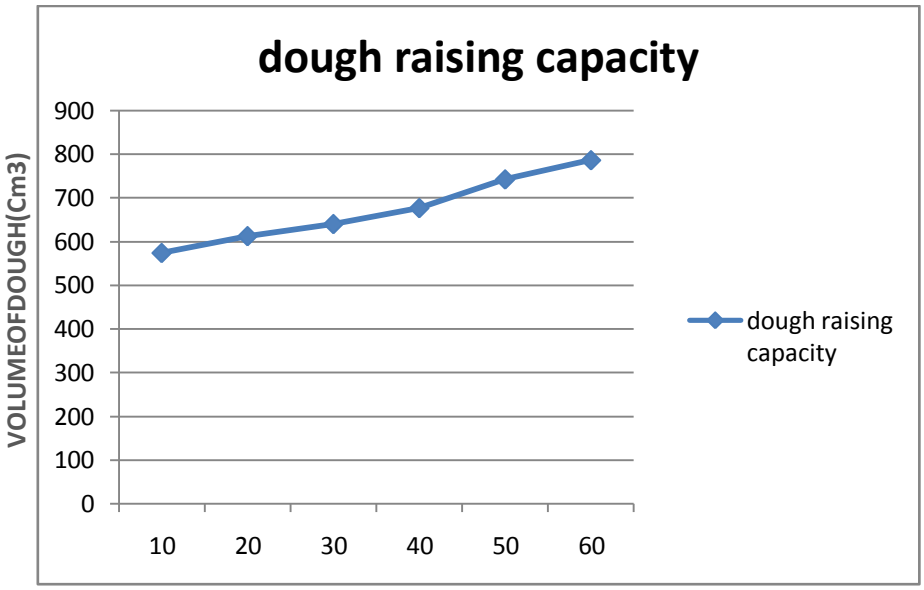
$$\% \text{ Moisture} = \frac{\text{Initial Sample Weight - Final Sample Weight}}{\text{Initial Sample Weight}} \times 100$$

When comparing the moisture content of the bread of standard values to experimental values is found to be low. Although experimental values were low of the moisture content when compared to the standard values, the result produced were beneficial overall because of the decrease in moisture content and water activity simultaneously, thus increasing the shelf life of the bread.

Ash refers to any inorganic material, such as minerals, present in food. The residue that remains after heating removes water and organic material such as fat and protein. Ash can include both compounds with essential minerals, such as calcium and potassium, and toxic materials, such as mercury. Generally, in the fresh food the ash content found be slightly high as 5, while some processed foods can have ash content of more than 10 percent. The ash content was determined by heating the bread and noted to be 4.3%, this value denotes that baked bread does not affect the texture.

Dough Raising Capacity

Figure 2: Dough raising capacity with increasing time



Doughing is an important part of the process of bread making, no matter which method is employed. If it is permitted to rise too much, the product will be coarse in texture. As has been noticed that best results are obtained if the bread dough is kept at a uniform temperature throughout its rising. The ability of water and yeast is used to raise the dough with the evolution of carbon dioxide was observed with increasing time interval which is elucidated in the given (Table- 1) and represent as graph in (Figure- 2). As shown in the graph volume of dough has increased in the interval of every 10 minutes.

Table 1: Dimensional changes after fermentation

Parameters	**Before Fermentation**	**After1 HourFermentation**
Height of dough(Cm)	4.5	5.2
Length of dough(Cm)	8.5	9.0
Breath of dough(Cm)	15	16.8
Volume of dough(Cm^3)	573.75	786.24

Discussion

Fungal spoilage is the main cause of substantial economic lossesin packaged bakery products and might also be regarded as sources of mycotoxins, involving public health problems. In this context, LAB may be considered as an alternative for bio-conservation. In this study, LAB strain (*Lactobacillusplantarum*-MTCCNo.1407) were selected for antifungal activity against *A.niger* (isolated from contaminated bread). In this work, *Lactobacillus plantarum* (regarded as antifungal positive strains) were used in the formulation of a mixed starter culture(LB) and used together with *Saccharomces cerevisiae* (commercial yeast) in bread elaboration.

Anti mi-microbial of *Lactobacillus plantarum* inhibits *Aspergillus niger, Fusarium graminearum* contaminants from flour, yeast and other sources. These metabolites may also exhibit some activity against spores, which may pass into a vegetative form. If these metabolites are heat stable, they may inhibit pathogen organisms contaminated through packaging and marketing, ands pore germination after baking.

Rapid acid production by lactic acid bacteria is a desired property for selection of starter cultures used for sourdough processes. Baking temperature may destroy the LAB. But antifungal activity can be retained at baking temperature. After fermentation, pH of the LAB may decrease to 3.5. At this pH antifungal activity of the bacteria increases. In addition, the fungal growth was delayed for 5-days when using the starter LB compared to Yeast(Y)-breads elaborated with (0.1–0.2%). Tests are carried out to predict the end use quality of bread. The functional tests such as moisture content test, ash content test, dough raising capacity of yeast and alkaline water retention test are performed to judge the quality of wheat flour to get best potential of flour when processed at industrial scale. These tests are useful in making compatible application of flour for a specific product. This avoids processing losses and helps in improving the overall quality of product.

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