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EFFECT OF DIFFERENT FOOD ENZYMES AS LEAVENING AGENT ON WHEAT FLOUR PROTEIN DURING FERMENTATION

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Abstract: The present study was undertaken for the characterization of wheat flour on the basis of protein content and dough quality and to evaluate the rheological changes of the dough after fermentation with α-amylase, protease, sodium acid pyrophosphate (SAPP) and yeast. In this experiment, three types of wheat flour (hard, soft and mixed) were used and the resulted gluten percentage were 1575, 10.14 and 5.78, respectively. Four types of dough samples were made. During fermentation of the dough, the temperature and p^H of the dough mass using different leavening agents viz. α-amylase, protease, SAPP and yeast have been found optimum 24 to 25 °C at p^H 5.9- 6.3; 25 to 26 °C at p^H 6.2; 26 °C at p^H 5.2 to 5.4 and 28 to 30 °C at p^H 4.9 to 5.3, respectively. After fermentation, the content of gluten in different processed dough samples were estimated as 8.32 %, 0.11%, 6.5% and 3.15 %, respectively. The use of α-amylase resulted crispiness crumb, protease resulted very small yellowish crumb product, SAPP resulted very good crumb mass with increased volume and yeast showed more elastic crumb texture with gas cell at desirable stage. The results of the study indicate that, soft wheat flour can be used for quality biscuit dough preparation and the use of particular leavening agents can bring desirable rheological changes during fermentation. The particular leavening agents can also help to produce well structured crumb texture, sufficient gas cell (porosity) and color which are few of the requirements for standard biscuit production.

Key wards: Dough, gluten, leavening, fermentation, enzymes, wheat flour

Introduction

Biscuit is one of the most common, relatively low cost traditional foods around the world. Biscuits and crackers are made from complex dough that undergoes suitable changes in their rheological properties during processing. The dough for biscuit and similar products consists of flour, water, yeast, salt (sodium and ammonium bi-carbonate, calcium phosphate, etc) and other ingredients such as sugar and fat. The quality of flour protein (gluten) is still an important factor in cookie and cracker dough rheology. Gluten is a combination of proteins, which form a large network during dough formation. Enzymes such as α-amylase, protease and oxidizing agent can directly or indirectly improve the strength of the gluten and consequently improve the quality of the finished biscuit. The α-amylases degrades the starch in wheat flour into small dextrins, thus allowing yeast to work continuously during dough fermentation. The result is improved biscuit volume and crumb texture. Staling is associated with the loss of freshness in terms of increased crumb firmness and decreased crumb elasticity. Staling is believed to be due to changes in starch structure during storage. Although the true mechanism of α-amylase, protease, or pentosanase in biscuit making has not been clearly demonstrated, it is well known that the addition of certain types of pentosanase at the correct dosage can improve dough machinability, yield more flexible and easier-to-handle dough. Bohn (1934) have reported that strong soft wheat flour works best as sponge flour; an intermediate strength of soft wheat flour works as a dough flour and very weak flour works for sweet goods. He also stated 'cracker sponge flour is often a strong, straight grade, untreated, soft wheat flour with a protein content of between 8.5% and 10.0% and an ash content of between 0.30% and 0.42%'.

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Barmore (1947) studied the viscosity and solubility of gluten fractions from wheat flour. Physical and chemical tests on these fractions were also discussed. Finley (1977) discussed the use of added calcium or ferric ions to increase the heat sensitivity of protein in gluten-starch wash water. Finney (1945) studied the variety and protein content of flour to determine their effect on baking absorption. Methods developed for estimating baking absorption were also described. Harris (1940) washed glutens from a series of 14 flours, which comprised samples milled from hard red spring and winter wheat, soft red winter wheat and durum wheat. The dried glutens were analyzed for moisture, ash and protein. The glutens were baked and final products evaluated for loaf volume, texture and color. Chinachoti and Steinberg (1984) have provided evidence for the interaction of sugar and starch. They found that a mechanical mixture of sucrose and starch gave absorption isotherms in good agreement with calculated values based on a mass balance. Matz and Matz (1978) reported that the quantity and quality of sweeteners have significant effects on cookie dough rheology, as well as on the texture, appearance and flavor of the finished product. The type and quantity of sweetener employed also affect dough machining properties and the response of the dough piece to oven conditions. Salgo (1981) studied wheat proteases and found two enzymes with p^H optima, p^H 3.8 and p^H 4.2**.** He reported that the enzymes were stable in the p^H 2.5 to p^H 5.0. Doescher and Hoseney (1985b) measured the changes in capillary viscosity of proteins extracted from cracker sponges with 1% SAPP and found a decrease within 18 h fermentation. This supported the theory of protein chain cleavage. Decreases in relative viscosity were also seen in sponges set at p^H 4.1 for 18 h. Doescher and Hoseney (1985a) modified and improved the procedure by developing inoculums to obtain the desired p^H reduction in the sponge. To produce the inoculums, flour, water, sugar and yeast were fermented for up to 36 h at 27 °C. A small amount of this slurry added to the sponge assured p^H values of 4.0 (or below) after 18 h. The objective of this research was to find the effect of various enzymes, chemicals viz. Sodium Acid Pyrophosphate (SAPP) and yeast on dough preparation and texture of biscuit in baking industry.

Materials and Methods

The basic ingredients required for biscuit dough preparation are wheat flour, sugar enzymes (α-amylase and protease), sodium acid pyrophosphate (SAPP), yeast and water. The wheat was cleaned, tempered and the percentage of moisture within the grain was adjusted before milling. They were passed through several reduction rollers. The grounded milling was separated into several streams of flour by sieving and bolting. Sugar was pulverized before mixing with a combined grinding machine.

Separation and estimation of gluten from wheat flour: starch was washed out from the mixture samples in beakers in order to obtain stiff ball of dough. The dough balls were soaked in water for one hour. The gluten allowed settling down at the bottom of beaker and dough was mashed with hands and kept for 15 minutes. Starch was removed by washing with running water and passed through a fine mesh sieve. Water was removed from the beaker and the gluten was sedimented as large bed. The coagulated gluten balls were filtered through filter paper to remove extra water. The gluten was put into the oven at 120 °C for 30 minutes. The temperature of the oven was gradually increased every 30 minute interval at 135, 150, 165, 180 and 195 °C. By this means gluten was heated for 3 h and was taken out from the oven. The dried gluten was weighed and the rheological changes i.e., crumb structure and texture was observed. Formulation of biscuit dough samples is shown in Table 1. Three types of wheat flour viz. hard, soft and mixed were taken into three different large size beakers. Two hundred grams of each type o flour was taken into separate beaker and was mixed with 350 ml water. Four types of samples were prepared by proper mixing 1 kg of standard quality wheat flour and 60 g of sugar with 450 ml of water in 4 separate small size containers (volume 5 kg). Then α-amylase, protease, SAPP and yeast were added in these samples.

Table 1. Formulation of biscuit dough samples.

Sample No.	Wheat flour (kg)	Sugar (g)	Water (ml)	α-amylase (g)	Protease (g)	SAPP (g)	Yeast (g)
S1	1	60	450	2	-	-	-
S2	1	60	450	-	1	-	-
S3	1	60	450	-	-	50	-
S4	1	60	450	-	-	-	60

In the first phase, all the starches and water were washed out from the mixture of the three types of samples and the gluten was separated for final assessment following AOAC method (Anon, 1990). In the second phase, all the starches and water were washed out from the mixture of two types of samples and gluten was

separated. In the third phase, four types of dough samples S1, S2, S3 and S4 were made carefully and α-amylase, protease, SAPP and yeast were then added in those samples subsequently. Fermentation of that dough was carried out following AOAC method (Anon, 1990). In the fourth phase, all the samples were taken from the containers that were kept for 16 to 18 h for fermentation to form sponge of the dough. One hundred and fifty g of each sample was then taken from those containers and gluten was separated from these samples following AOAC (Anon, 1990).

Results

The results of the experiments are shown in the following tables. Protein is an important nutrient content which has prediction of the end-use of wheat. Biscuit volume, crumb structure and crust texture are dirctly related to the protein content. Table 2 indicates that the percentage of gluten in hard wheat flour, soft wheat flour and mixed wheat flour were 15.76, 10.14 and 5.78 respectively.

Table2. Gluten percentage in three types of wheat flour.

Sample No.	Type and amount of wheat flour (g)	Amount of wetted gluten in sample (g)	Filter paper weight (g)	Dry weight of gluten (g)	Amount (wt) of gluten/ 200gm	Percentage (%) of gluten (g)
S*1	Hard Flour, 200	66.2005	2.3390	33.8650	31.526	15.763
S*2	Soft Flour, 200	47.1610	2.6100	22.8888	20.2788	10.1394
S*3	Mixed Flour, 200	29.9490	2.3685	13.9290	11.5605	5.7802

Table 3. Percentage of glutenin and gliadin in gluten from flour.

Sample No.	Type and amount of wheat flour (g)	Amount of wetted gluten in sample (g)	Amount of added ethyl alcohol (ml)	Extracted ethyl alcohol with gliadin (ml)	Percentage of wetted gliadin in sample (ml)	Percentage of wetted glutenin in sample (g)
S**1	Hard Flour, 100	28.1017	50.00	52.7991	2.7991	25.3026
S**2	Soft Flour, 100	14.5364	50.00	54.061	4.061	10.4754

Table 3 indicates that hard wheat flour contained 28.102% of gluten in which 2.8% gliadin and 25.303% glutenin were present and soft wheat flour contained 14.536% of gluten in which 4.061% gliadin and 10.475% glutenin were present.

Table 4. Effects of α-amylase, protease, SAPP and yeast on temperature and pH of the dough during fermentation.

Sample No.	Time (t = 0 h)		Time (t =1 hr)		Time (t =2 hr)		Time (t =3 hr)	
	Temp	p^H	Temp	p^H	Temp	p^H	Temp	p^H
S1	26.66^{0}C	5.9	25.55^{0}C	6.3	24.44^{0}C	6.2	25.55^{0}C	6.2
S2	25.55^{0}C	6.2	26.66^{0}C	6.2	25.55^{0}C	6.2	25^{0}C	6.2
S3	26.66^{0}C	5.3	26.66^{0}C	5.3	26.66^{0}C	5.2	26.11^{0}C	5.4
S4	28.33^{0}C	5.3	29.44^{0}C	5.1	30^{0}C	4.9	28.88^{0}C	5.0

Table 5. Gluten percentage in biscuit dough after fermentation.

Sample No.	Amount of wetted gluten in sample (g)	Filter paper weight (g)	Dry weight of gluten (g)	Weight (wt) of gluten (g)	Percentage (%) of gluten (g)
S1	27.3530	1.0051	9.3349	8.3298	8.3298
S2	1.3310	1.0019	1.1130	0.1111	0.1111
S3	19.3368	1.0025	7.5209	6.5184	6.5184
S4	9.9816	0.9870	4.1325	3.1455	3.1455

Fig. 1. Appearance of glutens after baking (S*1 high, S* 2 standard and S*3 low gluten content).

Fig.-2 Appearance of glutens after baking followed by Fermentation (S1 with α-amylase, S2 with Protease, S3 with SAPP and S4 with Yeast).

Table 4 shows that, during fermentation of the dough, the temperature and p^H of the dough mass for leavening agents viz.α-amylase, protease, SAPP and yeast was optimum i.e., 24 to 25 °C at p^H 5.9 - 6.3; 25 to 26 °C at p^H 6.2; 26 °C at p^H 5.2 –5.4 and 28 to 30 °C at p^H 4.9 - 5.3, respectively. After fermentation, the content of gluten in the processed dough was estimated at 8.32%, 0.11%, 6.5% and 3.15% respectively (Table 5). The results indicated that after fermentation, the percentage of gluten was high in sample 1, very low in sample 2, high in sample 3 and moderate in sample 4.

Discussion

It is observed from Fig. 1 that sample 2 gluten (isolated from soft flour) formed more uniformed crumb with aequate porosity and the crust of the gluten imparted desirable attractive color. The sample 1 gluten (isolated from hard flour) was much rigid and had smaller crumb structure. The sample 3 gluten (isolated from mixed flour) had dispersed crumb structure which was weak in strength.

From Fig. 2, it was found that sample 1 gluten was very much crunchy and crispy which turned to black color after baking and is the result of added α-amylase which acts on starch and converts it into sugar. The sample 2 gluten was very small yellowish color. Te reasons might be that the added protease breaks down the protein which is very much essential for the gluten polymerization during dough fermentation. The samle 3 gluten showed uniform crumb structure with attractive color. The increased volume was due to the result of added SAPP. The sample 4 glutenshowed highly developed crumb structure due to enzymatic reaction of the yeast which produced CO_2 gas during fermentation and helped in polymerization of the glutens in dough.

It was observed from Fig. 1 that gluten obtained from soft flour did produce uniform crumb structure with sufficient porisity with desirble physical appearance compared to guten obtained from hard and mixed flour samples which was much rigid, had smaller crumb structure (isolated from hard flwer) and had dispersed crumb structure with weak strength, respectively (isolated from mixed flour). The study reveals that use of α-amylase resulted crispiness crumb, protease resulted very small yellowish crumb product, SAPP resulted very good crumb mass with increased volume and yeast showed more elastic crumb texture with gas cell at desirable stage. The results of the study indicate that, soft wheat flour can be used for quality biscuit dough preparation and the use of particular leavening agents can bring desirable rheological changes during fermentation. The particular leavening agents can also help to produce well structured crumb, sufficient gas cell (porosity) and better crumb texture and color which is one of the requirements for standard biscuit production.

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