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Effect of Peroxidase on the Physico-chemical, Rheological Properties of Whole Wheat Flour Dough, and Quality Attributes of Chapati and Its Health Benefits

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Abstract: Additives are added to wheat flour to improve the quality of its products. Enzymes are preferred as additives over chemical agents, as they are safe and natural. Few studies indicate that incorporation of peroxidase in wheat flour influences the bread quality, however, no report is available on its influence on whole wheat flour and on chapati quality and hence, a study has been carried out on the effect of peroxidase on the protein characteristics and rheological properties of whole wheat flour dough, chapati quality and its health benefits. In the present study, addition of peroxidase to whole wheat flour increased water absorption, dough stability and overall quality of chapatis. Peroxidase treatment increased the puffed height of chapatis by 0.6 cm. Increase in disulfide content resulted in increase of molecular weight of non-gluten proteins on peroxidase treatment of the dough. However, no significant difference was observed in protein digestibility of chapatis, while glycemic index of chapatis prepared from peroxidase treated dough was significantly lower compared to that of control chapatis. Thus, peroxidase treatment improved the quality of chapatis and also the health benefits.
Keywords: Chapati; Peroxidase; Rheology; Health benefits.

1. Introduction

Different types of products are made using both refined as well as whole wheat flours due to their ability to form visco-elastic dough. Chapati is an unleavened flat product made from whole wheat flour consumed by Indians and Asian ethnic communities residing in other countries. The quality of chapati is mainly influenced by the dough quality, which in turn is influenced by constituents of whole wheat flour. Whole wheat flour from soft wheat are more suitable for cake and biscuit while medium strong wheat is more suitable for chapati [1].

In baking industry, various additives are added to wheat flour to make it more suitable for particular product. Additives such as bromates, azodicarbonamide are used for improvement of dough strength and in turn to improve the product quality [2]. As some of these chemical additives are reported to have toxic properties, enzymes are preferred as they are safe and natural. Peroxidase is an oxidative enzyme having substrates like phenols, arylamines, halides and electron donors like thiols [3-5]. Peroxidase treatment of dough shows positive effect in baking which is due to crosslinking of feruloylated arabinoxylans into larger aggregates, protein-protein crosslinks and also by formation of cross-links between arabinoxylans and proteins [6-9].

Earlier studies indicate that peroxidase influences the wheat dough and bread quality prepared from refined wheat flour [10- 12]. Adhesiveness (stickiness) of dough was reported to decrease upon treatment with peroxidase [8]. However, no studies were reported on the effect of peroxidase on chapati quality, which is prepared using whole wheat flour. As whole wheat flour contains bran and husk, the composition of proteins, pentosans and phenolics in whole wheat flour differs compared to refined wheat flour [13]. Therefore, the nature and extent of crosslinks in presence of peroxidase may vary compared to refined wheat flour. The whole wheat flour contains more non-gluten proteins compared to refined wheat flour. Also, the digestibility and consequently nutritional properties of product made with the peroxidase treated dough may also differ compared to the dough made with refined wheat flour [14].

Therefore, the present work was carried out to study the effect of peroxidase on protein characteristics, rheological properties of whole wheat flour dough, chapati quality, and digestibility of proteins and glycemic index of chapati prepared from whole wheat flour dough.

2. Material and methods

2.1 Wheat variety

Wheat variety NIAW34, a poor chapati making variety [15] was obtained from Agharkar Research Institute, Pune, India was ground to whole wheat flour using commercial disc mill.

2.2 Measurement of peroxidase activity

Horseradish peroxidase was procured from Sigma Aldrich, USA and the peroxidase activity was determined [16]. In brief, enzyme activity was measured 1% H_2O_2 and o-dianisidine as substrates in sodium acetate buffer at pH 4.8. One unit of peroxidase activity was defined as the amount of enzyme, which produced an increase of 1.0 U min^{-1}at 460 nm.

2.3 Chemical Properties

2.3.1 Isolation and estimation of soluble protein content

Whole wheat flour (10 g) was mixed with water alone or water containing peroxidase to prepare the dough. The dough thus prepared was rested for 30 min, freeze dried and used for further studies. Alternatively, gluten was prepared from dough from control and untreated doughs. Gluten was extracted using 0.5% SDS and the soluble protein content in the extract was estimated [17]. Bovine serum albumin was used as a standard protein.

2.3.2 Disulfide and thiol estimations

The disulfide and sulfhydryl content in the gluten suspension was determined by solid phase assay using $NTSB^{2-}$ (Disodium 2-nitro-5-thiosulphobenzoate) [18].

2.4 Gel filtration chromatography of salt soluble proteins of dough

Gel filtration chromatography was carried out for dough on Sephadex G-75 using 0.1M sodium chloride as eluent. Freeze dried dough (1g) was extracted with 10 ml of 0.1M NaCl for 1 h, and centrifuged at 10,000 x g for 20 min at 4ºC. The clear supernatant was loaded on to the column and proteins were eluted at a flow rate of 8 ml/h. The fractions were monitored at 280 nm using a spectrophotometer. The percentage yield of each peak fraction was calculated by comparing the absorbance of individual peak fraction with that of the sample loaded on the column.

2.5 Rheological Characteristics

Whole wheat flours were treated with 2,500, 5,000, 10,000 and 20,000U of peroxidase and the farinograph characteristics of control and treated doughs were done using Brabender Farinograph – E (Brabender OHG, Duisburg, Germany) [19].

2.6 Chapati making property

Chapati doughs were prepared by mixing whole wheat flour and water with the addition of 2,500 and 10,000 U of peroxidase using a Hobart mixer. Control chapati was prepared without the addition of peroxidase. The dough rested for 30 min at room temperature was divided into small portions (25g). Each portion was sheeted into a thickness of 1.5 mm and was cut into circular shape (12 cm diameter) using a die and baked on hot plate maintained at 215° C for 70 s on side 1 and 85 s on side 2. The chapati was then transferred to a heated gas tandoor (370°C) in such a way that side 1 was placed on the grill and heated for 10 s [20, 21]. The puffed chapati was then cooled, packed in polypropylene pouches until further use.

2.6.1 Measurement of puffed height of chapati

Puffed height of chapati was measured as soon as the chapati was removed from the gas tandoor as described by Haridas Rao et al [21].

2.6.2 Subjective evaluation of chapati

Chapati was evaluated by a panel of ten judges for color, appearance, tearing strength, pliability, mouthfeel, taste, aroma and overall quality. The panellists were provided quality description against each sensory attribute so that the scores can be enumerated.

2.7 Determination of protein digestibility

In-vitro digestibility of protein from peroxidase treated and untreated freeze dried chapati was determined according to Akeson & Stahman, [22]. The units of peroxidase used in the experiment was 2500 and 10000 U. *In*

vitro digestibility was described as percentage of total protein solubilised after enzymatic hydrolysis by pepsin and pancreatin.

2.8 Determination of glycemic Index

The glycaemic Index (*in-vitro*) of peroxidase treated and untreated chapati were done by using method described by Araya et al [23]. The units of peroxidase used in the experiment was 2500 and 10,000 U. Sample was incubated with enzyme mixture (amyloglucosidase, pancreatin and invertase) and subjected to glucose oxidase-peroxidase (GOD-POD) assay.

2.9 Statistical analysis and graphical representation

The experimental data were subjected to statistical analysis and their significance were determined using Duncan's new multiple range test (DMRT) [24]. The incremental areas under the blood glucose curves (AUC) were considered by using the software Graphpad Prism and GI was calculated as described by Wolever et al [25] using the following formula:

$$GI = \frac{\text{Incremental area of the test food}}{\text{Incremental area of the glucose}} \times 100 \quad (1)$$

3. Results and discussion

3.1 Influence of peroxidase on gluten properties

3.1.1 Thiol and disulfide contents of gluten isolated from untreated and treated dough with peroxidase

The whole wheat flour dough was treated with peroxidase (2,500U) and then the gluten was isolated from the dough. Control gluten was isolated from dough prepared without adding peroxidase. Peroxidase is an oxidative enzyme and it has been reported that it oxidizes the thiol (-SH) groups into disulfide (-S-S-) groups [16, 26]. Therefore, the thiol and disulfide contents in both treated and untreated gluten (Control) were determined. In the treated gluten, the –SH content decreased from 16.8 to 10.9 µmole/g and the disulfide (-S-S) groups content increased from 7.3 to 10.5 µmole/g (Table 1). Increase in S-S content was reported to increase the dough strength due to the cross-linking of proteins [27].

Table 1. Thiol, disulfide bond and soluble protein contents properties of gluten prepared from treated and untreated doughs

Variations	Soluble Proteins (%)	Thiol (µmole/g)	Disulfide (µmole/g)
Whole wheat flour	37.61^{a}±2.2	16.86^{a}±0.28	7.27^{a}±0.42
Whole wheat flour +H2O2+ Peroxidase	30.23^{b}±1.8	10.69^{b}±0.22	10.50^{b}±0.68

*Data expressed as mean ±SD, means followed by different letters in the same column differ significantly ($p<0.05$)

3.1.2. Effect of peroxidase on the solubility of gluten proteins

The soluble protein contents were determined in both control and enzyme treated glutens. The soluble protein in control gluten (isolated from untreated dough) was 37.61 g/100g, while it was decreased to 30.2g/100g in treated gluten (isolated from dough treated with peroxidise and H_2O_2). Cross-linking of proteins through disulfide bond formation might have decreased the soluble protein content of treated gluten. Peroxidase has been reported to improve the rheological properties of weak flour through cross-linking of gluten proteins [26]. Thus, the protein extractability decreased by about 20%, upon treatment of dough with peroxidase compared to control.

3.2. Effect of peroxidase on changes in molecular weight of non-gluten protein characteristics

Researchers treated the wheat glutenin subunits with peroxidase and reported that these subunits were cross-linked both by disulfide bonds and dityrosine linkages [16]. Weegles et al [28] reported that apart from gluten proteins, low molecular weight wheat proteins play an important role in controlling rheological properties of dough and addition of these proteins to medium wheat flours increased the loaf volume of bread. These low molecular weight proteins are soluble in water and salt solutions [29]. The whole wheat flour contains more non-gluten proteins compared to refined wheat flour and no studies were carried out on the effect of peroxidase on non-gluten proteins. Hence, a study on the effect of peroxidase on the MWs of salt soluble proteins like albumins and globulins was carried out.

Control and peroxidase treated doughs were extracted with dilute NaCl separately and centrifuged. The clear supernatants obtained were loaded on Sephadex G-75 column to know the changes in the molecular weight of

protein fractions after treatment with peroxidase. Results indicated that there is a change in the MW and yields of peak I and peak II proteins upon peroxidase treatment of dough compared to control (Figure 1). In case of peroxidase treated, the yield as well as the MW of peak I proteins increased compared to that of control. In case of control, the elution volume was 66 ml with a molecular weight around 60 kDa while in case of peroxidase treated dough the peak I had the elution volume of 50 ml with a molecular weight >80 kDa (Figure 1). Thus, the results indicate that upon treatment with peroxidase, the molecular weight of salt soluble proteins like albumins and globulins (non-gluten proteins) increased. This increase may be due to peroxidase mediated cross-linking of proteins. In the present study, it is reported that peroxidase treatment increased the molecular weight of salt soluble proteins as well as the yield of peak I (Table 2). Thus, results suggest that peroxidase treatment cross-links non-gluten proteins and this may lead to the alteration of dough properties.

Table 2. Percentage distribution and elution volume of different peaks obtained from gel filtration chromatography

Treatment	Peak I		Peak II	
	Elution Volume (ml)	Yield	Elution Volume (ml)	Yield
WWF (control)	63	25	124	60
WWF+ H_2O_2 +Peroxidase	49	42	117	40

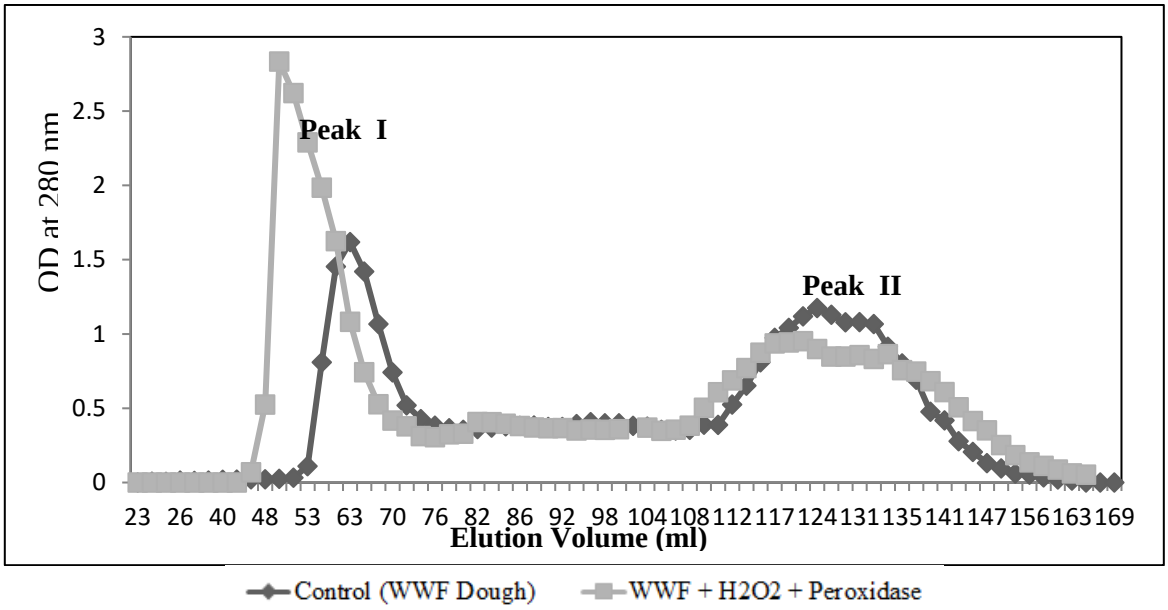

Fig. 1. Gel filtration chromatograms of crosslinked salt soluble proteins of dough (Column G-75; Eluent-NaCl)

3.3. Farinograph characteristics of peroxidase treated whole wheat flour dough

Rheological property of dough influences the product quality and the dough rheology depends on the protein characteristics. Farinograph characteristics are an important attribute in determining the rheological property of wheat dough. As peroxidase treatment of dough at 2500 U showed considerable changes in thiol and disulfide content as well as MW properties, effect of peroxidase on changes in farinograph characteristics of dough were carried out.

Earlier, Researchers carried out a detailed study on the effect of peroxidases on cross-linking of high and low MW glutenin subunits [16]. In their study, they used different levels of peroxidase to cross-link glutenin subunits and reported that the cross-linking of glutenin subunits increased in a concentration-dependent manner. Hence, we have used different levels of peroxidase to see the optimum changes in rheology.

Whole wheat flour was mixed by treating with 2500, 5000, 10000 and 20000 U of peroxidase and farinograph properties of dough was determined (Table 3). Water absorption (WA) increased from 75.3 (Control) to 77.7, 78.5, 78.7 and 79.8% on incorporation of peroxidase in the above mentioned levels, respectively. Dough development time (DDT) increased from 4.0 to 4.8, 4.8, 5.7 and 5.7 min on incorporation of peroxidase. Dough stability increased upon addition of peroxidase for all the variations from 2.0 to 2.4, 2.4, 3.0 and 3.2 min. There were changes in mixing tolerance index (MTI) of whole wheat flour obtained from all the variations (11, 30]. Farinograph water absorption is affected by changes in dough consistency. The higher the mixing dough consistency targeting the value above 500 BU, there is an urge to increase water to lower the dough consistency to the desired value. Consequently, water absorption is increased as the consistency is increased too. Researchers reported that peroxidase improve dough handling and dough tolerance [11]. Earlier workers also reported that the

rheological properties are improved as a result of different reactions in wheat dough that are promoted by enzyme catalysed reactions like disulfide bonding within the water soluble protein fraction [31], dityrosine bonding in gluten protein [32] oxidative gelation of wheat WEP and other enzymatic reactions that could have an effect in farinograph properties.

Dough development time and dough stability are important parameters and the result indicate that 2500 U and 5000 U of peroxidase showed similar result and 10,000 U and 20,000 U showed similar results with respect to these two parameters. Hence, further studies were carried out treating the dough with 2500 U and 10,000 U of peroxidase.

Table 3. Farinograph characteristics of whole wheat flour treated with peroxidase

Parameters	Control	2500 U*	5000 U*	10000 U*	20000 U*
Consistency (FU)	495	482	498	503	515
Water absorption (500 FU) (%)	75.3	77.7	78.5	78.7	79.8
Water absorption (14.0%) (%)	67.9	69.4	70.2	70.4	71.8
Dough development time (min)	4.0	4.8	4.8	5.7	5.7
Stability (min)	2.0	2.4	2.4	3.0	3.2
Tolerance Index (MTI) (FU)	73	75	76	77	79

*peroxidase units

3.4. Chapati quality of whole wheat flour treated with peroxidase

Chapatis were prepared from dough treated with 2,500 and 10,000 U of peroxidase. The puffed height of chapatis prepared from dough treated with peroxidase increased significantly (Table 4). The puffed height of chapatis prepared from dough treated with 2500U and 10000U peroxidase increased by 0.1 and 0.6 cm, respectively.

Sensory properties of chapatis prepared from dough treated with peroxidase did not show any significant improvement in the color and appearance (Table 4). Significant improvement in the tearing strengths of chapatis was observed in chapatis prepared from dough treated with peroxidase. Chapatis prepared from control dough were slightly brittle hence scored less; on the other hand, chapatis prepared from peroxidase treated dough improved the tearing strength, therefore had considerably high score.

Treating of chapati dough with peroxidase has affirmative effect on the flexibility of chapatis. Freshly prepared chapatis should be extremely pliable resulting in softer textured products. Significant development was observed in chapatis prepared from peroxidase treated dough. Sensory scores for pliability were 9.0 for control chapatis. These values increased to 9.5 and 10.0 for those prepared from peroxidase treated doughs. Earlier, it was reported that the addition of peroxidase improved dough properties, loaf volume and crumb softness in bread [11]. Treating of chapati dough with peroxidase did not show difference in taste and aroma of chapatis.

Chewing, biting and swallowing append to the attribute of eating quality. Sensory score for eating quality for control chapatis were 17.5. But these values ranged between 18.5 and 19.5 for those prepared from peroxidase treated doughs. Peroxidase treated doughs seemed to have better impact on chapatis than control. Overall quality of chapatis, which actually is inclined by all the above factors, improved when their respective dough was treated with peroxidase. The overall quality of chapatis made from dough treated with peroxidase increased significantly from 52.5 to 54.0 and 58.0 on 2500 and 10000 U of peroxidase, respectively. Treating of chapati dough with peroxidase showed a significant improvement in overall quality of chapatis. This enhancement could be credited to its effect on crosslinking of proteins and pentosans present in the whole wheat flour.

Table 4. Evaluation of chapati quality prepared from whole wheat flour dough treated with peroxidase

Variations	Puffed height (cms)	Appearance (10)	Tearing strength (10)	Pliability (10)	Aroma (10)	Eating quality (20)	Overall quality (60)
Control	$4.5^{a}\pm0.2$	$9^{a}\pm0.5$	$8^{a}\pm0.5$	$9^{a}\pm0.5$	$9^{a}\pm0.5$	$17.5^{a}\pm0.5$	$52.5^{a}\pm1.5$
2500 U	$4.6^{a}\pm0.3$	$9^{a}\pm0.5$	$8.5^{a}\pm0.5$	$9.5^{ab}\pm0.5$	$9^{a}\pm0.5$	$18.5^{b}\pm0.5$	$54.0^{b}\pm1.0$
10000 U	$5.1^{b}\pm0.2$	$10^{a}\pm1$	$9.5^{b}\pm0.5$	$10^{b}\pm0.5$	$9^{a}\pm0.5$	$19.5^{c}\pm0.5$	$58.0^{c}\pm1.5$

3.5. Digestibility of chapati prepared from dough treated with peroxidase (in-vitro)

Protein low digestibility leads to protein intolerance, which is a disorder that results from an adverse effect of the ingestion of food proteins. It is often associated with gastrointestinal symptoms [33]. Protein digestibility of chapatis prepared from dough treated with peroxidase was not significantly different from control chapati. Therefore, treatment of dough with peroxidase may not cause deleterious effects (Table 5).

Table 5. Digestibility studies and glycaemic indices of chapatis prepared from dough treated with peroxidise

Variations	Protein Digestibility (%)	Glycaemic Index (in-vitro) (%)
Chapatis (control)	81.7^{a}±1.5	49.9^{c}±0.4
Chapatis (2500 U peroxidase treated)	83.7^{a}±1.6	40.5^{b}±0.4
Chapatis (10000 U peroxidase treated)	84.1^{a}±0.9	35.5^{a}±0.3

*Data expressed as mean ±SD, means followed by different letters in the same column differ significantly ($p<0.05$)

3.6. Glycemic index of chapati prepared from peroxidase treated dough (in- vitro)

The Glycaemic Index (GI) (*in-vitro*) of chapatis treated with peroxidase treated dough was significantly lower than control chapatis (without treatment) (Table 5). Peroxidase catalyses the cross-linking of proteins as well as formation of cross-links between carbohydrates and proteins [6-8]. Compared to free carbohydrates, release of glucose from cross-linked starches by hydrolytic enzymes is slow, thereby lowering the blood glucose levels [34, 35]. Thorne et al [36] and Turco et al [37] have also reported that protein-starch interactions decreased glycaemic index of food. Foods low in GI may reduce the insulin demand due to low blood glucose [38], reduce blood lipid concentrations [39] and thereby could help prevent diabetes and cardiovascular diseases [40].

4. Conclusion

Peroxidase treatment of dough improved the overall quality of chapati by improving its puffed height pliability, tearing strength and chewiness. Peroxidase treatment of dough decreased the glycemic index of chapatis prepared from it. Decrease in glycemic index of a food product is an indicator of beneficial effect on health. Thus, peroxidase treatment has not only improved the quality of chapatis prepared from poor quality wheat but also improved the health benefits.

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