THE CORELATION BETWEEN QUALITY OF BREAD AND EXOGENUOUS ADDITION OF DIFFERENT TYPES OF α-AMYLASES

Gabriela Pop[1], David Krishok[2]

[1] "Ştefan cel Mare" University of Suceava, Faculty of Food Engineering, Str. Universitatii, no. 13, 720229, Suceava, Romania,; e-mail: gabipop@usv.ro
[2] Kansas State University, Department of Grain Science & Industry, Manhattan, KS 66506-2201, USA.

Abstract: *A normal flour, milled from sound wheat, contains significant amounts of β–amylase but a little or no α-amylase. B–amylase can produce some maltose to aid fermentation without the presence of α-amylase, but the amount produced is relatively small, so α-amylase must be added to sound flour from an outside source. Malted barley has traditionally served as the primary source of α-amylase, when the bread formulas were quite lean and contained relatively little sugar. Today, the primary reason for adding amylases to baked products is to improve the processing conditions as well as the overall quality of the baked product. Although barley malt is still used, supplementation with fungal and, to a lesser degree, bacterial amylases has become more common. The different sources of amylase activity are not equivalent since the amylase enzymes produced from these sources have different properties. While most amylase operate best under relatively acidic conditions (pH 5.0-6.5), the temperatures ranges over which they are able to operate are quite different.*

Our objectives were to study the effects of α-amylase addition on dough and bread attributes and to relate these performance differences to amylolytic mechanisms and to differences between types of amylolytic sources (malt, fungal and bacterial). Also the experiments wanted to see if we could obtain a significant increasing in the area of shelf life extension.

Key words: *α-amylase, β–amylase, bread, starch, hydrolysis, flour, malt, fungal, bacterial*

Introduction

Man's food habits have changed a lot during the last decades due to a series of factors such as urbanization, technological development and the time spend outside house, and the consumer's attitude has also changed as for the sensorial qualities of food products.

Therefore, in a period of time when food is dominated by "artificialized "(synthetic) products resulted from intensified processing, few characteristics of raw materials are still preserved, food influence on man' s health is a present time aspect

The necessity of providing food safety requires much transparency and awareness of minimizing any kind of risks. This necessity may be expressed by starting from the content of a food plate and going to a life existence itself.

Taking into consideration that cereals products provide over 50% of humankind's food sources, no effort is good enough to find new ways of superior development as regards raw materials, that may allow both production of food ranges adapted to local specific features, to consumer's taste and providing a superior quality from the point of view of its innocuousness and nutritional value.

Successful modification of either the baking process and/or the characteristics of finished cereal grain products must to be based on an understanding of the basic components of wheat flour and effects resulting from their enzymatic modification. A normal wheat flour is composed of approximately 81% starch, 11.5 % protein, 3.5% fibers and 0.5% ash. The best candidates for enzymatic modification are the starch, protein and fiber fraction.

Amylases are enzymes that catalyze the hydrolysis of the bonds between glucose units in amylose and amylopectin. α-amylase is an enzyme commonly found in flour that randomly attacks a starch polymer. It severs α-1,4 linkages anywhere in an amylose or amylopectin molecule, although it cannot hydrolyze α-1,6 linkages or any α-1,4 linkages that are closely proximity to a branch point. Consequently, if allowed to act long enough, α-amylase reduces starch to a series of branched and linear fragments, called dextrins. These dextrins may subsequently be hydrolyzed by β–amylase to yield maltose, and/or amyloglucosidase to yield glucose. Because starch exists as a tightly packed granule, amylases must act upon starch granules that are damaged (as many are during flour milling) or on granules that have been gelatinized by moisture and heat (such as when a dough is mixed and baked). The sugars resulting from amylase activity act as food for yeast in yeast-raised products. As a result, the presence of these enzymes in the proper proportion is critical to carbon dioxide generation.

Amylase also can affect the consistency of dough. Damaged starch granules absorb more water than intact granules. This ability is reduced when the damaged granules are acted upon by amylase. With their ability to immobilized water reduced, the damaged granules release free water which softens the dough and makes it more mobile.

A third function of amylases could be the ability to retard staling. Over time, crumb of baked products firms due to a complex set of changes that includes recrystallization (or retrogadation) of amylopectin in the starch. By hydrolyzing the amylopectin into smaller units, it seems that bacterial amylases can maintain softness and extend shelf-life.

The rate and extent to which the viscosity of the starch suspension is reduced indicates the level of α-amylase present. The Falling Number method measures the relative magnitude of the enzyme induced drop in viscosity as the amount of time required, including 60 seconds of stirring, for a plunger to sink through a viscometer tube column of the starch suspension being tested. Typical F.N. ranges for wheat are:

< 150 sec	Flour from sprout-damage wheat
220-250 sec	Optimal range for flour for bread making
>300 sec	Flour from sound, unsprouted wheat. Will require α-amylase supplementation

Like primary sources for α-amylases used in bakery foods we can use:

- *Malt ingredient*. When a cereal kernel becomes moist and germinates, it experiences a dramatic increase in α-amylase. Consequently, malting grains such barley or wheat can serve as the basis of many α-amylase ingredients like malt flour or malt extracts and syrups. Actually exist two types of malt syrup: diastatic and non diastatic.
- *Fungal amylase*. Certain fungal, such *Aspergillus oryzae* can synthesize α-amylase during growth. Culture of fungal are extracted, concentrated and dried to yield fungal amylases. Fungal amylases can be used to standardize wheat flour, but are most often added at the production facility to aid dough conditioning.

Bacterial amylase. Certain bacteria such as *Bacillus subtilis*, also synthesize α-amylase. This can be extracted and dried much like fungal amylases. Bacterial amylases tend to be more thermally stable and are, therefore, useful for maintaining softness in finished baked products.

Materials and methods

In order to obtain some available experimental data I used like control sample, wheat flour from Kansas State University Laboratory Mill. The technological characteristics of flour are shown in table 1.

Table 1 Analytical parameters of Control flour

Moisture %	Ash %	Wet gluten %	Protein %	Hydration capacity %	Falling Number sec	Zeleny index ml
13.42	0.68	31.1	13,3	62.6	329	36

The analytical flour quality was determined according to the international standard methods (ash content – ICC104/1, wet gluten – ICC105/2, protein content – ICC106/2, hydration capacity with Farinograph - ICC115/1 and Zeleny index – ICC116/1)

Like raw material I used also compacted fresh yeast (*Saccharomyces cerevisiae)* from Lallemand, Inc., Montreal, Canada, with 32.5% dry matter and 46.54% protein content (N x 6.25).

White pan bread for baking test was prepared from the following ingredients:

Wheat flour	100%
Water	52%
Sodium chloride	2%
Baker yeast	2,5% ,

by mixing with a spiral mixer for 4 minutes at 140 rpm and for 3 minutes at 280 rpm. The dough temperature was 26° C. The dough was allowed to rise for 40 minutes at 34° C. and, after degassing and molding by hand, for 65 minutes at 34° C. The bread was subsequently baked for 30 minutes at 230° C.

To the dough ingredients were added Malted flour with diastatic power 223 U (provided from American Institute of Baking, K.S.), NOVAMYL™ (a recombinant maltogenic amylase encoded by a DNA sequence derived from Bacillus strain NCIB 11837, described in U.S. Pat. No.4,598,048), and, Fungamyl 1600 S (a commercial α-amylase available from Novo-Nordisk a/s). The effect of the exo α-amylases added was evaluated by addition of 0.5% per the flour weight.

The enzymatic samples were added to the baking formula during the mixing stage. Bread volume was determined after 24 hours of cooling by means of rape seeds.

In order to analyze the crumb firmness we made tests on breads from each of the dough with Voland –Stevens crumb firmness analyzer in baking day and after 24, 48, 72 and 96 hours. Also we made bread scoring for each of the 3 samples.

The Falling Number values for the experimental flours improved with α-amylases were determined with AACC/No.56-81 method using a 1500 PERTEN Falling Number System.

We use a Chopin Alveograph (AACC/No.54-30A, ICC121, ISO 5530/4) to determine the relationship between elasticity of the dough and rising power in order to analyze the rheological characteristics of tested flours.

The experiments are made in the research laboratory of " Ştefan cel Mare" University of Suceava, Faculty of Food Engineering and Kansas State University, Manhattan, Kansas, U.S.A.

Results and discussions

The Control wheat flour used had been a high value of Falling Number that means a low amylase activity. To improve fermentation capacity of flour carbohydrates is necessary α-amylase adding. In order to observe what kind of sources are most proper to improve in the same time, the analytical proprieties of flour and quality of dough/bread we made a series of experiments. First of all we wanted to see how will affect the α-amylases sources the analytical parameters of flours. Flour quality parameters as determined by standard methods after mixing Control flour with enzymatic preparation in 0.5% reported to basic flour are shown in table 2.

Table 2 Analytical parameters of tested flours

Type of Flour	Moisture %	Ash %	Wet gluten %	Protein %	Hydration capacity %	Falling Number sec	Zeleny index ml
Control	13.42	0.68	31.1	13,3	62.6	329	36
Control + Malted flour	13.35	0.77	31,4	13,95	64,9	250	38
Control + FUNGAMYL™ 1600 S	13.35	0.68	30,89	12,37	61,6	246	35
Control + NOVAMYL™	13.65	0.67	30,94	12,72	61,6	251	35

Analytical characteristics of all flour samples are very similar except for the one with Malted wheat flour, when we can observe an increase of protein content.

Regarding the Falling Number it is obvious that the spectacular decrease is registered in sample with fungal α-amylase that means an expected increase of fermentative capacity of flour carbohydrates. Became possible to obtain a slightly larger total bread volume via increasing dough extensibility and hence improved gas retention during oven spring.

But, we have to take into consideration that an excessive α-amylase activity can yield doughs that show decreased optimum water levels, stickiness and slackness. The resulting bread may have reduced loaf volume with inferior grain and texture. That's why we continued the experiments with Chopin Alveograph tests (table 3).

Table 3 Average values of alveograph evaluations

Type of Flour	P, mmH_2O	L, mm	G	P/L	W, $10e^{-4}J$	Falling Number sec
Control	86	48	19	1.79	144	329
Control + Malted flour	90	123	25	0.73	354	250
Control + FUNGAMYL™ 1600 S	84	90	21	0.93	258	246
Control + NOVAMYL™	85	117	24	0,72	335	251

If we analyze the data recording by alveograph we can observe an interesting situation. From the point of dough quality, the best behavior was obtaining from Control flour improved with Malted wheat flour. The dough based on flour improved with α-amylase from malt flour had a better extensibility in correlation with a superior resistance/stability to stretching.

In fungal and bacterial α-amylases we also obtain an improver of workability of dough, in especially in NOVAMYL™ case when the recording extensibility is almost like in malt flour case. In correlation with Falling Number evolution we can say that all three sources could constitute a proper manner to make our Control flour good for baking.

Next step was to make baking tests and to evaluate the quality of breads (table 4).

Table 4 Quality of breads after 24 hours after baking

Type of Flour	Weight, g	Volume, cm^3/100g	Height, cm	H/D	Porosity (Crumb structure), %	Elasticity, %	Moisture, %	Bread note
Control	520	271	8.5	0.53	77	97	43.8	80
Control + Malted flour	519	299	10.3	0.54	82	98	43.79	85
Control + FUNGAMYL™ 1600 S	517	285	9.7	0,55	80	99	43.2	84
Control + NOVAMYL™	517	299	10.1	0.54	79	98	43.49	84

The obtaining scores are almost similar for all three samples with α-amylases add, that's why became very important to correlate the practical results with enzymatic preparation price. Regarding the evolution of crumb firming, the results appear in table 5.

Table 5 Crumb structure evolution

Type of Flour	Crumb structure	Crumb freshness 48h	Crumb freshness 72h	Crumb freshness 96h	Gummy crumb
Control	fine	240	160	160	no
Control + Malted flour	Fine	210	200	200	no
Control + FUNGAMYL™ 1600 S	Fine	210	155	135	no
Control + NOVAMYL™	fine	210	145	130	no

It appears from the tables above that, compared WITH Control flour, if we use malted flour, Fungamyl 1600 S and also the addition of NOVAMYL™ to dough leads to improved storage properties of the resulting bread without a concomitant gumminess of the crumb which only occurs a far larger dosage of the enzyme.

The bad results obtaining with malted flour could be explain through the quality of dextrins obtaining from starch hydrolyze.

Starch granules that have been hydrolyzed into dextrins by α-amylase cannot cross-link adjacent gluten polymers. The less cross-linked macro network is less rigid to the bite. α-amylase hydrolysis of swollen starch granules produces dextrins varying sizes.

Some of these appear to retard the rate of bread crumb firming.

Crumb firming appears to involve gradually developing cross – linkages between gluten protein polymers via starch granules and starch granules remain as intermediaries. Dextrins of a particular intermediate size (DP 3-9) appear to interfere with, and even block developing cross links at the interface between starch remnants and gluten (Martin and Hoseney, 1991). Such dextrins are formed by fungal and bacterial amylases, though not by malt. Malt produce larger dextrins of size > DP 9, which the writers state not only do not decrease the rate of firming, but which may in fact actually enhance bread firming rate by participating in the developing interaction between starch and gluten fibrils.

Conclusions

Analyzing the obtained results from the study about the possibilities to increase the quality of bread using α-amylases supplemented I can drop the following conclusions:

- All three sources of α-amylases could be a possibility to improve the wheat flour and bread quality;
- α-amylases from malt flour provide doughs with a great extensibility, but not a very good tolerance and also had no effect in bread staling;
- using flour supplemented with α-amylases all quality parameter of bread were improved (volume, porosity, elasticity, bread note);
- the sensorial indicators had also improved (crust and crumb color, especially);

bread supplemented with either bacterial or fungal α-amylase contain larger quantity of residual dextrins in the DP 3-9 range which could prevent the developing cross-links between gluten fibrils that rigidifies bread crumb structure to firm it.

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