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#### **Published**

With international search report.

(54) Title: BAKING IMPROVER/DOUGH CONDITIONER

(57) Abstract

A dough conditioner is described wherein the dough conditioner is soybean peroxidase or an aqueous extract of soybean hulls.

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#### "BAKING IMPROVER/DOUGH CONDITIONER"

#### Background of the Invention

This invention relates to the preparation and conditioning of dough and, more particularly, to the use of soybean peroxidase or soybean hull extract as a replacement for potassium bromate.

Dough conditioners as bakery additives have been highly beneficial to the food industry, particularly in the high-speed wholesale bakeries. They allow the baker to compensate for the inevitable differences in the flour and high energy input, so that process limits can be adhered to and maximum throughput can be attained while giving utmost stability during fermentation and good oven spring.

Surfactants and oxidants help provide the maximum strength in the gluten so that the dough will stand up to the physical stress put on it by automated production lines. Reductants and mix-time reducers help compensate for the flours that are too strong in their gluten characteristics or are bucky. Judicious application of the various conditioners available help produce consistent, profitable production of high quality bread and help the baker meet the needs and demands of the customer.

Potassium bromate has been used as an oxidant in flour doughs. Potassium bromate oxidizes the gluten in dough. This enables the dough to retain gas better and yields a taller, larger volume loaf. The result is so well known that it is simply called "the bromate effect." The history of potassium bromate usage in the baking industry is discussed by P. Ranum, "Potassium Bromate in Bread Making," Cereal Foods World, March 1992, Vol. 37, No. 3, pp 253-258.

There are several disadvantages to using potassium bromate as a dough conditioner. Potassium bromate is a strong oxidizer and can cause violent fires upon storage and even explosions upon contact with organic materials, particularly when the organic material is in a powder form such as flour. Toxicity of potassium bromate is also a major concern. been reported to be a carcinogen. As a result of the health concerns associated with potassium bromate, most of the countries in the European community have never permitted the use of potassium bromate and its use has now been banned in the United Kingdom, Japan and New Zealand. At the present time, there are no federal regulations governing the use of potassium bromate in the United States, however, the need for a suitable replacement is clear. The WHO banned the use of bromates and alternatives that will perform as well are sought. Due to the fact that most other approved oxidants are fast acting, it is logical to look for the answer and replacement for potassium bromate in oxidizing enzymes.

"Ascorbic acid is widely used as a flour improver in bread baking. According to Tsen ascorbic acid is first oxidized to dehydroascorbic acid, either spontaneously by atmospheric oxygen, or enzymatically by ascorbic acid oxidase. Dehydroascorbic acid is then reduced back to ascorbic acid with simultaneous oxidation of two sulphydryl groups to form disulphide bond by glutathione dehydrogenase." Chemistry and Physics of Baking, J.M.V. Bianshard, P.J. Frazier, T. Galliard, 1987, pp.114.

The two of the principal replacement oxidants for potassium bromate that have heretofore been used are ascorbic acid and azodicarbonamide (ADA). Ascorbic acid (vitamin C) has been used extensively around the world as a flour improver, particularly in those countries that do not allow potassium bromate. Compared to potassium bromate, ascorbic acid produces a smaller volume increase and is more expensive.

Azodicarbonamide (ADA) is a fast-acting oxidant that has been used since the 1960's. One problem with using ADA as a replacement for potassium bromate is its high reactivity. ADA can fully convert to biurea in sponges and flour doughs by reacting with the gluten and possibly with reducing substances produced by yeast. Unlike potassium bromate, ADA can react completely with ascorbic acid if both are present in a slurry, leaving no ADA to work at the dough-mixing stage when it is needed most. Because of this reactivity problem, ADA makes a poor replacement for potassium bromate in a yeast food. Other potential replacements for potassium bromate include iodates, sulfates, particularly, cupric sulfate, and certain enzymes.

This invention relates to the preparation of cereal products and baked products and improving their dough stability and quality using soybean peroxidase.

Some of the most common oxidizing enzymes are lipoxygenase, peroxidase, glucose oxidase, catalase, ascorbic acid oxidase, glutathione dehydrogenase, protein di sulphide isomerase, polyphenol oxidase, superoxide dismutase.

"Probably the most important oxidase in baking is lipoxygenase (E.C. 1.13.11.12) that catalyses the coupled oxidation by atmospheric oxygen of the carotene pigments of flour and of unsaturated fatty acids. While the principal effect of the use of lipoxygenase is a brightening of the bread's crumb color that results from the bleaching of the flour's carotene pigments. Fox and Mulvihill list among additional benefits of a finer crumb, better loaf volume, improved rheological properties through modification of protein sulfhydryl groups, improved tolerance, and off-setting of the deleterious effects of fast-acting oxidants." Baking Science & Technology. E.J. Pyler, 1988, Vol. 1, pp.175.

Most oxidases other than lipoxygenase form free radicals, at some stage, that are stable enough to take part in non-enzymatic reactions like cross-linking or oxidation of

sulfhydryl groups in the gluten network. This oxidation enhances dough stability during fermentation and increases oven spring causing the baked product to have higher volume.

"Polyphenol oxidase oxidize diphenolic groupings to quinones, which in turn may, in the right circumstances, engage in further reactions to form colored polymeric materials. This is often called "enzymatic browning" or "melaninization"." ENZYMES and Their Role in Cereal Technology, J.E. Kruger, D. Lineback, C.E. Stauffer, 1987, pp.245. Glucose oxidase, catalase and peroxidase enhance gluten protein oxidation by the ability of such enzymes to produce "active oxygen" in situ for flour protein oxidation.

Kieffer et al., <u>Z Lebensin Unters Forsch</u> (1981) 173: 376-379, report that dough and bread from wheat flours with poor baking properties can be improved by the addition of horseradish peroxidase at a concentration of 2.7 purpurogallin units/g. flour when used with hydrogen peroxide and catechol. H.W. Van Dam et al., "Yeast and Enzymes in Breadmaking," <u>Cereal Foods World</u>, March 1992, vol. 37, No. 3, pp 246, report that several redox enzymes such as glucose oxidase, polyphenol oxidase, ascorbate oxidase, lipoxygenase and peroxidase are favorable alternatives to potassium bromate. However, commercial quantities of effective enzymes in pure form are not yet available.

## Summary of the Invention

It is a primary object of the present invention to provide an alternative to potassium bromate for use in preparing and conditioning a flour dough.

It is a further object of the invention to provide new and improved methods and compositions for increasing gas retention and extending the volume of a flour dough during proofing and baking.

In accordance with the present invention, soybean peroxidase or soybean hull extract is used as a replacement for potassium bromate in the preparation and conditioning of dough. It has been found that soybean peroxidase is an effective oxidizing agent for use in dough making. Optionally, a peroxide and other common oxidizing agents such as ADA, sulfate, vitamin C, etc. may be used with the enzyme. Synergism may exist also with other baking additives such as fructose, glucose, etc., and other enzymes such as  $\alpha$ -amylase, prokinase, hemicellulase, etc.

Accordingly, one manifestation of the present invention is a flour mixture useful in preparing a bakery product, said mixture including flour and a dough conditioner, said dough conditioner comprising soybean peroxidase. In another manifestation of the invention, the dough conditioner additionally includes a peroxide and other oxidizing agents.

A further manifestation of the invention is a dough conditioner for use in preparing and conditioning a flour dough to improve gas retention and extend the volume of said flour dough during proofing as well as improving texture, crust, machinability, etc., said dough conditioner comprising soybean peroxidase and optionally a peroxide.

Yet another manifestation of the invention is a premixed dough conditioner for use in preparing and conditioning a flour dough to improve gas retention and stability, and extend the volume of said flour dough during proofing, said pre-mixed dough conditioner comprising soybean peroxidase, and at least one additive selected from the group consisting of a peroxide, emulsifiers, ascorbic acid, soybean flour, calcium propionate, malt flour, and mixtures thereof.

Still another manifestation of the invention is a method for making a bakery product from a flour dough having

sufficient gas retention and extended volume, said method comprising:

adding a dough conditioner including soybean peroxidase and optionally a peroxide to flour to prepare a flour mixture,

preparing a dough from said flour mixture, and baking said dough for a period of time and at a temperature sufficient to provide a bakery product.

A more particular method includes the steps of:
 mixing a dough comprising flour, water, salt, and a
dough conditioner which comprises soybean peroxidase and
optionally a peroxide to provide a dough;

forming said dough into dough bodies of a suitable size and shape for baking;

proofing said dough bodies for a period of time at a temperature and humidity sufficient to provide a fully raised dough; and

baking said proofed dough for a period of time and at a temperature sufficient to provide a bakery product having a browned crust and a fine, soft textured crumb.

Further manifestations of the invention reside in the use of an aqueous or alcoholic (e.g., ethanol) extract of soybean hulls as a dough conditioner. In addition to containing soybean peroxidase, this extract contains other enzymes which appear to be effective as dough conditioners. Other additives conventionally used in doughs include glucose, sugar, gums, inorganic salts, preservatives such as calcium proprionate, etc.

### **Definitions**

The term "dough" as used herein refers to any product prepared from a mixture of flour, salt and a liquid such as water or milk which has a consistency, prior to baking, which allows it to be kneaded or rolled. The term

includes doughs useful in preparing leavened and unleavened products such as breads, rolls, crackers, cookies, biscuits, buns, cakes, etc.

As used herein, the term "bakery product" refers generically to baked products including yeast bread, chemically leavened bread, unleavened breads, cookies, crackers, rolls, biscuits, buns, cakes, etc.

#### Detailed Description of the Invention

In accordance with the present invention, soybean peroxidase (SBP) or an aqueous extract of soybean hulls is used as an effective alternative to potassium bromate as an oxidant in the preparation and conditioning of dough for the baking industry.

Soybean peroxidase is abundantly available in purified form at economical prices. The SBP is stable under the conditions of dough development and provides, particularly in the presence of a peroxide such as calcium peroxide, the requisite ability to increase and retain dough stability and volume. Similar results can be achieved using the aqueous hull extract.

SBP is believed to catalyze a number of relevant alterations in dough components such as the oxidation of sulfhydryl (-SH) groups in gluten to disulfides (-S-S-), the crosslinking of pentosans through ferulic acid moieties as in hemicellulose, crosslinking of proteins through tyrosine residues, and the bleaching of carotenoids and chlorophyll. These activities contribute to the generation of dough with desirable rheological properties and color. Soybean peroxidase can be used in conjunction with and provide synergism with other oxidoreductases such as soybean lipoxygenase which is also used in the baking industry to bleach wheat flour and oxidize gluten. Soybean peroxidase

also provides an active catalyst which is resistant to protease activity of proteolytic catalysts used in dough development and its high temperature stability contributes to long shelf life and high activity during proofing. Furthermore, soybean peroxidase contributes to the production of desirable color and aroma in the finished bread product.

In accordance with the invention, the soybean peroxidase may be employed as the purified soybean peroxidase enzyme, as an aqueous or alcoholic soybean hull extract containing the enzyme alone or in combination with other enzymes extracted from the plant, or as ground soybean hulls containing the soybean peroxidase. The extract may be used wet or it may be dried to a powder.

Typically, the extract is obtained as follows: The soybean hulls, preferably ground, are extracted with hard water. The extraction is typically carried out in a suitable container using a mechanical stirrer. The ratio of hulls to water is typically about 1 pound hulls to 1 gallon water. The extraction of the ground hulls is carried out at about room temperature for a short period of time (15-30 min).

The soybean peroxidase may isolated from water by either freeze-drying or spray drying. Freeze-drying is a slower process, but usually provides quantitative recovery of the product. Spray drying is much faster, but often results in a partial loss of product activity.

As will be appreciated by persons skilled in the art, functional extracts can also be obtained using other extraction conditions. In any of these forms, the SBP may be introduced directly into either the flour or the mixed dough. While the direct use of the ground hulls as a source of the soybean peroxidase may not be desirable in every baking situation, it is a convenient way of providing the soybean peroxidase to the recipe, particularly since the hulls are

considered to be a good source of fiber, edible protein and hypocholesteremic polysaccharides.

In the extract, SBP is present in combination with other enzymes such as oxidoreductases and, more particularly, lipoxygenases, oxygenases, etc. which may also exhibit a conditioning effect.

The soybean peroxidase is used in an amount effective to provide the desired conditioning effect to the finished bread product. Typically, the amount of soybean peroxidase will be about 10 to 100 purpurogallin units/kg. flour and, preferably, about 10 to 40 purpurogallin units/kg. flour. The amount will vary with the nature of the flour and the type of bakery product desired. The aqueous hull extract can be used in an amount to provide the equivalent amount of SBP. When employing the hulls directly, the amount of hulls should be sufficient to provide the above amounts of soybean peroxidase.

The peroxide optionally used in conjunction with the soybean peroxidase can be any peroxide compatible with the peroxidase and with the preparation of the dough. Suitable peroxides include hydrogen peroxide, benzoyl peroxide, ethyl peroxide, ethylhydrogen peroxide, calcium peroxide, magnesium peroxide, sodium peroxide, potassium peroxide, or mixtures thereof, preferably, the peroxide is calcium peroxide or hydrogen peroxide.

The amount of peroxide used in the present invention is dependent upon the peroxide selected as well as the amount and activity of soybean peroxidase employed, flour quality and the nature of the dough and bakery product. Typically, the amount of peroxide will be about 0.1 to 0.5 g/kg flour and, preferably, about 0.2 to 0.4 g/kr flour.

In one embodiment of the invention, the SBP, peroxide and other dough conditioners and baking additives may be added to the flour mix at the bakery. It may also be

desirable to provide pre-packaged dry mixes or solutions of SBP alone or in combination with other conventional dough conditioners and additives provided that the combination of materials is sufficiently stable.

It may be desirable to provide a liquid or dry pre-mix in which both the peroxidase and peroxide are in a form so that any reactivity between the two ingredients would be prevented or, at least, significantly reduced until activated during preparation of the dough. The peroxidase or peroxide or both may be encapsulated in pressure-rupturable or shear-rupturable microcapsules or water soluble microcapsules or coatings so that the peroxidase and peroxide come together only upon breakdown of the microcapsules during mixing. Calcium peroxide is commercially available in an encapsulated or coated form useful in the present invention. There are numerous examples of such microcapsules in the literature. Such capsules are routinely used in the pharmaceutical industry and in carbonless paper products. Any other means which prevents reactive contact between the peroxidase and peroxide prior to their incorporation into the dough could be employed, e.g., a pre-mix formula wherein the peroxidase and peroxide are separated by a partition and, upon opening the packet, both the peroxidase and peroxide would be added to the dough recipe at the same time could also be used.

A dough conditioner system as contemplated herein may contain soybean peroxidase, emulsifiers, enzymes such as alpha-amylase, oxidizing agents such as ascorbic acid (Vitamin C), and a peroxide such as calcium peroxide. The concentration of each of the ingredients can vary depending on the baked product for which the pre-packaged mix is designed.

Typically, the concentrations of the ingredients in the dough conditioner or improver are as follows:

#### Cake Conditioner

	<u>Parts per</u> 100 parts flour
SBP	0.001
Glycerol monostearate (95-97%, acid value = 1.3-1.5)	0.3
Sodium stearoyl lactylate (SSL)	0.25
NaHCO <sub>3</sub>	0.02

#### Leavened Bread/Conditioner

Soybean peroxidase	(SBP)	0.005
Sodium stearoyl lactylate (SSL)		0.3
Alpha amylase		0.001
Vitamin C		20 ppm

#### Examples

In the following examples Basic Recipes 1, 2, and 3 were used to prepare baked products as indicated below. In the recipes, Improver No. 1 was made up of 0.3 parts sugar, 0.35 parts starch, 0.2 parts SSL, 0.1 part malt flour, 60 ppm ascorbic acid and 0.05 part alpha-amylases. Improver No. 2 was the same as Improver No. 1 except it contained 2 parts calcium peroxide and it did not contain the emulsifier SSL. The products were scored on a 5 point scale with a score of 5 being the best by a panel of 10 testers. The results are shown in the tables below. The volume of the product was measured using a conventional rapseed volumeter. Gas retention was measured using a Chopin rheofermentometer. Stability is the maximal development of the dough under a 1.5 kg weight after 3 hours fermentation in a temperature controlled cabinet.

## Basic Recipe No. 1 - Soft Rolls

The following ingredients were kneaded in a Hobart Mixer equipped with a "J" hook for 3 minutes at slow speed and 8 min. at high speed. The dough temperature was 29°C. The dough was allowed to stand 10 min. and then it was divided and rounded into 60 g. pieces. These pieces were allowed to rise 60 m in. at 35°C and 80% R.H. and then baked 15 min. at 180°C.

Bread Flour	100
Water	58
Sugar	3
Margarine	3
Salt	1.5
Instant Yeast	1
Improver (see Table)	1

## Basic Recipe No. 2 - Pan Bread ("Chala")

The following ingredients were kneaded in a Tweedy Mixer for 90 seconds. The dough temperature was 30°C. The dough was allowed to stand 10 min. and divided and rounded into 580 g. pieces. These pieces were rested 10 min., shaped and placed in a greased bread pan where the dough was allowed to ferment and rise 60 min. at 35°C and 80% R.H. The bread was baked with steam present at 200°C for 50 min.

Bread Flour	100
Water	55
Sugar	3
Margarine	3
Salt	1.5
Instant Yeast (Suspension)	1
Improver (see Table)	1

### Recipe No. 3 - White Bread

The foregoing ingredients were mixed in a Hobart Mixer equipped with a "J" hook. The dough temperature was 29°C. The dough was allowed to stand 10 min., divided and rounded into 300 g. pieces. After 10 min. the pieces were placed in aluminum bread pans and allowed to rise 60 min. at 35°C and 80% R.H. The bread was baked in a steam pastry oven for 25 min. at 200°C.

Bread Flour	100
Water	60
Margarine	1
Salt	2
Instant Yeast (Suspension)	1
Improver (see Table)	1

#### Summary of Baking Tests

	Improver No.	SBP (ppu)	Product	Volume	Stabil.	Gas Reten.	Score
1	1	2800	Rolls	1240	100	1280	4.5
3	1	2800	Bread	1080	-	-	4.5
2	1	2800	"Chala"	1200	-	-	5.0
1	2	2800	Rolls	1200	99	1250	4.5
3	2	2800	Bread	1100	-	_	4.5
2	2	2800	"Chala"	1140	<b>-</b>	-	4.5
3	1	0	Bread	960	80	1100	4.5
3	1	700	Bread	965	80	1100	4.5
3	1	1400	Bread	970	85	1150	4.5
3	1	2100	Bread	990	90	1150	5.0
3	1	2800	Bread	1000	90	1150	4.5
3	1	3500	Bread	1080	95	1200	4.5
3	1	4900	Bread	1100	95	1200	4.5
3	1	7000	Bread	1150	95	1200	4.5

Having described the invention in detail and referring to the preferred embodiments thereof, it will be apparent and variations are possible without departing from the scope of the invention defined in the appended claims.

What is claimed is:

1. A bakery mix useful in preparing a bakery product comprising flour and a dough conditioner, said dough conditioner comprising soybean peroxidase or an aqueous extract of soybean hulls.

- 2. The bakery mix of claim 1 wherein said soybean peroxidase is added to the mix as an aqueous extract derived from soybean hulls.
- 3. The bakery mix of claim 2 wherein said soybean peroxidase is added to said mix as ground soybean hulls.
- 4. The bakery mix of claim 1 wherein said dough conditioner further comprises at least one additive selected from the group consisting of peroxides, emulsifiers, ascorbic acid, soybean flour, calcium propionate, malt flour,  $\alpha$ -amylase and other enzymes and mixtures thereof.
- 5. The bakery mix of claim 1 wherein said soybean peroxidase is present in an amount of about 10 to 100 purpurogallin units/kg. flour.
- The bakery mix of claim 1 wherein said mix further comprises a peroxide selected from calcium peroxide, hydrogen peroxide, benzoyl peroxide, magnesium peroxide, ethyl hydrogen peroxide, sodium peroxide, potassium peroxide, or a mixture thereof.
- 7. The bakery mix of claim 6 wherein said peroxide is calcium peroxide.
- 8. The bakery mix of claim 1 wherein said mix contains yeast and said bakery product is a yeast-raised product.
- 9. The bakery mix of claim 1 wherein said mix contains sodium bicarbonate and said bakery product is a chemically leavened product.

10. The bakery mix of claim 7 wherein said mix contains encapsulated calcium peroxide.

- 11. A pre-mixed dough conditioner for use in preparing and conditioning a flour dough, said pre-mixed dough conditioner comprising soybean peroxidase or an aqueous extract of soybean hulls and at least one additive selected from the group consisting of peroxides, emulsifiers, ascorbic acid, soybean flour, calcium propionate, malt flour, and mixtures thereof.
- 12. The pre-mixed dough conditioner of claim 11 wherein said soybean peroxidase is present as an aqueous extract derived from soybean hulls.
- 13. The pre-mixed dough conditioner of claim 11 wherein said soybean peroxidase is present as ground soybean hulls.
- The pre-mixed dough conditioner of claim 11 wherein said conditioner additionally includes a peroxide selected from the group consisting of calcium peroxide, hydrogen peroxide, benzoyl peroxide, magnesium peroxide, ethyl hydrogen peroxide, sodium peroxide, potassium peroxide or a mixture thereof.
- The pre-mixed dough conditioner of claim 14 wherein said peroxide is encapsulated calcium peroxide.
- 16. A method for making a bakery product from a flour dough said method comprising:

adding a dough conditioner including soybean peroxidase or an aqueous extract of soybean hulls to a flour mixture,

preparing a dough from said flour mixture, and baking said dough for a period of time and at a temperature sufficient to provide a bakery product.

17. The method of claim 16 wherein said dough conditioner further comprises additional additives selected from the group consisting of peroxides, emulsifiers, ascorbic acid, soybean flour, calcium propionate, malt flour and mixtures thereof.

- 18. The method of claim 17 wherein said soybean peroxidase is an aqueous extract derived from soybean hulls.
- 19. The method of claim 17 wherein said soybean peroxidase is present as ground hulls.
- 20. The method of claim 17 wherein said soybean peroxidase is added to said flour mix in an amount of about 10 to 100 purpurogallin units/kg. flour.

### INTERNATIONAL SEARCH REPORT

International application No. PCT/US95/08482

A. CLASSIFICATION OF SUBJECT MATTER  IPC(6) :A23C 9/12 US CL :426/061						
According to International Patent Classification (IPC) or to both national classification and IPC						
B. FIELDS SEARCHED						
Minimum d	ocumentation searched (classification system follower	d by classification symbols)				
U.S. :	426/018, 019, 020, 021, 022, 027, 061, 062					
Documenta	tion searched other than minimum documentation to th	e extent that such documents are included	in the fields searched			
Electronic o	data base consulted during the international search (n	ame of data base and, where practicable	, search terms used)			
C. DOC	CUMENTS CONSIDERED TO BE RELEVANT					
Category*	Citation of document, with indication, where a	ppropriate, of the relevant passages	Relevant to claim No.			
Υ	US, A, 4,181,747 (KICKLE ET AL document.	) 01 January 1980, entire	1-20			
Y	US, A, 4,416,903 (COLE) 22 November 1983, entire 1-20 document.					
Υ	US, A, 5,108,765 (MAAT ET A document.	L) 28 April 1992, entire	1-20			
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Y	BROWN, M.A., 70(01):M0076 FST Millers' Journal, (1969) 72(8) 26-	•	1-20			
X Furth	er documents are listed in the continuation of Box C	See patent family annex.				
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## INTERNATIONAL SEARCH REPORT

International application No.
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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No	
Y	Cereal Foods World, Volume 23, No. 7, issued July 1978, H. Neukom and H.U. Markwalder, "Oxidative Gelation of Wheat Flour Pentosans: A New Way of Cross-Linking Polymers", pages 374-376.	1-20	
(	Cereal Foods World, Volume 28, No. 12, issued December 1983, C.E. Stauffer, "Dough Conditioners", pages 729-730.	1-20	