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(54) Title: CONTROLLED BROWNING BY BIOENHANCER

(57) Abstract: The invention discloses a composition comprising a blend of bioenhancers for inhibition of browning of whole wheat by a process of phenolic substrate modification. The blend of bioenhancers comprises pentosanases, proteolytic enzymes, redox enzymes, reducing agents, acidulants, stabilizers, surfactants and fillers.

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"CONTROLLED BROWNING BY BIOENHANCER"

Technical Field of Invention:

The present invention relates to the use of scientifically blended bioenhancers with browning inhibitors to irreversibly modify the phenolic substrate, which in turn inactivates poly phenol oxidase in whole wheat. The resultant product has controlled browning effect than that compared to natural whole wheat dough. The present invention attributes technologically and economically to the life of common man through the improvement and benefits brought about herein.

Background of Invention and Prior Art:

Browning of foods during processing and storage, especially during manufacture of meat, fish, fruit and vegetable products decreases the sensory properties of products due to associated changes in colour, flavour and softening, besides nutritional properties. Therefore, its control is essential to preserve the quality of the food. Appearance, flavour, texture and nutritional value are four attributes considered by consumers when making food choices. Appearance, which is significantly impacted by colour, is one of the first attributes used by consumers in evaluating food quality. Colour may be influenced by naturally occurring pigments such as chlorophylls, carotenoids and anthocyanins in food, or by pigments resulting from both enzymatic and non-enzymatic reactions.

Enzymatic browning is a significant problem in a number of important commodities, specially fruits such as apricots, apples, pears, peaches, bananas and grapes; vegetables such as potatoes, mushrooms ,lettuce and sea food. This discoloration limits the shelf life as well as the acceptance of the product.

The major enzymatic browning is caused by poly phenol oxidase enzyme present inherently in the fruits, vegetables, cereals etc. Mechanism of browning due to poly phenol oxidase can be explained as follows:

Enzymatic browning is the discoloration that results when monophenolic compounds of plants or shellfish, in the presence of atmospheric oxygen and poly phenol oxidase (PPO), are hydroxylated to ortho phenols, and the latter are oxidized to ortho quinines. The quinine condenses and reacts nonenzymatically with other phenolic compounds to produce dark brown, black or red pigment of intermediate structure.

Many approaches have been done in fruits but not in whole wheat. The present invention is related to the prevention of enzymatic browning in whole wheat dough by using a blend of bioenhancers.

Whole wheat is nutritious than white wheat. This is because whole wheat retains bran layer containing iron (non-haem), zinc, copper, thiamin, riboflavin and niacin which are water solubles. Whole wheat aleuronic layer of cells contains valuable nutrients like vitamins B complex, vitamin E and minerals.

The present invention is related to prevent the enzymatic browning in chapatti, breads, rotis, nans etc made with whole wheat dough. The whole wheat retains the nutritional value with improved visual appearance. The main basis of the present invention is to improve the organoleptic properties of whole wheat products and to retain its nutritional value.

Abstract of EP0903083 discloses process for inhibiting enzymatic browning and maintaining textural quality of fresh peeled potatoes, where a process is disclosed for inhibiting enzymatic browning in raw, peeled potatoes comprising dipping the potatoes in a solution of heated organic acids (45-65°C), followed by treatment in a weakly basic solution to neutralize the potato surface and treatment with reducing agents, and followed by storage in modified atmosphere packaging. The process tends to delay the onset of enzymatic browning and, once browning has begun, limits the extent of enzymatic browning.

Abstract of US6020018 discloses inhibition of enzymatic browning of raw fruit and/or vegetable juice. The juice is treated with at least one sulfated polysaccharide in an amount sufficient to inhibit browning. A promoter may also be present, said promoter is selected from the group consisting of chelating agents, acidulents, or mixtures thereof.

Abstract of WO8911227 describes inhibition of enzymatic browning, a process for inhibiting oxidative darkening of foods by treating the food with a protease effective to inhibit oxidative darkening of the food, a composition for inhibiting oxidative darkening and a kit for preparing the composition.

Abstract of US 4,814,192 discloses process for preserving raw fruits and vegetables, including juices, comprising treating the products with ascorbic acid-2-phosphate esters and ascorbyl-6-fatty acid esters, individually or in combination. The treatments may be applied in an aqueous carrier and may further comprise other browning inhibitors, polyphenol oxidase inhibitors, emulsifying agents, dispersing agents and complexing agents. Treatments tend to delay or prevent the onset of enzymatic browning or, once browning has begun, to limit the extent of enzymatic browning.

Object of the Invention:

The main object of present invention relates to the use of bioenhancers with browning inhibitor in the whole wheat to inactivate the poly phenol oxidase and thereby to reduce the undesirable browning.

The other object of present invention relates to the fact that the nutritional value of the whole wheat flour before and after treatment with formulation of bioenhancers with browning inhibitor is retained without any lowering or deterioration.

Summary of the Invention:

The object of the invention is to provide a process for modification of phenolic substrates as bioenhancers by inhibiting poly phenol oxidase activity in the whole wheat.

The bioenhancer comprises of specific pentosanases which act on pentosans like pectin, xylan and such like, proteolytic enzymes selected from neutral proteases, papain and such like, redox enzymes such as catalase, peroxidase and such like in combination with reducing agents such as cysteine, glutathione and ascorbic acid with chelating agents such as phosphates, EDTA and such like with some acidulants like fumaric acid, phosphoric acid and citric acid with some stabilizers.

Phenolic substrate modification by bioenhancers in whole wheat formulation comprises of bioenhancers such as pentosanases – 0.1-5%, proteolytic enzyme – 0.1-2%, redox enzymes – 0.1-3%, reducing agents such as L-cysteine, ascorbic acid 0.5-6%, stabilizers such as sodium and calcium salts at 7-10% with acidulants such as fumaric acid and maleic acid at 10- 20%, thus phenolic substrate modification by the use of bioenhancers in the range of 0.1 – 0.4 % reduces the rate of browning in the whole wheat flour preparation, yet retaining its nutritional value.

Detailed Description:

The present invention claims the composition which consists of blend of bioenhancers like specific pentosanases which act on pentosans like pectin, xylan, cellulose and proteolytic enzymes like neutral proteases, plant protease (papain, bromalein, ficin) and redox enzymes such as catalase, gluconase oxidase and lipoxidase. The bioenhancers are formulated in conjunction with browning inhibiting components like reducing agents namely cysteine, glutathione and ascorbic acid, chelating agents such as phosphates and EDTA. The rate of action of bioenhancers and browning inhibitors can be enhanced with acidulants like fumaric acid, phosphoric acid, ascorbic and citric acid which stabilize the whole browning inhibition in conjunction with calcium, sodium salts and emulsifying agents such as polysorbates.

The present invention discloses the range of composition of bioenhancer such as pentosanase – 0.1 - 5%, proteolytic enzyme – 0.1- 2%, redox enzymes 0.1- 3%, reducing agents 0.5-6%, stabilizers such as sodium and calcium salts at 7-10% with acidulants 10- 20% and white dextrin, defatted soya flour as inert filler: q.s.

(1) Bioenhancer

The mechanism of bioenhancers can be exploited for the control of undesirable enzyme activities to inhibit browning effect.

1. Substrate and/or product modification other than the target enzymes
2. Direct inactivation of the target enzyme.
3. Inactivation by secondary reactions of highly reactive products.

(i)Pentosanase:

It helps in selective inhibition of browning by irreversible modification of phenolic substrates. Due to distorted phenolic structure of substrate, the active site on the poly phenol oxidase cannot come in contact with the substrates to give 100% reaction thus helping in reducing the browning effect.

(ii)Proteolytic enzymes:

The plant proteases like ficin, papain, and bromelain are sulphydryl enzymes of broad specificity, which are very effective browning inhibitors. This inhibitory effect is thought to be due to either binding or hydrolysis at specific sites necessary for poly phenol oxidase activity.

(iii)Redox enzymes:

These bioenhancers are capable of methylating the 3 rd position of 3, 4 dihydroxy aromatic compounds. It was observed to cause irreversible modification of phenolic substrates, thus preventing them from serving as substrates for poly phenol oxidase, thus preventing browning reaction.

(2) Reducing agents

Reducing agents play a role in the prevention of enzymatic browning either by reducing O-quinones of the phenolic substrates to colorless diphenols, or by reacting irreversibly with O-quinones to form stable colorless products.

(i)Ascorbic acid is a strong reducing compound, which is acidic in nature, forms neutral salts with bases and is highly water soluble. Polyphenol oxidase inhibition by ascorbic acid has been attributed to the reduction of enzymatically formed O-quinones to their precursor diphenols. Ascorbic acid is irreversibly oxidized to dehydroascorbic acid during the reduction process, thus preventing browning reaction to the desirable extent.

(ii)Cysteine is an effective inhibitor of enzymatic browning. It is reported to be more effective than bisulphites. Cysteine forms intermediate complex with quinine, which serves as competitive inhibitor of polyphenol oxidase enzyme.

(3) Acidulants

Acidulants are generally applied in order to maintain the acidity for optimum catalytic activity of an enzyme. Acidulants such as citric acid, malic acid and phosphoric acid are capable of lowering acidity of the system, thus rendering poly phenol oxidase inactive.

(4) Chelators

Enzymes generally posses metal ions at their active sites. Removal of these ions by chelating agents can therefore render enzymes inactive.

Chelating agents like sodium salt of ethylene diamine tetra acetic acid, sodium tripolyphosphate with pro-oxidative agents such as copper and iron ions, act through an unshared pair of electrons in their molecular structures which captures the metal ion present in poly phenol oxidase and reduces the browning action.

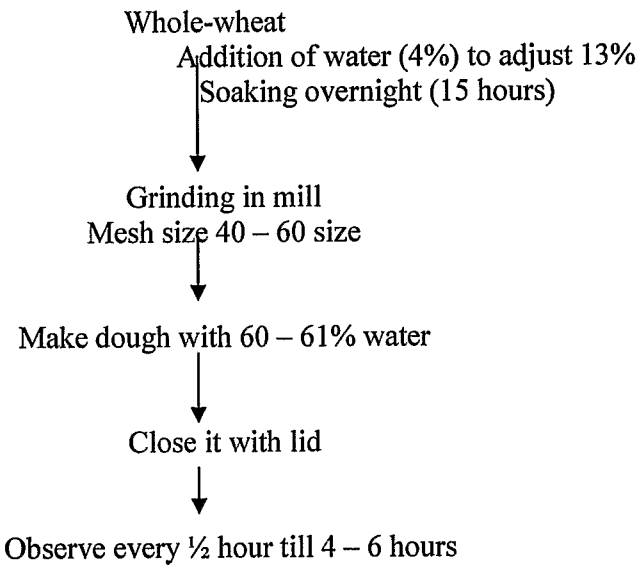
(5) Stabilizers:

Salts of sodium and calcium ions stabilize the composition of browning inhibitor containing bioenhancers.

According to the present invention, bioenhancers were prepared by fermentation using controlled conditions with the help of pressure, adjustments in temperature and using suitable fermentation medium referring to the environment. The fermentation process is carried out by using the fermentation substrate and the carbohydrate source that is metabolized by the fermenting microorganism(s). The fermentation media includes fermentation substrate and other raw materials used in the fermentation process. In the present invention, the fermentation media can bring out liquefaction and saccharification processes or other desired processes prior to or simultaneously with fermentation.

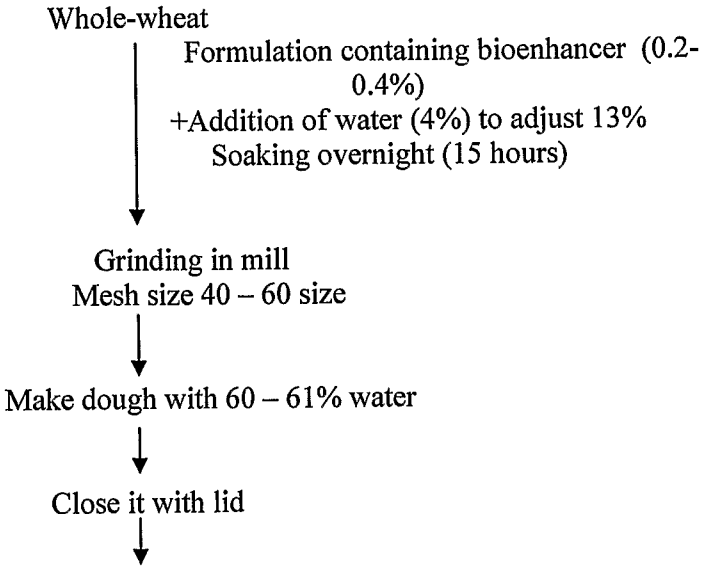
Schematic representation of process

Conventional process



Schematic representation of process

Bioenhancer Treatment



Observations and results:

Example 1: Lab trial results for the market wheat variety lokawan:

i) Lokawan variety

Effect of treatment of bioenhancer on Lokawan variety

Observations for 6 consecutive days are as follows:

Day 1 Observations

Hunter Lab Colorimeter 0th Hour Observations (1st Day)

Hunter Lab value	L Value	a value	b value
0 Hour Hunter Lab Colorimeter Reading			
Control wheat dough	54.98	6.25	17.58
Dampened wheat dough	56.15	6.78	18.36
Treated wheat dough	59.50	6.10	18.87
2 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	54.95	6.61	17.00
Dampened wheat dough	55.22	7.82	18.21

Treated wheat dough	58.86	6.14	18.45
4 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	54.79	6.44	17.78
Dampened wheat dough	54.98	6.63	16.84
Treated wheat dough	58.81	5.69	16.18
6 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	53.65	6.48	17.48
Dampened wheat dough	50.90	6.52	16.42
Treated wheat dough	58.04	5.80	16.52
	Control	Dampened	Treated
PPO activity in mg/units	2.45	1.75	0.5444
Loss on drying	7.35%	7.22%	7.31%
Residual enzyme level Amylase in SKB units	46.12	49.98	46.10
Residual enzyme level protease in PC units	122	110	98

Day 2 Observations

Hunter Lab Colorimeter after 24 hours Observations (2nd Day)

Hunter Lab value	L value	a value	b value
0 Hour Hunter Lab Colorimeter Reading			
Control wheat dough	58.33	7.02	18.98
Dampened wheat dough	57.51	6.03	17.78
Treated wheat dough	60.12	5.69	18.63
2 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	55.33	6.60	17.98
Dampened wheat dough	55.32	6.52	18.12
Treated wheat dough	58.66	6.19	18.48
4 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	56.77	6.52	18.01
Dampened wheat dough	56.22	6.42	18.12
Treated wheat dough	58.46	6.35	19.32
6 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	55.32	6.32	17.56
Dampened wheat dough	53.43	6.42	17.45
Treated wheat dough	58.22	5.84	18.82
	Control	Dampened	Treated
PPO activity in mg/units	2.61	2.51	1.2
Loss on drying	7.36%	7.25%	7.34%

Residual enzyme level Amylase in SKB units	49	50	48
Residual enzyme level protease in PC units	128	118	90

Day 3 Observations

Hunter Lab Colorimeter after 48 hours Observations (3rd Day)

Hunter Lab value	L Value	a value	b value
0 Hour Hunter Lab Colorimeter Reading			
Control wheat dough	57.46	6.33	18.34
Dampened wheat dough	55.84	6.53	18.05
Treated wheat dough	60.09	7.35	17.95
2 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	57.85	6.52	17.34
Dampened wheat dough	55.32	6.42	16.80
Treated wheat dough	59.05	7.23	16.84
4 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	56.68	6.50	18.54
Dampened wheat dough	55.70	6.72	18.68
Treated wheat dough	58.82	6.68	19.20
6 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	56.29	6.53	18.50
Dampened wheat dough	54.61	6.78	18.51
Treated wheat dough	58.55	6.71	19.00
	Control	Dampened	Treated
PPO activity in mg/units	2.9150	1.45	0.98
Loss on drying	7.47%	7.7%	7.61%
Residual enzyme level Amylase in SKB units	48	46	49
Residual enzyme level protease in PC units	118	120	121

Day 4 Observations

Hunter Lab Colorimeter after 72 hours Observations (4th Day)

Hunter Lab value	L Value	a value	b value
0 Hour Hunter Lab Colorimeter Reading			
Control wheat dough	58.44	7.05	19.02
Dampened wheat	57.81	6.02	17.79

dough			
Treated wheat dough	60.38	5.72	18.71
2 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	53.61	6.79	17.92
Dampened wheat dough	53.78	6.71	17.59
Treated wheat dough	59.80	5.52	18.12
4 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	52.22	7.20	17.82
Dampened wheat dough	52.89	6.72	17.52
Treated wheat dough	58.82	6.20	18.88
6 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	49.52	6.69	16.42
Dampened wheat dough	52.78	6.75	17.51
Treated wheat dough	58.05	6.12	18.56
	Control	Dampened	Treated
PPO activity in mg/units	2.9	2.3	0.666
Loss on drying	7.21%	7.19%	7.22%
Residual enzyme level Amylase in SKB units	48	46	49
Residual enzyme level protease in PC units	128	118	98

Day 5 Observations

Hunter Lab Colorimeter after 96 hours Observations (5th Day)

Hunter Lab value	L value	a value	b value
0 Hour Hunter Lab Colorimeter Reading			
Control wheat dough	56.82	6.62	18.21
Dampened wheat dough	56.75	6.72	18.44
Treated wheat dough	62.90	5.78	19.46
2 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	55.12	6.8	18.01
Dampened wheat dough	56.91	6.54	18.15
Treated wheat dough	61.54	5.96	19.52
4 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	55.62	6.82	18.18
Dampened wheat dough	56.92	6.64	18.65
Treated wheat dough	60.82	5.98	19.15
6 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	55.81	6.79	18.18
Dampened wheat dough	54.82	6.52	17.82
Treated wheat dough	59.39	5.62	18.61
	Control	Dampened	Treated
PPO activity	3.1	2.3	0.966

Loss on drying	7.62%	7.61%	7.74%
Residual enzyme level Amylase in SKB units	48	46	49
Residual enzyme level protease in PC units	118	116	92

Day 6 Observations

Hunter Lab Colorimeter after 120 hours observations (6th Day)

Hunter Lab value	L Value	a value	b value
0 Hour Hunter Lab Colorimeter Reading			
Control wheat dough	57.18	6.42	17.89
Dampened wheat dough	58.40	6.88	18.32
Treated wheat dough	60.81	6.18	18.80
2 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	55.11	6.29	17.11
Dampened wheat dough	57.62	6.69	18.32
Treated wheat dough	60.15	5.77	18.12
4 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	55.69	6.24	17.19
Dampened wheat dough	56.59	6.31	17.42
Treated wheat dough	59.20	5.92	18.25
6 Hours Hunter Lab Colorimeter Reading			
Control wheat dough	54.72	6.29	17.06
Dampened wheat dough	55.24	6.60	17.32
Treated wheat dough	58.18	5.94	18.02
	Control	Dampened	Treated
PPO activity in mg/units	2.08	1.6	0.66
Loss on drying	7.32%	7.21%	7.28%
Residual enzyme level Amylase in SKB units	50	50	49
Residual enzyme level protease in PC units	120	119	100

Graphical representation of reduction in ppo content:

Effect of ppo on lokawan wheat variety with bioenhancer

(refer to graph no 1)

Example 2: Effect of treatment of bioenhancer on Sarbathy variety

Observations for alternate days are as follows:

Hunter lab colorimeter observations for 0^{th} hour

Day 1 observations

Colour measurement of whole wheat (before grinding)by hunter lab colorimeter

0^{th} Hour Observation	L value	a value	b value
Sample			
Control whole wheat	44.61	7.04	15.51
Treated wheat (0.1%)	48.30	6.29	15.98
Treated wheat (0.2%)	49.34	6.02	15.91
Treated wheat (0.3%)	49.91	6.12	16.31
Treated wheat (0.4%)	50.32	5.81	16.18

Colour measurement of whole wheat dough by hunter lab colorimeter

24 Hour zeroth hour observation	L value	a value	b value
Control wheat dough	55.81	6.72	18.47
Treated wheat (0.1%)	59.21	6.26	17.68
Treated wheat (0.2%)	61.32	5.47	18.10
Treated wheat (0.3%)	61.58	5.6	18.28
Treated wheat (0.4%)	61.62	5.46	18.31
After 2 hours observation	L value	a value	b value
Control wheat dough	55.12	6.56	17.92
Treated wheat (0.1%)	58.21	6.21	17.70
Treated wheat(0.2%)	58.31	6.15	18.38
Treated wheat(0.3%)	58.40	6.22	18.36
Treated wheat (0.4%)	59.12	6.08	18.74
After 4 hours observation	L value	a value	b value
Control wheat dough	54.98	6.59	17.88
Treated wheat (0.1%)	57.15	6.15	17.49
Treated wheat (0.2%)	57.58	6.17	18.02
Treated wheat (0.3%)	58.82	5.63	17.73
Treated wheat (0.4%)	58.95	5.91	18.30
After 6 hours observation	L value	a value	b value
sample			
Control wheat dough	52.68	6.55	17.26
Treated wheat (0.1%)	54.56	5.9	16.58
Treated wheat (0.2%)	57.89	5.98	17.26

Treated wheat (0.3%)	58.12	6.06	17.99
Treated wheat (0.4%)	58.82	5.92	18.33

L equals 0-100 (0=black and 100=white)

a equals red to green (+ = red and - = green)

b equals yellow to blue (+ = yellow and - = blue)

PPO content in the whole wheat flour after 24 Hour observations

Sample	PPO mg/ units	% PPO
Control wheat	2.32 units	100%
Treated wheat (0.1%)	0.84165 units	35.36%
Treated wheat (0.2%)	0.825 units	34.66%
Treated wheat (0.3%)	0.708	29.74%
Treated wheat (0.4%)	0.416	17.479%

Loss on Drying for 24 hour

Sample	% LOD
Control wheat	9.4
Treated wheat (0.1%)	8.64
Treated wheat (0.2%)	8.63
Treated wheat (0.3%)	9.22
Treated wheat (0.4%)	9.4

Resudual Enzyme Level after 24 hours Samples

Sample	Amylase activity (SKB/GM)	Protease activity (PC/gm)
Control wheat	47	131
Treated wheat (0.1%)	44.33	97.88
Treated wheat (0.2%)	47	115
Treated wheat (0.3%)	44.33	92
Treated wheat (0.4%)	45	100

Note:

There are inherent enzymes like protease and amylase present in the whole wheat. The added Bioenhancer is not contributing any additional protease and amylase activity to the final wheat flour and thus maintaining the original rheological properties of the wheat flour.

Hunter Lab Colorimeter Observations After 72 Hour

Day 3 Observations

Colour measurement of whole wheat Dough by hunter lab colorimeter

72 HOUR zeroth hour observation	L value	a value	b value
Control wheat dough	59.08	5.98	17.41
Treated wheat (0.1%)	60.22	5.48	17.62
Treated wheat(0.2%)	63.12	5.14	18.10
Treated wheat(0.3%)	62.31	5.51	17.91
Treated wheat (0.4%)	62.48	5.32	18.16
After 2 hours observation	L value	a value	b value
Control wheat dough	52.61	7.07	16.72
Treated wheat (0.1%)	56.96	5.55	17.11
Treated wheat (0.2%)	57.98	5.73	17.57
Treated wheat (0.3%)	58.95	5.85	17.63
Treated wheat (0.4%)	58.91	5.65	17.72
After 4 hours observation	L value	a value	b value
Control wheat dough	51.44	6.90	16.35
Treated wheat (0.1%)	56.63	5.90	17.01
Treated wheat (0.2%)	57.92	5.87	17.69
Treated wheat (0.3%)	59.96	5.54	17.68
Treated wheat (0.4%)	59.98	5.65	17.95
After 6 hours observation	L value	a value	b value
Control wheat dough	50.78	6.67	16.05
Treated wheat (0.1%)	53.31	5.75	16.28
Treated wheat (0.2%)	56.98	5.51	16.72
Treated wheat (0.3%)	57.99	5.60	17.30
Treated wheat (0.4%)	58.62	5.78	18.03

L equals 0-100 (0=black and 100=white)

a equals red to green (+ = red and - = green)

b equals yellow to blue (+ = yellow and - = blue)

PPO content in the whole wheat for 72 Hour observations

Sample	PPO mg/ units	% PPO
Control wheat	2.48 units	100%
Treated wheat (0.1%)	0.833 units	33.58%
Treated wheat (0.2%)	0.833 units	33.58%
Treated wheat (0.3%)	0.75 units	30.24%
Treated wheat (0.4%)	0.5 units	20.16%

Loss on Drying for 72 Hour

Sample	% LOD
Control wheat	8.6

Treated wheat (0.1%)	8.28
Treated wheat (0.2%)	8.72
Treated wheat (0.3%)	8.42
Treated wheat (0.4%)	8.12

Residual Enzyme Level for 72 Hour Samples

Sample	Amylase activity (SKB/GM)	Protease activity (PC/gm)
Control wheat	49	120
Treated wheat (0.1%)	47	98
Treated wheat (0.2%)	48	117
Treated wheat (0.3%)	46	98
Treated wheat (0.4%)	49	119

Note:

There are inherent enzymes like protease and amylase present in the whole wheat. The added Bioenhancer is not contributing any additional protease and amylase activity to the final wheat flour and thus maintaining the original rheological properties of the wheat flour.

Hunter Lab Colorimeter Observations for 120 Hour

Day 5 Observations

Colour measurement of whole wheat Dough by hunter lab colorimeter

120 Hour zeroth hour observation	L value	a value	b value
Control wheat dough	56.82	6.32	17.87
Treated wheat (0.1%)	59.12	6.10	17.98
Treated wheat (0.2%)	60.60	5.08	17.34
Treated wheat (0.3%)	61.02	5.47	18.07
Treated wheat (0.4%)	61.12	5.26	17.81
After 2 hours observation	L value	a value	b value
Control wheat dough	57.65	6.45	18.36
Treated wheat (0.1%)	60.82	5.19	17.51
Treated wheat (0.2%)	61.42	5.42	17.87
Treated wheat (0.3%)	60.98	5.79	18.14
Treated wheat (0.4%)	60.17	5.72	18.23
After 4 hours observation	L value	a value	b value
Control wheat dough	57.58	6.15	17.93
Treated wheat (0.1%)	58.14	5.25	17.02
Treated wheat (0.2%)	58.08	5.31	17.33

Treated wheat (0.3%)	58.29	5.54	17.54
Treated wheat (0.4%)	58.51	5.73	18.03
After 6 hours observation	L value	a value	b value
Control wheat dough	56.68	6.05	18.06
Treated wheat (0.1%)	58.04	5.21	17.92
Treated wheat (0.2%)	59.98	5.43	17.73
Treated wheat (0.3%)	59.02	6.22	18.16
Treated wheat (0.4%)	59.08	6.14	18.66

L equals 0-100 (0=black and 100=white)

a equals red to green (+ = red and - = green)

b equals yellow to blue (+ = yellow and - = blue)

PPO content in the whole wheat for 120 Hour observations

Sample	PPO mg/ units	% PPO
Control wheat	1.99 units	100%
Treated wheat (0.1%)	1.1units	55.27%
Treated wheat (0.2%)	0.6665 units	33.49%
Treated wheat (0.3%)	0.5 units	25.12%
Treated wheat (0.4%)	0.4 units	20.10%

Loss on Drying for 120 hour

Sample	% LOD
Control wheat	8.53
Treated wheat (0.1%)	8.64
Treated wheat (0.2%)	8.56
Treated wheat (0.3%)	8.9
Treated wheat (0.4%)	8.78

Residual Enzyme Level for 120 Hour samples

Sample	Amylase activity (SKB/GM)	Protease activity (PC/gm)
Control wheat	51	88
Treated wheat (0.1%)	48	85
Treated wheat (0.2%)	50	83
Treated wheat (0.3%)	49	84.17
Treated wheat (0.4%)	50	84.81

Note:

There are inherent enzymes like protease and amylase present in the whole wheat. The added Bioenhancer is not contributing any additional protease and amylase

activity to the final wheat flour and thus maintaining the original rheological properties of the wheat flour.

Graphical representaion of redution in ppo content

Effect of ppo on sharbhati wheat variety with bioenhancer

(refer to graph no 2)

Example 3: Wheat variety: WH 147

Effect of treatment of bioenhancer on WH 147 variety

Observations are as follows:

Colour measurement of whole wheat before grinding by hunter lab colorimeter

Zeroth hour observation	l value	a value	b value
Sample			
Control whole wheat	46.11	7.77	16.84
Treated whole wheat	50.88	7.02	17.32

L equals 0-100 (0=black and 100=white)

a equals red to green (+ = red and - = green)

b equals yellow to blue (+ = yellow and - = blue)

Colour measurement of whole wheat Dough by hunter lab colorimeter

Zeroth hour observation	l value	a value	b value
Sample			
Control dough	61.17	5.82	19.20
Treated dough	63.03	5.99	20.24
After 3 hours observation	l value	a value	b value
Sample			
Control dough	59.12	4.62	15.49
Treated dough	61.82	5.12	18.72
After6 hours observation	l value	a value	b value
sample			
control dough	54.32	6.31	17.12
Treated dough	58.06	5.22	15.71

L equals 0-100 (0=black and 100=white)

a equals red to green (+ = red and - = green)

b equals yellow to blue (+ = yellow and - = blue)

Graphical representation of reduction in ppo content

Effect of ppo on WH 147 wheat variety with bioenhancer after 24 hours treatment (refer to graph no 3)

Example 4: Wheat variety: sure

Effect of treatment of bioenhancer on sure variety

Observations are as follows

Colour measurement of whole wheat before grinding by hunter Lab colorimeter

Zeroth hour observation	L Value	a value	b value
Sample			
Control whole wheat	44.89	7.50	15.89
Treated whole wheat	54.00	6.41	17.54

L equals 0-100 (0=black and 100=white)

a equals red to green (+ = red and - = green)

b equals yellow to blue (+ = yellow and - = blue)

Colour measurement of whole wheat Dough by hunter Lab colorimeter

Zeroth hour observation	L value	a value	b value
Sample			
Control dough	58.32	6.50	19.20
Treated dough	61.29	5.89	19.50
After 3 hours observation	L value	a value	b value
Sample			
Control dough	58.12	6.40	19.32
Treated dough	61.30	6.16	20.25
Sample			
control dough	56.23	6.22	18.14
Treated dough	59.68	6.14	19.13

L equals 0-100 (0=black and 100=white)

a equals red to green (+ = red and - = green)

b equals yellow to blue (+ = yellow and - = blue)

Graphical representation of reduction in ppo content

Effect of ppo on sure wheat variety with bioenhancer after 24 hours treatment

(refer to graph no 4)

Overall benefits of using bioenhancer in whole wheat atta are as follows:

- inhibits the poly phenol oxidase activity
- improves the colour of dough
- increases the water absorption of the flour
- Makes chapatti, rotis softer for longer time with improved shelf life
- Improves digestibility of the final product

Amount of polyphenol content in different varieties of whole wheat

Graphical representation is as follows

(refer to graph no 5)

Wheat Variety	Poly Phenol Content in Mg/ Units
Lokawan	2.9
WH 147	2.3
Sarbathy	2.5
Sure	2.2

Amount of polyphenol after treatment with bioenhancer in whole wheat varieties
(refer to graph no 6)

Wheat Variety	Poly Phenol Content in Mg/ Units
Lokawan	0.66
WH 147	0.45
Sarbathy	0.32
Sure	0.21

Comparison of ppo content in original and treated varieties of wheat

(refer to graph no 7)

Analysis of Wheat Flour

The analysis of wheat flour treated and untreated wheat flour for various parameters such as moisture, ash, gluten, protein etc for all four varieties are as follows:

Moisture analysis of control and treated wheat flour
(refer to Graph no 8)

Wheat variety	Control % moisture	Treated % moisture
Lokawan	13.1	13.1
Sarbathy	12.99	12.99
WH 147	13.45	13.45
Sure	12.98	12.98

Gluten analysis of control and treated flour
(refer to Graph no 9)

Wheat variety	Control % gluten	Treated % gluten
Lokawan	9.1	9.1
Sarbathy	8.9	8.98
WH 147	9	8.99
Sure	9.12	9.11

Protein analysis of control and treated wheat flour
(refer to Graph no 10)

Wheat variety	Control % protein	Treated % protein
Lokawan	9.2	9.2
Sarbathy	9.2	9.23
WH 147	8.9	8.99
Sure	7.68	7.66

Ash analysis of control and treated wheat flour
(refer to Graph no 11)

Wheat variety	Control % Ash	Treated % ash
Lokawan	1.2	1.2
Sarbathy	1	1
WH 147	1.1	1.1
Sure	1.23	1.23

Nutritional report for control and treated wheat flour

Nutritional report of Lokawan flour before and after treatment

Sample	Thiamine content in mg/100 g of flour
Whole Lokawan wheat flour untreated	0.592

Whole Lokawan wheat flour treated with 0.3% bioenhancer	0.593
Whole Lokawan wheat flour treated with 0.4% bioenhancer	0.592

Nutritional report of WH 147 flour before and after treatment

Sample	Thiamine content in mg/100 g of flour
Whole WH 147 wheat flour untreated	0.511
Whole WH 147 wheat flour treated with 0.3% bioenhancer	0.512
Whole WH 147 wheat flour treated with 0.4% bioenhancer	0.511

Nutritional report of Sarbathy flour before and after treatment

Sample	Thiamine content in mg/100 g of flour
Whole Sarbathy wheat flour untreated	0.536
Whole Sarbathy wheat flour treated with 0.3% bioenhancer	0.536
Whole Sarbathy wheat flour treated with 0.4% bioenhancer	0.536

Nutritional report of Sure flour before and after treatment

Sample	Thiamine content in ppm
Whole Sure wheat flour untreated	0.521
Whole Sure wheat flour treated with 0.3% bioenhancer	0.522
Whole Sure wheat flour treated with 0.4% bioenhancer	0.522

Sensory evaluation of chapattis prepared from treated and untreated whole wheat flour.

Chapatti preparation:

Chapattis are prepared from treated and control wheat flour.

Sensory evaluation

Each chapatti samples was evaluated by a panel of judges for various sensory attributes color, flavour, taste, texture, chewing ability, folding ability and appearance.

Results and Discussion:

The sensory evaluations of the chapattis prepared from different flour are mentioned below. The total pooled scores obtained by control and treated flour of chapattis for colour, flavour, taste, texture, chewing ability; folding ability and appearance were 34.47 and 46.65 respectively. These results revealed that chapattis prepared from treated whole wheat flour were ranked at the top than that of control. However, on the basis of scores assigned by the panel of trained judges to different sensory attributes, it is obvious that chapattis prepared from treated flour showed the better quality characteristics than that of control in the above mentioned properties.

Pooled data for sensory evaluation of chapattis

Sample	Colour	Flavour	Taste	Texture	Chewing ability	Folding ability	Total score of acceptability
Untreated wheat flour (control)	6.33	5.16	4.66	4.66	3.83	6.00	34.47
Treated wheat flour	6.50	6.66	7.00	6.83	6.33	7.00	46.65

Where the value of score represents 1= Very Poor; 7= Excellent.

Pooled data for sensory evaluation of chapattis prepared from control and treated flour (refer to Graph no 12)

Organoleptic properties of chapatti prepared from the wheat treated with bioenhancer

Observations:

Sensory evaluation of dough and chapatti

Sample	Particle size through 9xxx mesh	Water absorption ml/100g of atta	Dough colour		Rolling and sheeting characteristics	Stored chapatti after 4 hours	
			0 hour	After 4 hours		Appearance & hand feel	aroma
0 Hour Grinding Treatment							
1st day grinding control wheat	83.82	78	wheatish	Dark	Easy to roll	Not soft	

1st day grinding dampened wheat	83.58	75	wheatish	Dark	Easy to roll	Not soft	wheatish
1st day grinding treated wheat	85.73	73	wheatish	No Darkness	Easy to roll	soft	
24 Hours after Treatment							
2nd day grinding control wheat	84.31	75	wheatish	Dark	Easy to roll	Not soft	wheatish
2nd day grinding dampened wheat	84.57	75	wheatish	Dark	Easy to roll	Not soft	
2nd day grinding treated wheat	83.04	72	wheatish	No Darkness	Easy to roll	soft	
48 Hours after Treatment							
3rd day grinding control wheat	82.76	72	wheatish	Dark	Easy to roll	Not soft	wheatish
3rd day grinding dampened wheat	85.16	72	wheatish	Dark	Easy to roll	Not soft	
3rd day grinding treated wheat	84.92	69	wheatish	No Darkness	Easy to roll	soft	
72 Hours after Treatment							
4th day grinding control wheat	82.02	76	wheatish	Dark	Easy to roll	Not soft	wheatish
4th day grinding dampened wheat	82.63	74	wheatish	Dark	Easy to roll	Not soft	
4th day grinding treated wheat	81.53	69	wheatish	No Darkness	Easy to roll	soft	
96 Hours after Treatment							
5th day grinding control wheat	85.34	75	wheatish	Dark	Easy to roll	Not soft	wheatish
5th day grinding dampened wheat	86.13	75	wheatish	Dark	Easy to roll	Not soft	
5th day grinding treated wheat	83.72	68	wheatish	No Darkness	Easy to roll	soft	
120 Hours after Treatment							
6th day grinding control wheat	79.82	73	wheatish	Dark	Easy to roll	Not soft	wheatish
6th day grinding dampened wheat	79.91	73	wheatish	Dark	Easy to roll	Not soft	
6th day grinding treated wheat	79.96	68	wheatish	No Darkness	Easy to roll	soft	

Poly phenol oxidase activity determination was done as per method specified in "Enzyme and related biochemicals" by Worthington™

Amylase Method (Skbu/ Gm):

Reagents: Sodium acetate buffer, dilute iodine solution, buffered starch solution.
Enzyme solution: Estimate quantity of enzyme necessary to be in range and dissolve in water.

Procedure - Pipette 5.0 ml of dilute solution of iodine into series of test tubes. Pipette 10 ml of buffered starch substrate solution into a test tube. Equilibrate at 30°c for 15 min. at zero time add 5 ml of appropriate enzyme dilution into equilibrated buffered substrate solution and start the stop watch as zero time. After 15 min of reaction time, Pipette one of the hydrolysis material into 5 ml dilute iodine solution. Mix the tube and compare against color comparator. Near the end point the comparison should be made at 30 seconds interval.

Calculation: One unit of SKB activity is defined as that amount of enzyme to dextrinize 0.1 gm of soluble starch per 60 minutes under the condition of assay.

$$\text{SKBU/GM:} \quad \frac{0.2 \times 60}{0.1 \times \text{Time} \times 5} \times \text{DILUTION FACTOR}$$

Protease (PC/GM) was determined referring to Food Chemical Codex I. Thiamine was estimated referring to Analytical Methods of Vitamins. Determination of moisture, ash, gluten was as per aoac standard methods. Determination of proteins was carried out referring to Pearson's Composition and Analysis of Foods.

We claim:

1. A composition comprising a blend of bioenhancers wherein said composition inhibits browning of whole wheat by a process of phenolic substrate modification.
2. The composition as claimed in claim 1 wherein the blend of bioenhancers comprises pentosanases, proteolytic enzymes, redox enzymes, reducing agents, acidulants, stabilizers, surfactants and fillers.
3. The composition as claimed in claim 1 and 2 wherein said pentosanases are in the range of 0.1-5% by potency determination.
4. The composition as claimed in claim 1 and 2 wherein said proteolytic enzymes are selected from papain, bromelain and ficin in the range of 0.1-2% by potency determination.
5. The composition as claimed in claim 1 and 2 wherein said redox enzymes are selected from catalase, gluconase oxidase and lipoxidase in the range of 0.1-3% by potency determination.
6. The composition as claimed in claim 1 and 2 wherein said reducing agents are selected from L-cysteine and ascorbic acid in the range of 0.5-6% by weight.
7. The composition as claimed in claim 1 and 2 wherein said acidulants are selected from maleic acid and fumaric acid in the range of 10-20% by weight.
8. The composition as claimed in claim 1 and 2 wherein said stabilizers are selected from sodium and calcium salts in the range of 7-10% by weight.
9. A composition comprising a blend of bioenhancers wherein said composition inhibits browning of whole wheat by a process of phenolic substrate modification, wherein said process comprises the following steps:
   a. Adding formulation of bioenhancer to whole wheat and soaking overnight,
   b. Grinding in mill to obtain 40-60 mesh size,
   c. Making dough with 60-60% water and closing it with lid and
   d. Observing every half hour for four to six hours.
10. A composition comprising a blend of bioenhancers wherein said composition inhibits browning of whole wheat by a process of phenolic substrate modification as exemplified substantially in the foregoing examples 1-4.

FIGURE 1

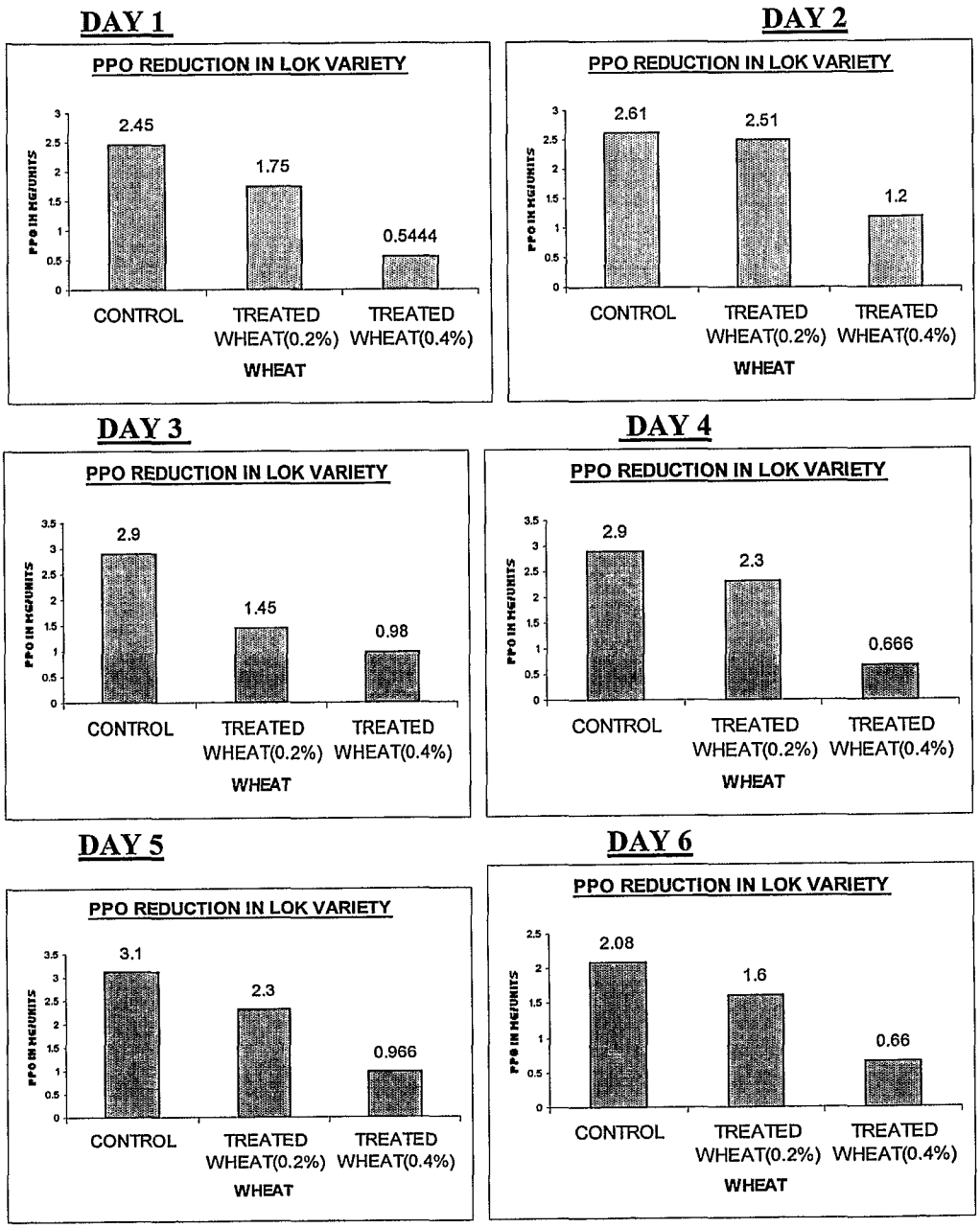
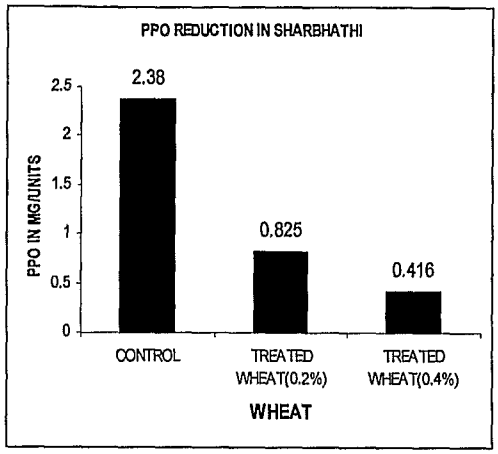
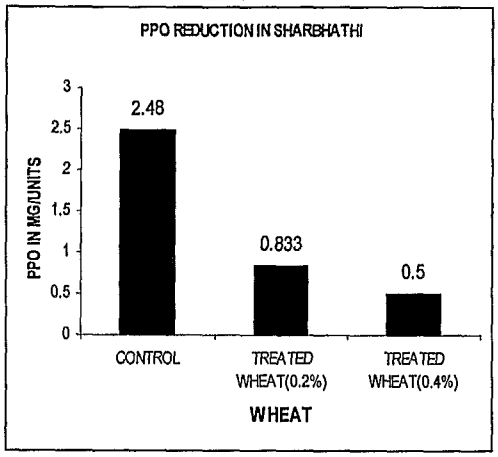

DAY 1
PPO REDUCTION IN LOK VARIETY
PPO IN MG/UNITS
2.45
1.75
0.5444
CONTROL
TREATED WHEAT(0.2%)
TREATED WHEAT(0.4%)
WHEAT
DAY 2
PPO REDUCTION IN LOK VARIETY
PPO IN MG/UNITS
2.61
2.51
1.2
CONTROL
TREATED WHEAT(0.2%)
TREATED WHEAT(0.4%)
WHEAT
DAY 3
PPO REDUCTION IN LOK VARIETY
PPO IN MG/UNITS
2.9
1.45
0.98
CONTROL
TREATED WHEAT(0.2%)
TREATED WHEAT(0.4%)
WHEAT
DAY 4
PPO REDUCTION IN LOK VARIETY
PPO IN MG/UNITS
2.9
2.3
0.666
CONTROL
TREATED WHEAT(0.2%)
TREATED WHEAT(0.4%)
WHEAT
DAY 5
PPO REDUCTION IN LOK VARIETY
PPO IN MG/UNITS
3.1
2.3
0.966
CONTROL
TREATED WHEAT(0.2%)
TREATED WHEAT(0.4%)
WHEAT
DAY 6
PPO REDUCTION IN LOK VARIETY
PPO IN MG/UNITS
2.08
1.6
0.66
CONTROL
TREATED WHEAT(0.2%)
TREATED WHEAT(0.4%)
WHEAT

FIGURE 2

DAY 1



DAY 3



Day 5

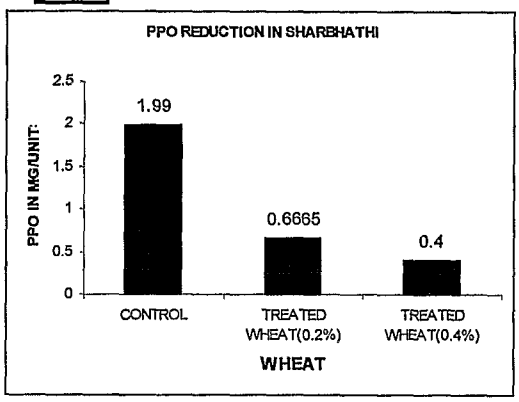


FIGURE 3

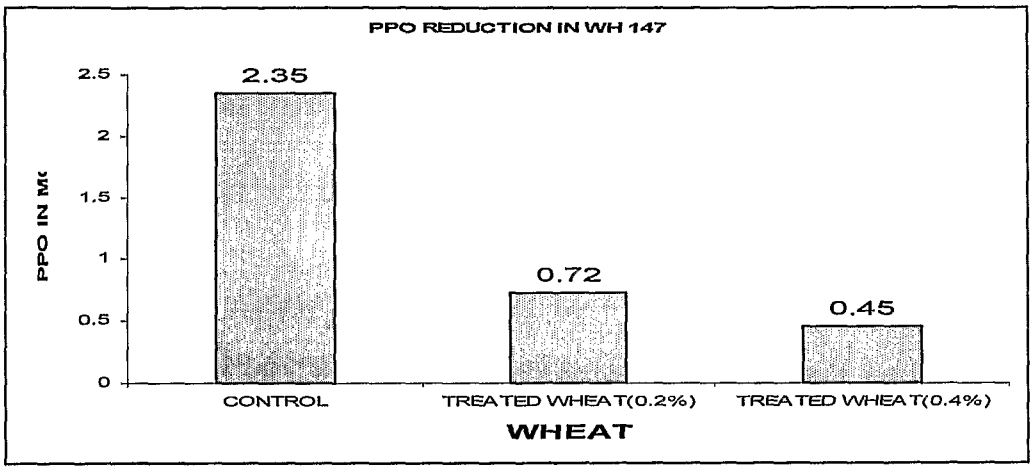

PPO REDUCTION IN WH 147
PPO IN M
2.5
2
1.5
1
0.5
0
2.35
0.72
0.45
CONTROL
TREATED WHEAT(0.2%)
TREATED WHEAT(0.4%)
WHEAT

FIGURE 4

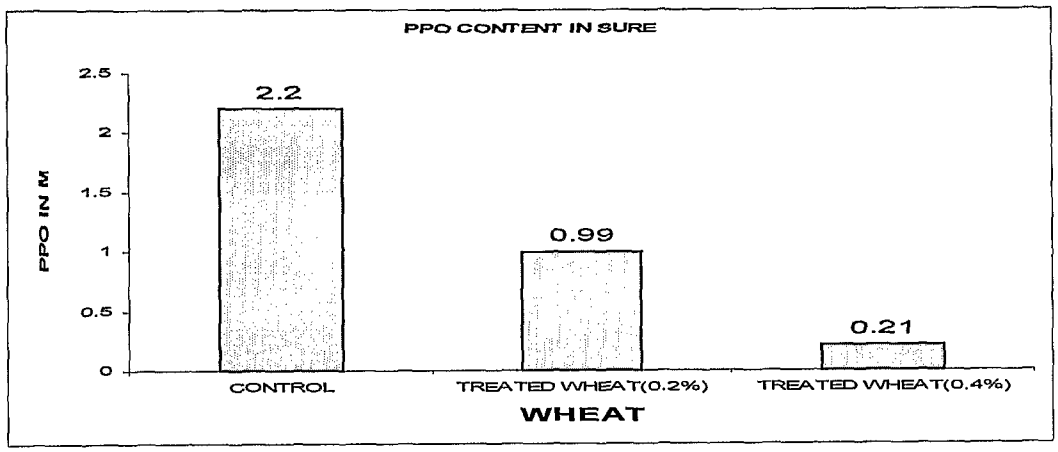

PPO CONTENT IN SURE
PPO IN M
2.5
2
1.5
1
0.5
0
2.2
0.99
0.21
CONTROL
TREATED WHEAT(0.2%)
TREATED WHEAT(0.4%)
WHEAT

FIGURE 5

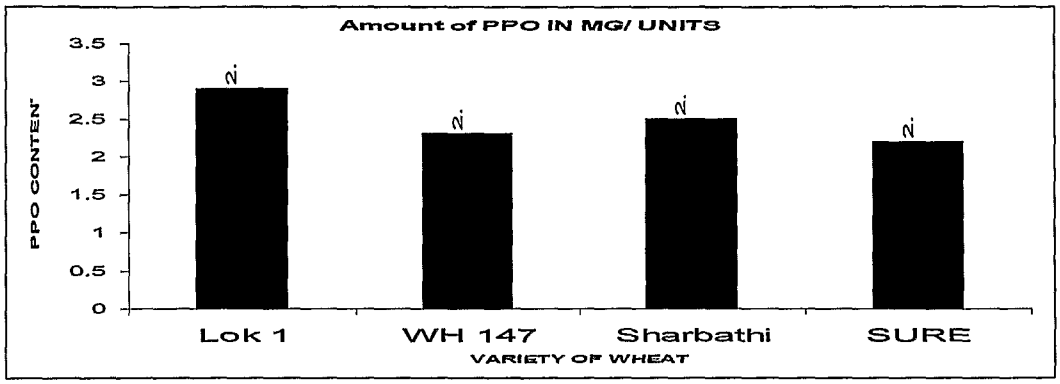

Amount of PPO IN MG/ UNITS
PPO CONTENT
3.5
3
2.5
2
1.5
1
0.5
0
Lok 1
WH 147
Sharbathi
SURE
VARIETY OF WHEAT

FIGURE 6

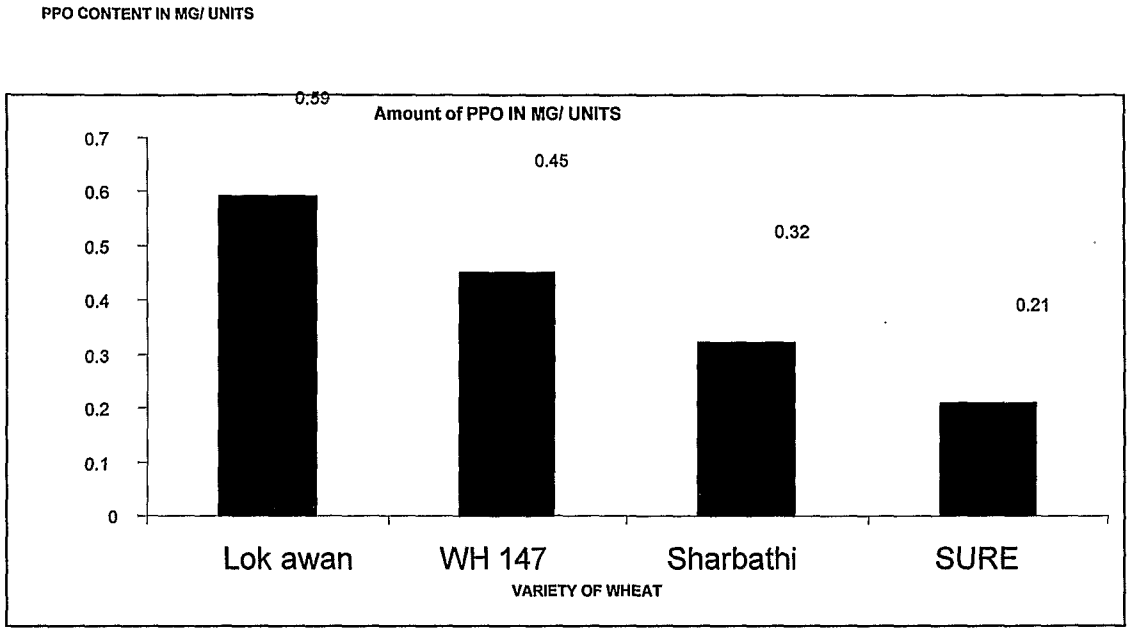

PPO CONTENT IN MG/ UNITS
Amount of PPO IN MG/ UNITS
0.59
0.45
0.32
0.21
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Lok awan
WH 147
Sharbathi
SURE
VARIETY OF WHEAT

FIGURE 7

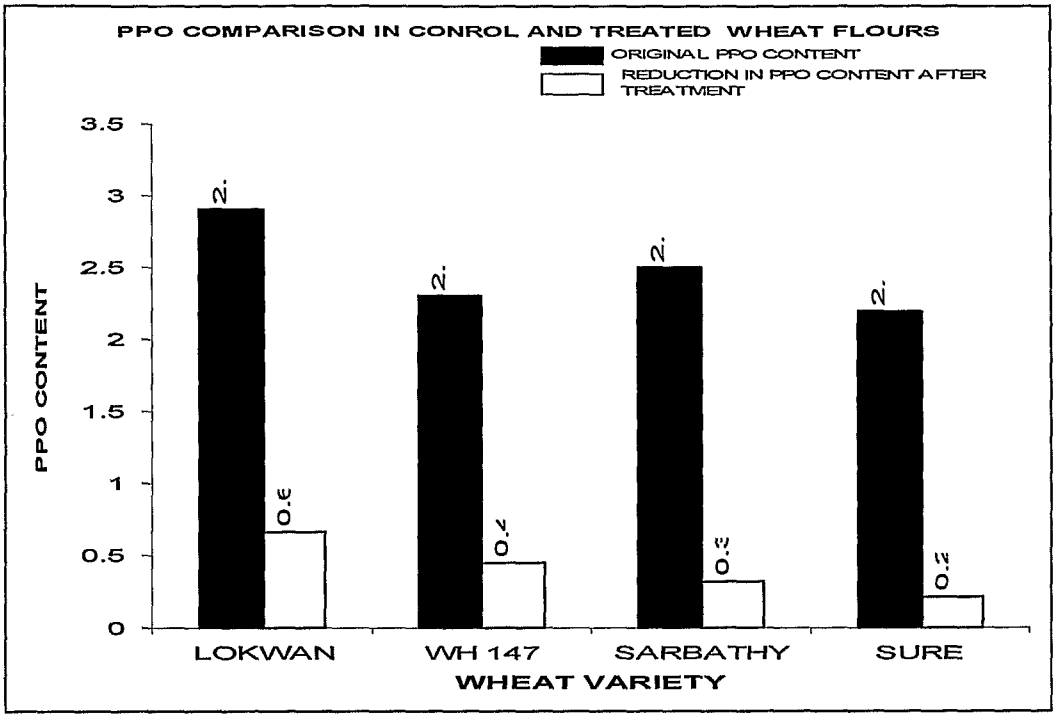

PPO COMPARISON IN CONROL AND TREATED WHEAT FLOURS
ORIGINAL PPO CONTENT
REDUCTION IN PPO CONTENT AFTER TREATMENT
PPO CONTENT
3.5
3
2.5
2
1.5
1
0.5
0
LOKWAN
WH 147
SARBATHY
SURE
WHEAT VARIETY

FIGURE 8

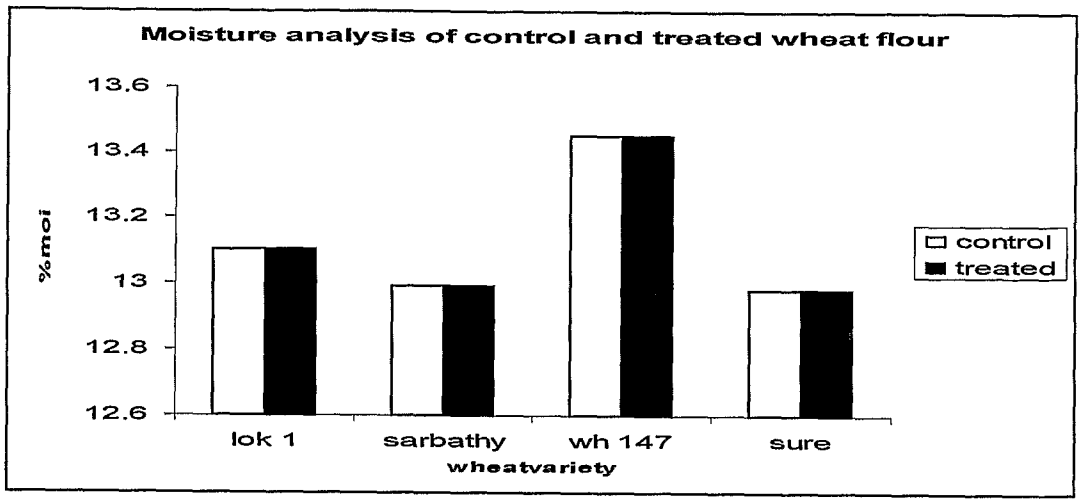

Moisture analysis of control and treated wheat flour
13.6
13.4
13.2
13
12.8
12.6
%moi
lok 1
sarbathy
wh 147
sure
wheatvariety
control
treated

FIGURE 9

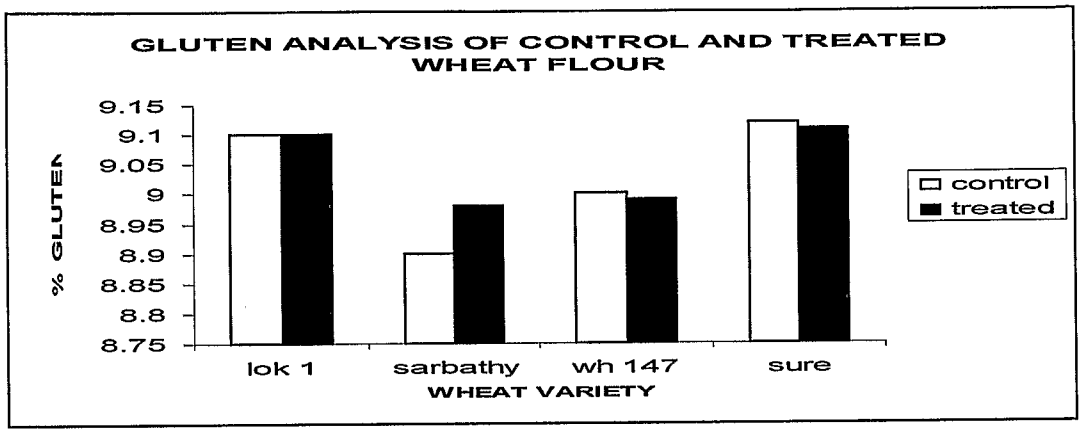

GLUTEN ANALYSIS OF CONTROL AND TREATED WHEAT FLOUR
% GLUTEN
9.15
9.1
9.05
9
8.95
8.9
8.85
8.8
8.75
lok 1
sarbathy
wh 147
sure
WHEAT VARIETY
control
treated

FIGURE 10

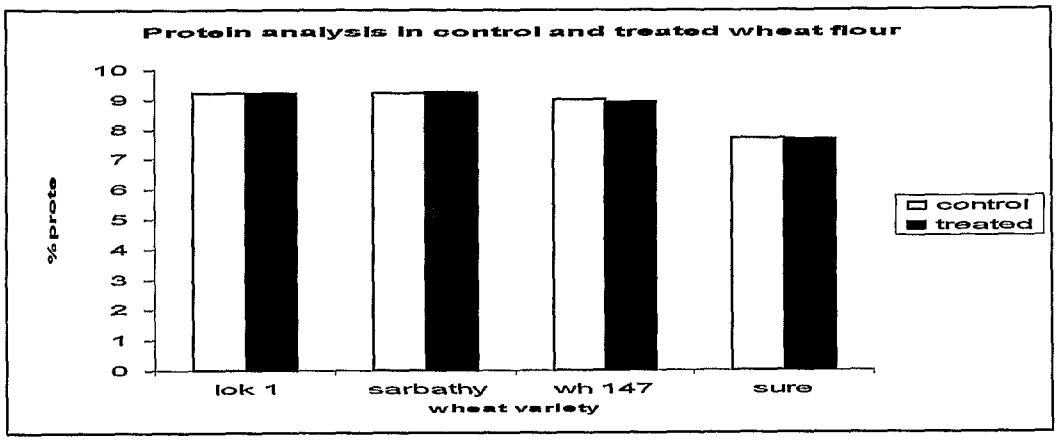

Protein analysis in control and treated wheat flour
% prote
10
9
8
7
6
5
4
3
2
1
0
lok 1
sarbathy
wh 147
sure
wheat variety
control
treated

FIGURE 11

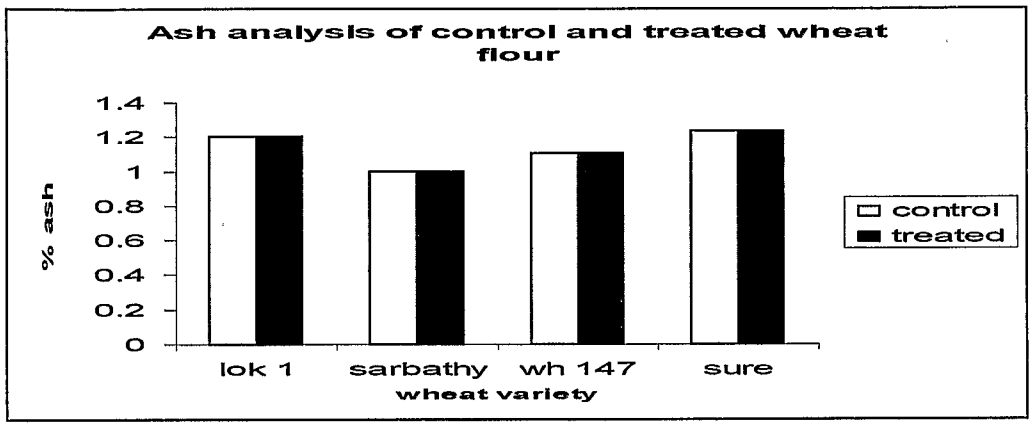
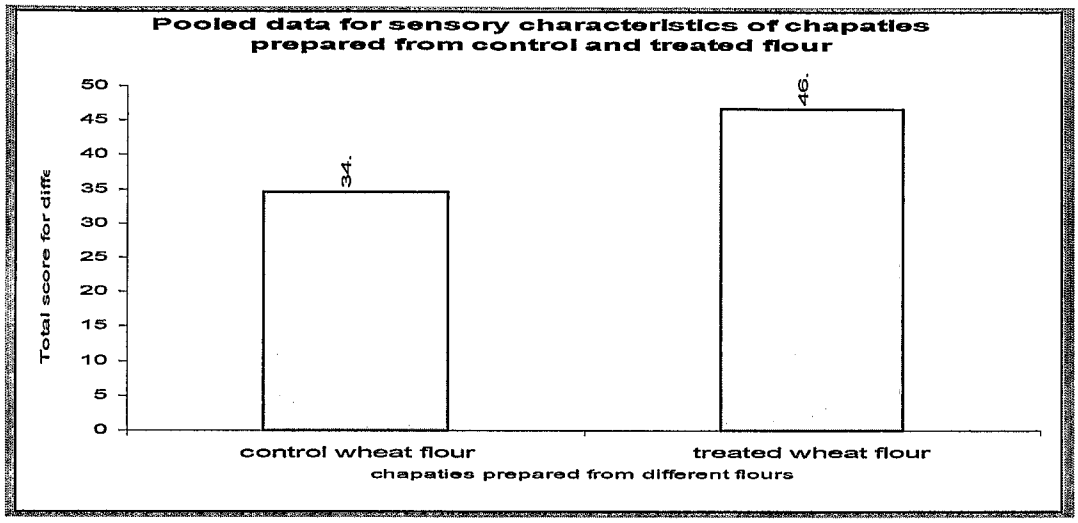

Ash analysis of control and treated wheat flour
1.4
1.2
1
0.8
0.6
0.4
0.2
0
% ash
lok 1
sarbathy
wh 147
sure
wheat variety
control
treated

FIGURE 12


Pooled data for sensory characteristics of chapaties prepared from control and treated flour
Total score for diffe
50
45
40
35
30
25
20
15
10
5
0
34.
46.
control wheat flour
treated wheat flour
chapaties prepared from different flours