Title: BREAD PREMIX AND BREAD PRODUCED UTILIZING SAME

Abstract: A bread premix is provided that comprises a mixture of amylases produced bacterially from a flour suspension and antimicrobial agents bacterially produced from a flour suspension. The mixture of amylases and the antimicrobial agents are preferably produced separately from each other after which they are mixed and bacterial activity in the mixture is terminated by virtue of an elevated temperature. The mixture of amylases is preferably produced from a slurry containing bread flour, an optional minor quantity of soy flour; and sugar with the mixture being stirred to maintain the condition of a suspension. The bacteria that are preferably selected from one or more of Lactobacillus acidophilus, Lactobacillus amylovorus, and Lactobacillus amylophilus act to ferment the slurry to produce enzymes (mainly amylases) that convert starch to fermentable sugars. The antimicrobial agents are preferably produced in a two-step fermentation process in the first of which Lactobacillus pentosus or an equivalent is firstly utilised to produce an antimicrobial peptide together with lactic acid and in the second of which Propionibacterium sp or an equivalent is employed to convert at least some of the lactic acid to propionic acid. A method of producing bread utilizing such a bread premix is also provided.
FIELD OF THE INVENTION

This invention relates to a bread premix that may, at least in some instances, be used to enhance the shelf life of bread produced utilizing it and that may, at the same time, or in the alternative, enable the omission of one or more ingredients that are regarded as chemical ingredients, in particular calcium propionate, with a view to providing a more natural product. Added to this, at least in some instances, there is typically at least some cost advantage that may be achieved in consequence of a simplification of the bread production procedure and, cost of the combined ingredients.

BACKGROUND TO THE INVENTION

Bread is normally produced by fermenting a mixture of bread flour, soya flour, spray-dried fats, sugar, enzymes, various micro-ingredients, and yeast. A typical formulation for a white bread mixture is, simply by way of example, given in the following Table 1.

<table>
<thead>
<tr>
<th>INGREDIENT</th>
<th>STANDARD FORMULATION (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread flour</td>
<td>100</td>
</tr>
<tr>
<td>Salt</td>
<td>1.980</td>
</tr>
<tr>
<td>Soy, full-fat, active</td>
<td>0.590</td>
</tr>
<tr>
<td>Sugar, brown</td>
<td>0.490</td>
</tr>
<tr>
<td>Calcium propionate</td>
<td>0.170</td>
</tr>
</tbody>
</table>
Fat powder* 0.083
Enzymes (complex)† 0.051
Ascorbic acid‡ 0.005
Carrier• 0.011
Vitamins and mineral salts# 0.120
Yeast 2.923
Water 56.41

*Fat powder = “Base”, containing vegetable fat, enzymes (mainly alpha-amylase with xylanase and lipase activity), ascorbic acid and a carrier

†Enzymes = from “Base”

‡Ascorbic acid =

•Carrier = from “Base”

#Vitamins and minerals =

electrolytes, iron, zinc oxide and a complex of vitamins (in this instance a mixture of 0.020% of an enrichment sold under the trade name KULUBRITE 10 by Idwala of South Africa and comprising calcium carbonate (mineral salts) with 95% activity, food grade; and 0.100% of an enrichment sold under the trade name ROCHE IS-324 by Roche Vitamins of South Africa and comprising mineral salts (electrolytic iron, zinc oxide), vitamins and food grade carrier and marketed was employed).

The components are mixed and the dough is allowed to prove for about 45 minutes at a temperature of about 45°C. During this time the enzymes added to the dough (mainly amylase and xylanase) convert the starch to
fermentable glucose and other sugars. The lipase added converts lipids (fats) to fatty acids and other fermentable carbohydrates. The yeast converts most of the fermentable carbohydrates to carbon dioxide (CO₂), which leads to leavening of the dough. The time needed to reach the desired (sometimes referred to as maximum) dough volume will be referred to as “proving time”. The bread is then baked at a temperature of initially about 220°C decreasing to about 200°C over the baking period of about 25 minutes.

Of the ingredients identified above the calcium propionate may be regarded as a chemical that contributes to, not only the cost of the bread, but also to the bread not being regarded as a natural product.

The calcium propionate is intended to serve as an anti-fungal agent to extend the shelf life of the bread. However, the shelf life of bread produced in this manner is usually only about 3 days at ambient temperature. Furthermore, it has been reported that calcium propionate may cause allergies as well as hyperactivity amongst children.

**OBJECT OF THE INVENTION**

It is, accordingly, an object of the invention to provide a pre-mix that enables at least the calcium propionate to be omitted from a bread recipe. It is a further object of the invention to provide a premix that, in some instances, provides bread having a somewhat extended shelf life. It is a still further object of the invention to provide a premix and bread production process utilizing same wherein, at least in some instances, bread production is facilitated, in particular, but not necessarily exclusively, in consequence of, at least in some instances, a shorter proving time.
SUMMARY OF THE INVENTION

In accordance with one aspect of the invention there is provided a bread premix comprising a mixture of amylases produced bacterially from a flour suspension and antimicrobial agents bacterially produced from a flour suspension.

Further features of the invention provide for the mixture of amylases and the antimicrobial agents to be produced separately from each other after which they are mixed and, as may be required, heated to terminate bacterial activity in the mixture; for the mixture of amylases to be produced from a slurry containing bread flour, an optional minor quantity of soya flour; and sugar with the mixture being stirred to maintain the condition of a suspension; for the bacteria employed in the production of the mixture of amylases to be selected from strains of bacteria that act to ferment the slurry to produce enzymes (mainly amylases) that convert starch to fermentable sugars; and for such bacteria to be selected from one or more of Lactobacillus acidophilus, Lactobacillus amylovorus, and Lactobacillus amylophilus.

Still further features of the invention provide for the antimicrobial agents to be produced from a slurry containing bread flour and sugar with the mixture being stirred to maintain the condition of a suspension; for the antimicrobial agents to include an antimicrobial peptide active against a range of spoilage bacteria including Bacillus species; for the antimicrobial agents to include propionic acid; and for the mixture of antimicrobial agents to be produced in a two-step fermentation process in the first of which Lactobacillus pentosus or an equivalent thereof is firstly utilised to produce an antimicrobial peptide together with lactic acid and in the second of which Propionibacterium sp or an equivalent thereof is employed to convert at least some of the lactic acid to propionic acid.
The bread premix produced as defined above is preferably maintained in the form of a slurry and stored, other than for immediate use, under reduced temperature prior to being incorporated in a bread mix.

The invention also provides a method of producing bread comprising mixing, in suitable proportions, a bread premix as defined above together with substantially standard ingredients in substantially standard proportions but wherein the resultant dough is devoid of at least added calcium propionate.

It has been found that a bread dough made according to this invention has a characteristic that proving time can be appreciably reduced, and by up to about 50%. It has also been found that baked bread originating as such bread dough has, at least in instances in which the antimicrobial agents are suitable, an extended shelf life of up to about six days.

In order that the above and other features of the invention may be more fully understood one embodiment of the invention at its present stage of development will now be described with reference to the accompanying drawing.

**BRIEF DESCRIPTION OF THE DRAWING**

The accompanying drawing is a schematic diagram illustrating the production of a bread premix.

**DETAILED DESCRIPTION WITH REFERENCE TO THE DRAWING**

**Preparation of bread pre-mix**

In this embodiment of the invention a bread pre-mix (slurry) is based on a mixture of bread flour, soya flour and sugar. Briefly, the slurry is fermented with a combination of selected strains of the bacteria *Lactobacillus*...
acidophilus, Lactobacillus amylovorus, Lactobacillus amyophilus, Lactobacillus pentosus and Propionibacterium sp. The Lactobacillus acidophilus, Lactobacillus amylovorus and Lactobacillus amyophilus strains produce enzymes (mainly amylases) which convert starch to fermentable sugars. The Lactobacillus pentosus strain produces an antimicrobial peptide active against a range of spoilage bacteria, including Bacillus species. The Propionibacterium sp. produces propionic acid from lactic acid, which is produced by the bacteria in the same fermentation step.

More particularly, the bread pre-mix (slurry) is prepared by combining two separately produced bacterial mixtures in two stirred containers (1) and (2) in a batchwise procedure as follows:

Into the one container (1) are introduced the following ingredients:-

70 g soya flour  
800 g bread flour  
200 g sucrose  
3 litres cold water  
3 litres warm water (60°C).

The conditions of the slurry premix are adjusted, as may be necessary, to a pH of about 5.5 and a temperature of 30-35 degrees centigrade.

This container is then inoculated with:-

300 ml L. amylovorus BPM1 (see below) (a combination of L. amylovorus and L. acidophilus) and

300 ml L. amyophilus BPM2 (see below), each at an approximate concentration of $10^7 - 10^8$ cells per ml. The mixture is then incubated for 24h at 37°C. During this fermentation process enzymes that are mainly amylases that convert starch to fermentable sugars are produced. The resultant slurry component may be termed the amylase containing slurry component.
Into the other container (2) are introduced the following ingredients:-

800 g bread flour
400 g sucrose
3 litres cold water
3 litres warm water (60°C).

The conditions of this slurry premix are also adjusted, as may be necessary, to a pH of about 5.5 and a temperature of 30-35 degrees centigrade.

This container (2) is then inoculated with:-

300 ml L. pentosus LPP

at an approximate concentration of \(10^7 - 10^8\) cells per ml and incubated for 12h at 37°C. During this part of the fermentation process an antimicrobial peptide is produced as well as lactic acid.

Thereafter, this container (2) is inoculated with:-

300 ml Propionibacterium sp. BPM3 (see below)

at an approximate concentration of \(10^7 - 10^8\) cells per ml and incubated for a further 12h at 37°C. During this part of the fermentation process lactic acid produced in the first part of the fermentation process carried out in this container is converted to the natural preservative propionic acid. The resultant slurry component may be termed the antimicrobial slurry component.

After fermentation of the two slurry components as described above, the contents of the two containers (1) and (2), are heated separately to 60°C in order to terminate bacterial activity and then cooled down to room temperature (25°C).

The two slurry components are combined in a single container (3) and 2.8 litres cold water (at 25°C) is added to the slurry mixture. During the combination of the two slurry components and its dilution with water, rapid stirring is carried out in order to avoid clotting. The resultant bread pre-mix
slurry may be stored at room temperature (25°C) overnight. Alternatively, if it is to be stored for any longer, it is stored in a chiller (4).

**Preparation of the starter cultures**

The starter cultures (listed below) are prepared in the following manner:

**Bacterial strains:**

(i) *Lactobacillus amylovorus* and *Lactobacillus acidophilus* (BPM1)

(ii) *Lactobacillus amylophilus* (BPM2)

(iii) *Lactobacillus pentosus* (LPP) (a relatively new strain that has been assigned the accession number PTA-1466 and the American Type Culture Collection (ATCC))

(iv) *Propionibacterium* sp. (BPM3)

All of the above four starter cultures were propagated separately in 10ml MRS (Merck) broth and incubated at 30°C, without aeration for 24 to 48 hours. Up-scaling to larger volumes was done by inoculating each of the 10ml cultures into 300ml MRS broth (Merck), followed by incubation at 30°C, without aeration for 24 to 48 hours. Larger volumes of the starter cultures were prepared by inoculating the 300 ml MRS broth cultures into a 10 litre slurry containing bread flour (13.30%, w/v), soy flour (1.17%, w/v), sucrose (3.33%, w/v) and water (for starter cultures BPM1 and BPM2) and bread flour (13.30%, w/v), sucrose (6.66%, w/v) and water (for starter cultures LPP and BPM3). Fermentation was carried out for 24 to 48 hours of growth, at conditions as described above. The period of fermentation was determined by monitoring the decrease in pH. At a pH of approximately 4.0, the 10 litre slurries containing the starter cultures were inoculated into 300 litre volumes with contents in the same ratio as described.

Long term storage of the starter cultures is effected in glycerol (40%, final concentration) at -20°C or as freeze-dried cultures at ambient temperature.
Identification of the starter cultures

The strains were identified by using the API 50 CHL carbohydrate fermentation system, as described by the suppliers (bioMerieux Marcy l'Etoile, France).


*Lactobacillus pentosus* LPP is described in international patent application publication number WO 00/59308. Starter culture BPM3 contains an unidentified combination of propionibacteria, most probably *Propionibacterium freudenreichii* as determined by sugar fermentations according to the API 50 CH system.

The ability to degrade starch and determination of the residual sugars (extracellular amylolytic activity) was recorded by using the iodine-starch colour reaction method, as described by Nakamura (International Journal of Systematic Bacteriology, 1981, vol. 31, pp. 56-63) and the microtiter plate assay, as described by Imam et al., 1991, Current Microbiology, vol. 22, pp. 365-370.
Incorporation of the pre-mix (slurry) into bread dough

The bread pre-mix (slurry) produced as described above may be added to the rest of the usual ingredients of a commercial bread recipe with the important difference that certain of the ingredients in the original bread recipe (as listed in Table 1 above), in particular calcium propionate has been omitted (see Table 2 below).

Table 2. Formulation of bread dough made with the pre-mix (slurry)\textsuperscript{a}

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>STANDARD FORMULATION (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread flour</td>
<td>100</td>
</tr>
<tr>
<td>Salt</td>
<td>1.980</td>
</tr>
<tr>
<td>Soy, full-fat, active</td>
<td>0.590</td>
</tr>
<tr>
<td>Sugar, brown</td>
<td>0.000</td>
</tr>
<tr>
<td>Calcium propionate</td>
<td>0.000</td>
</tr>
<tr>
<td>Fat powder\textsuperscript{*}</td>
<td>0.081</td>
</tr>
<tr>
<td>Enzymes (complex)\textsuperscript{†}</td>
<td>0.007</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.005</td>
</tr>
<tr>
<td>Carrier\textsuperscript{*}</td>
<td>0.015</td>
</tr>
<tr>
<td>Vitamins and mineral salts#</td>
<td>0.120</td>
</tr>
<tr>
<td>Yeast</td>
<td>2.974</td>
</tr>
<tr>
<td>Pre-mix (slurry)\textsuperscript{*}</td>
<td>3.800</td>
</tr>
<tr>
<td>Water</td>
<td>53.33</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Changes made to the recipe in Table 1 are indicated in bold.
Fat powder = "Base", containing vegetable fat, enzymes (mainly alpha-amylase with xylanase and lipase activity), ascorbic acid and a carrier (in this case KPS a product marketed by Germantown of New Zealand (available in South Africa from Goodman Fielder International (a vegetable fat and carrier (modified starch, antioxidant)) + Capz SS a product marketed by Innovative Ingredients of South Africa (an encapsulated enzymes system consisting of enzymes (mainly alpha-amylase and amylo-glucosidase with xylanase and lipase as side activity), hydrogenated palm fat and soy lecithin)

Enzymes = from "Base" (in this case a mixture of 0.001% Grind A5000 a product marketed by Danisco Cultor of South Africa (fungal alpha-amylase produced by the fermentation of a strain of Aspergillus oryzae) and 0.006% Capz SS)

Carrier = from "Base" (in this case KPS + Capz SS)

Vitamins and minerals = electrolytes, iron, zinc oxide and a complex of vitamins

Pre-mix slurry = as prepared in "Preparation of bread pre-mix above"

No sugar or calcium-propionate has been added to the recipe containing the bread pre-mix (slurry). Only 13.725% of the usual quantity of enzyme complex has been added to the recipe with the premix, compared to the normal bread recipe (without pre-mix).

Bread dough produced in this manner was found to require, at least in some instances, up to 50% less proving time than its prior art counterpart.

The bread dough proved to be particularly suitable for preservation
Baking trials

It was found that implementation of the invention could be carried out without any adverse affect on the texture and organoleptic quality of the final product and the baked bread had an extended shelf life of up to about six-days.

There are numerous ways in which the invention can be implemented and the premix described above, its production, and use are simply illustrative of the invention at its present state of development.

It is envisaged that many further modifications may well be possible to a standard commercial bread recipe within the scope of the invention.
CLAIMS:

1. A bread premix characterized in that it comprises a mixture of amylases produced bacterially from a flour suspension and antimicrobial agents bacterially produced from a flour suspension.

2. A bread premix as claimed in claim 1 in which the mixture of amylases and the antimicrobial agents are produced separately from each other after which they are mixed.

3. A bread premix as claimed in either one of claims 1 or 2 in which bacterial activity in the mixture has been terminated by virtue of an elevated temperature.

4. A bread premix as claimed in any one of the preceding claims in which the mixture of amylases is produced from a slurry containing bread flour, an optional minor quantity of soya flour; and sugar with the mixture being stirred to maintain the condition of a suspension.

5. A bread premix as claimed in any one of the preceding claims in which the bacteria employed in the production of the mixture of amylases are selected from strains of bacteria that act to ferment the slurry to produce enzymes (mainly amylases) that convert starch to fermentable sugars.

6. A bread premix as claimed in claim 5 in which the bacteria are selected from one or more of Lactobacillus acidophilus, Lactobacillus amylovorus, and Lactobacillus amylophilus.

7. A bread premix as claimed in any one of the preceding claims in which the antimicrobial agents are produced from a slurry containing bread
flour and sugar with the mixture being stirred to maintain the condition of a suspension.

8. A bread premix as claimed in any one of the preceding claims in which the antimicrobial agents include an antimicrobial peptide active against a range of spoilage bacteria including *Bacillus* species.

9. A bread premix as claimed in any one of the preceding claims in which the antimicrobial agents include propionic acid.

10. A bread premix as claimed in claim 9 in which the antimicrobial agents are produced in a two-step fermentation process in the first of which *Lactobacillus pentosus* or an equivalent thereof is firstly utilised to produce an antimicrobial peptide together with lactic acid and in the second of which *Propionibacterium* sp or an equivalent thereof is employed to convert at least some of the lactic acid to propionic acid.

11. A method of producing bread comprising mixing, in suitable proportions, a bread premix as claimed in any one of claims 1 to 10 together with substantially standard ingredients in substantially standard proportions but wherein the resultant dough is devoid of at least added calcium propionate.
