QUALITY WHEAT
CRC PROJECT REPORT

Project: 3.1.6

Investigations on Increasing the Conditioning Efficiency of Wheat

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Investigations on Increasing the Conditioning Efficiency of Wheat

EXECUTIVE SUMMARY OF RESULTS

A simple and quick method was found to monitor the rate of water penetration into wheat by measuring the hectolitre weight over a selected time interval. The efficiency of the conditioning process was investigated by looking at the effect that chemicals (Acetic acid, Sodium Hydroxide, Ethanol and Sodium Dodecyl Sulphate) and enzymes (Shearzyme, Pectinex SMASH and Viscozyme) had on the rate of water penetration into the wheat.
The use of chemicals and enzymes displayed a very similar pattern to water and therefore very little effect is seen on the rate of moisture penetration into the grain and hence the efficiency of the conditioning process.

INTRODUCTION

Conditioning of wheat is an important step in the preparation of wheat for milling. Depending on grain type wheat is usually conditioned to a moisture content of 13.5 – 17.5%. It is at this moisture content that the maximum flour yield is achieved with minimal bran contamination during milling.

The conditioning process involves two steps
1. The amount of water to be added
2. The time taken for wheat to equilibrate prior to milling

There have been numerous publications in regards to conditioning or tempering of wheat. The review of such literature has shown that there have been many studies on water penetration through the grains and the factors that affect the rate. However there is very little literature in regards to chemical conditioning although a few researchers used areosols and food acids as means of chemical conditioning.

Review of such literature can be divided into three groups:
1. Studies determining the rate of entry
2. Studies in the use of heat.
3. Studies in the use of additives

Determining the Rate of Entry

There have been numerous publications in regards to mode of entry, rate of penetration and the factors that affect them. Below is a table summarising work on the factors determining the rate of entry.

RESEARCHER	STUDY	CONCLUSION
Von Ugrimoff 1933	Water Entry	Water entered the grain through the germ region
Bure and Cosse 1949	Water Entry	Water entered the grain through the germ region
Moss 1973	Barriers to Water Penetration	Major barriers are outer cuticle and testa – contain waxy hydophobic cutins
Butcher and Stenvert 1973, Stenvert and Kingswood 1976, Moss 1977	Rate of Penetration	After 1 hour water entered the aleurone cell layer however 1-3 hour penetration occurs in the endosperm.
Campbell and Jones 1955	Factors Affecting Penetration: Increasing temperature	Temperatures between 20°C and 43.5°C were tested and found that a rise of 12°C causes a threefold increase in the movement of moisture.
Moss 1973, Lee and Stenvert 1973, Moss 1977	Open Bran Structure	A more open bran structure favors a rapid rate of penetration
Butcher and Stenvert 1973	Type of Wheat	Soft wheats show larger response moisture level as compared to hard.
Butcher and Stenvert 1973, Stenvert and Kingswood 1976 and Moss 1973.	Protein Content	Protein retards moisture content – the higher the protein content the structure of the endosperm is more ordered (relates to hardness) and therefore rate of penetration decreases.

The Use of Heat

Studies involving the use of heat to increase penetration can be divided into two groups. These groups are:

1. Warm Conditioning: which involves adjusting moisture at a temperature less than or equal to 46°C.
2. Hot Conditioning: which involves adjusting moisture at temperatures greater than 46°C.

The use of warm conditioning has shown to reduce lying time by increasing temperature and therefore increasing penetration into the grain such that an increase from 20°C to 43.5°C reduced lying time from 24h to 3h. It has also shown that the use of warm conditioning gave good or slightly better results than cold conditioning in regards to flour quality and millability.

The use of hot conditioning has hastened the penetration of water into the wheat such that at 70°C water penetration into the grain was complete within an hour however temperatures greater than 46°C may result in both a biochemical and physical change in the grain composition and therefore affect baking.

The Use of Additives

Studies involving the use of chemicals/additives as a medium for conditioning either with or without water have been very little in comparison to the use of water on its own. Several workers have added chemicals to water to decrease the lying time and to facilitate the separation of bran from adhering endosperm. Below is a table summarising the use of additives and its effect on wheat.

RESEARCHER	CHEMICAL USED	EFFECT
Sullivan	0.1% Aerosol OT (Sodium Dioctyl Succinate)	Time required for hard spring and winter wheats was reduced from 8-18 hours to 2-3 hours. No differences were recorded in milling yield, ash, protein or baking results after two different tempering methods.
Altrogge	2% of 5% Solution of Aqueous $NaHCO_3$	Solution was applied to German wheat that was pre-dampened to 16.5%. After 1 hour wheat became discoloured and varied from dark yellow to light green, however the wheat showed better loosening of the coat but gave poor milling results.
Fritsch and Cleve	Lactic Acids and Organic Acids	Improvement in baking qualities and protein however the results were not controlled.
Kranz	2% Aqueous Solution of Methylcellulose. Others include Gum Arabic, Agar, Ethyl Cellulose, Dextrins, Polyphosphates mixed with Formaldehyde to produce a film.	Grain dried to 14% and conditioned to 16% allowing the wheat to stand for ½ - 1 hour before milling. Bran considerably moist and tougher than endosperm hence achieving separation.
Robinson	Heat and an increase in the alkalinity of the water - 20°C and 400ppm NaOH.	Absorption of water is increased.

PROJECT AIMS

- To monitor the rate of water penetration into the grain.
- To investigate methods both chemical and biological which may reduce conditioning time and therefore increase the efficiency of milling.
- To investigate ways of increasing flour yield by enhancing bran separation from adhering endosperm.
- To investigate the effect of the new conditioning processes on flour quality, by-product quality and milling.

METHODOLOGY

Sample Selection

Two wheat samples a hard and a soft were used. Each of the samples were cleaned using a Simon Carter Day Dockage Tester and the following wheat quality tests performed: hectolitre weight, particle size index, NIR protein and moisture (using Infratec) (Table 1).

Table 1 : Wheat Quality Test Results

WHEAT	PSI (%)	TKW (g)
Hard	18	34.0
Soft	23	34.6

Determining the Rate of Moisture Penetration

Samples were conditioned using water and measured at varying time intervals. Moisture penetration was monitored using the sieve test and hectolitre weight test.

Sieve Test

300g of clean hard wheat was weighed into a bucket and conditioned to 16.5% by the addition of 20.4mL of water. Samples were shaken by hand for 5 minutes until all the water was absorbed by the grain. The time interval chosen was every 2 hours and then at 24 hours.

10g of wheat sample was weighed and ground using the KT30 Mill. The ground wheat sample was then sieved using a series of sieves (1mm, 500um, 250um and 75um) for 2 minutes at an amplitiude of 3mm using the Fritsch Analysette 3 Pro Vibratory Sieve Shaker.

Hectolitre Weight Test

600g of clean hard and soft wheat was weighed into a bucket and the rate of moisture penetration into the grain monitored according to the table below:

Table 2 : Selected Time Intervals for Determining the Rate of Moisture Penetration

TRIAL	WHEAT SAMPLE	CONDITIONING MOISTURE (%)	VOLUME OF WATER ADDED (ml)	TIME INTERVAL (hours)
1	Hard	16.5	45.0	0, 2, 4, 6, 8 and 24
	Soft	14.5 *	6.1	0, 2, 4, 6, 8 and 24
2	Hard	16.5	45.0	0, 1, 2, 3, 4, 5, 6, 7, 8 and 24
	Soft	15.5	14.8	0, 1, 2, 3, 4, 5, 6, 7, 8 and 24
3	Hard	16.5	45.0	0, 1/4, 1 /2, 3/4, 1, 1 1/4, 1 1/2, 1 3/4, 2, 3, 4, 5, 6, 7, 8 and 24
	Soft	15.5	14.8	0, 1/4, 1/2, 3/4, 1, 1 1/4, 1 1/2, 1 3/4, 2, 3, 4, 5, 6, 7, 8 and 24

* After the first trial water addition was adjusted for the soft wheat sample.

Reducing the Conditioning Time

Two methods were examined, a chemical and biological approach.

1. Chemical Approach

The use of chemicals involved the following applications

- Effect of pH

The effect of pH was trialed using acetic acid (acidic solution) and sodium hydroxide (basic solution).

Three solutions of acetic acid were prepared from a concentrated stock solution. The concentrations were as follows: 0.1M, 0.5M and 1.0M solution where the pH

range is between 2 and 3. A solution of 0.1M sodium hydroxide solution was also used which had a pH 13.

- Use of a Surfactant and an Alcohol

A 1%w/v solution of Sodium Dodecyl Sulphate (SDS) and a 20%v/v solution of Ethanol was used to see if the efficiency of the conditioning process could be increased.

2. Biological Approach

Three enzyme solutions that were chosen were Pectinex Smash, Shearzyme and Viscozyme. All enzymes are liquid solutions and are used in the starch/juicing industry.

Milling

Test millings were carried out on a hard wheat sample of 3kg quantity using the Buhler Test Mill MLU202. The time interval chosen were 0 (no water added), 1h, 2h, 3h, 4h, 6h, 8h and 24h.

The flour was evaluated by analytical and end product testing with the following tests outlined in the table below.

Table 3 : Analytical and End Product Tests

ANALYTICAL TESTS	END PRODUCT TESTS
Branscan	Pan Breads – Rapids
Minolta	Yellow Alkaline Noodle Sheet
Ash	
Colour Grade	
Protein	
Moisture	
Extensograph	
Farinograph	

RESULTS AND DISCUSSIONS

Determining the Rate of Penetration

Sieve Test

The sieve test results are given in table 4.

Table 4 Sieving Test Values for Hard Wheat Sample

SIEVE SIZE	t0	t2	t4	t6	t8	t24
1mm	0.1	0.2	0.1	0.2	0.2	0.1
500um	2.5	2.1	2.2	2.1	2.2	2.1
250um	3.9	3.9	3.6	3.7	3.7	3.7
75um	1.8	2.2	2.2	2.0	2.1	2.1
Base	1.3	1.1	1.3	1.5	1.3	1.4

The results obtained from this test were not sensitive to moisture penetration as little variation in particle size distribution was detected over the selected time period.

Hectolitre Weight Test

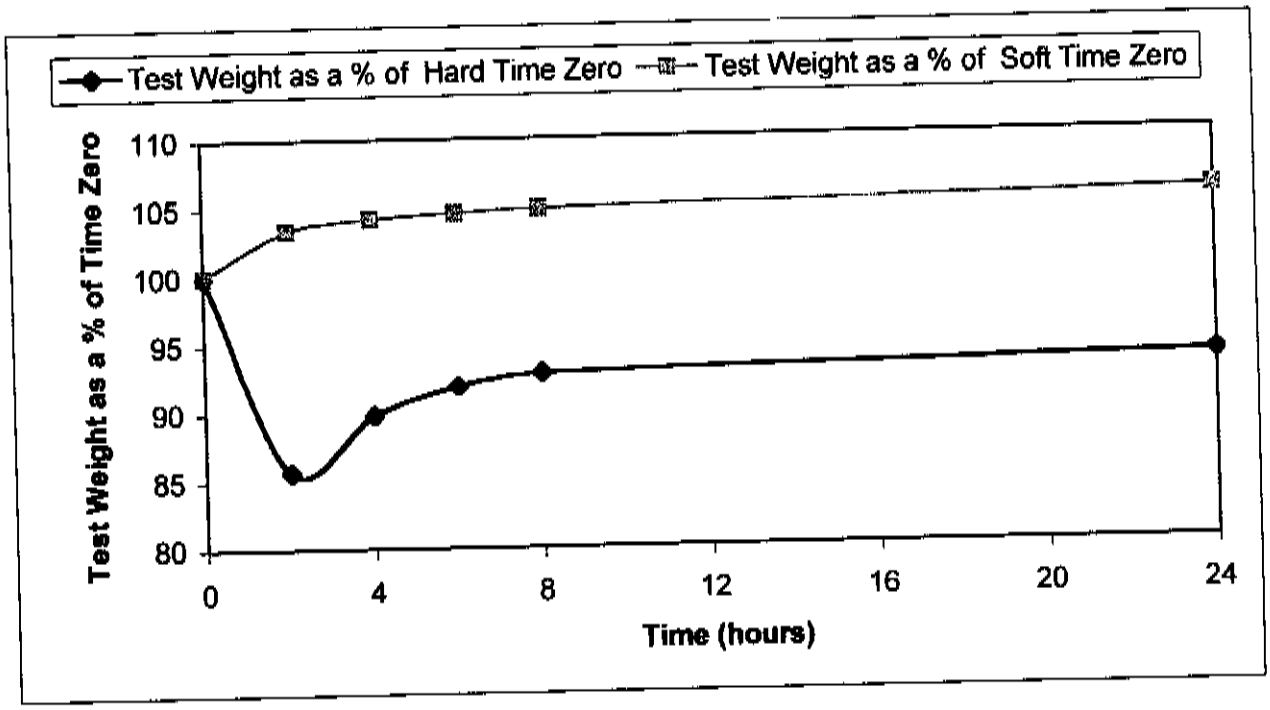
The results obtained from the hectolitre weight proved to be useful as moisture penetration into the grain could be monitored by observing the change in hectolitre weight over the selected time interval as outlined in table 2.

The hectolitre weight results for each of the trial is given in tables 5 to 7 with their corresponding figures (fig.1-3).

Table 5 : Average Hectolitre Weight Results for Trail 1

WHEAT TYPE	TIME (hours)	TEST WEIGHT AS A PERCENTAGE of TIME ZERO
HARD	0	100.0
	2	85.6
	4	89.8
	6	91.9
	8	92.8
	24	93.6
SOFT	0	100.0
	2	103.4
	4	104.2
	6	104.5
	8	104.8
	24	105.6

Figure 1: Moisture Penetration for a Hard and Soft Wheat Sample

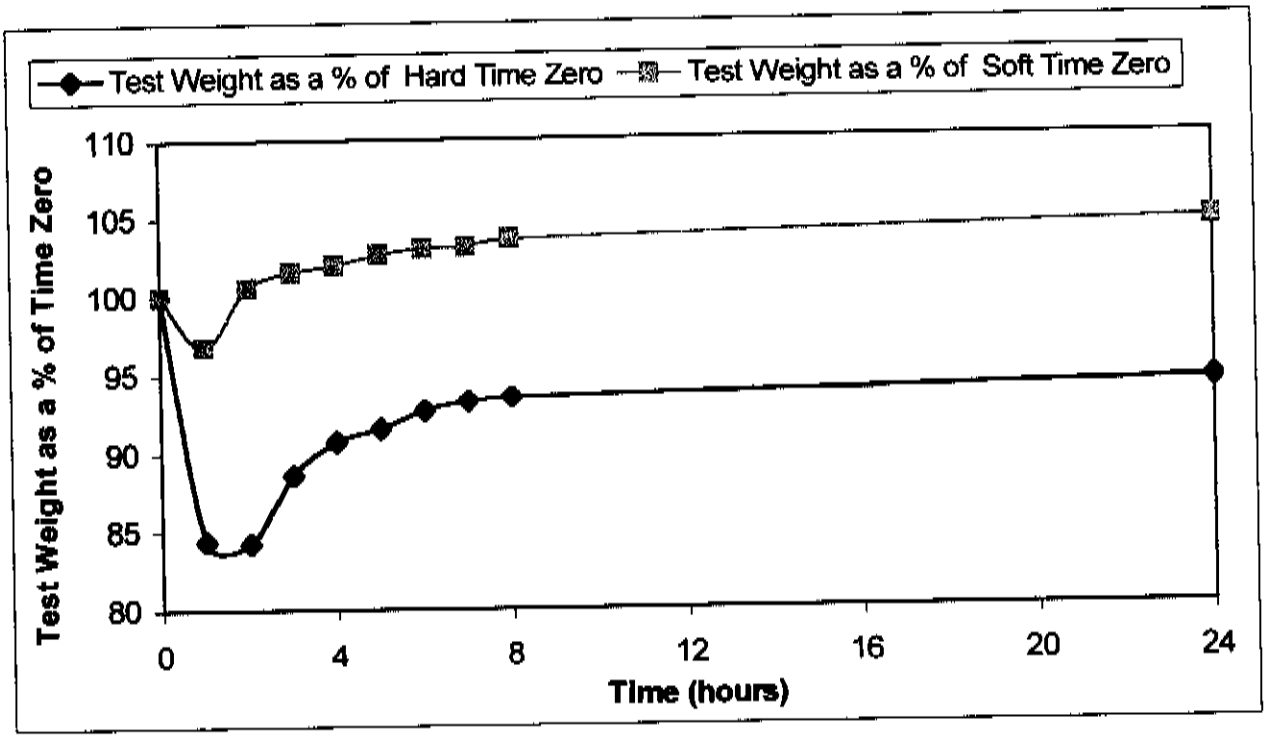


Over time hard wheat sample showed change in hectolitre weight as moisture penetrated through the grain while soft wheat sample showed a slight change in weight.

Table 6 : Hectolitre Weight Results for Trial 2

WHEAT TYPE	TIME (hours)	TEST WEIGHT AS A PERCENTAGE of TIME ZERO
HARD	0	100.0
	1	84.4
	2	84.3
	3	88.6
	4	90.7
	5	91.6
	6	92.7
	7	93.3
	8	93.5
	24	94.3
SOFT	0	100.0
	1	96.8
	2	100.5
	3	101.5
	4	102.0
	5	102.6
	6	103.0
	7	103.1
	8	103.6
	24	104.5

Figure 2 : Moisture Penetration for a Hard and Soft Wheat Sample (measured at each hour)

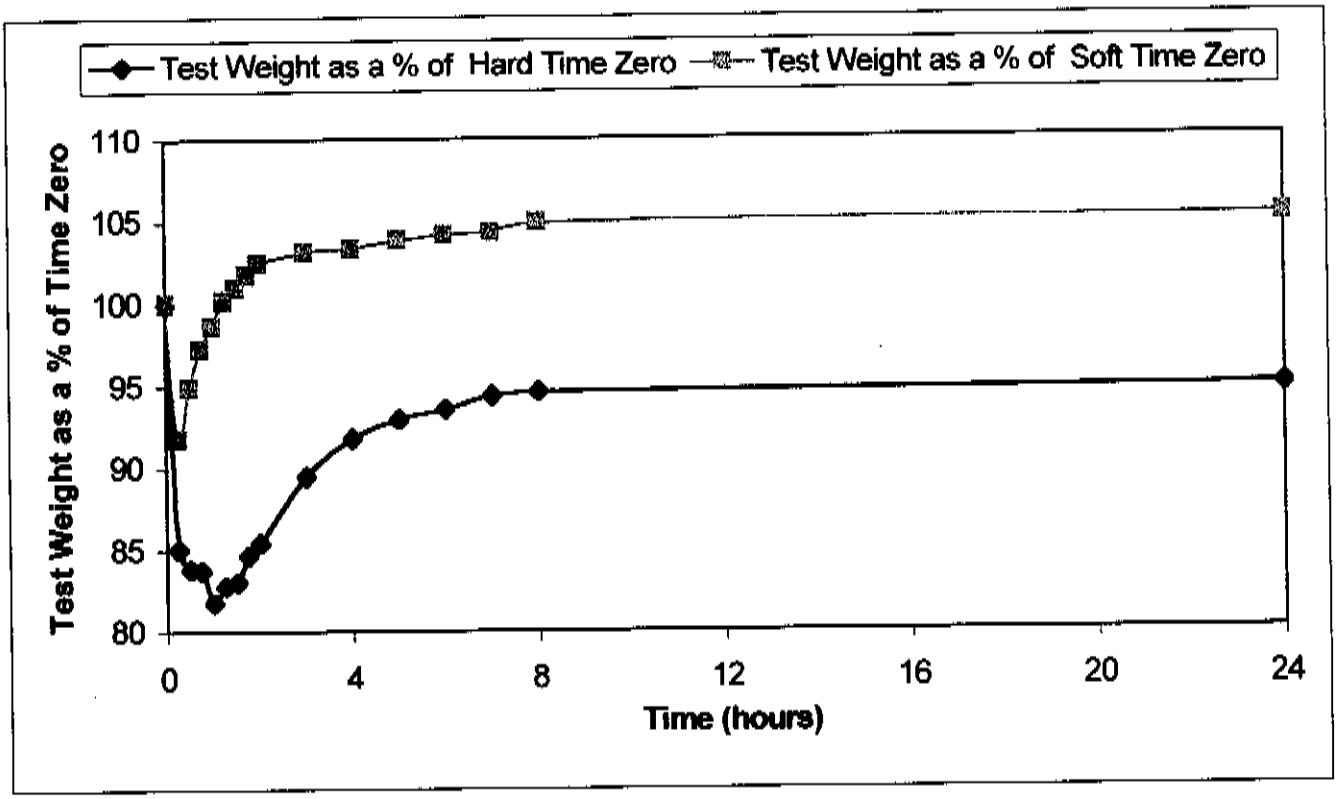


Within the first two hours of conditioning (the hard wheat sample) the hectolitre weight decreased from 100% to 84.3%. As time progressed (3-8 hours) hectolitre weight gradually increased. Within the first hour of conditioning (the soft wheat sample) the hectolitre weight decreased from 100% to 96.3%. As time progressed (2-8 hours) hectolitre weight gradually increased. The results show that the hectolitre weight is a useful method of monitoring moisture penetration where the first two hours are crucial as changes in the hectolitre weight (that is a decrease in weight) occurs before it begins to increase again.

Table 7 : Hectolitre Weight Results for Trial 3

WHEAT TYPE	TIME (hours)	TEST WEIGHT AS A PERCENTAGE of TIME ZERO
HARD	0	100.0
	1/4	85.1
	1/2	83.9
	3/4	83.8
	1	81.8
	1 1/4	82.9
	1 1/2	83.1
	1 3/4	84.7
	2	85.5
	3	89.5
	4	91.8
	5	92.9
	6	93.6
	7	94.4
	8	94.6
	24	94.9
SOFT	0	100.0
	1/4	91.8
	1/2	94.9
	3/4	97.2
	1	98.6
	1 1/4	100.2
	1 1/2	100.9
	1 3/4	101.7
	2	102.4
	3	103.1
	4	103.3
	5	103.8
	6	104.1
	7	104.3
	8	104.9
	24	105.1

Figure 3 : Moisture Penetration for a Hard and Soft Wheat Sample Over a 24 Hour Period



Within the first two hours after the sample was conditioned hectolitre weight decreased. This decrease is a result of water being taken up by the bran thus causing the bran to swell. As time progressed (3-8 hours) the hectolitre weight begins to increase. This increase in hectolitre weight is a result of water penetrating further into the grain and the bran layer becoming drier and swelling reduced. At some time between 8-24 hours, penetration occurs further into the endosperm where equilibrium is reached.

Differences between the hard and soft wheat samples can be explained by the difference in the physical properties of the two wheat types. In comparision to hard wheat, soft wheat is more porous and therefore absorbs water more quickly whereas hard wheat absorbs water within the bran and/or bran tissue layer and is therefore slower.

Reducing the Conditioning Time

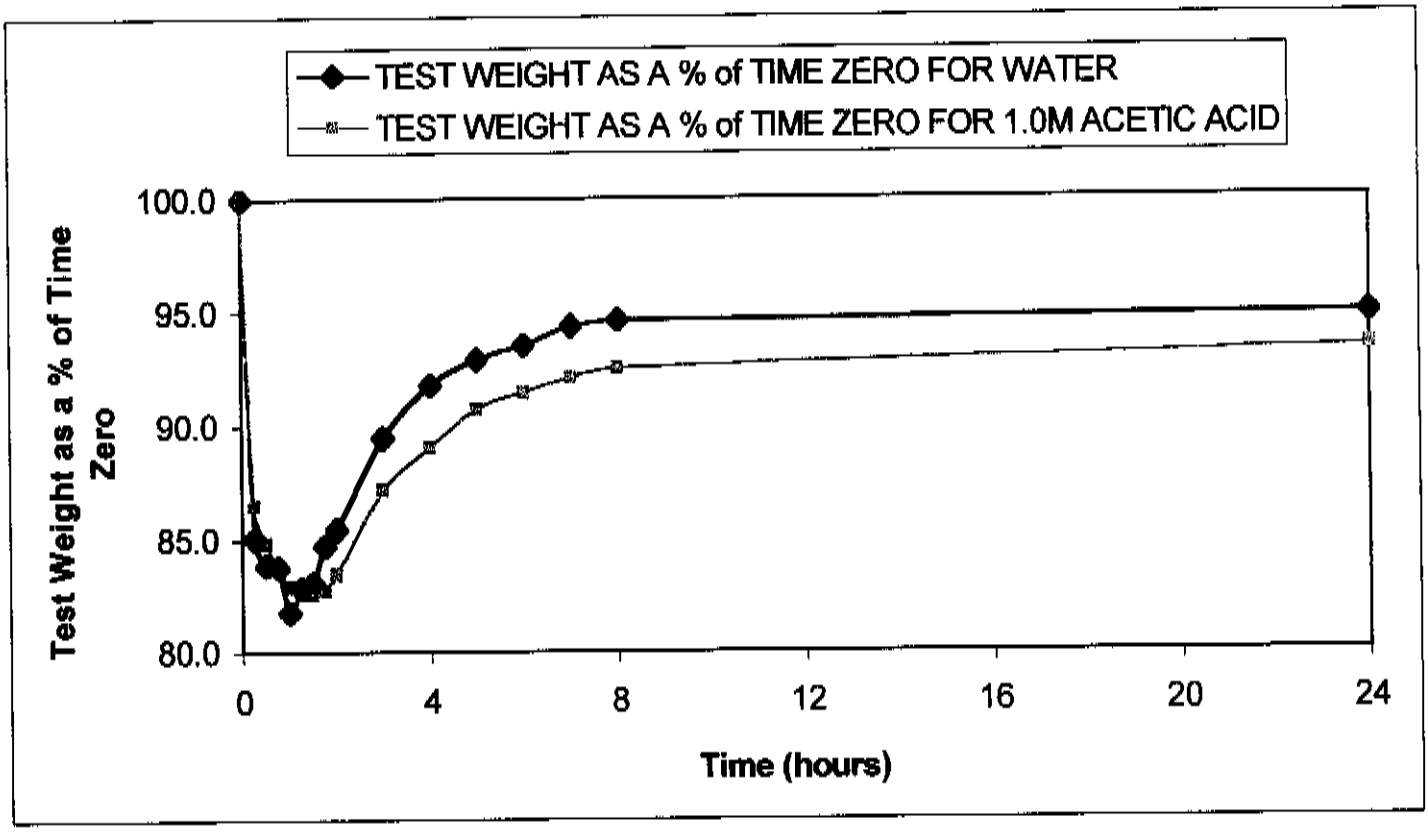
Effect of pH

The effect of pH on the conditioning time is given in table 8 and in the appendix at the back of the report. The results show that the use of an acidic solution in comparision to water gave a decrease in the hectolitre weight over time (figure 4) whereas the use of an alkaline solution in comparision to water also gave an increase in the test weight over time.

Table 8 : Effect of Varying pH on the Hectolitre Weight

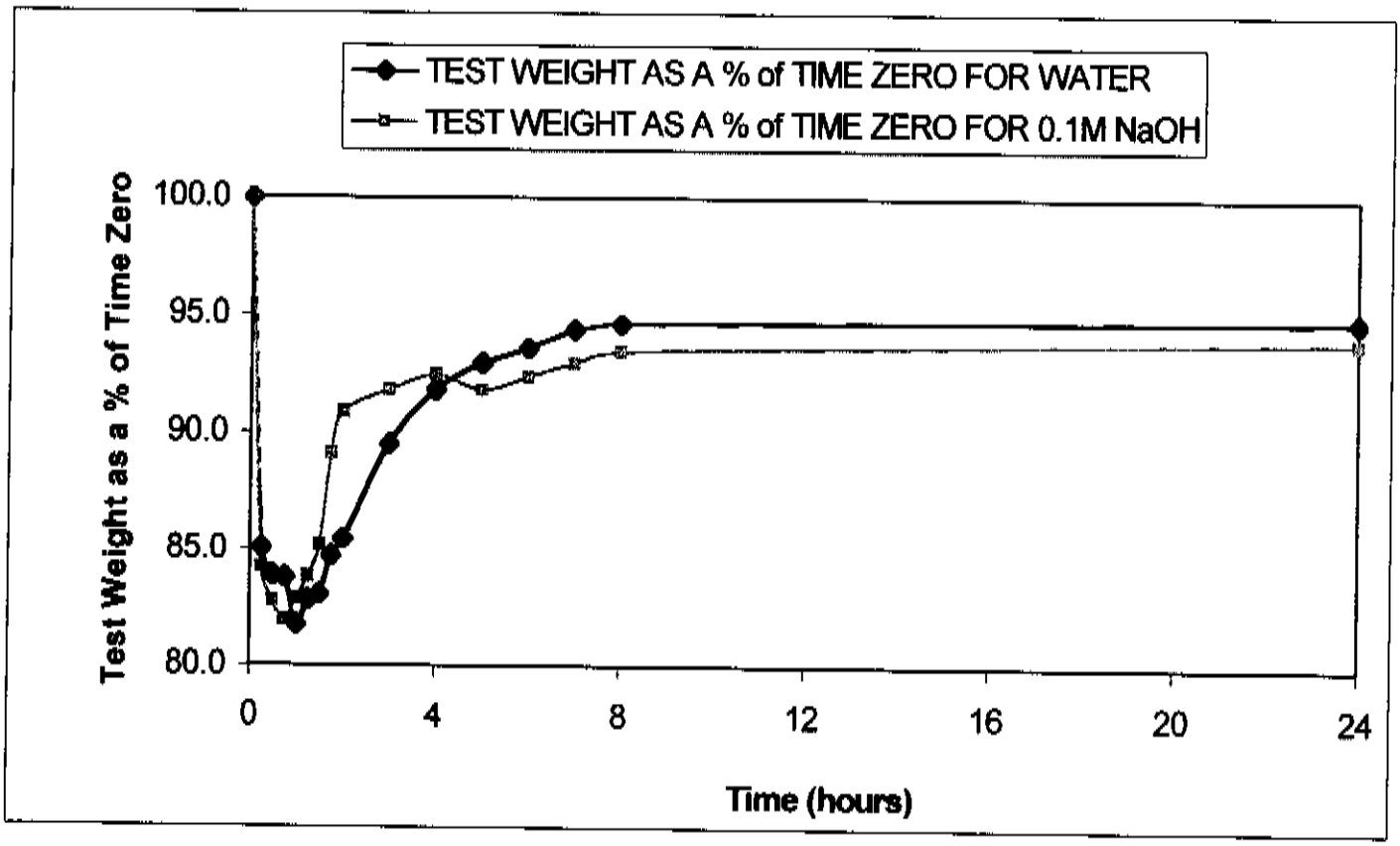
TIME (hours)	TEST WEIGHT AS A PERCENTAGE OF TIME ZERO FOR WATER	TEST WEIGHT AS A PERCENTAGE OF TIME ZERO FOR 1.0M ACETIC ACID SOLUTION	TEST WEIGHT AS A PERCENTAGE OF TIME ZERO FOR 0.1M SODIUM HYDROXIDE SOLUTION
0	100.0	100.0	100.0
1 ¼	85.1	86.5	84.2
1 ½	83.9	84.8	82.8
1 ¾	83.8	83.8	82.0
1	81.8	82.9	82.9
1 ¼	82.9	82.6	83.8
1 ½	83.1	82.6	85.2
1 ¾	84.7	82.8	89.1
2	85.5	83.5	90.9
3	89.5	87.2	91.8
4	91.8	89.1	92.5
5	92.9	90.8	91.8
6	93.6	91.5	92.3
7	94.4	92.1	93.0
8	94.6	92.5	93.4
24	94.9	93.9	93.9

Figure 4 : Moisture Penetration Over a 24 Hour Period for a Hard Wheat Sample Using 1.0M Acetic Acid



The use of 1.0M acetic acid solution in the hard wheat sample left a strong pungent odour in the wheat.

Figure 5 : Moisture Penetration Over a 24 Hour Period for a Hard Wheat Sample Using a 0.1M Sodium Hydroxide Solution.



The use of a 0.1M Sodium hydroxide solution in the hard wheat sample resulted in the discolouration of the wheat sample from a golden brown to a bright yellow orange colour with a strong pungent odour present in the sample.

Effect of a Surfactant and an Alcohol

The use of Sodium Dodecyl Sulphate (surfactant) and Ethanol on the rate of conditioning is very similar to the use of water alone (table 9) and therefore there was no effect on the lying time (figures 6-7).

Table 9 : Effect of SDS and Ethanol on the Hectolitre Weight

TIME (HOURS)	TEST WEIGHT AS A PERCENTAGE OF TIME ZERO FOR WATER	TEST WEIGHT AS A PERCENTAGE OF TIME ZERO FOR A 1% SDS SOLUTION	TEST WEIGHT AS A PERCENTAGE OF TIME ZERO FOR A 20% ETHANOL SOLUTION
0	100.0	100.0	100.0
1/4	85.1	85.4	84.7
1/2	83.9	83.7	82.8
3/4	83.8	82.3	81.6
1	81.8	82.2	81.4
1 1/4	82.9	81.9	82.2
1 1/2	83.1	82.1	82.2
1 3/4	84.7	84.2	83.3
2	85.5	85.5	85.0
3	89.5	89.4	89.0
4	91.8	91.6	90.9
5	92.9	92.5	92.0
6	93.6	93.3	92.7
7	94.4	93.7	93.0
8	94.6	93.8	93.3
24	94.9	94.5	93.9

Figure 6 : Moisture Penetration Over a 24 Hour Period for a Hard Wheat Sample Using 1% SDS Solution.

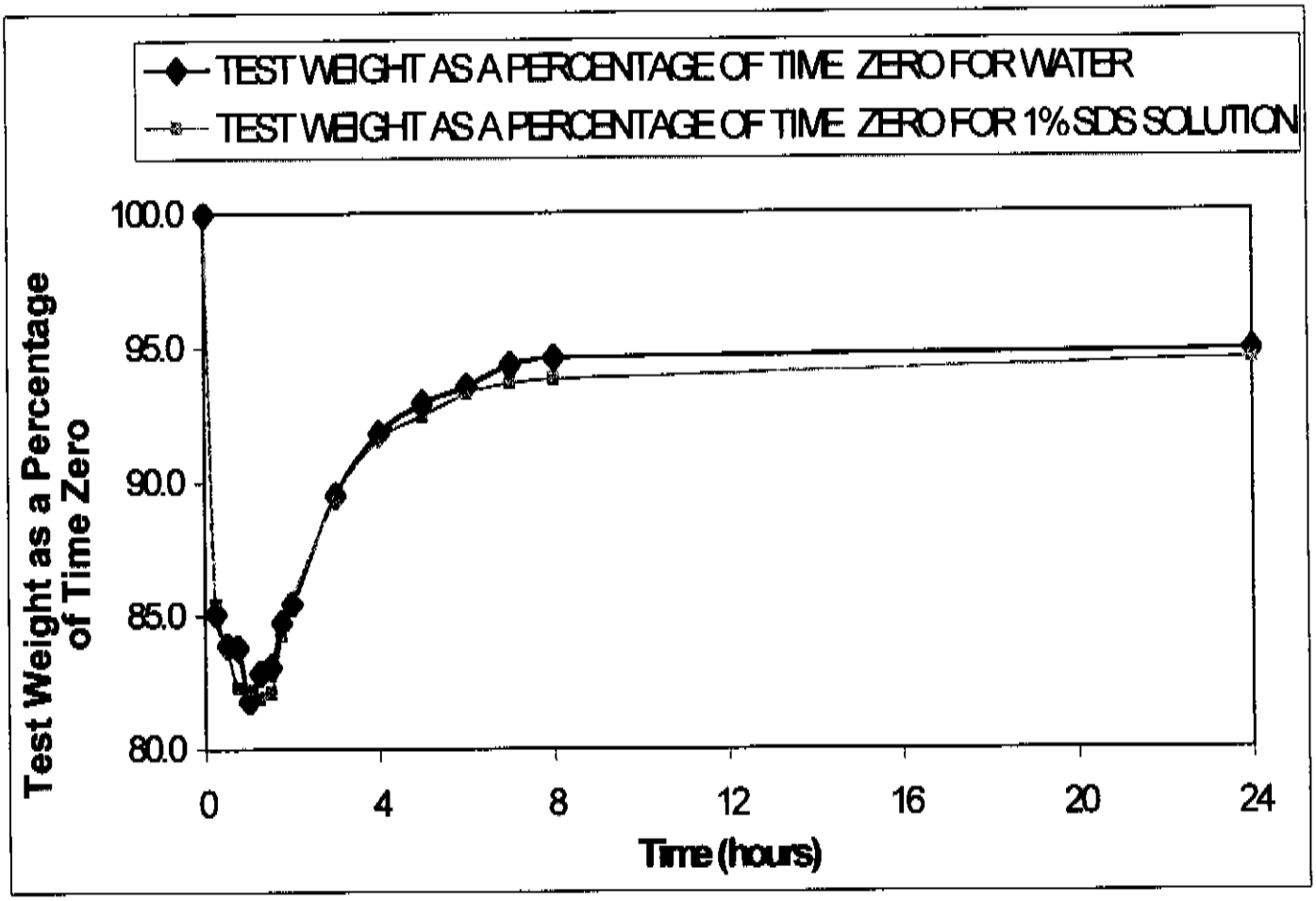
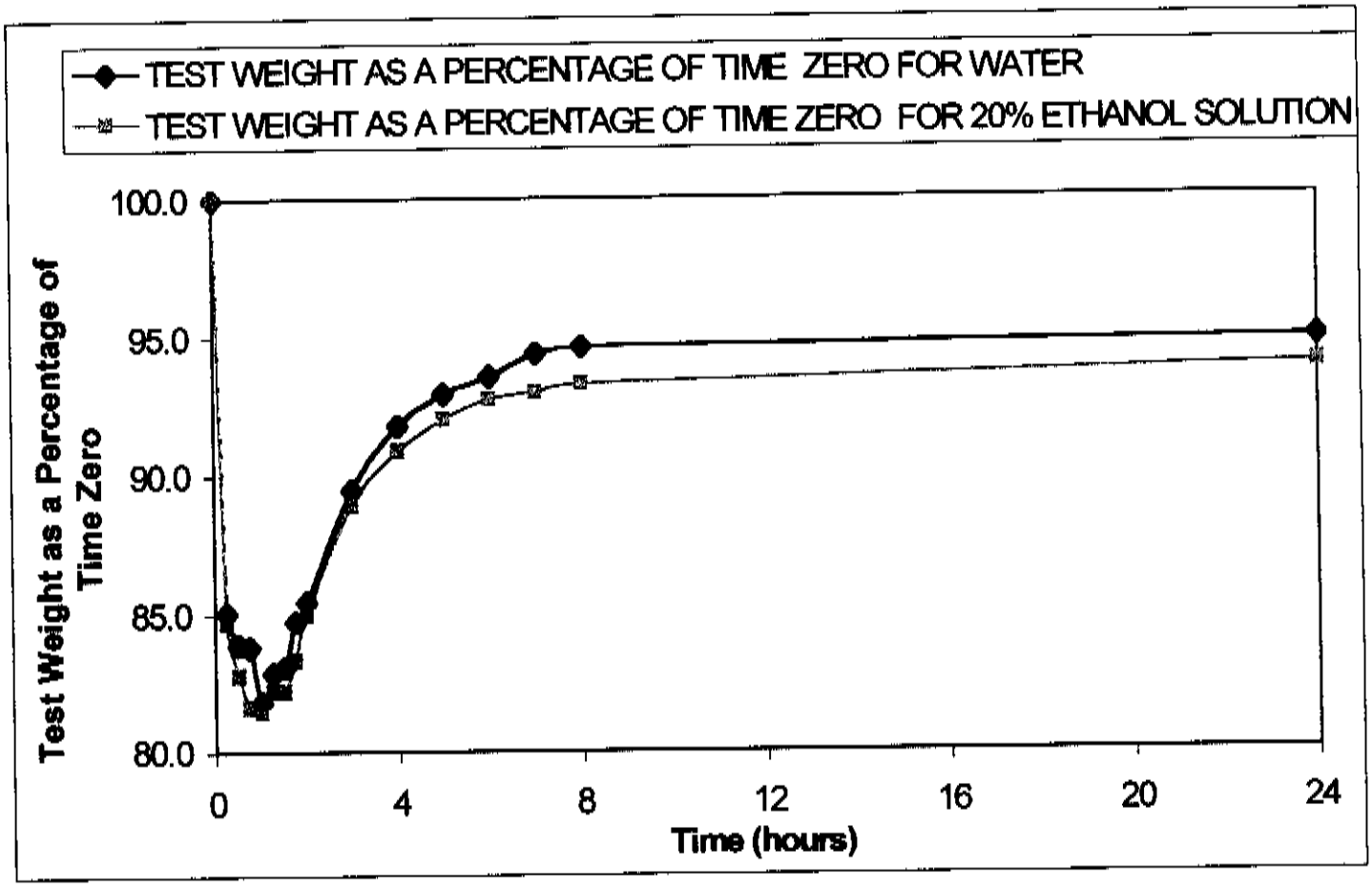


Figure 7 : Moisture Penetration Over a 24 Hour Period for a Hard Wheat Sample Using 20% Ethanol Solution.



In general the use of chemicals appeared to have slight effect on the lying time and hence the efficiency of the conditioning process.

Milling

Flour yield and flour quality results of selected tests are given in table 10 (for full flour quality results refer to appendix at the end of report).
The results show that with conditioning the number and area of specks present in the sample is reduced (figure 8), which in turn affects ash and colour (figure 9).

Table 10 : Flour Quality results

						FARINOGRAPH	
Time (hours)	Extraction (%)	Average Branscan (%)	Average Speck Count	Ash (%)	Colour Grade	Water Abs (%)	Development Time (mins)
0	79.5	0.46 ± 0.02	7.14 ± 0.55	0.59	1.8	62.6	4.8
1	75.9	0.28 ± 0.02	3.05 ± 0.35	0.57	0.9	59.2	7.2
2	76.3	0.22 ± 0.01	2.36 ± 0.26	0.52	0.4	59.8	7.0
3	77	0.16 ± 0.01	1.77 ± 0.29	0.50	-0.1	59.4	6.8
4	77.4	0.19 ± 0.01	2.14 ± 0.28	0.53	-0.4	59.7	7.9
6	77.5	0.19 ± 0.02	2.18 ± 0.28	0.50	-0.2	58.1	7.9
8	77.5	0.16 ± 0.01	1.62 ± 0.29	0.51	-0.2	57.9	9.2
24	78.1	0.18 ± 0.01	2.00 ± 0.29	0.55	-0.2	58.0	7.0

Figure 8 : Branscan Average % and Average Bran Speck Count

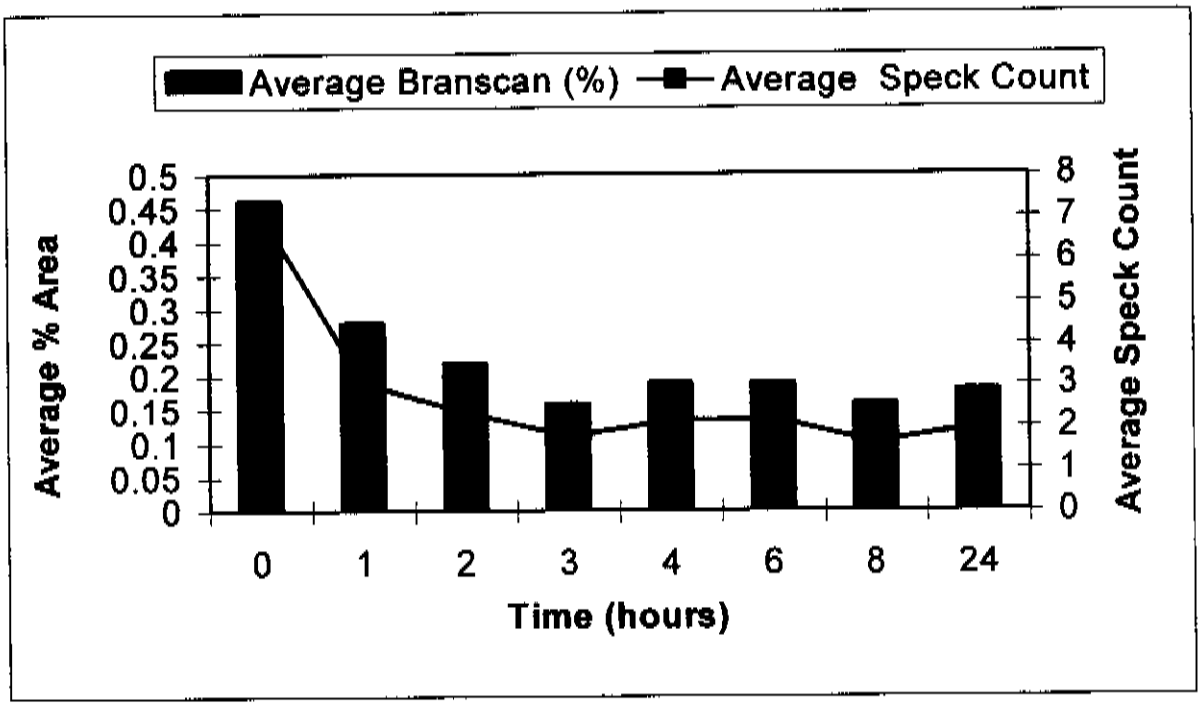
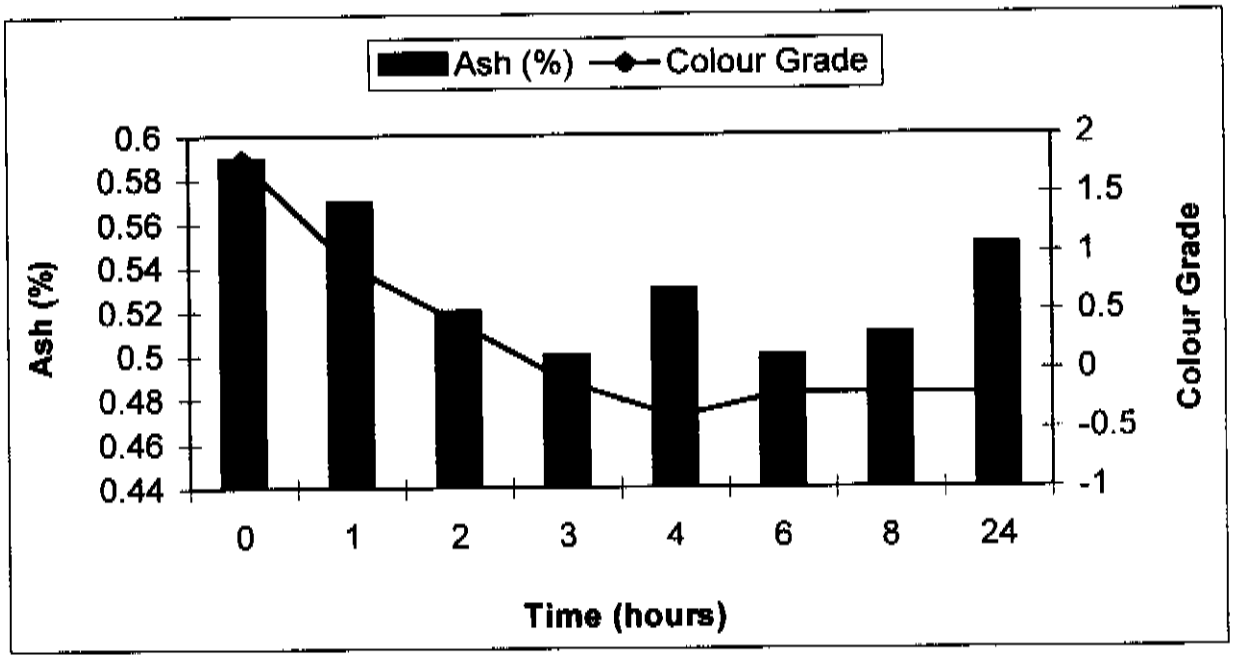
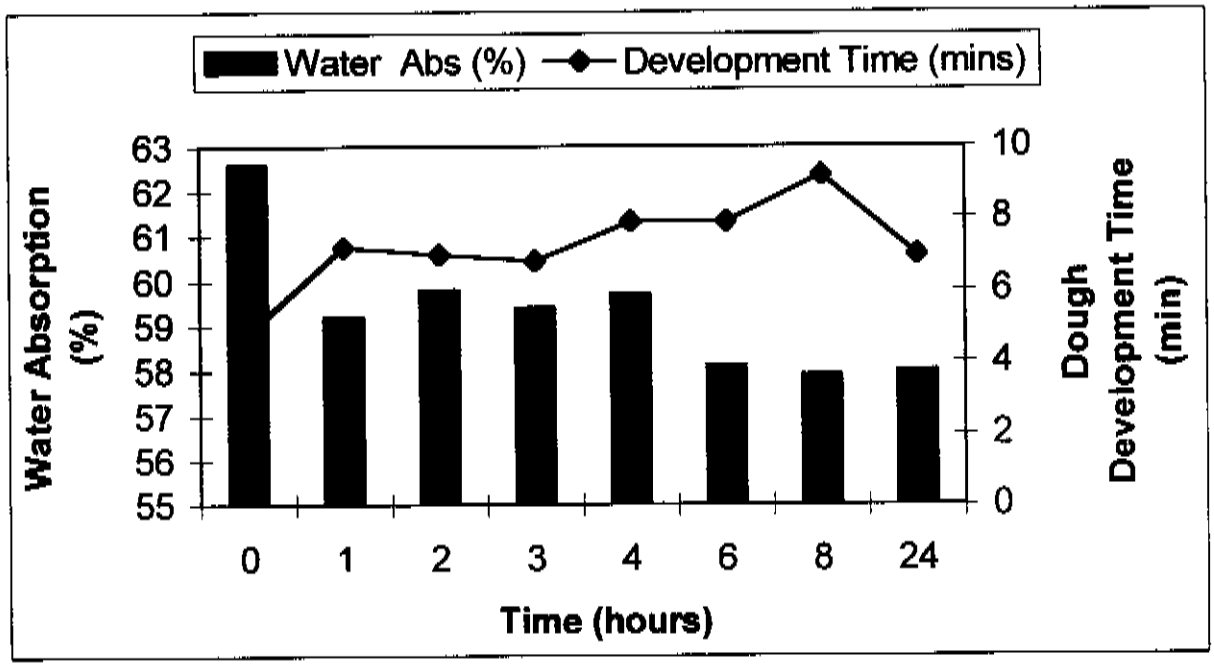


Figure 9 : Flour Ash and Colour Grade



Farinographs and extensographs show that there is an effect in dough characteristics with the addition of water but no trend with lying time after water addition (figure 10).

Figure 10 : Farinograph Water Absorption and Dough Development Time



Effect of Enzymes

The biological approach aims to see if the use of enzymes will have an effect on the rate of water penetration into the grain and hence the efficiency of the conditioning process.
Four enzymes were selected these were: Pectolyase, Polygalactouranse, Papain and Bromelain. The enzymes chosen are of plant origin and powder form.

A meeting was held at BRI in which CRC representatives from the milling industries attended. In this meeting a presentation was given on the purpose of the project, the experiments carried out, the results achieved and future work.

A representative from Weston's Milling suggested to look at enzymes which are available on a commercial level and gave details of an enzyme company – Novozymes Australia Pty Ltd.

With the support of a representative from Novozymes we were able to select three enzyme samples that are used in the starch/juicing industry. The enzymes are Pectinex Smash, Shearzyme and Viscozyme (all enzymes are liquid solutions). Below is a table describing the enzyme properties.

Table 11: Enzyme Description

ENZYME	DESCRIPTION	USES AND APPLICATIONS	TYPE
Pectinex Smash	The enzyme is a highly active pectolytic enzyme. The enzyme contains both pectolytic and hemicellulolytic activity which hydrolyses the methyl-esterified galcturonic acid residues in pectins; it also has the ability to break down cell walls.	The enzyme is used in the juicing industry to increase juice yield and is designed for the treatment of fruit and vegetable mashes in which both soluble and insoluble pectins are degraded.	The enzyme is a brownish liquid with a slight smell of fermeted products and a pH of 4.5
Viscozyme L	The enzymeis a multi-enzyme complex containing a range of carbohydrases such as arabanase, cellulase, ß-glucanase, hemicellulase and xylanase.	The enzyme is used in the starch, brewing and other related industries. The enzyme preparation is used in the breakdown of cell walls for the extraction of useful components from plant tissues and in the processing of cereal and vegetable products. The enzyme is also used as an ingredient detergent manufacturing.	The enzyme is a clear brown liquid with a slight smell of fermented products
Shearzyme 500L	The enzyme is a purified xylanase.	The enzyme is used in the starch industry.	The enzyme is a clear brown liquid with a slight smell of fermented products.

There is a patent that is titled ' A Process for Conditioning Grain' (for patent refer to appendix) which looks at how the conditioning time for wheat can be reduced by the use of enzymes. The experiment involved the use of two enzymes Cereszyme and Viscozyme where both the enzymes reduced the conditioning time of the wheat and increased the flour yield.

Reducing the Conditioning Time

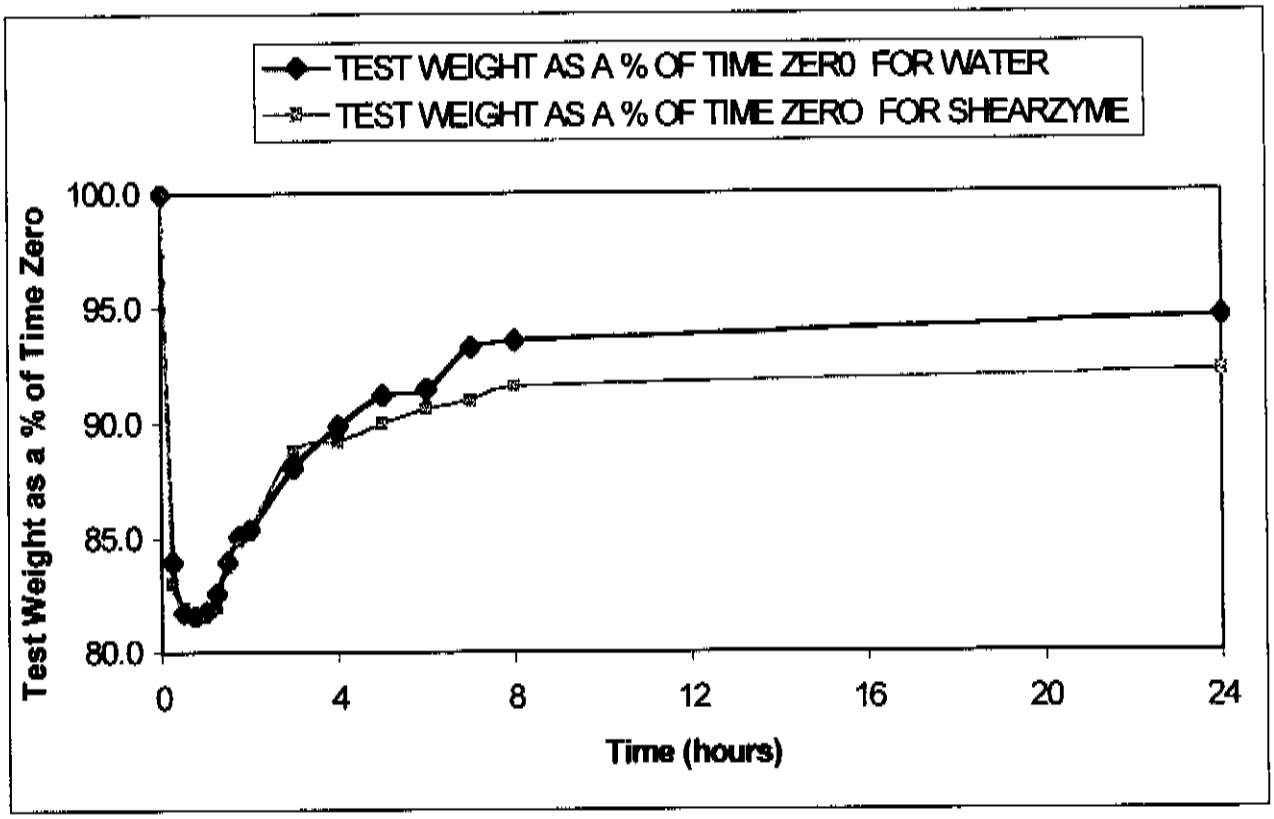
Effect of Enzymes

The use of the above enzymes on the conditioning time is given in table 12 and in the appendix at the back of the report. The results show that the use of enzymes (figures 11 – 13) is very similar to water.

Table 12 : Effect of Enzymes on the Hectolitre Weight

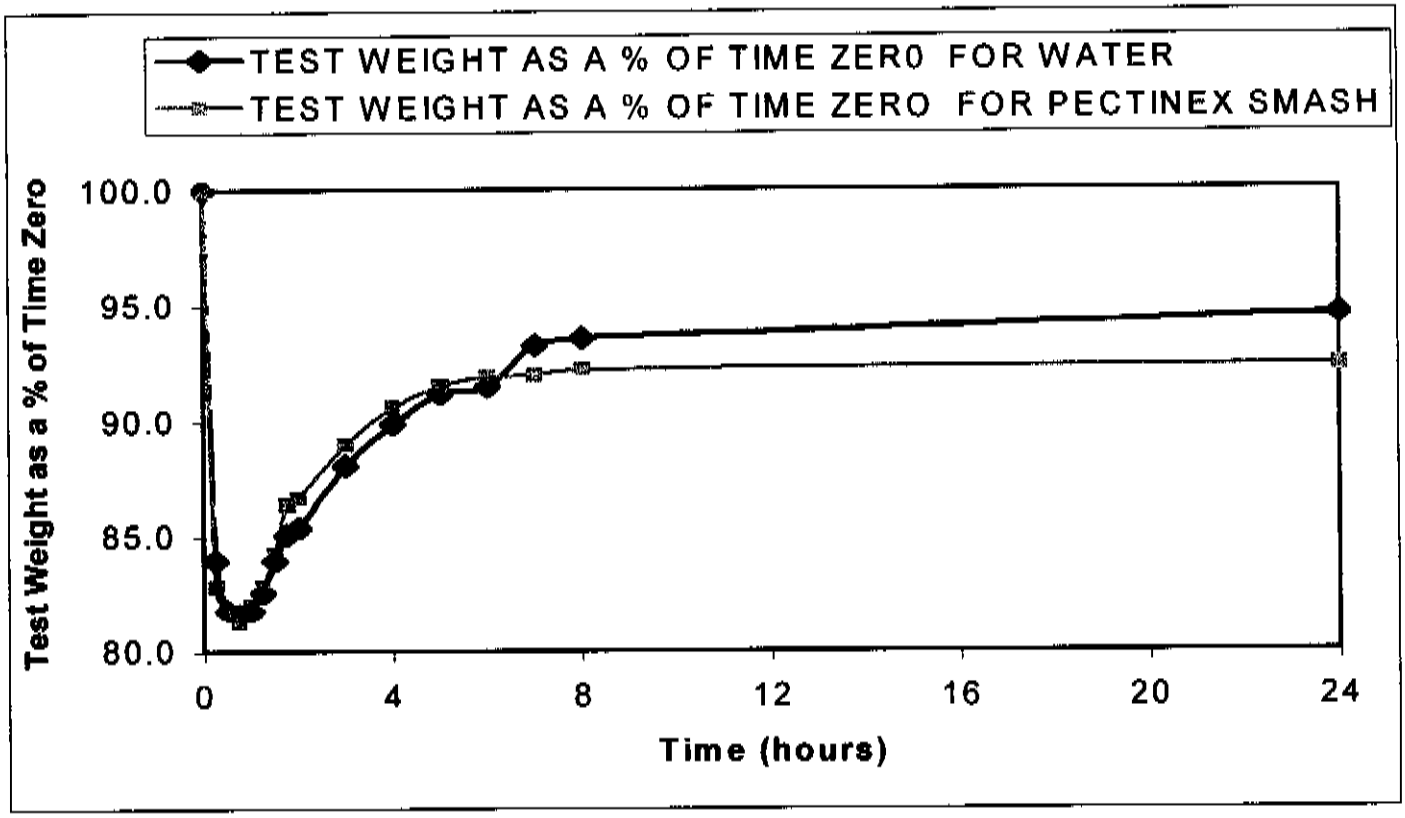
TIME (HOURS)	TEST WEIGHT AS A % OF TIME ZERO FOR WATER	TEST WEIGHT AS A % OF TIME ZERO FOR SHEARZYME 500L	TEST WEIGHT AS A % OF TIME ZERO FOR PECTINEX SMASH	TEST WEIGHT AS A % OF TIME ZERO FOR VISCOZYME L
0	100.0	100.0	100.0	100.0
¼	84.0	83.0	82.8	82.8
½	81.8	81.9	81.7	80.9
¾	81.6	81.6	81.3	81.0
1	81.8	81.7	82.0	81.4
1 ¼	82.6	82.0	82.8	83.2
1 ½	84.0	83.9	84.3	84.5
1 ¾	85.1	84.9	86.4	86.0
2	85.4	85.4	86.7	87.6
3	88.1	88.8	89.0	88.4
4	89.9	89.2	90.6	90.2
5	91.2	90.0	91.5	91.1
6	91.5	90.6	91.9	91.9
7	93.3	91.0	92.0	91.9
8	93.6	91.6	92.2	92.4
24	94.6	92.2	92.4	93.2

Figure 11 : Moisture Penetration Over a 24 Hour Period for a Hard Wheat Sample Using Shearzyme 500L



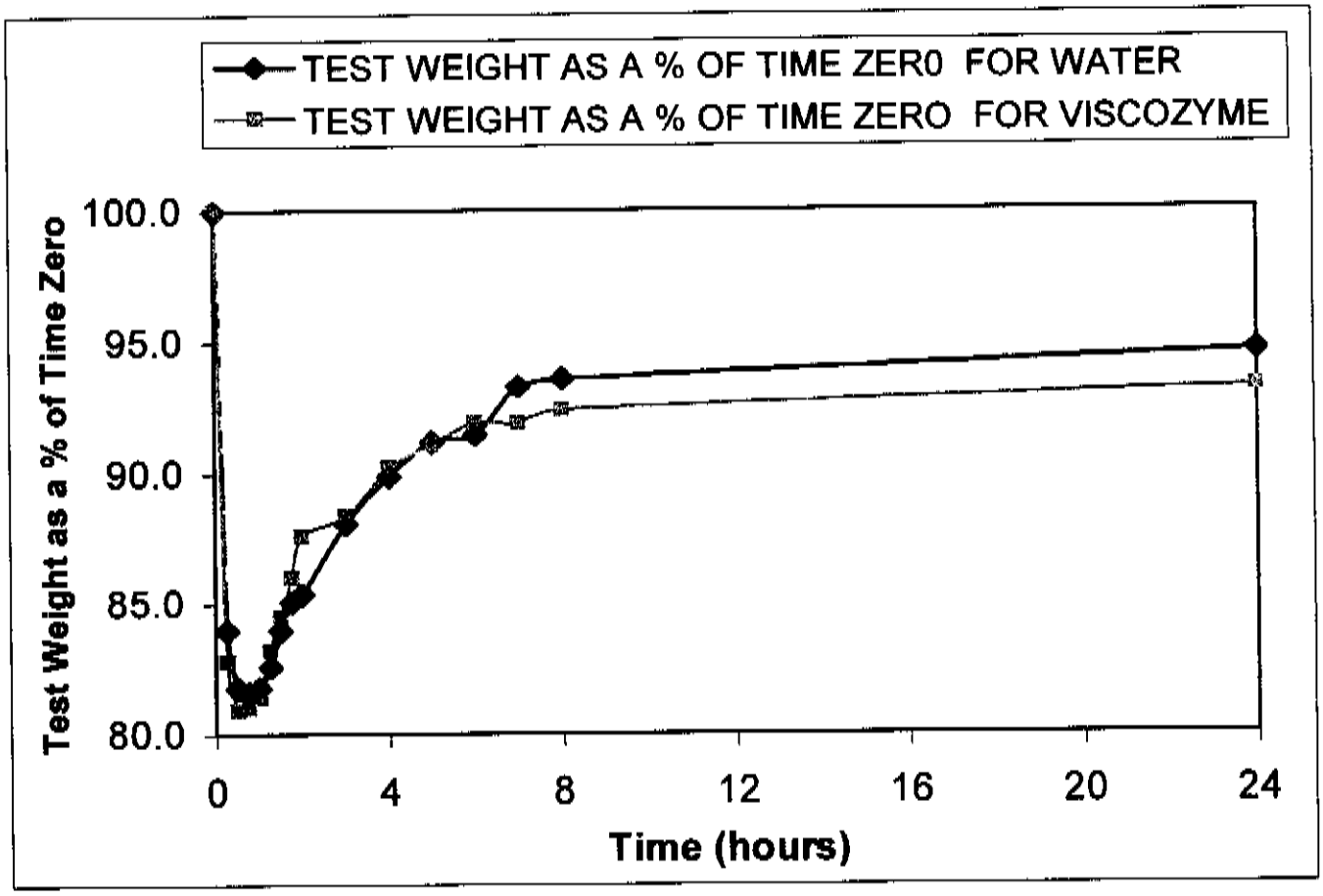
The use of Shearzyme 500L on the rate of moisture penetration into the grain is very similar to water and therefore there is no effect on the lying time.

Figure 12 : Moisture Penetration Over a 24 Hour Period for a Hard Wheat Sample Using Pectinex SMASH



The use of Pectinex SMASH on the rate of moisture penetration into the grain showed a slight increase in comparision to water .

Figure 13 : Moisture Penetration Over a 24Hour Period for a Hard Wheat Sample Using Viscozyme



The use of Viscozyme on the rate of moisture penetration into the grain is very similar to the use of water and therefore there was no effect on the lying time.

Milling

Test millings were carried out on a hard wheat sample of 2kg quantity using the Buhler Test Mill MLU202. The time interval chosen were 0 (no water added), 1h, 2h, 3h, 4h, 6h, 8h and 16h.

Flour yield and flour quality results of selected tests are given in tables 13 – 16 (for full report please refer to appendix).

Table 13 : Flour Quality Results for Water (Control)

TIME (hours)	FLOUR YIELD (%)	ASH (14% m.b)	COLOUR GRADE	BRANSCAN AVERAGE	
				AREA (%)	SPECK NUMBER
0	82.3	0.63	1.4	0.35 ± 0.02	4.68 ± 0.46
1	80.4	0.60	0.3	0.16 ± 0.02	2.05 ± 0.27
2	80.3	0.59	0.0	0.13 ± 0.01	1.68 ± 0.36
3	80.5	0.57	-0.1	0.17 ± 0.02	2.36 ± 0.38
4	80.7	0.56	-0.4	0.14 ± 0.01	2.05 ± 0.34
6	80.7	0.53	-0.4	0.19 ± 0.02	2.55 ± 0.31
8	80.6	0.58	-0.6	0.17 ± 0.02	2.18 ± 0.33
16	80.1	0.56	-0.8	0.11 ± 0.01	1.73 ± 0.26

Table 14 : Flour Quality results for Shearzyme

TIME (hours)	FLOUR YIELD (%)	ASH (14% m.b)	COLOUR GRADE	BRANSCAN AVERAGE	
				AREA (%)	SPECK NUMBER
0	82.3	0.63	1.4	0.35 + 0.02	4.68 + 0.46
1	79.5	0.56	-0.5	0.15 + 0.01	1.77 + 0.32
2	79.7	0.58	-0.7	0.14 + 0.01	1.50 + 0.23
3	80.1	0.57	-0.9	0.14 + 0.01	2.32 + 0.37
4	80.5	0.55	-0.7	0.15 + 0.01	2.45 + 0.31
6	80.6	0.57	-1.1	0.16 + 0.01	2.59 + 0.32
8	80.5	0.55	-0.9	0.14 + 0.02	1.36 + 0.25
16	80.1	0.57	-0.3	0.14 + 0.01	2.18 + 0.28

Table 15 : Flour Quality results for Pectinex Smash

TIME (hours)	FLOUR YIELD (%)	ASH (14% m.b)	COLOUR GRADE	BRANSCAN AVERAGE	
				AREA (%)	SPECK NUMBER
0	82.3	0.63	1.4	0.35 ± 0.02	4.68 ± 0.46
1	79.8	0.61	0.0	0.17 ± 0.02	2.32 ± 0.33
2	79.9	0.6	-0.3	0.18 ± 0.01	2.64 ± 0.35
3	80.0	0.58	-0.5	0.16 ± 0.01	2.41 ± 0.36
4	80.6	0.57	-0.3	0.16 ± 0.01	2.23 ± 0.29
6	80.8	0.59	-0.1	0.17 ± 0.02	2.55 ± 0.34
8	80.4	0.59	-0.2	0.15 ± 0.01	1.94 ± 0.34
16	80.1	0.55	-0.2	0.17 ± 0.01	2.27 ± 0.29

Table 16: Flour Quality results for Viscozyme

TIME (hours)	FLOUR YIELD (%)	ASH (14% m.b)	COLOUR GRADE	BRANSCAN AVERAGE	
				AREA (%)	SPECK NUMBER
0	82.3	0.63	1.4	0.35 ± 0.02	4.68 ± 0.46
1	79.8	0.57	-0.7	0.17 ± 0.01	2.14 ± 0.37
2	81.2	0.6	-0.3	0.20 ± 0.01	3.09 ± 0.35
3	80.6	0.59	-0.7	0.20 ± 0.01	3.73 ± 0.48
4	80.7	0.58	-0.5	0.19 ± 0.02	3.00 ± 0.31
6	80.7	0.59	-0.7	0.18 ± 0.01	2.73 ± 0.36
8	81.2	0.57	-0.6	0.20 ± 0.02	3.50 ± 0.41
16	82.0	0.56	-0.3	0.23 ± 0.02	3.75 ± 0.41

Flour Yield

The results show that Viscozyme showed an increase in flour yield when compared to the control. The use of Shearzyme and Pectinex SMASH gave flour yields very similar to the control.

Ash

The ash values for the enzyme treatment is very similar to the control.

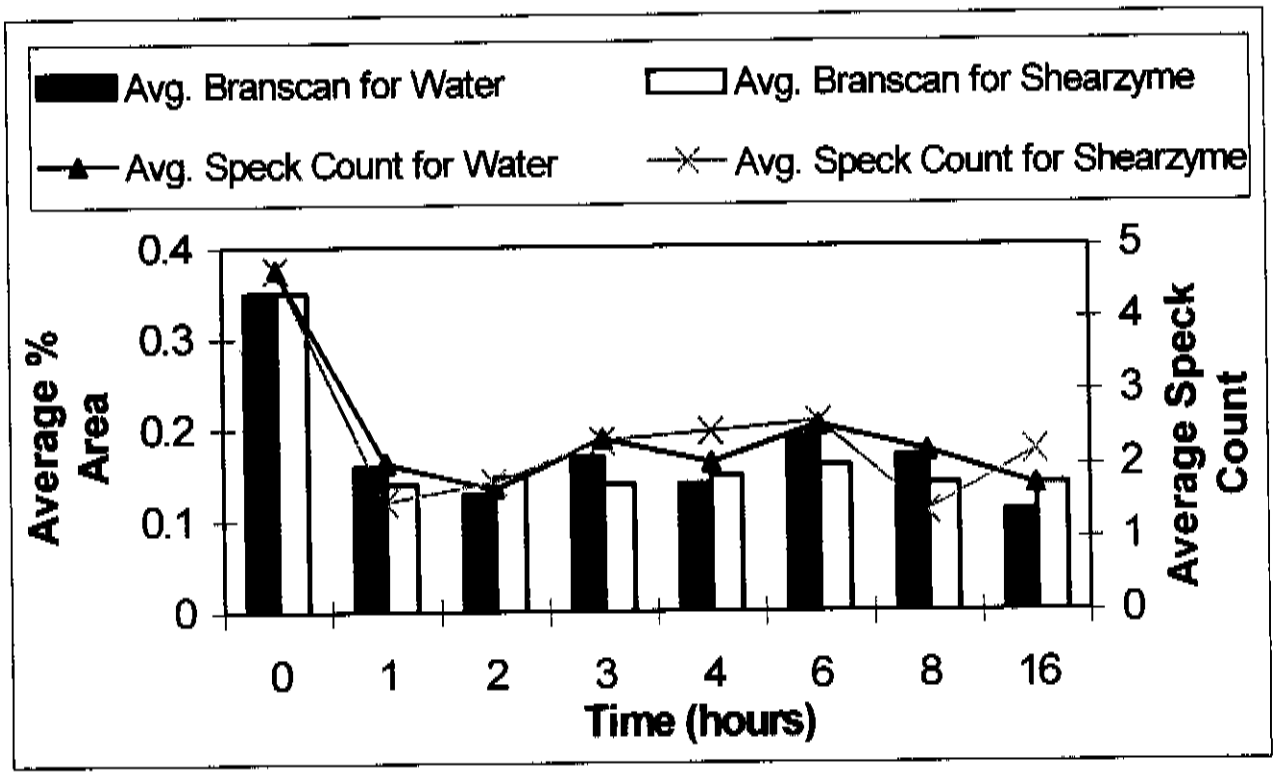
Colour Grade

Generally colour grade improved (values became lower) with conditioning compared with no conditioning.

Branscan

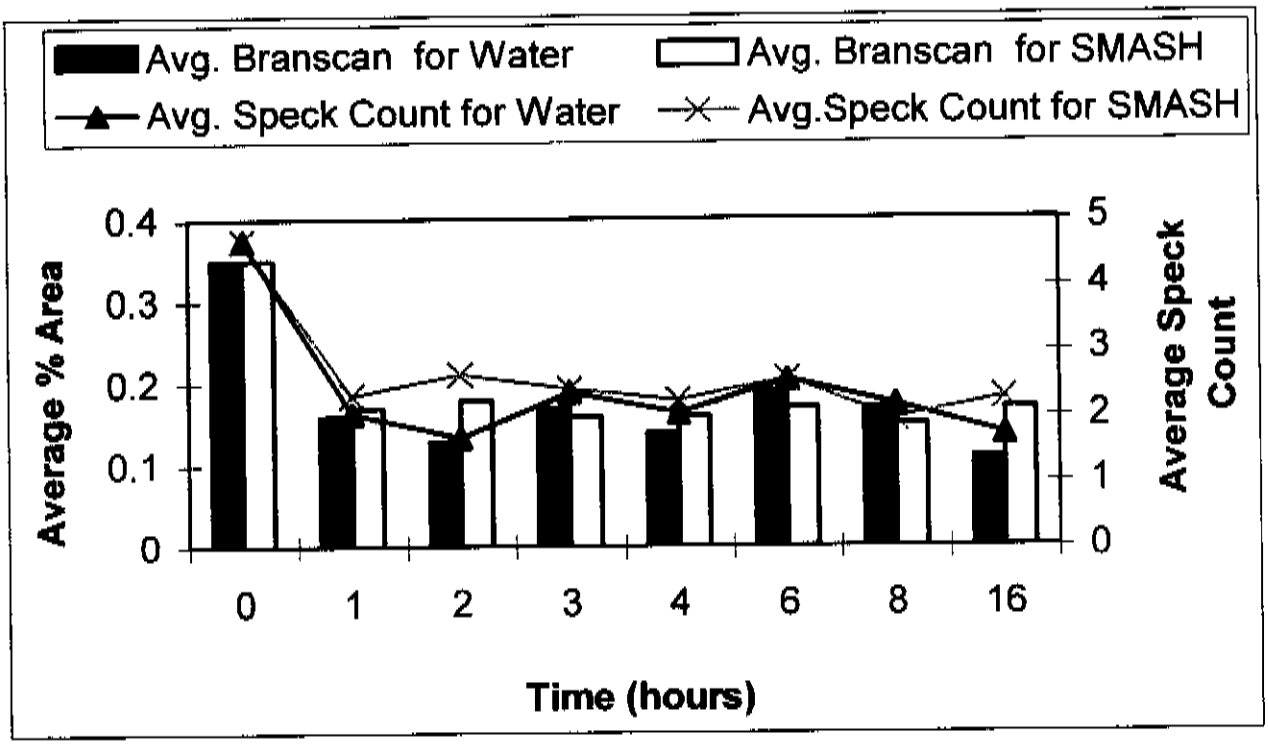
In general the enzyme treatment (Figures 14 – 16) gave a slightly higher speck value when compared to the control.

Figure 14 : Brancan Average % and Average Bran Speck Count for Shearzyme



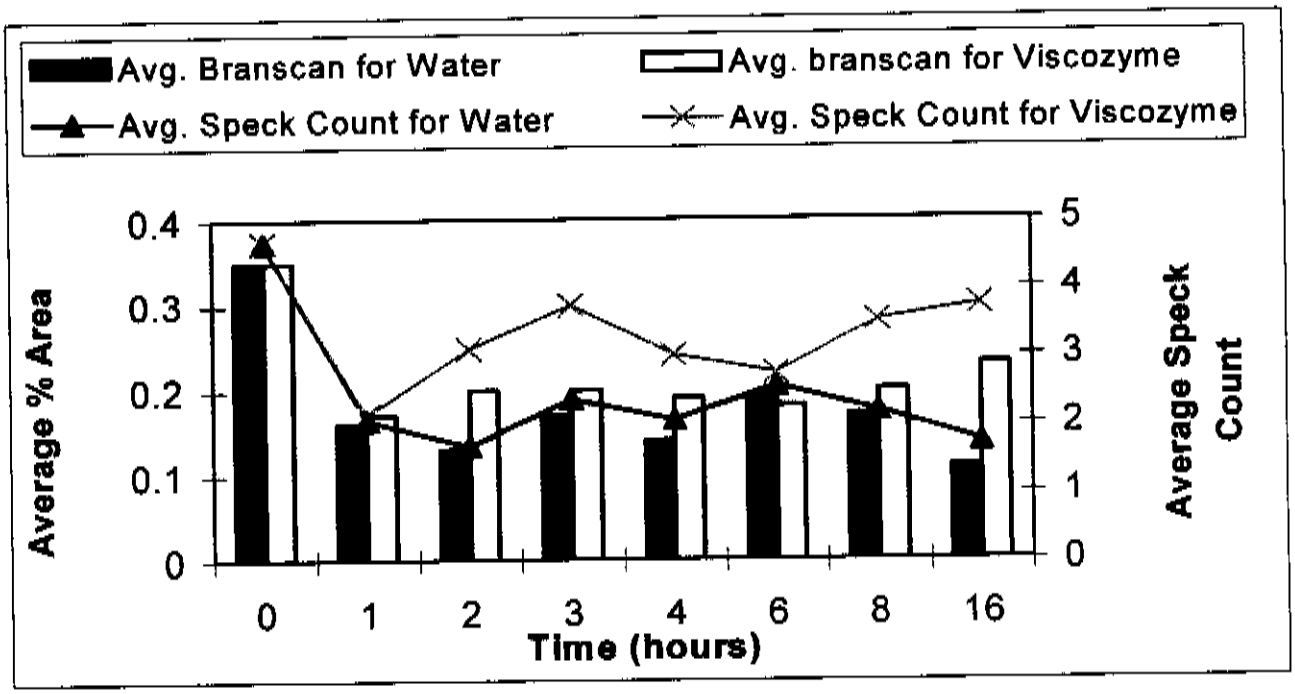
The above graph shows that the use of shearzyme as a tempering agent in the conditioning process resulted in a decrease in bran contamination during the first 2 hours while at the hours of 3, 4, 6 and 16 hours a slight increase in bran contamination was noticed.

Figure 15 : Branscan Average and Average Bran Speck Count for Pectinex SMASH



The results show that the use of Pectinex SMASH resulted in flour that is slightly higher in bran contamination (as indicated by the above graph) in comparison to the control. This slight increase may be a result of the enzyme activity; where the enzyme has some form of hemicellulolytic and pectolytic activity which may have degraded through the cell wall structure of the grain.

Figure 16 : Branscan Average % and Average Bran Speck Count for Viscozyme



The results show that the use of Viscozyme L resulted in a higher bran contamination when compared to water. This increase can be explained by the enzyme activity being the most active hence most likely shattering the cell wall structure of the grain.

Dough Rheology

Table 17 : Farinograph Water Absorption (%)

TIME (hours)	CONTROL	SHEARZYME 500 L	PECTINEX SMASH	VISCOZYME L
0	62.4	62.4	62.4	62.4
1	59.9	60.5	61.0	61.7
2	59.5	60.4	60.6	61.8
3	59.4	60.4	60.4	61.0
4	60.4	60.8	61.1	61.5
6	59.6	60.7	62.1	61.4
8	59.9	60.0	61.0	61.8
16	58.2	59.5	60.2	62.4

The results show that in comparision to the control the use of enzymes required more water hence having higher water absorption values. This increase is due to the presence of bran in the flour.

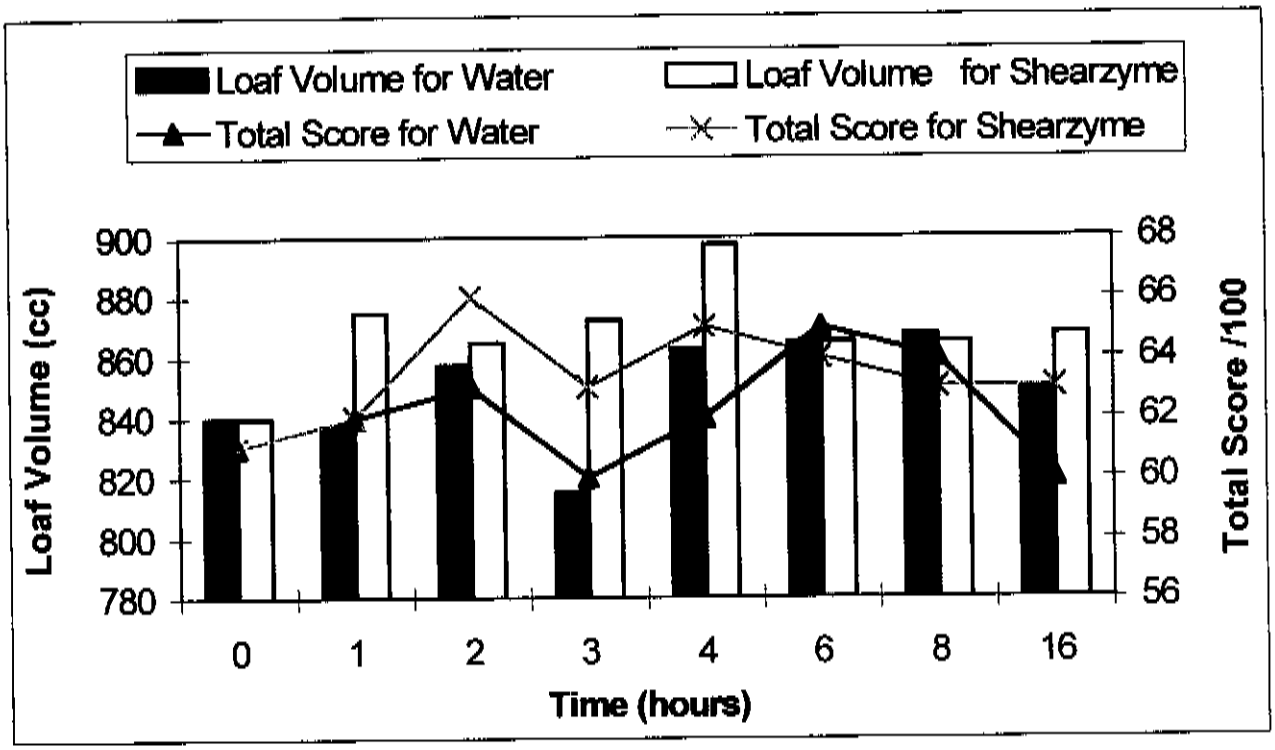
End Product Testing

End product testing was carried out on all flour samples to see if the use of enzymes had an effect on the flour quality and hence baking. Of the three enzymes used two gave interesting results.
In regards to flour quality Shearzyme had speck number very similar to water however in terms of baking the use of Shearzyme gave a higher volume loaf and a very similar score to the control.

Table 18 : Baking Data Results for the use of Shearzyme

TIME (hours)	LOAF VOLUME For WATER	LOAF VOLUME for SHEARZYME	TOTAL SCORE For WATER	TOTAL SCORE For SHEARZYME
0	840	840	61	61
1	837.5	875	62	62
2	857.5	865	63	66
3	815	872.5	60	63
4	862.5	897.5	62	65
6	865	865	65	64
8	867.5	865	64	63
16	850	867.5	60	63

Figure 17 : Rapid Dough Loaf Volume and Total Score

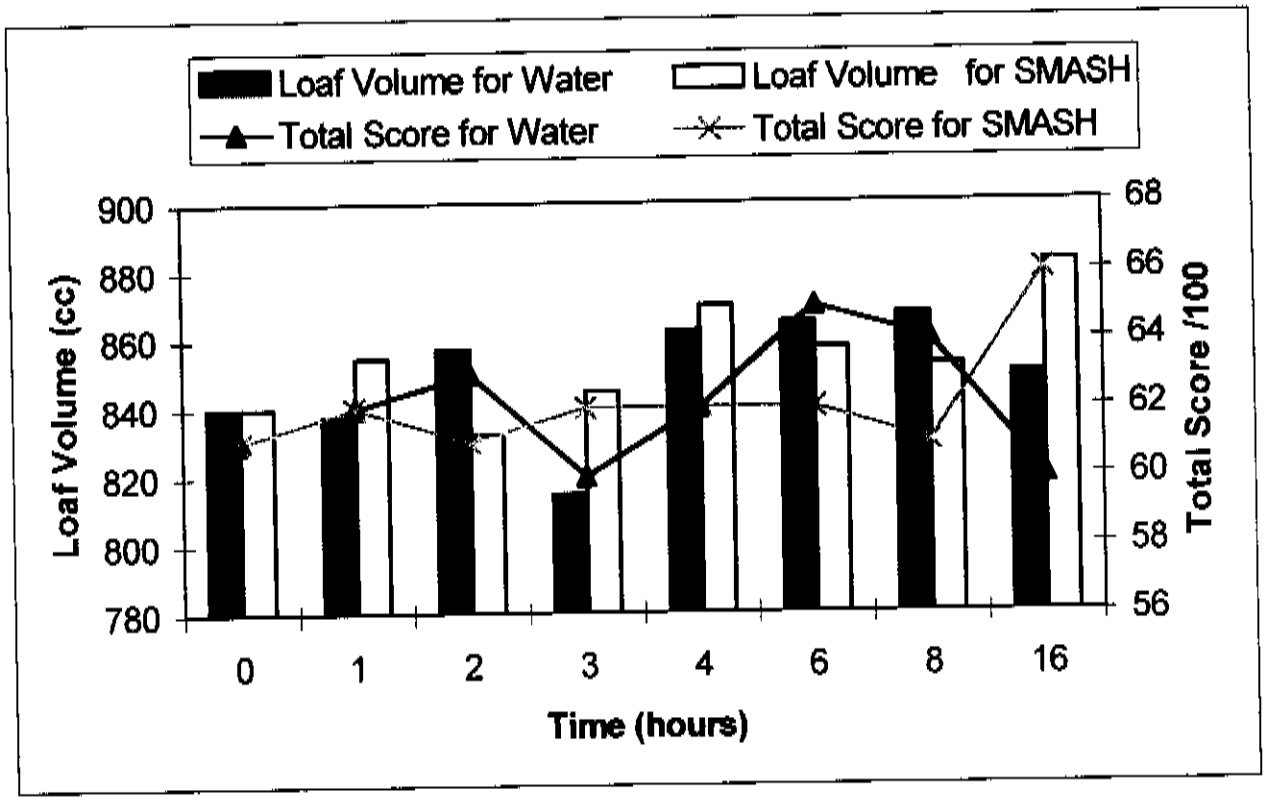


The baking results for the use of Pectinex Smash (table 19, figure 18) showed increases in the loaf volume during the follwing times 1, 3, 4 and 16 hours and the highest score at 16 hours.

Table 19 : Baking Data Results for the use of Pectinex SMASH

TIME (hours)	LOAF VOLUME for WATER	LOAF VOLUME for SMASH	TOTAL SCORE for WATER	TOTAL SCORE for SMASH
0	840	840	61	61
1	837.5	855	62	62
2	857.5	832.5	63	61
3	815	845	60	62
4	862.5	870	62	62
6	865	857.5	65	62
8	867.5	852.5	64	61
16	850	882.5	60	66

Figure 18 : Rapid Dough Loaf Volume and Total Score



CONCLUSION

Using hectolitre weight over a selected time interval allowed the moisture penetration into the grain to be monitored. This method prooved to be a quick, efficent and simple method.

A chemical and biological approach was adopted to see if the conditioning time can be reduced and hence increase the milling efficiency. The use of chemicals and enzymes diplayed a very similar pattern to water and therefore very little effect is seen on the rate of moisture penetration and hence the efficiency of the conditioning process.

A patent titled 'A process for Conditioning Grain' investigated conditioning time for wheat. It was found that Cereszyme and Viscozyme L reduced the conditioning time and increased flour yield. However this study found that Viscozyme L resulted in an increase in flour yield and was also associated with an increase in bran contamination when compared to the control. A major reduction in conditioning time was not observed.

ACKNOWLEDGEMENTS

We wish to thank Novozymes for the donation of the enzymes and CRC for funding this project.

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APPENDIX ONE

Standard Approach – Use of Water

Table 1 : Test Weight Measured at a 2 Hour Interval for a Hard Wheat Sample

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	79.88	79.95	79.92
2	68.35	68.54	68.45
4	71.60	71.95	71.78
6	73.27	73.56	73.42
8	74.03	74.31	74.17
24	74.78	74.89	74.84

Table 2 : Test Weight Measured at a 2 Hour Interval for a Soft Wheat Sample

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	69.35	69.84	69.60
2	71.51	72.39	71.95
4	72.43	72.59	72.51
6	72.59	72.92	72.76
8	73.45	73.47	72.96
24	73.40	73.61	73.51

Table 3 : Test Weight Measured at a 1 Hour Interval for a Hard Wheat Sample

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	79.88	79.95	79.92
1	68.50	66.44	67.47
2	67.31	67.38	67.35
3	70.90	70.76	70.83
4	72.38	72.64	72.51
5	72.98	73.40	73.19
6	74.03	74.14	74.09
7	74.35	74.72	75.54
8	74.63	74.81	74.72
24	75.26	75.51	75.39

Table 4 : Test Weight Measured at a 1 Hour Interval for a Soft Wheat Sample

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	69.35	69.84	69.60
1	67.50	68.24	67.37
2	69.74	70.19	69.97
3	70.39	70.88	70.64
4	70.48	71.44	70.96
5	71.25	71.58	71.42
6	71.60	71.76	71.68
7	71.55	71.94	71.75
8	71.71	72.45	72.08
24	72.61	72.85	72.73

Table 5 : Test Weight Over a 24 Hour Period for a Hard Wheat Sample

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	79.88	79.95	79.92
1/4	68.31	67.66	67.99
1/2	67.32	66.76	67.04
3/4	67.43	66.51	66.97
1	65.28	65.46	65.37
1 1/4	66.51	65.92	66.22
1 1/2	66.25	66.55	66.40
1 3/4	67.45	67.98	67.72
2	68.42	68.17	68.30
3	71.37	71.71	71.54
4	73.27	73.47	73.37
5	74.14	74.42	74.28
6	74.68	74.88	74.78
7	75.30	75.56	75.43
8	75.48	75.77	75.63
24	75.76	75.86	75.81

Table 6 : Test Weight Over a 24 Hour Period for a Soft Wheat Sample

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	69.35	69.84	69.60
¼	63.56	64.17	63.87
½	66.04	66.04	66.04
¾	67.46	67.68	67.68
1	68.47	68.84	68.66
1 ¼	69.44	70.02	69.73
1 ½	70.05	70.40	70.23
1 ¾	70.55	71.07	70.81
2	70.93	71.62	71.28
3	71.48	72.06	71.77
4	71.71	72.10	71.91
5	72.17	72.31	72.24
6	72.39	72.54	72.47
7	72.78	72.41	72.60
8	72.71	73.29	73.00
24	72.99	73.33	73.16

Table 7 : Flour Data

Time (hours)	Flour Yield (%)	Protein (N x 5.7) (14% m.b)	Moisture (%)	Ash (14% m.b)	Colour Grade	Minolta Tristimulus Colour				Branscan Average	
						L	a	b	L-b	Area (%)	Speck Number
0	79.5	13.4	12.4	0.59	1.8	90.34	0.34	9.64	80.70	0.46 ± 0.02	7.14 ± 0.55
1	75.9	13.0	15.0	0.57	0.9	91.19	0.33	9.23	81.96	0.28 ± 0.02	3.05 ± 0.35
2	76.3	13.1	14.5	0.52	0.4	91.51	0.27	8.84	82.67	0.22 ± 0.01	2.36 ± 0.26
3	77.0	12.9	14.7	0.50	-0.1	91.77	0.19	8.70	83.07	0.16 ± 0.01	1.77 ± 0.29
4	77.4	13.1	14.8	0.53	-0.4	91.92	-0.10	8.66	83.36	0.19 ± 0.01	2.14 ± 0.28
6	77.5	12.9	15.4	0.50	-0.2	91.87	-0.10	8.63	83.24	0.19 ± 0.01	2.18 ± 0.28
8	77.5	12.8	15.4	0.50	-0.2	91.93	-0.18	8.58	83.35	0.16 + 0.01	1.62 + 0.29
24	78.1	12.9	15.0	0.55	-0.2	91.91	-0.21	8.74	83.17	0.18 + 0.01	2.00 + 0.29

Table 8 : Dough Rheology Data

	Farinograph		Extensograph	
Time (hours)	Water Abs (%)	Development Time (min)	Extensibility (cm)	Maximum Resistence (BU)
0	62.6	4.8	26.0	275
1	59.2	7.2	26.2	495
2	59.8	7.0	26.3	470
3	59.4	6.8	26.2	445
4	59.7	7.9	26.2	430
6	58.1	7.9	26.4	490
8	57.9	9.2	26.2	445
24	58.0	7.0	23.8	465

Chemical Appraoch

Table 9 : Test Weight Over a 24 Hour Period for a Hard Wheat Sample

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	79.88	79.95	79.92
1/4	68.31	67.66	67.99
1/2	67.32	66.76	67.04
3/4	67.43	66.51	66.97
1	65.28	65.46	65.37
1 1/4	66.51	65.92	66.22
1 1/2	66.25	66.55	66.40
1 3/4	67.45	67.98	67.72
2	68.42	68.17	68.30
3	71.37	71.71	71.54
4	73.27	73.47	73.37
5	74.14	74.42	74.28
6	74.68	74.88	74.78
7	75.30	75.56	75.43
8	75.48	75.77	75.63
24	75.76	75.86	75.81

Table 10 : Test Weight Over a 24 Hour Period Using 0.1M Acetic Acid

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	79.88	79.95	79.92
1/4	68.98	69.28	69.13
1/2	67.62	69.01	68.32
3/4	66.23	66.48	66.36
1	66.32	66.39	66.36
1 1/4	65.62	64.81	65.22
1 1/2	65.25	67.64	66.45
1 3/4	66.18	67.48	66.83
2	67.01	68.27	67.64
3	70.19	70.42	70.31
4	71.99	72.29	72.14
5	73.15	73.32	73.24
6	73.73	74.03	73.88
7	74.20	74.42	74.31
8	74.52	74.60	74.56
24	74.77	74.89	74.83

Table 11 : Test Weight Over a 24 Hour Period Using 0.5M Acetic Acid Solution

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	79.88	79.95	79.92
1/4	69.01	68.94	68.98
1/2	67.94	68.22	68.08
3/4	66.13	66.28	66.21
1	66.39	66.02	66.21
1 1/4	66.25	65.33	66.79
1 1/2	66.30	65.99	66.15
1 3/4	66.80	66.36	66.58
2	66.36	66.67	66.52
3	69.75	70.16	69.96
4	71.51	71.76	71.64
5	72.71	72.90	72.81
6	73.40	72.90	73.15
7	73.86	73.98	73.92
8	74.07	74.31	74.19
24	74.95	75.04	75.00

Table 12 : Test Weight Over a 24 Hour Period Using 1.0M Acetic Acid

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	79.88	79.95	79.92
1/4	69.28	68.96	69.12
1/2	67.83	67.78	67.81
3/4	66.73	67.15	66.94
1	66.11	66.41	66.26
1 1/4	65.70	66.27	65.99
1 1/2	66.13	65.90	66.01
1 3/4	66.11	66.16	66.14
2	66.79	66.65	66.72
3	69.67	69.67	69.67
4	71.14	71.20	71.17
5	72.48	72.57	72.53
6	72.98	73.24	73.11
7	73.47	73.70	73.59
8	73.86	74.01	73.94
24	74.65	74.60	74.63

Table 13 : Test Weight Over a 24 Hour Period Using 0.1M Sodium Hydroxide

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	79.88	79.95	79.92
1/4	67.55	67.08	67.32
1/2	66.27	66.04	66.16
3/4	65.95	65.09	65.52
1	64.70	64.74	64.72
1 1/4	65.14	65.21	65.18
1 1/2	66.02	66.44	66.23
1 3/4	67.02	66.97	67.00
2	67.83	68.29	68.06
3	71.14	71.23	71.19
4	72.54	72.68	72.61
5	73.19	73.50	73.35
6	73.80	73.98	73.89
7	74.26	74.33	74.30
8	74.52	74.79	74.66
24	74.96	75.19	75.08

Table 14 : Test Weight Over a 24 Hour Period Using 1% w/v Sodium Dodecyl Sulphate

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	79.88	79.95	79.92
1/4	67.98	68.50	68.24
1/2	67.04	66.78	66.91
3/4	65.72	65.76	65.74
1	65.02	66.30	65.66
1 1/4	65.37	65.53	65.45
1 1/2	65.61	65.60	65.61
1 3/4	67.22	67.43	67.33
2	68.52	68.19	68.36
3	71.41	71.55	71.48
4	72.98	73.45	73.22
5	73.84	73.94	73.89
6	74.42	74.72	74.57
7	74.74	75.00	74.87
8	74.91	75.02	74.97
24	75.58	75.53	75.56

Table 15 : Test Weight Over a 24 Hour Period Using 20% v/v Ethanol

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	79.88	79.95	79.92
1/4	67.73	67.61	67.67
1/2	66.39	65.90	66.15
3/4	65.25	65.21	65.23
1	64.84	65.32	65.08
1 1/4	65.99	65.39	65.69
1 1/2	65.62	65.97	65.80
1 3/4	66.30	66.83	66.57
2	67.71	68.15	67.93
3	71.00	71.20	71.10
4	72.55	72.75	72.65
5	73.40	73.77	73.55
6	73.91	74.33	74.12
7	74.31	74.35	74.33
8	74.47	74.65	74.56
24	75.00	75.14	75.07

Biological Approach – Enzyme Treatments

Table 16 : Test Weight Over a 24 Hour Period Using Water

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	83.35	83.27	83.31
1/4	70.02	69.98	70.00
1/2	68.08	68.27	68.18
3/4	68.19	67.76	67.98
1	68.08	68.19	68.14
1 1/4	68.70	68.94	68.82
1 1/2	69.67	69.51	69.59
1 3/4	70.79	70.97	70.88
2	70.79	71.55	71.17
3	73.29	73.56	73.43
4	74.88	74.88	74.88
5	75.76	76.23	76.00
6	76.25	76.37	76.24
7	77.71	77.73	77.72
8	77.96	78.06	78.01
16	75.58	75.99	75.79
24	78.80	78.89	78.85

Table 17 : Test Weight Over a 24 Hour Period Using Shearzyme 500L

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	83.35	83.27	83.31
1/4	69.31	69.00	69.16
1/2	67.89	68.53	68.21
3/4	67.59	68.47	68.03
1	67.71	68.38	68.05
1 1/4	68.24	68.45	68.35
1 1/2	69.81	69.91	69.86
1 3/4	70.63	70.77	70.70
2	71.02	71.20	71.11
3	73.52	72.54	73.94
4	74.21	74.35	74.28
5	74.81	75.18	75.00
6	75.37	75.53	75.45
7	75.95	75.67	75.81
8	76.37	76.27	76.32
16	75.32	75.39	75.36
24	76.85	76.73	76.79

Table 18 : Test Weight Over a 24 Hour Period Using Pectinex SMASH

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	83.35	83.27	83.31
1/4	69.17	68.80	68.99
1/2	68.08	68.1	68.09
3/4	67.69	67.69	67.69
1	68.42	68.17	68.30
1 1/4	69.05	68.98	69.02
1 1/2	70.18	70.35	70.72
1 3/4	71.73	72.25	71.99
2	72.17	72.32	72.25
3	74.23	74.03	74.13
4	75.39	75.51	75.45
5	76.18	76.27	76.23
6	76.51	76.55	76.53
7	76.78	76.48	76.63
8	76.78	76.85	76.82
16	76.09	76.00	76.05
24	76.94	76.97	76.96

Table 19 : Test weight Over a 24 Hour Period Using Viscozyme L

Time (hours)	Hectolitre Weight (kg/HL) Duplicate One	Hectolitre Weight (kg/HL) Duplicate Two	Hectolitre Weight (kg/HL) Average
0	83.31	83.27	83.31
1/4	68.91	69.01	68.96
1/2	67.50	67.31	67.41
3/4	67.48	67.55	67.52
1	67.82	67.87	67.85
1 1/4	69.28	69.35	69.32
1 1/2	70.42	70.35	70.39
1 3/4	71.34	71.97	71.66
2	72.59	73.29	72.94
3	73.54	73.71	73.63
4	75.04	75.18	75.11
5	75.88	75.93	75.91
6	76.69	76.37	76.53
7	76.44	76.67	76.56
8	77.01	76.92	76.97
16	75.77	75.86	75.82
24	77.62	77.61	77.62

Table 20 : Flour Data

Time (hours)	Flour Yield (%)	Moisture (%)	Starch Damage (%)	Ash (14% m.b)	Colour Grade	Minolta Tristimulus Colour				Branscan Average	
						L	a	b	L-b	Area (%)	Speck Number
0	82.3	12.3	4.6	0.63	1.4	90.86	0.25	9.85	81.01	0.35 ± 0.02	4.68 ± 0.46
1	80.4	14.1	4.6	0.60	0.3	91.27	0.19	9.36	81.91	0.16 ± 0.02	2.05 ± 0.27
2	80.3	14.1	4.6	0.59	0.0	91.66	0.15	9.16	82.50	0.13 ± 0.01	1.68 ± 0.36
3	80.5	14.1	4.8	0.57	-0.1	91.71	0.12	9.14	82.57	0.17 ± 0.02	2.36 ± 0.38
4	80.7	13.9	4.9	0.56	-0.4	91.61	0.15	9.09	82.52	0.14 ± 0.01	2.05 ± 0.34
6	80.7	14.0	4.4	0.53	-0.4	91.75	0.10	9.08	82.67	0.19 ± 0.02	2.55 ± 0.31
8	80.6	14.0	4.4	0.58	-0.6	91.80	0.11	9.07	82.73	0.17 ± 0.02	2.18 ± 0.33
16	80.1	14.7	4.0	0.56	-0.8	91.89	0.06	9.04	82.85	0.11 ± 0.01	1.73 ± 0.26

Table 21: Flour Data

Time (hours)	Flour Yield (%)	Moisture (%)	Starch Damage (%)	Ash (14% m.b)	Colour Grade	Minolta Tristimulus Colour				Branscan Average	
						L	a	b	L-b	Area (%)	Speck Number
0	82.3	12.3	4.6	0.63	1.4	90.86	0.25	9.85	81.01	0.35 ± 0.02	4.68 ± 0.46
1	79.5	13.9	4.8	0.56	-0.5	91.62	0.14	9.21	82.41	0.15 ± 0.01	1.77 ± 0.32
2	79.7	13.9	4.4	0.58	-0.7	91.79	0.11	9.00	82.79	0.14 ± 0.01	1.50 ± 0.23
3	80.1	13.7	4.6	0.57	-0.9	91.62	0.10	9.06	82.56	0.14 ± 0.01	2.32 ± 0.37
4	80.5	13.6	4.6	0.55	-0.7	91.71	0.08	9.06	82.65	0.15 ± 0.01	2.45 ± 0.31
6	80.6	13.9	4.6	0.57	-1.1	91.79	0.09	9.06	82.73	0.16 ± 0.01	2.59 ± 0.32
8	80.5	14.2	4.8	0.55	-0.9	91.88	0.08	8.99	82.89	0.14 ± 0.02	1.36 ± 0.25
16	80.1	14.5	4.4	0.57	-0.3	91.83	0.10	8.96	82.87	0.14 ± 0.01	2.18 ± 0.28

Table 22 : Flour Data for Pectinex SMASH

Time (hours)	Flour Yield (%)	Moisture (%)	Starch Damage (%)	Ash (14% m.b)	Colour Grade	Minolta Tristimulus Colour				Branscan Average	
						L	a	b	L-b	Area (%)	Speck Number
0	82.3	12.3	4.6	0.63	1.4	90.86	0.25	9.85	81.01	0.35 ± 0.02	4.68 ± 0.46
1	79.8	13.5	4.8	0.61	0.0	91.58	0.19	9.15	82.43	0.17 ± 0.02	2.32 ± 0.33
2	79.9	13.4	4.9	0.6	-0.3	91.67	0.12	9.10	82.57	0.18 ± 0.01	2.64 ± 0.35
3	80.0	13.5	4.8	0.58	-0.5	91.67	0.13	9.13	82.54	0.16 ± 0.01	2.41 ± 0.36
4	80.6	13.3	4.9	0.57	-0.3	91.80	0.07	9.01	82.79	0.16 ± 0.01	2.23 ± 0.29
6	80.8	12.9	4.8	0.59	-0.1	91.59	0.12	9.18	82.41	0.17 ± 0.02	2.55 ± 0.34
8	80.4	13.4	4.6	0.59	-0.2	91.66	0.10	9.16	82.50	0.15 ± 0.01	1.94 ± 0.34
16	80.1	13.7	4.6	0.55	-0.2	91.65	0.14	9.06	82.59	0.17 ± 0.01	2.27 ± 0.29

Table 23 : Flour Data for Viscozyme L

Time (hours)	Flour Yield (%)	Moisture (%)	Starch Damage (%)	Ash (14% m.b)	Colour Grade	Minolta Tristimulus Colour				Branscan Average	
						L	a	b	L-b	Area (%)	Speck Number
0	82.3	12.3	4.6	0.63	1.4	90.86	0.25	9.85	81.01	0.35 ± 0.02	4.68 ± 0.46
1	79.8	13.2	5.2	0.57	-0.7	91.57	0.17	9.20	82.37	0.17 ± 0.01	2.14 ± 0.37
2	81.2	13.1	5.2	0.60	-0.3	91.53	0.20	8.99	82.54	0.20 ± 0.01	3.09 ± 0.35
3	80.6	13.4	4.8	0.59	-0.7	91.72	0.12	9.10	82.62	0.20 ± 0.01	3.73± 0.48
4	80.7	13.3	4.8	0.58	-0.5	91.70	0.15	8.96	82.74	0.19 ± 0.02	3.00 ± 0.31
6	80.7	13.4	4.8	0.59	-0.7	91.71	0.09	9.05	82.66	0.18 ± 0.01	2.73 ± 0.36
8	81.2	12.9	4.8	0.57	-0.6	91.64	0.11	9.08	82.56	0.20 ± 0.02	3.50 ± 0.41
16	82.0	12.4	4.4	0.56	-0.3	91.58	0.14	9.16	82.42	0.23 ± 0.02	3.75 ± 0.41

Table 24 : Dough Rheology Data

Time (hours)	Farinograph		Extensograph		RVA		
	Water Abs (%)	Development Time (min)	Extensibility (cm)	Maximum Resistence (BU)	Peak Viscosity RVU	Break Down RVU	Final Viscosity RVU
0	62.4	6.2	26.2	445	347	134	360
1	59.9	9.0	26.0	540	350	136	360
2	59.5	10.0	26.4	485	351	122	369
3	59.4	9.9	24.7	590	354	128	369
4	60.4	9.2	26.5	550	357	127	371
6	59.6	9.1	26.5	490	340	118	360
8	59.9	9.5	25.8	500	344	119	360
16	58.2	8.6	25.9	550	336	115	361

Table 25 : Dough Rheology Data

Time (hours)	Farinograph		Extensograph		RVA		
	Water Abs (%)	Development Time (min)	Extensibility (cm)	Maximum Resistence (BU)	Peak Viscosity RVU	Break Down RVU	Final Viscosity RVU
0	62.4	6.2	26.2	445	347	134	360
1	60.5	9.5	26.4	445	326	113	353
2	60.4	10.0	26.5	445	320	105	351
3	60.4	10.0	26.4	520	324	113	349
4	60.8	9.4	26.4	485	328	119	351
6	60.7	10.0	26.1	465	315	103	345
8	60.0	9.6	26.4	470	327	117	347
16	59.5	10.6	26.0	425	319	106	347

Table 26 : Dough Rheology Data

Time (hours)	Farinograph		Extensograph		RVA		
	Water Abs (%)	Development Time (min)	Extensibility (cm)	Maximum Resistence (BU)	Peak Viscosity RVU	Break Down RVU	Final Viscosity RVU
0	62.4	6.2	26.2	445	347	134	360
1	61.0	9.9	26.0	465	338	120	358
2	60.6	9.0	25.9	500	345	115	369
3	60.4	10.2	25.9	535	345	120	366
4	61.1	9.2	24.9	545	353	127	369
6	62.1	9.0	26.6	490	365	131	379
8	61.0	8.8	24.2	495	345	123	363
16	60.2	9.8	26.5	435	350	121	370

Table 27 : Dough Rheology Data

Time (hours)	Farinograph		Extensograph		RVA		
	Water Abs (%)	Development Time (min)	Extensibility (cm)	Maximum Resistence (BU)	Peak Viscosity RVU	Break Down RVU	Final Viscosity RVU
0	62.4	6.2	26.2	445	347	134	360
1	61.7	10.3	26.2	490	355	125	373
2	61.8	8.5	26.2	485	355	133	364
3	61	8.3	25.1	520	351	123	369
4	61.5	10.1	26.5	475	351	124	370
6	61.4	8.8	26.3	470	353	125	368
8	61.8	8.6	25.8	530	360	130	374
16	62.4	8.6	26.5	460	357	128	376

Table 28 : Baking Data

Time (hours)	Farino Water Abs %	Bakery Water Abs %	Volume cc	Volume Score /36	Oven Spring /10	External App'ance /10	Internal Texture /15	Internal Structure /15	Crumb Colour /14	Total /100	Minolta		
											L	a	b
0		58	840	27.6	4	6	7.5	7.5	8.4	61	72.59	-0.84	10.05
1		58	837.5	27.5	4	6	7.5	9.0	8.4	62	75.36	-1.10	10.25
2		58	857.5	28.3	5	6	7.5	7.5	8.4	63	74.70	-1.13	9.14
3		58	815	26.6	3	5	7.5	9.0	8.4	60	77.52	-1.15	10.29
4		58	862.5	28.5	4	6	7.5	7.5	8.4	62	74.09	-1.16	8.88
6		58	865	28.6	5	5	9.0	9.0	8.4	65	75.80	-0.98	9.69
8		58	867.5	28.7	4	6	7.5	9.0	8.4	64	77.35	-1.14	10.37
16		58	850	28.0	4	5	7.5	7.5	8.4	60	76.93	-1.15	10.17

Table 29 : Baking Data

Time (hours)	Farino Water Abs %	Bakery Water Abs %	Volume cc	Volume Score /36	Oven Spring /10	External App'ance /10	Internal Texture /15	Internal Structure /15	Crumb Colour /14	Total /100	Minolta		
											L	a	b
0		58	840	27.6	4	6	7.5	7.5	8.4	61	72.59	-0.84	10.05
1		58	875	29.0	3	5	7.5	9.0	8.4	62	75.31	-1.04	9.14
2		58	865	28.6	5	6	9.0	9.0	8.4	66	75.76	-1.10	9.20
3		58	872.5	28.9	4	5	7.5	9.0	8.4	63	74.78	-1.03	9.79
4		58	897.5	28.9	5	5	7.5	9.0	8.4	65	74.91	-1.15	9.02
6		58	865	28.6	4	6	7.5	9.0	8.4	64	72.33	-1.05	8.63
8		58	865	28.6	3	6	7.5	9.0	8.4	63	72.73	-1.06	8.58
16		58	867.5	28.7	3	6	7.5	9.0	8.4	63	73.82	-1.20	8.26

Table 30 : Baking Data

Time (hours)	Farino Water Abs %	Bakery Water Abs %	Volume cc	Volume Score /36	Oven Spring /10	External App'ance /10	Internal Texture /15	Internal Structure /15	Crumb Colour /14	Total /100	Minolta		
											L	a	b
0		58	840	27.6	4	6	7.5	7.5	8.4	61	72.59	-0.84	10.05
1		59	855	28.2	4	5	7.5	9.0	8.4	62	77.11	-1.01	9.95
2		59	832.5	27.3	4	5	7.5	9.0	8.4	61	75.95	-1.03	9.80
3		59	845	27.8	4	5	7.5	9.0	8.4	62	73.90	-1.06	10.43
4		59	870	28.8	4	6	7.5	7.5	8.4	62	76.78	-1.10	9.11
6		59	857.5	28.3	4	6	7.5	7.5	8.4	62	73.82	-1.04	8.96
8		59	852.5	28.1	3	6	7.5	7.5	8.4	61	75.59	-1.07	9.59
16		59	882.5	29.3	5	5	9.0	9.0	8.4	66	78.09	-0.85	9.90

Table 31 : Baking Data

Time (hours)	Farino Water Abs %	Bakery Water Abs %	Volume cc	Volume Score /36	Oven Spring /10	External App'ance /10	Internal Texture /15	Internal Structure /15	Crumb Colour /14	Total /100	Minolta		
											L	a	b
0		58	840	27.6	4	6	7.5	7.5	8.4	61	72.59	-0.84	10.05
1		59	857.5	28.3	4	5	7.5	9.0	8.4	62	73.67	-1.04	9.86
2		59	840	27.6	3	5	7.5	7.5	8.4	59	74.41	-1.08	10.08
3		59	852.5	28.1	3	5	7.5	7.5	8.4	60	76.97	-1.03	10.15
4		59	857.5	28.3	3	5	7.5	7.5	8.4	60	77.61	-0.95	10.40
6		59	847.5	27.9	3	5	7.5	9.0	8.4	61	74.56	-1.14	9.90
8		59	845	27.8	3	5	7.5	9.0	8.4	61	75.66	-1.04	9.99
16		59	845	27.8	3	5	7.5	9.0	8.4	61	74.74	-1.03	9.59

APPENDIX TWO

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(54) Title: A PROCESS FOR CONDITIONING GRAIN

(57) Abstract

By the addition of enzyme(s) the efficiency of the conditioning process for the treatment of grain prior to milling can be substantially improved thereby providing for a substantial increase in the yield of flour, and/or reduction of the conditioning time, and/or improvement of the rheological properties of the flour/bran produced.

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Title: A Process for Conditioning Grain

FIELD OF THE INVENTION

The present invention relates to a process for conditioning grain in connection with the milling of flour. The invention furthermore relates to compositions for use in such a process.

BACKGROUND OF THE INVENTION

The milling of grain into flour etc. generally comprises a number of steps of which the first step is the conditioning of the grain to prepare it for the actual grinding and separation processes that ultimately provide for a number of final products, such as flour(s), and bran(s).

A number of conditioning processes have been developed for the industrial milling of wheat. The object of conditioning, the central feature of which is the addition of water to grain, is to modify the wheat kernel so that milling can be performed under optimal conditions. Water is added, usually to obtain a moisture content of =16%, and, after storage, which generally lasts several hours, additional water is often added before milling. The optimal amount of water as well as tempering time depends on the properties of the grain. When used, heat is also an important factor, with the mode of application varying as to the process. Conditioning influences not only milling quality but also the technological quality of the end flour product.

The primary aim of conditioning is to change the mechanical characteristics of the different tissues of the kernel and thereby improve the separability of the endosperm from the outer layers of the grain, notably the bran. The addition of water also triggers a number of biochemical reactions in the kernel, thereby modifying characteristics of its components. These modifications can be amplified by increasing the temperature and the moisture content.

However, improvements in the conditioning of grain in order to obtain an improved separation between the various components, and especially improvements in the yield of the flour in relation to the yield of the bran, are still in demand.

SUMMARY OF THE INVENTION

It has now surprisingly been found that by adding enzyme(s) the efficiency of the grain conditioning process can be substantially improved thereby providing for a substantial increase in the yield of flour, and/or reduction of the conditioning time, and/or improvement of the properties of the flour/bran produced.

DETAILED DESCRIPTION OF THE INVENTION

According to the invention a process for the conditioning of grain is provided, wherein the grain is treated with an enzyme preparation.

It has been found that the process of the invention enhances the yield of flour obtained thereby increasing the value of product, as well as improving the rheological properties of the flour, thereby increasing the usefulness of the product so obtained.

Preferably, the process of the invention provides an enhanced yield of flour.

Accordingly, an embodiment of the invention relates to a process for the conditioning of a grain of the invention, wherein an increased yield of flour is obtained as compared to the yield of flour obtained when performing the same process without the addition of the enzyme preparation.

Examples of such increased flour yields, obtained by a process of the invention, are demonstrated in working example 1 and 3 herein (*vide infra*).

A further advantage that may be a reduced conditioning time while the present flour yield is still obtained thereby increasing the throughput in the milling plant.

See working example 2 (*vide infra*) for an example of such a reduced conditioning time while the present flour yield is still obtained.

One example of an enzyme preparation according to the invention is an enzyme complex as described in US Patent No. 4,478,939 or equivalent enzyme complexes. Another example of an enzyme preparation according to the invention is at least one enzyme activity, or a mixture of the enzyme activities selected

from the group comprising proteases, cellulases, pectinases, hemicellulases, xylanases, glucanases, β-glucanases, glucose oxidases, laccases, and amylases.

Such enzyme complexes are available from Novo Nordisk A/S and are known under trade names such as Cereszyme™ or Viscozyme®, or as SP249; or they can be produced, for example as described in US Patent No. 4,478,939, by fermentation of micro-organisms such as fungi, especially filamentous fungi, such as those belonging to the genus *Aspergillus*, especially *A. aculeatus*, and *A. japonicus*.

The single enzyme activity or the preparations comprising mixtures of such activities can be produced according to known techniques.

Among the activities mentioned above it has been found that the most important ones are pectinase, hemicellulase, xylanase, cellulase, glucose oxidase, and laccase activities.

When performing the process of the invention it is preferred that the enzyme preparation is added in an amount of between 1 g or cm^3 enzyme preparation per t of grain and 50000 g or cm^3 enzyme preparation per t of grain, preferably between 10 g or cm^3 enzyme preparation per t of grain and 2000 g or cm^3 enzyme preparation per t of grain, and most preferably between 100 g or cm^3 enzyme preparation per t of grain and 2000 g or cm^3 enzyme preparation per t of grain.

It has been found that the best economy in respect of performing the complete milling process including the grinding of the grain into flour and bran is obtained if the process of the invention is performed for a time period of from about 4 hours to about 32 hours, preferably from about 6 hours to about 24 hours.

Generally, the preferred conditioning time depends on the actual type of grain and in particular whether it is a soft, mid hard or hard grain.

In the present context a "soft grain" denotes a grain with the following average characteristics: W=80-150, P/L=0.2-0.5 as measured on an Alveograph (W: strength; P: Tenacity; L: extensibility);

60

a "mid hard grain" denotes a grain with the following average characteristics W=150-300, P/L= 0.5-0.8 as measured on an Alveograph (W: strength; P: Tenacity; L: extensibility); and

a "hard grain" denotes a grain with the following average characteristics: W=300-400, P/L= 0.8-1 as measured on an Alveograph (W: strength; P: Tenacity; L: extensibility).

For further details concerning the measurement of said grain characteristic with an Alveograph reference is made to the Materials and Methods section herein (*vide infra*).

Preferably, when the grain is a soft or mid hard grain the process of the invention is performed for a time period of from about 4 hours to about 18 hours, preferably from about 4 hours to about 12 hours; and

when the grain is a hard grain the process of the invention is performed for a time period of from about 12 hours to about 32 hours, more preferably from about 12 hours to about 28 hours and most preferably from about 15 hours to about 24 hours.

The process of the invention has been found to provide fine results if the grain has a humidity of 10% to 50%.

In this context humidity means % water present in the grain during the conditioning process.

Also, it has been found that the process of the invention works best if the treatment is performed at a temperature between 5°C and 60°C, preferably between 10°C and 40°C, and more preferably between 20°C and 30°C.

The process of the invention can be used for the conditioning of any cereal grain, such as barley, rye, corn, rice,or legume grain, such as alfalfa, soy beans.

Preferably the grain is wheat.

Examples of industries which advantageously may use a process of the invention are industries such as the

i) the milling industry for *e.g.* getting a higher yield of flour;

ii) the brewing industry to *e.g.* get an improved malt from barley; and

iii) the starch industry to e.g. get a higher yield of flour or to modify the flour composition such as an fibre enrichment of the flour.

Accordingly, further embodiments of the invention relate to use of a process for the conditioning of grain, according to the invention, in the milling industry; the brewing industry and/or the starch industry.

MATERIALS AND METHODS

Grain: Wheat that has a humidity or moisture content below 16% as available on the market.

Enzyme preparations:

Cereszyme® (Novo Nordisk A/S, Denmark): A mixture of hemicellulase, cellulase and pectinase activities with other minor activities.

Viscozyme® (Novo Nordisk A/S, Denmark): An enzyme complex produced by a filamentous fungus of the genus *Aspergillus*, especially from the species A. *aculeatus*.

Activities present in Viscozyme®

Pectinases:

Pectin lyase, pectin esterase, polygalacturonase I, polygalacturonase II, polygalacturonase III, rhamnogalacturonan acetyl esterase, rhamnogalacturonase I, rhamnogalacturonase II, pectin acetyl esterase, rhamnosidase(s), galacturonosidase(s), glucoronosidase(s).

Proteases:

Protease I, protease II, exo peptidases.

Esterases, Lipases:

lipase(s), phospholipase(s), feroylic acid esterase.

Amylases:

α-amylase(s), β-amylase(s), α-glucosidase(s), β-glucosidase(s).

Chitinases:

endo chitinase, exo chitinase.

Hemicellulases, Cellulases:
Arabinase, arabinofuranosidase I, arabinofuranosidase II, endoglucanase I, endoglucanase II, endoglucanase III, endoglucanase IV, galactanase, α-galactosidase, β-galactosidase, mannanase, xylan acetyl esterase, xylanase I, xylanase II, xylanase III, xylo galacturonase, mannosidase, manan acetyl esterase, cellobioase(s).

Assessment of flour quality:
Flour Yields:
A CHOPIN Laboratory Mill (model CD1) available from Chopin, France was used for the milling of the grain after conditioning. The mill separates the ground wheat into two fractions, flour and bran.

Rheological properties:
The rheological properties of the flour produced were examined using an Alveograph (available from CHOPIN, France) in accordance with the manufacturer's protocol.

These measurements provided the following properties:

P: Tenacity
L: Extensibility
W: Strength

expressed in mm.

EXAMPLES

EXAMPLE 1

Wheat treatment with Cereszyme® during conditioning time:

Three tests were performed using the following amounts and conditions:

1: 600 g of wheat, wetted to 16% humidity, resting 24 hours (standard).

2: 600 g of wheat, wetted to 16% humidity, resting 24 hours + 400 cm^3 Cereszyme® per ton grain

3: 600 g of wheat, wetted to 16% humidity, resting 6 hours + 400 cm^3 Cereszyme® per ton grain

The results from the milling in the Chopin mill and the Alveograph measurements are indicated in Table I and Table II, respectively.

Table I: Flour Yields

Test No.	Bran g	Flour g	Conditioning time Hours	Yield Improvements %
1.	205	350	24	-
2.	187	381	24	+8.9%
3.	189	365	6	+4.5%

Table II: Rheological properties

Test No.	P	L	P/L	W	W Improvement %
1.	55	116	0.52	206	-
2.	60	118	0.56	222	+7%
3.	-	-	-	-	-

From Tables I and II it is seen that the conditioning process of the invention provides for a substantial improvement in respect of both yield and rheological properties of the flour produced. It can also be seen that even when reducing the conditioning time substantially (24 hours ⇒ 6 hours) a substantial improvement was obtained in the yield.

EXAMPLE 2

Wheat treatment with Viscozyme® during conditioning time:

Two types of wheat grain ((A) German wheat check and (B) French wheat check) were tested by conditioning samples thereof

without enzyme for 14 hours and with an enzyme product (Viscozyme®) for different periods.

Both types of wheat are considered mid hard wheat with average characteristics W=150-300, P/L= 0.5-0.8, measured on an Alveograph as described above.

The tests were performed by using 500 cm^3 enzyme per ton grain

Tables III and IV show the results.

Table III Flour yields

Grain A + Enzyme	Resting time (hours)	Yield %	Moisture	Protein/ dry subst.
None	14	74	15.3	13.89
SP249	4	75.8	15.8	13.77
SP249	8	75.8	16	13.62
SP249	12	72.4	15	13.82
SP249	16	74.2	16.1	13.6
SP249	20	76	15.9	14
SP249	24	63.3	15.8	13.86
Grain B + Enzyme				
None	14	70.4	15.3	10.62
SP249	4	70.2	15.5	10.24
SP249	8	71.8	16.2	10.21
SP249	12r	73.8	15.8	10.39
SP249	16	71	15.7	9.96

Table IV Rheological Properties

Grain A + Enzyme	Falling	W	P/L	W 2 hours	Degradation 2 hours
None	351	295	0.56	307	0
SP249	324	356	0.61	326	8.4
SP249	342	353	0.64	342	3.1
SP249	341	340	0.5	313	7.9
SP249	306	366	0.56	352	4
SP249	346	333	0.61	322	3.3
SP249	360	363	0.57	314	13.5
Grain B + Enzyme					
None	278	200	0.6	182	9
SP249	272	256	0.72	241	5.9
SP249	289	241	0.61	227	5.8
SP249	275	248	0.53	217	12
SP249	289	205	0.64	181	12

The results confirm once more that a considerably shorter conditioning time can be used by the process of the invention and still obtain the same yields as obtained by the traditional conditioning time.

Viscozyme is a multi-enzyme complex comprising a wide range of carbohydrases, including arabinase, cellulase, β-glucanase, hemicellulase and xylanase. A more detailed listing of the activities present are given above.

EXAMPLE 3

Evaluation of the influence on yields of flour a hard wheat pre-treated with Viscozyme during conditioning time.

Wheat: GAZUL, hard wheat, average characteristics: W=300-400, P/L= 0.8-1.

Enzyme Dose: 400cm3 of Viscozyme pr 1ton of wheat.

Conditioning time: 24 hours

Assessments: Alveograph parameters: W: Baking Strength, P: Tenacity, L: Extensibility, P/L: ratio. (see above)

Table V: Yields of flour produced from wheat processed.

	W	P	L	P/L	YIELDS %
Wheat	393	85	147	0.57	73.4
Wheat + VISCOZYME	348	78	143	0.54	78.2

Technical Conclusions:

The process of the invention significantly increases the flour yield obtained from the hard wheat.

PATENT CLAIMS

1. A process for the conditioning of a grain characterised in that the grain is treated with an enzyme preparation.

2. The process of claim 1, wherein said treatment is performed for a period of time from about 4 hours to about 32 hours, preferably from about 6 hours to about 24 hours.

3. The process of claim 1 or 2, wherein said grain is a soft grain or a mid hard grain and wherein said treatment is performed for a period of time from about 4 hours to about 18 hours, preferably from about 4 hours to about 12 hours.

4. The process of claim 1 or 2, wherein said grain is a hard grain and wherein said treatment is performed for a period of time from about from about 12 hours to about 32 hours, more preferably from about 12 hours to about 28 hours and most preferably from about 15 hours to about 24 hours.

5. The process of any of claims 1 to 4, wherein said enzyme preparation comprises at least one enzyme activity selected from the group comprising proteases, cellulases, pectinases, hemicellulases, xylanases, glucanases, β-glucanases, glucose oxidase, laccase and amylases.

6. The process of claim 5, wherein said at least one enzyme activity is chosen from the group comprising pectinase, hemicellulase, xylanase, cellulase, glucose oxidase, and laccase activities.

7. The process of claims 5 or 6, wherein said enzyme preparation is an enzyme complex known as SP 249 or by the trade names Cereszyme® or Viscozyme®.

8. The process of claims 5 or 6, wherein said enzyme preparation is an enzyme complex that can be produced as described

in US Patent No. 4,478,939 by fermentation of microorganisms such as fungi, especially filamentous fungi, such as those belonging to the genus *Aspergillus*, especially *A. aculeatus*, and *A. japonicus*.

9. The process of any of claims 1 to 8, wherein said grain is selected from the group comprising cereal grain, barley, rye, corn, rice, and legume grains, such as alfalfa, soy bean, and preferably wheat.

10. The process of any of claims 1 to 9, wherein said grain has a humidity content of 5% to 50%, preferably from 10% to 40% or from 12% to 30%.

11. The process of any of claims 1 to 10, wherein said treatment is performed at a temperature between 5°C and 60°C, preferably between 10°C and 40°C, and more preferably between 20°C and 30°C.

12. The process of any of claims 1 to 11, wherein said treatment is performed by the addition of said enzyme preparation in an amount of between 1 g or cm^3 enzyme preparation per t of grain and 50000 g or cm^3 enzyme preparation per t of grain, preferably in an amount of between 10 g or cm^3 enzyme preparation per t of grain and 2000 g or cm^3 enzyme preparation per t of grain, and most preferably in an amount of between 100 g or cm^3 enzyme preparation per t of grain and 2000 g or cm^3 enzyme preparation per t of grain.

13. The process of any of the preceding claims, wherein an increased yield of flour is obtained as compared to the yield of flour obtained when performing the same process without the addition of the enzyme preparation.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 98/00460

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: B02B 1/00, C12S 3/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: B02B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	File WPI, Derwent accession no. 89-237436, Godo Shusei KK: "Grain milling improvement - by admixing cellulose with pulverising;" & JP,A,1171647, 890706, DW8933 --	1-13
X	WO 8504556 A1 (LEWIS, VICTOR, MARCUS), 24 October 1985 (24.10.85), see example 2 --	1-12
X	US 5662901 A (JAMES F. TOBEY, JR. ET AL), 2 Sept 1997 (02.09.97) --	1-12

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 February 1999	08 -02- 1999
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

21/12/98

International application No.
PCT/DK 98/00460

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8504556 A1	24/10/85	AU 583817 B	11/05/89
		AU 4082485 A	17/10/85
		EP 0181874 A,B	28/05/86
		US 4810506 A	07/03/89
US 5662901 A	02/09/97	NONE	

APPENDIX THREE

Starch / 2001-11709-01

Product Sheet

Shearzyme® 500 L

Description

Shearzyme 500 L is a purified xylanase produced by a genetically modified strain of *Aspergillus oryzae* (host), which has received the gene for xylanase from a strain of *Aspergillus aculeatus* (donor).

Product Properties

Appearance

Shearzyme 500 L is a brown liquid with a density of approx. 1.2 g/ml.

Product type

Shearzyme 500 L	Declared activity: 500 FXU(S)/g

Activity

The endo-xylanase activity (Fungal Xylanase Unit) is measured relative to a Novozymes FXU enzyme standard.
See the Analytical Method for further information.

Food-grade status

Shearzyme 500 L complies with FAO/WHO JECFA and FCC recommended purity specifications.

Standard Packaging

See the standard Packaging List for more packaging information.

Application

Shearzyme 500 L has a high specificity towards the soluble pentosan fraction in wheat. Shearzyme 500 L is virtually free of amylase and protease side activities.

Shearzyme 500 L is used in wheat separation, the process where flour is separated into gluten and starch. Shearzyme 500 L is added "upfront" in the process, whereby a range of advantages are obtained: better separation resulting in purer fractions; increased plant capacity and reduced processing times; reduced water and energy consumption.

Reaction Parameters

Activity and stability

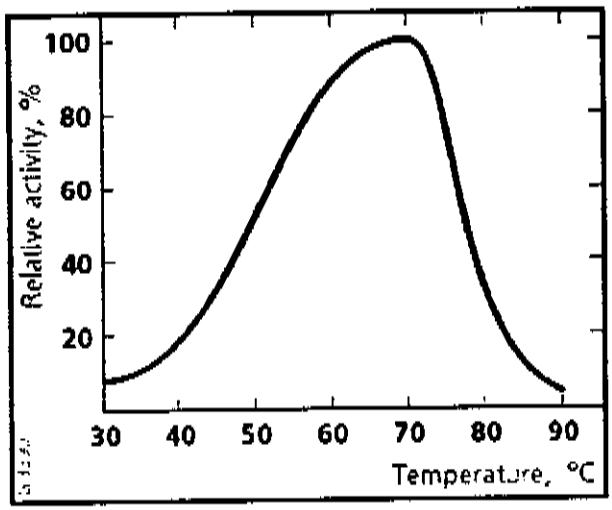

Fig. 1. The effect of temperature on activity of Shearzyme 500 L.

Substrate: Azo-wheat arabinoxylan
pH: 4.0
Reaction time: 10 minutes

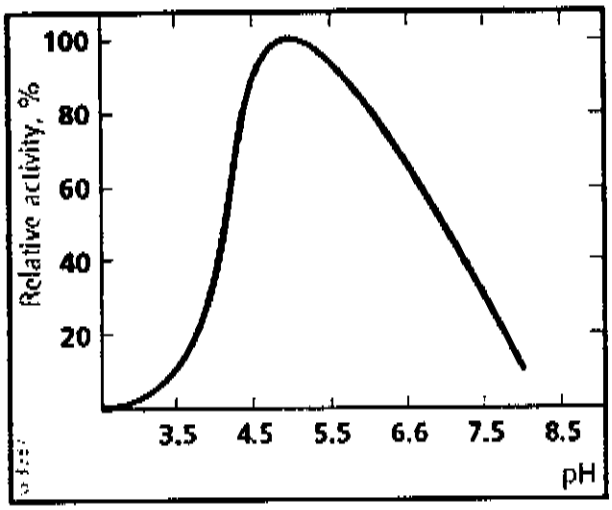

Fig. 2. The effect of pH on activity of Shearzyme 500 L.

Substrate: Azo-wheat arabinoxylan
Temperature: 70°C (158°F)
Reaction time: 10 minutes

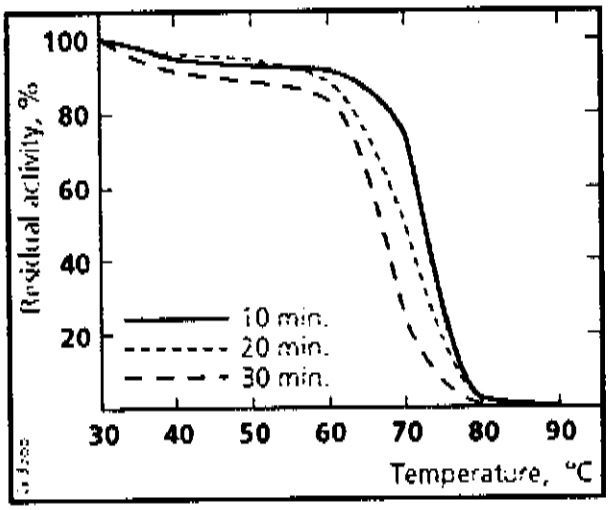

Fig. 3. The effect of temperature on stability of Shearzyme 500 L.

Substrate: Azo-wheat arabinoxylan
pH: 5.0

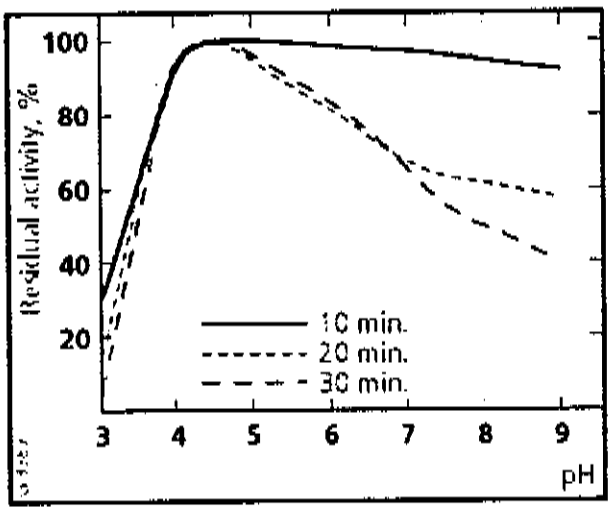

Fig. 4. The effect of pH on stability of Shearzyme 500 L.

Substrate: Azo-wheat arabinoxylan
Temperature: 50°C (122°F)

Safety

Enzymes are proteins and inhalation of dust or aerosols may induce sensitization and may cause allergic reactions in sensitized individuals. Some enzymes may irritate the skin, eyes and mucous membranes upon prolonged contact.

The product may create easily inhaled aerosols if splashed or vigorously stirred. Spilled product may dry out and create dust.

Spilled material should be flushed away with water (avoid splashing). Left-over material may dry out and create dust.

A Material Safety Data Sheet is supplied with all products. See the Safety

Manual for further information regarding how to handle the product safely.

Storage

Enzymes gradually lose activity over time depending on storage temperature. Cool and dry conditions are recommended. When stored in closed containers at 25°C (77°F), the product will maintain its declared activity for 3 months. When stored in closed containers at 0-10°C (32-50°F), the product will maintain its declared activity for 6 months. Extended storage and/or adverse conditions, including higher temperature or high humidity, may lead to higher dosage requirement.

Laws, regulations and third party rights may prevent customers from importing, processing, applying and/or reselling certain products in a given manner. It is the responsibility of the customers that their specific use of products from Novozymes does not infringe relevant laws and regulations and, furthermore, does not infringe patents or other third party rights.

The contents of this document are subject to change without further notice.

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Product Specification

Enzyme Business

Shearzyme 500 L

Valid from 2001-9-12

Appearance:	Light brown to brown liquid
Enzyme:	Endo-xylanase
Diluents:	Glycerol, Sorbitol
Preservatives:	Potassium sorbate, Sodium benzoate

Analysis name	Lower limit	Upper limit	Unit
Fungal Xylanase Units FXU	500	570	/g
Density	-	-	g/ml
Total Viable Count	-	50000	/g
Coliform Bacteria	-	30	/g
Enteropathogenic E.Coli	None Detected		/25g.
Salmonella	None Detected		/25g.

The product complies with the following FCC and JECFA requirements: Antibiotic activity (Not detected), Mycotoxins (Not detected), Heavy metals (< 30 ppm), Lead (< 5 ppm), Arsenic (< 3 ppm).

Enzyme Business

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Novo Allé
2880 Bagsvaerd
Denmark

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Fax +45 4444 1021
Telex 37560

MSDS

Enzyme Material Safety Data Sheet

1. Identification of the Preparation and of the Company

Hazardous according to criteria of Worksafe Australia

Commercial product name:	Shearzyme 500 L
Description:	Aqueous enzyme preparation
Appearance:	Clear brown to brown liquid
Responsible company:	Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd
Information phone number:	+ 45 88 24 99 99
Importer:	Novozymes Australia Pty. Ltd. 22 Loyalty Road North Rocks N. S. W.
Phone:	02 9630 8466
Fax:	02 9683 1170

2. Information on Ingredients

Chemical characterization of active component:	Enzyme protein
Synonyms:	Xylanase endo-1,4-
IUB number:	3.2.1.8
CAS number:	9025-57-4
EINECS number:	232-800-2
Hazardous ingredients:	Enzyme protein (1-10%)
Non-hazardous ingredients:	Up to 100%
Classification of preparation:	Xn (harmful), R-42 , R-36/37/38
Exposure limit:	Not established
Hazchem code:	No Hazchem code allocated

UN number:	No UN number allocated
Poisons schedule Number:	No poisons schedule number allocated
Use:	Processing aid for food productionICATION

3. Hazards Identification

Liquid enzyme preparations are dustfree preparations. However, inappropriate handling may cause formation of dust or aerosols. For appropriate handling, see section 6 and 7. Inhalation of enzyme dust or aerosols resulting from inappropriate with handling may induce sensitisation and may cause allergic reactions in sensitised individuals. Prolonged skin contact may cause minor irritation.

4. First Aid Measures

Skin contact:	Wash skin with plenty of water.
Eye contact:	Rinse eyes with plenty of water.
Ingestion:	Rinse mouth and throat thoroughly with water. Drink water.
Inhalation:	Remove from exposure. If symptoms of irritation or sensitisation occur (shortness of breath, wheezing or laboured coughing), call a doctor.

5. Fire-fighting Measures

Protection against fire and explosion:	No special requirements
Suitable fire extinguishing media:	Water, foam
Non-suitable media:	None
Special exposure hazards:	None

6. Accidental Release Measures

Spilled preparation should be removed immediately to avoid formation of dust from dried preparation. Take up by mechanical means, preferably by a vacuum cleaner equipped with a high-efficiency filter. Flush the remainder carefully with plenty of water. Avoid splashing and high-pressure washing (avoid formation of aerosols). Ensure sufficient ventilation. Wash contaminated clothing.

7. Handling and Storage

Avoid formation of aerosols and dust from drying out of spilled preparation. Avoid splashing and high-pressure washing. Ensure good ventilation of the room when handling this preparation. Store container in a cool place.

8. Exposure Control/ Personal Protection

RECOMMENDED PERSONAL PROTECTION EQUIPMENT:

Respiratory protection:	Respirator with P3 filter
Hand protection:	Impermeable gloves
Eye protection:	Protective glasses or eye shield
Clothing:	Wear suitable protective clothing

9. Physical and Chemical Properties

Appearance:	Clear brown to brown liquid
Odour:	Slight fermentation odour

pH, boiling point, melting point, flash point, ignition temperature, vapour pressure, density and solubility are not relevant to safety. For further information see the Product Specification and Product Sheet for this preparation.

10. Stability and Reactivity

This material is stable under normal conditions of use.

Conditions to avoid:	None
Materials to avoid:	None
Hazardous decomposition products:	None

11. Toxicological Information

Inhalation of aerosols or dust resulting from inappropriate handling may induce sensitization and may cause allergic reactions in sensitized individuals. Prolonged skin contact may cause minor irritation. Oral rat LD-50 > 2g/kg b.w. classifies the preparation as "non-toxic".

12. Ecological Information

LC-50(fish) > 100 mg/l, EC-50(daphnia) > 100 mg/l and IC-50(algae) > 100 mg/l, which classifies the preparation as "non-dangerous" to the environment.

The preparation is biodegradable.

13. Disposal Considerations

No special disposal method required, except as in accordance with current local authority regulations.

14. Transport Information

UN No.:	Not applicable
Sea:	Not applicable
Road/Rail:	Not applicable
Air:	Not applicable

15. Regulatory information

The preparation is a hazardous preparation.

Labelling:	Xn (harmful)
R-42	May cause sensitization by inhalation
R-36/37/38	Irritating to eyes, respiratory system and skin.
S-23	Do not breathe spray.
S-24	Avoid contact with skin
S-26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S-36/37/39	Wear suitable protective clothing, gloves and eye/face protection.

16. Other Information

As of the date of issue the information contained in this Enzyme Material Safety Data Sheet is believed to be true and correct. However, the accuracy or completeness of this information and any recommendations or suggestions are made without warranty or guarantee. Since the conditions of use are beyond the control of our company, it is the responsibility of the user to determine the conditions for safe use of this preparation. The information in this data sheet does not represent analytical specifications, for which please refer to our Product Specification.

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Fruit & Vegetable / 2001-07241-02

Product Sheet

Pectinex® SMASH

Description

Pectinex SMASH is a highly active pectolytic enzyme preparation produced by submerged fermentation of a selected strain of *Aspergillus aculeatus* and by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism. This enzyme preparation contains pectolytic and a range of hemicellu-lolytic activities. Pectinex SMASH also hydrolyzes the methylesterified galacturonic acid residues in pectins. It has the ability to break down plant cell walls.

Product Properties

Product Type

Pectinex SMASH is a brownish liquid with a slight smell typical of fermented products and with a pH of approx. 4.5.

Activity

Pectinex SMASH has a standard activity of 22,000 PG/ml (pH 3.5). For the polygalacturonase the standard activity is determined by measuring the viscosity reduction in a solution of pectic acid at pH 3.5 and 20°C (68°F). See the Analytical Method for further information.

Solubility

The active components of Pectinex SMASH are readily soluble in water in all concentrations that occur in normal usage. Any turbidity which may occur in the enzyme preparation has no influence on the volumetric activity or handling characteristics of the product.

Food-grade status

The product complies with the specifications recommended by FAO/WHO JECFA and FCC for food-grade enzymes, supplemented by maximum limits of 10 2 moulds per gram. The product is bottled aseptically after sterile filtration and therefore practically germ-free.

Packaging

See the standard Packaging List for more packaging information.

Application

The preparation is specially designed for the treatment of fruit and vegetable mashes and the maceration of plant tissues. Soluble and insoluble pectins as well as haze-provoking polysaccharides are also efficiently degraded. Pectnix SMASH applied on fruit and vegetable mashes and/or pomaces leads to drastically increased capacities in solid/liquid separation (e.g. press, decanter) and higher juice yields.

Reaction Parameters

Pectinex SMASH Activity

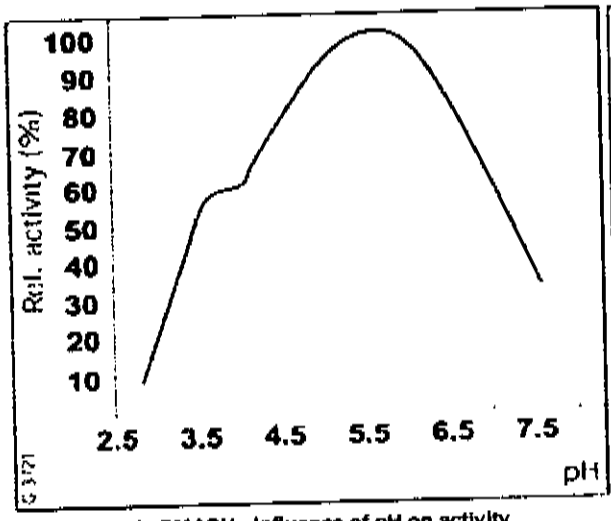

Fig. 1. Pectnix SMASH - Influence of pH on activity.
Substrate: 1.8% polygalacturonic acid
Temperature: 20°C
Novozymes method used at various pH values

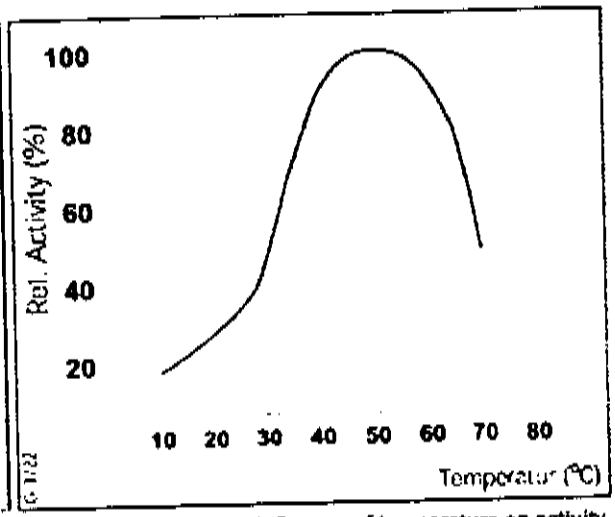

Fig. 2. Pectnix SMASH - Influence of temperature on activity
Substrate: 1.8% polygalacturonic acid
pH: 3.5
Novozymes method used at various temperatures

Safety

Enzymes are proteins and inhalation of dust or aerosols may induce sensitization and may cause allergic reactions in sensitized individuals. Some enzymes may irritate the skin, eyes and mucous membranes upon prolonged contact.

The product may create easily inhaled aerosols if splashed or vigorously stirred.

Spilled material should be rinsed away with water (avoid splashing). Left-over material may dry out and create dust.

A Material Safety Data Sheet is supplied with all products. See the Safety Manual for further information regarding how to handle the product safely.

Storage

When the product is stored at a temperature of 20°C (68°F), the declared activity is maintained for three months. For longer storage periods, a loss in activity of 1-2% per month may occur. When stored at 0-10°C (32-50°F), this product will maintain the declared activity for at least one year.

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Product Specification

Enzyme Business

Pectinex Smash

Valid from 2001-6-28

It is a brown liquid pectinase preparation prepared by standardization a concentrated and purified extract of a selected strain of Asp.aculeatus in pure culture. The pectinesterase is from Asp.aculeatus produced by submerged fermentation of a genetically modified Aspergillus oryzae. It contains 17% potassium chloride and 15% glycerol as stabilizers, and no preservatives. The density is about 1.16.

Analysis name	Lower limit	Upper limit	Unit
Poly Galactoronase at pH 3.5	22000		/ml
Total Viable Count		50000	/g
Coliform Bacteria		30	/g
Enteropathogenic E.Coli	None Detected		/25g.
Salmonella	None Detected		/25g.

Unless seperate agreement refers, the information contained in this specification is subject to change without notice.
The product complies with the following FCC and JECFA requirements: Antibiotic activity (Not detected), Mycotoxins (Not detected), Heavy metals (< 30 ppm), Lead (< 5 ppm), Arsenic (< 3 ppm).
Unless seperate agreement refers, the information contained in this specification is subject to change without notice.
The product complies with the following FCC and JECFA requirements: Antibiotic activity (Not detected), Mycotoxins (Not detected), Heavy metals (< 30 ppm), Lead (< 5 ppm), Arsenic (< 3 ppm).

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Fruit & Vegetable / 24/05/2001

MSDS

Enzyme Material Safety Data Sheet

1. Identification of the Preparation and of the Company

Hazardous according to criteria of Worksafe Australia

Commercial product name:	Pectinex SMASH
Description:	Aqueous enzyme preparation
Appearance:	Clear brown liquid
Responsible company:	Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd
Information phone number:	+ 45 88 24 99 99
Importer:	Novozymes Australia Pty. Ltd. 22 Loyalty Road North Rocks N. S. W.
Phone:	02 9630 8466
Fax:	02 9683 1170

2. Information on Ingredients

Chemical characterization of active component:	Enzyme protein
Synonyms:	Mix: Polygalacturonase/Pectinmethylesterase
IUB number:	3.2.1.15/3.1.1.11
CAS number:	9032-75-1/9025-98-3
EINECS number:	232-885-6/232-807-0
Hazardous ingredients:	Enzyme protein (1-10%)
Non-hazardous ingredients:	Up to 100%
Classification of preparation:	Xn (harmful), R-42 , R-36/37/38
Exposure limit:	Not established
Hazchem code:	No Hazchem code allocated

UN number:	No UN number allocated
Poisons schedule Number:	No poisons schedule number allocated
Use:	Processing aid for food productionICATION

3. Hazards Identification

Liquid enzyme preparations are dustfree preparations. However, inappropriate handling may cause formation of dust or aerosols. For appropriate handling, see section 6 and 7. Inhalation of enzyme dust or aerosols resulting from inappropriate with handling may induce sensitisation and may cause allergic reactions in sensitised individuals. Prolonged skin contact may cause minor irritation.

4. First Aid Measures

Skin contact:	Wash skin with plenty of water.
Eye contact:	Rinse eyes with plenty of water.
Ingestion:	Rinse mouth and throat thoroughly with water. Drink water.
Inhalation:	Remove from exposure. If symptoms of irritation or sensitisation occur (shortness of breath, wheezing or laboured coughing), call a doctor.

5. Fire-fighting Measures

Protection against fire and explosion:	No special requirements
Suitable fire extinguishing media:	Water, foam
Non-suitable media:	None
Special exposure hazards:	None

6. Accidental Release Measures

Spilled preparation should be removed immediately to avoid formation of dust from dried preparation. Take up by mechanical means, preferably by a vacuum cleaner equipped with a high-efficiency filter. Flush the remainder carefully with plenty of water. Avoid splashing and high-pressure washing (avoid formation of aerosols). Ensure sufficient ventilation. Wash contaminated clothing.

7. Handling and Storage

Avoid formation of aerosols and dust from drying out of spilled preparation. Avoid splashing and high-pressure washing. Ensure good ventilation of the room when handling this preparation. Store container in a cool place.

8. Exposure Control/Personal Protection

RECOMMENDED PERSONAL PROTECTION EQUIPMENT:

Respiratory protection:	Respirator with P3 filter
Hand protection:	Impermeable gloves
Eye protection:	Protective glasses or eye shield
Clothing:	Wear suitable protective clothing

9. Physical and Chemical Properties

Appearance:	Clear brown liquid
Odour:	Slight fermentation odour

pH, boiling point, melting point, flash point, ignition temperature, vapour pressure, density and solubility are not relevant to safety. For further information see the Product Specification and Product Sheet for this preparation.

10. Stability and Reactivity

This material is stable under normal conditions of use.

Conditions to avoid:	None
Materials to avoid:	None
Hazardous decomposition products:	None

11. Toxicological Information

Inhalation of aerosols or dust resulting from inappropriate handling may induce sensitization and may cause allergic reactions in sensitized individuals. Prolonged skin contact may cause minor irritation. Oral rat LD-50 > 2g/kg b.w. classifies the preparation as "non-toxic".

12. Ecological Information

LC-50(fish) > 100 mg/l, EC-50(daphnia) > 100 mg/l and IC-50(algae) > 100 mg/l, which classifies the preparation as "non-dangerous" to the environment.

The preparation is biodegradable.

13. Disposal Considerations

No special disposal method required, except as in accordance with current local authority regulations.

14. Transport Information

UN No.:	Not applicable
Sea:	Not applicable
Road/Rail:	Not applicable
Air:	Not applicable

15. Regulatory information

The preparation is a hazardous preparation.

Labelling:	Xn (harmful)
R-42	May cause sensitization by inhalation
R-36/37/38	Irritating to eyes, respiratory system and skin.
S-23	Do not breathe spray.
S-24	Avoid contact with skin
S-26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S-36/37/39	Wear suitable protective clothing, gloves and eye/face protection.

16. Other Information

As of the date of issue the information contained in this Enzyme Material Safety Data Sheet is believed to be true and correct. However, the accuracy or completeness of this information and any recommendations or suggestions are made without warranty or guarantee. Since the conditions of use are beyond the control of our company, it is the responsibility of the user to determine the conditions for safe use of this preparation. The information in this data sheet does not represent analytical specifications, for which please refer to our Product Specification.

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Special Food / 2001-08285-01

Product Sheet

Viscozyme® L

Description

Viscozyme L is a multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, beta-glucanase, hemicellulase and xylanase. The enzyme also has activity against the branched pectin-like substances found in soybean cell walls. The enzyme preparation is produced from a selected strain of *Aspergillus aculeatus*.

Product Properties

Appearance

Viscozyme L is a clear brown liquid with a density of approx. 1.2 g/ml.

Activity

Viscozyme ..... 100 FBG/g.

FBG = Fungal Beta-Glucanase Units.

See the Analytical Method for further information.

Other characteristics

The ability of Viscozyme L to function at low temperatures will result in reduced energy consumption in the extraction of materials from plant cells. In addition, the absence of significant levels of amylase and lipase activities means that these major components will not be affected during the extraction process.

The optimal conditions for Viscozyme L with its several and complex activities are a pH range of 3.3-5.5 and a temperature of 25-55°C.

Food-grade status

Viscozyme complies with the recommended purity specifications for food-grade enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC), supplemented with a maximum limit of 10^2/g for moulds.

Packaging

See the standard Packaging List for more packaging information.

Application

Viscozyme L is a special enzyme preparation used in the breakdown of cell walls for the extraction of useful components from plant tissue and in the processing of cereal and vegetable materials.

The multi-component nature of Viscozyme is of particular use in the processing of plant materials in the alcohol, brewing, starch and related industries. The ability of the enzyme to liberate bound materials and to degrade non-starch polysaccharides can be used to improve starch availability in fermentation and to generally reduce viscosity and hence improve extraction yields.

For the reduction of beta-glucans and viscosity a dosage of 0.02-0.1% of grist weight is recommended. For other applications a general recommendation is 0.05-0.1%.

Safety

Enzymes are proteins. Inhalation of dust or aerosols may induce sensitization and may cause allergic reactions in sensitized individuals. Some enzymes may irritate the skin, eyes and mucous membranes upon prolonged contact. This product may create easily inhaled aerosols if splashed or vigorously stirred. Spilled product may dry out and create dust.

Spilled material should be flushed away with water. Avoid splashing. Left-over material may dry out and create dust. Wear suitable protective clothing, gloves and eye/face protection as prescribed on the warning label. Wash contaminated clothes.

Handling Precautions

Viscozyme L is non-flammable, completely miscible with water and safe when used according to directions. Observe standard handling precautions to avoid direct contact with the product or inhalation of dust from the dried product. In case of accidental spillage and contact with the skin or eyes, rinse promptly with water.

A Material Safety Data Sheet is supplied with all products. See the Safety Manual for further information regarding how to handle the product safely.

Storage

Enzymes gradually lose activity over time depending on storage temperature and humidity. It is recommended to store the product under cool and dry conditions in closed containers at 0-10°C (32-50°F) (e.g. in the hop storage room). Extended storage and/or adverse conditions incuding higher temperature or high humidity, may lead to a higher dosage requirement. Further information on product stablility is available on request.

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Product Specification

Enzyme Business

Viscozyme L

Valid from 2001-9-25

Appearance:	Brown liquid
Enzyme:	Beta-glucanase
Diluents:	Sucrose, Sodium chloride
Preservatives:	Potassium sorbate

Analysis name	Lower limit	Upper limit	Unit
Betaglucanase Units FBG	100		/g
Total Viable Count	-	50000	/g
Coliform Bacteria	-	30	/g
Enteropathogenic E.Coli	None Detected		/25g.
Salmonella	None Detected		/25g.

The product complies with the following FCC and JECFA requirements: Antibiotic activity (Not detected), Mycotoxins (Not detected), Heavy metals (< 30 ppm), Lead (< 5 ppm), Arsenic (< 3 ppm).

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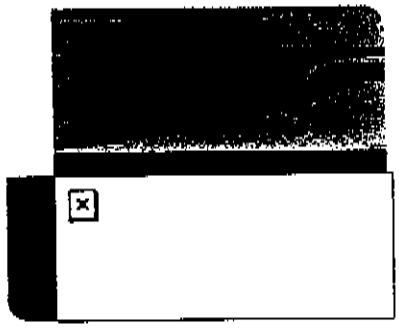
Special Food / 06/11/2001

MSDS



Enzyme Material Safety Data Sheet

1. Identification of the Preparation and of the Company

Hazardous according to criteria of Worksafe Australia

Commercial product name:	Viscozyme L
Description:	Aqueous enzyme preparation
Appearance:	Clear brown liquid
Responsible company:	Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd
Information phone number:	+ 45 88 24 99 99
Importer:	Novozymes Australia Pty. Ltd. 22 Loyalty Road North Rocks N. S. W.
Phone:	02 9630 8466
Fax:	02 9683 1170

2. Information on Ingredients

Chemical characterization of active component:	Enzyme protein
Synonyms:	Beta-glucanase endo-1,3(4)-
IUB number:	3.2.1.6
CAS number:	62213-14-3
EINECS number:	263-462-4
Hazardous ingredients:	Enzyme protein (1-10%)
Non-hazardous ingredients:	Up to 100%
Classification of preparation:	Xn (harmful), R-42 , R-36/37/38
Exposure limit:	Not established
Hazchem code:	No Hazchem code allocated

UN number:	No UN number allocated
Poisons schedule Number:	No poisons schedule number allocated
Use:	Processing aid for food productionICATION
	Additive for stock feed

3. Hazards Identification

Liquid enzyme preparations are dustfree preparations. However, inappropriate handling may cause formation of dust or aerosols. For appropriate handling, see section 6 and 7. Inhalation of enzyme dust or aerosols resulting from inappropriate with handling may induce sensitisation and may cause allergic reactions in sensitised individuals. Prolonged skin contact may cause minor irritation.

4. First Aid Measures

Skin contact:	Wash skin with plenty of water.
Eye contact:	Rinse eyes with plenty of water.
Ingestion:	Rinse mouth and throat thoroughly with water. Drink water.
Inhalation:	Remove from exposure. If symptoms of irritation or sensitisation occur (shortness of breath, wheezing or laboured coughing), call a doctor.

5. Fire-fighting Measures

Protection against fire and explosion:	No special requirements
Suitable fire extinguishing media:	Water, foam
Non-suitable media:	None
Special exposure hazards:	None

6. Accidental Release Measures

Spilled preparation should be removed immediately to avoid formation of dust from dried preparation. Take up by mechanical means, preferably by a vacuum cleaner equipped with a high-efficiency filter. Flush the remainder carefully with plenty of water. Avoid splashing and high-pressure washing (avoid formation of aerosols). Ensure sufficient ventilation. Wash contaminated clothing.

7. Handling and Storage

Avoid formation of aerosols and dust from drying out of spilled preparation. Avoid splashing and high-pressure washing. Ensure good ventilation of the room when handling this preparation. Store container in a cool place.

8. Exposure Control/Personal Protection

RECOMMENDED PERSONAL PROTECTION EQUIPMENT:

Respiratory protection:	Respirator with P3 filter
Hand protection:	Impermeable gloves
Eye protection:	Protective glasses or eye shield
Clothing:	Wear suitable protective clothing

9. Physical and Chemical Properties

Appearance:	Clear brown liquid
Odour:	Slight fermentation odour

pH, boiling point, melting point, flash point, ignition temperature, vapour pressure, density and solubility are not relevant to safety. For further information see the Product Specification and Product Sheet for this preparation.

10. Stability and Reactivity

This material is stable under normal conditions of use.

Conditions to avoid:	None
Materials to avoid:	None
Hazardous decomposition products:	None

11. Toxicological Information

Inhalation of aerosols or dust resulting from inappropriate handling may induce sensitization and may cause allergic reactions in sensitized individuals. Prolonged skin contact may cause minor irritation. Oral rat LD-50 > 2g/kg b.w. classifies the preparation as "non-toxic".

12. Ecological Information

LC-50(fish) > 100 mg/l, EC-50(daphnia) > 100 mg/l and IC-50(algae) > 100 mg/l, which classifies the preparation as "non-dangerous" to the environment.

The preparation is biodegradable.

13. Disposal Considerations

No special disposal method required, except as in accordance with current local authority regulations.

14. Transport Information

UN No.:	Not applicable
Sea:	Not applicable
Road/Rail:	Not applicable
Air:	Not applicable

15. Regulatory information

The preparation is a hazardous preparation.

Labelling:	Xn (harmful)
R-42	May cause sensitization by inhalation
R-36/37/38	Irritating to eyes, respiratory system and skin.
S-23	Do not breathe spray.
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16. Other Information

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APPENDIX FOUR

INVESTIGATIONS ON INCREASING THE CONDITIONING EFFICIENCY OF WHEAT.

J.Moawad[1,2] and M.D.Southan[1,2]

[1]BRI Australia Ltd., North Ryde, NSW 1670, Australia
[2]Quality Wheat CRC, North Ryde, NSW 1670, Australia

INTRODUCTION

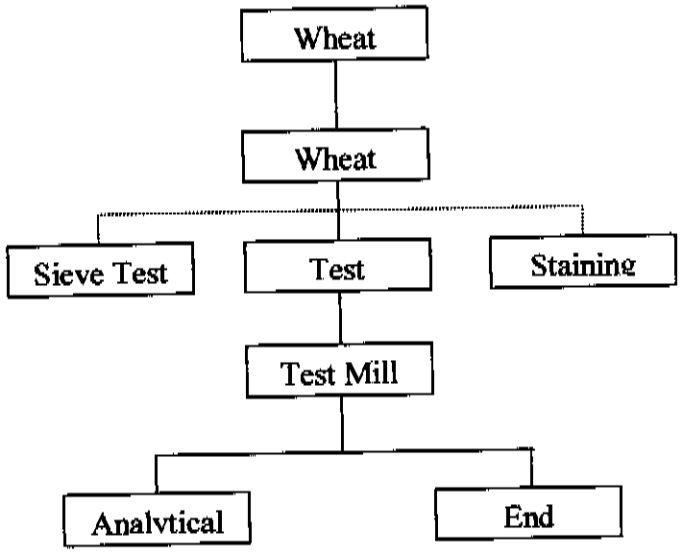
Conditioning of wheat is an important step in the preparation of wheat for milling. Depending on grain type wheat is usually conditioned to a moisture content of 13.5 – 17.5%. It is at this moisture content that maximum flour yield is achieved with minimal bran contamination during contamination.

The conditioning process involves two steps
1. The amount of water to be added
2. The time taken for wheat to equilibrate prior to milling.

AIM

Aim of the project is to look at ways of making the conditioning process more efficient by adopting either a chemical or biological approach, however before investigating a way to reduce the conditioning time a method needs to be found for monitoring the rate of water penetration through the grain.

METHOD



RESULTS AND DISCUSSION

Figure 1 is a plot showing the rate of penetration into the grain by observing change in test weight over a 24 hour time interval. Within the first 2 hours after the sample was conditioned test weight had decreased. This decrease is a result of water being taken up by the bran thus causing

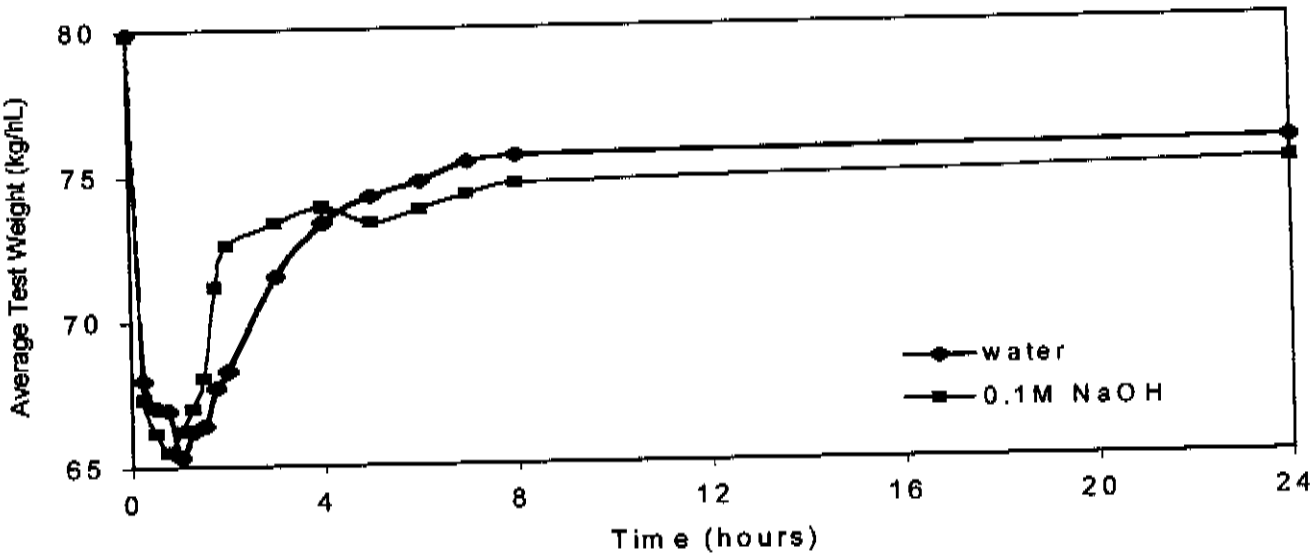


Figure 1 Moisture penetration over a 24 hour period for a hard & soft wheat sample.

the bran to swell. As time progressed (3-8 hours) test weight begins to increase. This increase in test weight is a result of water penetrating further into the grain and the bran layer becoming drier and swelling reduced. At some time between 8-24 hours penetration occurs further into the endosperm where equilibrium is reached.

Chemicals were used to see if the conditioning process could be made more efficient. The chemicals used included Sodium Hydroxide, Acetic Acid, Sodium Dodecyl Sulphate and Ethanol. Figure 2 shows an increase in the test weight (during the first 4 hours) before it starts to decrease.

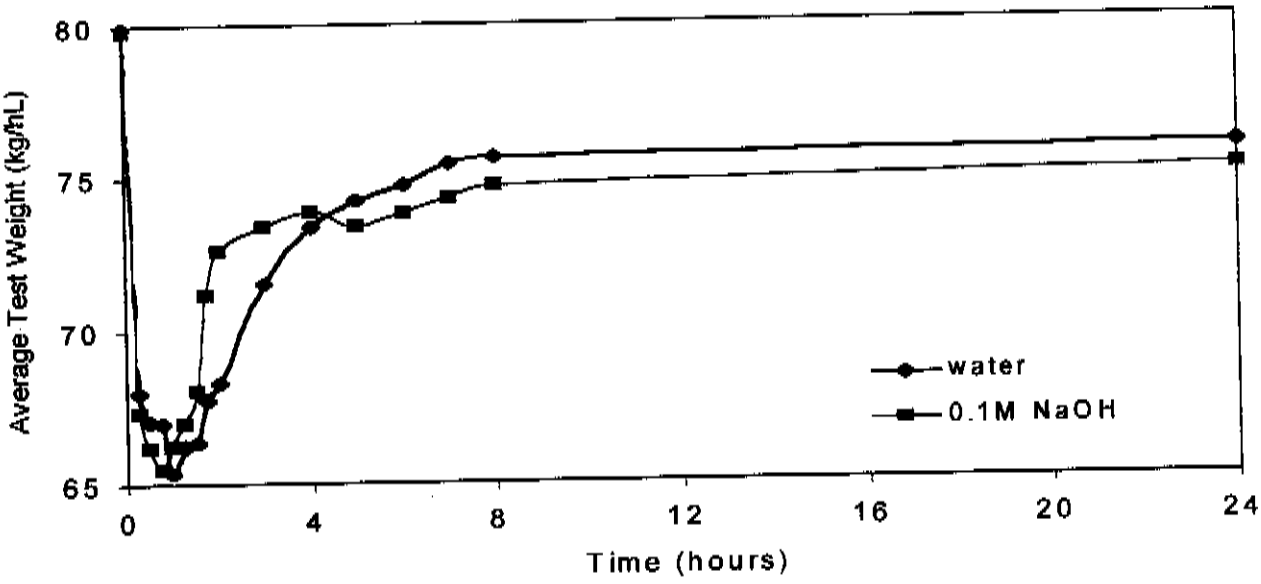


Figure 2 Moisture Penetration Over a 24 Hour Period Using a 0.1M Solution of Sodium Hydroxide

The overall use of chemicals did not appear to have a major effect on the conditioning time and displayed a very similar pattern to water.

Enzymes were used to see if the conditioning process could be made more efficient. The enzymes used included Shearzyme 500L, Pectinex SMASH and Viscozyme L. The use of Pectinex SMASH on the rate of conditioning (Figure 3) showed a slight increase in the test weight in comparison to water.

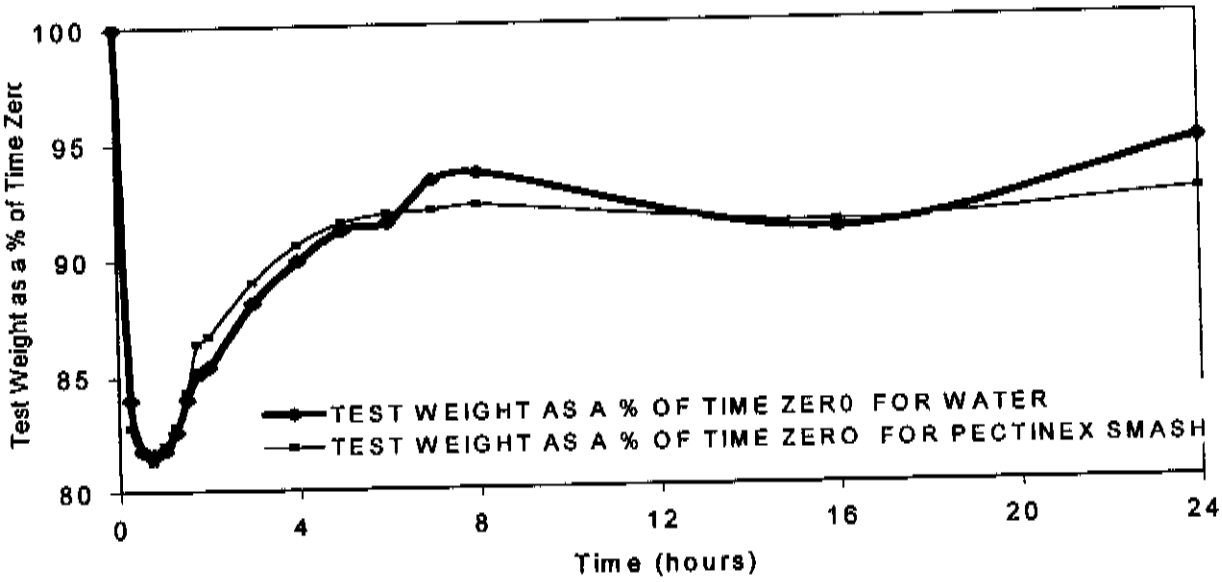


Figure 3 Moisture penetration over a 24 hour period using Pectinex SMASH.

Figure 4 is a plot comparing milling yield with water and Pectinex SMASH. Results show that flour yield for the enzyme treatment within the first four hours and the eighth hour is lower in comparison to the control while at time six the flour yield for the enzyme treatment is slightly higher than the control.

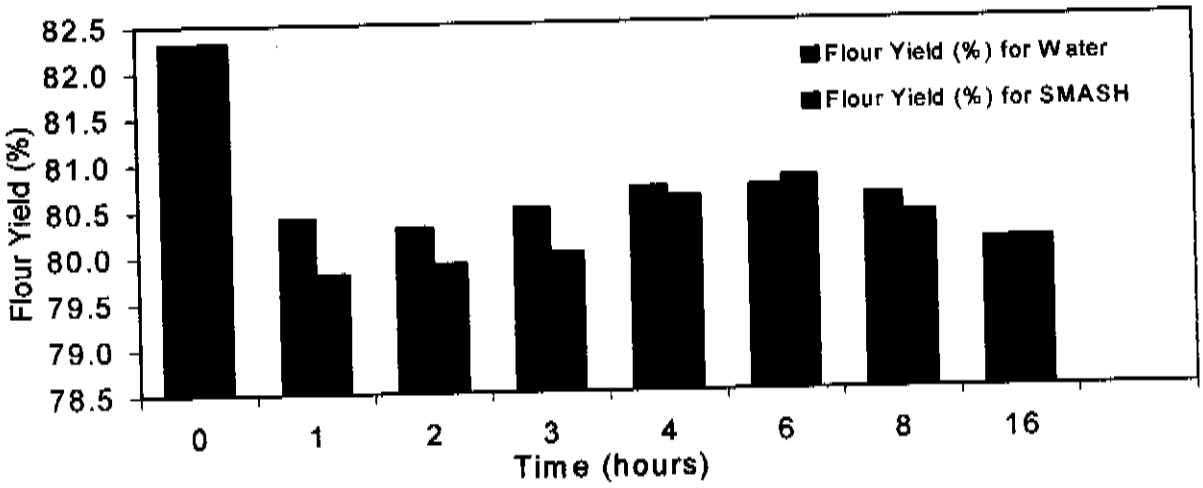


Figure 4 Comparing milling yield with water and Pectinex SMASH.

The branscan results for (Figure 5) shows that the use of the enzyme treatment when compared to the control resulted in a slightly higher bran contamination present in the flour. This may be partially due to the fact that the enzyme has both hemicellulolytic and cellulolytic activity, which may have penetrated through the cell wall structure of the wheat causing the bran to defragmant

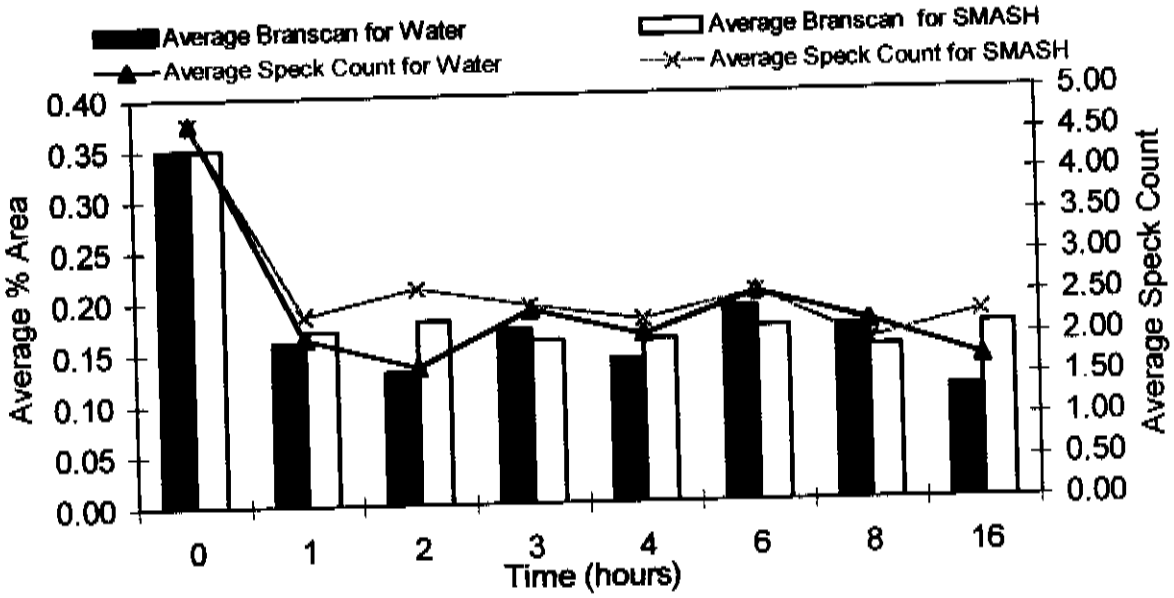


Figure 5 Branscan Average % and Average Bran Speck Count

CONCLUSION

The use of chemicals and enzymes displayed a very similar pattern to water and therefore very little effect is seen on the rate of moisture penetration into the grain. A patent titled 'A Process for Conditioning Grain' written by Novozyme investigated conditioning time for wheat. It was found that Cereszyme and Viscozyme L reduced conditioning time and increased flour yield. However this study found that Viscozyme L resulted in an increase in flour yield and was also associated with an increase in bran contamination when compared to the control. A major reduction in conditioning time was not observed.

ACKNOWLEDGMENTS

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