Evaluation of the Effect of Hexose Oxidase on the Bread Baking Properties of Flour from Three Kenyan Wheat Varieties

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Abstract: ***This study determined the effects of hexose oxidase on dough and bread made from wheat varieties grown in Kenya. Flour from Duma, Njoro 2 and Robin wheat varieties were treated with HOX at 0, 20, 40, 60 and 80 HOX units/kg of flour. The effect of this treatment on water absorption, dough development time, dough stability, mixing tolerance index, dough elasticity, dough resistance to extension and bread volume was studied. The control bread was prepared from untreated and potassium bromate ($KBrO_3$) treated dough. The findings indicate that HOX did not affect the water absorption, dough development time and the mixing tolerance index of all four but it increased the dough stability of Duma wheat. Addition of HOX to the dough also resulted in selective increase of dough strength and a reduction in dough elasticity. All HOX treated loaves had increased loaf volume. Treatment of dough with $KBrO_3$ at 75ppm resulted in an increase in bread volume for Duma wheat while a decrease in bread volume was observed for Njoro 2 and Robin wheat.***

Keywords: Hexose oxidase, Kenyan wheat varieties, dough characteristics, bread quality

1. Introduction

A majority of bread is conventionally produced from wheat flour. Wheat flour comprises of carbohydrates, proteins, lipids, minerals and water. The composition varies depending on genetic make-up, environmental conditions and the crop management practices used during growth [1][2][3]. This variation in the chemical composition of flour affects the dough rheological and baking properties of the flour thus results to variation of the quality of the baked product.

Bread improvers are preparations intended to simplify the production of baked goods, to compensate for changes in processing properties due to fluctuations in raw materials and to improve the quality of bakery products. Dough and bread properties that can be modified by the use of bread improvers include: dough stickiness, dough strength, flour water absorption, bread volume, bread crust and crumb color, crumb texture, bread shape or even bread shelf life[4][5][6]. One category of these bread improvers are the oxidants [7]. These promote the cross-linking of various protein fractions in flour notably the high molecular weight glutenin subunit resulting in increased dough strength. Increased dough strength up to a certain level is associated with desirable qualities such as increased bread volume, improved crumb texture and improved appearance of the baked product[8][9].

Treatment of dough using enzymes that perform function similar to chemical dough oxidants is becoming a common practice in the baking industry. This is mainly due to the perception that enzymes are natural and non toxic food components and that they do not remain active in the final product and, therefore, do not have to appear on the label [9][10][11]. One such enzyme is glucose oxidase. This enzyme catalyzes the oxidation of β-D-glucose to D-glucono- δ-lactone and the concomitant reduction of molecular oxygen to hydrogen peroxide. The effect of GO on dough and bread quality appear to be affected by the chemical composition of the flour used [12] [13]. The use of GO has one limitation, it requires the presence of glucose as a substrate in order to be effective and generally the glucose content in cereal flours is low [14]. Addition of glucose to dough increases the cost of production.

Hexose oxidase catalyzes the oxidation of various mono and oligosaccharides to their corresponding lactones and in the process, hydrogen peroxide is generated. Incorporation of this enzyme in dough is expected to result in generation of hydrogen peroxide just as glucose oxidase does. This enzyme however does not require the addition of glucose since it is able to utilize maltose that is naturally present in dough. [15] demonstrated that addition of HOX enhances the cross-linking of flour proteins and the gelation of pentosans resulting in dough of desirable qualities. This results in baked products of improved volume and texture. However, these studies did not report the effect of HOX on flour with different chemical composition or on flour obtained from different wheat varieties. It is known that the effect of a bread improver is dependent on the quality of flour or the wheat variety from which the flour was obtained [16][17]. It was, therefore, important to determine how HOX will affect the dough and bread made from wheat varieties grown in Kenya.

2. Materials and Methods

Kenyan wheat varieties namely Robin, Duma and Njoro 2 were sourced from the cereal breeding section of Kenya Agricultural Research Institute, Njoro, Kenya. Milling of the wheat samples was done according to the AACC Method 26-21.02. HOX enzyme was provided by Danisco. Proximate analysis of flour samples (Moisture, ash, protein and wet gluten) were determined by using Standard AACC methods [18]. The rheological behavior of dough from different wheat varieties supplemented with different concentration of HOX was evaluated by use of a farinograph and an alveograph following the AACC Method 54-21 and Method 54-30 respectively [18]. The straight dough method was used for making bread. The ingredients used included; 100g wheat flour, 3g shortening, 3g milk powder, 0.03g malt, 4g sugar, 2g salt, 2g yeast, water according to the farinograph, and the bread improver where appropriate (i.e. potassium bromate 75ppm ,or HOX at 20, 40, 60 and 80 EU/kg of flour). Potassium bromate ($KBrO_3$) was used for the purpose of comparison. After baking, the loaves were evaluated by determining the bread volume by the rapeseed displacement method and the specific volume of the bread was determined from the bread volume and weight. Analysis of variance was done and the means were separated using Tukey test.

3. Results and Discussions

3.1 Proximate composition of flour

Data on the proximate composition of flour from different wheat varieties used in this study is shown in table 1. Statistical analysis revealed that there are significant variations in the composition of flour from the three wheat varieties. In terms of the crude protein content, Duma had a significantly higher protein content followed by Njoro 2 while Robin had the lowest protein content. Likewise in terms of the gluten content Duma had higher gluten compared to Njoro 2 and Robin.

3.2 Farinograph properties

3.2.1 Effect of HOX on the water absorption of flour from different wheat varieties

Results on the effect of HOX on the water absorption (WA) of flour from different wheat varieties are shown in table 2. WA refers to the amount of water that is required to form dough of a predetermined consistency. With reference to the farinograph WA, WA refers to the amount of water required to center the farinograph curve on the 500 brabender-unit. On this line, the dough has maximum consistency and produces baked products of superior quality. Results from our study revealed that HOX did not significantly affect the WA of all flours.

3.2.2 Effect of HOX on the dough development time of different wheat variety

Results on the effect of HOX on the dough development time (DDT) of flour from different wheat varieties are shown in table 3. DDT refers to the time required to mix the dough in order to generate dough of known optimum consistency. Supplementation of HOX at high doses to wheat flour from Njoro 2 and Duma resulted in an increase in the DDT while the DDT of Robin was not affected.

3.2.3 Effect of HOX on dough stability of dough made from different wheat varieties

Results on the effect of HOX on the stability of dough made from different wheat varieties are shown in table 4. The effect of HOX on dough stability seems to be affected by the type of flour used. Addition of HOX at high doses to flour from Duma wheat varieties significantly increased the dough stability. The stability of dough from Njoro 2 flour was not affected while that of Robin was unpredictable when for the different levels of HOX added.

3.2.4 Effect of HOX on the dough mixing tolerance index

The dough MTI is an index that is used to determine the rate at which dough loses its stability as a result of over mixing. Results of how HOX affected the MTI of different wheat varieties is shown in table 5. Generally, HOX had no significant effect on the MTI of dough from all the wheat varieties used.

3.3 Alveograph properties

3.3.1 Effect of HOX on dough elasticity (L) and resistance to extension (P) of dough made of flour from different wheat varieties

The result for the effect of HOX on dough elasticity, resistance to extension and the P/L ratio are shown on table 6, 7 and 8. When HOX was added to the dough, it significantly reduced the elasticity of dough made Robin and Njoro 2 wheat. The extent of the decrease in dough elasticity appears to be affected by the amount of HOX added. However the elasticity of dough made from Duma wheat was not affected by levels of HOX used in this study. Considering the alveograph P value, addition of HOX significantly increased the dough strength of Duma and Robin wheat four while the increase for Njoro 2 flour was not statistically significant. The amount of HOX required to obtain maximum resistance vary among the wheat varieties. For Duma the amount required is 20 HOX units/kg flour while Robin required 40 HOX units/ kg. The effect of HOX on the P/L ratio is shown in table 8 below. Addition of increasing levels of HOX to dough made from Robin and Njoro 2 wheat resulted in a more predictable increase P/L ratio as compared to addition to Duma wheat.

3.4 Bread evaluation

3.4.1 Effect of HOX and $KBrO_3$ on bread volume of bread made from Duma, Robin and Njoro 2 wheat varieties

The effect of HOX and $KBrO_3$ on the loaf volume of bread made from different wheat varieties is shown in table 9. Addition of 75ppm $KBrO_3$ to flour from Robin and Njoro 2 wheat varieties resulted in a significant decrease in the bread volume. On the other hand addition of $KBrO_3$ to flour from Duma wheat resulted to a significant increase in bread volume. Addition HOX to flours resulted to significant increase in the bread volume of bread made from all the wheat varieties. The increase in volume was however not linear to the amount of HOX used. There are also variations in the concentration of HOX required to produce optimum bread volume. The concentrations are 60, 20 and 20 HOX units/kg of flour for Duma, Njoro 2 and Robin wheat flour respectively.

3.5 Discussions

Results from proximate analysis have shown that there are significant variations in the compositions of wheat flour obtained from different wheat varieties. However these compositions of may vary depending on the environmental conditions and crop management practices during growth [1] [2] [3].

Findings by different researchers have given conflicting reports on the effect of oxidants including enzymes, performing similar functions, on the WA of flour. Results by [19][20] found that the use of glucose oxidase (GO) (an enzyme that is functionally similar to HOX) in dough increased in the water absorption of flour and a drier and less sticky dough was formed. [12] demonstrated that addition of GO at 0.005% to dough significantly increased the amount of water absorbed by the flour. However, their finding also revealed that addition of GO at lower or higher levels than 0.005% did not affect the WA of the flour. Similar results were obtained by [13] who found out that HOX increased the WA of flour at specific concentration only, below or above this concentration the WA of flour is not affected. On the other hand, findings by [21] did not show any increase in water absorption when GO or Laccase (an oxidizing enzyme) was applied in flour. Results from our study revealed that HOX did not affect the WA of all flours. Our results agree with the findings of [21]**.**

Addition of oxidative bread improvers to dough is known to enhance the formation of inter and intra molecular covalent bonds among the various proteins in wheat flour dough. Depending on the type of enzyme used, dityrosine, disulphide or glutamyl-lysine linkages can be formed [14][22]. [23] demonstrated that treatment of glutenin protein with hydrogen peroxide and a subsequent separation of the gluten proteins on SDS-PAGE results in the formation of higher molecular weight proteins which either remain on top of the stacking gel or just enter the separating gel. Cross-linking of various protein fractions in dough is known to reduce the elasticity of dough as well as increase the force/pressure required to extend the dough[7][15][16]. The increase in dough strength and decrease in dough elasticity observed in our findings could be attributed to the cross linking of the various protein by the H_2O_2 generated by the added HOX.

The effect of oxidative bread improvers on the bread volume appear to be affected by the quality of flour used. [12][13][14][19][20] demonstrated that the addition of GO which is an enzyme that is functionally similar to HOX resulted to an increase in the bread volume. This effect was pronounced when GO was used with other synergistic enzyme such as xylanase and hemicellulase. This effect was attributed to the increase in dough strength which allowed for better gas retention during baking. On the other hand [11] [21] [24], noted that addition of GO either did not affect the bread volume or it negatively affected the volume. This decrease in bread volume in was attributed to over strengthening of the dough which resulted in the dough not rising adequately during the proofing stage. Over strengthening of dough could explain why the bread volume of Robin and Njoro 2 decreased after addition of 75ppm $KbrO_{3.}$ [15][13] demonstrated that addition of HOX to flour resulted in significant increase in the bread volume, however finding by[15] showed a linear positive relationship between the bread volume and the amount of HOX used. [13], noted that the increase in bread volume was only at a specific HOX concentration, above this concentration, the volume decreases. Findings of this work revealed that HOX increases the volume of bread made from Duma, Njoro 2 and Robin wheat. Our findings agreed with the findings of [13] and partially with those of [15].

4. Conclusions

Results of this research have given an indication that HOX can be used to modify the dough characteristics and therefore the bread characteristics of bread made from Duma, Njoro 2 and Robin wheat varieties. HOX appears to increase the dough stability and dough the strength while reducing the dough elasticity. The result of these dough modifications are manifested as a significant increase in bread volume. However these results cannot be generalized for all wheat varieties as HOX affects the dough from different wheat varieties in different ways. Lastly, the levels of oxidative bread improvers to be used for a particular type of flour should be determined experimentally before use. In our case, addition of 75ppm $KBrO_3$ resulted in an increase in the volume of bread made from Duma wheat while it reduced the bread volume of Robin and Njoro 2 wheat. Similarly 60, 20 and 20 HOX units/kg of flour was required to produce bread with increased volume for Duma, Robin and Njoro 2 wheat varieties respectively.

5. Acknowledgement

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6. Future Scope of the Study

Hexose oxidase (HOX) is an enzyme which could be used effectively to replace chemical oxidants such as potassium bromate in bread dough making. Enzyme such as the hexose oxidase would be preferred to the addition of chemical improvers as they do not remain active in the final product and, therefore, do not have to appear on the label of processed food. There will be a future need to undertake trials at commercial level where hexose oxidase would be used in large scale dough preparations and monitor its ability to enhance the properties observed in the study such as machinability, dough strength and crumb firming in wheat dough. It will also be of future research interest to investigate the possibilities of using enzymes such as Hexose oxidase for gluten hydrolysis in other wheat foods. There is also a need to study the exact mechanism in which HOX utilizes maltose in wheat dough.

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Tables Captions

Table 1

Variety	*Protein*	*Gluten*	*Ash*	*Moisture*
Duma	15.51±0.27^a	36.62±3.10^a	0.80±0.00^a	11.78±0.33^c
Robin	11.63±0.59^c	24.84±5.22^c	0.71±0.06^b	12.26±0.36^a
Njoro 2	14.31±0.13^b	29.81±0.53^b	0.81±0.03^a	11.84±0.32^{bc}

Proximate composition of flour obtained from different wheat varieties.

Table 2

Variety\ WA	*Control*	*20 HOX*	*40 HOX*	*60 HOX*	*80 HOX*
Duma	67.70± 0.30^a	67.00± 0.40^a	67.60± 0.40^a	68.00± 0.40^a	67.30± 0.70^a
Robin	65.30± 0.40^a	64.30± 0.30^a	64.90± 0.50^a	65.07± 0.35^a	65.03± 0.70^a
Njoro 2	65.30± 0.50^a	65.30± 0.10^a	64.70± 0.30^a	64.70± 0.20^a	65.30± 0.50^a

The effect of HOX on WA of flour obtained from different wheat varieties.

Table 3

Variety	Control	20 HOX	40 HOX	60 HOX	80 HOX
Duma	7.00 ± 0.5^{a}	8.00 ± 1.50^{a}	7.00 ± 2.00^{a}	8.00 ± 1.5^{a}	10.00 ± 1.00^{a}
Robin	2.50 ± 0.50^{a}	2.67 ± 0.29^{a}	2.50 ± 0.00^{a}	2.50 ± 0.00^{a}	2.50 ± 0.00^{a}
Njoro 2	10.50 ± 1.50^{ab}	9.00 ± 1.50^{b}	12.00 ± 2.00^{ab}	10.50 ± 2.00^{ab}	14.50 ± 0.87^{a}

The effect of HOX on the DDT of dough made from different wheat varieties.

Table 4

Variety	Control	20 HOX	40 HOX	60 HOX	80 HOX
Duma	11.00 ± 2.00^{b}	11.00 ± 2.50^{b}	12.00 ± 1.50^{b}	12.50 ± 1.50^{b}	19.00 ± 0.50^{a}
Robin	8.83 ± 1.26^{a}	8.33 ± 0.29^{ab}	6.00 ± 0.87^{c}	6.67 ± 0.29^{bc}	7.33 ± 0.29^{abc}
Njoro 2	10.50 ± 1.00^{a}	13.50 ± 2.00^{a}	13.50 ± 0.50^{a}	14.50 ± 2.00^{a}	11.67 ± 3.01^{a}

The effect of different levels of HOX on the dough stability of dough from different wheat varieties.

Table 5

Variety	Control	20 HOX	40 HOX	60 HOX	80 HOX
Duma	30.00 ± 10.00^{a}	30.00 ± 0.00^{a}	30.00 ± 10.00^{a}	30.00 ± 10.00^{a}	13.33 ± 5.77^{a}
Robin	23.33 ± 5.78^{ab}	16.67 ± 5.78^{b}	36.67 ± 5.78^{a}	36.67 ± 5.78^{a}	26.67 ± 11.55^{ab}
Njoro 2	30.00 ± 10.00^{a}	26.67 ± 15.28^{a}	30.00 ± 10.00^{a}	10.00 ± 0.00^{a}	30.00 ± 20.00^{a}

The effect of HOX on the MTI of dough made from different wheat varieties

Table 6

Variety	Control	20 HOX	40 HOX	60 HOX	80 HOX
Duma	113.0 ± 8.00^{a}	103.0 ± 2.00^{a}	127.7 ± 1.53^{a}	100.0 ± 20.88^{a}	106.0 ± 12.77^{a}
Robin	89.67 ± 10.02^{a}	70.33 ± 5.51^{ab}	62.33 ± 2.52^{b}	62.33 ± 6.03^{b}	51.00 ± 10.39^{b}
Njoro 2	63.00 ± 4.00^{bc}	74.33 ± 3.51^{a}	71.00 ± 3.00^{ab}	51.33 ± 3.51^{d}	60.67 ± 0.58^{c}

HOX effects on the elasticity (L value) of dough made from Duma, Robin and Njoro 2 wheat varieties

Table 7

Variety	Control	20 HOX	40 HOX	60 HOX	80 HOX
Duma	101.57 ± 2.54^{b}	121.37 ± 3.36^{a}	84.70 ± 8.80^{c}	88.73 ± 3.54^{bc}	93.87 ± 7.16^{bc}
Robin	59.77 ± 6.63^{b}	71.50 ± 7.21^{b}	96.07 ± 0.64^{a}	87.63 ± 7.16^{a}	91.30 ± 5.50^{a}
Njoro 2	73.70 ± 3.97^{ab}	56.87 ± 5.50^{b}	115.13 ± 2.77^{a}	112.20 ± 4.40^{a}	124.67 ± 8.82^{a}

HOX effect on the alveograph P value of dough made from Duma, Robin and Njoro 2 wheat varieties

Table 8

Variety	Control	20 HOX	40 HOX	60 HOX	80 HOX
Robin	0.67 ± 0.01^{c}	1.02 ± 0.02^{bc}	1.54 ± 0.06^{a}	1.42 ± 0.26^{ab}	1.83 ± 0.32^{a}
Duma	0.90 ± 0.05^{b}	1.18 ± 0.06^{a}	0.66 ± 0.08^{c}	0.91 ± 0.18^{b}	0.89 ± 0.05^{b}
Njoro 2	1.18 ± 0.14^{bc}	$0.78\pm$ 0.0.02^{c}	1.63 ± 0.11^{ab}	2.81 ± 0.06^{a}	2.06 ± 0.16^{a}

HOX effect on the P/L ratio of dough made from Duma, Robin and Njoro 2 wheat varieties

Table 9

Variety	Control	Potassium bromate	20 HOX	40 HOX	60 HOX	80 HOX
Duma	596.7 ± 15.3^{c}	655.0 ± 13.2^{a}	603.3 ± 22.6^{c}	590.0 ± 10.0^{c}	645.0 ± 13.2^{ab}	606.7 ± 11.6^{bc}
Robin	541.7 ± 10.4^{bc}	455.0 ± 18.0^{d}	581.7 ± 2.9^{a}	550.0 ± 10.0^{b}	555.0 ± 8.7^{ab}	515.0 ± 13.2^{c}
Njoro 2	593.3 ± 11.6^{b}	515.0 ± 15.0^{c}	633.3 ± 12.6^{a}	600.0 ± 10.0^{ab}	578.3 ± 2.9^{b}	600.0 ± 17.3^{ab}

HOX effect on the loaf volume of bread made from Duma, Robin and Njoro 2 wheat varieties

Author Profile

Nicholas Maikweki has a BSc degree in Chemistry and is waiting to finalize his Msc degree in food science at Egerton University. He has been involved in the research on the ability of Hexose oxidase for improving machinability, dough strength and crumb firming in wheat dough. Mr Maikweki continues to work as a teaching and research assistant in the department of food science- Egerton University Kenya.

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