The effect of selected enzymes on the quality and structural attributes of white salted and yellow alkaline Asian noodles

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

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Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged.

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Thank you all very much!

love

Lara

Publications

Most of the work reported in this thesis has previously been presented in the following papers:

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Cato, L. and Small, D. M. 2003. The impact of selected enzymes on structure, colour and texture of yellow alkaline noodles. Proceedings of the 12th World Congress of Food Science and Technology. Held July 2003 in Chicago, USA.

Cato, L. and Small, D. M. 2003. The impact of biochemical interactions and processing on the structure and quality attributes of Asian noodles. Proceedings of the 36th Australian Institute of Food Science and Technology (AIFST) annual meeting 'Food – Leading the way - Foods for Life'. Held from the 24th until the 27th of August 2003 in Melbourne, Australia.

Cato, L. Bui, L. and Small, D. M. 2004. Catalysts on the menu: role of enzymes in foods. Chemistry in Australia 71 (3) 6-7. (refereed paper)

Cato, L., Halmos, A. L. and Small, D. M. 2004. Measurement of peroxidase activity in Australian white wheat flours and yellow alkaline noodles: The impact of peroxidase on the texture and colour of noodle products. Proceedings of the 37th AIFST annual meeting 'Innovation Concept Creation Commercialisation'. Held from the 25th until the 28th of July 2004 in Brisbane, Australia.

Cato, L., Halmos, A. L. and Small, D. M. 2004.The effect of exogenous α-amylases on the quality characteristics of white salted noodles made from Australian white wheat flours. Proceedings of the Joint meeting of American Association of Cereal Chemists (AACC) and Tortilla Industry Association (TIA). 89th annual meeting of the AACC and the 15th annual convention and trade exposition of the TIA. Held from the 19th until the 22nd of September 2004 in San Diego, USA. p 86.

Cato, L., Halmos, A. L. and Small, D. M. 2004. Measurement of α-amylase in Australian white wheat flour and evaluation of its impact on the quality parameters of yellow alkaline noodles. Proceedings of the Joint Symposium Food Structure & Functionality Forum – European Section of the American Oil

Chemical Society (AOCS), 'Conference on Food Structure and Food Quality'. Held from the 3rd until the 7th of October 2004 in Cork, Ireland. p 6.

Cato, L., Halmos, A. L. and Small, D. M. 2005. Measurement of lipoxygenase in Australian white wheat flour: The effect of lipoxygenase on the quality properties of white salted noodles. Proceedings of the 4th European Symposium on Enzymes in Grain Processing (ESEGP). Held from the 5th until the 8th of June 2005 in Nantes, France.

Cato, L., Halmos, A. L. and Small, D. M. 2005. The effect of lipoxygenase on the processing characteristics and quality attributes of yellow alkaline noodles. Proceedings of the International Association for Cereal Science and Technology (ICC) – Jubilee Conference 'Cereals the Future Challenge'. Held from the 3rd until the 6th of July in Vienna, Austria. Published by ICC – International Association for Cereal Science and Technology, Vienna, Austria 2005, p 69.

Cato, L., Halmos, A. L. and Small, D. M. 2005. The effect of peroxidase on the texture and colour of white salted noodles. Proceedings of the 55th Australian Cereal Chemistry Conference, 'Connect 2005 – Chemical Challenges for the 21st Century'. Held from the 3rd until the 6th of July in Sydney, Australia.

Cato, L., Halmos, A. L. and Small, D. M. 2005. The impact of ascorbic acid oxidase on the texture and colour of white salted noodles. Proceedings of the 38th AIFST annual meeting 'Passion 4 Food – Skills for Success'. Held from the 10th until the 13th of July 2005 in Sydney, Australia.

Cato, L., Halmos, A. L. and Small, D. M. 2006. Impact of α-amylase on quality characteristics of Asian white salted noodles made from Australian white wheat flour. Cereal Chemistry (American Association of Cereal Chemists, St Paul) 83(5):491-497.

Cato, L., Halmos, A. L. and Small, D. M. 2006. Measurement of lipoxygenase in Australian white wheat flour: the effect of lipoxygenase on the quality properties of white salted noodles. Journal of the Science of Food and Agriculture 86:1670-1678.

Abstract

Wheat and wheat products represent a major food staple consumed around the world. Asian noodles account for the end-use of at least twelve percent of all wheat produced globally. Whereas there has been extensive research into the role and significance of enzymes in the utilisation of wheat flour in bread-making, less is known of their role in Asian noodles. Accordingly, this study has been based on the hypothesis that some enzymes will have a significant impact on the quality characteristics of at least some styles of Asian noodle products.

Five enzymes were selected for study: α-amylase, lipase, lipoxygenase, peroxidase and ascorbic acid oxidase. The focus has been on the processing of white salted and yellow alkaline styles of Asian noodles and the role of the enzymes in relation to the quality attributes of these products has been systematically investigated. The quality aspects encompass colour and colour stability, texture, cooking properties as well as structural characteristics of the products. As a part of the preliminary phases of the investigation, procedures for analysis and assessment of flours and noodles have been evaluated. In particular, for the textural properties of noodles, results were obtained with the TA-XT2 Texture Analyser using both a flat cylinder probe, to measure noodle hardness, and also a cutting blade measuring noodle firmness. In addition, various approaches were trialled for sample preparation and presentation in the use of scanning electron microscopy for the investigation of noodle structure.

In order to measure the activity of the enzymes in flours and noodle products, assay procedures were set up and validated. These were then used for the analysis of a series of commercial flours and the levels of activity in each of the flours was relatively low indicating that they had been milled from wheat which had not been subjected to pre-harvest sprouting.

α-Amylase was measured using the Ceralpha method and two different sources of exogenous α-amylase (bacterial and barley) were added to noodle formulations. In preliminary experiments various levels of α-amylase incorporation were compared and

the impact on texture measured. Both sources of α-amylase resulted in softer noodle products. Adverse effects of the preparations on colour were observed in fresh noodles, although the differences were less obvious when noodles were cooked or dried immediately after preparation. Cooking losses were higher in noodles incorporating amylase, particularly the bacterial preparation. These impacts were reflected in changes in the appearance of starch granules in scanning electron micrographs of the noodles.

Three different lipase preparations were studied and their incorporation had only minor effects on texture of noodles. Addition of wheat germ lipase resulted in slightly softer noodles, fungal lipase caused slightly harder noodles, while addition of porcine pancreas lipase gave harder noodles in the raw state and softer noodles after cooking. Similarly variable results were observed when colour and colour stability were evaluated, and there were no adverse effects upon cooking quality of Asian noodles.

Two different preparations of horseradish peroxidase were investigated and both resulted in adverse effects on colour including at all stages of storage. One of the preparations resulted in softer noodles when texture was measured using the cylinder method and in firmer noodles when the blade attachment was applied. Neither the surface appearance of noodles nor the cooking properties were altered by the addition of peroxidase to the formulations.

Different levels of addition of ascorbic acid oxidase from *Cucurbita* species showed only minor effects on characteristics for both styles of noodles. Incorporation of this enzyme resulted in lower lightness values but there was little effect on yellowness. Discolouration of noodle sheets was faster and more obvious at 25°C and compared to the storage of noodles at 4°C. The cooking qualities of noodles did not change upon addition of the oxidase.

Activity of the enzyme lipoxygenase was measured spectrophotometrically using linoleic acid as substrate. Upon addition to the noodle formulations the enzyme preparation from soy bean resulted in slightly harder and firmer noodles. Colour and colour stability were not enhanced by the addition of lipoxygenase and significantly higher yellowness values were measured in some samples. This enzyme did not adversely impact upon the cooking or structural properties of either style of noodles.

Some of the enzymes studied here demonstrated undesirable impacts on one or another aspect of noodle quality, particularly producing darkening or soft textural characteristics. Enzymes that might usefully be considered at lower levels of addition are ascorbic acid oxidase, porcine pancreas lipase and lipoxygenase. These three had no negative effects upon texture, structure or cooking quality of noodles. Visually the colour properties were not adversely impacted and instrumental assessment indicated brighter noodle sheet colours. At lower levels of addition, these three enzymes provide enhancement of noodle quality. On the other hand peroxidase, the two amylases and lipases affected the colour and colour stability of noodles. It was observed that the amylase preparations did result in pronounced softening of noodles. However, the data indicate that the adverse impact attributed to this enzyme when flour from sprouted wheat is used in noodle processing, are probably due to enzyme activities other than α-amylase.

In conclusion, the application of two different approaches to the instrumental measurement of noodle texture indicates that both are useful, generally providing similar relative rankings of the softness and firmness characteristics of the products. In addition, there are specific techniques available to allow the convenient application of scanning electron microscopy to the study of Asian noodles, including fresh and cooked products. The results obtained when selected enzymes were added to noodle formulations demonstrate that the stability of the enzymes during the processing varies depending upon the pH of the products and the pH characteristics of the particular enzymes. In terms of the quality attributes investigated here, there is potential for the use of commercial enzyme preparations as ingredients in the manufacture of both white salted and yellow alkaline Asian noodles.

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Abbreviations

a*	redness (component of colour measure using Minolta Chroma Meter)
AACC	American Association of Cereal Chemists
AAO	ascorbic acid oxidase
ANZ	Australia New Zealand
AOAC	Association of Official Analytical Chemists
b*	yellowness (component of colour measure using Minolta Chroma Meter)
BGF	Baker's strong flour (see Chapter 7, page 86) for details
BRI	Bread Research Institute of Australia
CU	Ceralpha units (of α-amylase activity)
Da	Daltons (units of relative molecular mass)
DHAA	dehydroascorbic acid
DR	dried noodles
EC	Enzyme Commission
E	energy (measured in the context of texture analysis)
ESEM	environmental scanning electron microscope
EU	European Union
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration (United States of America)
FFA	Free fatty acid(s)
FR	refrigeration temperature (4°C)
g	acceleration due to gravity
HPLC	high-performance liquid chromatography
ICC	International Association for Cereal Science and Technology
L*	lightness value (component of colour measure using Minolta Chroma Meter)
LAA	L-ascorbic acid
LOX	lipoxygenase

Mag	magnification
N	Newtons (units of force used in texture analysis)
n	the number of replicate analyses used in calculation of individual results
N/A	not applicable
PF	P-farina flour (see Chapter 7, page 86) for details
POX	peroxidase
PP	porcine pancreas (source of lipase preparation)
PPO	polyphenol oxidase
r^2	coefficient of determination for a regression line
rpm	revolutions per minute
RT	Room temperature (25°C)
SEM	scanning electron microscope
sd	standard deviation
TA-TX2	Texture analyser ((see Chapter 7, page 88) for details
U	Unitsof enzyme activity (as defined for the particular enzyme – see Chapter 7)
UV	ultraviolet
UW	Ultra White flour (see Chapter 7, page 86) for details
WG	wheat germ (source of lipase preparation)
WS	white salted
WSN	white salted noodles
YAN	yellow alkaline noodles

Explanatory notes

The purpose of these notes is to briefly describe the approaches adopted during the preparation of this thesis. They relate to the nomenclature of enzymes and other chemical compounds, spelling, units of measurement, the expression of analytical results, as well as the referencing of literature sources:

1. In the naming of chemicals including enzymes, the most recent recommendations of the Enzyme Commission have been followed (see Chapter 2).
2. Where alternative spellings are in common use then the British rather than the American approach has been adopted in the text. Examples include the term colour (rather than color), words ending with –ise (rather than –ize) and some technical terms.
3. Where possible, for the presentation of results SI units have been used. However for the presentation of enzyme activity data two approaches have been followed.
4. Generally experimental data is presented on a 14% moisture basis rather than a fresh weight (or 'as is') basis unless otherwise clearly specified. The reason that this approach was adopted has been to facilitate direct comparisons of results obtained at different processing stages during manufacture of Asian noodles.
5. The moisture contents and the time required for cooking to the optimum point were routinely measured for each noodle sample prepared and for the different stages of processing.
6. In the citation and listing of references and information sources, the current recommendations of the American Association of Cereal Chemists (AACC International) for the journal entitled Cereal Chemistry (Cereal Chem. 2005) have been applied throughout (see page).

Chapter 1

Introduction

The purpose of this Chapter is to provide an overview of the research program described in this thesis on the effect of selected enzymes on the quality attributes of Asian noodles. During the development of this project the following issues were taken into account:

- Wheat and wheat products are a significant proportion of food consumption around the world. Furthermore, Asian noodles are an important part of the diet in many countries.
- There is considerable scientific evidence concerning the contribution of selected enzymes during the baking process. However, much less is known of the application of these in flour-based products other than bread. In particular, at this point in time relatively little is known of the role or potential of enzymes in influencing quality attributes of Asian noodle products.
- Wheat sprouting is an important issue in Australia and around the world. The detrimental effect of sprout damaged wheat on noodle processing has been linked with discoloration but relatively little has been reported in the literature in terms of the impact on textural properties. As significant amounts of wheat are sprout damaged in most years in Australia alone, it would be of great benefit for the grain industry if means could be found to overcome this problem.
- Enzymes are generally considered to be relatively unstable during processing of foods. This sensitivity is primarily due to the differences in pH in various food systems and the influence heating applied during processing, although enzymes can also be influenced by light, oxidation and other food ingredients.
- Asian noodles represent a major end use of wheat with an estimated proportion of more than twelve percent of total world wheat production used for these products. There are many different styles of noodles in Asia but the three main groups are the traditional yellow alkaline types (sometimes also referred to as Chinese noodles),

white salted styles (including the traditional Japanese styles, Udon and Somen) and the newer instant noodle products.

- There are wide regional variations in consumer preferences for these types of products and the evaluation of product quality presents challenges due of its subjective nature.
- A range of ingredient formulations is used in noodle manufacture and this influences product quality. There are two distinct aspects of quality: appearance and eating quality. Colour and brightness are important factors of the appearance while eating quality reflects the textural properties of noodles. In addition, there are many variations in the specific processing steps applied during noodles making, drying as well as storage.
- Due to the importance of texture of these products there is a requirement to establish the usefulness of mechanical tests in order to replace human sensory evaluation for the evaluation of Asian noodles.

Accordingly, the underlying hypothesis of this project is that at least some enzymes will have a significant effect on the quality attributes of the various styles of Asian noodle products.

Therefore, this research seeks to investigate selected enzymes naturally present in the flour, and also study the effect of incorporating exogenous enzymes from various sources into the formulations of Asian noodle products.

Chapter 2

Background and literature review: the significance, sources and activity of selected enzymes

The purpose of this chapter is to provide background and review the relevant scientific literature on selected enzymes relevant to the research described in this thesis. The areas covered are the chemical structures of the enzymes, their use as processing aids in foods as well as the factors influencing activity and stability under conditions encountered during food processing. Following general descriptions, the specific roles of the enzymes in cereal based foods are reviewed.

2.1 General introduction to enzymes

Enzymes are proteins with catalytic properties and power of specific activation (Tucker 1995).

$$\text{Substrate(s)} \xrightarrow{\text{enzyme}} \text{product(s)}$$

Enzyme levels are generally measured by determining the action they have on substrates, reflecting the relative specificity of enzymes for one or a very limited number of substrates. Many assays of enzymes in foods are carried out to determine freshness (e.g. oxidative enzymes in fruits), to detect treatments including pasteurisation (e.g. phosphatases or lactoperoxidase), or to detect the onset of microbial degradation. The three primary factors that may influence enzymes in food systems are pH, temperature and the presence of endogenous inhibitors. As the responses of different enzymes to pH vary widely it is important to determine the optimal pH for each particular enzyme to be assayed (Tucker 1995). There are many enzymes that are used during the processing of foods and examples are presented in Table 2.1.

The Enzyme Commission has assigned each enzyme three designations: a systematic name, a trivial name and an Enzyme Commission number (EC). Usually the systematic name is composed of parts, the first being the name of the substrate(s), the second part has an -ase ending and it is based on the six types of chemical reactions catalysed.

Table 2.1 Examples of some enzymes commonly used in food processing

Common name	Classification	Common sources	Systematic Name (IUB)	EC number
α-Amylase	hydrolase	*Aspergillus niger, A oryzae, Rhizopus oryzae, Bacillus subtilis*	1,4-α-D-glucan glucanohydrolase	3.2.1.1
β-Amylase	hydrolase	barley malt	1,4-α-D-glucan maltohydrolase	3.2.1.2
Catalse	oxidoreductase	*A niger,* bovine liver, *Micrococus lysoddeikticus*		1.11.1.6
Amylogluco sidase	hydrolase	*A niger, A oryzae, Rhizopus oryzae*	1,4-α-D-glucan glucohydrolase	3.2.1.3
Glucose Oxidase	oxidoreductase	*A niger*	β-D-glucose: oxygen oxidoreductrase	1.1.3.4
Lactase	hydrolase	*A niger, A oryzae*	β-D-galactoside galactohydrolase	3.2.1.23
Lipase	hydrolase	edible forestomach tissue, animal pancreatic tissue	carboxylic-ester hydrolase	3.1.1.1
		A niger, A oryzae	triacylglycerol acylhydrolase	3.1.1.3
Pectinase	hydrolase or lyase	*A niger*	poly(1,4-α-D-galacturonide) glycanohydrolase	3.2.1.15
		Rhizopus oryzae	pectin pectylhydrolase	3.1.1.11
			poly(1,4-α-D-galacturonide) lyase	4.2.2.2
Proteases	hydrolase	*A niger, A oryzae, B subtilis*	none	3.4.21.14 3.4.24.4
Rennet	hydrolase	stomach of ruminant animals	none	3.4.23.4
		Mucor miehei		3.4.23.6

Note: Adapted from Food Chemical Codex 1981

The numbering system designates enzymes with a number containing four digits separated by periods and preceded by the letters EC. This is derived from a classification with six main groups of enzymes, derived from the chemical reactions catalysed:

- Oxidoreductases – enzymes which oxidise or reduce substrates by transfer of hydrogens or electrons or simply by use of oxygen. An example is hydrogen peroxide oxidoreductase (catalase, EC 1.11.1.6). $H_2O_2 + H_2O_2 \rightarrow O_2+2H_2O$
- Transferases – enzymes that remove groups (not H) from substrates and transfer them to acceptor molecules (not H_2O_2). An example is ATP:D-glucose 6-phosphotransferase (glucokinase, EC 2.7.1.2). ATP + D-glucose → ADP + D-glucose 6-phosphate
- Ligases – enzymes which catalyse covalent linking of two molecules. An example includes ammonia ligase (asparate-ammonia ligase, EC 6.3.1.1). ATP + L-aspartame + NH_3 → AMP + pyrophosphate + L-asparagine.
- Lyases – enzymes which remove groups from their substrates to leave double bond or which add groups to double bonds. Typical example is S-malate hydro-lyse (fumarate hydratase, EC 4.2.1.2).
- Hydrolases – enzyme systems where water plays a role in breaking of covalent bonds of the substrate. An example is triacylglycerolase (triacylglycerol lipase, EC 3.1.1.3).
- Isomerases – enzymes which facilitate isomerisation of a substrate. An example is alanine racemase (alanine racemase, EC 5.1.1.1 (Whitaker 1996; Nebesny et al 2002).

The majority of enzymes of interest in food processing are hydrolases, with oxidoreductases also being of significance.

2.1.1 Enzyme kinetics

Enzyme kinetics is the study of enzyme reactions. Activity of an enzyme depends upon various parameters including enzyme concentration, substrate concentration, pH, temperature as well as the presence of inhibitors and co-factors. For most enzymatic reactions the speed of the reaction is directly proportional to the concentration of the amount of enzyme present. This forms the basis of most methods used to determine enzyme concentration in unknown samples. The velocity of an enzymatic reaction decreases after the initial stages and this may be due to various causes. The most important reasons are the depletion of substrate and the inhibition of the enzyme by the formation of end products (Reed 1975).

2.1.1.1 Enzyme concentration

In general the rate of enzymic reactions are directly proportional to enzyme concentration ([E]) (Fig 2.1). The relationship between initial velocity (v_o) and [E] is linear when other factors are kept constant (Fig 2.2). Exceptions to this rule are limitations of substrate, conversion of substrate to product, coupled enzyme reactions (reactions where the product of one enzyme is the substrate for another enzyme) and also the presence of irreversible inhibitors (Whitaker 1996).

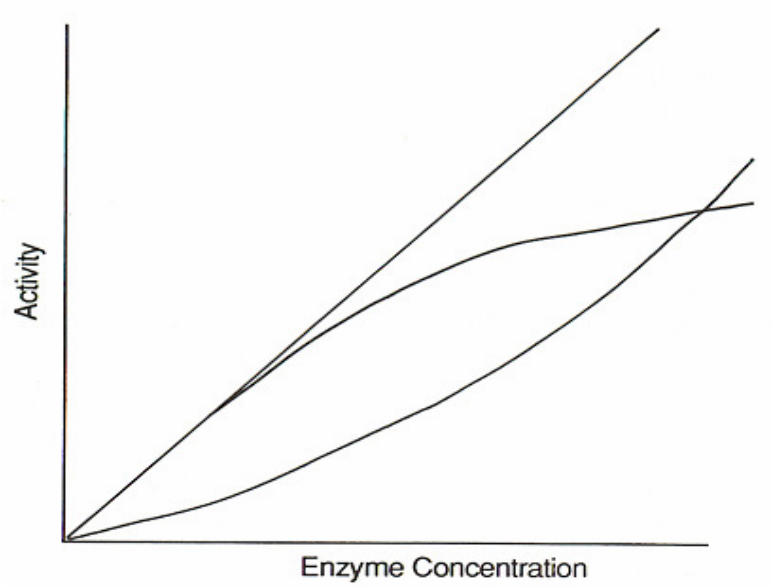


Fig 2.1 Typical patterns for activity (reaction rate) obtained when enzyme concentration is varied
Source: Tucker 1995

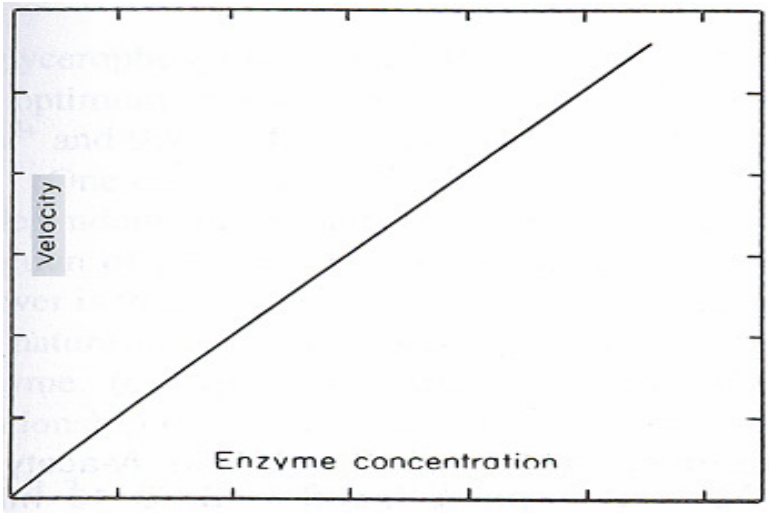


Fig 2.2 Linear relationship between enzyme concentration and velocity (reaction rate)
Source: Fennema 1996

2.1.1.2 Substrate concentration

The rate of an enzymically catalysed reaction depends primarily on substrate concentration. As substrate concentration increases the enzyme activity tends towards the maximum. The typical enzyme – substrate equation is as follows:

$$E+S \leftrightarrow ES \rightarrow E+P$$

The conditions applying to this equation are:

- the concentration of substrate must be higher than that of the enzyme;
- the rate constant for the release of the product must represent the limiting rate for the reaction as a whole and E and ES are essentially at equilibrium; and
- The concentration of P must be zero, i.e. there is no reverse reaction.

The effect of substrate concentration on the initial velocity of an enzyme reaction is very important for the practical use of enzymes as well as to a fundamental understanding of enzymatic reactions (Reed 1975).

2.1.1.3 pH effects on the activity of enzymes

Enzymes are said to be ampholytes, that is both their acidic and alkaline groups dissociate. In most cases the change in enzymatic activity at different pH levels is caused by changes in the ionization of the enzyme, substrate or the enzyme – substrate complex (Reed 1975). The effect of pH on an enzyme reaction is crucial for most

enzyme activities, since substrate binding and catalysis are dependent on the charge distribution of substrate and enzyme molecules. Some enzymes are active over a broad range of pH values, although most are active only over a relatively narrow range. The pH optimum varies for different enzymes (Table 2.2). In addition, enzyme stability is affected by pH and typically the stability range tends to be much greater than the activity range. Both pH impacts have to be considered during processing (Tucker 1995).

The complexity of pH effects on enzyme – catalysed reactions is shown in Fig 2.3. Enzymes are not stable at all pH levels, therefore it is important to determine the pH range of enzyme activity (Fennema 1996). Another issue is that the pH optimum may differ for different substrates. In addition, the pH activity curves are not always similar for two enzymes that hydrolyse the same linkage in a substrate. These differences in pH optima for enzymes that are acting on similar substrates are of considerable importance for the food industry. For most applications in food systems the pH cannot be readily adjusted to the pH optimum of an enzyme. Therefore an enzyme generally has to be chosen on the basis of its activity at the natural pH of the food. There are some exceptions where pH adjustment is possible including the production of glucose by the enzyme – enzyme process where starch is first adjusted to a pH between 5 and 7 for optimal hydrolysis by bacterial amylase (Reed 1975).

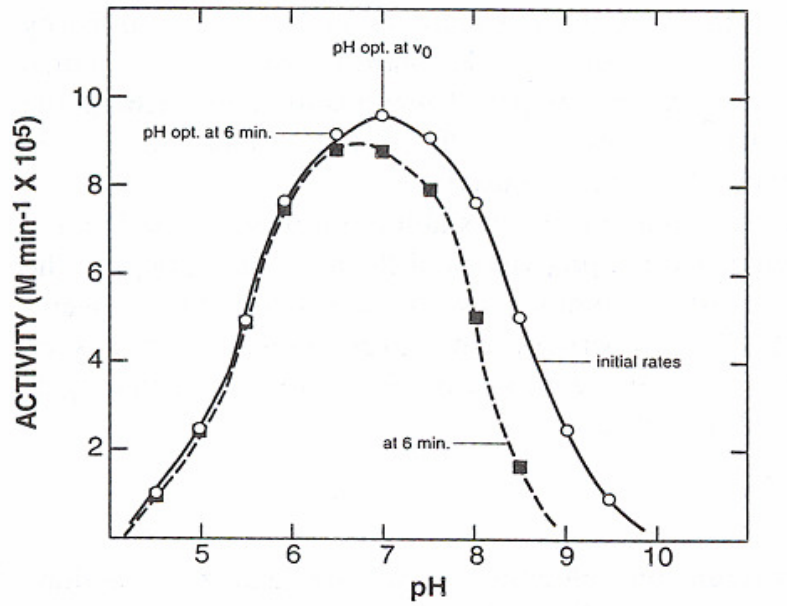


Fig 2.3 The effect of pH on the rate of enzymically catalysed reactions
Source: Whitaker 1996

Table 2.2 General properties of some enzymes used in food processing

Enzyme	Source	pH	Temperature (°C)	Km
α-Amylase (EC 3.2.1.1)	*B stearothermophilis*	4.6	65	1.0×10^{-3} gmL^{-1}
	P saccharophilia	5.9	25	6.0×10^{-4} gmL^{-1}
β-Amylase (EC 3.2.1.2)	sweet potato	4.8	35	7.3×10^{-5} M
Ascorbic acid oxidase (EC 1.10.3.3)	*A aerogenes*	6.5	37	4.0×10^{-3} M
	squash	5.6	25	2.4×10^{-4} M
Polyphenol oxidase (EC 1.10.3.1)	potato	7.0	-	2.2×10^{-5} M
	mushroom	7.0	-	2.4×10^{-4} M
Glucose oxidase (EC 1.1.3.4)	*P notatutm*	5.6	30	9.6×10^{-3} M
	A niger	5.6	25	3.3×10^{-2} M

Note: Source: Tucker 1995

2.1.1.4 Temperature effect on the activity of enzymes

Generally, the rate of enzyme reaction increases as temperature increases (Fig 2.4). This relationship can be described using the following equation:

$$d\ln k / dT = E / RT_2$$

where k is the rate constant, T is temperature (in °K), E is the activation energy and R the gas constant. Temperature not only effects reaction rate but also enzyme stability. The temperature stability of enzymes varies greatly (Table 2.2). Heat is often used to inactivate endogenous enzymes (Tucker 1995). At higher temperatures the curve deviates from linearity reflecting instability at higher temperatures (Fig 2.4; Reed 1975).

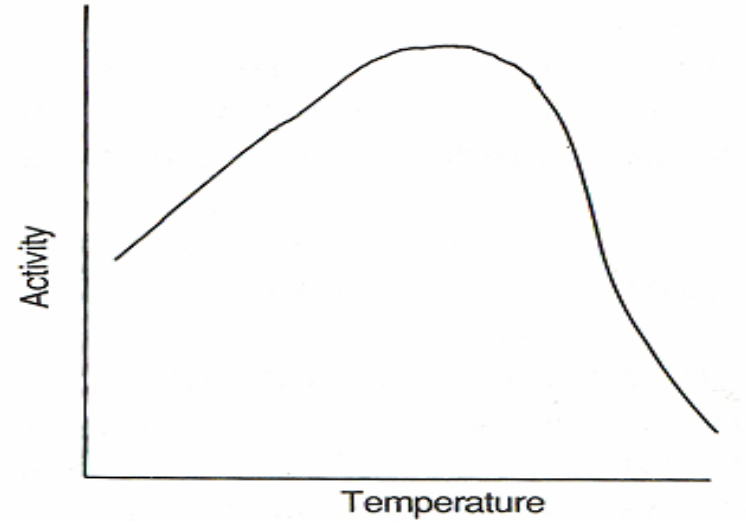


Fig 2.4 The typical influence pattern of temperature on enzyme activity
Source: Tucker 1995

2.1.2 Enzyme inactivation, control and inhibition

Enzymes are heat labile and heat denaturation of the enzyme ultimately results in a loss of its catalytic properties. The rate of inactivation increases with increasing temperature. In addition higher temperatures increase the rate of enzymatic reactions. Since higher temperatures increase the rate of inactivation of the enzyme these two factors have opposite effects on the rate of the enzymatic reaction. Optimum temperatures for enzymatic reactions have to be interpreted with a great deal of caution, since the time of an enzymatic reaction has a considerable effect on optimum temperature. Shorter reactions have higher temperature optima than longer reactions. In general for most enzymes used in food production heat inactivation becomes important at temperatures above 45°C (Reed 1975).

Enzymes are required for many reactions associated with reproduction, growth and maturation of all living organisms. However, enzyme activity may result in undesirable effects, for example the browning of fruits and vegetables caused by polyphenol oxidase. Enzymes may continue to catalyse reactions in foods and food ingredients leading to loss of texture, colour, flavour and overall nutritional quality. Therefore it is important to control enzyme activity. Enzyme inhibitors are widely used as means of controlling enzyme activity. Any compound that decreases the rate of enzyme reaction when added to the enzyme – substrate system is referred to as an enzyme inhibitor and these may be synthetic or natural. In addition, changes in pH, temperature as well as

denaturation of enzymes due to high pressures, irradiation or microbial action can lower enzyme activity. All of these strategies are valid ways of controlling enzyme activities in foods (Whitaker 1996).

2.2 α-Amylase

α-Amylase belongs to the group of amylolytic enzymes, which hydrolyse starch. Other members of the group include β-amylase α-glucosidase, pullulanase and cyclodextrin glycosyltransferase. α-Amylase (EC 3.2.1.1) (1,4 - α-D-glucan glucanohydrolase) is an endoglucosidase that cleaves the α-1,4 glucosidic bond of the substrate, to yield dextrins and oligosaccharides with the C1-OH in the α-configuration. α-Amylases typically have a molecular weight of approximately 50kDa and require Ca^{2+} for stability and activity. There are three sources of commercial preparations of α-amylase: cereal, bacterial and fungal. The pH optimum of the enzyme depends on the source e.g. 4.8 – 5.8 for *A oryzae*, 5.8 – 6.0 for *B subtilis*, and 5.5 - 7.0 for *B licheniformis*. Similarly temperature optima also vary: 70 – 72°C for the α-amylase produced by *B subtilis,* and 90°C for that from *B licheniformis* (Wong 1995).

Starch constitutes approximately 80% in the endosperm of the wheat grain. α-Amylase is a critical enzyme in remobilisation of the endosperm since it initiates hydrolyses of the starch granules. Wheat grain tissues can synthesises two groups of α-amylases, or α-amylase isoenzymes that differ in genetic control as well ad physical and chemical properties. Perhaps the most important group in grains are the high pI group (germination or malt α-amylases) that are products of the *α-Amy1* genes located on the group 6 chromosomes of wheat and that are normally synthesised in the grain during the early stages of germination. The second group referred to as low pI (pericarp or green α-amylases), are found in the pericarp of immature grains and at low levels in mature grains and are also synthesised during the later stages of germination. The synthesis of the low pI isoenzymes is controlled by *α-Amy2* genes located on the 7 chromosomes of wheat. Under ideal growing conditions, ripe wheat grains contain a small amount of low pI α-amylase and no high pI and following milling the flour can be used to produce variety of processed foods including pan breads, flat breads, noodles cakes and biscuits. Under non-ideal growing conditions, or as a result of adverse genotype × environment interactions, variable amounts of either or both groups of α-amylases may be retained or

synthesised prior to harvest and as a consequence the grain may be unsuitable for processing. Flat breads and sourdough breads are more tolerant to α-amylase, whereas Japanese Udon noodles are very sensitive. High levels of α-amylase activity in ripe wheat grain can arise via a number of mechanisms. First the low pI α-amylase that is synthesised in the green (chlorophyllous) pericarp of immature grains may be retained rather than being gradually degraded as the grain matures. Second, a small number of wheat genotypes have a genetic defect referred to as late maturity α-amylase (LMA) pr prematurity α-amylase (PMAA) that is reflected in the synthesis of high pI α-amylase in the aleurone tissue during the latter stages of grain development. Finally adverse weather conditions (rain accompanied with high humidity) at maturity and prior to harvest may trigger grins to commence germination whilst still in the spike. This phenomenon known as pre harvest sprouting is known is relatively common in many parts of the world. Whilst LMA and sprouting are readily distinguish both in genetic control and in the pattern of enzyme distribution within the grain, the α-amylase isoenzymes involved are similar, as are the effects on the quality of end products (Mares et al 2004).

Successful modification of the baking process and the characteristics of finished cereal grain products should be based on an understanding of the basic components of wheat flour and the effects resulting from their enzymatic modification. Hard wheat flour (70 % extraction rate) is composed of approximately 82% starch, 12.5% protein, 3.5% fibre, 1.5% lipid and 0.5% ash; soft wheat flour has approximately 8-10% protein and correspondingly more starch. The more obvious targets for enzymatic modification are the starch, protein and fibre fractions. Most flours, milled from sound wheat, contain significant amounts of β-amylase but little α-amylase.

Of the common commercial preparations, fungal amylase is the most susceptible to heat, being inactivated at a temperature of approximately 60°C. Cereal amylases remain active until the temperature reaches around 70°C, and so they can cause further hydrolysis after the starch has gelatinized. The thermal stability of α-amylase from various sources is compared in Fig 2.5. Bacterial amylases are the most heat stable, with some remaining active throughout the bread baking cycle (Mathewson 2000).

α-Amylases of different origins vary in their ability to cleave linear maltodextrins with lower degrees of polymerization (DP) and how readily they break linkages in the vicinity of the α-1,6-branch points in amylopectin or glycogen. The level of endogenous α-amylase in cereal grains and products significantly affects the industrial utilization of these commodities (McCleary and Sturgeon 2002). The enzyme has a central role in the mobilization of starch reserves in the germination of cereals and many legumes and it is a key quality parameter in the commercial utilization of most cereals (McCleary et al 2002).

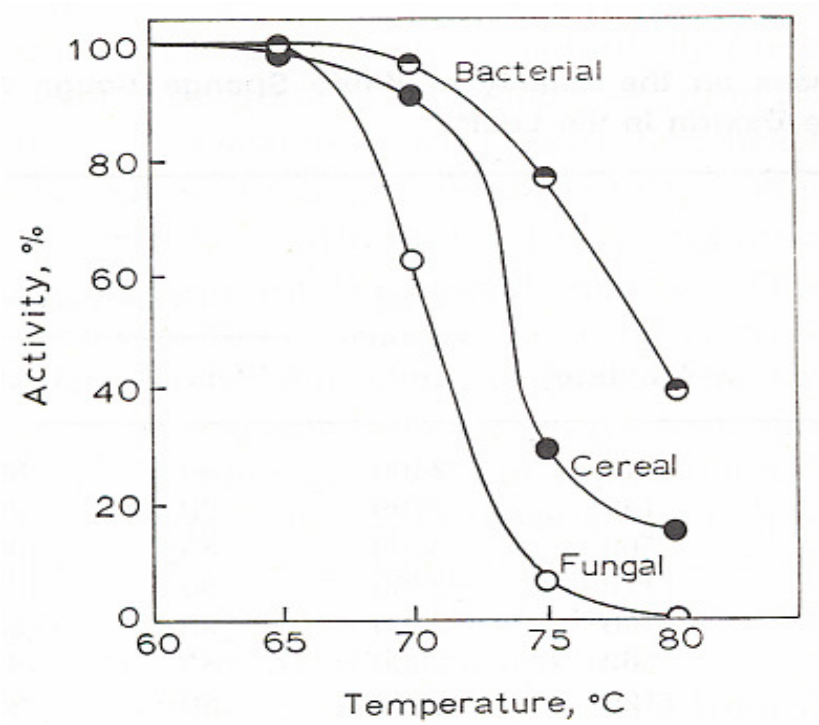


Fig 2.5 Thermal stability of α-amylase from different sources
Source: Reed 1975

The enzyme preparation from *A oryzae* has long been approved by the U.S. Food and Drug Administration for general use in food systems (Meyrath and Volavsek 1975). The organism commonly used for production of bacterial α-amylase is *B subtilis.* Although these enzymes have been approved for use in bread production, it is very difficult to satisfactorily control the level of bacterial α-amylase in bread making. The levels of this enzyme that improve texture and loaf volume result in soft and gummy crumb characteristics.

2.3 Lipases

Lipases (EC 3.1.1.3) are a group of lipolytic enzymes, more fully described as triacylglycerol acylhydrolase (Wong 1995). Lipase catalyses the hydrolysis of triacylglycerols, diacylglycerols and, in some cases, monoacylglycerols (Urquhart et al 1984; Thomson et al 1999). It is noted that in this thesis the term acylglycerol is used in place of the superseded term glyceride. Lipases exhibit maximum activity at oil – water interfaces and it is known that reverse reactions can be catalysed when there is limited moisture present. It has been reported that in cereals lipase is present in rice and oats but not in ungerminated wheat, corn scutellum, rye or barley (Matlashewski et al 1982).

Lipolytic enzymes can be defined as carboxylesterases that hydrolyse acylglycerol molecules. Those hydrolysing acylglycerols having less than ten carbon atoms in a fatty acid chain are referred to as esterases or carboxylases (EC 3.1.1.1). On the other hand, the enzymes hydrolysing acylglycerols having ten or more carbon atoms in the chain are known as lipases or triacylglycerol acylhydrolases (EC 3.1.1.3). Esterases are active in aqueous solutions while true lipases are more active in oil – water interfaces. The specificity of lipolytic enzymes is controlled by three factors:

- the molecular properties of the enzyme;
- the structure of the substrate; and
- the binding of the enzyme to the substrate (Chen et al 2003).

Lipases are capable of catalyzing a variety of reactions, many of which have great potential importance in industry (Melo et al 2000; Schmid et al 1995; Mukataka et al 1989).

The lipase activity of oat kernels was studied at different pH values, different stages of development as well as the distribution within the kernel (Ekstrand et al 1992). Although the fat content of oats is relatively high, the lipid composition is similar to that of other cereals. The activity of lipase is high at early stages of kernel development and increases upon germination. Lipase activity in oats is associated with the parts located close to the surface of the caryopsis. The postharvest and storage of oats grains may damage the tissues and this would increase hydrolysis as the contact between the enzyme and substrate would be facilitated. Lipase inactivation during the production of

oaten products for human consumption is necessary in order to minimise fat hydrolysis and oxidation, both of which would lead to rancidity, the main limiting factor in storage of oat products. Steam treatment can be used for partial or total lipase inactivation.

The applications of lipases are diverse and are summarised in Table 2.3 (Thomson et al 1999; Pancreac and Baratti 1996; Otero et al 1995; Winkler and Stuckmann 1979).

Table 2.3 Common applications of lipases

Areas of application	Effects obtained	Products
Dairy	Hydrolysis of milk fat	Flavouring agent
Bakery	Flavour & shelf life	Bakery products
Brewing	Aroma & fermentation	Alcoholic beverages
Salad dressing	Improvement of egg quality	Mayonnaise, whippings
Meat & fish processing	Flavour & removal of excess fat	Meat & fish products
Fats and oils	Transesterification of natural oils	Cocoa butter
Chemical synthesis	Synthesis of esters	Esters
Detergents	Removal of stains & lipids	Laundry detergents
Medical	Blood triacylglycerol assay	Diagnostics
Cosmetic	Removal of lipids	Broad area of cosmetics
Pharmaceuticals	Digestion of oils &fats	Digestive aids
Leather	Removal of fats from animal skin	Leather products

Note: Source: Thomson et al 1999

In breadmaking, there is an effect of lipid hydrolysis on baking quality of (Ekstrand et al 1992; Mosinger 1965). Lipase activity is barely detectable in the mature seeds of many cereals and generally appears only after germination. In wheat, lipase activity is initially very low, but increases upon germination. Peanut lipase also increases during

maturation and development. Changes in the composition of triacylglycerols during maturation have been reported for safflower and oats, although this might simply reflect increasing synthesis of the enzyme rather than a physiological role during seed maturation (Urquhart et al 1984).

Lipases are widely used as catalysts to split acylglycerols under mild conditions. Treatment of tallow with lipase yields fractions of fatty acids rich in oleic acid, which has wide application in many industries. The remaining acylglycerols (the unreacted residues) are rich in stearic and palmitic acids and are used as bread softeners in th baking industry (Piazza et al 1989).

Gélinas et al 1998 studied bleaching effects of lipase in bread doughs and reported that complete bleaching was seen when linoleic acid (90-200mg) with 325-815U of lipase/100g of flour was used. They stated that under the conditions studied in doughs, lipase showed greater potential for bleaching than peroxides, because lower concentrations of linoleic acid were required by the lipase (Gélinas et al 1998).

In cereals lipase is present in oats, rice but not (or at very low levels) in ungerminated wheat, barley or rye. However, a study by Matlashewski et al 1982 suggested that the lipase activity was still very low even after germination (Matlashewski et al 1982). During germination the role of lipase is to breakdown oil storages from cellular reserves called lipid bodies. Since the active site for lipase is the oil – water interface, a stable and large surface area of lipid globules in an ester emulsion is important for detecting lipase activity (Hoppe and Theimer 1996). Considerable efforts have been made to determine the role of lipases in dietary fat digestion and the molecular and kinetic events occurring during the catalytic process at lipid – water interactions (Pencreac'h et al 2002). The reaction takes place at the interface of the substrate lipid in contact with an aqueous buffered solution of lipase. Fuse et al 2000 reported that for an efficient progress of the reaction it is necessary to keep substrate lipid highly dispersed in the mixture during incubation. In addition the emulsifying agent is liable to change the fat digestive potency. Thus they studied flow injection analysis with electrochemical detection (FIA/ECD) as a very sensitive method with high reproducibility for determining lipase activity in pharmaceutical preparations. The small sample size allows the use of olive oil substrate without emulsifying agent (Fuse et al 2000).

During germination of oilseeds, the physiological role of lipase is the breakdown of storage oils. These cellular oils are stored in cell compartments called oleosomes or lipid bodies. These lipid bodies can be stabilized by proteins called oleosins and these prevent coalescence. The main site of action for lipase is the oil – water interface, and therefore a large and stable surface area of lipid globules in a neutral ester emulsion is important for assaying lipase activity. For this reason triacylglycerols have been found as an ideal substrate. In a study by Hope and Theimer 1996 the possibility of determining low lipase activity in non- fatty tissues was demonstrated for the first time. Hope and Theimer based lipase activity measurements on the free fatty acids released from triacylglycerols, like triolein by enzymatic hydrolysis. They reported that the stabilisation of the emulsified substrate by gum Arabic and the detergent (desoxycholate) was important to measure lipase activity in a pH –stat. In addition they concluded that complete ionization of free fatty acids was performed at pH 9.0, furthermore the addition of $CaCl_2$ tends to decrease the lipase activity while addition of NaCl had the opposite effect on lipase activity (Hope and Theimer 1996).

Lipases have been widely investigated and their structures elucidated. There are close similarities in the three-dimensional structures of some of the lipases studied and these are shown in Fig 2.6.

2.3.1 Pancreatic lipases

The enzyme is produced by acinar cells of the pancreas and is released with other enzymes into the duodenum where it plays an important part in the digestion of lipids. Pancreatic lipase hydrolyses triacylglycerols more rapidly than monohydric alcohols. It does not possess absolute specificity for short or long chain fatty acids nor for saturated or unsaturated fatty acids. Long chain polyunsaturated fatty acids are hydrolysed at a slower rate. It has been shown that essentially no activity is lost when free SH groups are substituted by phenylmercuric radicals (Shahani 1975).

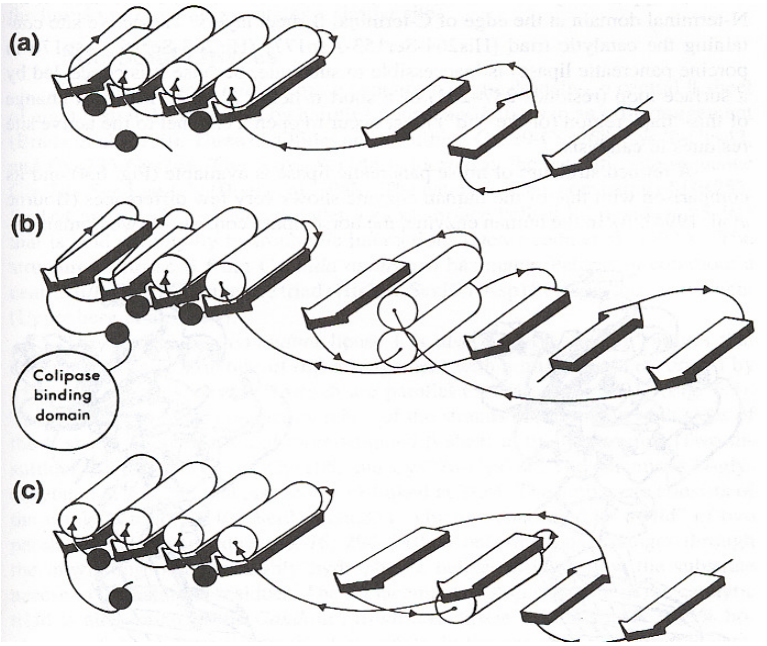


Fig 2.6 **Three dimensional structures of α/β fold of lipases**
a) ***Rhizomucor miehei***
b) **Human pancreatic**
c) ***Geotrichum candidum***.
Source: Wong 1995

2.3.2 Microbial lipases

Bacterial lipases are primarily extracellular and produced during early stationary and late log phases of growth. Many microorganisms are able to produce more than one type of extracellular lipase that can hydrolyse different chain lengths of fatty acids, but especially short chains. The levels of lipase produced and the ratio between extracellular and intracellular lipase produced depends on factors including temperature, pH, lipid and nitrogen sources, the availability of oxygen, concentration of inorganic salts. Polysaccharides, for example glycogen and gum Arabic are reported to be good media for lipase production. (Chen et al 2003). The structural characteristics of some microbial lipases are known (Fig 2.7). The properties of bacterial lipases are listed in Table 2.4 and these demonstrate considerable variation in the characteristics.

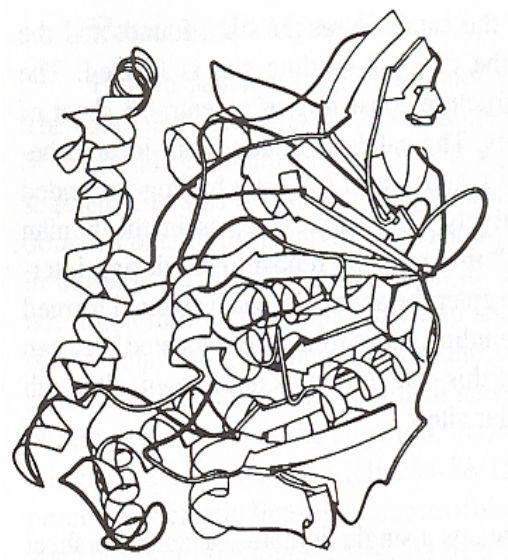


Fig 2.7 View of lipase structure from *Geotrichum candidum* (side view of 11-stranded mixed β sheet
Source: Wong 1995

Table 2.4 The properties of various bacterial lipases

Source	Molecular mass (kDa)	Substrate specificity
Staphylococus aureus	76	Broad
B subtilis	19.4	1,3 position & C8
Streptomyces species	27.9	ND
Aeromonas hydrophila	71.8	C6 - C8
Propinibacterium acnes	41.2	Broad
Pseudomonas aeruginosa species 109	30	C4 – C6
P. aeruginosa	29	1,3 position & C18
P. alcaligines	30	1,3 position & C12 – C18
P. species ATCC 21808	35	C8 – C 10
P. fluorescens SIKW1	48	1,3 position & C6 – C8

ND Not determined
C# Number of carbon atoms in the chain
Source: Thomson et al 1999

2.3.3 Cereal lipases

Wheat and other cereal grains deteriorate during storage. When there is high humidity, high moisture content of the grain and high temperature the deteriorative changes are even faster. In addition various chemical changes occurring during grain deterioration are prominent. Hydrolysis of fats with an increase in free fatty acids is rapid and occurs during the early stage of spoilage. The germ portions of cereal grains contain higher levels of lipolytic activity than the endosperm (Shahani 1975). Lipase activity from whole oat seeds is dependent upon calcium ion in a two phase olive oil – water system (Piazza et al 1989).

2.4 Lipoxygenase

Lipoxygenase (LOX) (EC 1.3.11.12) is a dioxygenase that catalyzes the oxygenation of polyunsaturated fatty acids containing a cis, cis-1,4-pentadiene system to hydroperoxide as follows:

$$CH_3\text{-}(CH_2)n\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{-}(CH_2)n\text{-}COOH$$
$$\downarrow \; + O_2$$
$$CH_3\text{-}(CH_2)n\text{-}CH{=}CH\text{-}CH{=}CH\text{-}CH\text{-}(CH_2)n\text{-}COOH$$
$$|$$
$$OOH$$

The enzyme is widespread in plants and those from different sources differ in pH and temperature optima and substrate requirements. The most widely studied and used is the LOX from soybean and its structure is shown in Fig 2.8. It has MW of ~ 98,000 Da; pH optimum of 9.0 and co-oxidation activity. Soybean shows co-oxidation activity on β-carotene. This ability to bleach pigments is widely applied in commercial bread making where soy-bean flour is added to supply this enzyme (Wong 1995). LOX is naturally present in flour where it catalyses oxidative reactions of great importance for the rheological properties of the dough. It has been found that LOX is at least partially denatured in the initial stages of mixing and dough development (Rakotozafy et al 1999).

In breadmaking LOX increases the amount of free lipids in the dough and modifies dough properties by increasing mixing tolerance and relaxation times with the

consequence of enhanced volume (Eskin and Grossman 1977; Veldnik et al 1977; Gibian and Vandenberg 1987). Some modification of bred aroma is also due to LOX actions (Baianu et al 1982; Eriksson 1968). Cereal lipids are rich in polyunsaturated fatty acids, making them good substrates for LOX actions (Rutgersson et al 1997). The enzymatic bleaching of carotene by coupled oxidation with unsaturated fats is also significant in the loss of yellow colour during pasta processing due LOX enzymes (McDonald 1979).

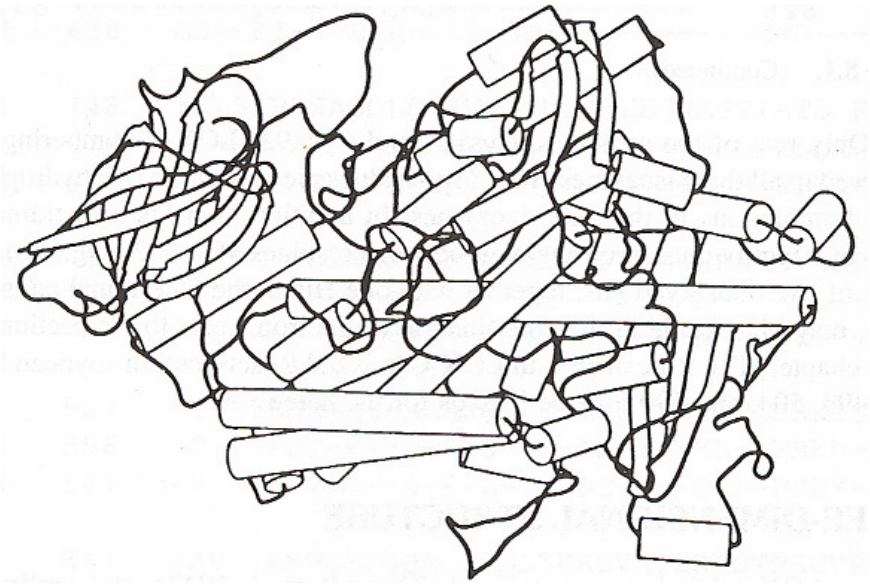


Fig 2.8 Diagram of the structure of soybean LOX
Source: Wong 1995

2.5 Polyphenol oxidase

Polyphenol oxidase (PPO) (EC 1.10.3.1) is also known as tyrosinase or catechol oxidase. It plays an important role in determining the quality of fruit and vegetable during processing and storage. It also has a desirable action in tea, coffee, prune, black raisins and black figs. It is most widely found in mushrooms and it has a MW of approximately 128.0 kDa (Wong 1995; Anderson and Morris 2003; Marsh and Galliard 1986).

PPO catalyses two different types of reactions both involving phenolic compounds. These reactionc include hydroxylation of monophenols to *o*-diphenols and the removal of hydrogens from *o*-diphenols to produce an *o*-quinone. (Reed 1975).

The range of optimum pH for PPO activity ranges from 5 to 7, at lower pH the activity cannot be established, also at higher pH autooxidation of the substrate makes it impossible to measure PPO activity with significant level of activity. PPO has been of interest to food processors since it causes browning in food systems. Enzymatic browning results from the polymerization of quinones formed from simple mono or dihydric phenols (Scott 1975). PPO catalyses the oxidation of phenolic compounds in presence of molecular oxygen. They are widely distributed in plants and cause enzymatic browning in food material through an initial oxidation of phenols into quinines. Quinones can spontaneously undergo polymerisation or condensation with amino acids or proteins via their amino groups and form complex brown polymers.

In wheat it has been reported that PPO activity is affected by cultivar and also growing location. A number of studies indicate that high levels of PPO in the grain endosperm of bread wheat have a deleterious effect, causing discolouration in particular in chapattis and Asian noodles. A correlation between *o*-diphenol oxidase activity and flour colour has been reported as well as non significant correlation of L* values and PPO activity in the flour. These findings are not contradictory since water must be added to flour thus facilitating enzymatic browning. There are also reports that due to the low levels of PPO in durum semolina it is unlikely that this enzyme alone is a major factor determining the colour of spaghetti. PPO activity increases upon germination and grain dissection has indicated that a large part of PPO was in the immature wheat kernel was present in the endosperm. Thus as phenolic compounds are abundant in immature endosperm it has been proposed that the action of PPO on these phenols could lead to the formation of quinonoid compounds (Feillet et al 2000).

The amount of PPO in bread wheat flour depends upon the milling of the wheat. PPO activity increases with increasing bran contamination and has a linear correlation with ash content. Furthermore, since PPO activity is distributed in the different regions of the durum wheat grain as well as in bread wheat grain regions, PPO may be involved in pasta brownness if semolina is contaminated by outer regions of the grain. However, whether such brownness is the result of inherent brownness of the milling streams or from the action of PPO is yet to be determined (Feillet et al 2000; Kruger et al 1994;

Vadlamani and Seib 1996; Kruger 1974; Bhattacharya et al 1999; Demeke et al 2001; Jukanti et al 2004).

2.6 Peroxidase

Oxidoreduction enzymes in wheat flour as well as in baking additives, have always been of interest to cereal chemists. As a member of this group, LOX has already been described. The effects of peroxidase (EC 1.11.1.7) (POX) are much less thoroughly documented, although they are reported to have a relatively high level of activity in wheat flour (Kruger and LaBerge 1974; Kieffer et al., 1982). More generally this enzyme has often been used as a genetic marker and indicator of food quality. It seems to be crucial for colour quality, as it effectively bleaches carotenoids (Iori et al 1995).

Among numerous oxidising agents, peroxidase appears as the most efficient catalyst for the oxidative gelation of wheat flour water-soluble pentosans. There are two mechanisms proposed for the oxidative crosslinking of pentosans. One is based on the formation of a diferulate bridge between two adjacent ferulic acid residues involved in crosslinking of arabinoxylan chains. Another mechanisms involves the addition of a protein radical to the active double bond of ferulic acid esterified to the arabinoxylan fraction being involved in a crosslinking between a protein and a pentosan chain. However, the action of POX on bread making still remains unanswered because the formation of the primary substrate (hydrogen peroxide) remains uncertain (Delcros et al 1998).

Wheat has a pH optimum of 4.5 and in the pH range of wheat doughs (5 – 6), the activity is considerably lower. In contrast, horseradish POX retains 80 % of its maximum activity at the pH of dough. The addition of horseradish POX in combination with H_2O_2 leads to strengthening of wheat doughs. It has also been reported that H_2O_2 can be replaced by the H_2O_2 producing systems glucose oxidase and glucose (Van Oort et al 1997).

POX is found naturally distributed in plants (horseradish, tobacco, potato, turnip), as well as microorganisms (yeast). The native enzyme horseradish POX has the ferric iron

co-ordinated to the four nitrogens of the pyrrole ring of the protoporpyrin IX (Fig 2.9). The two reaction steps involved in the mechanism of horseradish POX are:

- a two electron oxidation of the ferric enzyme to HRP-I, with the POX substrate cleaved at the O-O bond,
- a one-electron reduction of HRP-I by electron donor substrates to the enzyme via compound HRP-II (Wong 1995).

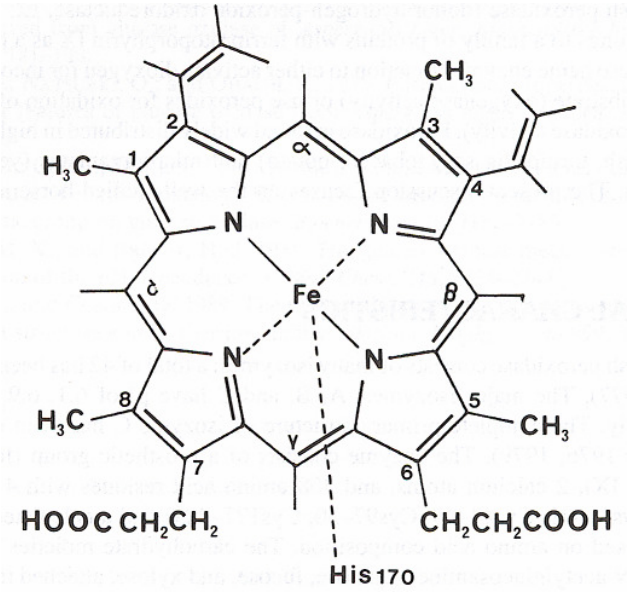


Fig 2.9 Diagram showing the position of the iron group of horseradish POX
Source: Wong 1995

The mechanism of horseradish POX is well understood (Figs 2.10 & 2.11). These show the conversion of the native enzyme to HRP-I and HRP-II and reaction mechanism of horseradish POX showing the pathways involving the two electron oxidation of the native enzyme to the HRP-I and the two step one electron reduction of HRP-I to HRP-II respectively.

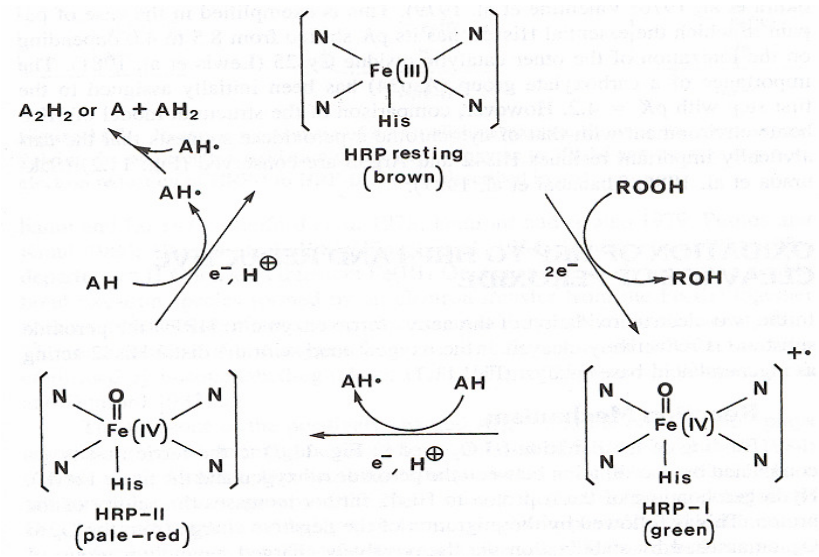


Fig 2.10 Representation of a general mechanism of horseradish POX (conversion of native enzyme to HRP-I and HRP-II
Source: Wong 1995

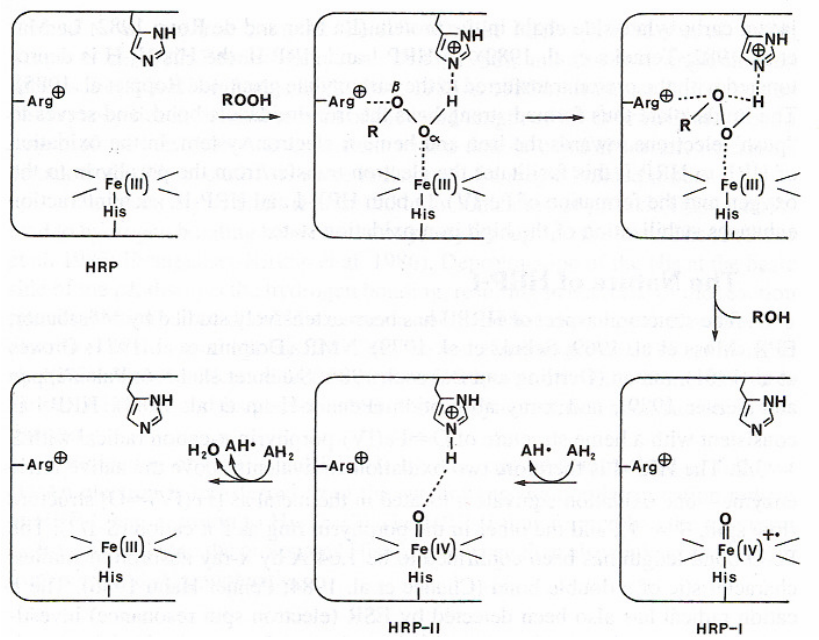


Fig 2.11 The mechanism of horseradish POX (conversion of HRP-I and HRP-II)
Source: Wong 1995

2.7 Ascorbic acid oxidase (AAO)

AAO (EC 1.10.3.3) is found in plants and is a copper-containing blue oxidase that catalyses the aerobic oxidation of ascorbic acid to dehydroascorbic acid (DHA) via a free radical intermediate. Although no definite function has been assigned to the enzyme, AAO is of special interest because of the generally high concentrations of

ascorbate in plant cells, the high reactivity of the ascorbate/DHA redox pair, and its cell wall localisation. The cell wall is a complex and dynamic structure consisting of several classes of polysaccharides, proteins (enzymes as well as structural proteins), and small molecules, offering opportunities for interaction with ascorbate/DHA (Roux et al 2003; Billaud et al 2003; Yahia et al 2001; Nakamura and Kurta 1997a; Nakamura and Kurta 1997b).

The mechanism of the ascorbic acid improver effect in bread making has been largely determined, but the extent to which ascorbic acid oxidation to dehydroascorbic acid in dough is mediated by enzymatic reactions is still not clear (Every et al 1995; Every et al 2003). The enzyme appears to be heat labile and specific for L-ascorbic acid with an optimum pH of 6.6. It is also believed that AAO has a role in the development of the citrus flavour (Scott 1975; Strothkamp and Dawson 1977; Tono and Fujita 1982; Grant and Sood 1980; Grant 1974; Honold and Stahmann 1968).

2.8 Enzymes as food processing aids in cereal foods

Enzymes are used today in a variety and growing number of industries and these range from the animal feed industry and alcohol manufacture, to dairy processing and textiles. They only catalyse single type reaction their commercial use is very specific. Endogenous enzymes present in cereal grains have an important role in food processing e.g. in baking, beer production. In addition to the endogenous enzymes, exogenous enzymes from fungal and bacterial sources are widely added to food systems. The use of calf rennet in cheese making, fermentation in wine making are just some of examples of an age old practice of using enzymes to improve food processing quality. However, currently relatively few enzymes are used in food manufacturing. Some examples include

- α-Amylase - used in bread making to ensure supply of fermentable sugar for yeast growth; conversion of starch to dextrin in corn syrup production
- β-Amylase - used in the making of high maltose syrup
- Lipase - often used for flavour development in cheese making; fat production
- Glucose oxidase - used to prevent Maillard reactions in eggs products by converting glucose to gluconic acid (Wong 1995).

Enzyme activity is probably the most important information when assessing an enzyme. The international unit for enzyme activity is the Katal (kat) and it is defined as the enzyme activity which will transform one mole of substrate per second under optimum conditions. However, in practice units that reflect the preferences of the typical enzyme application are preferred. For example in baking α-amylase activity would be expressed in 'SKB' units whereas in brewing industry traditionally the same enzyme activity would be expressed in 'Degrees Lintner'. There are many different methods used to measure α-amylase activity thus yielding different units of activity. Unfortunately there is no mean of comparing results obtained using different assay procedures unless conducting more practical work. Also at the moment there is no requirement to list the enzyme or enzyme activity on the label, this should also be considered since enzymes have become prominent in food industry and are being added to different food products on a regular basis. Therefore, the consumers would perhaps like to know what they are eating.

Overall enzymes have a very important role on the quality of foods. Enzymes can also be added to foods during processing. One of the most common uses of enzymes in food systems is the production of high-fructose corn syrup. Heat stable α-amylase, glucoamylase and glucose isomerase are involved as in the following reaction

$$\text{starch} \xrightarrow{\alpha\text{-amylase}} \text{dextrins} \xrightarrow{\text{glucoamylase}} \text{glucose} \underset{\text{isomerase}}{\overset{\text{glucose}}{\rightleftharpoons}} \text{fructose}$$

(Whitaker 1996; Nickerson and Ronsivalli 1978).

2.8.1 Endogenous enzymes and food quality

Endogenous enzymes are those enzymes that are naturally found in the ingredients of a food. These enzymes could either be complementary or deleterious in relation to cereal quality. During maturation of cereal grains large number of enzymes form in the outer layer of the wheat plant including many endo enzymes including α-amylase and endoproteases. These enzymes reach a peak during grain development but then disappear after maturation. At the same time there is higher concentration build up of

exo –enzymes including β-amylase and aminopeptidases. Most of the time these enzymes do not cause any concerns during food processing unless specific conditions such as pre-harvest sprouting occur (this will be discussed further in Chapter 3) (Kruger 1997).

Enzymes are widely added to foods to change or control their characteristics. The quality of many foods is judged primarily upon colour. For example meat must be red, enzyme – catalysed reactions in meat can alter oxidation – reduction reactions and water content and thus effect the colour of meat. In fruits and vegetables upon ripening chlorophyll levels decrease due to enzyme actions. Three main enzymes responsible for pigmentation in fruits and vegetables are lipoxygenase, chlorophyllase and polyphenol oxidase. In addition to colour texture is also very important quality attribute in foods (Bernier and Howes 1994).

2.8.2 Enzymes as processing aids in the baking industry

Bread baking is a process that has been used for thousands of years. However, it was only in this century that greater knowledge became available about the processes taking place during dough fermentation and baking. Nowadays a range of enzymes is used to enhance bread quality (Mutsaers 1997). Wheat is the main ingredient of many baked products and it is also very variable. The miller is the first who must cope with all the requirements set by different applications of the flour. The use of enzymes in breadmaking technology is mainly concerned with the optimisation of dough properties which reflect the quality characteristics of fresh and or stored baked goods. Enzymes commonly used by the baking industry are listed in Table 2.5. In addition, to the traditional starch – hydrolyzing enzymes, amylolytic / dextrinising and debranching enzymes, enzymes able to degrade non starch polysaccharides and lipid as well as gluten modifying enzymes have proved to be effective for dough conditioning and strengthening, along with enhancement of yeast activity and bread flavour (Collar et al 2000; Martin et al 2004).

One of the oldest applications of enzymes in baking is the application of malt. This is used in breads to improve volume, colour and crumb characteristics of the products. Although malt contains a range of enzymes including proteases and pentosanases, the desirable effects are primarily due to amylases. Amylases are used to improve dough

handling, loaf volume and product quality particularly keeping quality (Hamer 1991). Bacterial maltogenic α-amylase has been demonstrated to have antistaling effects, improving crumb elasticity and effects the initial crumb softness by the action of degrading amylose and amylopectin at the gelatinisation temperatures by producing α-maltose (Collar et al 2000).

Fungal amylase from *A oryzae* was the first microbial enzyme used in bread baking. It continues to be used as a replacement for malt flour which may contain unwanted side activities including protease. In addition thermostable amylases from *Bacillus* spp are active on gelatinized starch and therefore improve crumb softness (Mutsaers 1997). The low levels of α-amylase in ungerminated wheat requires supplementation of the flour for best effect of this enzyme in the production of baked goods. The two primary functions of α-amylase in baking are the provision of fermentable sugars for yeast activity and continued gas production. It also affects dough properties, improving the structure and the keeping quality of baked goods. A certain amount of starch is damaged when wheat is milled. The level of damaged starch normally present in bread flour is important to support proper and efficient fermentation if the starch can be converted to maltose. The β-amylase naturally found in flours does not attack undamaged starch and therefore cannot convert enough starch to maltose. α-Amylase does attack damaged starch and converts it to dextrins, which are then hydrolysed by β-amylase to maltose. Therefore it is essential to supplement bread flour either at the mill or at the bakery with α-amylase. α-Amylase has a marked effect on the viscosity and softness of a dough. The addition of α-amylase to a dough yields bread with a softer crumb and a slower rate of firming (Barrett 1975).

Another enzyme widely used in bread making are the proteases. These are readily added to the formulation to shorten the time required for mixing, but also to improve dough consistency. Proteases are used to assure dough uniformity and enhance textural and flavour properties (Hamer 1991). Glucose oxidase is also widely used in bread making. The reaction catalysed by glucose oxidase is the formation of hydrogen peroxide in the dough. This is achieved by oxidation of glucose by molecular oxygen. It is the combination of glucose oxidase and endo-xylanase effectively improves loaf volume. Glucose oxidase also improves mixing tolerance of doughs (Mutsaers 1997). In general

oxidizing agents have a beneficial effect on dough properties including volume, textural properties and crumb structure.

Various peroxidases (horse radish and soybean) have been found to perform equally well as glucose oxidase in bread systems. Peroxidase results in finer crumb structure and thus a better appearance of breads (van Oort 1997). In addition to these enzymes lipase is also widely employed in bread making. The substrate for lipase in wheat flours are endogenous wheat lipids. Some of the effects of lipase on the baked loaves include improved crumb structure, dough stability, crumb softness and longer shelf life (Poulsen and Soe 1997). Its main application, however, is in crumb softening. Lipase hydrolyses triacylglycerols to free fatty acids and mono and di acylglycerols and mono acylglycerols are effective crumb softeners. To achieve the best effect, however, lipase should be combined with fat (Mutsaers 1997). Lipase has also been shown to have a significant positive effect on the crumb structure, improved dough stability and shelf life (Poulsen and Soe 1997). The use of exogenous lipases in bread has been claimed to retard bread staling and improve dough rheological properties. A good conditioning effect, improved rheological characteristics and dough stability during over-fermentation, along with better crumb structure in breads without the use of shortenings was demonstrated for some particular lipase preparations (Collar et al 2000).

Lipoxygenase has been used for bleaching through the oxidation of carotenoid pigments, resulting in whiter bread crumb. It also gives greater mixing tolerance and the reaction mechanism probably involves formation of free radicals leading to the formation of protein disulfide bonds and ultimately increased dough strength (Van der Lugt 1997).

In bread baking main purpose of using enzymes is to reduce the rate of staling in bread. Some enzymes commonly used include debranching enzymes (1,6 splitting) in addition to α-amylase, genetically engineered α-amylase that rearranges the branched chains of amylopectin to produce linear polymer, a mixture of cellulase, β-glucanase and pentosanases, a mixture of cellulase, hemicellulases and pentosanases used together with fungal α-amylase (Whitaker 1996). In addition, special xylanases can improve the baking process of wheat breads by stabilizing the dough structure against over-

fermentation, freezing and thawing. Most often used are the baking – active xylanase from *Aspergillus* spp. This dough stabilization by xylanases is also important in the making of high fiber bread. In combination with ascorbic acid resulting baked loaves are high in volume, fine and soft crumb and prolonged shelf life (Sprössler 1997).

It is common practice to add soybean LOX to bread formulase as part of enzyme active bread improver. The effects include better dough rheology (tolerance to over mixing), but also positive effect on stalling rates and loaf colour. However, the molecular basis of bread improvement through LOX activity still remains unclear, although it is likely that it involves the formation of intermolecular disulphide bridges between the high molecular weight (HMW) glutenin polypeptides in wheat endosperm. It is believed that LOX supports this behaviour by production of a network of disulphide bonds (Fig 2.12) (Casey 1997).

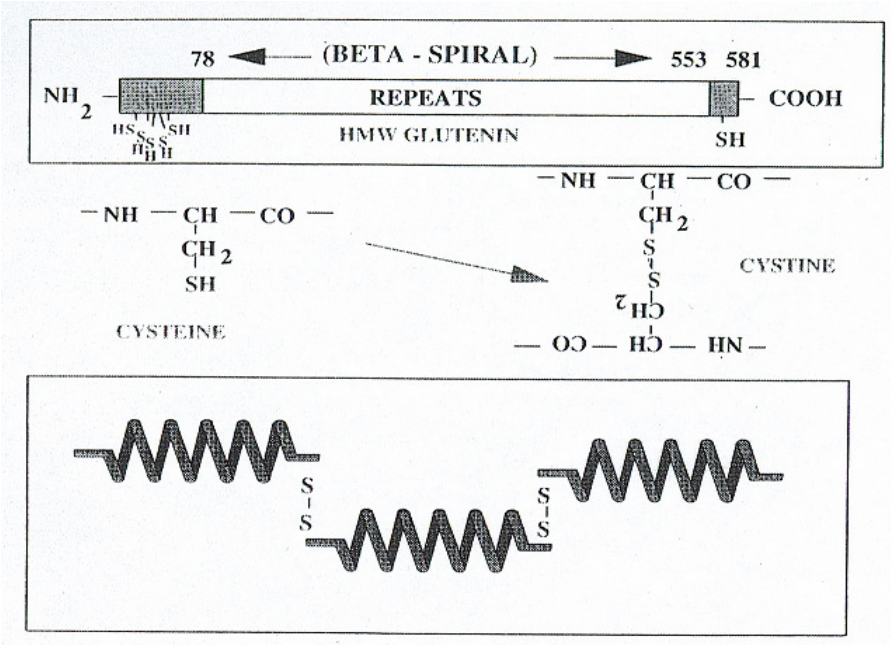


Fig 2.12 Schematic representation of the co-oxidative formation of intermolecular disulphide bonds between wheat HMW glutenin subunits, catalysed by LOX
Source: Casey 1997

Table 2.5 Some enzymes commonly used in baking

Enzyme group	Main purpose
Amylase	Anti-staling, improve fermentation, colour
Proteases	Improve rheological properties of doughs, mixing time, flavour,
α-Glutamyl transferase, glutathione oxidase, cysteinyl glycine dipeptidase	Positive effect on loaf volume, crumb structure, dough elasticity
Pentosanases	Main action in rye breads shorter development time
Sulfhydryl oxidase	Improves dough strength by formation of –S-S- bonds
Peroxidase	Dough development, proofing time, shape
Xylanase	Colour, shape, bread volume, proofing time

Note: Source: Whitaker 1996; Van Oort 1997

Glucose oxidase has also been shown to be effective as a gluten strengthener. The beneficial effects might be attributed to the formation of disulfide bridges between proteins in the gluten fraction, to pentosan gelation and or to protein pentosan coupling. The great effect of this enzyme as an oxidative bread improver is due to its higher affinity for glucose and its ability to utilise maltose (Collar et al 2000).

2.9 The significance of enzymes in cereal grain processing

The growth in value of enzymes for grain processing has been steady and positive. Tables 2.6 and 2.7 show enzyme sales for grain processing and the distribution of sales respectively.

Table 2.6 The increase in enzyme sales for grain processing over recent years

Year	Sales margin (∈) m	Share of total enzyme sales (%)
1991	84	22
1996	230	28
2001	510	26

Note: 1 Source: Godfrey 2003
2 Values are presented in millions of Euros

Table 2.7 Distribution of enzymes sold for grain processing for the year 2001

Industry sector	Proportion of total enzyme sales (%)
Starch	52
Animal feed	28
Protein	1.5
Nutraceutical	1.5
Flavour	2
Baking	8

Note: Source: Godfrey 2003

Starch processing uses the largest collection of different enzymes. Generally, these enzymes are used to accurately direct the hydrolysis of starch and or synthesis of derivatives to generate products with special properties. Baking application accounts for 8 % of the industry sector sales (Table 2.8). Enzymes from this group particularly include α-amylases, xylanase, pentosanase group and other members of the hemicellulase group. The enzymes used are low priced and high volume GM versions of the traditional enzyme preparations (Godfrey 2003). Furthermore enzymes are used in wheat grain processing to achieve effective separation between endosperm and bran during milling processing, obtained by optimum grain conditioning that leads to an increase of the friability differences between endosperm and outer layers (Lullien-Pellerin et al 2003; Tikhomirow et al 2003; Muñoz and Barceló 1996; Wilkström and Eliasson 1998).

2.10 Amylases in baking

It is believed that there are two main roles of amylases in baking these being flour standardisation (optimisation of α-amylase activity in the flour) and antistaling of baked goods. The most widely used amylase in baking is the fungal α-amylase from *A oryzae*. It is usually supplemented to the flour in order to optimise α-amylase activity. In contrast to malt amylases from either barley or wheat malt, fungal amylase does not have negative effects on bread crumb within the normal dosage range. Fungal α-amylase has an adequate thermostability and it accumulates short malto-oligosaccharides whereas malt amylases have a higher thermostability and release

longer maltodextrins. Therefore malt amylases are more prone to negative effects upon overdosing.

The second important use of amylases in baking is antistaling or improving the fresh keeping of baked goods. Amylases used for antistaling purposes (Table 2.8) have different functions. The effect of fungal amylases here is limited due to their low thermostability. Reduced bread crumb firmness after baking and during storage is more pronounced when using endo-amylases with high thermostabilty including that from *B amyloliquefaciens*. However, there is a very narrow range of optimal dosage therefore exo-amylases such as maltogenic amylases are often preferred for antistaling purposes (Kragh 2003; Sahström and Bråthen 1997).

Table 2.8 Functional properties of amylases used to delay staling of baked goods

Enzyme source	Main action mechanism	Thermostability	Softening effect	Resilience effect
A. oryzae α-amylase	endo	low	+	little
A. niger amylases	endo	intermediate	++	little
B. amyloliquefaciens α-amylase	endo	very high	++++	negative
Maltogenic α-amylase	endo	high	++++	positive
Plant β-amylases	exo	low	++	moderately positive

Note: Source: Kragh 2003

2.11 Declaration of food additives: Legal and safety implications

Enzymes are used widely in food processing and their legal/safety status depends on the application as well as the regulations of the country involved. The factors that play a major role when establishing how an enzyme is to be regulated include:

- The purpose for which the enzyme is to be used;
- The type of food in which the enzyme is to be incorporated;
- The country (regulations differ from country to country);
- The origin of the enzyme (e.g. some countries will have special requirements for genetically modified enzymes).

Within the European community, the definition of processing aids according to the Additive Directive is as follows: “Processing aid – is any substance not consumed as a food ingredient by itself, therefore intentionally used in the processing of raw materials to fulfill a certain technological purpose during treatment and which might result in the technically unavoidable presence of residues of the substance in the final product, provided that these residues do not present any health risk and do not have any technological effect on the final product” (Praaning 1997).

For enzymes this might lead to some confusion, as a particular enzyme could fall under both categories, depending on the application. If it has a technological function in the finished product it will fall under the Additive Directive, whereas, if it does not have a technological function in the finished product it is than a processing aid and therefore it is exempt from the Additive Directive. Many enzymes are considered processing aids as small quantities remain in the final product but the are often no longer active. Some cases when an enzyme falls under the Additives Directive include:

- lysozyme (preservative in cheese)
- invertase (soft centered confectionary)
- glucose oxidase (antioxidant for drinks and sauces).

It must be added that the same enzymes might also be used as processing aids when used in different food applications. In addition there is no overall EU regulations for food enzymes. Most of them are exempted from the EU Food Additive Directive, because they are used as processing aids and not as additives being active in the finished food product (Praaning 1997).

In Australia and New Zealand, food additives must be declared in accordance with the ingredient labeling requirements of the Food Standards Code. This states “enzymes need only be declared by the class name ‘enzyme’ and not by specifically declaring the name of the enzyme”. On the other hand where they are processing aids these do not need to be referred to in labeling the product.

2.11.1 Health and legal aspects of enzymes in food systems

Enzymes are found in all fresh foods including nuts, milk, cheese, fruits and vegetables, meats and eggs. Pure enzymes are generally not toxic but enzymes and other associated proteins might result in allergy reactions if consumed in foods. Many commercial enzymes used in food processing are not consumed in their active form, since they are denatured during food processing. Exceptions to this include partial activity of bacterial amylase in baked goods and partial survival of papain in beer production. Dry concentrated enzyme powders might also cause allergies. However, total amounts of enzyme concentrations in food systems are relatively small. For example bread on average contains approximately 15ppm of a fungal amylase concentrate (5000 units per gram), which corresponds to 15ppb of pure fungal amylase. There are no known cases nor implications that enzymes used in the production of food are harmful *per se* (Tucker 1995).

In summary, enzymes are widely used in food processing. In the utilisation of cereal grains, enzymes have a number of key functions. Although some enzymes have been thoroughly researched, there are many potential applications which remain to be investigated, particularly in products other than baked breads.

Chapter 3

Background and literature review: sprouting of cereal grains

In the context of the increases in enzyme activity which occur upon germination, described in the previous chapter, the purpose is now to provide literature review and background knowledge on the sprouting of cereal grains, along with the resultant problems encountered during processing of wheat based products.

3.1 Sprouting of grain

Sprouting in grains can occur once the kernel has reached maturity; thus, when a mature kernel is subjected to increased moisture and suitable temperature it begins to sprout. The physiological changes needed to produce a new plant require energy and nutrients, therefore, the wheat kernel produces enzymes to breakdown starch (amylases), oil (lipases) and protein (proteases). If the moisture levels in the wheat grain decrease sufficiently, the sprouting process is suspended, and in cases where there are no visible signs of sprouted kernels this is referred to as incipient sprouting.

Wheat sprouting prior to harvest can result in serious negative quality characteristics. The impact of sprouting on foods produced from wheat depends on the amount of enzymes produced and the extent to which breakdown has occurred in the kernel. However, even very low levels of pre harvest sprouting can affect the market value of the grain (Donelson et al 2001). Preharvest sprouting reduces grain quality and ultimately milling and baking quality parameters of grain end products (Paterson et al 1989). These changes depend upon a number of factors including soaking duration, temperature, wheat variety and also sprouting duration (Singh et al 2001). Sprouting is part of life cycle of wheat and is necessary to form grains which will mature into the kernel that eventually will be used for food production. However, the wheat must be harvested when the kernel is an integral whole. Thus sprouting affects quality of wheat based products due to higher activity and or increased levels of some endogenous enzymes. Therefore, the market value of sprouted wheat is reduced and this results in economic losses. The incidence of sprouting might have economic consequences at

many stages. Firstly the occurrence of sprouting might not be sufficient to eliminate wheat from being used for food purposes. At a second stage, however, the sprouting might be so severe so that the wheat can no longer be used for food but might be diverted for feed production. In the worst case the sprouting might be so severe that the wheat must be discarded.

Wheat production and trade data show that China along with USA and India are the largest producers of wheat, while USA, Canada and Australia are largest exporters of wheat (Table 3.1). Although there are no recent published data indicating the extent of economic losses due to sprouting, earlier estimates (Wahl and O'Rourke 1994) demonstrate that:

- the frequency with which sprouting occurred varied from three years in tenup to instances of annual occurrence

- the classes of wheat impacted vary although in most cases all wheat produced is subjected to sprouting and

- the estimates of economic losses are considerable.

Table 3.1 World wheat production and export[a]

	Crop year				
Country	**2000**	**2001**	**2002**	**2003**	**2004**
United States					
Production	60,757.49	53,261.98	44,062.70	63,813.91	58,737.80
Export	29,604.24	27,537.17	26,875.76	28,896.14	33,124.26
Canada					
Production	26,535.50	20,630.20	16,197.50	23,552.00	25,860.40
Export	19,564.02	18,431.83	13,073.05	12,608.47	16,270.45
Australia					
Production	22,108.00	24,299.10	10,132.00	25,700.00	22,605.00
Export	18,129.59	15,974.93	15,155.50	9,889.65	18,939.74
China					
Production	99,636.13	93,873.23	90,290.26	86,458.26	91,952.24
Export	541.5	1,093.73	1,397.60	2,977.54	1,627.41
Russian Federation					
Production	34,455.49	46,982.12	50,609.10	34,104.29	45,412.71
Export	698.07	1,926.60	10,503.28	8,076.11	5,031.79
India					
Production	76,368.90	69,680.90	72,766.30	65,129.30	72,060.00
Export	1,173.56	3,024.87	4,312.21	4,875.79	2,230.01

Note a Units are in 1000 Tonnes
Source: http://faostat.fao.org/site/336/DesktopDefault.aspx?PageID=336

The areas most commonly affected include the Pacific Northwest, upper Midwest and Northwest in the USA, Australia and central Canada. The economic losses and effects differ depending if blending is allowed or not. In Australia and Canada for example the presence of even low levels of sprout damage may result in the grain being used for feed production only. In addition, the strategy of blending is not regarded as a suitable or practical solution to the problem. (Wahl and O'Rourke 1994).

Preharvest sprouting and scab of wheat (*Triticum aestivum*) causes harvest losses, loss of seed viability, reduced test weight and reduced flour quality resulting primarily from

increased protease and α-amylase activity. Scab damaged kernels have dull, chalky appearance and contains visible fungus in the germ. Incidence of scab occurs from field infection by mould *Fusarium graminerarum* during anthesis of kernel filling, due to damp and cool conditions during the maturing and harvesting. The presence of scab adversely affects flour ash, flour colour, dough properties as well as loaf volume. In most cases this results in shriveled kernels and mycotoxin deoxynivalinol (Martin et al 1998; Dowell et al 1999).

The degree of sprouting has an important bearing on the utilization of wheat. The starch in wheat is degraded and free sugars increase during sprouting due to the higher activity of amylolytic enzymes. The extent of starch degradation depends upon sprouting time. The sprouting has been reported to adversely affect the rheological properties determined using the Farinograph and Visco-amylograph. The adverse effect of sprouting has been reported in bread, cake, noodles and spaghetti making (Singh 2001; Bean et al 1974a; Hatcher and Symons 2000; Edwards et al 1989; Bean et al 1974b; Sorrells et al 1989; McCrate et al 1981; Dick et al 1974). Sprouting also lowers test weight and flour yield, impacting on the grade and value of grains. The effect on baking properties is manifested by lower absorption (water added to the formulation, which reduces bread yield), significantly reduced mixing strength and tolerance, and sticky dough. It might also have an impact on loaf volume, crust properties such as crumb strength, texture and crumb gumminess, the later causing problems with slicing and shelf life

The degradation of starch and higher enzyme activity has also been reported to reduce the quality of Asian noodles (reduced yield, texture and colour). In the processing of pasta, sprouting increases semolina specks counts, reduces shelf life of dried pasta (due to cracking), increases cooking loss and in turn gives softer cooked pasta (www.ag.ndsu.nodak.edu/aginfo/smgrains/fallnum.htm).

3.2 Test for sprout damage estimation

Most frequently used analytical procedures in wheat evaluation include: test weight, kernel hardness, Zeleny sedimentation, moisture content, Falling Number, flour yield, protein content, SDS sedimentation, while typical tests used in flour evaluation include:

ash content, gluten index, extensograph, farinograph, amylograph, Rapid Visco Analyser (RVA), starch damage, alveograph, wet gluten content and mixograph. The Mixograph is popular in the USA. The RVA is an Australian invention, which is rapidly gaining acceptance world-wide. It is particularly useful in identifying flours suitable for noodle-making. Cereal technologists have striven for over 100 years to relate the physico-chemical properties of wheat flours to functional properties, in terms of suitability for the preparation of the very wide range of foods for which wheat is used.
A full picture of the baking properties of wheat flours can be obtained by determining the gelatinisation characteristics of flours. An important phase is the heating up period of the dough in the baking process, when swelling of the starch and gelatinisation are in full course. The change in viscosity during the heating of the dough is partially caused by amylases. The degree of viscosity change will depend on amylase and the resistance of the starch against decomposition. Amylograph tests must be carried out at a temperature similar to the first stage of the baking process during which the dough is subjected to a constantly increasing temperature (from 25°C at a rate of 1.5°C per minute up to a maximum of 94°C), and the starch in aqueous environment is swelling and gelatinising. During this period changes in viscosity are continually measured and recorded. The resulting diagram indicates the process of gelatinisation of the starch under the influence of amylase activity.

α-Amylase activity is conveniently measured by Falling Number apparatus. The time taken for a plunger to fall through a suspension of starch gelatinized by heating in a water bath is recorded in seconds. A Falling Number (FN) of 400 or more seconds indicates a very sound starch, with practically no α-amylase present. FN values of 350, 300, 250, 200, 150 seconds or less indicate increasing -amylase activity. The lowest FN possible is 60 seconds. On the high side, FN values of over 500 have been reported for some wheats from Australia, Argentina, most Mediterranean countries, India and Saudi Arabia. α-Amylase activity is not additive, and a small amount of the enzyme exerts a disproportionately large influence on flour functionality. Red wheats are less susceptible to sprouting than are white wheats, but a degree of sprout tolerance has been introduced to the newest western Canadian Soft White Spring wheat varieties. The measurement of α-amylase it he flours from weather damaged (pre harvest sprouting) wheat and barley grains presents a special problem. The levels of the enzyme to be measured are low and extracts of the flour contain high levels of starch which acts as an alternative substrate.

However, due to the industrial importance of pre harvest sprouting numerous methods have been developed and continue to be developed. The traditional Falling Number Method and Amylograph Viscosity Method are widely used by millers bakers and plant breeders. Where large numbers of assays need to be performed various improved iodine-based or dye labelled substrate methods are employed. Detailed evolution of Phadebas (dye-labelled starch) method by Barnes and Blakeney (1974) clearly demonstrated a good correlation between this method and the Falling Number and Amylograph methods.

Sprouted wheat grains can contain high levels of α-amylases (Finney 2001; Henry and McClean 1986; Zawistowski et al 1993; Henry et al 1992). Since sprout damaged wheat kernels can vary enormously in the activity of α-amylases a precise and rapid method is desirable for sprout damage screening. Furthermore certain wheat genotypes can have elevated levels of so called late maturity α-amylase in ripe grains even in the absence of sprout damage. In addition, if wheat grain has suffered frost damage it would have increased levels of α-amylase activity (Kruger 1997). There are many procedures widely used to estimate pre harvest sprouting damage in wheat grains including the Falling Number method, the Rapid Visco Analyser method and the Brabender Amylograph – Viscograph method. These methods involve heating of wheat meal or flour in a water suspension, to 96°C, thus heating starch past its gelatinisation temperature and causing the suspension to thicken. The amylolytic enzymes present and α-amylase in particular hydrolyse the gelatinized starch granules and the viscosity of the suspension is reduced. Pre harvest sprouting increases the levels of amylolytic enzyme activity and reduces viscosity. It has been reported that there is an important uncontrolled source of variation in the pasting viscosity methods a large natural range in the pasting viscosity among sound samples (not sprouted). It is therefore difficult to directly compare the degree of pre harvest sprouting between samples (Donelson et al 2001).

The Falling Number and α-amylase methods can help to identify most sprout damaged wheat that would cause problems for commercial bakeries, but these methods cannot identify all samples that might result in breads with sticky dough properties. In addition the bake test is used as well to established the levels of sprout damage. It was further

shown that changes of the activity of α-amylase in milling fractions upon storage affects the Falling Number and bake test assessments of sprout damage in stored flour and grains (Moot and Every 1990). On the other hand it has been argued that the level and impact of sprout damage is not fully realized until it is processed into bread or pasta. The Falling Number test does not directly measure amylase activity, but rather changes in the physical properties of the starch portion of the wheat kernel caused by these enzymes during the test. More correctly, the Falling Number is a measure of the flour paste liquefaction (due to α-amylase activity) by a decrease in starch viscosity while heating to 100°C.

Various studies have shown that wheat meal pasting properties can be influenced by other parameters than only α-amylase activity. Lenartz et al 2003 have reported that low Falling Number values do not always correspond to high levels of α-amylase activities. They suggested that low values might be due to starch structure, the type of enzyme present, and also to the non-starch polysaccharide composition of the flour (Lenartz et al 2003 RAEGP). In general for wheat a Falling Number value of 350 seconds or higher indicates low enzyme activity and relatively sound wheat. As the amount of enzyme activity increases, the Falling Number decreases. Values below 200 seconds indicate high levels of enzyme activity. Many wheat buyers write minimum tolerances of 300 to 350 into purchase contracts (www.ag.ndsu.nodak.edu/aginfo/smgrains/fallnum.htm).

Since wheat is not only used for bread making, higher levels of α-amylase may be tolerable for other end-products. For example in pasta (spaghetti) there is low water absorption and large particles of semolina inhibit the migration of enzyme and the interaction with its substrate. Researchers from USA and France have reported that sprout damage in durum wheat causes uneven extrusion, difficulties during drying, starching of pasta strands and poor storage stability (Donnelly in Feillet and Dexter 1996). However, some other studies appear to contradict these findings (Dick et al 1980; Combe et al 1988 and Dexter et al 1990, Feillet and Dexter 1996) and reported that sprout damage had little effect on spaghetti cooking quality. They further stated that combined effects of low water content in the dough and relatively rapid loss of moisture during the drying process prevent the α-amylase from degrading starch during the processing of pasta. Furthermore, it was stated by (Kruger and Matsuo 1982, Feillet and

Dexter 1996) that the α-amylase is rapidly denatured during the cooking process. Others (Grant et al 1993, Matsuo 1982, Feillet and Dexter 1996) have stated that severe sprout damage might have slight effects on pasta firmness and cooking loss.

Pre-harvest sprouting is accompanied by a number of biochemical changes in the grain, but the most pronounced is the increase of α-amylase (Corder and Henry 1989). Bread made from sprouted wheat flour has poor loaf volume and crumb color (Chakraborty et al 2003). Further, it has been reported that noodles made from sprouted wheat flour have poor color and cooking qualities (Edwards et al 1989; Hatcher and Symons 2000). Edwards et al 1989 reported that noodles made from rain damaged whet were rated significantly lower than controls for both types of noodles. Noodles made from rain damaged wheat were darker than their sound controls (Edwards et al 1989). On the other hand it has been suggested that the impact of α-amylase in raw Japanese noodles might be limited by the small amount of mechanically damaged starch available to act as a substrate (Bean et al 1974). It has been suggested that it is not α-amylase alone that affects quality attributes of noodles. PPO is of significance since colour is a primary consideration in these products. This enzyme is primarily located in the bran and, as with α-amylase can be at least partially separated during milling. This is why millers producing flour for noodle making use lower extraction rate, often around 60%. However, PPO differs from α-amylase in it cannot be readily solubilised. Also there are genotypic variations which can be as important as phenotypic variations due to sprout damage. Furthermore it appears that PPO is not synthesized *de novo* during germination. Another question is that of bran PPO having been studied when it may be the endosperm form of PPO that has the greatest impact on quality of food products (Kruger 1997).

In summary, sprouting is a significant global issue. The changes occurring in the grain often have an adverse impact on baked and other products made from flour. The increase in α-amylase and the presence of other enzymes is thought to be important for the attributes of Asian noodle products. However, there is little published information that clearly establishes the relative significance of the effects.

Chapter 4

Background and literature review: The utilisation of wheat for Asian noodle products

The purpose of this chapter 4 is to provide background on the utilisation of wheat flour for manufacture of Asian noodles. The areas reviewed encompass a brief comparison of durum pasta and Asian noodles, the significance of Asian noodles in Australia and aboard, ingredients and processing methods as well as the different styles of Asian noodles.

4.1 Introduction to wheat

Wheat has been cultivated as a grain crop for longer than any other. In addition to consumption in the form of bread, many other products are made: pasta, and even beer and whiskey. The major end uses of wheat and flour are presented in Table 4.1. Accordingly the quality of wheat is defined by a large number of different food products which contain flour, starch, bran and gluten (Morris 2004; Morris 1998; Azudin 1998; Jones et al 1999; McLean et al 2005; He et al 2005).

Table 4.1 Uses of Australian wheat in the manufacture of various products in international markets

Product	Estimated relative proportion (%)
Middle Easter flat breads	32
Chinese noodles (yellow alkaline)	22
Japanese noodles (white salted)	11
Pan breads	21
Steam breads	4
Cakes & biscuits	2
Animal feed	8

Note: Source: McMaster and Gould 1998

4.2 Australian wheat utilisation

Australian wheats have white grain characteristics and are particularly well suited for processing of Asian noodles (Martin and Stewart 1994) particularly Hokkien, instant, fresh and Udon noodles. Wheat used for the production of white salted noodles (WSN) is blend of soft grained wheat varieties, these varieties result in products having low flour ash, bright and creamy colour, good stability and eating quality. On the other hand wheat for the production of yellow alkaline noodles is selected from the prime hard and hard wheat varieties as well as Australian Premium White. This hard grained, high protein content wheat results in yellow alkaline noodles (also known as Chinese style) with excellent brightness, a stable yellow colour and good eating characteristics. Typical ingredient formulations used for the two general styles of Asian noodles and preferred quality characteristics of these noodle types are shown in Table 4.2 (Kruger et al 1996).

Table 4.2 Main ingredients and quality attributes of WSN and YAN
Source: Miskelly 1996

	General class name	
	Salted	Alkaline
Colour	white or creamy white	yellow
Major raw materials	flour, water, sodium chloride	flour, water, alkaline salts, e.g., sodium carbonate
Countries consumed	Japan, Korea, northern China	Malaysia, Singapore, Indonesia, Thailand, Taiwan, Hong Kong, southern China, Japan
pH of noodles	6.5–7	9–11
Organoleptic qualities	soft, elastic texture	firm, elastic texture

4.3 Pasta production from wheat

In order to effectively discuss the utilisation of wheat for processing of Asian noodles it is important to briefly discuss pasta products as these two groups of products appear to be similar. Pasta products have been known to Mediterranean civilisation for many centuries and are enjoyed today all over the world. They are popular due to their versatile, natural and wholesome food properties (Feillet and Dexter 1996). The word pasta, however, is derived from the Italian word for paste meaning dough and is used to describe products fitting the Italian style of extruded foods including spaghetti, macaroni and lasagne. It is well distinguished from the Oriental style of sheeted and cut foods called noodles (Sissons 2004). The main ingredients in pasta dough are durum semolina and water. Pasta is sold fresh, frozen or dried. Pasta comes in many shapes, sizes and colours.

The colour and crispiness of dried pasta determine quality of these types of products (Hensen and Brismar 2003; Dexter et al 1983; Vignaux et al 2005; Rathi et al 2004; Güler et al 2002; Taha 1992; Taha et al 1992a,b). Pasta should be slightly golden and translucent (Mayo Clinic, University of California & Dole Food Company, Inc. 2002). The raw materials most commonly used for dried pasta production include durum wheat semolina, water, and any other variable ingredient such as eggs, vitamins or vegetables in powder form e.g. dehydrated spinach, tomato etc.

The major differences between durum wheat semolina and wheat flour is the particle size, semolina is milled in coarser particles around 200 - 700μm, while flour is usually milled in particles maximum 150μm. In pasta making it is necessary to have just the right amount of water in order to transform the semolina into a dough to go through the extrusion process and subsequent drying operations. In general durum wheat semolina absorbs less water than wheat flour. Water is believed to be very important to the quality of the end product (Irie et al 2004; Guan and Seib 1994). Typically water for pasta production should be low in sodium, magnesium and calcium ions, because these can influence pasta's colour as well as flavour. In addition to this the temperature of water also plays an important role for the quality of the final product. Most of the pasta producers use water at a temperature of 35 - 45°C because it is absorbed faster by the

semolina. The extrusion process allows continuous kneading and shaping of the dough to be carried out simultaneously. This is also the main difference of pasta as compared to the noodle production (as noodles are typically rolled and sheeted). Extrusion is the most important operation in the pasta making as it determines firstly the quality of the end product and secondly it also regulates the rate of production (Agnesi 1996; Manthey et al 2004; Ponsart et al 2003; Smewing 1997). Finally after first and second drying phases the pasta products undergo a cold – air treatment for product stabilisation purposes (Pollini 1996).

The colour and appearance of pasta are two important quality factors. The appearance of pasta is determined by:

- colour (depends largely on the characteristics of wheat used for the production of pasta)
- surface texture (related mainly to the conditions of the die)
- strength and stability of pasta strands (related to the drying and extrusion conditions)
- specks (related to the presence of damaged kernels and improper cleaning and mill settings).

The colour of pasta products results from a desirable yellow component (related to the presence of carotenoid pigments) and an undesirable brown component (two phenomena seem to be involved: the presence of naturally coloured proteins and enzymatic oxidation). Under certain conditions during the drying process (using too high temperatures) a red hue might develop in the pasta products (related to the loss in lysine caused by high temperatures and longer drying periods) (Dexter et al 1984, Feillet and Dexter 1996). A bright yellow colour in semolina ensures a good colour in the pasta that is preferred by the consumers. The main pigments in durum wheat responsible for the yellow colour are xanthophylls and lutein. These can be partially degraded by the enzyme lipoxygenase during the processing. However, relatively high temperatures during the processing / drying of pasta denature the enzyme and make it ineffective. In addition, a brown pigment caused by a copper protein complex causes a browning effect on the semolina which leads to a dull appearance in the pasta (Sissons 2004; Ingelbrecht et al 2001, Sözer and Kaya 2003; Brijs et al 2004).

In addition to colour, the cooking quality is also of great importance for consumers thus also for wheat producers, breeders and pasta processors. Cooking quality of pasta is usually described by the following characteristics:

- minimum, maximum and optimal cooking time (starch gelatinisation, time required for optimum textural properties)
- water uptake characteristics
- textural properties
- surface characteristics
- aroma, taste , flavour (Feillet and Dexter 1996; Táha 1992a; Táha 1992b Guan and Seib 1994; Taha et al 1992a; Taha et al 1992b; Manthey and Schorno 2002; Vetrimani et al 1999; Abecassis et al 1989; Dexter et al 1997; Edwards et al 1993; Pszczola 2001; Vernière and Feillet 1999; Cole 1991; Del Nobile et al 2005; Macche-Rezzoug and Allaf 2005; Zweifel et al 2003).

Although pasta and noodle products might look alike they differ widely in the raw ingredients used as well as in the processing steps applied (Table 4.3). The Mediterranean region remains the world's largest producer of durum wheat (55 -60% of world production) (Sissons 2004).

4.4 Asian noodles

Because of their range and versatility, noodles, whether made from rice, wheat or even mung beans are one of the most popular foods in the world. They are inexpensive and easy to cook, an excellent base for hundreds of more strongly flavoured ingredients and this means that they can be used equally in to spicy soups, savoury stir-fries and even sweet deserts. Noodles are served in more unusual forms including wontons and noodle cakes (Collado and Corke 1996; Collado and Corke 1997; Collado et al 1997; Collado et al 2001; Chan 2004; Collado and Corke 2004). Noodles have been part of Asian cuisine for centuries and continue to play a central role in many dishes in Asian but also they are widely consumed all over the world (Huang and Morrison 1988).

Table 4.3 Comparison of pasta and Asian noodle products

	Pasta products	**Asian noodles**
Wheat used for the production	*Triticum durum* commonly referred to as durum wheat	*Triticum aestivum* also known as breadwheat
Desired grain hardness	very hard	medium
Ploidy of wheat	4N	6N
Primary raw ingredient of dough	semolina	flour
Granularity of primary ingredient	relatively coarse	very fine desirable
Other ingredients	egg (optional)	common salt and/or alkaline salts (many other optional ingredients)
Preferred colour	yellow	depends on the type of noodles consumer preferences range from white to yellow
Dough preparation	mixing of a crumbly dough	mixing of a crumbly dough
Basic processes steps	extrusion typically applied in commercial production; sheeting and cutting may be used for home and small scale operations	repeated sheeting, then resting followed by further sheeting and cutting into strands using cutting rolls
Typical shapes of products	various including strands (spaghetti, fettuccini), macaroni, shells and sheets (lasagne)	commonly strands: cross sections typically square or rectangular
Typical cooking times	typically ten minutes or longer	Usually less than 10 minutes; as short as 2 minutes but processing might include steaming or frying and or deep frying

Note: Information from McKean 1999 and Kruger et al 1996

Wheat noodles are prepared from a dough containing flour water and salt and or alkaline salts. The processing steps of making bread and noodles are summarised in Fig. 4.1. Other ingredients often found in Asian noodles include sodium silicate, sodium polyphosphate, lecithin, fat, variety of starches and gums (Oh et al 1983). Asian noodles differ widely in the raw ingredients used, mode of preparation, width of the strands, type of noodle, colour and texture. The final product might be raw, lightly boiled and coated with oil, dried, steamed and fried or steamed and dried (Kruger et al 1992; Inazu et al 2002; Yun et al 2002; Lii and Chang 1981; Kruger et al 1998; Panglili et al 2000; Chompreeda et al 1988; Kubomura 1998; Kim 1996; Collado and Corke 1996; Kim et al 1996; Brillantes 1992; Galvez et al 1995; Sing and Chauhan 1989; Huang 1996; Miskelly 1993; Jeffers et al 1979). Flour properties important for the production of Asian noodles include milling properties and grain hardness (flour yield and particle size), starch characteristics such as amylose – amylopectin ratio, starch pasting properties and flour swelling power, protein content and protein quality properties such as sedimentation, mixograph and alveograph, colour characteristics (pigmentation and discolouration) (Kashem and Mares 2002; Oda et al 1980; Panozzo and McCormick 1993; Mares and Campbell 2001; Lorenz et al 1994; Preston et al 1986; Nakamura 2001; Lang et al 1998; Huang and Morrison 1988; Zhao et al 1998; Wang et al 1996; Kruger et al 1992; Toyokawa et al 1989; Toyokawa et al 1989; Jensen et al 2002).

Textural properties are a critical characteristic of Asian noodles (Baek et al 2001; Kruger et al 1998; Nagamine et al 2003). Starch and protein play a major role in determining textural properties of Asian noodles (Baik et al 1994; Hatcher et al 1999; Chong et al 2002; Wu 2002; Crosbie 1991; Crosbie et al 1992; Edwards et al 1996; Bhattacharya and Corke 1996; Ross et al 1997; Miskelly and Moss 1985; Moss and Miskelly 1984; Miskelly 1984; Moss 1982; Moss 1980; Batey et al 1997; Bhattacharya and Corke 1996; Edwards et al 1996; Chung and Kim 1991; Beta and Corke 2001; Seib et al 2000; Crosbie et al 1999; Akashi et al 1999; Noda et al 2001; Briney et al 1998; Hatcher et al 2002; Wrigley et al 1996; Singh et al 1989; Collado et al 2001; Galvez et al 1994; McCormick et al 1991; Kim and Seib 1993; Bejosano and Corke 1998; Kadharmestan et al 1998; Muhammad et al 1999; Collado et al 1997; Collado and Corke 1997; Habernicht et al 2002).

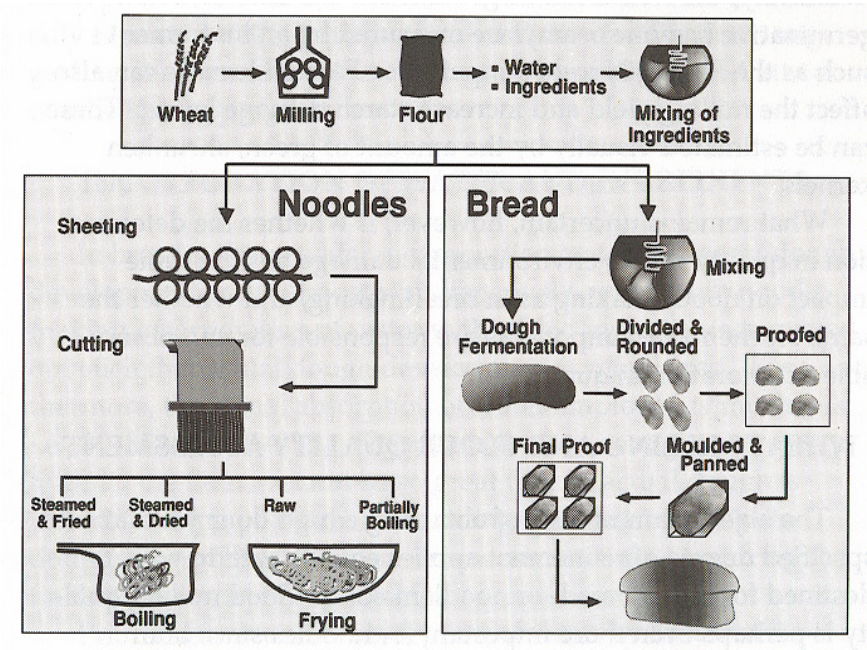


Fig. 4.1 Similarities and differences between bread and noodle making processes
Source: Kruger 1996

In addition, noodles are an important part of the diet of Asia (approximately 40% of the flour used goes for noodle production). Today China is one of the world greatest wheat producers with wheat production of 105 million tonnes in 1993-1994. Furthermore, China is also one of the largest wheat importing countries (Huang 1996). Noodles are classified according to the raw materials used, the size of noodle strands, by the manufacturing steps and the type of the product on the market shelves (Nagao 1996). Furthermore, in terms of processing for most noodles the dough is sheeted and cut into strands. Most of noodles on the market today are made by machine, having similar methods of mixing, sheeting and combining of two sheets, rolling and cutting. The processing of various types of noodles is shown in Fig.4.2.

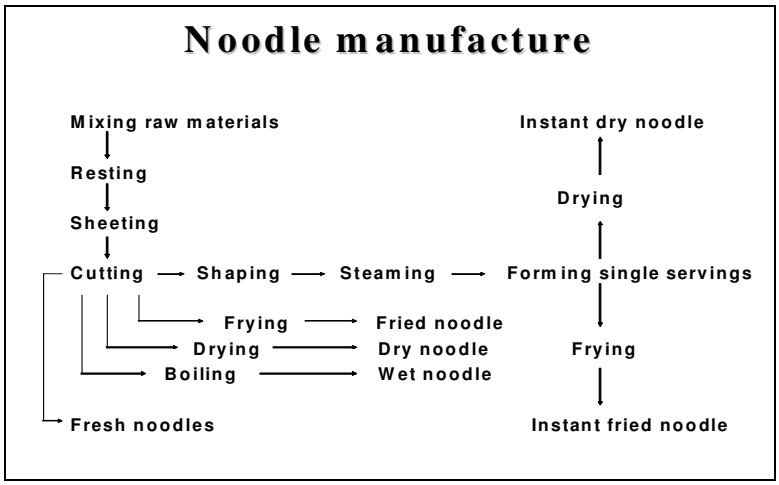


Fig. 4.2 Processing steps of noodles making
Adapted from Nagao 1996 and Hoseney 1994

4.5 Raw materials and their effects on noodle quality

Wheat

The general consideration in selecting wheats for the milling of flours for noodle production is that it should be well-filled, of good appearance, and not damaged by weather (heavy rain falls) or drying. In addition wheat used for noodle processing should have an appropriate balance of protein content as well as have an appropriate protein and starch quality for the particular types of noodles. Excessive levels of α-amylase (through pre-harvest sprouting and or late maturity α-amylase can have deleterious effects on processing and on the quality of noodles including increased boiling losses and decreased eating quality (Edwards et al 1989; Bhattachary et al 1999; Abdul-Hussain and Paulsen 1989; Rosell et al 2002; Hatcher and Kruger 1997; Castello et al 1999; Castello et al 1998; Marsh and Galliard 1986; Hareland 2003). Also high levels of protease, associated with rain damage, might lead to increased breakage of noodles during drying as well as poor colour in both yellow alkaline and white salted noodles. The milling process is also important, the highest quality noodle flours are associated with low extraction milling and low ash levels in the flour (Collado and Corke 2004; Seib 2000).

Flour

The main influence on noodle appearance are flour extraction levels and ash content. Higher flour extraction will produce noodles with duller appearances, thus lower extraction levels and ash levels are preferred in order for noodles to retain a clean,

bright appearance after being cooked. Raw noodles, however, discolour with time. This has been associated with PPO activity, flour extraction rate, weather damage and variety. The yellowness of the flour is mainly due to the presence of xanthophyll pigments, has a significant effect on the colour of both raw and cooked noodles (Baik et al 1994; Lamkin et al 1981).

Flour and noodle colour

Colour is one of the most important considerations in assessing flour quality. There are many factors affecting flour colour. Increased extraction rate was found to be main cause of colour deterioration in Japanese noodles, where flour colour is regarded as a prime factor in assessment of commercial value. During drying a brown colour may develop, which is due to the action of tyrosinase located in the bran layers of the wheat grain and it is involved in oxidation of phenols to quinones which are subsequently converted to dark coloured melanins by polymerisation and interactions with proteins. Granularity influences the colour of dry flour, a flour with finer particle size is brighter and whiter. Flour colour grade is strongly associated with protein and is correlated more closely with protein than with ash. Brightness is inversely proportional to the protein content. Discolouration was associated with the degree of darkening developed by gluten washed from the samples on drying under standard conditions. Flour carotenoids, principally xanthophyll, together with flavone compounds are responsible for the yellow colour of the flour. Advantage is taken of the natural colour of the flavones in the production of YAN, where an alkaline solution is used to produce the traditional yellow colour. Kansui also improves the flavour and texture of the noodles. Significant varietal differences in the yellow pigment content of flours milled from Australian wheat varieties resulted in no significant relationship between yellow pigment and protein content or extraction rate (Miskelly 1984).

For WSN, protein content of the flour was the single most important factor other than flour colour affecting the colour of the fresh noodle sheets. Flour of lower protein content gave the whitest noodles. High brown pigment levels also contributed to discolouration associated with the brightness of the fresh noodle. After boiling noodle colour became inversely related to the yellow pigment of the flour and the correlation with the protein was no longer significant. For WSN an increase in the level of the flour protein or brown pigment decreases flour brightness and produced dull noodles.

However, neither of the effects of brown pigment nor that of protein was found to be significant once the noodles had been boiled, presumably because the melanins and pigments dissolved in the cooking water. The colour of boiled noodles was then limited by the yellowness of the flour (Miskelly 1984).

For YAN, raw noodle colour was found to be inversely related to protein content and brown pigment. Apart from the extraction rate, flour colour is dependent on other flour characteristics including protein content (being the single most important determinant of flour brightness), mineral content and starch damage also affected the brightness, latter suggesting that wheat hardness and granularity of the flour may be important. Entire starch granules reflect more light and this reflectance decreases with increasing starch damage in the milling process.

Brightness of YAN was inversely related to the protein and brown pigment level. The brightness and freedom form any discolouration which developed after processing was important and in addition the type of yellowness which developed after reaction of the flavones with Kansui (Miskelly 1984; Ohm et al 2005).

For instant noodles the yellowness of the noodle sheet was highly correlated with increased milled yield, whereas brightness of the noodle sheet decreased with increasing yield. Increased flour extraction rate resulted in higher level of germ, hence more flavone compounds available for reaction with Kansui to produce more yellowness, the brightness of the product decreased owing to discolouration from bran and other components high in mineral (Miskelly 1984).

Flour and noodle texture

The main flour characteristics that affect textural properties of noodle products include protein content, total starch content and / or the amount of damaged starch, although present at relatively low levels in flour lipid content might also play an important role in determining the textural characteristics of noodles. Flour protein provides the basic structure of many cereal foods including noodle strands. There is conflicting evidence in the literature regarding the relative importance of protein content and gluten composition. Increased protein content has been related with harder noodle products (Park and Baik 2004a; Crosbie 1994; Nagamine et al 2003; Park and Baik 2004b).

Starch has also been reported to have a significant effect on noodle texture (Miura and Tanii 1994; Inazu et al 2001; Moss et al 1987; Moss 1984; Elbers et al 1996; Crosbie and Lambe 1993; Crosbie et al 2002; Crosbie 1991; Kim and Seib 1993). Recent studies indicate that flour with low swelling starch is required to give the firmness and lack of stickiness in some types of YAN, however, this requirements might not be necessary for all types of YAN. Recently, the genetic underpinning of the main differences in starch characteristics for wheats preferred for Udon (type of WSN) have been elucidated. The increased relative proportion of the highly branched amylopectin leads to greater swelling of starch during cooking (gelatinisation), which can be seen as increased flour swelling volume (FSV), and as the unique soft and elastic texture desired in Udon noodles as well as in some other types of specialised noodle products (Crosbie and Ross 2004; Baik et al 1994; Moss 1971; Ross et al; Seib et al 2000; Kruger 1994).

Elasticity and springiness of Asian noodles

Elasticity and springiness are important component of texture in Asian noodles. Often these sensory descriptions are used interchangeably and often times they are used to describe what is believed to be quite different mouthfeel characteristics. New evidence is showing that these descriptions are not describing the same textural attribute and that their use in a loosely interchangeably fashion may lead to confusion. Instrumental testing has shown that springiness is related to firmness, while elasticity has no particular relationship to firmness or softness (Oh et al 1983; Oh et al 1985a; Oh et al 1985b; Oh et al 1985c; Oh et al 1986; Rho et al 1988; Hatcher et al 1999). No current instrumental methods or techniques appear adequate to measure mouthfeel and elasticity, partly because the terms are not well defined in practice and partly because they may use insufficient deformation of the sample. The use of intensity rating sensory assessments provides a critical interface between consumer preference assessment, mechanical testing and scientific understanding of the roles of various food components. This is also the case for noodle texture which is generally defined by the sensory descriptors firmness / softness, elasticity and surface smoothness along with the subsidiary descriptors, stickiness, chewiness and cohesiveness.

Satisfactory instrumental methods (uniaxial cutting or compression) exist for determination of noodle firmness / softness, however, although instrumental methods for determination of noodle elasticity exist none are universally accepted. The most

profound drawback to widespread adoption of any mechanical method has been the difficulty of correlating with sensory intensity ratings of noodle elasticity (Ross and Crosbie 1997).

Impact of sodium chloride

Typical levels of sodium chloride in noodles are in the range of 2 - 4 %. High levels of sodium chloride p to 8 % can be used in some types of dried noodles. Sodium chloride has an impact on the rate of drying, it also modifies enzymatic activity and prolongs shelf life. Sodium chloride is also related to reduced cooking time and a softer mouth feel of noodle products.

Impact of alkaline salts (Kansui)

Common alkaline salts used in the formulation of YAN include sodium carbonate, potassium carbonate, sodium bicarbonate, often sodium hydroxide is also used in some parts of Asia. Often a combination of the above ingredients is also used. The addition of alkaline salts to the noodle formulation has an impact on noodle textural properties mainly due to its effects on gluten and starch properties. It has been reported that noodles made using sodium hydroxide has a greater impact on starch gelatinisation than sodium carbonate. In addition noodles made with sodium hydroxide are reported to have a softer texture compared to noodles made with sodium carbonate this is likely to be associated with increased starch swelling (Crosbie and Ross 2004).

Alkaline salts increase the Farinograph water absorption and generally decrease the mixing requirement. Dough become tougher and shorter. In the noodle manufacturing process, the alkaline salts serve initially to toughen the dough, which then appears to become weaker and more extensible with successive passes through the combining rolls (Moss et al 1986). The summarised findings are presented in Table 4.4. It was found that alkaline noodles varied between 9.9 and 11.4 pH units compared to 6.2 pH units for the salted noodles. They all had longer cooking time than those with NaCl and had a higher water uptake giving a higher yield of boiled noodles. The microscopic appearances of the starch at the reduction stage was similar in all three samples this lack of change under vary alkaline conditions may be related to the low water content of these doughs. The more alkaline noodles were most attractive in appearance but on cooking had softer textures and stickier surfaces than less alkaline noodles.

Table 4.4 The impact of alkaline treatments typically used in YAN

Alkaline salt (type & concentration)	Impact on YAN
NaCl (1%)	Noodles were white or creamy in colour.
$NaCO_3$ (0.6 %) + K_2CO_3 (0.4 %)	Noodles had green hue. Clear yellow.
Na_2CO_3 (0.9 %) + K_2CO_3 (0.1%)	Noodles had green hue.
NaOH (1%)	Sticky on the surface after cooking and showed signs of breakdown indicating a slower advancement of the cooked zone into the centre of the noodle. No discolouration. Dough appeared tougher. More of the starch granules were distorted or had burst. Voids in the centre more numerous and larger.

Note: Adapted from Moss et al 1986.

Impact of other ingredients often used in noodle processing

Other ingredients that are commonly added to the noodle formulation include starches, vegetable gums, emulsifiers, enzymes, colourings etc. Modified starches are often used in frozen noodles as well as instant noodles to enhance the freeze – thaw stability or slow retrogradation therefore improve the textural properties, and to increase the rate of rehydration respectively. Vegetable gums that typically have an application in noodle technology include guar gum, alginates, xanthan gum and carrageenan gum and are commonly added to the noodle formulation to make noodles firmer, reduce water – holding capacity, to prevent ice crystal formation in frozen noodles and also to reduce fat uptake in deep fried (instant) noodles. Monoglycerides are most commonly used as emulsifiers in noodle formulations to improve dough sheeting properties or to restrict starch swelling on cooking. More recently enzymes have been introduced to various noodles in order to improve textural properties as well as colour characteristics. Lipases have been reported to have a significant impact on reduced surface stickiness, and increased firmness, while amylases have been applied to the surface of cooked noodles as well to reduce the surface stickiness in pre-cooked noodles. Noodle colour can be adjusted / improved by the addition of food colourants such as β-carotene. In some parts of Asia (Japan) riboflavin (vitamin B_2) might be used in YAN to improve the yellow colour of noodle products. On the other hand in South East Asia, tartrazine is commonly added to the formulation of Hokkien noodles (type of YAN) also to enhance the yellow colour (Crosbie and Ross 2004 EGS; Solah and Crosbie 1998; Yoenyongbuddhagal and

Noomhrom 2002; Hatcher et al 2005; Tam et al 2004; Corke and Bhattacharya 1999; Oleson 1998; Nagao 1998; Wu et al 1998; Miskelly 1998; Hou 2001; Lee et al 2002; Williams et al 1999; Lee et al 1998; Chompreeda et al 1987; Lii and Chung 1981; Crisanta et al 1992; Waniska et al 1999; Ge et al 2001; Morris et al 2000; Sunhendro et al 2000; Bhattachary et al 1999).

4.6 Yellow Alkaline Noodles

Among all the types of noodles consumed around the world Chinese noodles might be the most common (Nagao 1996). There are three main subcategories of alkaline noodles. Cantonese noodles which are sold uncooked, Hokkien noodles which are sold partially cooked (parboiled) and instant noodles which are steamed and fried or steamed and dried (Morris et al 2000). They are made from flour (100 parts), water (32-35 parts) and alkaline salts (1-2 parts) (Nagao 1996). The alkaline salts also called Kansui or lye water, is usually a mixture of potassium and sodium carbonates, but sodium bicarbonates, or sodium hydroxide can also be used, impact the flavour and the distinct yellow colour of Chinese noodles. In general Chinese noodles should be firm and elastic having a smooth surface. Colour preferences of noodles vary with region but a bright yellow product free from specks and discolouration is highly desirable.

Flour protein requirement for Chinese noodles in Japan is between 11-12.5%, in other parts of Asian these noodles can be made with flour proteins as low as 9.5. Flour with lower protein content results in softer and quite stickier noodles. On the other hand protein content and colour of noodles have an inverse relationship. Water absorption also plays an important role for noodle quality as too much water absorption causes sticky dough while too little water absorption results in a stiff dough (Lang et al 1998). Typical ash content of the flour used for Chinese noodle production is around 0.33-0.38%. Basically any hard wheat flour can be used in the production of Chinese noodles (Nagao 1996). The unique quality of Chinese noodles is due to the addition of alkaline salts referred to as Kansui or lye water. The addition of alkaline salts results in characteristics flavour, firm elastic textural properties and bright yellow colour (Miskelly 1996; Hatcher 2005; Hatcher et al 2004). The yellow colour of alkaline noodles is attributed to the presence of naturally occurring flavones in flour, recently identified as apigeninglycosides. Colourless at acidic or neutral pH, these compounds

become yellow under alkaline pH. Undesirable noodle colours are generally recognised as time dependent darkening phenomena. Poor colour develops or increases over time. In this context, the converse of discolouration, colour stability is considered desirable trait of a flour or wheat cultivar. Colour stability is especially important for Cantonese noodles because they are stored uncooked. The presence of phenolic substrates oxidising enzymes, those generically referred to as polyphenol oxidase (PPO) are generally invoked to explain the time – dependent darkening of alkaline noodles.

Cultivars of wheat and their flours have variable levels of PPO that tend to be correlated with the rate of development or absolute level of undesirable dull brown and grey colours (Morris et al 2000). In addition to alkaline salts and enzymatic activities yellowness and brightness of Chinese noodles are effected also by bran specks. Raw noodles tend to darken with time this phenomena being more obvious with YAN than with the WSN. The alkaline salts in addition also modify starch pasting properties as well as cooking characteristics of noodles. pH of YAN is usually between 9 and 11 as compared to the WSN pH which lies between 6 and 7. Alkaline salts also increase water absorption and decrease the mixing requirement resulting in a tougher noodle dough (Miskelly 1996).

Preparation of YAN – laboratory scale

YAN are typically made from flour (100 parts), water (32 parts) and alkaline salts [Na_2CO_3 (0.9 parts) and K_2CO_3 (0.3 parts)]. The ingredients are usually mixed for one minute at low speed, one minute at fast speed and three minutes at low speed. The mixture is sheeted between steel rolls 2.75mm apart, folded once sheeted, folded twice. Sheeted and then allowed to rest for 30min. The single sheet of dough is then passed through the rollers four times with the clearance successively reduced to 2.5, 2.0, 1.5 and 1.0mm. The dough sheets are subsequently cut into strips 1.5mm wide. After standing for 3hr at 25°C the noodles are boiled for the optimum time and evaluated. To be regarded as satisfactory, YAN must have an attractive appearance not only when freshly made but also for the next 24hr as in practice they may be kept for consumption until the next day. In South East Asian where noodles are an important component of the diet, clear pale yellow noodles, free of any darkening or discolouration are preferred. They must also give a satisfactory ‘al dente’ reaction on bitting although this is a subjective judgment with preferences varying from person to person and place to place.

Sufficient firmness to give a clean bite without being though is required, as is some degree of springiness or elasticity.

Noodle Colour

Noodles must have an attractive appearance at the time of manufacture, after a day or so of storage and after boiling. There are two distinct aspects of colour: brightness (or freedom of discolouration) and yellowness (Valdamani and Seib 1997; Miskelly and Moss 1985; Hatcher and Symons 2000a; Hatcher and Symons 2000b; Yun et al 1997).

In contrast to bread crumb colour, a light yellow to creamy colour is desirable in some noodles and other pasta products. The yellowish wheat pigments that are extractable with organic solvents are usually referred to as carotenoids because the provitamin A carotene had first (incorrectly) been reported as the principal pigment. The designation carotenoids is still retained for the totality of carotene, the hydroxylated xanthophylls (lutein) and the mono and di-esters of lutein. Another important class of wheat pigments is the flavones, principally tricin. Trace amounts of other xanthine compounds and chlorophyll decomposition products have been reported (Kruger and Reed 1988). The flavone pigments are also widely distributed in plants. The group of flavones includes yellow to brown pigments with the following molecular structure: 5,7,4-trihydroxy 3,5-dimethoxy flavone. In Table 4.5 the distribution of flavones and carotenoids in the components of the kernels of hard red spring wheat is shown. Of the total content of flavones (expressed as tricin) about one third is contained in the endosperm and two thirds in the bran and embryo. The colour of flour is affected by numerous variables. The most important are wheat variety, the effect of milling (rate of extraction, conditioning, and particle size) storage of flour and the effect of bleaching treatments. Year of harvest and location also affect the flour colour (Kruger and Reed 1988).

Table 4.5 The distribution of flavones and carotenoids in hard red spring wheat

Carotene & Xanthophyll	Starchy endosperm		Embryo		Bran		Whole wheat	
	µg / g	%	µg / g	%	µg / g	%	µg / g	%
Carotene	0.09	5.7	0.72	10.0	0.04	4.3	0.18	10.0
Xanthophyll	0.84	53.5	5.78	80.3	0.42	45.1	0.99	55.0
Xanthophyll Ester	0.64	40.8	0.69	9.6	0.47	50.5	0.63	35.0
Total	1.57		7.19		0.93		1.80	
Flavones as tricin	0.7		52.8		6.5		2.3	

Note: Adapted from Kruger and Reed 1988.

4.7 White salted noodles

The best known type of WSN are referred to as Udon and are a popular wheat food in Japan. Production of Japanese WSN consumes approximately 450kT of flour annually in Japan. The most important characteristic of these noodles is colour closely followed by eating quality (noodle texture), taste, surface appearance and weight and volume after cooking. They should be smooth, soft and elastic in texture with clean, bright appearance and a cream colour. Similar noodles are also made in China and Korea, where the quality characteristics preferences is for firmer textured noodles in China and for whiter noodles in both Korea and China. Wheat quality requirements for WSN are bright and creamy colour, smooth and glossy surface appearance, soft texture with a slight surface firmness and elasticity. Texture is probably the most important criteria for judging the eating quality of noodles. Starch is the main component of wheat flour and its properties are important to the quality of WSN. Most starch consists of the polysaccharide molecules amylose and amylopectin. The ratio of amylose to amylopectin affects the physiochemical properties of starch. Wheat flour with lower amylose content was reported to be suited for making noodles with better eating quality. Zhao et al 1998 reported that the granule-bound starch synthase (GBSS) in the starch granules of what flour was related to the noodle quality. However, Batey et al 1997

reported a non-linear relationship between amylose content and noodle eating quality. A clear relationship between amylose content and noodle quality has not been demonstrated.

WSN made from wheat flour with different amylose content exhibited clearly different rheological properties. Noodles of low amylose lines showed lower storage shear modulus (*G'*) obtained by dynamic viscoelasticity measurements than those of lines with higher amylose content. Noodles made from waxy lines showed very low *G'* and a higher frequency dependence of lines *G'*. Noodles of waxy lines showed unique characteristics for compressive stress. This demonstrates that the texture of waxy line noodles was very soft but difficult to cut completely. Waxy wheat flour has unique characteristics for noodle texture (Sasaki et al 2004).

Cooking time is especially important for textural properties of WSN. Undercooked noodles are hard and give a raw flour taste and unpleasant mouthfeel when bitten, while overcooked noodles are soft and soggy to handle and easily broken into small pieces. Major flour constituents (protein and starch) undergo physical and chemical changes such as denaturation of protein and gelatinisation of starch during the cooking process. Surface structure is easily disrupted in Cantonese noodles of low protein content, which leads to reduced cooking time for gelatinisation of starch granules inside noodle strands. Therefore, it is highly possible that cooking time of noodles may be estimated by monitoring the changes in physiochemical properties of protein and starch during cooking. Cooking time of dry WSN determined by squeezing noodle strands during cooking was shorter in noodles prepared from soft wheat flours than in noodles from hard wheat flours.

The positive relationship between protein content and cooking time was reported in WSN and in Cantonese noodles. The effect of starch characteristics of wheat such as amylose content, starch swelling and pasting properties on cooking time of WSN has not been investigated, while desirable functional properties of starch for the textural quality of WSN have been reported. Optimum cooking time of WSN varied widely, from 8min to 17.4min in wheat flours of diverse protein and starch amylose content. Cooking time of WSN was significantly influenced by and correlated with protein content of flours, with the exception of waxy wheat flours. Cooking time of noodles

prepared from wheat flour with high protein content was longer than for noodles from low protein content with similar amylose content. WSN prepared from waxy double null partial waxy and commercial wheat flours of reduced starch amylose content generally required shorter cooking time than those prepared from wheat flours of wild type in starch amylose content. In addition to the sensory test or the method of squeezing noodle strands and observing the disappearance of the white core during cooking, the optimum cooking time of noodles can be estimated objectively by monitoring the changes in amylograph onset temperature of noodle during cooking (Park and Baik 2004a,b,c).

The soft white wheats having moderately high dough strength and starch pasting have been reported to be responsible for superior noodle quality. These pasting properties include high starch paste peak viscosity, low gelatinisation temperature, short time to peak viscosity and high breakdown (Konik and Miskelly 1992). Japanese noodles are made from soft wheat flour of a medium protein content and they usually contain flour (100 parts), water (28-45 parts) and salt (2-3 parts) (Nagao 1996). Although it is quite difficult to express preferred quality characteristics of Japanese noodles, texture is probably most important, followed by the colour and surface appearance (Toyokawa et al 1989).

A positive correlation was found between the desired softness and elasticity in the cooked noodles and 1. a low pasting temperature, 2. a high pasting peak, 3. a high breakdown in the amylograph. The eating quality of Chinese noodles was correlated with starch that showed a low pasting consistency. It is hypothesised that non-starch lipids are elevated in soft wheat flours renowned for noodle quality. Those lipids restrict starch swelling during cooking of noodles, thereby increasing their elasticity. In alkaline noodles made from hard wheat flours that contain an average level of lipid, starch swelling appears to be inhibited by the carbonate ions in the alkaline salts (Kim and Seib 1993).

4.7 Instant noodles

Instant noodles appeared on the market in 1958. Currently, instant noodles are consumed in more than 80 countries. Typically they would contain 10-30g salt per kg flour. Either sodium chloride (salt) or Kansui can be used, although the level of Kansui is typically between 1-2g per kg of flour. For oil fried noodles the level is 3-6g per kg. The content of the liquid is usually 0.3-0.4kg per kg of flour (Kubomura 1998). Instant noodles have distinctive flavour and texture due to unique processing, steaming and frying and to the residual oil in the noodles. Instant noodles should have porous spongy structure as well as pregelatinsed starch through the steaming process. During the frying process many tiny holes are created as water is quickly dehydrated and replaced by the oil on the surface of the noodles, serving as a channel for water during cooking. Frying and steaming processes an enable quick serving compared with other types of noodles and the processes are important in governing the quality of instant noodles (Park and Baik 2004).

For these noodles, mixing time is approximately 15-20 min. During this time all ingredients are uniformly distributed and the flour particles are hydrated. During the rolling process the dough is passed through two rotating rollers in two pieces that are than combined into one noodle belt or sheet. This sheeting step is called ‘compounding’. The sheet is folded and passed through rollers again this step may be repeated several times. It is critical not to decrease gap between rolls as this would not allow proper gluten development.

After compounding noodle sheet is reduced in thickness are cut into strands. Noodles then pass through a steam machine, exposing them to temperatures of approximately 100°C for 1-5min. During steaming the starch gelatinises and the noodle texture is improved. If heating during the steaming step is insufficient the noodles become fragile and fall to pieces easily. On the other hand, excessive heating causes non uniform drying and the noodles become hard and gluey. In terms of flavouring liquid seasoning can be used to flavour oil fried noodles. After flavouring, the moisture content is adjusted on the noodle conveyor by ventilation or natural drying. Noodles are cut and modified each portion is 40-70 cm in length.

Dehydration is achieved by frying in oil, drying in hot air or freeze drying. More than 80% of instant noodles are dried by frying. Before frying moisture content of instant noodles is approximately 30-50% frying at 140-160°C for 1-2min decreases moisture content to 2-5%. Oil content of these types of noodles is approximately 15-20%. The heating contributes to the gelatinisation of starch and noodles become more porous. The cooking and boiling before eating restores their characteristic flavour and texture.
Hot air dehydration is not as good for restoration as oil heat dehydration. In hot air dehydration noodles are held at 70-90°C for 30-40min to achieve 8-12% moisture content. The longer dehydration time may cause shrinkage of the noodle structure, which later effects cooking time and final texture. However, without oil these noodles are less prone to oxidation. The noodles are then quickly cooled and checked for weight, colour, shape, moisture content and general quality. In drying noodles the water activity must be less than 0.7 at 20°C and salt content les than 4.9%. Generally, the moisture content of instant noodles is 10% and the water activity is 0.5-0.6 thus providing microbial stability.

When water is added to the flour the dough becomes very soft and sticky. This is the desired texture for noodle making. The gluten structure develops giving elasticity. Starch also plays a role in noodle structure and texture development. Noodles may also contain starches that contribute to texture improvement eg potato starch, waxy corn, barley, rice and tapioca starch. Advantage of these starches is that they quickly gelatinise and maintain high maximum viscosity that contributes to the texture. Starch levels are typically 5-15%. Starch can also be used to prevent adhesion between the processing belt and the product. In some cases sago starch is used as a dusting powder. Modifying starches are used in some applications because they are acid resistant, heat resistant, display less retrogradation and exhibit good water holding ability.

Water addition, kneading and sheeting result in development of gluten network, which contributes to the viscoelasticity of the dough and increases smoothness of the noodle surface. The dough becomes more cohesive after kneading, the sheen and transparency of the noodle improve, the boiling is decreased and the size and shape of the noodles are retained after boiling. Addition of more water results result in soft and uniform dough

formed quicker. However, with less than 35% water the dough exhibits resistance and requires more kneading and takes much longer to form.

Addition of salt enhances flavours, improves texture and decrease the boiling time. Kansui on the other hand interacts with gluten, producing a gum-like texture that is typical of noodle texture. It also contributes to the development of the yellow colour and enhances the flavour. Emulsifiers are also often used, including glycerin fatty ester, sucrose fatty ester and lecithin. They are often used to improve texture and structure, prevent foaming in the noodles and improve cooking quality. Other additions may include wheat gluten (1-5%) is used to improve the texture of Japanese buckwheat and Chinese noodles as well. Dehydrated whole egg powder may be used at 0.5 – 2.0% to prevent bubbles from forming in the noodles (Kubomura 1998; Kim 1996; Shin and Kim 2003).

In summary, Asian noodles represent a major end use of wheats globally and also of Australian wheat production. The various types of product can be distinguished from the traditional pasta foods made from durum semolina. Although there is increasing evidence regarding the significance of flour characteristics and ingredient formulation upon product attributes, there is relatively little information on the effects of enzymes or on their potential to enhance the consumer appeal of noodles.

Chapter 5

Background and literature review: procedures for the measurement of selected enzymes

The purpose of this chapter is to provide a brief background on the methods available for measurement of selected enzymes in food systems.

5.1 Overview of measurement methods

The most fundamental procedure in enzyme assays is generally the measurement of concentration, either of product formed or substrate disappearance. Most enzyme measurement methods can be divided into two main groups: direct and indirect methods. Direct methods are usually quicker and give a result which is related to the concentration of the product or the substrate with no need for intermediate manipulation. An example of a direct method would be change in absorbance as recorded by the spectrophotometer. Direct methods can be divided into optical and potentiometric methods. Table 5.1 summarises some most commonly used direct methods in enzymology.

Indirect methods on the other hand require an incubation period (usually for the fixed period of time) in addition to further manipulation of values before a final result can be related to the product or substrate concentration. An example of indirect enzyme measurement method is the assay of amylase where an increase in concentration of reducing sugars is measured by a chromogenic reaction followed by the quantitation in the spectrophotometer and finally reference to the standard curve (Stauffer 1989). Indirect methods used to measure an enzyme activity can be divided into chemical reactions and separation of product and these are described briefly in Table 5.2.

Table 5.1 Direct enzyme assays and main actions involved

Direct enzyme methods	Main actions used in assays
Optical methods	
Spectrophotometry	Ultra violet and visible lights used to measure absorbance in terms of either product formation or substrate disappearance
Spectroflurometry	It irradiates the sample with monochromatic light of a wavelength which is absorbed by the compound of interest. The compound emits light at a higher wavelength filtered through optical filters thus the lower wavelength is removed and so only the emitted light is directly proportional to the concentration of the compound.
Nephelometry	The detector is placed perpendicular to the direction of the incident light falling on the cuvette thus responds to the fraction of light scattered at 90°C. The scattered light is proportional to the concentration of particles and the square of the particle volume.
Optical rotation	The action of optical rotatory dispersion / circular dichroism (ORD / CD) is very similar to measuring absorbance changes in a spectrophotometer. Usually used for studies of protein and polymer configurations, but also for carbohydrases or other enzymes producing optical rotatory change during catalysis.
Colorimetric using indicators	The rate of change of in $[H^+]$ can be followed by using an indicator dye, i.e. due to the change in pH change in the absorbance due to the dye can be measured (suing titration of the dye with an acid).
Potentiometric methods	
Ion electrodes	These methods depend on the change in electric potential between two electrodes as some property of a given solution changes. Examples include pH electrodes, Ca^{2+} electrodes, O_2 electrodes and other specific ion electrodes.

Note: Adapted from Stauffer 1989

Table 5.2 Summary of indirect methods used to measure enzyme activity

Indirect enzyme methods	Main actions used in assays
Chemical Reactions	
After fixed time incubation of enzyme with its substrate the reaction is usually stopped by raising the temperature, adjusting the pH or enzyme inactivation due to the addition of inhibitors.	
Colorimetry	These reactions produce chromophore which cane be quantified using a spectrophotometer. The reaction is usually calibrated by running a standard curve.
Titrimetry	Many chemical products may be estimated by titration. Most are either acid –base or redox titrations.
Separation of product	
Typically some form of separation is applied to the enzyme reaction mixture. In some cases the separation step is also the end point of assay.	
Precipitation	Certain unreacted substrates might be precipitated thus the small fragments remaining in solution may be measured following centrifugation or filtration.
Chromatography	The techniques of chromatography range from paper chromatography to high pressure liquid chromatography, from silica gel columns to gas phase chromatography.

Note: Adapted from Stauffer 1989

5.2 Review of methods for assay of α-amylase

The enzymology of amylases has a very long history going back to the early work on the diastase enzymes found in barley malt. One of the difficulties is that when amylase assays specify ‘starch’ or ‘soluble starch’ the amylose/amylopectin ratios of different starches varies and might influence the measurement of enzyme action. A wide range of approaches to amylase assay have been reported (Christophersen et al 1998; Ali et al 2001; Corder and Henry 1989; Verity et al 1999; Barnes and Blakeney 1974; McCleary et al 2002; Mathewson 2000; McCleary and Strugeon 2002; Asp et al 1988; Crosbie et al 1999) and the more commonly used are now described briefly. It is noted that the

extraction buffer used for enzyme preparation in most methods incorporates low concentrations of calcium ions which enhance the stability of the enzyme.

Reduced starch method

Common assays of α-amylase involve reduced starch and the neocuproine acid for analysis of products by colour development (Stauffer 1989). α-Amylase can also be assayed by using the Somogy – Nelson colour reaction method. Purified amylose is generally used as the substrate (Stauffer 1989). Reducing sugar assays cannot be readily used to measure α-amylase in cereals because of the high levels of reducing sugars present in the extracts. Attempts to remove these sugars might lead to loss of some enzyme activity (McCleary and Sturgeon 2002; Henry et al 1992). Furthermore, an assay based on cross linked soluble starch coupled to a soluble dye has also been used widely. The cross linking is analogous to the 1,6 linkages in the structure of β-limit dextrin, thus this substance is resistant to β-amylase attack, and therefore the colour of the filtered solution is proportional to the α-amylase activity. The extracting solution used was made of NaCl 5g and $CaCl_2$ 0.2g made up to a liter with distilled water, the substrate used were Phadebas tablets (Barnes and Blakeney 1974; RACI 1995).

Fluorigenic limit dextrin method

Marchylo and Kruger 1978 described the action of α-amylase on a fluorigenic limit dextrin as the basis for a sensitive assay. The substrate used is a limit dextrin anthranilate and following incubation, the reaction is stopped by addition of phosphate buffer, the insoluble starch removed by centrifugation and the fluorescence of the supernatant measured.

Continuous assay

In addition to these methods Marshall and Christian 1978 described a continuous assay for pancreatic α-amylase which monitors the decrease in absorbance due to the starch – iodine complex. The substrate used is a 1% solution of soluble starch in water, The enzyme α-amylase can also be measured by using the coupled enzyme assays. The maltose formed during the action of α-amylase on starch can be measured using enzymes: glucosidase, mutarotase and glucose dehydogenase (Stauffer 1989).

Gel diffusion method

Mestecky et al 1969 described a gel diffusion assay based upon the reaction of iodine with unhydrolysed starch, measuring the diameter of a clear zone after incubation on an agar (Stauffer 1989). Tipples described a viscosity measurement of cereal α-amylase using a small volume of Ostwald viscometer. The substrate used was potato starch and outflows times were measured periodically. It was suggested that a 10% outflow time decrease would be close to theoretical considerations (Stauffer 1989). Starch solutions have a high viscosity at relatively low concentrations this viscosity decreases quite rapidly as molecules are cleaved by endo-acting α-amylase (McCleary and Sturgeon 2002).

Falling Number method

This method has been used for a long time and is based on detecting sprouted grains and thus estimating the levels of the enzyme present. The original design used grain or flour (which acts as a substrate) and contains enzyme as well. A 7g sample is suspended in 25mL of water and heated to 100°C to gelatinise the starch. The viscosity of the slurry is established as the time required for a plunger to fall a set distance through it (Stauffer 1989).

The Ceralpha method

One of the most advanced substrate preparations for measuring α-amylase is the nitrophenyl-maltodextrins. The reaction is terminated by the addition of an alkaline solution which develops colour of the nitrophenyl ion. Due to the presence of exo-acting starch – degrading enzymes the method could not initially be used on cereals. The introduction of 'non-reducing-end'–blocked nitrophenyl-maltodextrins (McCleary and Sturgeon 2002) made this approach an effective alternative. The Ceralpha method is described in detailed in Chapter 7 as this method was used in this study.

5.3 Assay procedures for lipases

Many enzymes in food systems are able to catalyse the hydrolysis of esters. These are basically divided into two groups: one involves esters of carboxylic acids and the other phosphate esters. The carboxyl esters hydrolyses are specific for soluble esters called

esterases, or for insoluble fatty acids esters named lipases. The clear difference between those two was first based upon water solubility of the substrate. It was shown by Sarda and Desnuelle 1989, that pancreatic lipase reacted with fatty acid esters only when solubility concentration exceeded and a lipid/water interface was formed (Stauffer 1989). The most common substrates used for measurement of lipase are triacylglycerols. When these are used as substrates the hydrolysis is best measured in terms of free fatty acids (FFA) formed. The most convenient method is to extract the FFA into an organic phase, form a copper soap and measure the light absorption of the copper soap or of a coloured complex of the copper with diethyldithiocarbamate (DDC). This particular method was used in this study and will be described in further detail in Chapter 7.

Several other methods have been developed for the measurement of lipase activity. Many of the methods available are based on the principle of product appearance as distinct from substrate depletion.

Titrimetric methods

Titrimetric methods for measuring the activity of lipases appear to offer adequate sensitivity in many applications. The assays require no sophisticated equipment and the analysis is straightforward, however, titrimetric methods tend to be time consuming and this might make them unsuitable for large – scale screening of lipase activity (Thomson et al 1999). An example of a titrimetric method is as follows:

Lipase is incubated with a buffered substrate for a fixed period of time, reaction is then stopped by the addition of acid, the FFA extracted with isooctane and then titrated with 0.01M KOH in methanol to a phenolphathalein end-point. A variation on this assay uses continuous titrimetric assay, where the pH of the assay is monitored and base such as 0.01N NaOH is used to keep the pH constant (Stauffer 1989).

Turbidimetric methods

A successful lipase assay depends mainly on the formation of a substrate emulsion. Therefore it is possible to monitor the rate of clearing thus measure the lipase activity. Under optimum conditions, the lower limit of detection is 1μg of lipase per milliliter (Thomson et al 1999). The basis of a turbidimetric assay is that triacylglycerol

emulsions will have decreased light scattering properties as the substrate is hydrolysed by lipase. (Stauffer 1989).

Fluorometric methods

Lipase activity has also been measured using fatty acids esterified to umbelliferone where conjugate cleavage is monitored spectrofluorometrically. These methods offer the advantage of increased sensitivity (Thomson et al 1999) of approx three orders of magnitude greater than titration assays (Stauffer 1989).

Spectrophotometric methods

In a study by Pencreac'h et al 2002 an ultraviolet spectrophotometric assay was used to measure lipase activity using long – chain triacylglycerols from *Aleurites fordii* seeds. This assay is based upon the difference between molar extinction coefficients of the two types of α-eleostearic acid present the one which is esterified into triacylglcerols and the one which is released in the reaction medium. This difference makes the variations in the UV absorption spectrum of the reaction medium occurring upon enzymatic triacylglycerols hydrolysis.

The study by Melo et al 2000 describes a spectroscopic analysis of thermal stability of the *Chromobacterium viscosum* lipase. The thermal stability of the lipase from *Chromobacterium viscosum* was analysed by the loss of activity, fluorescence, circular dichroism and static scattering measurements. They studied lipolytic activity in a closed reaction vessel at 60°C, using triolein as substrate.

Colorimetric methods

These were developed due to the demand to increase the speed and sensitivity of free fatty acids assays. These methods are based on the complexation of free fatty acid assays on the formation of complexes of free fatty acids with a divalent metal (usually copper) followed by spectrophotometric analysis of the organic phase. In general these methods are not expensive, but are very reliable and convenient. (Thomson et al 1999).

Conductance methods

Conductance changes in the reaction medium are observed through hydrolysis of triacetin by lipase. Long – chain triacylglycerols are not recommended for use in these

methods due to their poor water solubility and the sensitivity of the assay being further reduced by the limiting equivalent conductivity of the anion, which decreases with increased number of carbons in the chain. Good sensitivity and high reproducibility were obtained by these methods and addition of calcium ions had no effect on the on the assays. Since the conductivity of ions vary significantly with temperature, the temperature of the assay must then be controlled within a narrow range (Thomson et al 1999).

Chromatographic methods

Chromatographic methods of lipase analysis have been based on quantification of hydrolytic products of the lipase reaction by gas chromatography (GC) or high performance liquid chromatography (HPLC). GC with flame ionization detection is usually preferred because HPLC suffers from the low sensitivity of available detectors. A disadvantage of GC analysis is the need to derivatise the fatty acids prior to chromatographic separation, however, this has been solved by the use of kits designed for this purpose. Capillary GC with this form of detection gives the best results for the analysis of fatty acids and methyl esters and extremely small samples can be analysed (Thomson et al 1999).

Surface tension methods

Since lipases are active at interfaces it is possible to monitor enzyme activity by looking at the changes in surface pressure. Using monomolecular film technology it is possible to measure interfacial parameters including surface pressure or molecular area of the substrate that influences lipase activity. In addition the oil – drop method can also be used to monitor changes in interfacial tension lipolysis, where the decrease in interfacial tension over a period of time can be used to follow lipase kinetics (Thomson et al 1999).

Test kits

Test kits available from major chemical suppliers are also used to measure lipase activity. Most of the kits currently available have been designed for clinical analysis. Some are based on enzymatic clearing of an emulsion where the decrease in absorbance values can be used to determine lipase activity in samples. Others are based on the titrimetric determination of fatty acids released from olive oil triacylglycerols using

thymolphthalein for the end point indication. Alternatively, the formation of a quinone diimine dye may be monitored.

NMR methods

It has been noted that under certain conditions which include water content and solvent type in the medium, lipases can function as synthetic enzymes. Therefore a nuclear magnetic resonance (NMR) method has been developed to measure lipase activity. The NMR method is based on the changes in the ratio of ester to alcohol in the reaction mixture. A relatively crude substrate can be employed for this method (Thomson et al 1999).

5.4 Assays of ascorbic acid oxidase (AAO)

A simple spectrophotometric method is based on absorbance of ascorbic acid at 265nm where the reaction product (dehydro ascorbic acid) is not absorbing. Typically citrate – phosphate buffer is used at pH 5.6 and the rate of disappearance of the ascorbate is observed at 265nm and it can be converted to molar terms using the molar absorptivity of ascorbate (Stauffer 1989).

A continuous spectrophotometric assay of AAO has been thoroughly described (Every et al 1995; Every et al 1996) and this is outlined in Chapter 7 as the approach was adopted after slight modification for this study.

5.5 Assays of peroxidase (POX)

The general assay for measuring POX has not changed much from the original method proposed in 1950's. The reaction mixture usually contains 0.01M phosphate buffer pH 7.0 with 0.74mM H_2O_2 and 20mM pyrogallol. As soon as the enzyme is added the increase in the absorbance is monitored at 430nm due to the formation purpurogallin. Bovaird et al 1982 studied various experimental effects on the POX reaction and proposed their optimized assay as containing 0.1M phosphate buffer pH 5.0, 3mM o-phoenylenediamine and 3.2mM H_2O_2 used as substrate. They assayed the enzyme at 435nm.

Assays used for POX activity determination as published by Delcros et al 1998 and Ioru et al 1995 were slightly modified and used in this study and this is described in detail in Chapter 7.

5.6 Assays of lipoxygenase (LOX)

The enzyme lipoxygenase catalyses the oxidation of unsaturated fatty acids and their esters having the *cis, cis* – 1,4 – pentadiene system. The most commonly used methods were the traditional manometric techniques. Mitsuda et al (1967) used polarpgraphic means to measure the activity of LOX. with detection of the oxygen concentration in the reaction medium.

Spectrophotometric determination of LOX

The spectrophotometric method is based on the absorbance of the conjugated dienes at 234 nm formed when the substrate is oxidized. This procedure is simple and rapid but turbid solutions cannot be assayed. However, kinetic studies can be performed with clear solutions.

Blain & Todd (1995) described the application of an LOX assay based on destruction of carotene. The assay is based on the addition of the enzyme solution to a suspension of linoleate and carotene in buffer. The mixture is allowed to act for a specified time the reaction is then stopped by the addition of sodium hydroxide and the residual carotene is estimated colourimetrically against a distilled water blank. A control differing to the reaction only in that the enzyme solution is added after the sodium hydroxide is also tested against distilled water and carotene destruction calculated by subtraction. The whole assay operates at a pH value at which the primary substrate, linoleate and the enzyme are in different phases, and any natural surface active factors occurring in crude extracts of LOX might affect dispersion and reaction interface. Nevertheless, Blain & Todd (1995) concluded that valuable information can be obtained by studies of oxidation of secondary substrates such as carotene, in addition to that of the primary substrates, such as linoleate by LOX.

5.7 Some challenges in the choice of enzyme assays

In reviewing the literature on enzyme assays, it is clear that there are some major challenges involved. Some of these are:

- specific substrates have to be used for specific enzymatic action
- the great sensitivity often required and the dependence upon temperature and pH particularly
- cloudy extracts which cause interference in spectrophotometric methods.

In the assessment of enzyme levels in Asian food products, various evaluation trials had to be designed in order to ensure that enzyme assay methods were able to reproducibly measure activity levels.

Chapter 6

Summary of background and description of the project aims

The purpose of this chapter is to summarise the context in which this project has been developed and to describe the aims of the research program.

6.1 Summary of current situation and significance of the project

In Australia there are approximately 40,000 farms which produce wheat as one of their main grain crops. Australia is the fourth largest wheat exporter, following the United States, Canada and the European Community. Most of our production is exported and wheat is a significant part of the national economy. Asia represents an important and valued market for Australian wheat. Since the Asian region is the world's most dynamic growth area, the Australian wheat industry is keen to remain part of this development (Martin and Stewart 1994).

Almost one eight of all wheat produced globally is used for Asian noodle production. In addition, it has been estimated that one third of all Australian wheat exports if used for the production of Asian noodles (Wrigley 1994) as these products are a staple food in many Asian countries. Furthermore, the consumption of noodles is increasing in Australia due to the popularity of Asian cuisines. Over recent years there have been advances in our understanding of consumer preferences for different styles of noodles. In addition, the impact of flours having different characteristics has been studied. Some of the factors determining the colour and textural properties of particular products are: the manipulation of pH using kansui and the pasting properties of the starch present in the flours.

In recent years, scientists have turned their attention from understanding flour quality solely as it relates to bread because of the growing appreciation that wheat is used for other end products including noodles, steamed breads and flat breads. In examining the scientific literature, it is evident that there is a paucity of knowledge about noodle products compared to bread. However, a certain amount of the biochemical information

and procedures developed for testing of breadmaking quality may relate to noodlemaking. Another issue is that there are many alternative processes that can be applied in noodle manufacture prior to cooking and consumption (Oh e*t al*., 1985).

The contribution of naturally occurring enzymes during the baking process is well established and in addition the potential of selected enzymes is being realised in the baking process. However, the application of some of these in products other than bread has not been thoroughly explored. At this point in time relatively little is known of either the role or potential of enzymes in influencing quality attributes of Asian noodle products.

Up to this time most enzymatic studies on noodles have been concerned with colour, particularly discolouration during processing and also during storage and the resultant appearance of undesirable brownness in the dried form of noodles. Furthermore, the detrimental effect of sprout damage on noodle processing was first reported in 1974, when studies of Bean et al (1974a, 1974b) indicated that amylase might be an important quality issue when present at higher levels. The authors suggested that factors including proteases, solubilised carbohydrates, or other modified constituents could play a significant role in noodle dough properties. It was also concluded that the effects of sprout damage might be corrected by adjusting salt and pH. Another consideration is that any softening effect on the texture of cooked noodles attributed to the action of some enzymes may not necessarily be detrimental since textural properties between soft and firm vary even between regions within a country as well a among counties.

Pre harvest sprouting of grains is a major problem for farmers, millers and various other members of the food industry. The use of flour from sprouted wheat in bread making is associated with a sticky dough. In addition baked products have damp and gummy crumb and are darker in colour and inferior in texture compared to bread from sound wheat. Noodles made from sprouted wheat flour show discoloured spots and may be darker than their sound controls, unattractive and less yellow in both the raw and cooked state (Moss and Orth 1987). Thus sprouted wheat is not acceptable for either bread or noodle production and is classified as lower grade. However, the molecular basis for the observations on noodle characteristics are not yet well understood.

In conclusion, as Asian noodles represent the end use of at least one eighth of all the wheat grown globally, it is important to better understand the factors influencing product quality. In addition, there is need to explore the potential of enzymes to enhance the quality and production of the many different styles of Asian noodle products.

Manufacturing of Asian noodles is well established around the world and the production has been increasing in Australia during the recent decades. Furthermore, it is now recognized, that the value of noodles production in Australia exceeds $A50 million annually (McKean 1999). Therefore, it is important to understand factors influencing quality of these products to be able to expand the production even further.

6.2 Hypothesis

This study has been based upon the hypothesis that as it has been shown that enzymes have an important function in baking, it might be expected that they will have a significant impact on the important attributes of quality of Asian noodle products.

6.3 Project aims

The overall aim of this project has been to investigate whether various enzymes play a significant role in determining quality of the major styles of Asian noodle products. The different styles of noodles studied have been yellow alkaline noodles, and white salted noodles. The enzymes investigated have been lipoxygenase, lipase, glucose oxidase, ascorbic acid oxidase, α-amylase and peroxidase.

The levels of endogenous enzymes were investigated and the impact of these during noodle making studied in relation to the molecular interactions occurring and for impact on overall product characteristics. The significance of elevated enzyme levels in flour that results from pre-harvest sprouting were also investigated as there is anecdotal evidence that this can have an adverse impact on noodles.

The specific aims of this project have been to:

1. Develop our knowledge on the molecular interactions occurring in the manufacture of different noodle products;

2. Provide a basis of adding novel ingredients to enhance product qualities;

3. Evaluate the undesirable effects of enzymes from sprouted grain and identify the most important factors detracting from the appeal in these products;

4. Provide a basis for greater control of product quality during manufacture of these products;

5. Develop existing knowledge of the structural properties of fresh raw, fresh cooked, dried and dried cooked noodles and importance of selected enzymes and / or other functional food ingredients on the quality attributes of Asian noodle products.

Chapter 7

Materials and methods

The purpose of this chapter is to describe the chemicals, reagents, equipment and methods used during this study. This includes procedures applied in the sampling and preparation of noodles, methods for measurement of selected enzymes in food systems, along with details of calculations for individual enzyme activity.

7.1. Materials

The chemicals including enzyme preparations used in product formulations and analytical procedures were of highest purity available or analytical grade, unless otherwise stated. The details of the enzymes and reference used are presented in Table 7.1. The reference flour used in the study was the Royal Australian Chemical Institute, Cereal Chemistry Division Check Sample for 2001 and the specifications supplied with the sample are presented in Table 7.2. The details of laboratory chemicals used together with the respective suppliers are listed in Table 7.3. Similarly data on commercial flours are provided in Table 7.4.

Table 7.1 Enzyme preparations used in the study

Enzyme preparation	Description	Activity[a]	Supplier
α-Amylase (barley malt)	A-2771, Lot No 033K0975; Type VIII-A from Barley Malt	1.8 units / mg solid; one unit will liberate 1.0 mg of maltose from starch in3 min @ pH 6.9 @ 20°C	Sigma Chemical Co, USA
α-Amylase (*B subtilis*)	Amylase LT 23, Lot No PIFB 901;	23,000 KMWU / g. Assay is based on the measurement of starch colouration by iodine.	Enzyme Solutions Pty. Ltd, Australia
Ascorbic Acid Oxidase	A-0157. Lot No 11K4025; from *Cucurbita sp.*,	205 units / mg solid; one unit will oxidase 1.0 μmole of L-ascorbic acid to D-ascorbic acid per min @ pH 5.6 @ 25°C	Sigma Chemical Co, USA
Lipase PP	L-0382, Lot No 081K7663; Type VI-S from Porcine pancreas	52,900 units / mg solid; one unit will hydrolyse 1.0 micro – equivalent of fatty acid from olive oil in 1h @ pH 7.7 @ 37°C (30 min incubation)	Sigma Chemical Co, USA
Lipase WG	L-3001; Lot No 033K7063; from wheat germ	8.6 units / mg protein; one unit will hydrolyse 1.0 micro – equivalent of fatty acid from triacetin in 1hr @ pH 7.4 @ 37°C (60 min incubation)	Sigma Chemical Co, USA
Lipase fungal 8000	Lot No FR 9061A;	80.000 LU / g. The assay is based on a 30min hydrolysis of a defined olive oil emulsion at pH 6.0 and 37°C. The resulting fatty acids are titrated to a phenolphthalein end point. Ten Lipase Units (LU) is that amount of activity required to liberate one micro-equivalent of fatty acid per minute under the conditions of the assay	Enzyme Solutions Pty. Ltd, Australia
Lipoxygenase	Lot No 7609E; code 100825; from Soy beans	250,000 U / mg one unit represents the increase in the absorbance of 0.001 / min @ 234 nm; pH 9.0 @ 25°C due to linoleate oxidation	MP Biomedicals. Inc. Germany

Table 7.1 Enzyme preparations used in the study (continued)

Enzyme preparation	Description	Activity[a]	Supplier
Peroxidase	Lot No R9284; from horseradish	100-150 purpurogallin units / mg solid; one unit represents the amount of enzyme causing the production of one milligram of purpurogallin in 20 seconds at 20°C	MP Biomedicals. Inc. Germany
Peroxidase	P-6140; Lot No 051K7490; Type X from horseradish	291 purpurogallin units/mg protein; one unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds @ pH 6.0 @ 20°C	Sigma Chemical Co, USA
Poly Phenol Oxidase	T-3824, Lot No 023K7024 from mushroom	2,130 units / mg solid; one unit equals to ΔA280 of 0.001 per min @ pH 6.5 @ 25°C in 3 ml reaction mix containing L-tyrosine	Sigma Chemical Co, USA

Note Description presented as product number, batch or lot number
a Activity as stated by the manufacturer

Table 7.2 Details of flour check sample

Analysis	Average	Std. Dev.	n
Kjeldahl protein (%)	12.20	0.13	8
Dumas protein (%) Kjeldahl calibrated	12.24	0.16	4
Dumas protein (%) Chemically calibrated	12.49	0.08	4
Oven moisture (%)	13.97	0.13	11
Ash (%)	0.55	0.03	8

Table 7.3 Details of chemicals and suppliers

Supplier	Chemicals
Sigma Chemical Co, USA	Ammonium hydroxide (A-6899, 118H4355), Bromophenol blue (2383, B82), Glutathione (G-4626, 88H7440), Hydrochloric acid (101256J, K26945452), L-Ascorbic acid (A-7630, 110K1256), Potassium ferricyanide (P-8131, 54H3488), Trichloroacetic acid (T-6399, 39H0412)
BDH Laboratory Supplies, England	12-methyl-1-propanol (100625K, H728341-920), Acetic acid (100015N, K27574117012), Acetonitrile (152856K, 034210 221), di-Sodium hydrogen orthophosphate (30158.5000, 22160), Ferrous sulphate (101124V, TA711641511), Formic acid (101156G, K25587943), Glycerol (101118.4k, 27685), MeOH (15250, L050502), n-Hexane (103876Q, I870778919), Octan-1-ol (294084 B), Orthophosphoric acid (153153N, K29299573128), Papain (390303G, F389661883), Potassium chloride (10198.7X, 30523), Potassium hydroxide (10210.5000, 21336), Sodium acetate (010236.5000, 20876), Sodium chloride (10241.AP, 26194), Sodium dihydrogen orthophosphate (30132, 18560), Sulphuric acid (303246E, Z50960792), Toluene (010284.2500, 20985), Triethylamine (153293B, L456341 028)
Ajax Chemicals, Melbourne	Sodium hydroxide (10252.7R, 24444), Glass wool (1755, 70147712), Sodium carbonate (1225, 216302)
Megazyme	Total starch assay kit – (AA/ AMG) No: K- TSTA; Amylazyme – α-Amylase Assay Procedure / Kit AMZ 7/98
Fluka Chemie Gmbh, Germany	Acid phosphatase (85433020-16/28, 108227)
EM Science, USA	*iso*-propyl alcohol (PX 1838-1, UN 1219)
CSR, Melbourne	Ethanol (UN 1170)

Note Description presented as chemical name (product number, batch or lot number)

Table 7.4 Description of flour samples used for this study

Flour	Description	Supplier
Maximus	Baker's Strong (BGF) Code: 36360	Allied Defiance, NSW
P-farina (PF)	Extra-white flour Product code: 36347	Allied Defiance, NSW
Ultra White (UW)	Gem of the West	Manildra, Auburn, Sydney

7.2 Apparatus and auxiliary equipment

The items of equipment used, together with the details of manufacturers and model numbers are presented in Table 7.5.

7.3 Laboratory procedures for manufacture and processing of Asian noodles

7.3.1 Description of flour samples used in preparation of particular types of noodle samples in the laboratory

A series of flour samples having different characteristics and suited to different end uses were used in this study (compare with Table 7.4). These were used for the assays of various enzymes as well as for the preparation of laboratory noodles. In the study presented here P-Farina and Ultra White flours were used for the production of white salted noodles while Baker's Strong flour was used in the manufacture of yellow alkaline noodles (Martin and Stewart 1994).

In studies of noodles prepared in the laboratory, a number of batches of the same noodle style were made and the samples of these batches were analysed at least in triplicate. The averages of the results from the analysed data were calculated and are presented here, representing the value of each batch of noodles prepared.

7.3.2 General procedures applied in the preparation of noodle samples

Noodle samples were prepared using procedures based on those previously described (Moss 1982; Moss et al 1987; Crosbie et al 1999; Lee et al 1987) In general, the noodle making methods for the two styles have four common steps: mixing, sheeting, cutting and drying. The preparation of each of noodles are now described in more detail.

Table 7.5 Description of equipment used

Equipment	Manufacturer/supplier	Model No
Water bath (thermostatically controlled)	Thermoline Scientific Instruments Pty Ltd, Melbourne	BTC 9090
Minolta Chroma Meter	Minolta Camera Co Ltd, Osaka, Japan	CR300
Cary Spectrophotometer (UV-VIS)	Varian Australia Pty Ltd, Melbourne	1E
Ultra-Turrax homogeniser	Janke and Kunkel, Stanfen, Germany	T 25
Kenwood mixer	Kenwood Ltd, Britain	KM210, Serial no. 0309397
Noodle maker	Domestic 'spaghetti machine' Imperia, Italy	MOD 150, design no. 1048534
Cutting attachment for noodle maker	Imperia, Italy	MOD 150
Scanning electron microscope (SEM) and Environmental scanning electron microscope (ESEM)	Philips	XL 30
Kjeldahl protein	Tecator, Sweden	1003 digestor and 1012 distillation
Texture Analyser (TA-XT2)	Stable Microsystems, England	TA-XT2
Oven	Memmert, GmbH, Germany	Type: UML 500, F No: 891319, NIN 12880-KI
Centrifuge	Beckman Instrument, Inc, Germany	GS-15R, 360904, series GYD 95H13.
Spectrofluorimeter	Perkin-Elmer Corp, UK	LS 50, Serial No. 26309.
Grinder	Philips, China.	HR 2185
Sieve (500 μm)	Endecotts Ltd. London, England	Part No. 667924, 737176

7.3.3 Preparation of white salted noodles

Ingredients used for white salted noodles

White salted noodles were prepared from either P-farina or Ultra White as these had processing characteristics generally suitable for this style of noodles (Shelke et al 1990; Moss 1984; Yunt et al 1996; Wang and Seib 1996; Konik et al 1992; Yun et al 1997; Wills and Woontton 1997; Yun et al 2002; Chong et al 2002; Epstein et al 2002; Lai and Hwang 2004; Zhang et al 2005; Park and Baik 2002; Baik and Lee 2003; Guo et al 2003; Kojima et al 2004; Martin et al 2004; Park et al 2003; Wang et al 2004; Batey et al 1997; Nagao et al 1976; Black et al 2000; Jun et al 1998; Wotton and Wills 1999; Inazu and Iwasaki 2000; Lee et al 1987; Yamaguchi and Allen 1998; Wardle et al 1996; Crosbie et al 2002). The basic ingredients used for making all white salted noodles were: 200.0g flour, 69.0g water and 8.0g sodium chloride. In some instances, enzymes were also incorporated into the formulation as described in the results section.

Method for white salted noodles

Mixing: Sodium chloride was first dissolved in the water and this solution was added to the flour over a period of 30s in a Kenwood mixer set on speed one. Timing of mixing then began when all the liquid had been added. The mixer was set at the lowest setting (speed 1) for 1min then it was stopped so that the dough material adhering to the bowl and beater could be scraped down. After that, the speed of the mixer was increased smoothly to setting 2 and allowed to mix for a further 4min. After a total of 5min mixing (1 plus 4min), the resultant dough had a crumbly consistency similar to that of moist breadcrumbs (Hatcher and Preston 2004).

Rolling: The dough was first formed into a dough sheet by a process of folding and passing the crumbly dough through the rollers of the noodle machine several times. For this combining step the rollers were set at the maximum gap available, corresponding to 2.7mm. Typically three passes were required although up to 5 passes were used where necessary in order to give a uniform sheet which held together as a single dough piece. Then this combined sheet was allowed to rest for 30min. For this purpose, the sheet was sealed in a plastic bag to exclude moisture loss after resting. The thickness of the sheet

was reduced stepwise by passing between the rollers of the noodle machine. The roll gap settings used were: 2.2, 1.8 and 1.4mm.

Cutting: The sheet was cut into strands using the cutting roll attachment of the noodle machine having a cutting width of 2.0mm. The noodle strands were then cut into 25cm lengths using a knife before drying.

Drying: The fresh noodles were arranged upon trays lined with aluminium foil. The noodles were placed loosely in order to facilitate effective drying and the trays were stored in a fan forced oven at 40°C for 30hr. The product was then allowed to cool for 30min in the ambient conditions of the laboratory prior to being placed in airtight plastic bags or containers for storage (Inazu et al 2003; Inazu and Iwasaki 1999).

7.3.4 Preparation of yellow alkaline noodles

Ingredients used for yellow alkaline noodles

The flour used for preparation of yellow alkaline noodles was Baker's Strong flour based on the slightly higher protein content compared to other flours. The basic formulation was: 200.0g flour, 70g water and 2.0g Kansui (sodium : potassium carbonate; 9 : 1).

Method for yellow alkaline noodles

The procedure for making yellow alkaline noodles was basically the same as that described above for white salted noodles with the following modification:

Mixing: alkaline salt (rather than common salt used for white noodles) was dissolved in the required volume of water (Allen et al 1999; Zhao and Seib 2005).

7.3.5 Enzyme addition to noodle formulations

Samples of noodles were also prepared in which additional enzymes had been incorporated into the formulation. Each of two different noodle styles was fortified under controlled conditions. This was achieved by first dissolving the enzyme in water and a suitable volume was then used as part of the liquid portion of the formulation. Other aspects of noodle preparation followed the procedures described above. It is noted that here the fortification levels are expressed on the basis of the fresh weight of flour.

The levels of each enzyme added to the noodle formulation are detailed and discussed in results and Discussions Chapters.

7.4 Basic methods for characterisation of flours and noodle samples

In the analysis of all samples, multiple analyses were carried out as described for the individual analysis procedure. In all cases the results for at least duplicate measurements of individual samples were assessed statistically and are reported as the mean value ± standard deviation. In reporting data, the latter is abbreviated as sd and the number of replicate determinations is referred to as n.

7.4.1 Determination of moisture content

The moisture contents of samples (flours, doughs, fresh noodles, dried noodles, and cooked noodles) were measured following the air oven method (AOAC 2000; RACI 1995; AACC 2002; Hunt and Pixton 1954). For each sample analyses were carried out in triplicate. It is noted that samples were not ground prior to analysis. Empty aluminum moisture dishes with lids were first placed into a pre-heated oven set at 130 ± 3°C. After 1hr, the empty dishes were taken from the oven and cooled in a desiccator containing active silica gel desiccant for a period of 20min and then weighed. Sub-samples (approximately 2.0g) were accurately weighed into the pre-weighed dishes. Then the covered dishes containing the samples were placed into the oven with the lids placed under the respective dishes and dried at 130 ± 3°C. The process of drying, cooling and weighing was repeated after 1hr until a constant weight was attained. The loss in weight was used to calculate the moisture content of the samples using the following equation:

$$\text{Moisture content} = (\%)\ \frac{\text{Loss in weight of dish, lid and sample upon drying}}{\text{Initial weight of sample}} \times 100$$

7.4.2 Measurement of the pH of flours and noodle samples

The measurement of pH values of flour and noodle samples was based on the method described by the by the AACC standard procedure (AACC 1994b). For this, a sample (10.0g) was thoroughly blended in 100mL of water using the Ultra-Turrax homogeniser. The mixture was then allowed to settle for approximately 60min after which the

supernatant liquid was decanted and tested with a calibrated pH meter. All analyses were carried out in triplicate.

7.4.3 Protein content determination

The nitrogen content of flour and noodle samples was measured using the Kjeldahl method and the protein content was then estimated from the nitrogen value using the following formula:

Protein content = Nitrogen content × protein conversion factor (5.70) (RACI 1995; AACC 2002).

For each batch of samples one blank and one reference were used. Reference of known chemical composition (glycine) was used. One gram of sample was put onto a filter paper and transferred into a Kjeldahl tube, two Copper catalyst tablets were added per each tube, followed by 12.5mL of sulphuric acid. The samples were digested @ 420°C for an hour. After cooling period of 30min 50mL of distilled water was added to each tube. Samples were then distilled as follows:
25mL of boric acid solution was measured into a receiver flask and samples were collected into it after being distilled with concentrated sodium hydroxide. These were titrated using 0.1M HCl to the neutral grey end point. The nitrogen content was calculated as follows:

Nitrogen content = (sample titre – blank titre) / 1000 × (Molecular weight of nitrogen / sample weight) × 100 × moles per liter of HCl × protein conversion factor

7.4.4 Total starch content determination

The total starch was determined using the Megazyme Total Starch kit (AACC Method 76.13; AOAC Method 996.11 and ICC Standard Method 168). The procedure described in the methods was followed rigorously and the following calculation used to calculate the total starch content of the flour samples:

Starch = $\Delta_E \times F \times 1000 \times (1/1000) \times (100/W) \times 162/180$

$= \Delta_E \times F/W \times 90$

Where:

Δ_E = Absorbance read against the blank;

F = (1000µg of glucose) / (Absorbance of 100µg of glucose) [conversion from absorbance to µg];

1000 = Volume correction (0.1mL taken from 100mL);

1/1000 = Conversion from milligrams to micrograms;

100/W = Factor to express 'starch' as a percentage of flour weight;

W = The weight in milligrams ('as is basis') of the flour analysed;

162/180 = Adjustment from free glucose to anhydro glucose (as occurs in starch);

Starch % (dry wt. basis) =

Starch % as is × 100 / (100 – moisture content (%) (Megazyme 2003).

7.4.5 Cooking loss and cooking weight characteristics of Asian noodles

Cooked weight and cooking loss were determined by methods modified from Grant et al 2004 and Chakraborty et al 2003. Noodles (10g) were cooked in 300mL of distilled water to their optimum cooking time, rinsed with distilled water and left to drain for two minutes at room temperature, reweighed and results reported as % increase on cooking. The residual cooking water was dried in an oven at 110^{o}C and results reported as % weight lost during cooking.

7.4.6 Measurement of noodle colour

The colour of noodles was determined by using Minolta Chroma Meter (CR300). The instrument was first calibrated using the white calibration tile supplied by the manufacturer. For analysis, the three different colour parameters, *L**, *a** and *b** were recorded. The *L** value measures the degree of whiteness/darkness and the higher the *L** value, the lighter the colour. The *a** value indicates the balance between redness and greenness of the sample with positive values corresponding to red colours and negative to green. The *b** value indicates the balance between yellowness (+) and blueness (-). For *a** and *b** readings, values closer to zero indicate less intense colour whereas readings further from zero correspond to more intense chroma characteristics. Multiple

sets of readings (n=10) were taken on all samples by moving the measuring head on a random basis to different locations on the surface of the sample between readings. Mean values are reported. Colour was measured at time zero (just after manufacture) and then every hour for the total period of 9hr and subsequently at the 24^{th}, 48^{th}, 72^{nd} and 96^{th} hour. The colour was evaluated on noodle sheets stored in plastic bags and stored at 4°C or 25°C. Colour was also recorded on dried noodles just after the process of drying was finished), and on cooked noodles as well (immediately after cooking; for this purpose noodles were tightly packed between two clear plastic plates and the readings were taken) (Morris et al 2000; Hatcher and Symons 2000; Bhattacharya et al 1999a; Baik et al 1995; Miskelly 1984; Hatcher et al 1999; Crosbie et al 1996; Martin et al 1996; Ward et al 1996; Allen et al 1996a; To and Quail 1998; Lee et al 2002; Allen et al 1996).

7.5 Textural profile of laboratory made noodles (TA-XT2)

Textural properties of noodles were examined using the textural analyser (TA-XT2) equipped with the Texture Expert Exceed software package (Stable Micro Systems 1995) using two attachments a cylinder probe (P/45) measuring compression force (N) representing back tooth action and subsequently measuring noodle firmness and a flat (stainless steal) cutter / blade (7 x 11.5 x 0.3cm) both measuring maximum cutting force (N) representing front tooth action (Tang et al 1999; Rice, and Caldwell 1996; Kim et al 1998). Measurements were done on raw noodle samples (at time zero and at 24hr following storage under defined conditions) and also on cooked noodles. Cooking was performed at time zero and at 24hr following storage under defined conditions. Texture was analysed within 30min of cooking. Noodle samples were cooked to their optimum cooking time determined earlier.

Cylinder method

Two noodle strands were tested at each time (placed close to each other as possible) making sure that the strand test region was positioned centrally under the probe. For probe calibration – 15mm return trigger path was used (Stable Micro Systems LTD 1995). Analysis was performed in duplicate batches, each batch consisting of 20 compressions. The following settings were used: mode: measures force in compression; option: return to start; pre-test speed: 2.0mm/s; test speed: 2.0mm/s; post-test speed: 2.0mm/s; strain: 75%; trigger type: auto – 10g; data acquisition rate: 200pps. The results

using the cylinder probe will be discussed as noodle hardness, force required to compress noodles to 75% of their original thickness.

Blade method

Again two batches were analysed, each batch consisting of 15 cuttings. Settings were as follows: mode: measure maximum cutting force; option: return to start, pre-test sped: 0.5mm/s, test speed: 0.17mm/s; post-test speed: 10.0mm /s, distance 4.5mm; trigger type: button; data acquisition rate: 400pps; test set-up: five strands of noodles adjacent to one another centrally under the knife blade, with the axis of the product at right angles to the blade (Stable Micro Systems LTD 1995; AACC 16-50, 1995). The results using the cutting blade will be discussed as noodle firmness, which is the maximum cutting force required to penetrate noodle stripes. For all measurements the TA – XT2 was equipped with a 5kg load cell.

7.6 Structural assessment of noodles prepared in the laboratory

The surface structure of noodles was examined using the Philips XL 30 scanning electron microscope. Scanning electron microscope (SEM) mode was used for cooked and dried noodles, while environmental scanning electron microscope (ESEM) mode was used for raw noodles. Low vacuum mode (10kV) was used in the SEM mode for noodles examined with no preparation, while high vacuum mode (20 or 30kV) was applied to noodles examine in the SEM mode following sample preparation (freeze-drying and / or gold coating). Cooked noodles were freeze-dried immediately after cooking and gold coated before examining in high vacuum mode. Some cooked noodle samples were also examined suing the Cryo mode. This method of examination involved freezing samples in the liquid nitrogen immediately after cooking, transferring them into the closed chamber to minimise any possible changes to the sample surface. The sample was then fractured and subsequently heated up in the microscope chamber for a determined amount of time to allow defrosting of ice that might have accumulated on the surface. The sample was finally sputtered with gold for 120s and viewed (Dexter et al 1979).

7.7 Preparation of samples for enzyme assays

For analysis purposes, flours did not require pre-treatment, while noodle products were prepared as follows:

Grinding

All the noodles investigated, were ground to pass through a 35-mesh screen (sieve aperture: 500μm) prior to extraction of enzymes.

Cooking

The noodle samples were cooked by placing a small amount (approximately 10g) into a saucepan of gently boiling water (300mL). After every minute, a strand of noodle was removed, immediately placed into water which was at ambient temperature. The strand was then squeezed between two microscope slides. Noodles were considered to be fully cooked at the point where the uncooked core had just disappeared, that is a uniform colour and appearance was observed upon squeezing. The optimum cooking time of both commercial and laboratory product varied considerably depending upon the cross-sectional sizes and also the style of noodles.

7.7.1 Extraction of enzymes from flour and noodle samples

Homogenisation

A small quantity of sample (1-6g) (depending on the assay and enzyme analysed) (including, dough, ground noodles, fresh noodles, and cooked noodles) were weighed and blended with a buffer (the type of the buffer and its preparation and the final pH varied again depending upon enzyme being analysed; details are given for each individual enzyme analysed below). The volume used was that sufficient to allow effective maceration of the particular sample.

7.8 Procedures and calculations applied for enzyme assays

Due to the potential sensitivity of the enzymes to light and temperature, all procedures were performed in the absence of direct light or subsequently at lower temperatures. In addition, samples, sample extracts and buffers were covered with aluminium foil to

exclude light and brown glassware was used wherever possible. Except where otherwise indicated, all steps in analytical methods were performed without delay.

7.8.1 Procedures used in the validation of enzyme assays methods

In all cases, a variety of approaches were used to ensure the validity of the methods and the resulting analytical data. In most cases assays were performed at different pH values and for a varying lengths of time.

7.8.2 Calculation of enzyme activity values to a dry weight basis

The results obtained for enzyme activity in flour and noodle samples were routinely adjusted by calculation to a 14% moisture basis. The purpose was to facilitate the direct comparison of the results particularly for different sample types. All samples analysed for enzyme activity were also tested for moisture content. The following general equation was applied:

$$\text{Enzyme activity (adjusted to a constant moisture basis)} = \text{Enzyme activity (as is basis)} \times \frac{100 - 14}{100 - \text{actual moisture of sample}}$$

In some cases the data were recalculated to a dry weight basis (where the constant moisture figure is zero) so the equation was used in the form:

$$\text{Enzyme activity (adjusted to a dry basis)} = \text{enzyme activity (as is basis)} \times \frac{100}{100 - \text{actual moisture of sample}}$$

7.8.3 Presentation of analytical results for enzyme activity

In the analysis of samples for enzyme activity, at least duplicate sub-samples of each sample was extracted on different days. In addition, multiple analyses were performed on each extract obtained. The results of replicate analyses of each sample have been calculated and presented as mean values ± sd. These calculations were carried out using Microsoft® Excel 2000 software. In the evaluation of results obtained when reference

materials were repeatedly analysed, the coefficient of variability of a series of values was also calculated using the following formula:

$$\text{Coefficient of variability (percent)} = \frac{\text{standard deviation}}{\text{mean value}} \times 100$$

7.9 Procedures for assay of enzyme activities

Preparation of buffers

All buffers were prepared according to Dawson et al (1986) unless otherwise stated. Buffers from the kits were used where possible. Each buffer was usually a mixture of two components and the pH was adjusted.

7.9.1 α-Amylase assay

The enzyme α-amylase was assayed following the Ceralpha procedure from Megazyme (Megazyme 2002; RACI 1995). This procedure employs as substrate, the defined oligosaccharide – a non-reducing-end blocked *p*-nitrophenyl maltoheptaoside (BPNPG7) in the presence of high levels of a thermostable α-glucosidase. Extraction buffer used contained the following: sodium malate 1M, sodium chloride 1M, calcium chloride 40mM and sodium azide 0.1%. The concentrated buffer was diluted (50mL of buffer to 1000mL of water) according to manufacture's instructions and kept at 4°C. The stopping reagent contained 20% (w/v) Tri-sodium phosphate solution, pH 11. The stopping reagent was diluted (25mL to 500mL of water) and kept at room temperature. The substrate used in the assay was blocked *p*-nitrophenyl maltoheptaoside (BPNPG7, 54.5mg) and thermostable α-glucosidase (125U at pH 6.0). It was diluted in 10mL of water and frozen until being used.

α-Amylase extraction

For enzyme extraction 3.0g of flour or grinded noodle samples were weighed into a 50mL capacity flask. To this 20.0mL of extraction buffer (pH 5.4) were added and the content was vigorously stirred. The mixture was incubated for 20min at 40°C, with occasional mixing. At the end of the 20min the mixture was centrifuged at 25,000*g* for 10min at 25°C.

α-Amylase assay procedure

All reagents used here as well as enzyme extracts were pre-incubated at 40°C for five minutes before being used. Enzyme extract 0.2mL was mixed with the substrate 0.2mL and incubated at 40°C for exactly 20min. After the 20min have elapsed the reaction was stopped by the addition of stopping reagent (3.0mL) and the mixture was vigorously mixed (on a vortex mixer). Subsequently the absorbance of the mixture was read against the blank at 400nm. One blank was done for each batch of samples being analysed. The blank was obtained by mixing 0.2mL of the substrate and 3.0mL of the stopping reagent. At the end of the incubation 0.2mL of the enzyme extract was added.

Calculation of α-amylase activity

One unit of α-amylase activity is termed a Ceralpha unit and it is defined as the amount of enzyme in the presence of high levels thermostable α-glucosidase, that is required to release one micromole of *p*-nitrophenol from BPNPG7 in one minute under the defined conditions.

Units / g flour or noodle sample:

$= (\Delta E_{400}$ / incubation time)× (total volume in cell / aliquot assayed) × (1 / E_{mM}) × (extraction volume / sample weight) × dilution

where,

$\Delta E_{400 =}$ absorbance – absorbance blank

Incubation time = 20min

Total volume in the cell= 3.4mL

Aliquot assayed = 0.2mL

E_{mM} = of *p*-nitrophenol (at 400nm) in 1 % tri-sodium phosphate = 18.1

Extraction volume = 20mL per 3.0g

Dilution = if any

Thus,

Units (CU) / g of flour / noodle sample:

$= (\Delta E_{400} / 20) \times (3.4 / 0.2) \times (1 / 18.1) \times (20 / 3.0)$

$= \Delta E_{400} \times 0.313$ (Megazyme 2002).

Falling Number assay

The Falling Number was measured following the RACI and AACC official methods (RACI 1995; AACC 2002). The test was done by mixing ground sample and water in a test tube to form a slurry. The tubes were placed in a boiling water bath (100°C) and automatically stirred for 60 seconds prior to the period of the test. Results (in units of seconds) were read directly from the instrument.

7.9.2 Ascorbic acid oxidase assay

For the assay of ascorbic acid oxidase activity a continuous spectophotometric method was used (Every et al 1995; Every et al 1996; Jin and Quail 1999).

Ascorbic acid oxidase extraction

Flour or noodle samples (6g) were mixed with 1.0M Na_2SO_4 (18mL) for 20min at 25°C, the pH was adjusted to 8.3 with 0.2M NaOH. The mixture was immediately centrifuged at 18,000*g* for 10min at 25°C. The supernatant was then again adjusted to pH 6.3 with 0.1M H_2SO_4 and recentrifuged. The clear part under the lipid layer was removed (6.0mL) and mixed with an equal volume of 0.05M $NaHPO_4$ – citric acid buffer (pH 6.3).

Ascorbic acid oxidase assay procedure

The substrate used was L-ascorbic acid (L-AA) (from Sigma Aldrich). For assay of ascorbic acid oxidising activity, crude extracts (1mL) were placed in the reference and sample cells at 25°C and the spectrophotometer was set to zero. Ascorbic acid (50μg) was rapidly mixed into the test cell and the decline in absorbance at 300nm for ascorbic acid in crude extracts was recorded for a period of three minutes using the enzyme kinetics mode of the spectrophotometer.

Calculation of ascorbic acid oxidising activity

One unit of ascorbic acid oxidising activity is defined as the amount of activity that oxidises one microgram of LAA to dehydroascorbic acid (DHAA) in one minute at 25°C.

The activity of ascorbic acid was calculated as follows:

U / g flour or noodle sample = (ΔAbs / min) / (Abs at 0min) × (µg AA / ml assay volume) × (ml extraction mixture) / g sample) × dilution factor

= (ΔAbs / min) / (Abs at 0 min) × X × dilution factor.

Where;

X = (ml of extraction mixture / g of sample) this ranged from 116 – 128 for flour and noodle samples.

For flours a 1 in 10 dilution was used whereas for noodle samples a 1 in 5 dilution was performed.

7.9.3 Lipase assay

The activity of lipase (liberation of free fatty acids (FFA) from emulsified olive oil) was assayed using the spectrophotometric method and modified copper soap solvent extraction. This was modified from the previously published methods of Heltved 1974, Shipe et al 1980, Matlashewski et al 1982, Gélinas et al 1998, Urquhart et al 1983, Chen, et al 2003, Piazza et al 1989, Urquhart et al 1984, Ekstrand et al 1992, Thomson et al 1999, Pencreach et al 2002, Fuse et al 2000, Melo et al 2000, Hoppe and Theimer 1996, Ciuffreda et al 2001, Haltved 1984 and Myrtle and Zell 1975.

Lipase extraction

Flour and ground noodle samples were defatted before extraction. Five gram samples were defatted twice with acetone (50mL) at - 18°C and once with diethyl ether (50mL) at room temperature. The residual ether was removed in vacuo. The copper reagent used here was a mixture containing 5mL of triethanolamine and 10mL of Cu $(NO_3)_2$. $3H_2O$, diluted to 100mL with saturated NaCl solution, the pH was adjusted to 8.3 with 1M NaOH solution and the solution was stored in the dark at room temperature. The colour reagent was a 0.5% sodium diethyl dithiocarbamate solution in n-butanol. The stopping reagent used was a mixture of propan – 2 – ol : heptane : 0.1M H_2SO_4 (4 : 1 : 0.2 v/v). Defatted samples (0.5g) were mixed with (0.5mL) 0.04M imidazole – HCl buffer, pH 7.0, and an emulsion (0.5mL) containing 0.02g of a mixture of olive oil – Tween 80 (1 : 0.04 v/v) in 0.04M imidazole buffer pH 7.0. The mixture was briefly mixed using a vortex and incubated at 30°C for 30min. After 30min. of incubation the reaction was stopped by adding 5.0mL of the stopping reagent followed by 2.0mL of water and

3.0mL of heptane. After mixing the phases were allowed to separate (15min, standing at room temperature was allowed) and 2.5mL of the upper heptane layer was then taken for the FFA analyses by the copper – soap method. Zero time incubation or the blank was obtained by addition of the stopping reagent (5.0mL), water (2.0mL) and heptane (3.0mL) to the samples before the addition of the substrate and buffer solution.

Calibration curve

To obtain a calibration curve (see Fig. 10.1 Chapter 10) for the assay a weighed amount of palmitic acid was dissolved in heptane. A range of concentrations of palmitic acid used ranged from zero, to 1.0. Absorbance values (A_{440nm}) of the coloured complex between copper soaps and diethyldithiocarbamate was compared with those given by standards containing measured amounts of palmitic acid and the lipase activity was calculated as μg of FFA released per minute per g of defatted flour under defined conditions.

Analysis of FFA

The upper / heptane phase (2.5mL) obtained after stopping the reaction was mixed with an equal volume of copper reagent. After mixing using the vortex the mixture was allowed to separate into two phases again (10minutes standing at room temperature was allowed again). The organic phase (2mL) was removed and mixed with 0.1mL of the colour reagent. The absorbance was read at 440nm using the Ultra visible spectrophotometer Cary 1C against the blank within half an hour after mixing with the colour reagent occurred.

Calculation of lipase activity

One unit of lipase activity is defined as number of micromoles of fatty acids released by lipase per g of defatted flour / noodle sample under the defined conditions. The absorbance obtained for the coloured copper complex was converted to the number of micromoles of fatty acids using the conversion factor of 1.5054 obtained from the calibration curve (Fig. 10.1).

Where;

there are x μmoles of fatty acids in a tube

so x μmoles Fatty acids in 2.0mL of the heptane layer

so (x μmoles /2) × 2.5 – in 2.5mL of organic layer
so in total there were 8.0mL of organic solvent
thus (x μmoles × 2.5) / (2 × 2.5) × 8 gives the number of micromoles of fatty acids released by lipase per g of sample under defined conditions.

7.9.4 Lipoxygenase assay

The activity of the enzyme lipoxygenase was determined by modification of methods by Eriksson 1967; Surrey 1964; Rani et al 2001; Hsieh et al 1984, Blain and Todd 1955, Faubion and Hoseney 1981, Delcros et al 1998, McDonald 1979, Biawa and Bamforth 2002, Miller and Kummerow 1948, Shiiba et al 1991, Rutgersson et al 1997, Hilhorst et al 1999, Gélinas et al 1998, Wallace and Wheeler 1975, Galliard 1986, Nicolas et al 1982, Borrelli et al 1999 and Zimmerman and Vick 1970. Lipoxygenase activity was determined spectrophotometrically at room temperature. The substrate used was prepared as follows:
Tween 20 (0.5mL) was dissolved in borate buffer (10mL) pH 9.0, vigorous mixing was avoided to prevent bubbling of a solution. Linoleic acid (0.5mL) was added drop wise and the mixture was thoroughly mixed as to disperse the acid into a fine emulsion. To this emulsion 1M NaOH (1.3mL) was added and the mixture was agitated until a clear solution was obtained. Finally borate buffer (90mL) was added and the total volume made up to 200mL with water. The pH was adjusted to 7.0 with HCl.

Lipoxygenase extraction

Lipoxygnease was extracted with 0.1M phosphate buffer pH 7.0. Flour or noodle samples (2.0g) were mixed with the phosphate buffer (10mL) and agitated on ice for 30min. The mixture was then ultra turraxed 2 × 15s and centrifuged immediately at 25,000*g* for 20min at 4°C. The assay was completed within half an hour of extraction.

Lipoxygenase assay

Enzyme extract (0.5mL) was mixed with the substrate (0.5mL) with oxygen passed continuously through the mixture. In the preliminary test samples were taken out periodically at zero, two, five, seven and ten minutes and assayed. Linear relationship was established and further analyses were done by taking samples out after five minutes of incubation. To the enzyme extract and substrate absolute alcohol (2.0mL) and 60%

alcohol were then added to stop the reaction and make up the total volume of 10.0mL. The mixture was then centrifuged at 25,000*g* for 10min at room temperature. Optical density of clear solutions was read at 234nm against a control using simple reads program at an ultra visible spectrophotometer Cary 1C. The controls were obtained by adding absolute alcohol (2.0mL) to the enzyme extract, after mixing the tubes were allowed to stand for five minutes, then 60% alcohol (7.0mL) was added followed by the substrate, the mixture was finally centrifuged following the same conditions as described for the samples. When necessary further dilutions were made with 60% alcohol.

Calculation of lipoxygenase activity

A unit of lipoxygenase activity is defined as that amount of activity which will produce an optical density of one at 234nm in one minute in a total volume of 10.0mL of 60% alcohol.

7.9.5 Peroxidase assay

The enzyme peroxidase was assayed using spectrophotometrically by measuring the slope from the linear increase in absorbance at 470nm due to oxidation of guaiacol to tetrahydroguaiacol in the presence of H_2O_2. The adopted method was a modification of published methods by Iori et al 1995, Tanaka et al 1990, Vermulapalli et al 1998, Vermulapalli and Hoseney 1998, Wikström and Eliasson 1998 and Edwards et al 1989.

Peroxidase extraction

Flour or noodle samples (1.0g) were homogenized in 0.1M acetate buffer (10.0mL) pH 4.2 and ultra turrexed 2 × 15s, agitated for 20minutes on ice and centrifuged immediately at 30,000 *g* for 20minutes at 4°C. The extracts were diluted (with 0.1M acetate buffer pH 4.2) flour samples were diluted 1 : 10, while noodle samples were diluted 1:5.

Peroxidase assay

Enzyme extract (0.5mL) was mixed with acetate buffer (1.10mL) 0.1M, pH 4.2 and guaiacol (1.10mL) 0.08M and $CaCl_2$ (0.05mL) 2.0M and the activity of POX was measured spectrophotometrically at 470nm and at ~ 25°C upon the addition of 0.25mL

of H_2O_2 (0.03M) into the sample cell. The linear increase in absorbance was monitored for three minutes as increase in the absorbance at 470nm.

Calculation of peroxidase activity

One unit of peroxidase activity is defined as increase in absorbance per g of sample per minute. Where;

The change in the absorbance was due to the enzyme present in one milliliter of dilute preparation then per 10.0mL of the original supernatant enzyme activity is

$(\Delta A \times 10 \times 10)$ / sample weight (g).

7.10 Statistical analysis of results

One way analysis of variance and Tukey's test were applied using SPSS software version 13.0 (SPSS Inc, Chicago, Illinois).

Chapter 8

Results and discussion: Preliminary assessment of procedures for evaluation of textural and structural characteristics of WSN and YAN

The purpose of this chapter is to describe and discuss the results obtained during the evaluation of procedures for measurement of textural properties and the structure of the two types of Asian noodles studied here.

8.1 Introduction

There is a wide range of different types of foods and correspondingly a variety of methods have been used to measure the textural attributes in an objective way

Although many different procedures have been described, force measurement is the most common of the approaches used for texture measuring instruments. Force has the dimensions mass × length × time^{-2} and the standard unit for force is Newton (N). Force measuring tests include: puncture, compression-extrusion, cutting-shear, compression, tensile, torsion, bending and snapping and deformation.

Following a thorough review of the broad range of options available and particularly those that have been used for assessment of Asian noodles and similar styles of products, it was decided that two sets of measurements would be made on experimental products. Accordingly, in this study the Texture Analyser (TA-XT2) (Stable Micro Systems) has been used to measure the textural properties of Asian noodle products. Two distinct attachments have been used and different textural characteristics have been measured. Firstly a flat cylinder probe P/45 has been used to measure the hardness of noodle samples (by measuring the maximum compression force, when samples are compressed to 75% of their original height). Secondly an uniaxial blade (7 × 11.5 × 0.3 cm) has been used to measure the firmness of noodle products (by measuring the maximum cutting stress when the sample has been cut through to the 75% of its original thickness).

8.2 Comparison of results for assessment of texture of Asian noodles

The initial purpose here has been to provide results and correlations for the two type of measurements. One can be related to the action of the molar teeth, whereas, the second translates to action of the front teeth or to the first bite). It is believed that both of these measurements can be related to other rather characteristics – chewiness of noodles. This study also looked at raw and cooked noodles, although some reports have been made that measuring the textural properties using TA-XT2 is rather difficult and not meaningful as noodle products are consumed cooked (and this attribute is more important from the consumer point of view). Raw noodles also tend to bend during compression (resulting in a higher force being measured). The settings used are described in Chapter 7. Typical plots obtained from each type of measurement are shown in Figs 8.1 and 8.2.

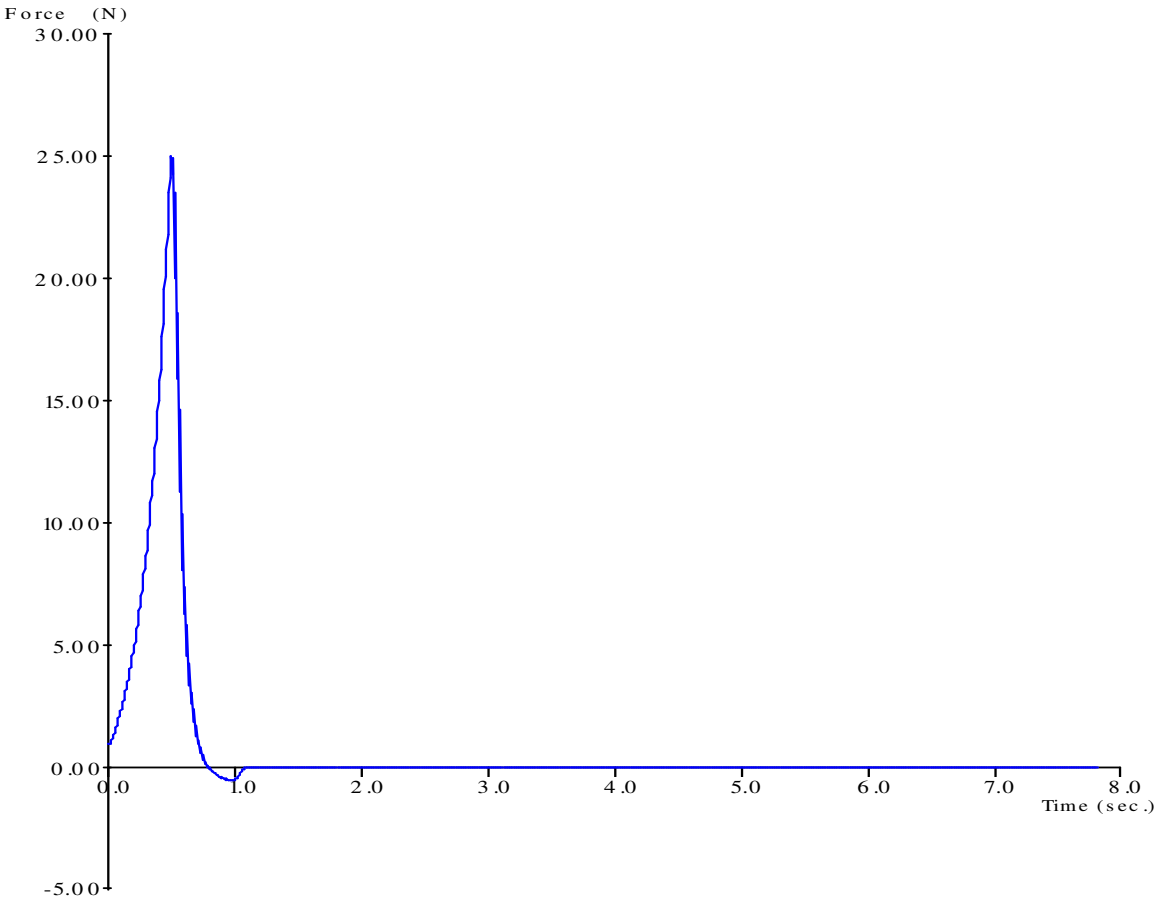


Fig. 8.1 Typical plot obtained using the flat cylinder probe P/45 and the TA-XT2
TA-XT2 Stable Microsoft Systems

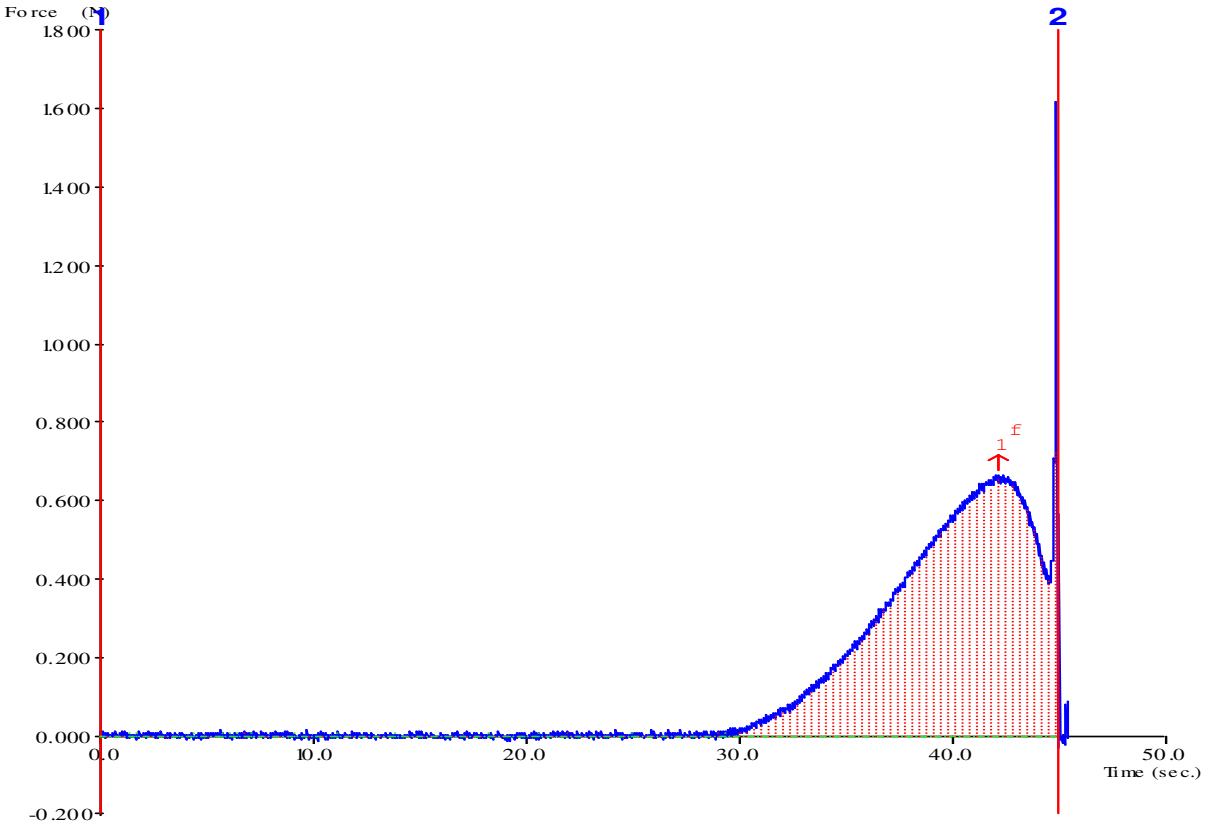


Fig. 8.2 Typical plot obtained using the blade attachment of the TA-XT2
TA-XT2 Stable Microsoft Systems

When correlations were compared from the two methods used to measure textural properties of noodles two properties obtained from these methods were compared here (total energy to compress vs total energy to work and maximum compression force vs maximum cutting force. Correlations obtained from the two methods used to measure textural properties were highly positive when texture was measured on dried / cooked noodles (for both of these parameters). A positive correlation of 0.9248 from energy to work vs energy to compress and r^2 of 0.9458 for maximum cutting force vs maximum compression force was seen (Fig. 8.3). Positive correlation was also seen for fresh cooked noodles for all methods evaluated (Fig. 8.4). However, negative correlation between the properties measured was seen when the texture of raw noodles was correlated (Fig. 8.5). This indicates need for methods and attachments which will allow to measure textural properties of raw noodles as well. These although not as meaningful as textural properties of cooked noodles (final product as consumed by the customers) this measurements could be used as indicative results for textural characteristics of noodles.

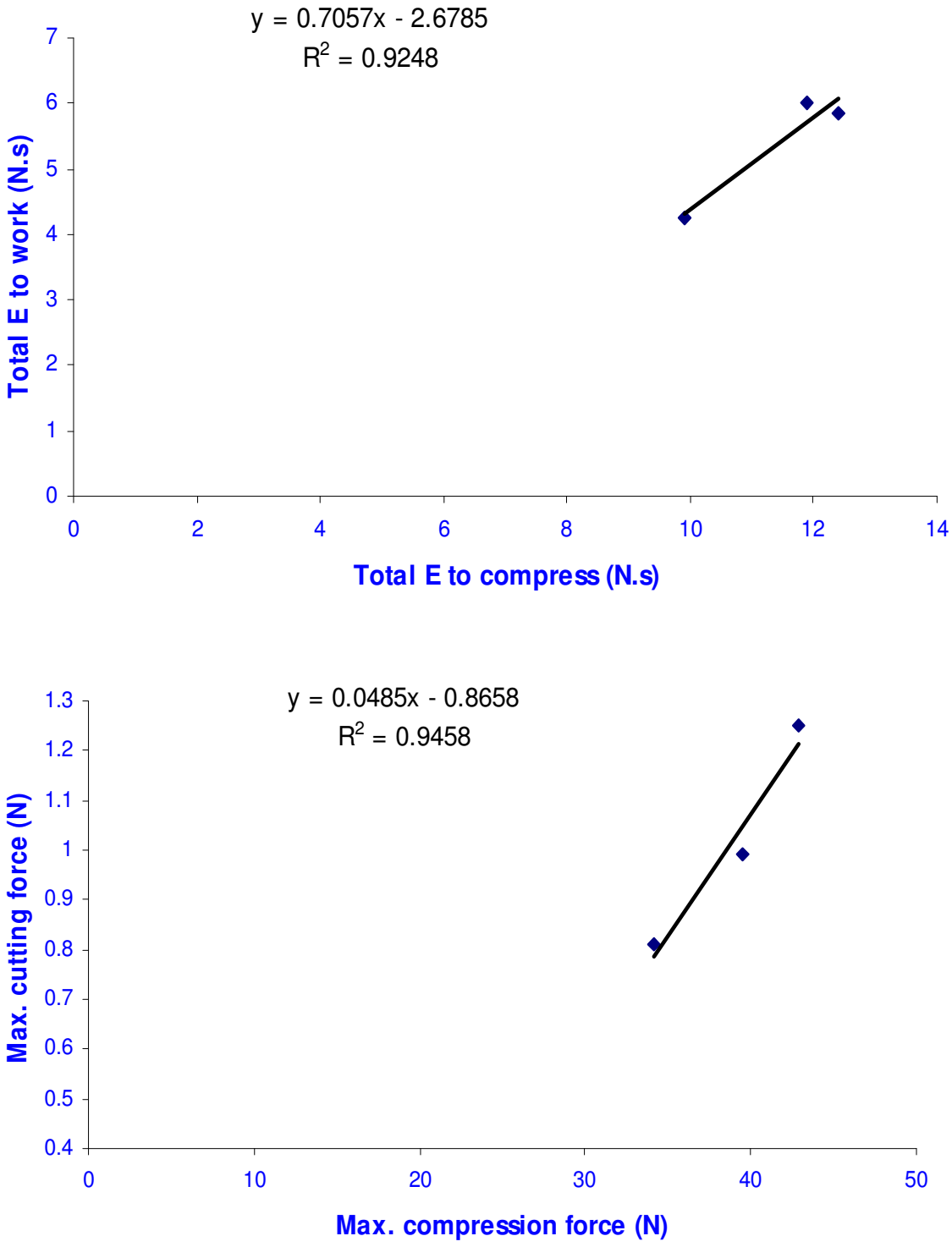


Fig. 8.3 Correlation for textural measurements obtained on dried cooked noodles

Data based on the mean values for firmness and hardness measurements of WSN (made from UW and PF) and YAN (made from BGF)

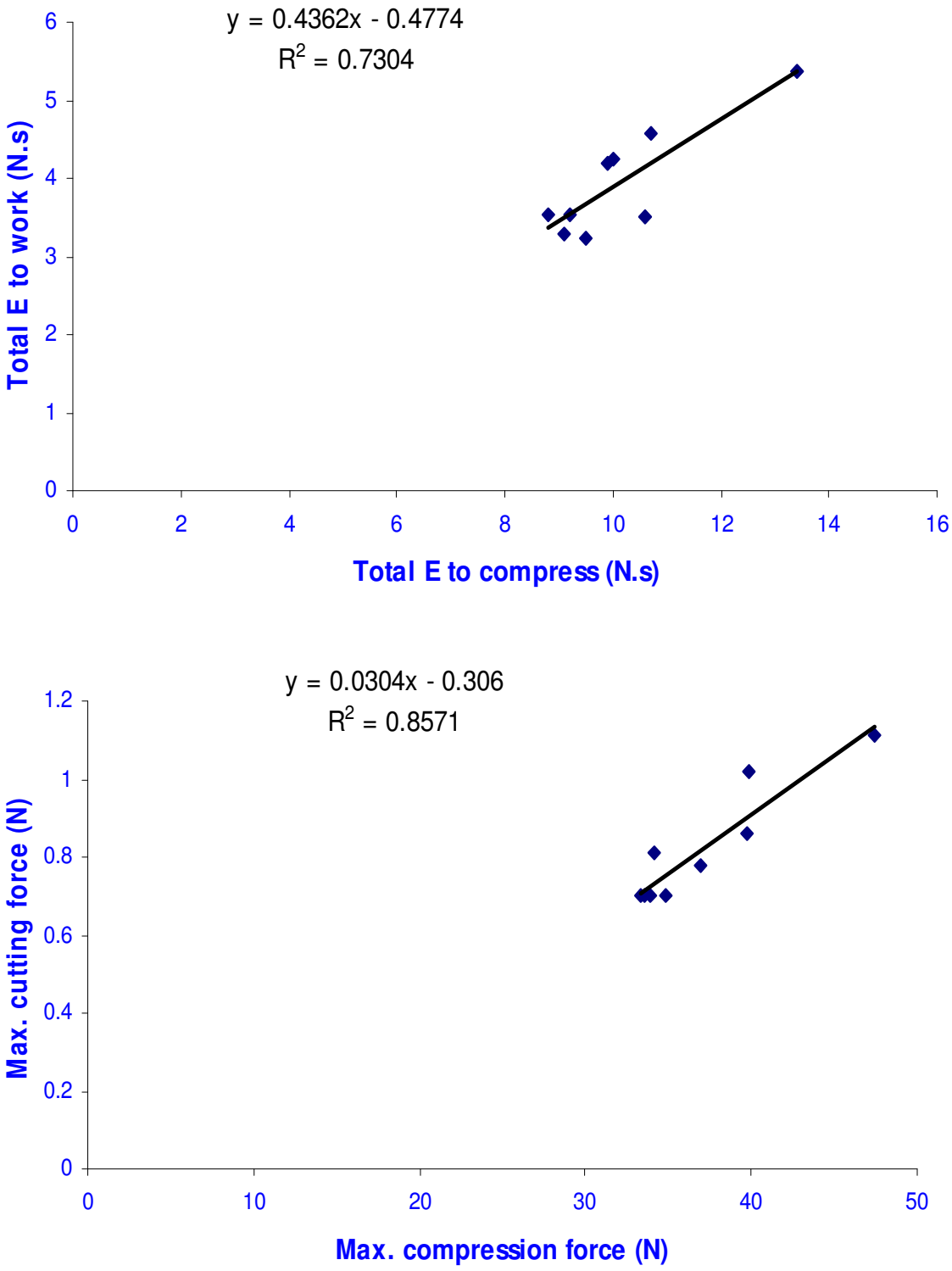


Fig. 8.4 Correlation for textural measurements obtained on fresh cooked noodles

Data based on the mean values for firmness and hardness measurements of WSN (made from UW and PF) and YAN (made from BGF)

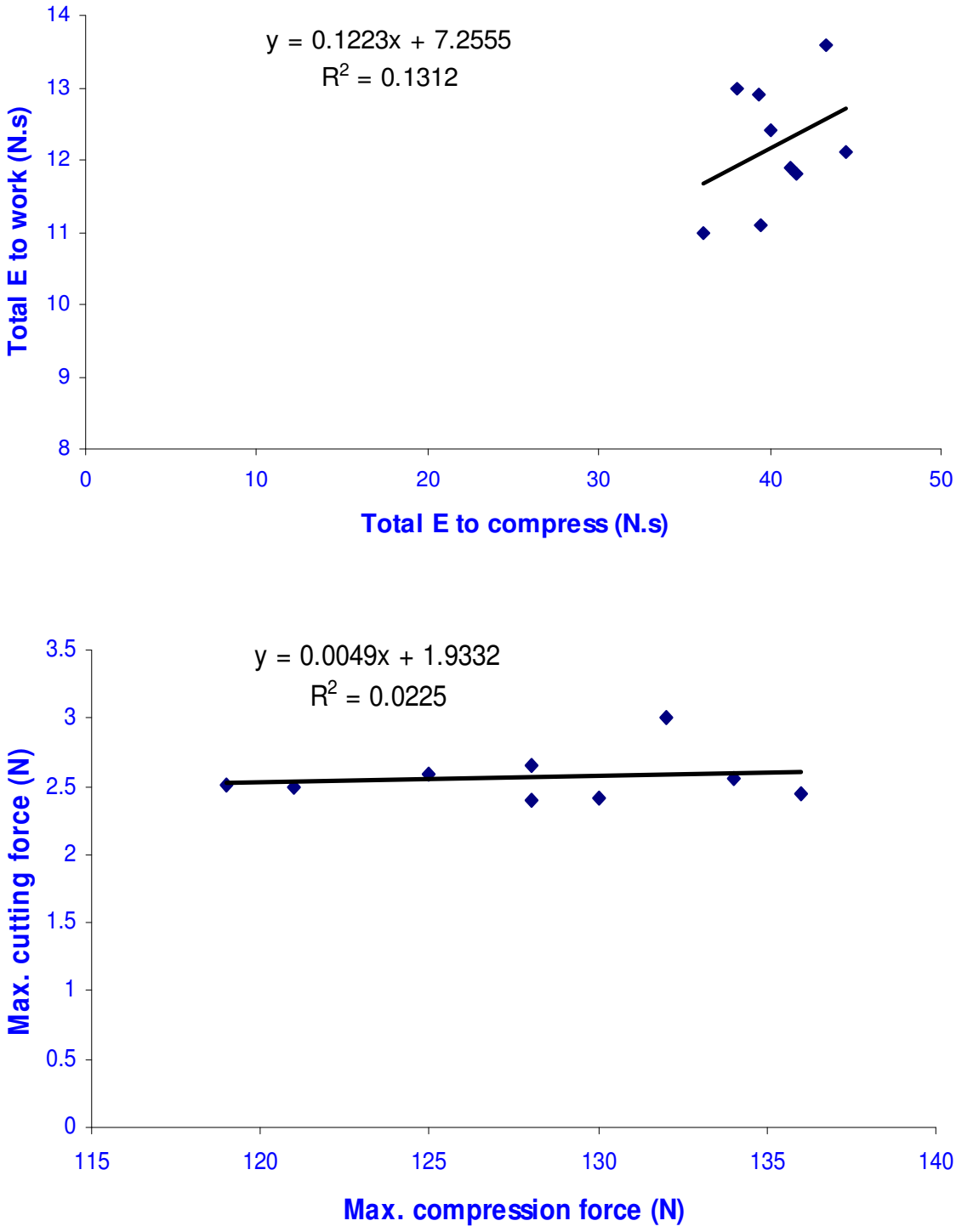


Fig. 8.5 Correlation for textural measurements obtained on raw fresh noodles
Data based on the mean values for firmness and hardness measurements of WSN (made from UW and PF) and YAN (made from BGF)

8.3 Introduction to assessment of noodle structures using scanning electron microscopy

Scanning electron microscopy (SEM) uses electrons rather than light to form an image. This allows the surface structures of foods to be viewed and was selected here as a means of comparing enzyme treatments and the impact of cooking of the various noodle samples.

In addition to SEM, Environmental SEM was also used (ESEM). This newer technique allows examination of specimens surrounded by a gaseous environment. The specimen in the microscope does not need to be conductive and therefore does not need to be coated with a conductive material. This mode was used fresh raw noodles, which were viewed without any further preparation.

The Cryo mode of operation of the SEM was also used. This mode was used for cooked noodles. For this, samples were freeze dried using liquid nitrogen and further preparation took place within the chamber of the instrument. This involved coating with a thin layer of gold.

8.4 Comparison of different modes of SEM

Three different modes of the SEM were used and examples of the resultant images are presented in Figs. 8.6-8.8. It is noted that further information on the preparation of the noodles under laboratory conditions is provided in Chapter 7 and also in subsequent chapters of this thesis. In evaluating the optional approaches in electron microscopy, firstly, the SEM mode was trialed using both low and high vacuum conditions and in both cases it was possible to view samples without any further preparation. Selected images are compared in Fig. 8.6. Based upon these, it was concluded that the images obtained using the high vacuum were of higher resolution and were also clearer.

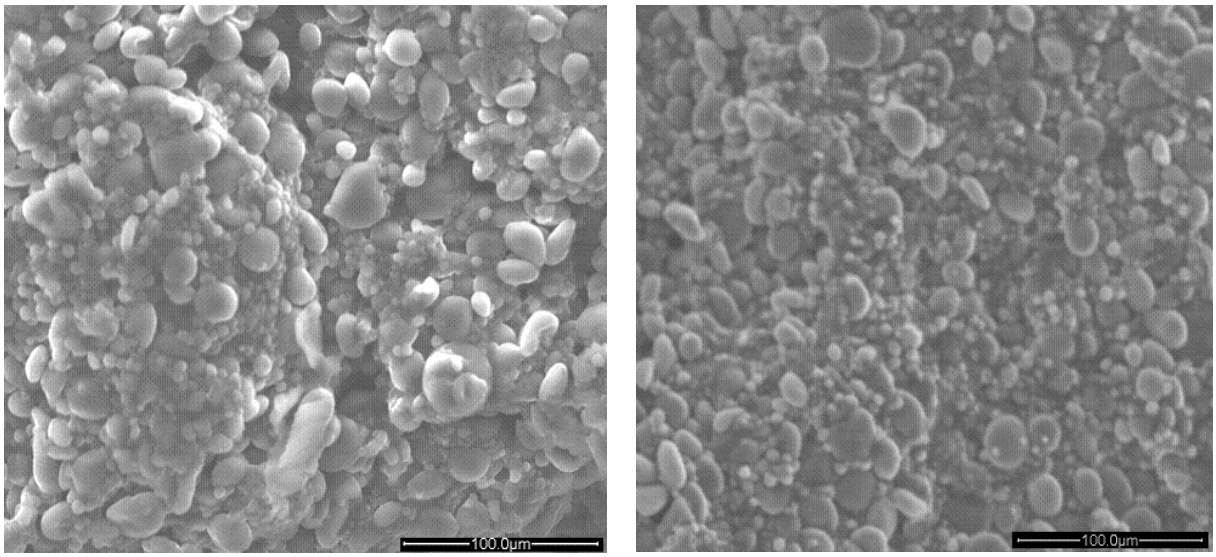


Fig. 8.6 SEM images of dried WSN (made from PF)
High vacuum 30kv (left hand side image) vs low vacuum 20kv (right hand side image); Mag. × 400

Samples of cooked noodles were gold coated prior to viewing using high vacuum SEM. These were compared with uncoated samples (Fig. 8.7) and no clear advantages or disadvantages of gold coating were seen.

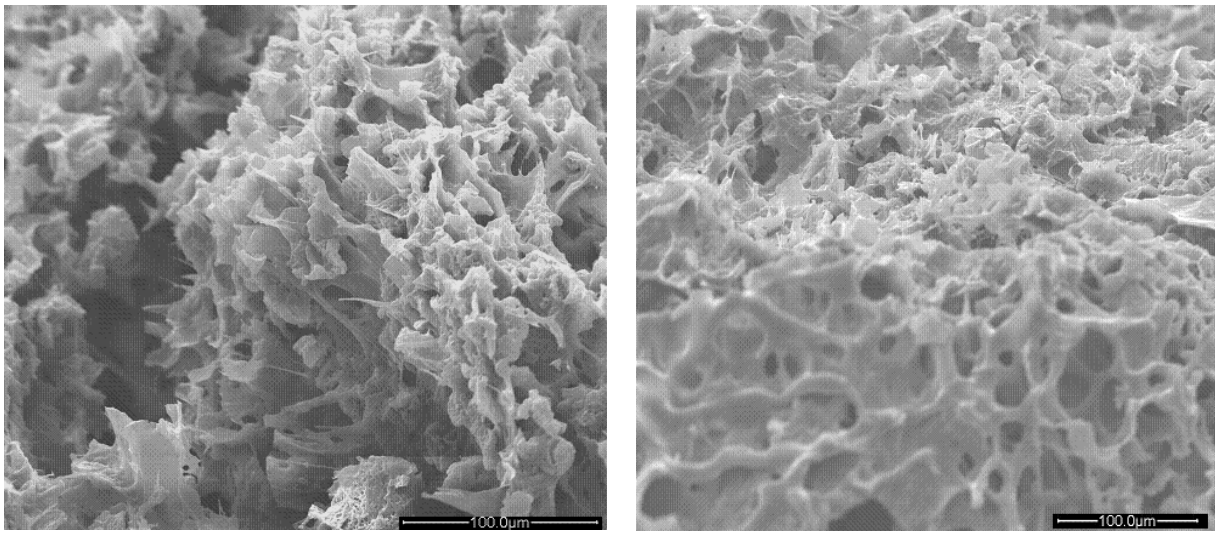


Fig. 8.7 SEM images of dried and cooked WSN (made from PF).
High vacuum 30kv gold coating (left hand side image) vs high vacuum 30kv no coating (right hand side image); Mag. × 400.

A further comparison was made in which the Cryo mode of the SEM was used to view noodle samples. This technique allows use of higher magnification (Fig. 8.8). Here the images are magnified up to 6,000 ×, thereby providing finer detail in the structures to be viewed.

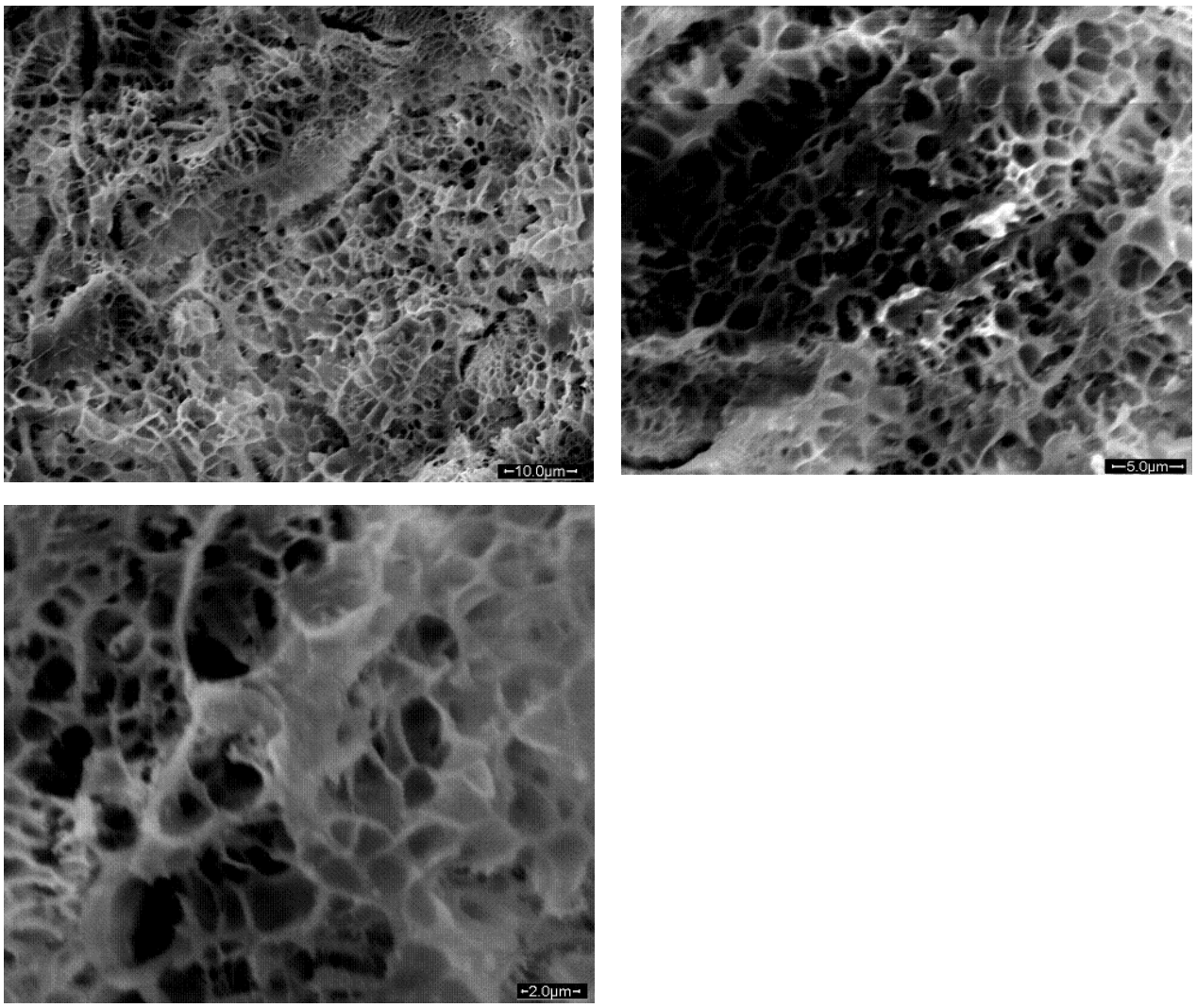


Fig. 8.8 SEM images (CRYO mode) of dried and cooked WSN (made from PF)
Cryo mode high vacuum 30kv (top left Mag. × 1500; top right Mag. × 3000; bottom lower Mag. × 6000)

8.5 Conclusions on the use of SEM for noodle samples

SEM is a useful technique in studying structure and was shown here to be very useful in evaluation of structural properties of noodle products. It was also demonstrated that high vacuum gives enhanced images in comparison with the low vacuum mode. However, there were no clear advantages or disadvantages seen in using gold coating.

In addition, the use of the Cryo mode proved to be a particularly useful technique for freshly cooked and dried and cooked noodles showing the structure of noodle products in finer detail.

Chapter 9

Results and discussion: the preliminary analysis of flours and Asian noodles prepared in the laboratory

The purpose of this chapter is to describe and discuss the results obtained during the preliminary analysis of flours used in this study and also of the Asian noodles prepared on a laboratory scale

9.1 Introduction

Three different flours obtained from commercial flour mills in Melbourne have been studied here. The flours selected were Baker's strong (BGF), P-Farina (PF) and Ultra White (UW) (see also Chapter 7) and these have been characterised for various physical and chemical properties including moisture, protein, ash and total starch contents as well as flour particle size, colour properties and Falling Number.

In addition, two different styles of Asian noodles, WSN and YAN, prepared under controlled laboratory conditions have also been analysed for a similar range of analyses as well as pH.

9.2 Preliminary analysis of flour and Asian noodles

Moisture content was determined following the AACC (44-16) method. Table 9.1 shows the data on moisture content obtained from triplicate analysis. The total moisture content was calculated by the weight difference of samples before and after drying.

These data show relatively little variation in moisture contents of the two styles of noodles at corresponding stages of processing. The primary value of these results has been in the calculation of enzyme activity content results to either a dry matter or 14 % moisture basis. These calculations were used in all subsequent phases of this research in order to facilitate the comparison of results at different stages of processing.

Table 9.1 Moisture content of the flours studied and Asian noodles prepared under controlled laboratory conditions

Sample[a]	Moisture content (%)
BGF flour	11.90 ± 0.16
PF flour	11.29 ± 0.25
UW flour	11.29 ± 0.25
PF-WSN dough	30.92 ± 0.01
PF-WSN fresh cooked	72.39 ± 0.15
PF-WSN fresh day 1	30.50 ± 1.99
PF-WSN fresh 24h FR	30.33 ± 1.06
PF-WSN fresh 24h RT	30.56 ± 1.01
PF-WSN dried	8.83 ± 0.07
PF-WSN dried cooked	72.21 ± 0.30
UW-WSN dough	30.30 ± 0.22
UW-WSN fresh cooked	73.83 ± 0.65
UW-WSN fresh day 1	30.04 ± 1.10
UW-WSN fresh 24h FR	30.01 ± 1.05
UW-WSN fresh 24h RT	30.05 ± 1.01
UW-WSN dried	8.67 ± 0.23
UW-WSN dried cooked	71.66 ± 0.22
YAN dough	33.31 ± 0.11
YAN fresh cooked	73.31 ± 0.34
YAN fresh day 1	29.44 ± 1.26
YAN fresh 24h FR	29.45 ± 1.11
YAN fresh 24h RT	29.51 ± 1.15
YAN dried	9.27 ± 0.07
YAN dried cooked	75.44 ± 0.24

a Abbreviations used are: BGF, Baker's strong flour; PF, P-Farina; UW, Ultra White; WSN, white salted noodles; YAN, yellow alkaline noodles; FR, storage at 4°C; RT, storage at room temperature (~ 25°C)

Note: Results are the mean of triplicate analyses and are expressed as mean ± standard deviation values in units of g per 100g

Ash contents of flour and noodle samples were measured using the AACC method. Five grams samples were incinerated using a furnace set at 590°C for a period of 12hr. The results are shown in Table 9.2.

Table 9.2 Ash content of the flours studied and Asian noodles prepared under controlled laboratory conditions

Sample[a]	Ash content (%)
BGF flour	0.68 ± 0.05
PF flour	0.69 ± 0.07
UW flour	0.60 ± 0.08
PF-WSN fresh day 1	2.73 ± 0.07
PF-WSN fresh 24h FR	2.75 ± 0.03
PF-WSN fresh 24h RT	2.77 ± 0.02
PF-WSN dried	2.70 ± 0.05
UW-WSN fresh day 1	2.69 ± 0.04
UW-WSN fresh 24h FR	2.71 ± 0.03
UW-WSN fresh 24h RT	2.75 ± 0.06
UW-WSN dried	2.81 ± 0.04
YAN fresh day 1	2.43 ± 0.15
YAN fresh 24h FR	2.51 ± 0.07
YAN fresh 24h RT	2.53 ± 0.06
YAN dried	2.55 ± 0.09

a Abbreviations used are: BGF, Baker's strong flour; PF, P-Farina; UW, Ultra White; WSN, white salted noodles; YAN, yellow alkaline noodles; FR, storage at 4°C; RT, storage at room temperature (~ 25°C).

Note: Results are the mean of triplicate analyses and are expressed as mean ± standard deviation values in units of g per 100g. All data is reported on a 14% moisture basis.

9.3 Protein and starch analyses

Protein content of flour and noodle samples was determined following the Kjeldahl procedure (as described in Chapter 7). Table 9.3 shows the protein content of samples tested. Baker's strong had a higher protein content when compared to the other two flours analysed. On the basis of the protein content BGF flour was chosen for the preparation of YAN as higher protein content is desirable for this type of product to obtain highest quality. Subsequently, PF and UW have been chosen for the manufacture of WSN, as they contained lower protein content as required for these types of products. An ideal protein content for YAN is between 11 and 12.5 % whereas ideal protein content for WSN is in the range of 8 to 9.5 %.

Table 9.3 Protein content of the flours studied and Asian noodles prepared under controlled laboratory conditions

Sample[a]	Protein content (%)
BGF flour	11.77 ± 0.28
PF flour	9.96 ± 0.22
UW flour	10.36 ± 0.20
PF WSN fresh day 1	9.98 ± 0.33
PF WSN fresh 24h FR	9.79 ± 0.31
PF WSN fresh 24h RT	9.81 ± 0.33
PF WSN dried	9.80 ± 0.29
UW WSN fresh day 1	10.39 ± 0.13
UW WSN fresh 24h RT	10.38 ± 0.21
UW WSN fresh 24h FR	10.39 ± 0.22
UW WSN dried	10.36 ± 0.19
YAN fresh day 1	11.70 ± 0.18
YAN fresh 24h FR	11.72 ± 0.11
YAN fresh 24h RT	11.69 ± 0.08
YAN dried	11.73 ± 0.05

a Abbreviations used are: BGF, Baker's strong flour; PF, P-Farina; UW, Ultra White; WSN, white salted noodles; YAN, yellow alkaline noodles; FR, storage at 4°C; RT, storage at room temperature (~ 25°C).

Note: Results are the mean of triplicate analyses and are expressed as mean ± standard deviation values in units of g per 100g. All data is reported on a 14% moisture basis.

The total starch contents of flour samples was measured using the Megazyme total starch kit as described in Chapter 7. The results are presented in Table 9.4.

Table 9.4 Total starch content of the flours analysed

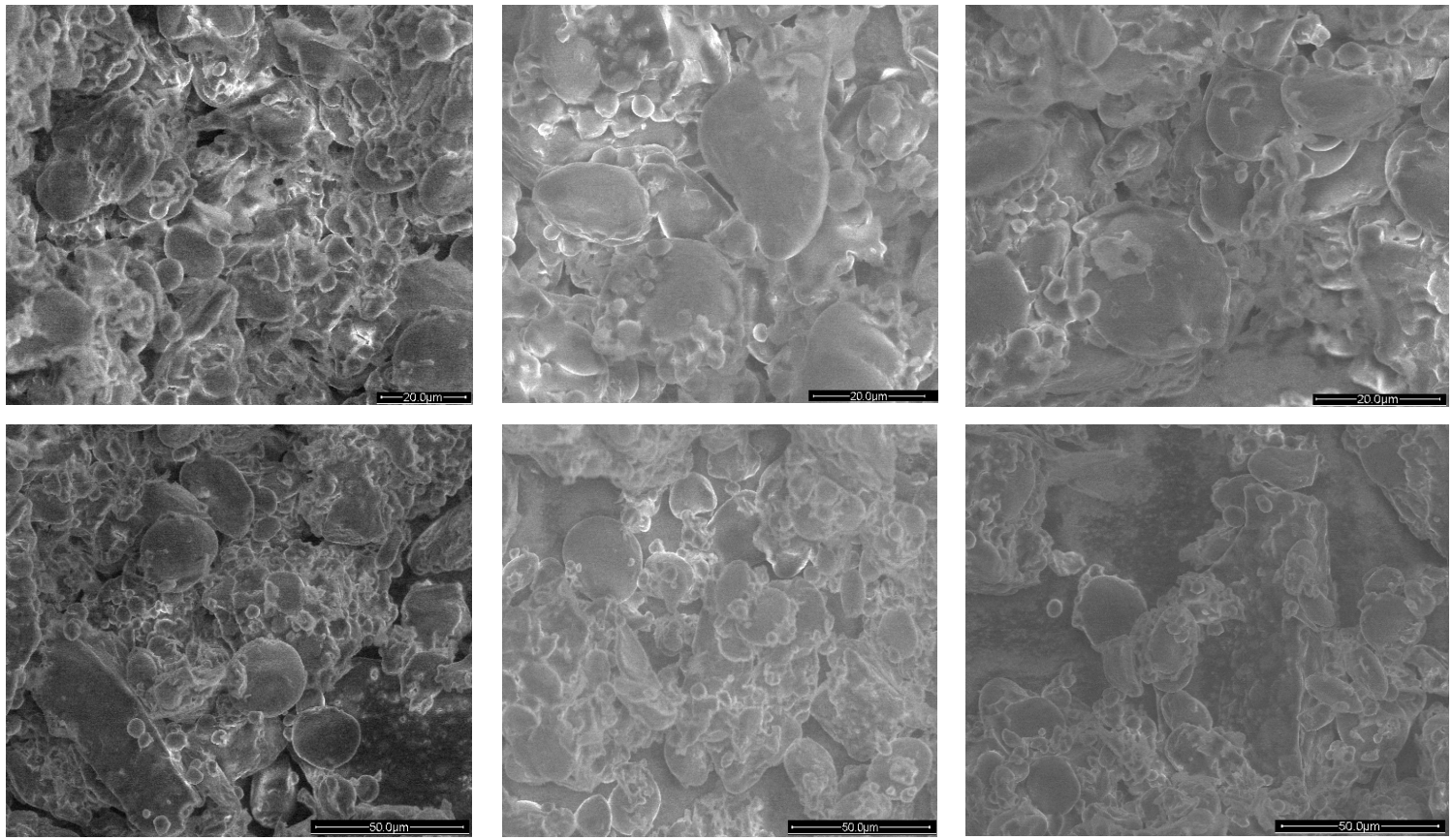
Sample[a]	Total starch (%)	Total starch (dry wt basis, %)
BGF flour	64.9 ± 3.9	73.6 ± 3.9
PF flour	63.7 ± 3.9	71.9 ± 3.9
UW flour	66.5 ± 4.3	75.0 ± 4.3
Starch reference	103.9 ± 3.5	N/A

a Abbreviations used are: BGF, Baker's strong flour; PF, P-Farina; UW, Ultra White flour; N/A, not applicable.

Note: Results are the mean of triplicate analyses and are expressed as mean ± standard deviation values in units of g per 100g.

9.4 SEM appearance of the flours used in this study

The appearance of the three flours studied have been evaluated by viewing with the scanning electron microscope (SEM) using a variety of magnifications (Fig. 9.1). Starch granules having typical circular or lenticular shape can be clearly seen on the surface of all three images. Starch granules appear slightly larger in PF and UW flours as compared to BGF.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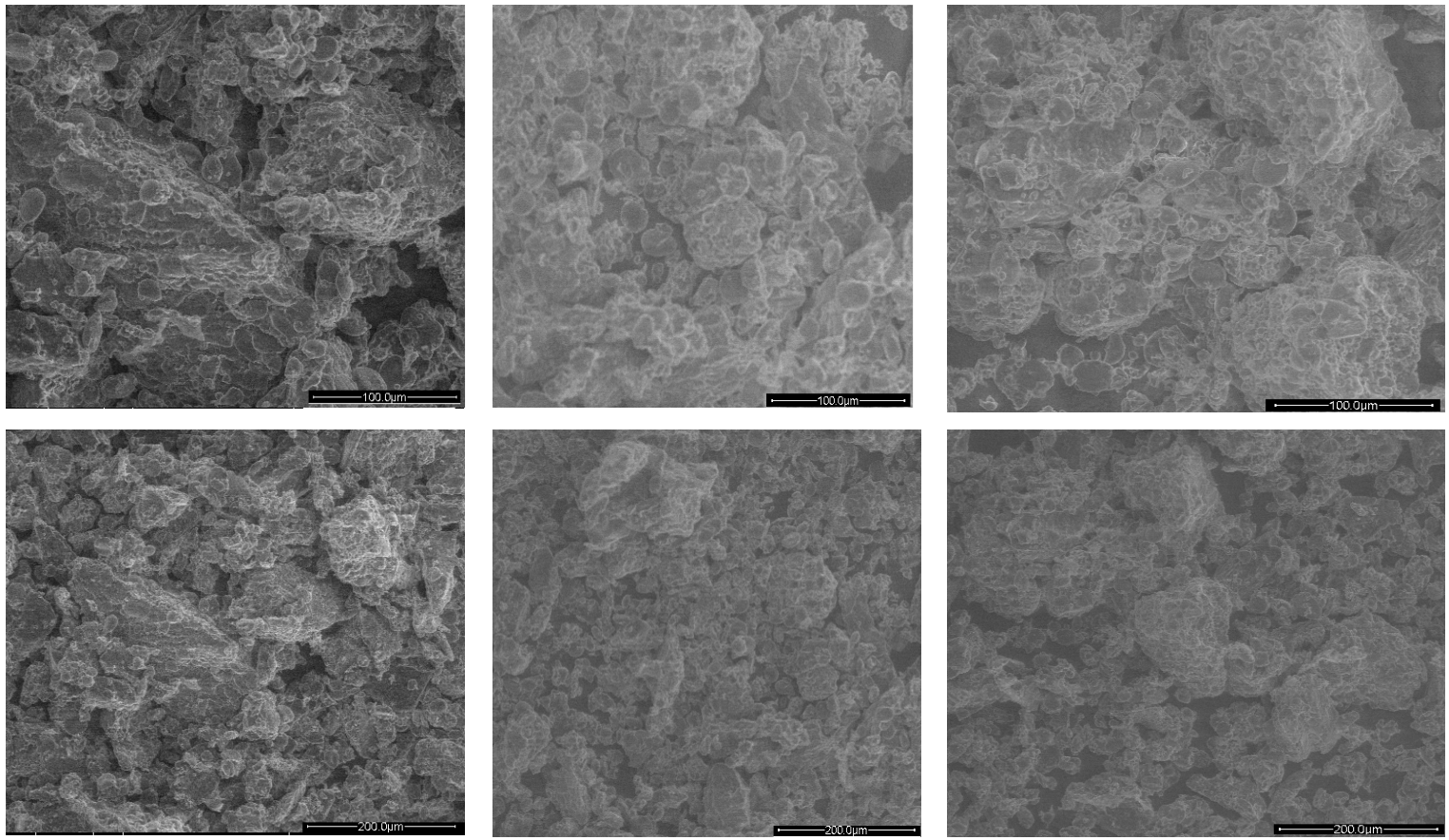


Fig. 9.1 SEM[a] images of the three flours used in the current study shown at four different magnifications
BGF, PF and UW from left to right

a SEM, 0.5 Torr, 23°C, 5.0 spot size, 30kV.
Mag. × 1100 (20µm); Mag. × 800 (50µm); Mag. × 400 (100µm); Mag. × 200 (200µm)

9.5 Falling Number

Falling Number was measured following the standard AACC method as described in Chapter 7. This procedure is based on a sample size of 7.00 g and the results were also compared to a newly proposed method on the basis of a 6.00 g sample size (Hatcher 2004). The impact of kansui, common salt, and the different α-amylases were measured. Noodles supplemented with α-amylases have been analysed for Falling Number as well and compared to the enzyme-free control. Prior to analyses noodles were ground so as to have a particle size distribution similar to that of flour particles. The Falling Number results are presented in Table 9.5.

The internationally recognised Falling Number test is based upon the ability of α-amylase to liquefy a starch gel. The activity of the enzyme is reflected in the time it takes to stir and allow a standardised stirrer to fall through a set distance. The method is based on a 7.00 g sample size and the modified procedure was evaluated that follows the standard method with the exception that 6.00 g of material is used. Preliminary evaluation of this method suggested that it offered significant benefits for the reduction of time for carrying out the official method, since this would be of immense benefit for the grain industry in this study the two methods were compared. This comparison is shown in Table 9.6. This experiment was done as part of the 'modified Falling Number international collaborative test' to evaluate the potential of a shortened Falling Number test. These results indicate (as supported by recently published results where 5.50g vs 6.00 vs 7.00g of samples have been investigated) that the time saving benefits of using a smaller sample size (6.00g) can be anticipated to provide a useful tool to segregate grain on the basis of Falling Number, where estimating sample sprout damage within a critical time period is important. However, where time is not critical, the official method 7.00g (14% mb) offers the best differentiation of sprout damage (Hatcher 2005; Finney 2001).

Table 9.5 Falling Number results of flour and noodles and influence of kansui, salt and α-amylases on Falling Number values

Sample[a]	Falling Number[b]
Flour	
BGF	547 ± 28
PF	567 ± 33
UW	620 ± 20
BGF + kansui	515 ± 7
BGF + barley α-amylase	62 ± 0
BGF + bacterial α-amylase	62 ± 0
BGF + barley α-amylase + kansui	420 ± 8
BGF + bacterial α-amylase + kansui	62 ± 0
PF + salt	555± 5
PF + barley α-amylase	62 ± 0
PF + bacterial α-amylase	132 ± 8
PF + salt + barley α-amylase	62 ± 0
PF + salt + bacterial α-amylase	148 ± 8
UW + salt	617 ± 1
UW + barley α-amylase	62 ± 0
UW + bacterial α-amylase	138 ± 2
UW + salt + barley α-amylase	62 ± 0
UW + salt + bacterial α-amylase	145 ± 2
Noodles	
YAN	370 ± 16
PF WSN	252 ± 6
UW WSN	249 ± 12
YAN + barley α-amylase	363 ± 13
YAN + bacterial α-amylase	146 ± 4
PF WSN + barley α-amylase	62 ± 0
PF WSN + bacterial α-amylase	62 ± 0
UW WSN + barley α-amylase	62 ± 0
UW WSN + bacterial α-amylase	62 ± 0

a Abbreviations used are: BGF, Baker's strong flour; PF, P-Farina; UW, Ultra White; WSN, white salted noodles; YAN, yellow alkaline noodles.
b The unit for Falling Number values is seconds.
Note: Results are the mean of triplicate analyses and are expressed as mean ± standard deviation. All data is reported on a 14% moisture basis.

Table 9.6 Comparison of the results obtained with the standard (7.00g) Falling Number[b] method and a new proposed (6.00g) method

Flour[a]	7.00 g sample size (as is)	6.00 g sample size (as is)
BGF	534 ± 28	421 ± 21
PF	550 ± 33	454 ± 19
UW	602 ± 20	498 ± 25

a Abbreviations used are: BGF, Baker's strong flour; PF, P-Farina; UW, Ultra White.
b Unit for Falling Number is second.
Note: Results are the mean of triplicate analyses and are expressed as mean ± standard deviation.

9.6 Flour colour properties

Colour properties of flours studied were measured using the Minolta Chroma Meter (CR300). Table 9.7 shows the colour characteristics of flours studied, L* values (whiteness), b* values (yellowness) and a* values measuring redness of flour samples. BGF flour was slightly darker compared to the other two flours analysed, as indicated by lower L* values and higher a* and b* values. This also reflects slightly higher protein content on this flour, as colour and protein content are directly related, i.e. the higher the protein content the lower the whiteness values of the flour and higher b* values i.e. yellowness of the flour.

Table 9.7 Colour characteristics of the three flours studied

Flour sample[a]	L*	a*	b*
BGF	90.76 ± 0.08	- 0.55 ± 0.03	+ 9.44 ± 0.11
PF	92.96 ± 0.08	- 0.12 ± 0.01	+5.32 ± 0.05
UW	92.63 ± 0.02	+ 0.1 ± 0.03	+ 5.13 ± 0.06

a Abbreviations used are: BGF, Baker's strong flour; PF, P-Farina; UW, Ultra White.
b L*, (whiteness); a*, (redness); b*, (yellowness).
Note: Results are the mean of triplicate analyses and are expressed as mean ± standard deviation.

In Figures 9.2a-9.2c the relationship between protein content and the colour of flours is shown. The relationship between colour of the noodle sheets and protein content was also analysed. It is known that higher protein wheats result in slightly darker flours. Hence, this is one of the reasons lower or medium protein content flours are preferred for WSN.

Highly positive correlations were found for between protein content and all three colour measurements recorded. Highest one was the correlation between L* values (whiteness) and the protein content of flours where an r^2 value of 0.9948 was recorded. An r^2 of 0.9383 and 0.7258 was recorded for correlations between b* values (yellowness) and a* values (redness) and the protein content of flours respectively (Figs. 9.2a-9.2c). Least correlation was seen between a* values and the protein content of flours, however, this value as indicating redness / greenness is not of great importance / indication for Asian noodle products as are L* and b* Minolta values.

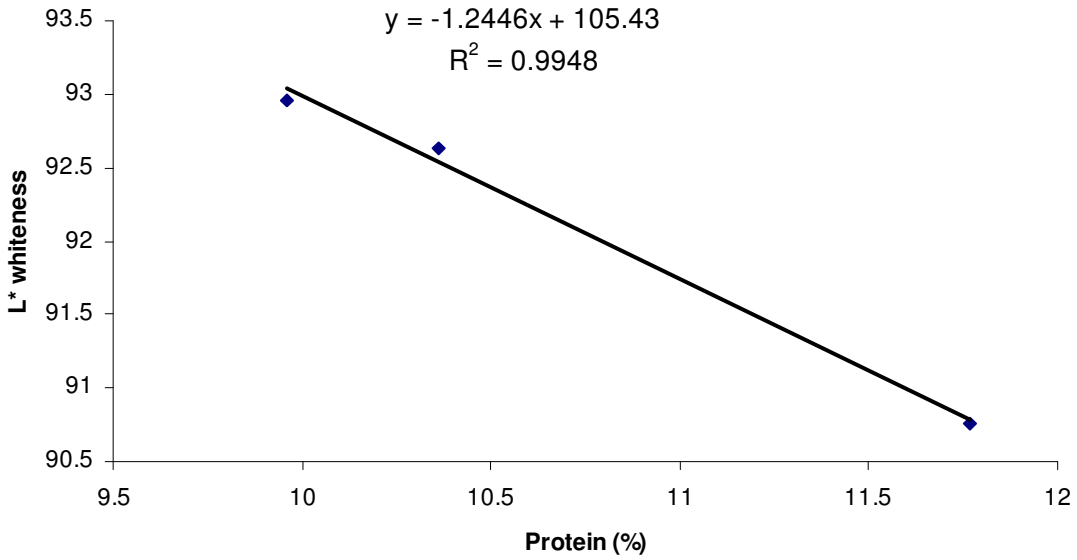


Fig. 9.2a Relationship between flours protein content and colour characteristics L* values (whiteness)

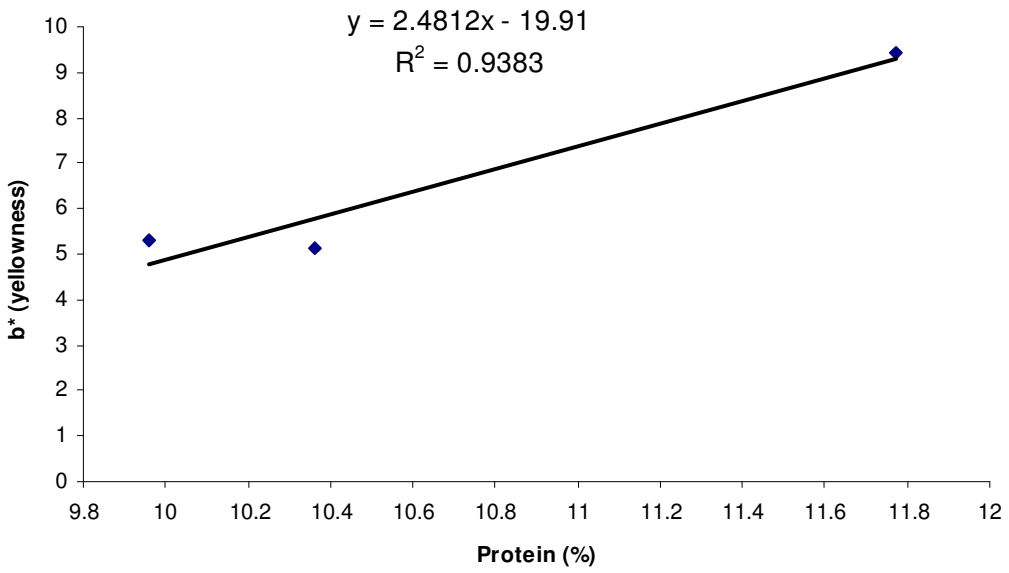


Fig. 9.2b Relationship between flour protein content and colour characteristics b* values (yellowness)

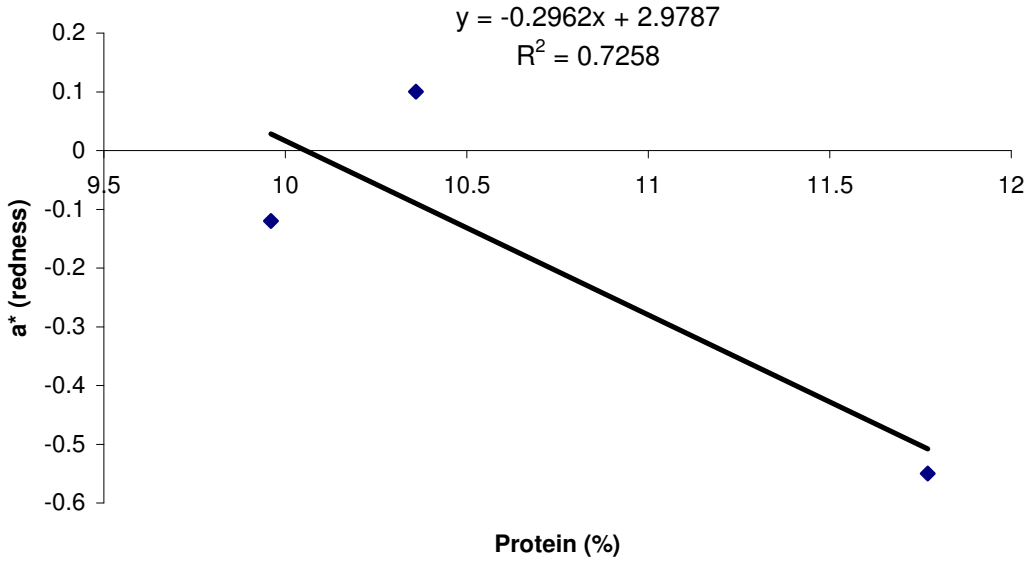


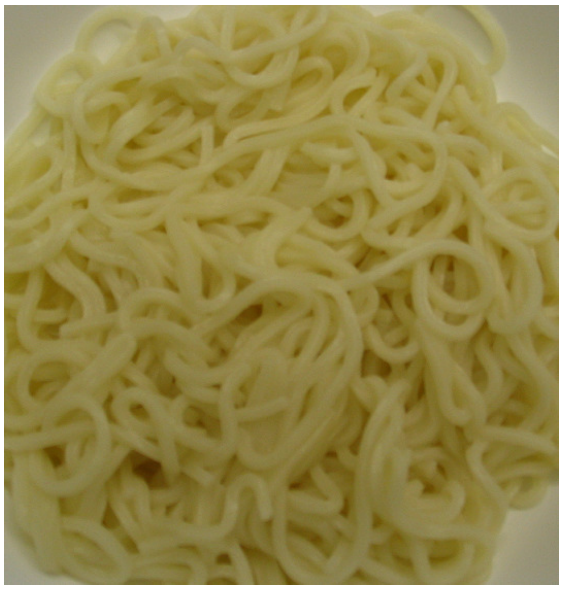
Fig. 9.2c Relationship between flour protein content and colour characteristics a* values (redness)

9.7 The preparation of two styles of Asian noodles under laboratory conditions

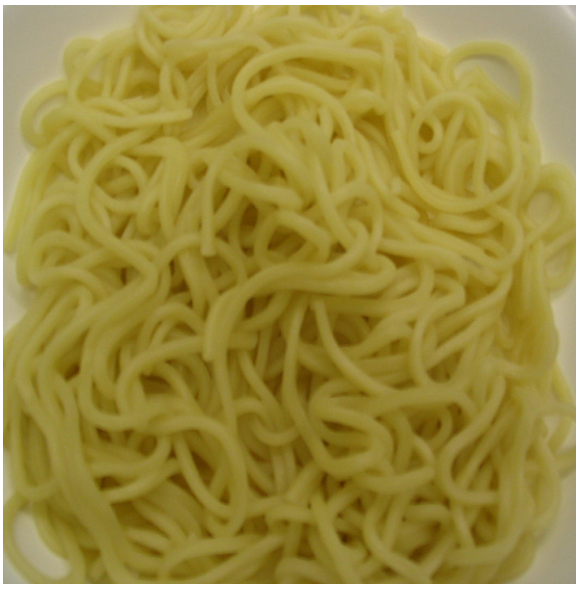
Laboratory methods for noodle preparation were selected and set up to reflect, as far as possible, typical commercial formulations and processing practices for each of the two styles of noodles. The procedures were based upon published approaches and are described in detail in Chapter 7. It is emphasised that for all steps here the water used

was distilled grade. The basis of this choice was to minimise the potential impact of impurities in the water upon the assays of enzymes. For both styles of Asian noodles, samples of the noodles prepared in the laboratory were taken at each stage of processing and also following cooking to the optimum point which was assessed for each batch of noodles prepared. In Fig 9.3 noodles prepared in the laboratory are shown.

The pH value of noodles was also measured and typical results were approximately 6 for the WSN and approximately 10 for YAN. The pH vales measured for all samples including flour, noodles at different stages of preparation and noodles supplemented with enzymes are shown in Table 9.8.



WSN UW; freshly cooked



YAN BGF; freshly cooked

Fig. 9.3 Images of noodles prepared in the laboratory
Note: Images taken with a Cannon digital camera. WSN made from UW and YAN made from BGF

Table 9.8 pH characteristics of flours and noodles

Samples[a]	pH value
Flour	
BGF	6.13
PF	6.07
UW	5.86
Dough	
YAN	10.05
PF WSN	6.16
UW WSN	6.16
Noodles fresh	
YAN	10.05
PF WSN	6.16
UW WSN	6.16
Noodles dried	
YAN	10.03
PF WSN	6.09
UW WSN	6.05
Noodles cooked	
YAN	10.05
PF WSN	6.16
UW WSN	6.16
Noodles treated with barley α-amylase	
YAN	9.94
PF WSN	5.74
UW WSN	5.73
Noodles treated with bacterial α-amylase	
YAN	9.93
PF WSN	5.72
UW WSN	5.75

Table 9.8 pH characteristics of flours and noodles (continued)

Sample[a]	pH value
Noodles treated with POX	
YAN	9.97
PF WSN	5.88
UW WSN	5.86
Noodles treated with LOX	
YAN	10.16
PF WSN	6.01
UW WSN	5.98
Noodles treated with AAO	
YAN	10.02
PF WSN	6.03
UW WSN	6.01
Noodles treated with fungal lipase	
YAN	9.94
PF WSN	5.61
UW WSN	5.55
Noodles treated with PP lipase	
YAN	9.85
PF WSN	6.07
UW WSN	6.04
Noodles treated with WG lipase	
YAN	9.87
PF WSN	5.70
UW WSN	5.65

a Abbreviations used are: BGF, Baker's strong flour; PF, P-Farina; UW, Ultra White; WSN, white salted noodles; YAN, yellow alkaline noodles; POX, peroxidase, AAO, ascorbic acid oxidase; LOX. lipoxygenase; PP, porcine pancreas; WG, wheat germ.
Note: Data presented on a 'as is' basis.

These pH measurements indicate that the addition of various enzyme preparations did not influence the pH, i.e. the pH values were not shifted in one or the other direction upon the addition of various enzymes. As expected the addition of alkaline salts (kansui) was a major ingredient that shifted a pH to the alkaline side of the scale. The

addition of kansui shifted the pH to around 10.0 as compared to values for WSN just below neutral (approximately 6) (Table 9.8). In addition differences in processing and boiling of noodles did not cause any changes in the pH of noodles made in the laboratory.

Cooking characteristics, particularly the yield of noodles after cooking, in addition to the textural and colour properties of noodles are important quality attributes. Accordingly, cooking properties of noodles were measured using an evaporation method and results expressed as proportion lost upon cooking and as water uptake during cooking, both expressed as a percentage. The results (Table 9.9) show significantly higher cooking loss for YAN as compared to WSN, which is again probably due to the alkaline slats in the formulation for YAN. Furthermore, higher losses are recorded for dried as compared to fresh noodles, which might be explained by the fact that these types of noodles have longer optimum cooking time (6.30min for WSN and 9.00min for YAN) compared to fresh noodles (2.30min for WSN and 4.30min for YAN).

Table 9.9 Cooking characteristics of Asian noodles prepared in the laboratory

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh WSN-PF	2.30	45.5[a]	5.16[a]
fresh WSN-UW	2.30	47.8[ab]	5.64[a]
fresh YAN	4.30	48.1[b]	7.90
dried WSN-PF	6.30	74.5[a]	9.48[a]
dried WSN-UW	6.30	75.2[ab]	9.39[a]
dried YAN	9.00	76.0[b]	13.40
RT WSN-PF	2.30	44.7[a]	5.47[a]
RT WSN-UW	2.30	46.6[ab]	5.66[a]
RT YAN	4.30	47.8[b]	7.88
FR WSN-PF	2.30	46.8[a]	5.55[a]
FR WSN-UW	2.30	44.9	5.50[a]
FR YAN	4.30	47.3[a]	8.37

a Abbreviations used are: PF, P-Farina; UW, Ultra White; WSN, white salted noodles; YAN, yellow alkaline noodles; RT, room temperature; FR, refrigeration temperature

Note: Means followed by the same letter within the same column and treatment (fresh, dried, RT and FR) are not statistically different at $p<0.05$.

In summary, a series of commercial flours have been selected and analysed for a series basic parameters. These samples have then been used for the preparation of the two styles of Asian noodles and the products characterized. The results form the basis upon which further comparisons are to be made during subsequent stages of this investigation as enzyme preparations are added to noodles formulations.

Chapter 10

Results and discussion: the enzyme activities of flours and Asian noodles prepared in the laboratory

The purpose of this chapter is to describe and discuss results obtained for enzyme activity of flours and two types of Asian noodles prepared in the laboratory. Following validation, for each enzyme assay, all flours used in this study and all noodle samples stored under different storage conditions have been analysed for the following enzyme activities: α-amylase, lipase, ascorbic acid oxidase (AAO), lipoxygenase (LOX), and peroxidase (POX).

10.1 Introduction

Although clean sound flour has relatively low levels of enzymes their role in food processing might be of great importance. Enzymes studied here and enzymes in general all have differing units and one cannot readily compare the amounts or levels of enzymes present or the activity of particular enzyme to another. The units of enzymes studied varied for the different enzymes. These were the Ceralpha Unit for α-amylase, to increase in absorbance over a period of time for AAO and POX, to micro moles of hydroperoxide formed, and micro moles of free fatty acids produced for LOX and lipase respectively. More information, including assay procedures, is provided in Chapter 7. In the preliminary phases of this study, enzyme activities have been measured for all flours used, as well as noodles made and stored under different storage conditions and finally assays were performed on the supplemented samples as well.

10.2 Assay of α-amylase

The level of endogenous α-amylase in cereal grain products significantly impacts the industrial use of these commodities. In bread making for example, the level of α-amylase should be sufficient to produce saccharides which can be absorbed and utilized by the yeast, but on the other hand if the levels are too high excessive starch dextrinisation will occur and lead to sticky crumb and various problems during

processing. On average clean sound wheat flour will contain significant amounts of β-amylase bit very little α-amylase. Tables 10.1 and 10.2 show the activity of α-amylase in the flours and noodles studied here. The Amylase HR reagent, used here to measure the activity of α-amylase was suitable to measure α-amylase from all three sources (bacterial, fungal and cereal) however, assay conditions needed to be modified to suit each particular enzyme preparation. In addition Amylase HR reagent is specific for α-amylase and absolutely resistant to attack by *exo* enzymes including β-amylase, amyloglucosidase and α-glucosidase.

Table 10.1 α-Amylase activity of flours studied

Sample	α-Amylase activity (CU)[b]
BGF	0.1055 ± 0.004
PF	0.0757 ± 0.004
UW	0.0768 ± 0.004
Wheat standard[a]	0.12 ± 0.01

a Wheat standard was supplied by Megazyme stated α-amylase activity = 0.12 units
b Unit for α-amylase is Ceralpha Unit (see Materials & Methods; Chapter 7)

Although no losses in the levels of endogenous α-amylase were recorded during noodle processing and after the storage of noodle products under previously defined conditions, once noodles have been supplemented with exogenous α-amylase slight losses were recorded for both barley malt α-amylase and that of bacterial origin. The losses associated with these enzymes preparation occurred after the drying of noodle products (Table 10.2), this indicates that slightly higher temperatures (conditions of drying process were 40°C for 30hr) might have caused some loss of α-amylase activity in both preparations. Although slightly higher activity was measured in samples supplemented with α-amylase from barley malt as compared with noodles treated with α-amylase of bacterial origin greater differences in textural and structural properties were seen in samples treated with the latter enzyme (Chapters 12 & 13). This might indicate that bacterial α-amylase probably had more influential role on starch degradation than α-amylase from barley malt.

Table 10.2 Activity of α-amylase of noodles prepared in the laboratory

Treatment[a]	α-Amylase activity (CU)[b]
Controls	
Day1 WSN-PF	0.083 ± 0.002
Day1 WSN-UW	0.081 ± 0.002
Day1 YAN	0.129 ± 0.003
24hr RT WSN-PF	0.088 ± 0.002
24hr RT WSN-UW	0.081 ± 0.002
24hr RT YAN	0.134 ± 0.005
24hr FR WSN-PF	0.083 ± 0.007
24hr FR WSN-UW	0.091 ± 0.0002
24hr FR YAN	0.134 ± 0.005
Dried WSN-PF	0.063 ± 0.03
Dried WSN-UW	0.066 ± 0.01
Dried YAN	0.095 ± 0.03
Barley (72×10^2 U / batch added)	
Fresh WSN-PF	1.794 ± 0.05
Fresh WSN-UW	1.795 ± 0.12
Fresh YAN	0.146 ± 0.01
Dried WSN-PF	1.368 ± 0.06
Dried WSN-UW	1.375 ± 0.12
Dried YAN	0.114 ± 0.03
Bacterial[c]	
Fresh WSN-PF	1.188 ± 0.16
Fresh WSN-UW	1.229 ± 0.28
Fresh YAN	1.840 ± 0.32
Dried WSN-PF	0.924 ± 0.29
Dried WSN-UW	0.913 ± 0.41
Dried YAN	1.402 ± 0.25

a Abbreviations are: WSN, white salted noodles; YAN, yellow alkaline noodles; PF, P-Farina flour; UW, Ultra white flour; RT, storage at room temperature; FR, storage at 4°C

b Unit for α-amylase is Ceralpha Unit; (see Materials & Methods, Chapter 7)

c Levels of addition were: WSN – 2.3 KMWU / batch; YAN – 2.3×10^3 KMWU / batch

Table 10.3 α-Amylase activity of wheat flours reported in the literature

Material	α-Amylase (CU)[a]	No. of labs
White wheat flour A	0.10	15
White wheat flour B (with fungal α-amylase)	0.14	15
Milled malt A	375	12
Milled malt B	117	15
Medium strong wheat	**α-Amylase (mg Maltose)[c]**	
Wheat	4.20 ± 0.032	
Break 1	2.74 ± 0.080	
Break 2	2.60 ± 0.050	
Break 3	2.67 ± 0.060	
Break 4	3.12 ± 0.080	
Break 5	3.71 ± 0.080	
Reduction 1	4.10 ± 0.090	
Reduction 2	3.71 ± 0.090	
Reduction 3	4.20 ± 0.100	
Reduction 4	4.30 ± 0.085	
Reduction 5	5.80 ± 0.120	
Reduction 6	5.90 ± 0.110	
Atta	4.98 ± 0.100	
Semolina	3.40 ± 0.070	
Bran flakes	3.88 ± 0.090	
Bran rough	3.70 ± 0.095	
Bran deluxe	3.56 ± 0.045	

a Adapted from McCleary *et al* 2002. Unit for α-amylase is Ceralpha Unit per gram
c Adapted from Rani *et al* 2001. Unit for α-amylase is mg maltose liberated in 3 minutes at 37°C by 1.0ml of enzyme solution. Values are expressed per gram of protein

10.3 Assay of lipase

Lipase has been widely used in the baking industry as a processing aid. Its actions upon fats and oils added to the bread formulation are highly desirable as it results in softer and uniform breadcrumb and develops a desirable flavour and aroma. Although there if

no addition of fats or oils in the noodle processing thus no effect of lipase upon quality of these products might be expected, the low levels of fat present in the flour originally and addition of exogenous lipase might lead to desirable changes in textural and structural properties of noodles. Relatively low levels of lipase activity have been measured in the flour samples studied and no losses were recorded during noodle processing and storage. A plot of a typical standard curve used for calculation of lipase activity is shown in Fig. 10.1. Here a range of concentrations (0.0 to 1.0 micromoles of palmitic acid were made, and release of free fatty acid measured upon reaction with coupled cooper mixture, resulting in a plot that has been used to calculate ($y = 1.5054 \times X$) the amount of free fatty acids produced in each gram of defatted sample in one minute. Prior to lipase assays all samples were defatted with acetone (-18°C twice, followed by diethyl ether once). Three different lipase preparations (lipase from porcine pancreas, fungal lipase and lipase from wheat germ) were used in this study and their role upon quality attributes of Asian noodle products has been analysed (Chapters 14 & 15).

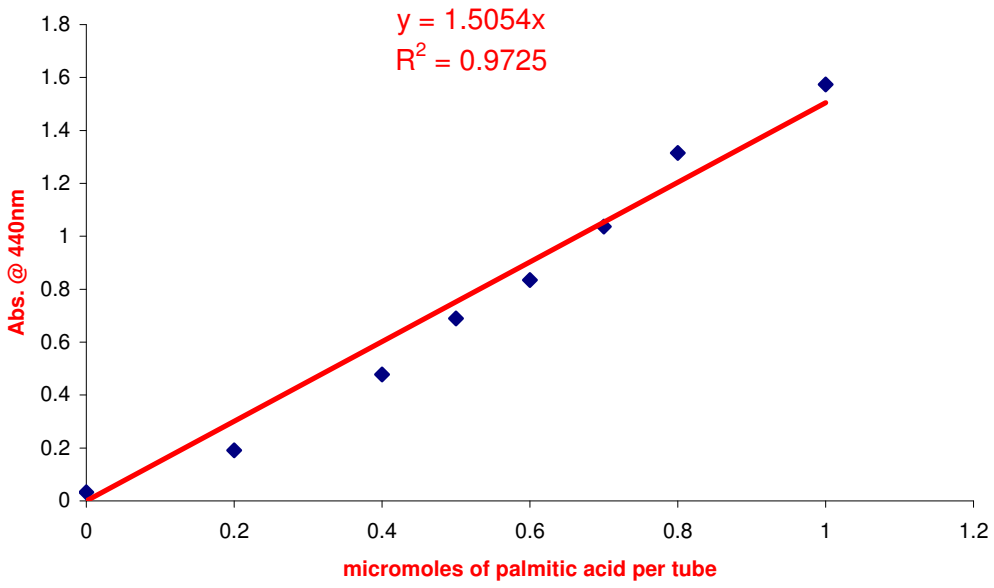


Fig. 10.1 A typical standard curve obtained for lipase assay

Table 10.4 Activity of lipase in the three flours studied

Sample[a]	Lipase activity[b]
BGF	1.49 ± 0.24
PF	1.53 ± 0.23
UW	1.56 ± 0.25

a Abbreviations are: BGF, Bakers strong flour; PF, P-Farina flour; UW – Ultra white flour

b Unit for lipase activity is micromoles of FFA produced per gram of defatted flour per minute

The three flours analysed had similar lipase activity, ranging from 1.49 ± 0.24 for BGF to 1.56 ± 0.25 for UW (Table 10.4). Similar levels and not much variation were also found in noodles prepared in the laboratory. Slightly lower levels of lipase were recorded for dried noodles, compared to samples stored under different conditions (Table 10.5). Samples treated with three different lipases showed two to three times higher activities as compared to the control. Considering the relatively high levels of addition it can be concluded that not all lipases were recovered in the final products and that the stability of endogenous lipases seems to be greater than that of added (exogenous) lipases studied here. There seems to be no impact of alkaline salts upon the activity of lipases already present in the flours, as lipase activity for BGF was 1.49 ± 0.24, and subsequently the lipase activity of YAN was 1.45 ± 0.18, which indicates that no enzyme got inactivated during the processing and especially after the addition of alkaline salts to the formulation (Tables 10.4 & 10.5). Noodles supplemented with three different lipases, all had higher activities than control samples. However, the measured activities were approximately two-fold of the control samples, considering relatively high levels of supplementation greater differences were expected. Since similar results were recorded for both WSN and YAN it seems that the action of alkaline salts is not the only parameter that effected the stability of the added enzymes. Some lipase inactivation also occurred during the processing of noodles especially during the drying procedures.

Table 10.5 Lipase activity of noodles prepared in the laboratory

Treatment[a]	Lipase activity[b]
Controls	
Day1 WSN-PF	1.84 ± 0.22
Day1 WSN-UW	1.81 ± 0.2
Day1 YAN	1.45 ± 0.18
24hr RT WSN-PF	1.87 ± 0.22
24hr RT WSN-UW	1.81 ± 0.22
24hr RT YAN	1.61 ± 0.14
24hr FR WSN-PF	1.98 ± 0.18
24hr FR WSN-UW	1.78 ± 0.21
24hr FR YAN	1.72 ± 0.27
Dried WSN-PF	1.19 ± 0.39
Dried WSN-UW	1.03 ± 0.21
Dried YAN	1.18 ± 0.13
Porcine Pancreas (2×10^6 U / batch added)	
Fresh WSN-PF	3.51 ± 0.16
Fresh WSN-UW	3.69 ± 0.22
Fresh YAN	2.58 ± 0.58
Dried WSN-PF	3.15 ± 0.50
Dried WSN-UW	3.13 ± 0.53
Dried YAN	2.09 ± 0.71
Fungal (20×10^4 LU / batch added)	
Fresh WSN-PF	3.77 ± 0.81
Fresh WSN-UW	4.01 ± 0.55
Fresh YAN	2.88 ± 0.61
Dried WSN-PF	3.24 ± 0.33
Dried WSN-UW	3.17 ± 0.52
Dried YAN	2.99 ± 0.22

Table 10.5 Lipase activity of noodles prepared in the laboratory (continued)

Treatment[a]	Lipase activity[b]
Wheat Germ (8.19×10^2 U / batch added)	
Fresh WSN-PF	2.11 ± 0.29
Fresh WSN-UW	2.13 ± 0.33
Fresh YAN	1.75 ± 0.54
Dried WSN-PF	2.02 ± 0.4
Dried WSN-UW	1.94 ± 0.43
Dried YAN	1.35 ± 0.26

a Abbreviations are: WSN, white salted noodles; YAN, yellow alkaline noodles; PF, P-Farina flour; UW, Ultra white flour; RT, storage at room temperature; FR, storage at 4°C
b Unit for lipase is micromole FFA formed per gram; (see Materials & Methods, Chapter 7)

10.4 Assay of LOX

The flours analysed contained relatively low levels of LOX (Table 10.6) similar to data reported on Indian wheat by Rani *et al* 2001. The endogenous enzyme remained active during noodle preparation, with no losses under any of the storage conditions studied (Table 10.7). Indicating that even, addition of alkaline salts (as in the case of YAN) and relatively high pH of the final product, did not lower activity of LOX. Supplemented noodles had slightly higher LOX activity, however, not as high as expected, considering relatively high levels of enzyme addition. This might suggest again, that exogenous LOX is less stable than endogenous LOX (already present in wheat flour) (Table 10.7).

Table 10.6 Activity of lipoxygenase in the flours studied

Sample[a]	Lipoxygenase activity[b]
BGF	28.55 ± 0.08
PF	29.27 ± 0.06
UW	34.92 ± 0.09

a Abbreviations are: BGF, Bakers strong flour; PF, P-Farina flour; UW, Ultra white flour

b Unit for lipoxygenase activity is given in micro moles of hydroperoxide formed per gram per minute under assay conditions

10.5 Assay of POX

The activity of POX activity was determined by measuring the slope from the linear increase in the absorbance at 470nm. The increase in the absorbance was due to the oxidation of guaiacol to tetrahydroguaiacol in the presence of H_2O_2. The reaction was started when enzyme extract was added to the typical mixture in a cell containing guaiacol in acetate buffer, calcium chloride and H_2O_2. The increase in the absorbance was monitored for a period of two minutes at room temperature at 470nm. A typical POX assay plot is shown in Fig. 10.3.

Although POX belongs to one of the most studied plant enzymes, due to its genetic, analytical and physiological implications (Iori *et al* 1995) its role in pasta and Asian noodle products has not been investigated. Oxidising agents have a beneficial effect on dough properties and attributes such as volume, texture and crumb structure of the baked products made from wheat flour. Replacement of chemical oxidizers by enzymes including POX, LOX or glucose oxidase, has a benefit of more specific and better controlled oxidation processes. It was reported nevertheless, that POX is responsible for colour quality of wheat processed products, as it effectively bleaches carotene (Hsieh and McDonald 1984). It has also been reported that POX negatively affects the yellow colour intensity of flour and products made from flour (Iori *et al* 1995).

Table 10.7 Lipoxygenase activity of noodles prepared in the laboratory

Treatment[a]	Lipoxygenase activity[b]
Controls	
Day1 WSN-PF	29.52 ± 0.12
Day1 WSN-UW	33.63 ± 0.06
Day1 YAN	28.35 ± 0.41
24hr RT WSN-PF	30.06 ± 0.13
24hr RT WSN-UW	33.33 ± 0.11
24hr RT YAN	28.60 ± 0.08
24hr FR WSN-PF	30.44 ± 0.09
24hr FR WSN-UW	33.75 ± 0.08
24hr FR YAN	28.46 ± 0.05
Dried WSN-PF	28.55 ± 0.16
Dried WSN-UW	32.05 ± 0.22
Dried YAN	28.22 ± 0.31
Lipoxidase (3.70×10^3U / batch added)	
Fresh WSN-PF	31.64 ± 0.13
Fresh WSN-UW	37.25 ± 0.08
Fresh YAN	31.45 ± 0.11
Dried WSN-PF	30.33 ± 0.05
Dried WSN-UW	36.13 ± 0.25
Dried YAN	30.25 ± 0.31

a Abbreviations are: WSN, white salted noodles; YAN, yellow alkaline noodles; PF, P-Farina flour; UW, Ultra white flour; RT, storage at room temperature; FR, storage at 4°C

b Unit for lipoxygenase is given in micro moles of hydroperoxide formed per gram per minute under assay conditions

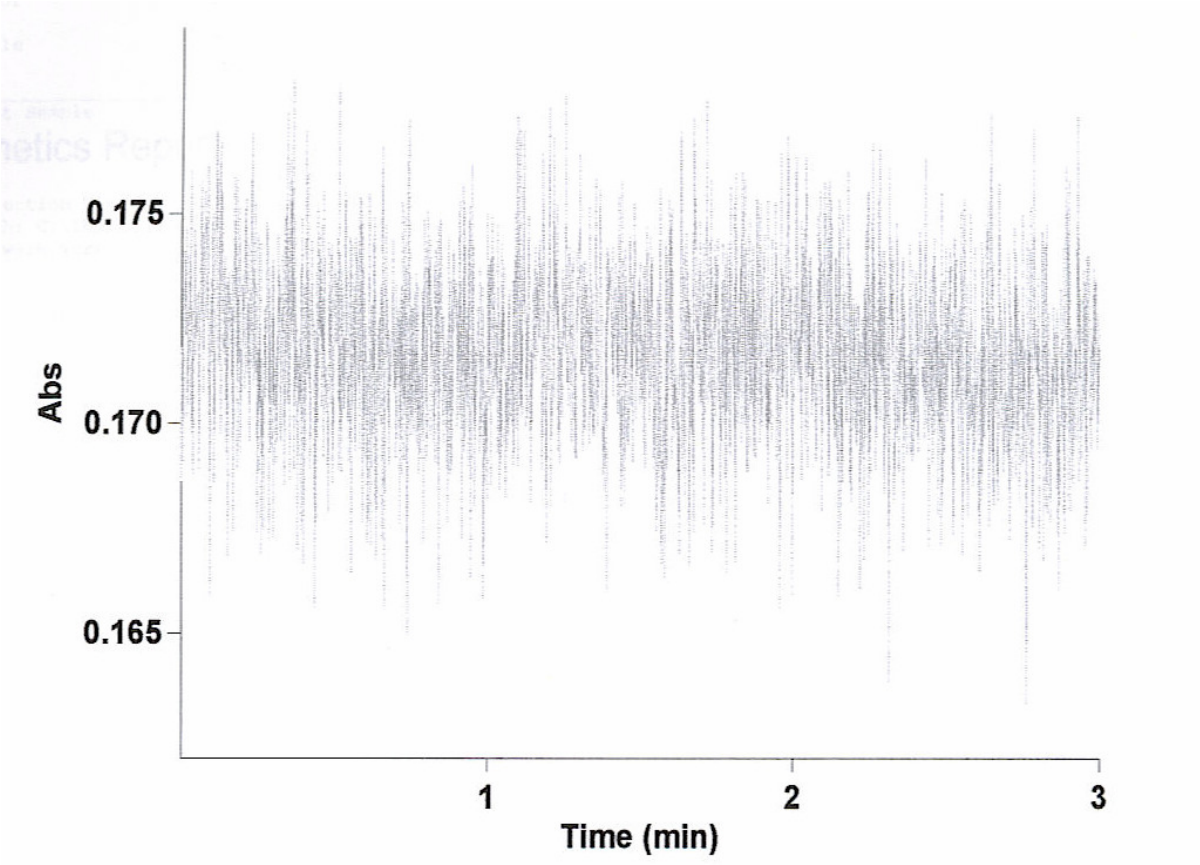


Fig. 10.2 A typical lipoxygenase assay plot
Assays performed by Ultra Visible Spectrophotometer (UV-VIS)

In relation to LOX activity, it was observed by Hsieh and McDonald 1984 that purified LOX (L-1) from durum wheat endosperm also showed POX activity. Since both LOX and POX are both metalloenzymes with one iron atom per molecule having polypeptide chains with different structures and masses, have active sites containing the iron atom organized differently and have ability to catalyse distinct reactions. It is know that these reactions being of an oxidative type, involve oxygen of different origins. The study by Iori *at al* 1995 suggested that different carotene bleaching agents are present in the crude extracts of several durum wheat semolinas and these bleaching activities were highly correlated with POX activity. Thus, they suggested that in durum wheat semolina there is more than one enzyme responsible for yellow colour bleaching during processing of what products. Furthermore, Iori *at al* 1995 reported very low levels of LOX and very high of levels of POX and these are in accordance with the results presented here as well (Table 10.3 and 10.5). The effect of POX on the colour of Asian noodles is presented in Chapters 16 & 17.

Table 10.8 LOX activity reported in literature[a]

Wheat material	LOX activity[b]
Wheat	13.10 ± 0.14
Break 1	9.90 ± 0.13
Break 2	8.17 ± 0.09
Break 3	4.20 ± 0.05
Break 4	10.86 ± 0.14
Break 5	9.60 ± 0.14
Reduction 1	2.70 ± 0.09
Reduction 2	2.71 ± 0.07
Reduction 3	8.40 ± 0.10
Reduction 4	11.30 ± 0.13
Reduction 5	9.13 ± 0.12
Reduction 6	10.12 ± 0.12
Atta	20.52 ± 0.20
Semolina	4.86 ± 0.08
Bran flakes	19.50 ± 0.60
Bran rough	27.20 ± 0.42
Bran deluxe	11.00 ± 0.15

a Adopted from Rani *et al* 2001
b Unit for LOX is amount of hydroperoxide formed (micromoles) per minute. Values expressed per gram of protein

Table 10.9 POX activity in the flours studied

Sample[a]	POX activity[b]
BGF	425 ± 16
PF	323 ± 15
UW	343 ± 19

a Abbreviations are: BGF, Bakers strong flour; PF, P-Farina flour; UW, Ultra white flour
b Unit for POX activity is increase in the absorbance at 470nm per minute per gram of sample

Relatively high POX activity was found in the flours analysed compared to the levels of other enzymes present (Table 10.5). Baker's strong flour was found to have higher POX activity compared to the other two flours analysed (Table 10.9). However, slightly lower activity was recorded in the noodles made in the laboratory and analysed on day one of preparation and following the storage under defined conditions. In some cases loss of POX activity was recorded after storage and after drying of noodle products (Table 10.6).

10.6 Assay of AAO

Here a continuous spectrophotometric assay has been used to measure the activity of AAO in flour and noodle samples. This method was used to measure the disappearance of LAA based on measurement of absorbance at 290nm for total period of three minutes at 25°C. A typical AAO assay is shown in Fig. 10.4, and these plots were used to calculate the change in absorbance per unit time and subsequently express the activity of AAO as amount of enzyme able to convert one micromole of LAA into DHAA per minute per gram of sample.

Up to now, the main function of AAO and DHAA is considered to be linked with the cell wall since AAO is found primarily in the cell wall and is most active there. The bread improving action of LAA is well established. The first step in this process is the oxidation of LAA to DHAA and the presence of AAO in wheat flour is an effective catalyst for this action.

Table 10.10 POX activity of noodles prepared in the laboratory

Treatment[a]	POX activity[b]
Controls	
Day1 WSN-PF	269 ± 10
Day1 WSN-UW	305 ± 10
Day1 YAN	371 ± 17
24hr RT WSN-PF	314 ± 17
24hr RT WSN-UW	286 ± 7
24hr RT YAN	327 ± 13
24hr FR WSN-PF	273 ± 7
24hr FR WSN-UW	298 ± 10
24hr FR YAN	322 ± 10
Dried WSN-PF	260 ± 33
Dried WSN-UW	292 ± 17
Dried YAN	305 ± 22
POX 72 × 10^3 U / batch added [c]	
Fresh WSN-PF	701 ± 13
Fresh WSN-UW	763 ± 15
Fresh YAN	773 ± 6
Dried WSN-PF	668 ± 23
Dried WSN-UW	767 ± 17
Dried YAN	738 ± 12

a Abbreviations are: WSN, white salted noodles; YAN, yellow alkaline noodles; POX, peroxidase; PF, P-Farina flour; UW, Ultra white flour; RT, storage at room temperature; FR, storage at 4°C

b Unit for POX is increase in the absorbance at 470nm per minute per gram of sample

c POX added was from MP Biomedicals, the amount added was 72 × 10^3 U of POX per batch of noodles (200g of flours)

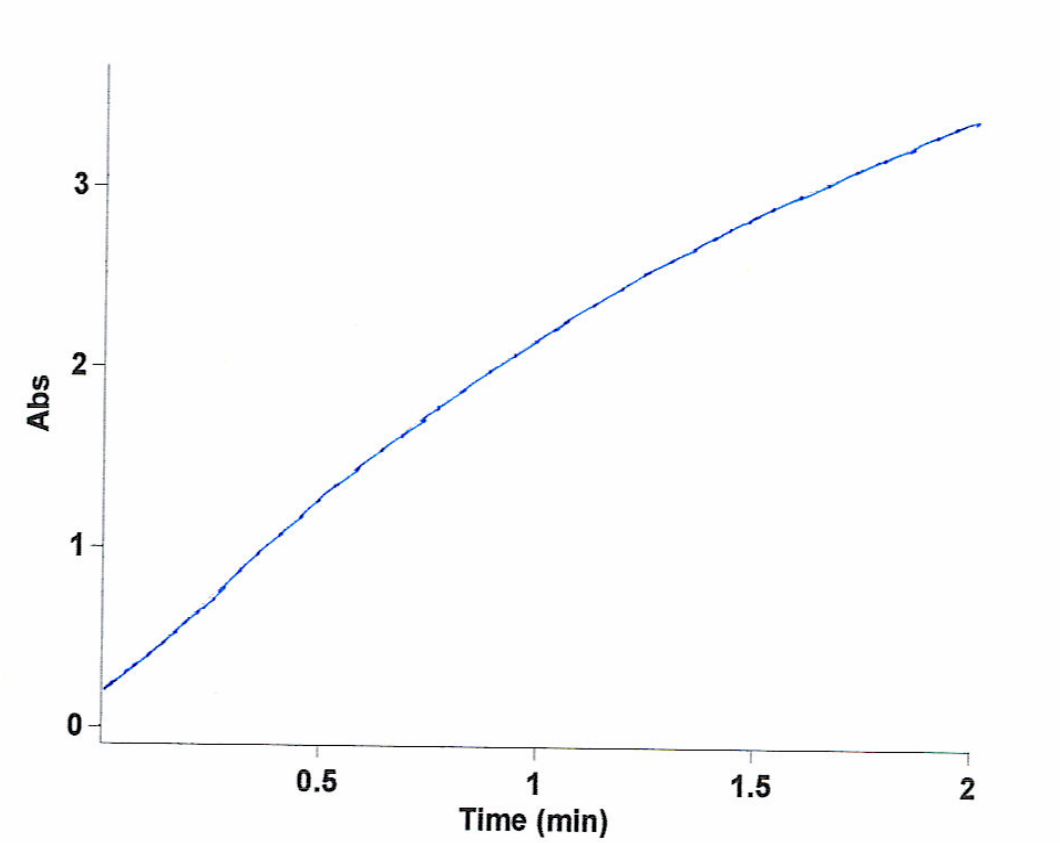


Fig. 10.3 A typical POX assay plot
Assays were performed spectrophotometrically (UV- VIS)

It was reported by Every *et al* 1996 that the level of AAO in wheat was not critical to the ascorbic acid improver effect, provided sufficient oxygen was mixed into the dough by high speed mixers. They showed that there is a wide variation in AAO activity in different wheat cultivars and furthermore in different lines of the same cultivar. On the other hand, one might assume that due to large variations between cultivars the level of AAO in some wheats might be a limiting factor for the AA improver effect.

Table 10.11 Activity of wheat POX reported in the literature[a]

Wheat material	POX activity [b]
Wheat	2496 ± 93.0
Break 1	751.0 ± 44.0
Break 2	1893.0 ± 75.0
Break 3	1832.0 ± 81.0
Break 4	1468.0 ± 51.0
Break 5	741.0 ± 38.0
Reduction 1	1647.0 ± 52.0
Reduction 2	1104.0 ± 28.0
Reduction 3	2041.0 ± 75.0
Reduction 4	923.0 ± 75.0
Reduction 5	2164.0 ± 28.0
Reduction 6	847.0 ± 40.0
Atta	3723.0 ± 97.0
Semolina	597.0 ± 26.0
Bran flakes	4107.0 ± 74.0
Bran rough	1444.0 ± 60.0
Bran deluxe	5183.0 ± 84.0

a Adapted from Rani *et al* 2001
b Unit for POX is change in absorbance of 1.0 per minute. Values are expressed per gram of protein

Table 10.12 AAO activity in the flours studied

Sample[a]	AAO activity (U/g)[b]
BGF	32.5 ± 4.9
PF	31.5 ± 4.4
UW	38.6 ± 6.0

a Abbreviations are: BGF, Bakers strong flour; PF, P-Farina flour; UW, Ultra white flour

b Unit for AAO U/g one unit of ascorbic acid oxidising activity is defined as the amount of activity that oxidises one microgram of LAA to dehydroascorbic acid (DHAA) in one minute at 25°C

All three flours studied had similar levels of AAO activity (Table 10.12). Only minor losses of activity were recorded in dried noodle samples (Table 10.13). Otherwise processing and storage of noodles at room and refrigeration temperatures seemed to have no effect on the activity of AAO.

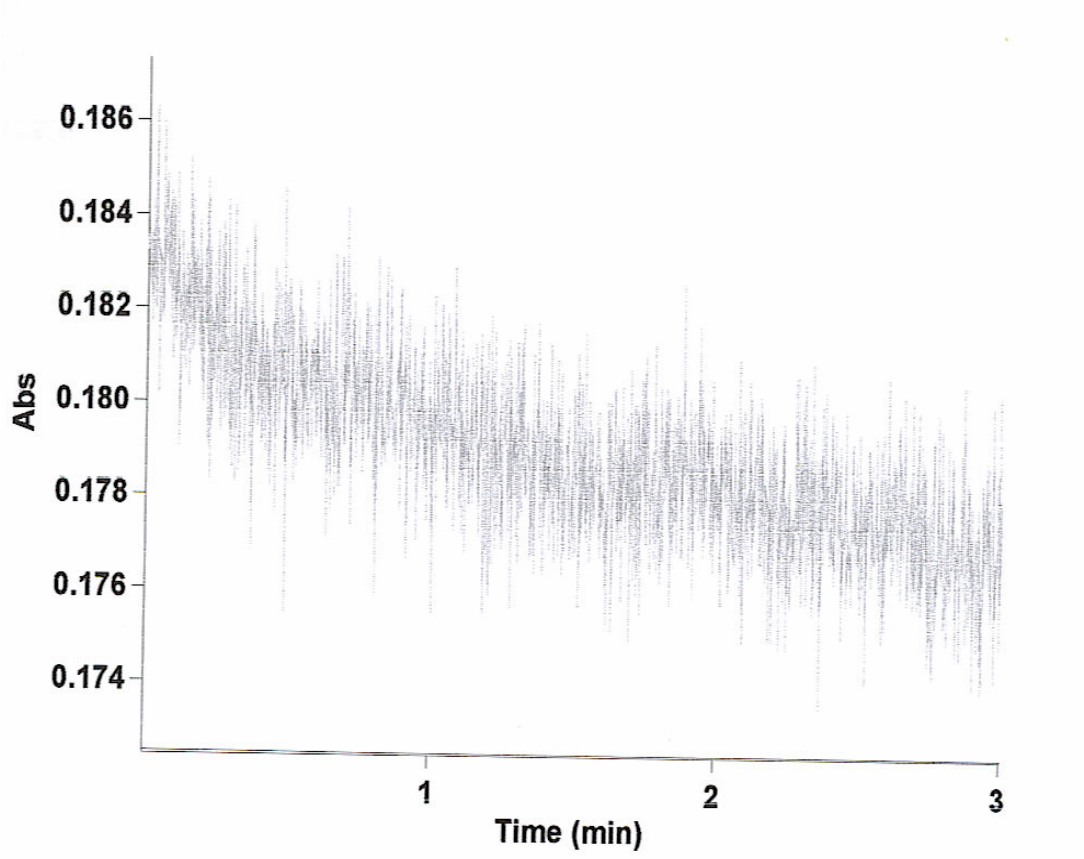


Fig. 10.4 A typical AAO assay plot
Assay performed on the Ultra Visible Spectrophotometer

Table 10.13 AAO activity of noodles prepared in the laboratory

Treatments[a]	AAO activity (U/g)[b]
Controls	
Day1 WSN-PF	38.5 ± 4.1
Day1 WSN-UW	37.6 ± 3.7
Day1 YAN	42.2 ± 2.4
24hr RT WSN-PF	33.7 ± 4.1
24hr RT WSN-UW	35.0 ± 2.1
24hr RT YAN	32.3 ± 3.0
24hr FR WSN-PF	34.3 ± 2.7
24hr FR WSN-UW	37.3 ± 4.4
24hr FR YAN	34.2 ± 3.9
Dried WSN-PF	25.6 ± 3.1
Dried WSN-UW	26.7 ± 6.4
Dried YAN	27.7 ± 6.2
AAO (1.1×10^2U / batch added)	
Fresh WSN-PF	41.4 ± 2.1
Fresh WSN-UW	39.3 ± 2.1
Fresh YAN	43.0 ± 1.1
Dried WSN-PF	27.1 ± 3.1
Dried WSN-UW	28.5 ± 2.1
Dried YAN	29.2 ± 3.0

a Abbreviations are: WSN, white salted noodles; YAN, yellow alkaline noodles; AAO, ascorbic acid oxidase; PF, P-Farina flour; UW, Ultra white flour; RT, storage at room temperature; FR, storage at 4°C

b Unit for AAO U/g one unit of ascorbic acid oxidising activity is defined as the amount of activity that oxidises one microgram of LAA to dehydroascorbic acid (DHAA) in one minute at 25°C

Table 10.14 Activity of wheat AAO reported in the literature[a]

Wheat cultivar[b]	AAO activity[c]
Domino (8)	10.5
Otane (8)	7.1
Norseman (8)	6.9
Batten (8)	6.1
Tancred (8)	5.4
Amethyst (8)	5.3
Orua (6)	5.2
Dartagnan (7)	4.6
Sapphire (8)	3.7
Karamu (4)	3.5
Brock (7)	3.2

a Adapted from Every *et al* 1996
b The number of samples tested for each cultivar is shown in parentheses. New Zealand wheat cultivars harvested in 1992
c Unit for AAO is the amount of enzyme that oxidizes 1.0 µg of AA to DHAA in one minute at pH 6.2 and 25°C

10.7 Enzyme contents of wheat flour

In analysing the flour samples it was found that the enzyme levels were relatively low in all cases other than for POX. Relative high amounts of this enzyme were measured (enzyme extracts needed to be diluted ten fold in case of flour samples and five fold for noodle samples so that the absorbance could be recorded). Thus, the flour sample was tested repeatedly over a period of three weeks and repeats of 50 assays were done in each case. The amounts present in the flour extracts were approaching the limit of detection in the case of LOX and AAO, thus different approaches and different extractions were also tried, and the most satisfactory results were obtained using the approaches described in Materials & Methods (Chapter 7).

In considering the current analytical results there is little published information on the relative amounts of different enzymes in white flours. The available data are summarised in Tables 10.3, 10.8, 10.10, 10.11 and 10.14. However, it should be noted that a direct comparison is not possible in most cases since, different approaches and different units are used for expression of enzyme activity. Nevertheless, it is a useful to

look at the range of different assays and indicate the most suitable assay and unit for a range of cereal enzymes.

10.8 The stability of enzymes in two styles of Asian noodles

In selecting a suitable level of fortification for the current study, consideration was given to the amounts already present in the flour, and a staring point of addition was to add approximately 20 times higher amounts of exogenous enzymes. Regulatory framework applying in Australia does not exist for enzymes studied here. In all cases the enzyme preparation was prepared and added separately to either the salt or kansui solution, as appropriate in the particular formulation.

A series of supplemented products was prepared in the laboratory and samples were analysed for the appropriate enzyme activity. Similar patterns of activity were obtained for the various fresh and dried noodles. In addition similar patterns were found for both white salted and yellow alkaline noodles.

The results demonstrate that, overall, considerable stability of all enzymes was recorded for both types of Asian noodles studied here. The similarities in the results for these two styles of noodles indicate that for enzymes studied here the pH of the noodles is not a primary determinant of enzyme stability. Whilst lower temperatures as compared to drying at 40°C caused no loss, it does appear that drying is a factor influencing the overall rate of enzyme loss in some cases.

10.9 General discussion and summary of results for enzymes in two styles of Asian noodles

The overall conclusions from the analyses of selected enzymes in the current study are that the levels of the enzymes flour are relatively low (with exception of POX). The incorporation of selected enzymes into Asian noodle formulations resulted in enhanced levels in the final fresh and dried products. The retention in the two styles of noodles was similar. Some losses occurred during drying of noodles.

Chapter 11

Results and discussion: the effect of exogenous α-amylases on the quality parameters of Asian noodles prepared in the laboratory: White salted noodles

The purpose of this chapter is to describe and discuss the results obtained during the preparation of noodles supplemented with α-amylases from barley malt or of bacterial origin. White salted noodles were made from P-Farina and Ultra White flours, these two flours were chosen as they had slightly lower protein content as preferred for this style of noodles. Noodle samples were supplemented with high amounts of α-amylases from either source and subsequently the impact of the addition was analysed. In particular noodle textural, structural and colour properties were studied.

11.1 Introduction

White salted noodles (WSN) are an important staple food in many countries around the world. WSN noodles are typically made using only flour, water and salt. None-the-less there is considerable variation in WSN, due to the characteristics of the raw materials used, product shape, as well as processing methods. This partially reflects varying regional preferences. One popular form which has received considerable attention is Japanese Udon which are softer and more elastic than WSN from other parts of Asia (Epstein et al 2002). The quality of WSN has various aspects: appearance, eating quality, taste and cooking properties (Toyokawa et al 1989). Colour and brightness are important, while eating quality is the primary criterion for consumer acceptance. Although preferences vary, generally softness, tenderness and some feeling of elasticity are desirable (Jeffers et al 1979) and these depend upon the protein content of the flour.

Whilst the significance of enzymes for bread quality has been researched in considerable detail, less is known of their role, influence and potential in noodle products. Accordingly the objectives of this study have been to examine the effect of selected exogenous α-amylases on textural and colour properties of WSN.

11.2 Levels of α-amylase in flour and stability during noodle processing

As expected, the flours contained low levels of α-amylase, as indicated by high Falling Number and confirmed by direct assay. The endogenous enzyme remained active during noodle preparation, with no losses under any of the storage conditions studied. This probably results from the presence of only salt as an ingredient that might influence enzyme stability. Accordingly the pH of the WSN samples was measured and found to be 6.1. These results are similar to those found when bacterial α-amylase was incorporated into yellow alkaline noodles (YAN). In that case the product pH is relatively high (10.05) due to the alkaline salts. While a resultant loss in activity might be expected, it was found that the level of bacterial α-amylase remained unchanged [see Chapter 12].

11.3 The effect of α-amylase on textural properties of WSN

When WSN were prepared, the addition of the two enzyme preparations resulted in similar patterns for the two flours. In preliminary studies, various addition levels of either of the α-amylase preparations were evaluated (Figs. 11.2 & 11.3) and texture measured using only the cylinder probe (P/45).

Table 11.1 Activity of α-amylase in control and supplemented noodle samples stored under different conditions and respective Falling numbers

Treatments[a]	α-Amylase (C/U)[b]	Falling Number (s)[c]
Controls		
Day1 WSN-PF	0.067 ± 0.002	N/A
Day1 WSN-UW	0.066 ± 0.002	N/A
24hr RT WSN-PF	0.067 ± 0.002	N/A
24hr RT WSN-UW	0.066 ± 0.002	N/A
24hr FR WSN-PF	0.067 ± 0.007	N/A
24hr FR WSN-UW	0.0738 ± 0.0002	N/A
Dried WSN-PF	0.22 ± 0.03	238 ± 6
Dried WSN-UW	0.21 ± 0.01	235 ± 12
Barley (72×10^2U / batch added)		
Fresh WSN-PF	4.62 ± 0.05	N/A
Fresh WSN-UW	4.68 ± 0.12	N/A
Dried WSN-PF	4.63 ± 0.06	62
Dried WSN-UW	4.67 ± 0.12	62
Bacterial (23KMWU / batch added)		
Fresh WSN-PF	3.06 ± 0.16	N/A
Fresh WSN-UW	3.18 ± 0.28	N/A
Dried WSN-PF	3.12 ± 0.29	62
Dried WSN-UW	3.09 ± 0.41	62

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour; UW, Ultra white flour; RT, storage at room temperature; FR, storage at 4°C, N/A, not applicable

b Unit for α-amylase is amount of enzyme, in the presence of excess thermostable α-glucosidase releasing one micromole of *p*-nitrophenol from BPNPG7 in one minute at 40°C (Ceralpha Units)

c Unit for Falling Number is second.

Table 11.2 Effects of α-amylases on the textural properties measured by TA-XT2 for WSN made from UW

Treatments[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.6^{a}	130^{a}	11.8^{a}	2.42^{a}
	barley	31.8^{b}	112^{b}	8.32^{b}	2.35^{a}
	bacterial	39.3^{ac}	128^{a}	10.53^{a}	2.44^{a}
	RT control	43.3^{a}	134^{a}	13.6^{a}	2.55^{a}
	barley RT	34.4^{b}	118^{b}	11.0^{a}	2.53^{a}
	bacterial RT	31.6^{b}	113^{b}	7.81^{b}	2.27^{a}
	FR control	44.5^{a}	136^{a}	12.1^{a}	2.44^{a}
	barley FR	30.4^{b}	110^{b}	9.72^{ab}	2.35^{a}
	bacterial FR	35.5^{bc}	120^{ab}	7.49^{b}	2.27^{a}
Cooked	control	8.8^{a}	33.3^{a}	3.54^{a}	0.70^{a}
	barley	5.5^{b}	27.1^{ab}	2.98^{a}	0.62^{a}
	bacterial	5.6^{b}	27.1^{a}	1.71^{b}	0.40^{b}
	RT control	9.2^{a}	34.8^{a}	3.54^{a}	0.70^{a}
	barley RT	4.5^{b}	18.5^{c}	2.16^{b}	0.47^{ab}
	bacterial RT	4.1^{b}	22.1^{bc}	0.98^{b}	0.27^{b}
	FR control	10.0^{a}	37.0^{a}	4.24^{a}	0.78^{a}
	barley FR	4.2^{b}	16.6^{c}	3.43^{a}	0.67^{a}
	bacterial FR	5.1^{b}	25.7^{b}	1.34^{b}	0.35^{b}
Dried[b]	control	11.9^{a}	39.5	6.01	0.99^{a}
	barley	9.20^{ab}	28.9^{a}	3.78^{a}	0.75^{ab}
	bacterial	7.80^{b}	32.5^{a}	2.57^{a}	0.50^{b}

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Table 11.3 Effects of α-amylases on the textural properties measured by TA-XT2 for WSN made from PF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.2[a]	128[a]	11.9[a]	2.40[a]
	barley	38.6[a]	127[a]	8.10[a]	2.22[a]
	bacterial	35.2[a]	115[b]	11.4[a]	2.50[a]
	RT control	38.1[a]	119[ab]	13.0[a]	2.51[a]
	barley RT	43.0[a]	136[a]	9.70[a]	2.40[a]
	bacterial RT	32.5[a]	110[b]	12.4[a]	2.61[a]
	FR control	39.4[a]	121[ab]	12.9[a]	2.50[a]
	barley FR	42.8[a]	136[a]	7.71[a]	2.21[a]
	bacterial FR	32.8[a]	111[b]	12.3[a]	2.60[a]
Cooked	control	9.10[a]	33.6[a]	3.30[a]	0.70[a]
	barley	6.42[b]	25.8[ab]	2.30[a]	0.52[ab]
	bacterial	4.01[b]	20.1[bc]	1.70[b]	0.40[ab]
	RT control	9.50[a]	33.9[a]	1.23[b]	0.30[b]
	barley RT	6.50[b]	27.2[abc]	1.90[b]	0.50[ab]
	bacterial RT	3.90[b]	21.4[bc]	1.70[b]	0.40[ab]
	FR control	9.91[a]	34.1[ab]	4.20[a]	0.81[ab]
	barley FR	6.20[b]	24.8[bc]	3.31[a]	0.70[ab]
	bacterial FR	4.52[b]	22.8[bc]	1.80[b]	0.42[ab]
Dried[b]	control	9.90[a]	34.1[a]	4.25[a]	0.81[a]
	barley	9.50[a]	33.9[a]	5.70[a]	1.01[a]
	bacterial	7.54[a]	31.3[a]	2.11	0.52[a]

a Abbreviations are: WSN, white salted noodles; PF, P- Farina flour; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

11.4 α-Amylase from barley malt

The starting point of addition was 3.60×10^2U per batch of noodles. This level was chosen as it was estimated to provide levels of α-amylase approximately 20 times higher than the endogenous activity measured in the flour. Higher rates of incorporation were also used (18×10^2U, 36×10^2U and 72×10^2U per 200g flour). The highest level of addition showed the greatest difference between the control and supplemented treatments when assessed visually and also in terms of textural characteristics measured instrumentally. Accordingly this level was chosen for further experimentation.

Subjective assessment indicated differences with enzyme supplemented noodles feeling lighter and softer than controls. These observations were supported by texture analysis using the TA-TX2. For this, two approaches were used and the results are presented in Tables 11.2 & 11.3. In all cases the noodles supplemented with enzyme were softer than the controls. In comparing the effect of different storage temperatures, no significant difference was found in textural characteristics. This indicates that the enzyme effects are minimal as it might be expected that the higher activity at elevated temperatures would have greater impact. Enzyme assay data demonstrated no loss in activity during storage, even during drying at 40^{o}C for 30hr (Table 11.1).

Greater textural differences were seen for cooked samples, and in all cases enzyme supplemented noodles were significantly softer than the controls. Again storage conditions appeared to have no impact on texture. Texture was analyzed by the two different procedures: noodle firmness (blade / cutting attachment) and hardness (a flat cylinder probe P/45). Similar observations were made and prior to cooking, noodles were softer for products incorporating either of the enzyme supplements. More pronounced effects were seen for cooked noodles treated with either of the enzymes. These had softer textural characteristics and differences were particularly obvious at time zero and after storage at 4°C (Tables 11.2 & 11.3). The measurements taken in the two approaches were positively correlated (r^2 vales were 0.97 and 0.93 for UW and PF flour respectively) hardness as measured using the flat cylinder probe versus firmness of noodles as measured using the blade attachment of the TA-XT2 (Fig 11.1).

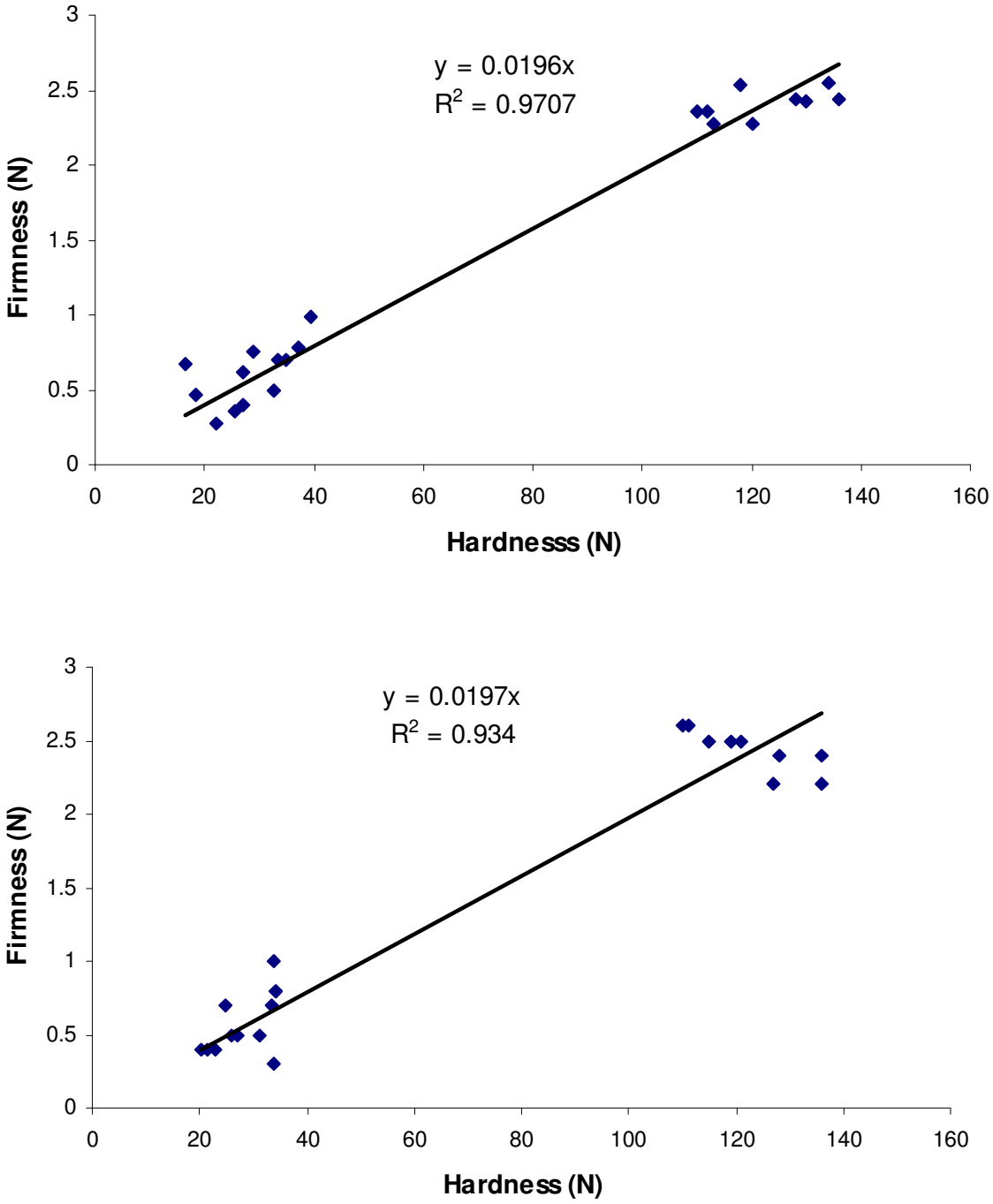


Fig 11.1 Correlation between hardness and firmness of WSN

Data based on the mean values of firmness and hardness measured with the TA-XT2 on cooked and dried and cooked WSN (made from UW and PF, controls and amylase treatments)

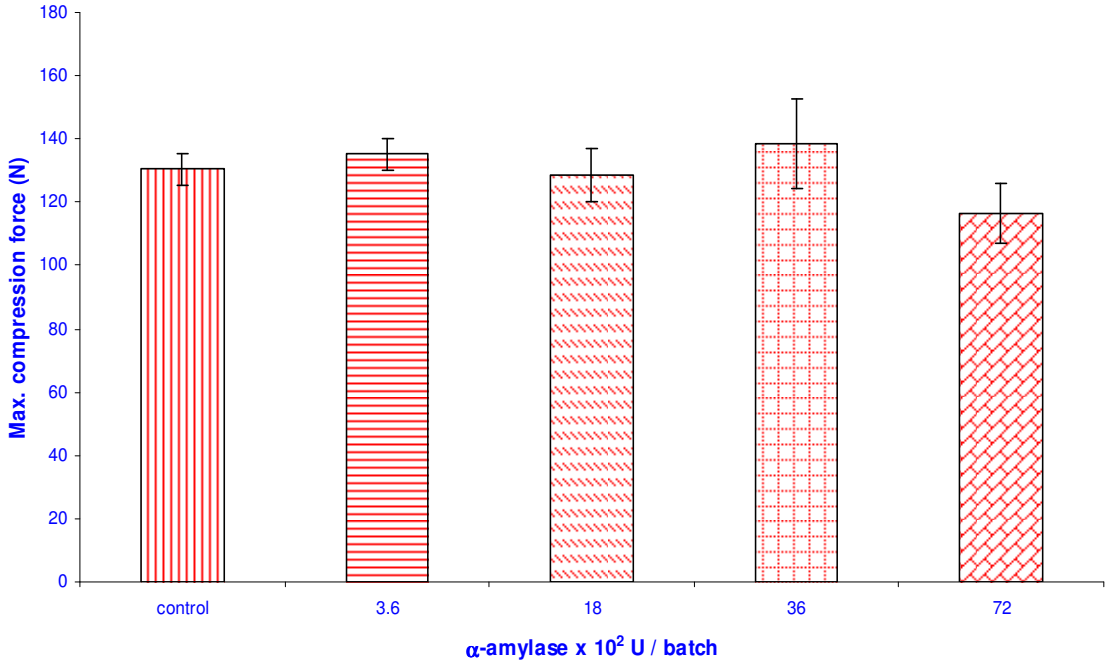


Fig 11.2 Hardness characteristics of fresh raw WSN (made from UW) prepared with varying levels of α-amylase from barley malt
Error bars represent standard deviation values. Measurements were taken with the flat cylinder probe (P/45)

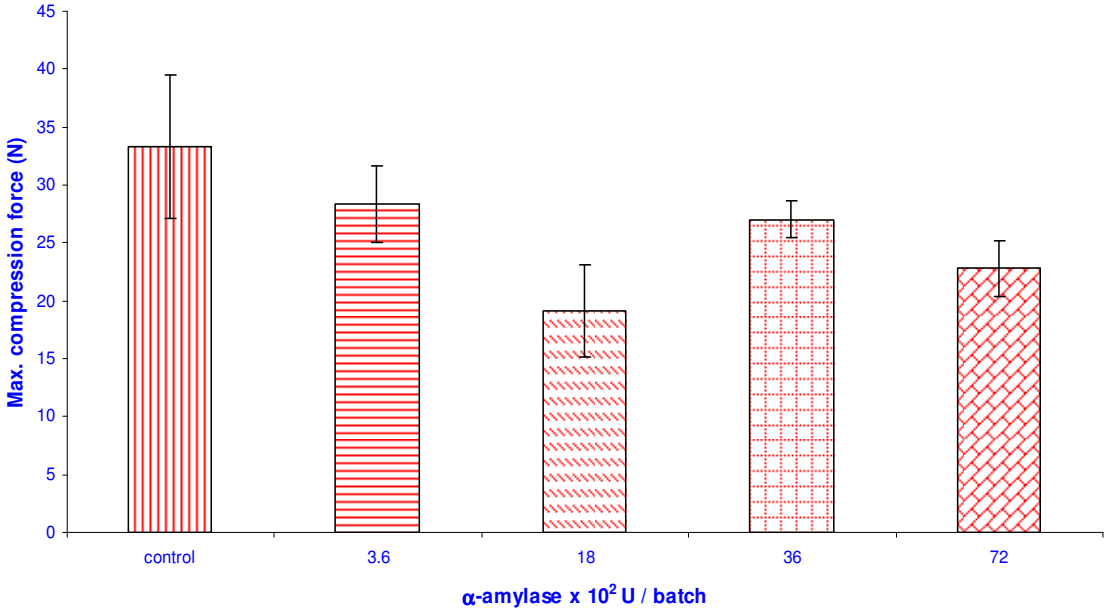


Fig 11.3 Hardness characteristics of fresh cooked WSN (made from UW) prepared with varying levels of α-amylase from barley malt
Error bars represent standard deviation values. Measurements were taken with the flat cylinder probe (P/45)

11.5 α-Amylase of bacterial origin

Different levels of addition were compared (Fig 11.3). Even with the lowest amounts of bacterial α-amylase, raw noodle strands were visibly different from controls appearing transparent and of very light yellow colour. They also felt relatively soft and light on touch. These observations were supported by the TA-XT2 analysis with both attachments providing lower values of firmness and hardness for the treated samples (Tables 11.2 & 11.3). When noodles incorporating the lowest level of enzyme were cooked the noodle strands felt stickier than the control, tending to adhere to each other and were softer (Tables 11.4 & 11.5). At higher levels of addition, strands either disintegrated or formed an amorphous mass similar in appearance to a gluten ball and it was impossible to separate the strands.

There was a general pattern that following storage at room temperature, treated noodles were softer than the controls and also that room temperature storage resulted in softer noodles than refrigeration. In addition, where drying was used, firmer product textures were observed. The impact of amylases on textural attributes was partially counteracted by the inclusion of the drying step.

Bean et al 1974 reported that in the case of raw Japanese noodles α-amylase activity might be limited by the small amount of mechanically damaged starch available to act as a substrate, thus only minor effects of α-amylase on dough properties might be expected (Bean et al 1974). However, in the current study, textural differences were seen especially when bacterial α-amylase was incorporated into the noodle formulation. This might either indicate that even low amounts of mechanically damaged starch are sufficient for this enzyme to impact on the texture. It is also possible that some enzyme other than amylase might be present and be influencing the noodle attributes, as it is unlikely that the enzyme preparations are completely devoid of other activities.

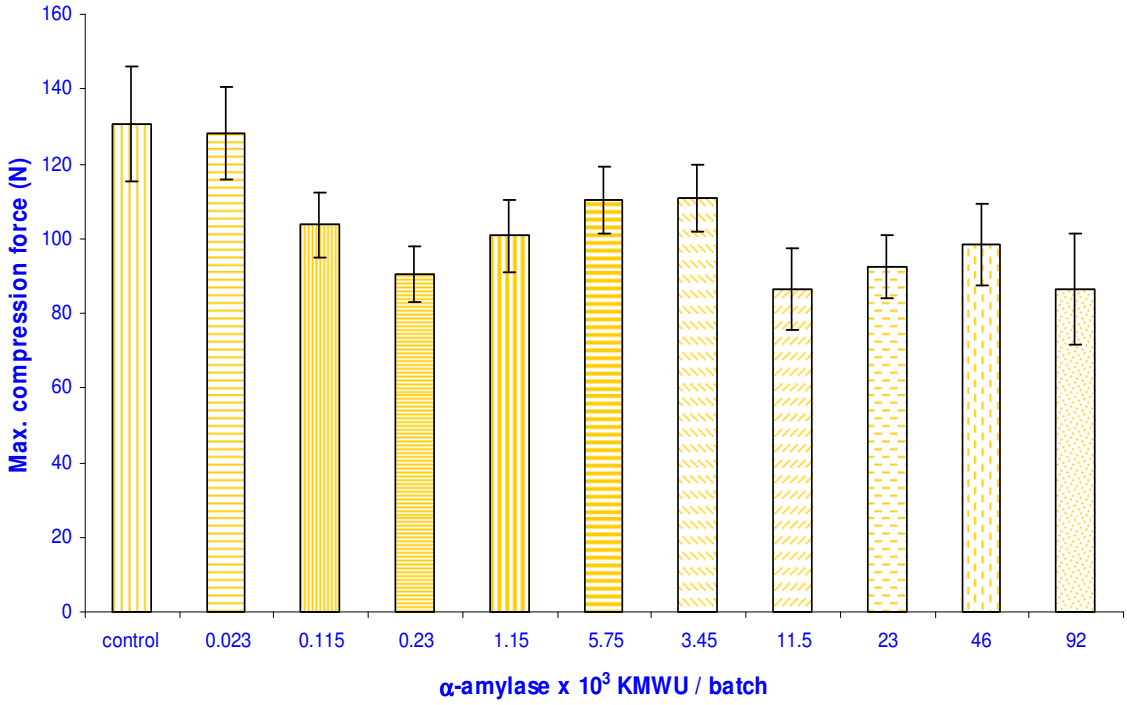


Fig 11.4 Hardness characteristics of fresh raw WSN (made from UW) prepared with varying levels of α-amylase of bacterial origin
Error bars represent standard deviation values. Measurements were taken with the cylinder probe (P/45)

11.6 The effect of α-amylase on cooking yield

For the various treatments water absorption and cooking loss were also determined (Tables 11.4 & 11.5). Significantly higher cooking losses were observed in noodles treated with bacterial α-amylase compared to both control samples and noodle treated with barley α-amylase. This was found to be true for both fresh and dried noodles. The differences in water uptake and more importantly in yield upon boiling might partially explain the textural characteristics of cooked noodles treated with either of the enzyme preparations.

Table 11.4 The impact of α-amylase addition on cooking quality of WSN made from UW flour

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	2.3	58.7[a]	6.93[a]
fresh barley	2.3	54.8[a]	9.04[a]
fresh bacterial	2.3	44.1	19.3
dried control	6.3	70.8[a]	8.84
dried barley	6.3	67.8[a]	14.47
dried bacterial	6.3	60.1	27.5

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour

Table 11.5 The impact of α-amylase addition on cooking quality of WSN made from PF flour

Sample [a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	2.3	56.32[a]	6.38[a]
fresh barley	2.3	53.65[a]	9.41[a]
fresh bacterial	2.3	44.13	20.39
dried control	6.3	70.25[a]	8.94[a]
dried barley	6.3	68.82[a]	12.32[a]
dried bacterial	6.3	61.16	26.85

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour

11.7 Relationship between α-amylase and colour properties of WSN

Discolouration of noodles sheets occurred for all samples including controls (Figs. 11.5 – 11.8). In each case the changes observed did not result in the development of speckiness. Darkening happened at a faster rate and was observed earlier in noodles stored at 25°C as compared to those stored at 4°C. In addition, these effects were accentuated in both enzyme treatments. Control and barley treatments were yellow and dark yellow respectively at time zero and after 96 hours the colour became even darker

yellow to brown. On the other hand bacterial α-amylase treatments were clear yellow and became darker (more yellow) but were transparent from preparation and throughout storage.

The colour attributes of boiled noodles were measured when fresh products were cooked immediately following preparation. The colour of dried noodles was also measured (Figs. 11.9 & 11.10) and there was considerable variation in the colour values for the various treatments. Noodles made with α-amylase from barley malt were found to be darker, by visual observation than either control samples or noodles made with bacterial α-amylase. This was supported by instrumental readings and similar observations were made for dried noodles (Figs. 11.9 & 11.10).

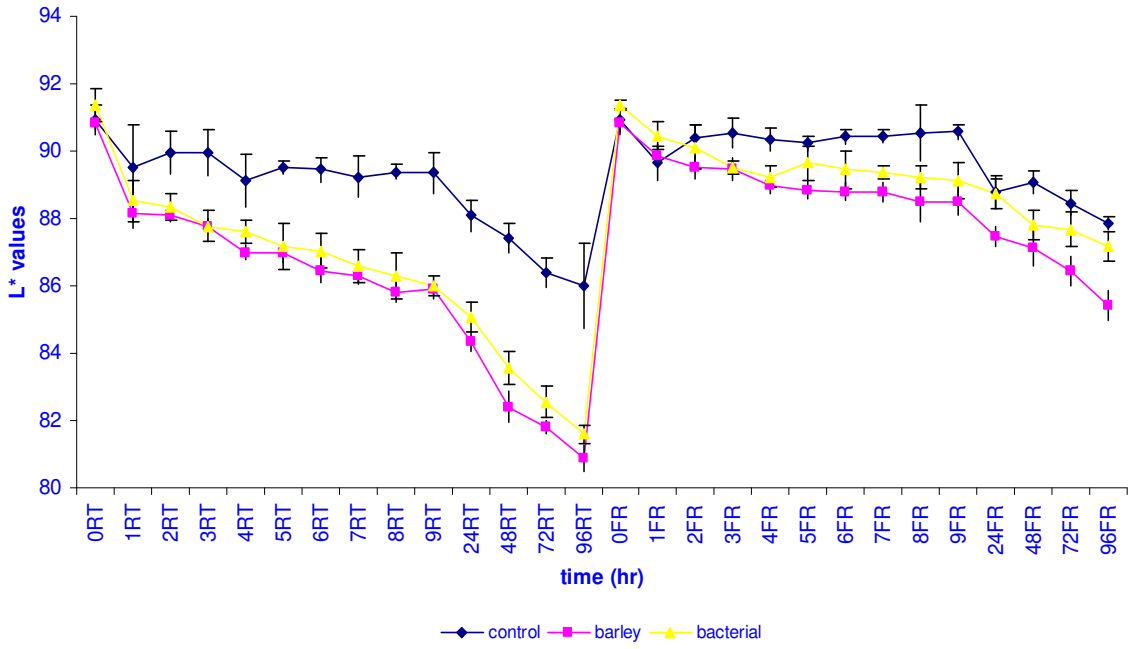


Fig 11.5 Colour characteristics (L* values [whiteness]) of WSN made from PF stored at 25°C and 4°C
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

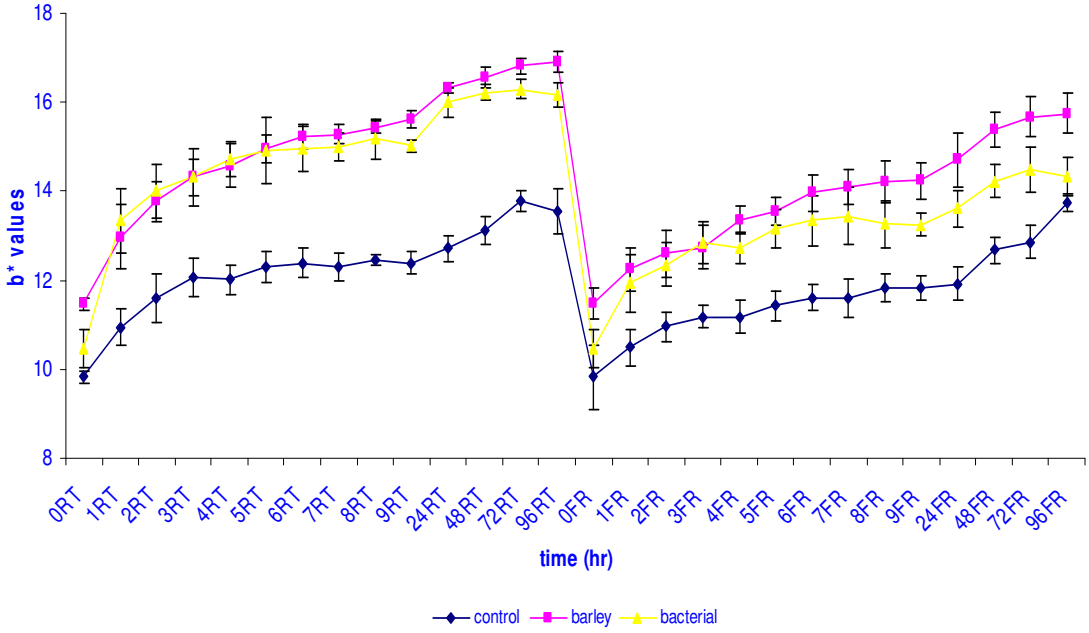


Fig 11.6 **Colour characteristics (b* values [yellowness]) of WSN made from PF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

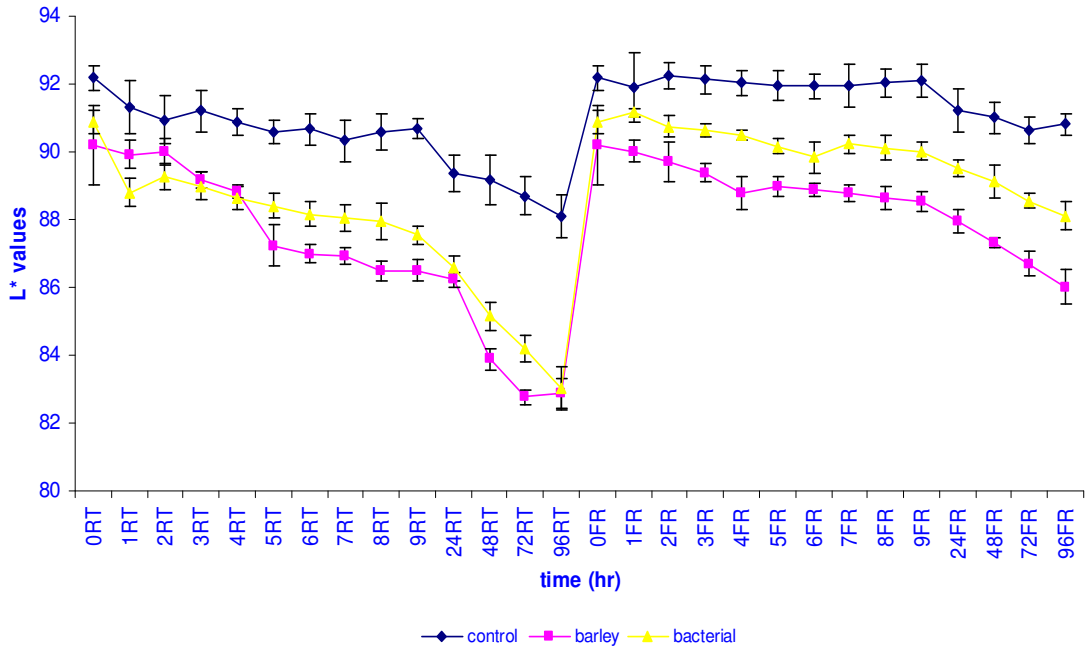


Fig 11.7 **Colour characteristics (L* values [whiteness]) of WSN made from UW stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

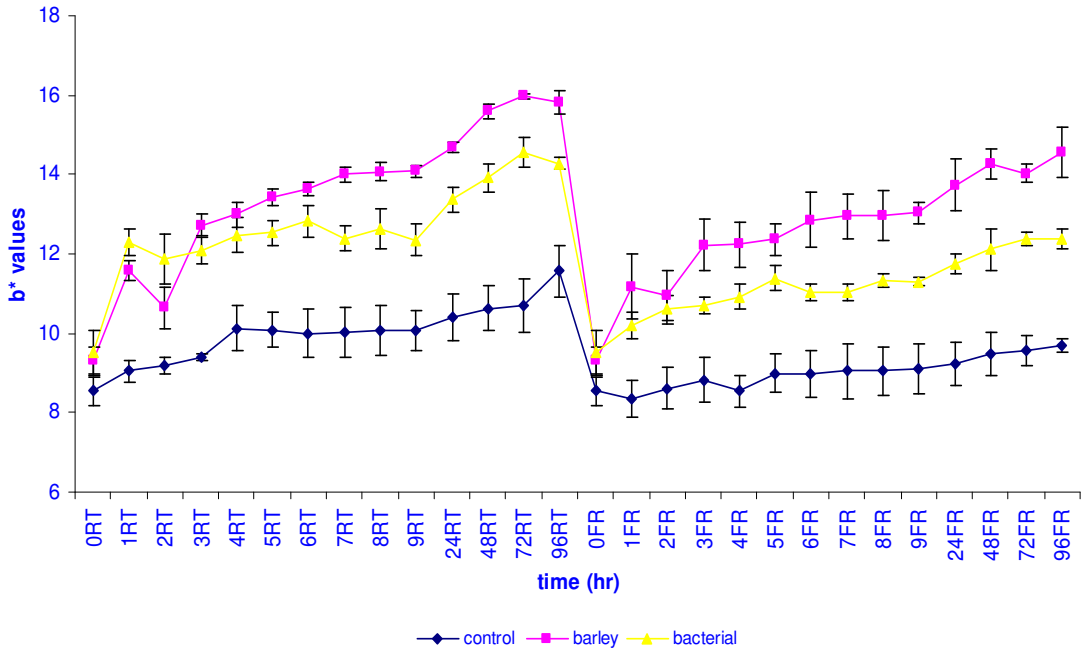


Fig 11.8 **Colour characteristics (b* values [yellowness]) of WSN made from UW stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

Fig 11.9 **Colour characteristics (L* whiteness / brightness) of dried & fresh cooked WSN made from PF & UW**
Error bars represent standard deviation values

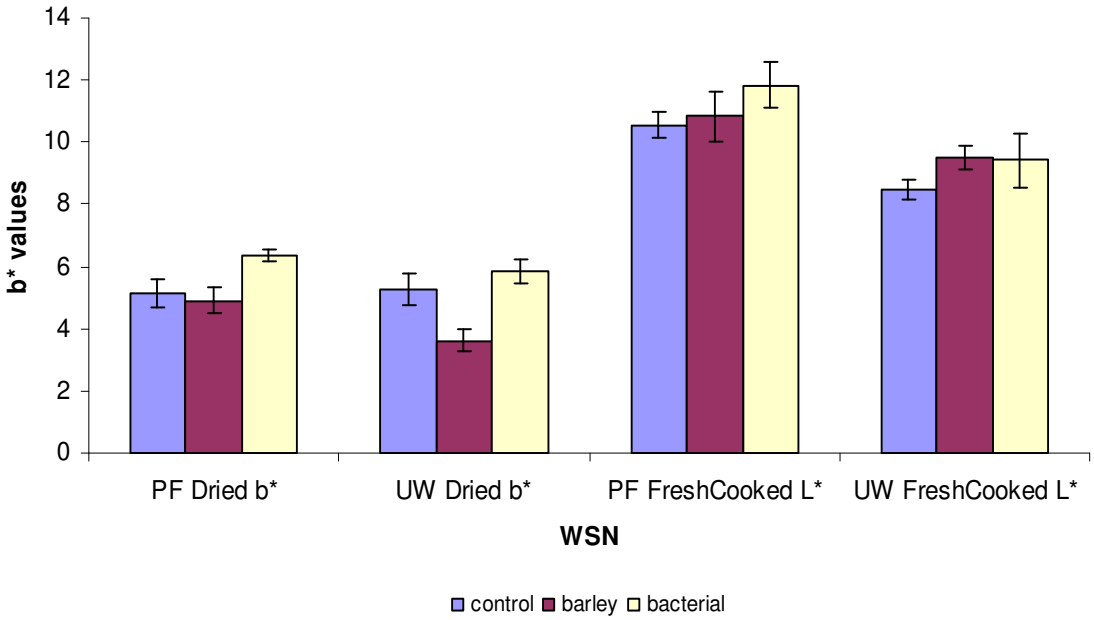


Fig 11.10 Colour characteristics (b*values yellowness) of dried & fresh cooked WSN made from PF & UW
Error bars represent standard deviation values

11.8 Structural properties of WSN

The surface structure of raw noodles was studied using the ESEM mode of an electron microscope. In the case of all samples of raw noodles granular starch can be clearly seen, although it appeared more disrupted in noodles incorporating α-amylase of bacterial origin (Fig 11.11). The control showed compact, lenticular to circular shaped starch granules. When looking at the samples treated with α-amylase from barley malt it can be seen that slightly amorphous and not as compact images are obtained. Noodles incorporating α-amylase of bacterial origin showed even more hollow structure with many smaller starch granules interspersed with gel-like regions. This is consistent with some of the starch granules having been hydrolyzed or damaged by α-amylase.

Upon cooking most of the starch gelatinises and therefore the granular appearance is no longer visible. Some evidence of granular structure remains in control samples, where fairly evenly hollow pockets can be seen. Relatively larger pockets in the network can be seen in samples treated with α-amylase from barley malt. Again the greatest difference among the treatments was seen in noodles incorporating α-amylase of bacterial origin. Here, relatively large pockets can be seen while the whole structure appears disrupted and not as compact as either control or samples treated with α-amylase from barley malt (Fig 11.11). The images of dried and dried and then cooked WSN are shown in Fig 11.12. Here the difference between the treatments is not as visible, indicating less change occurred when noodles were dried immediately after preparation. When looking at the images of dried noodles taken using the SEM mode of an electron microscope, compact, lenticular to circular shaped starch granules can be seen in all samples, perhaps with slight starch gelatinisation already happening in the sample treated with bacterial α-amylase where more starch paste can be seen between smaller starch granules. Upon cooking, however, in the control samples there is still visible evidence of granular form of starch, while in the barley and bacterial α-amylase treatments all of the starch appears completely gelatinised and it is hardly visible across the protein network.

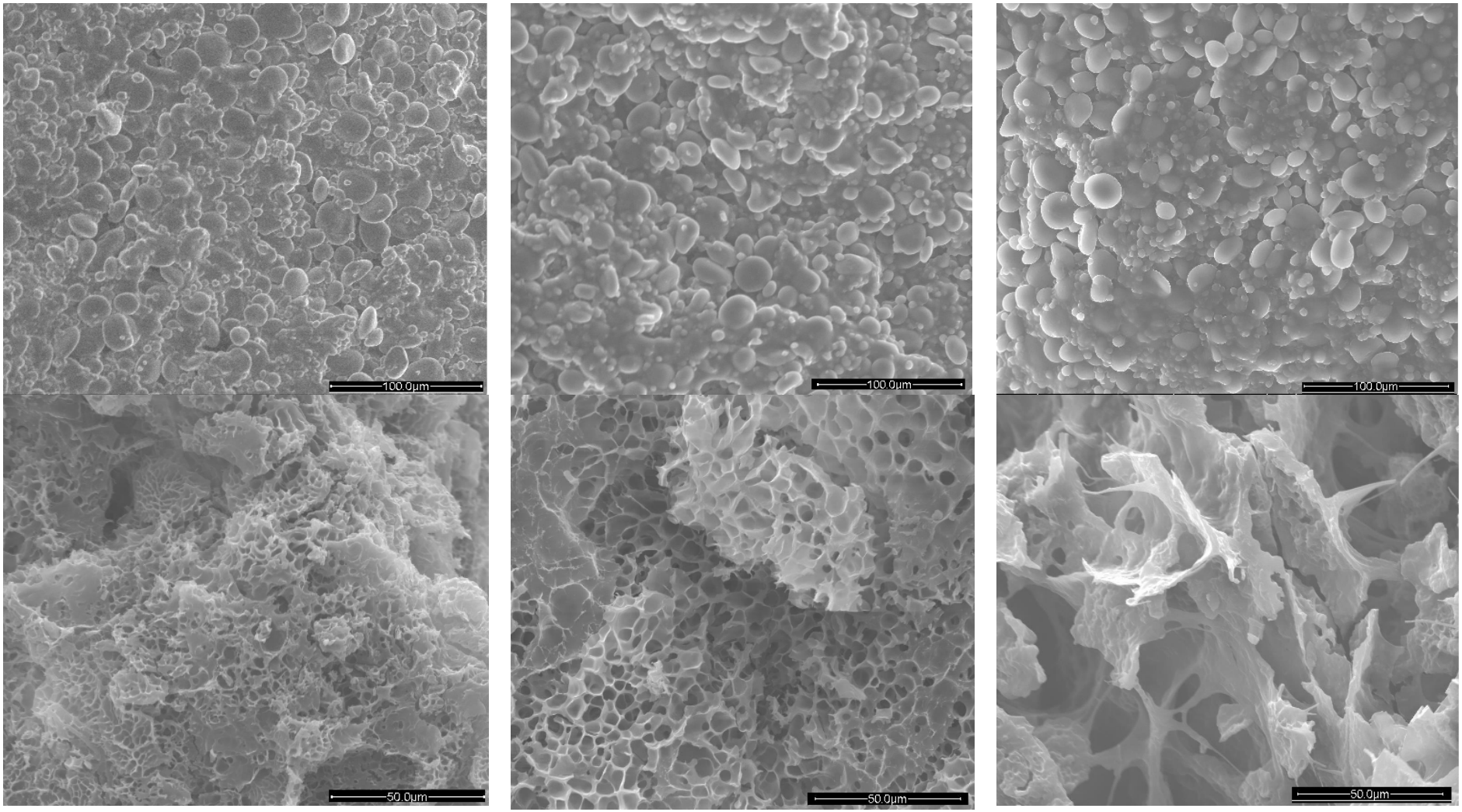


Fig 11.11 **ESEM[a] images of raw noodles [control, barley and bacterial treatments, from left to right (top)] & SEM[b] images of cooked noodles [control, barley and bacterial treatments, from left to right (lower)]**

a ESEM, 6.0 Torr, 4°C, 800× Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 600× Mag., 5.0 spot size, 30kV

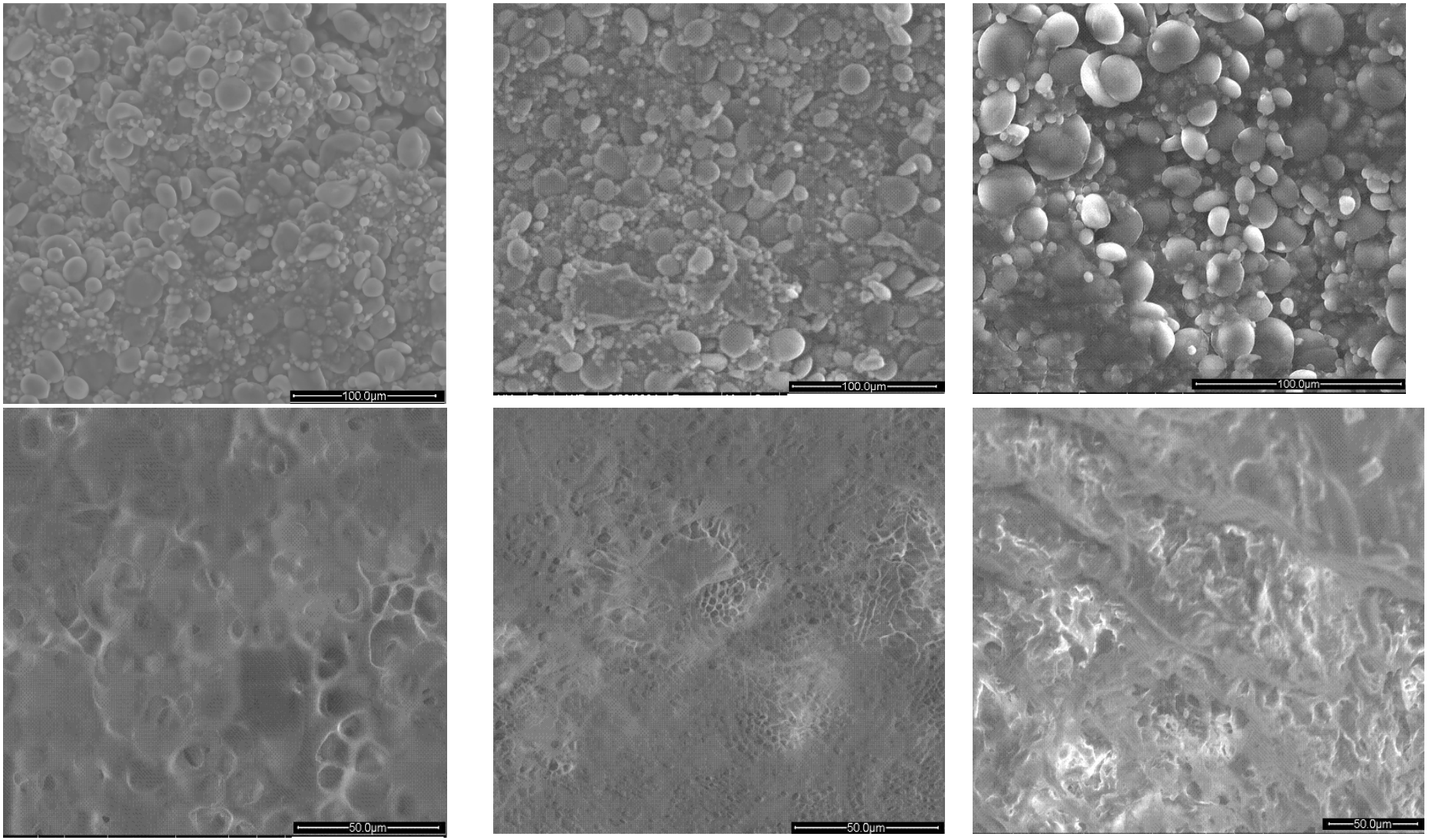


Fig 11.12 SEM[a] images of dried (top) and dried cooked (lower) WSN noodles control barley and bacterial treatments (from L to R)

a SEM, 0.5 Torr, 23°C, 800× Mag. (top); 600× Mag.(lower), 5.0spot size, 30kV

11.9 Summary of results

When α-amylase preparations sourced from a bacterium and from barley were incorporated into a WSN formulation, the product firmness and hardness characteristics were altered, particularly at higher levels of addition. Directly comparing enzyme activity levels is difficult due to the differing characteristics of enzymes. However, when similar amounts of the two amylases were directly compared, the bacterial enzyme had more obvious effects on both the raw and the cooked noodles. At lower levels of addition, particularly for the barley enzyme, the enhancement of the product softness may be of practical significance.

The assessments of texture obtained here using two different probes do provide measurements indicating that enzyme incorporation results in some softening. A reasonable level of correlation was found between primary measurements from the two approaches.

The changes observed in colour characteristics during storage probably reflect the presence of enzymes activities other than α-amylase. The similarities in colour for treated and control products after cooking indicates that there is potentially adverse impact of amylase on colour whilst textural enhancement was observed for noodles cooked immediately following preparation and also for those dried prior to cooking.

ESEM & SEM images revealed that α-amylase of bacterial origin might have greater impact upon starch than α-amylase from barley malt, as more of the starch appeared to be damaged or hydrolyzed in both raw and cooked samples. While samples incorporating α-amylase from barley malt showed fairly similar images to the control with perhaps more amorphous structure, greater changes to the structural appearance were seen in samples incorporating α-amylase of bacterial origin. The addition of bacterial α-amylase resulted in high cooking losses having an adverse impact on the cooking quality of WSN.

Pre-harvest sprouting of wheat results in rapid synthesis of α-amylase. It has been thought that this enzyme plays a primary role in rendering sprouted wheat unsuitable for

noodle manufacture. In the current study, the addition of the exogenous amylases at levels that resulted in Falling Number values of 62 did not necessarily result in difficulties in handling or processing of the noodles. In addition, the textural characteristics of the noodles were enhanced despite the incorporation of amylase, particularly from barely.

It is concluded that the action of α-amylase is not the primary factor influencing texture of WSN prepared from flour milled from sprouted wheat. In addition the results here show that α-amylase incorporation has the potential to enhance product quality if other enzyme activities are not present to influence colour or appearance. Barley amylase probably offers greater prospects for textural enhancement.

Further studies are warranted to investigate the most suitable levels of addition of enzymes in conjunction with investigation of potential sources having appropriate purity characteristics. In addition, it is recommended that additional studies be made into the varying approaches to textural analysis available for assessment of Asian noodle products including sensory evaluation.

Chapter 12

Results and discussion: the effect of exogenous α-amylases on the quality parameters of Asian noodles prepared in the laboratory: Yellow alkaline noodles

The purpose of this chapter is to describe and discuss the results obtained during the preparation of yellow alkaline noodles made from Baker's strong flour and supplemented with α-amylases from barley malt or of bacterial origin.

12.1 Introduction

It is known upon that textural properties in addition to colour are the two most important quality properties of YAN (Miskelly 1984). The objectives of this phase of the study were to examine the textural, structural and colour properties of YAN when selected α-amylases have been added to the noodle formulation.

12.2 Levels of α-amylase in flour and stability during noodle processing

As indicated by the enzyme assay data and confirmed by the Falling Number test, the flours were sound and thus contained minimal amounts of α-amylase. The endogenous enzyme remained active during noodle preparation, with no losses under any of the storage conditions studied (Table 12.1). The pH of YAN was measured and found to be 10.05 thus some loss might have been expected under such high pH conditions.

Table 12.1 Activity of α-amylase in control and supplemented YAN samples stored under different conditions and respective Falling numbers

Treatment[a]	α-Amylase (C/U)[b]	Falling Number (s)[c]
Controls		
Day1	0.106 ± 0.003	N/A
24hr RT	0.110 ± 0.005	N/A
24hr FR	0.110 ± 0.005	N/A
Dried	0.10 ± 0.03	370 ± 16
Barley (72×10^2 U / batch)		
Day1	0.12 ± 0.01	N/A
Dried	0.12 ± 0.03	363 ± 13
Bacterial (23×10^2 KMWU / batch)		
Day1	1.51 ± 0.32	N/A
Dried	1.48 ± 0.25	146 ± 4

a Abbreviations are: YAN, yellow alkaline noodles; RT, storage at room temperature; FR, storage at 4°C, N/A, not applicable

b Unit for α-amylase is amount of enzyme, in the presence of excess thermostable α-glucosidase releasing one micromole of *p*-nitrophenol from BPNPG7 in one minute at 40°C (Ceralpha Units)

c Unit for Falling Number is second.

12.3 The effect of α-amylase on textural properties of YAN

In preliminary experiments a series of levels of α-amylases were tried, and for this, textural properties were measured using only the flat cylinder probe (P/45). For α-amylase from barley malt only at higher levels were any changes observed, while α-amylase of bacterial origin had an adverse impact on the textural properties of YAN even when relatively small amounts were added to the formulation.

12.4 α-Amylase from barley malt

The highest level of α-amylase from barley malt addition to the WSN (72×10^2U per batch of noodles, i.e. 200g of flour) tried in this study, was used for YAN. This level of addition showed greater differences (for cooked noodles) between the control and the enzyme treated noodles when assessed visually and also in terms of textural characteristics measured with the TA-XT2 (Figs 12.1 & 12.2). While in raw noodles

even at these higher levels no impact on the activity of α-amylase was measured (Table 12.1). In the case of raw there was no obvious difference measured with either of the two attachments evaluated. This indicates that in the raw form, supplementation with α-amylase from barley malt did not have an adverse effect on the texture of YAN in terms of hardness or firmness. In some instances cooked noodles treated with α-amylase from barley malt appeared slightly softer in terms of hardness (property measured using P/45) when compared to the control, but these were not significantly different from the control in terms of noodle firmness (measured using the blade). In addition when comparing the effect of different storage temperature, no significant difference was found, although it might have been expected that the enzyme would show more activity at higher temperatures (Figs 12.1 & 12.2; Table 12.2). Drying of noodles seemed to have no impact on the activity of α-activity (Table 12.1).

The relatively little or no changes between controls and noodles supplemented with α-amylase from barley malt can be explained by the fact that most of the α-amylase appeared to be inactivated, probably due to relatively high pH which is due to the kansui used in the formulation. Both the Falling Number values obtained for dried noodles and the results of the enzyme assays on supplemented samples suggest that there was no activity after processing, as compared to the samples treated with bacterial α-amylase (Table 12.1).

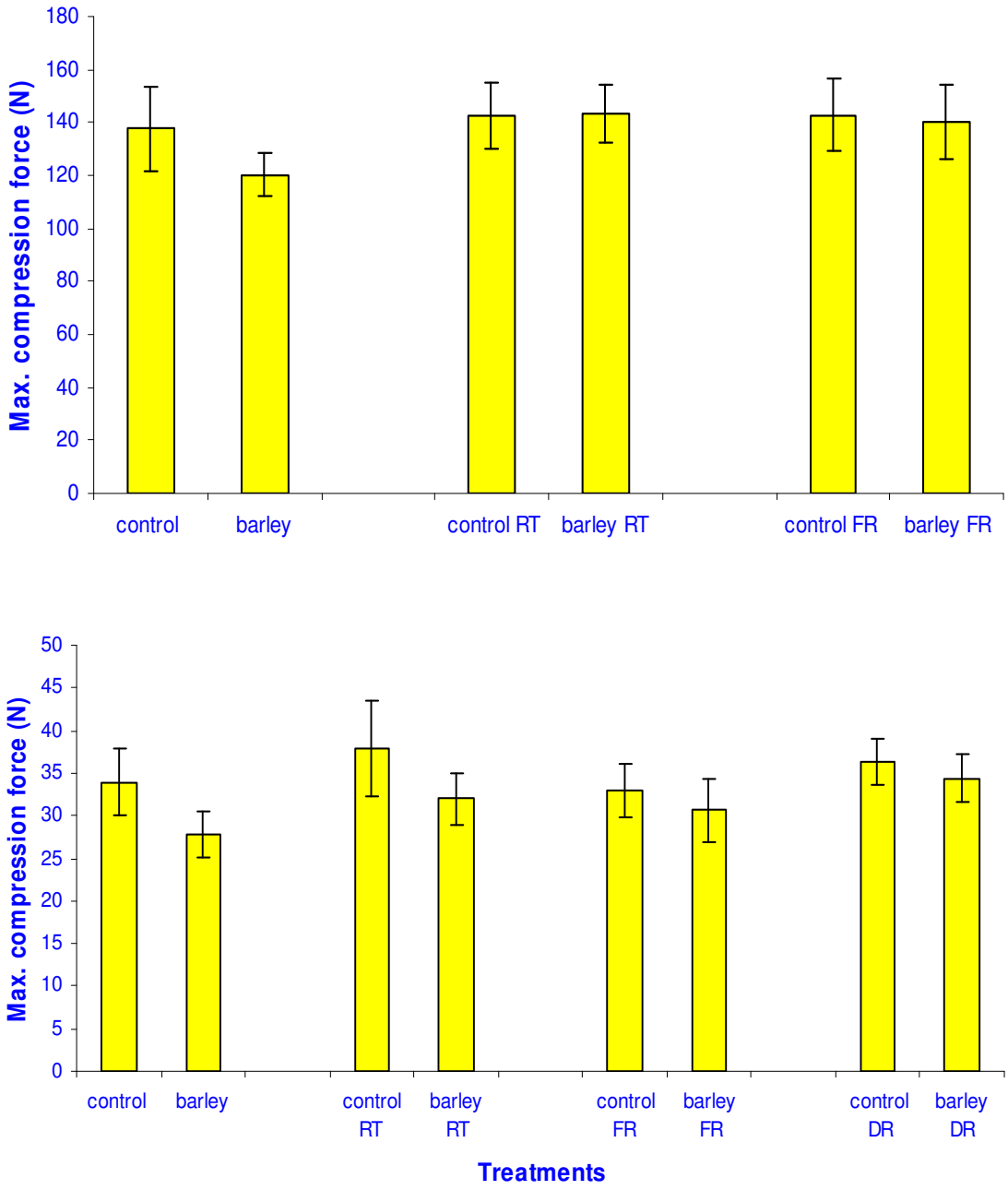


Fig 12.1 **Hardness characteristics of fresh raw (top) and cooked (bottom) YAN treated with α-amylase from barley malt. Impact of various levels of the enzyme on the textural properties of YAN**

Error bars represent standard error values. Measured using the flat cylinder probe (P/45)

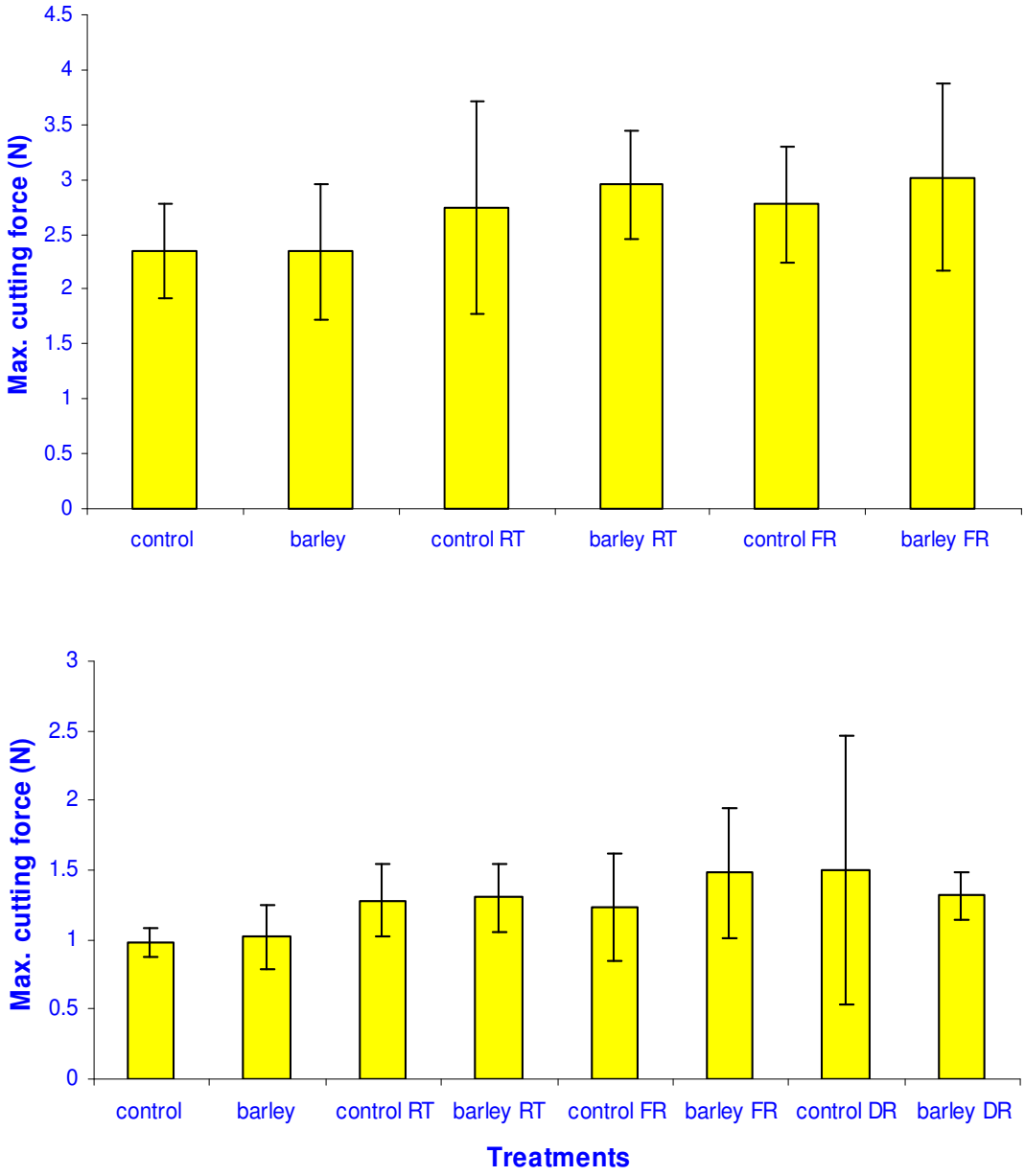


Fig 12.2 **Firmness characteristics of fresh raw (top) and cooked (bottom) YAN treated with α-amylase from barley malt. Impact of various levels of the enzyme on the textural properties of YAN**

Error bars represent standard error values. Measured using the blade

12.5 α-Amylase of bacterial origin

Different levels of addition were tried and even the lowest amounts of bacterial α-amylase added to the noodle formulation, samples were visibly different from controls (transparent and very light / pale yellow in colour) feeling very soft on touch. These observations were supported by the TA-XT2 analysis for cooked samples only. As for noodles treated with α-amylase from barley malt, in the raw form treatment with α-amylase of bacterial origin gave samples not distinctly different from controls (Figs 12.3 & 12.4). Higher levels of addition (higher than 2.3×10^3KMWU per batch) resulted in product disintegration during the cooking process. Noodles either 'dissolved' in water or formed a structure similar to a gluten ball at lower levels of enzyme addition and it was not possible to separate them into strands. In terms of the storage conditions, it was observed that storage at 4°C resulted in slightly firmer and harder noodle strands as compared to the storage at 25°C (Figs 12.3 & 12.4; Table 12.2).

The correlation investigated between the two methods used to evaluate textural properties of YAN here was found to be relatively high. An r^2 value of 0.81 was obtained when firmness was plotted against hardness (Fig 12.5), indicating that both methods are giving relatively reliable results in measuring the textural properties of Asian noodles.

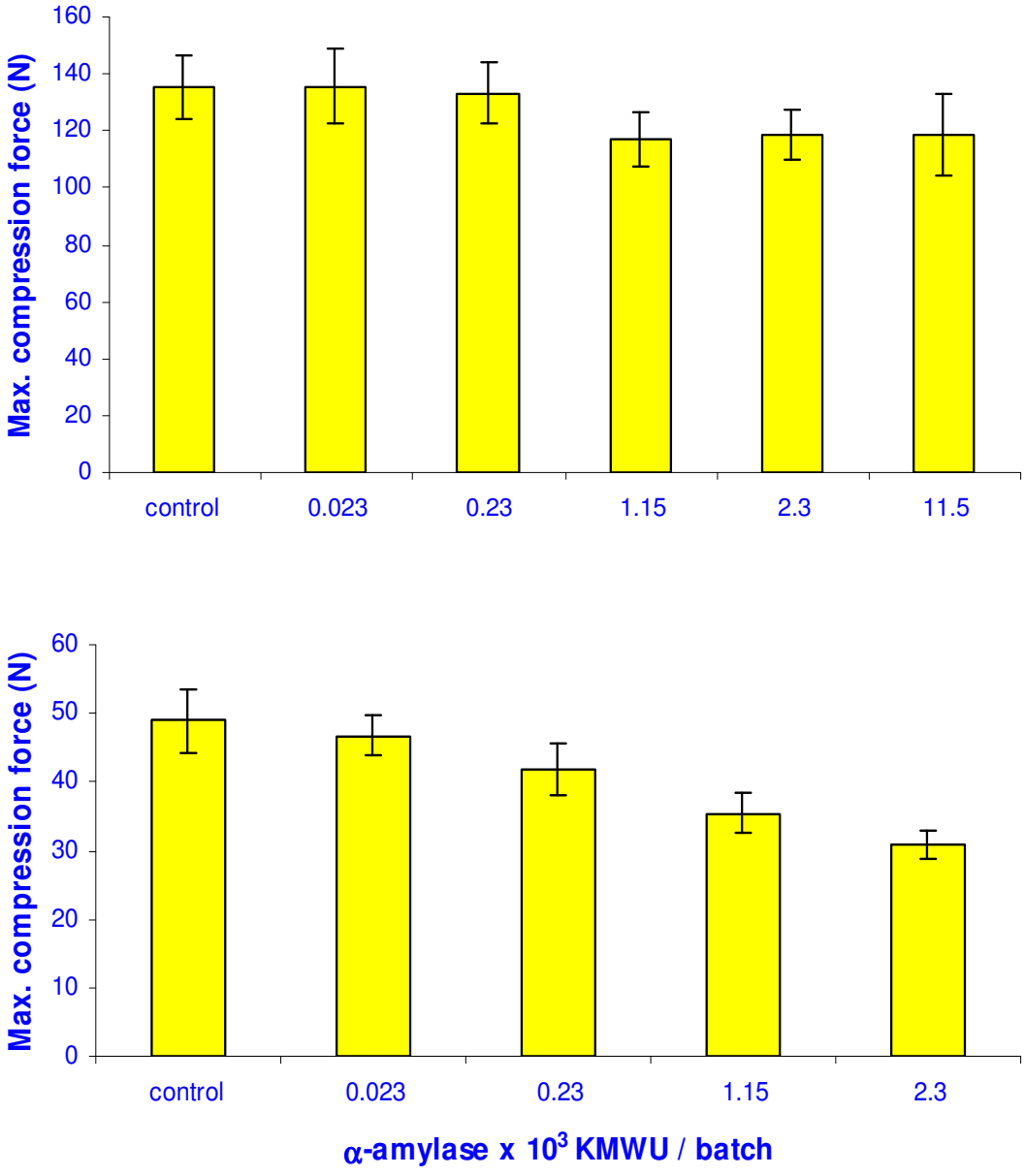


Fig 12.3 **Hardness characteristics of fresh raw (top) and fresh cooked (bottom) YAN treated with α-amylase from bacterial origin. Impact of various levels of the enzyme on the textural properties of YAN**
Error bars represent standard error values. Measured using the flat cylinder probe (P/45)

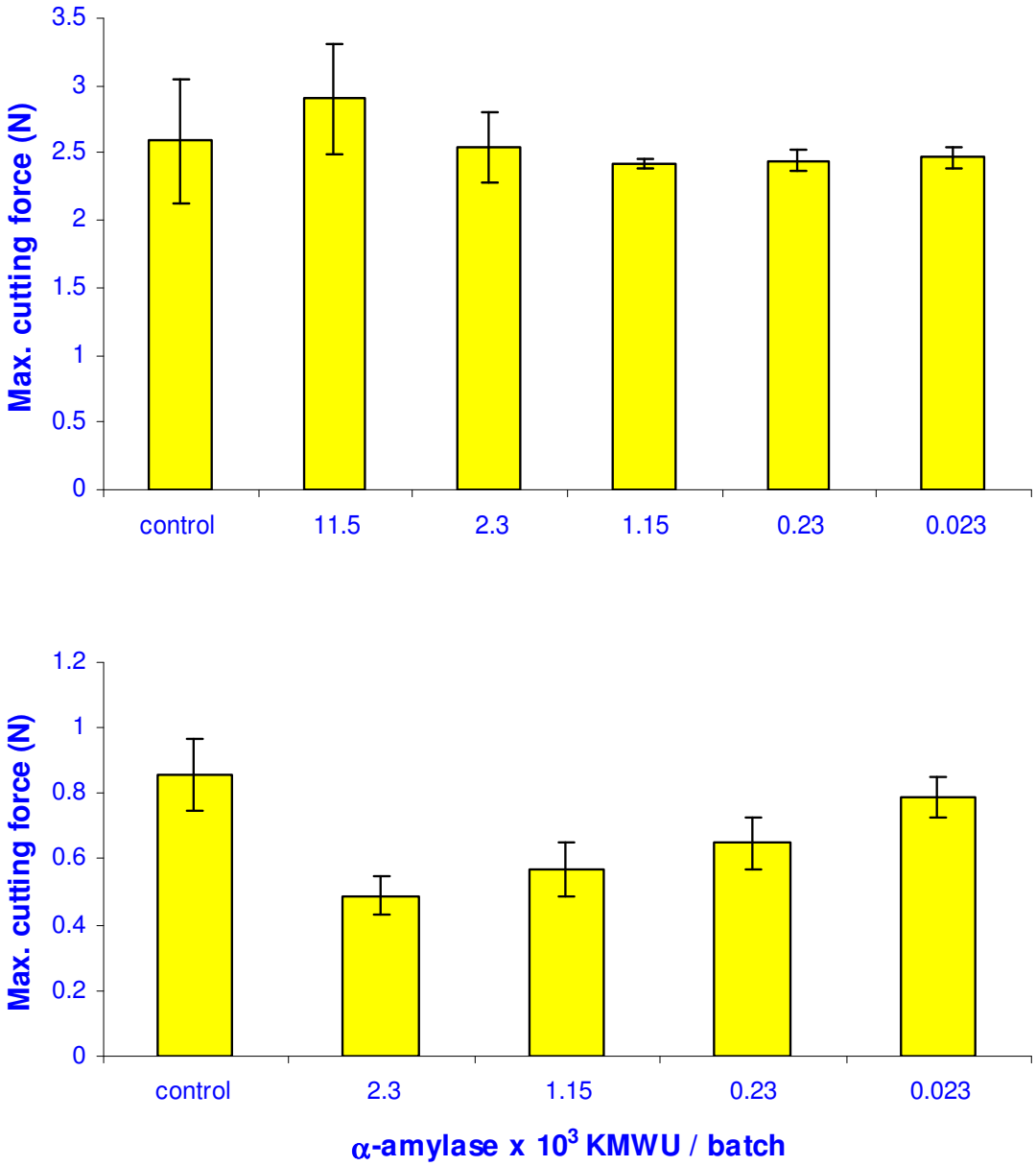


Fig 12.4 **Firmness characteristics of fresh raw (top) and fresh cooked (bottom) YAN treated with α-amylase from bacterial origin. Impact of various levels of the enzyme on the textural properties of YAN**
Error bars represent standard error values. Measured using the blade attachment

Table 12.2 Effects of α-amylases on the textural properties of YAN

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	36.1[a]	125[a]	11.0[a]	2.59[a]
	barley	35.3[a]	120[a]	9.18[a]	2.34[a]
	bacterial	36.2[a]	123[a]	10.6[a]	2.54[a]
	RT control	40.1[a]	132[a]	12.4[a]	3.01[a]
	barley RT	45.7[a]	143[b]	12.7[a]	2.05[a]
	bacterial RT	30.3[a]	124[a]	11.9[a]	2.84[a]
	FR control	39.5[a]	128[a]	11.1[a]	2.66[a]
	barley FR	44.8[a]	140[b]	11.7[a]	3.02[a]
	bacterial FR	32.1[a]	115[a]	12.8[a]	3.02[a]
Cooked	control	10.6[a]	39.7[a]	3.50[a]	0.86[a]
	barley	7.44[a]	27.8[b]	4.49[ab]	1.02[a]
	bacterial	6.38[a]	30.1[ab]	1.67[c]	0.49[a]
	RT control	10.7[a]	39.9[a]	4.58[ad]	1.02[a]
	barley RT	9.13[a]	32.0[ab]	6.55[bde]	1.30[b]
	bacterial RT	3.69[b]	19.5[c]	1.52[c]	0.44[a]
	FR control	13.4[a]	47.4	5.36[ae]	1.11[a]
	barley FR	8.69[a]	30.7[ab]	7.27[e]	1.48[b]
	bacterial FR	4.91[b]	23.3[bc]	2.49[ac]	0.62[a]
Dried [b]	control	12.4[a]	42.9[a]	5.84[a]	1.25[a]
	barley	10.1[a]	34.3[b]	6.31[a]	1.32[a]
	bacterial	11.4[a]	39.6[ab]	4.39[a]	0.81[a]

a Abbreviations are: YAN, yellow alkaline noodles; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum

Note: Means followed by the same letter are not statistically different ($p<0.05$) within the same column and treatment (raw-cooked-dried)

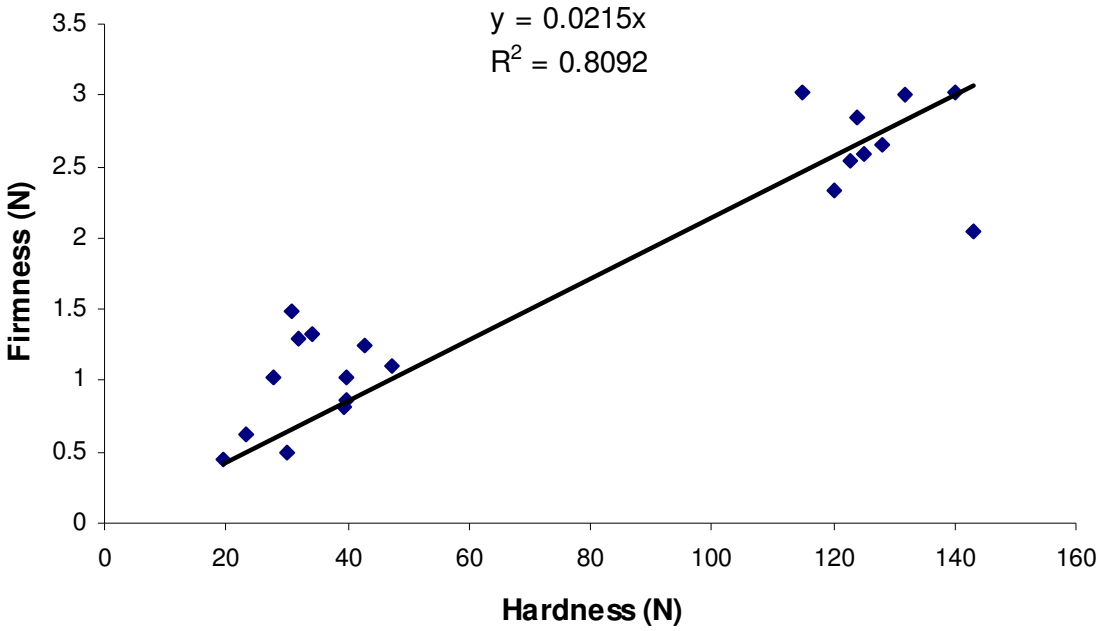


Fig12.5 Correlation between firmness and hardness measurements using the TA-XT2

Data based on the mean values for firmness and hardness measurements of YAN (made from BGF, controls and amylase treatments). Cooked and dried and cooked YAN

12.6 The effect of α-amylase on cooking yield

Water absorption and cooking loss values of YAN treated with either amylase were also determined (Table 12.3). High cooking losses were recorded for noodles treated with the bacterial α-amylase. This was the case for both fresh and dried noodles (Table 12.3). These differences in water uptake and more importantly differences in yield lost upon boiling might partially explain the differences seen in textural characteristics of cooked noodles treated with the enzyme preparations.

Table 12.3 Cooked weight and cooked loss of YAN made from BGF flour with and without α-amylase addition

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	4.30	24. 9	9.63[a]
fresh barley	4.30	56. 2	11.21[a]
fresh bacterial	4.30	44.7	22.16
dried control	9.00	37.5	12.70[a]
dried barley	9.00	69.2	16.23[a]
dried bacterial	9.00	59.7	26.23

a Abbreviations are: YAN, yellow alkaline noodles; BGF, Baker's strong flour
Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

12.7 Relationship between α-amylase and colour properties of YAN

Discolouration of noodle sheets occurred in all samples including the control. Darkening of noodle sheets happened at a faster rate and also earlier in noodle sheets stored at 25°C as compared to storage at 4°C. In addition, discolouration was faster and it occurred earlier in noodle sheets treated with either of the enzyme preparations as compared to the control (Figs 12.6 & 12.7). No specks were observed in any of the noodle sheets. Control noodles and noodles incorporating α-amylase from barley malt were yellow and dark yellow in colour respectively at time zero and after 96 hours the colour noodles became even darker yellow to brown. The noodle sheets incorporating α-amylase of bacterial origin, were pale yellow in colour and transparent like at time zero, after the storage period of 96 hours noodle sheets became darker yellow but stayed transparent throughout the storage.

The colour of cooked noodles also changed although colour was only measured immediately after cooking. Noodles incorporating α-amylase from barley malt were found to be darker, by visual observation than either control samples or noodle samples incorporating α-amylase of bacterial origin, these observations were supported by readings taken instrumentally (Figs 12.8 & 12.9). Similar observations were also found for dried noodles (Figs 12.8 & 12.9). As stated earlier most of the α-amylase appeared to be inactivated in the noodle samples incorporating α-amylase from barley malt,

however, greater change in colour over a period of time was seen when these noodles were compared to the control samples (Table 12.1). One explanation could be that the enzyme preparation used were not 100% pure and that other enzymes activities and side activities might have been present.

12.8 Structural properties of YAN

The surface structure of raw noodles was studied using the ESEM mode of an electron microscope. In all samples of raw noodles granular starch can be clearly seen. The appearance of control showed compact, lenticular to circular shaped starch granules. When compared to noodles incorporating α-amylase from barley malt it can be seen that granules are more amorphous and not as compact. Noodles treated with α-amylase of bacterial origin show even more hollow structure with many smaller starch granules and amorphous material in between, this might then indicate that here some of the starch might have been used up or damaged by α-amylase (Fig 12.10). Relatively little protein network is visible on the surface of raw YAN. Upon cooking most of the starch gelatinises and therefore no granular form is visible any longer. Quite even hollow pockets of gluten surrounded by protein can be seen in the control, while some larger empty pockets between protein network are seen in noodles incorporating α-amylase from barley malt. Again noodles treated with bacterial α-amylase show greater differences in structure with an even more amorphous surface appearance (Fig 12.10). Similarly for dried products all samples show an amorphous structure, although some granular formations of starch is still visible on the surface, Again the greatest changes were seen in YAN treated with bacterial α-amylase when compared to the control and noodles incorporating α-amylase from barely malt. In addition to the relatively amorphous structure seen in these samples, a gel like mass between the starch granules that are still there, can also be seen (Fig 12.11).

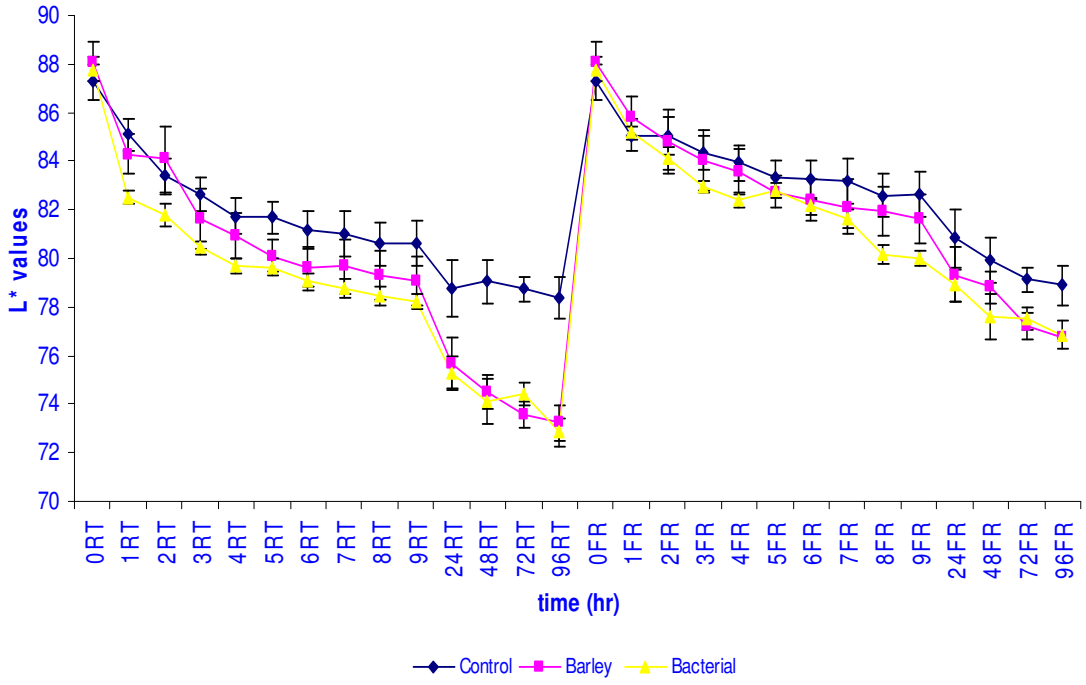


Fig 12.6 **Discolouration of noodle sheets stored at 25°C and 4°C recorded as L* values (whiteness) over a period of 96hr**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

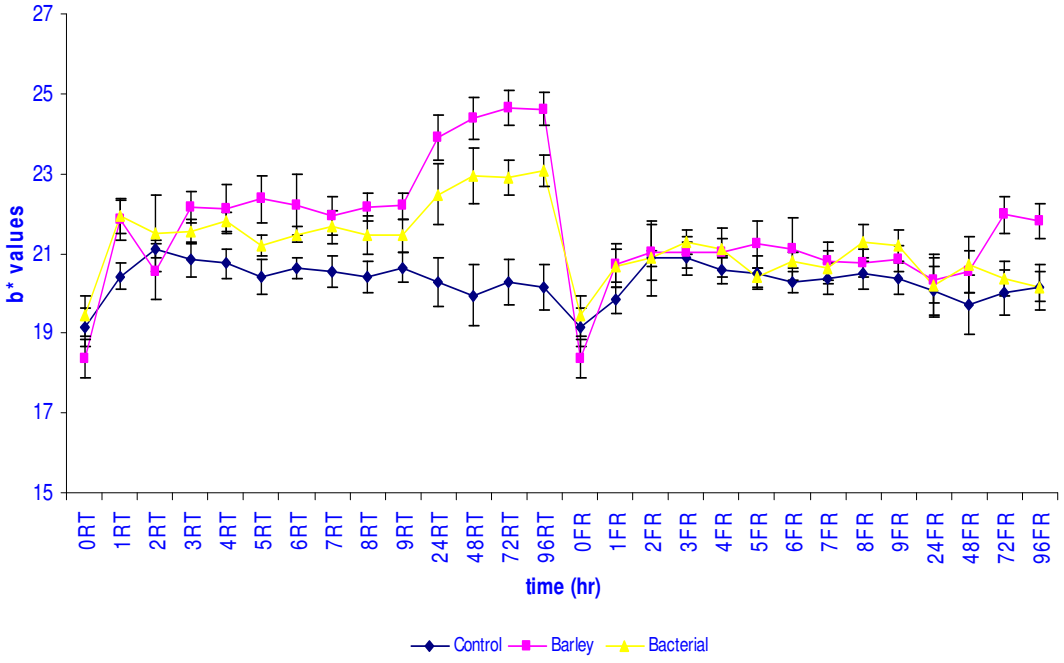


Fig 12.7 **Discolouration of noodle sheets stored at 25°C and 4°C recorded as b* values (yellowness) over a period of 96hr**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

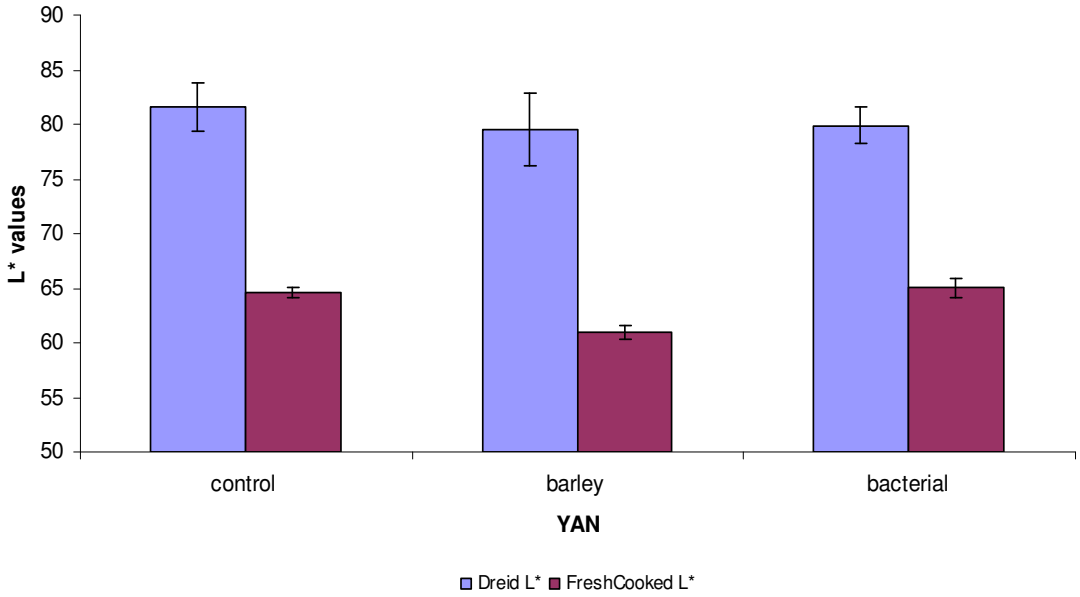


Fig 12.8 Colour characteristics (L* whiteness / brightness) of dried and fresh cooked YAN made from BGF
Error bars represent standard deviation values

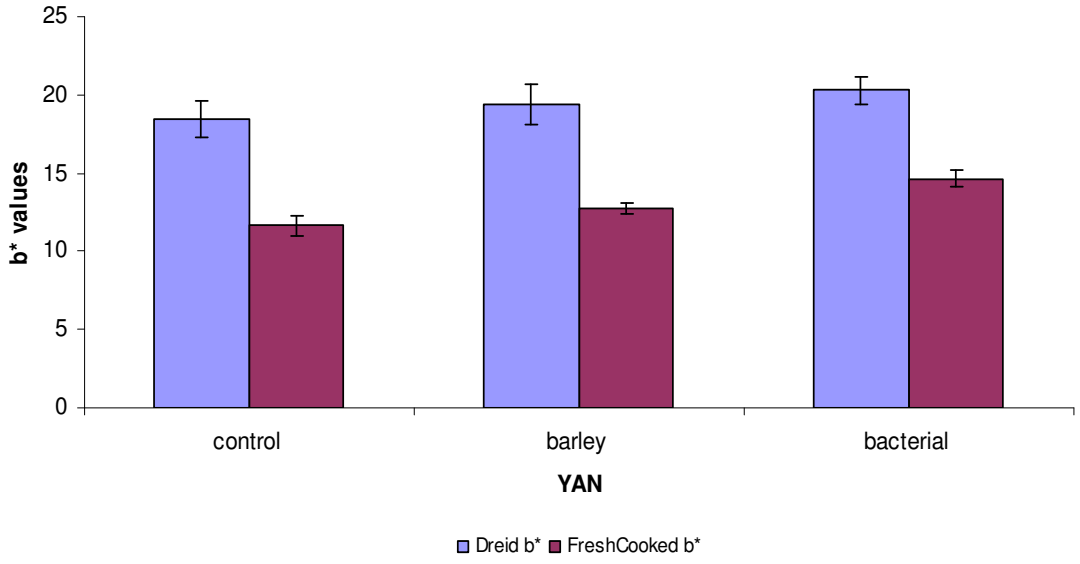


Fig 12.9 Colour characteristics (b* yellowness) of dried & fresh cooked YAN made from BGF
Error bars represent standard deviation values

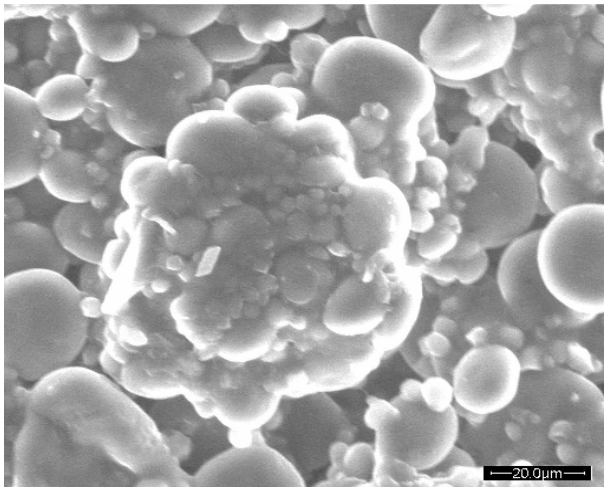
12.9 Summary of results

The use of either barley or bacterial α-amylase resulted in significantly softer noodles (in case of bacterial α-amylase mushy noodles were observed after cooking). Some variations in terms of firmness and hardness was seen between noodles stored at different temperatures, slightly firmer noodles were observed after storage at 4°C. Only relatively low amounts of bacterial α-amylase can be used for noodle-making (as at higher level noodles tend to dissolve upon cooking). On the other hand lower amounts of α-amylase from barley malt did not have any effect on the textural properties of YAN.

Significantly higher cooking losses were seen in YAN incorporating bacterial α-amylase, while treatment with α-amylase from barley origin had slightly higher cooking losses compared to the control, but the differences measured were not significant.

Darkening of noodles is not a desirable characteristics therefore other methods of enzyme manipulation would have to bee investigated to overcome this problem. Darkening of YAN sheets was observed in all samples but was more obvious in noodles stored at 25°C. Noodles treated with bacterial α-amylase were not as dark in colour as the barley treatments but rather transparent and very light yellow but tend to get darker upon storage.

ESEM & SEM images showed that bacterial α-amylase might have greater effect on starch than barley α-amylase, as more of the starch was being damaged in both raw and cooked samples. On the other hand, barley treatments showed fairly similar images to the control with perhaps more amorphous structure.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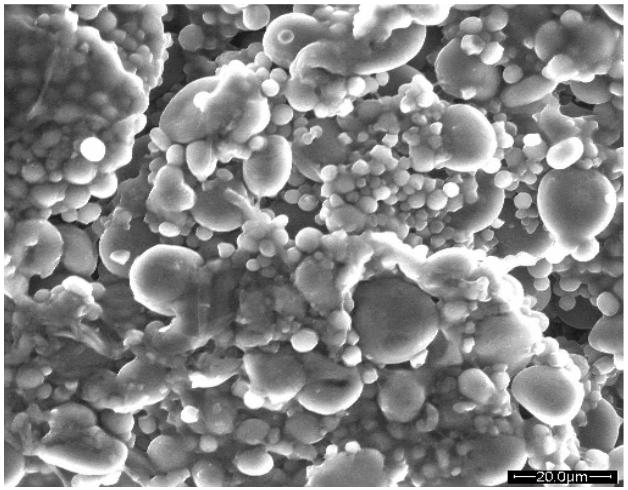
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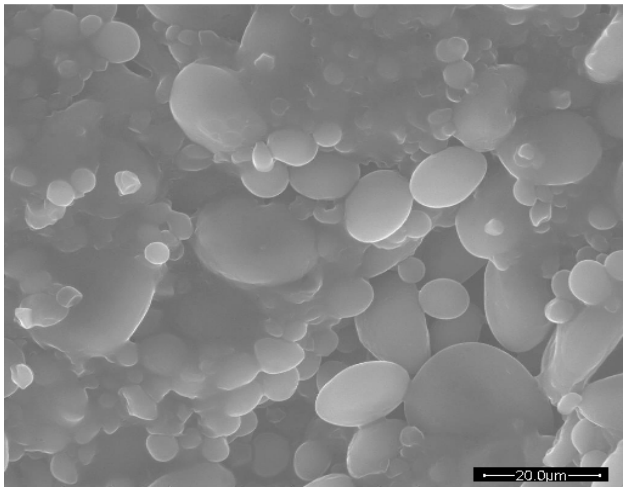
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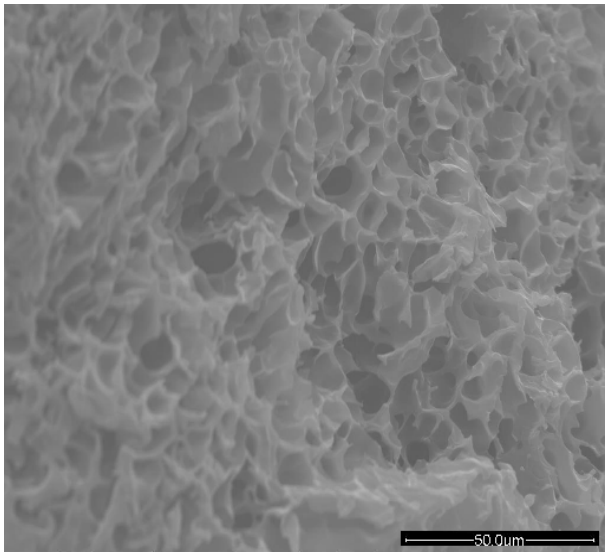
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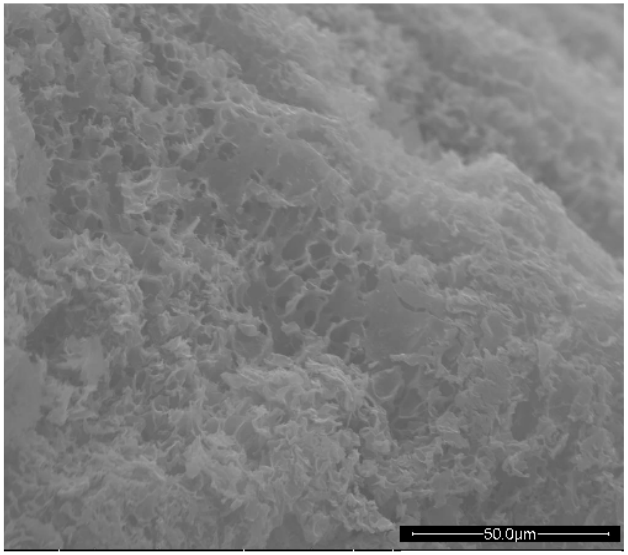
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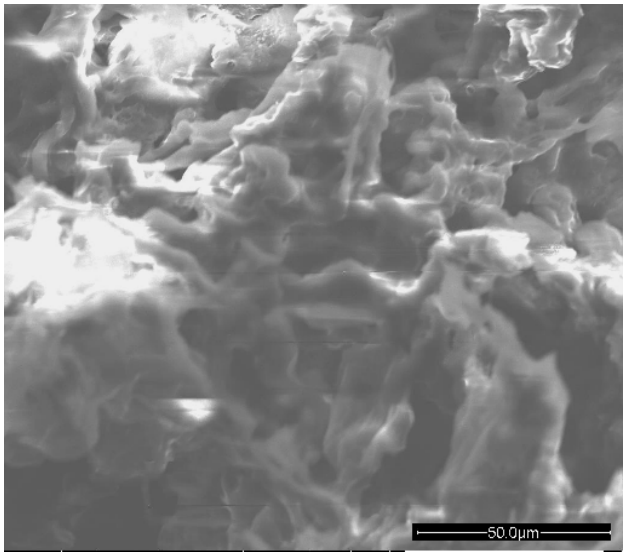
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Fig 12.10 **ESEM[a] images of raw YAN [control, barley and bacterial treatments, left from top to bottom] & SEM[b] images of cooked YAN [control, barley and bacterial treatments, right from top to bottom]**

a ESEM, 6.0 Torr, 4°C, 800× Mag., 5.0spot size, 30kV
b SEM, 0.5 Torr, 23°C, 600× Mag., 5.0spot size, 30kV

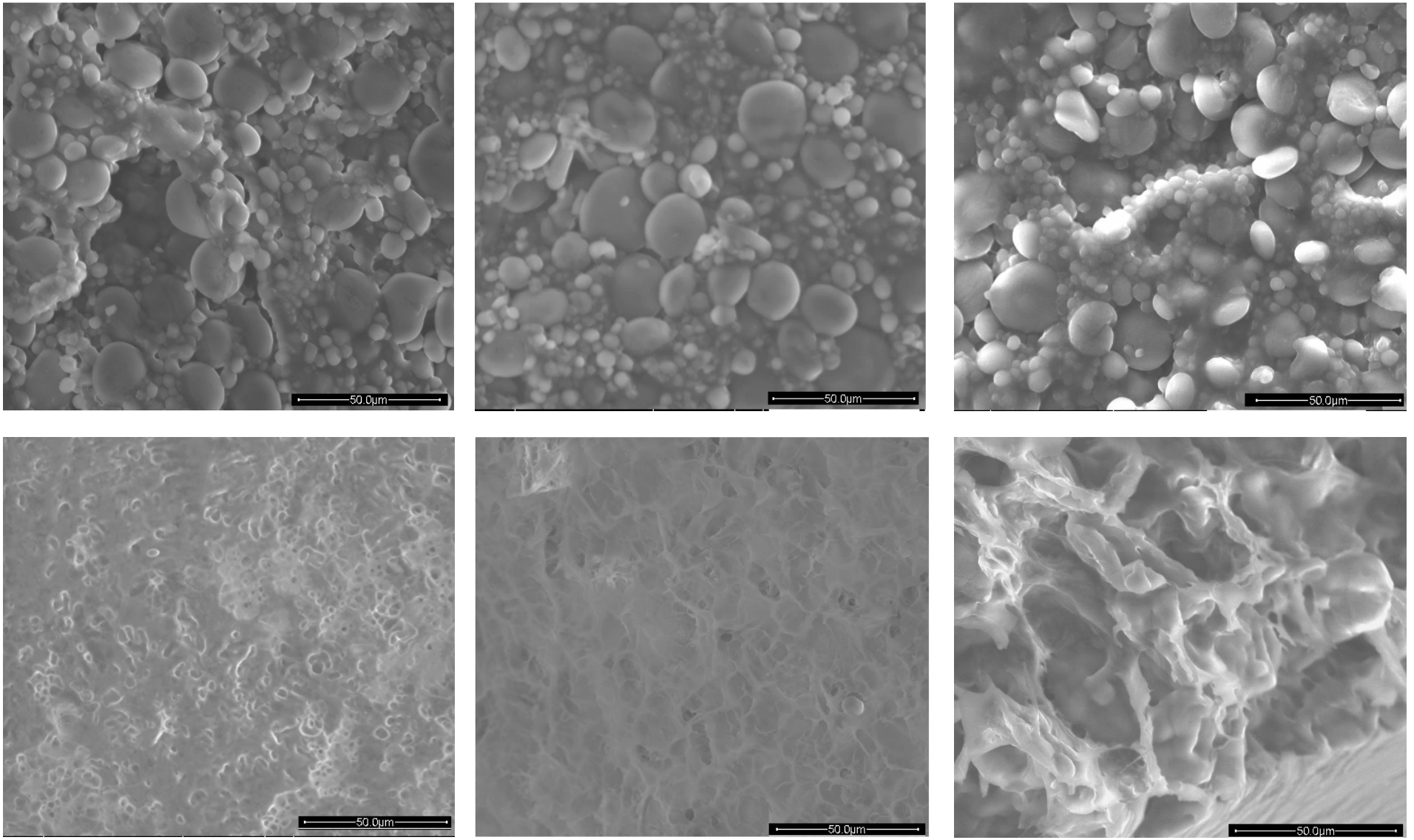
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Fig 12.11 **SEM[a] images of dried (top) and dried cooked (lower) YAN noodles control barley and bacterial treatments (from left to right)**

a SEM, 0.5 Torr, 23°C, 800× Mag., 5.0spot size, 30kV

Chapter 13

Results and discussion: the effect of exogenous lipases on the quality parameters of Asian noodles prepared in the laboratory: White salted noodles

The purpose of this chapter is to describe and discuss the results obtained during the preparation of noodles supplemented with lipases from wheat germ, porcine pancreas and fungal origin. Noodle samples were supplemented with various levels of lipases from all three sources studied and the highest level of addition was chosen in all cases for subsequent analysis of the impact on textural, structural and colour characteristics.

13.1 Introduction

Various lipases have been described in plants; some with acid pH optima that hydrolyse both mono and triacylglycerols and others with alkaline pH optima that act on monoacylglycerols. In wheat grains lipase is not detectable, but activity increases dramatically on germination (Urquhart et al 1984; Matlashewski et al 1982). It has been demonstrated that lipase activity appears after 12hr of imbibition of water in wheat and increases up to the sixth day of germination. From 12hr until 120hr of germination, there is a 15-fold increase in lipase activity (Heltved 1984).

The application of lipase in the baking industry has been documented, with positive effects on crust as well as breadcrumb including softer texture and more uniform structure. Lipase has also been reported to have some bleaching action in the dough (Gélinas et al 1998). Its influence in noodle processing and its impact on noodle quality characteristics has not been documented. Accordingly three different lipases have been evaluated here and their impact upon the quality attributes of WSN studied. The lipases selected were from porcine pancreas (PP), wheat germ (WG) and a fungal source. Furthermore, the objectives of this phase of the study have been to examine the effect of these lipases on processing and quality attributes of WSN samples prepared in the laboratory.

13.2 Levels of lipase in flour and stability during noodle processing

Relatively low levels of lipase activity were found in the flours studied, furthermore, no losses were recorded during noodle processing and storage under different condition (Table 13.1). The levels of naturally present lipase in the flours ranged from 1.49 ± 0.24 for BGF to 1.53 ± 0.23 for PF and 1.56 ± 0.25 for UW. Again relatively low levels of this enzyme as well indicate sound flours, and it has been reported that lipase is so low or virtually undetectable in sound wheats (Urquhart et al 1984; Matlashewski et al 1982). Significant increase in the activity was seen upon addition of lipase preparations to the noodle formulation indicating relative stability of the preparations upon processing and storage. A two to three fold increase in activity was seen when exogenous lipases were added and the impact of these upon quality attributes of WSN are discussed in this Chapter.

Table 13.1 Activity of lipase in control and supplemented WSN stored under different conditions

Treatment[a]	**Lipase activity** [b]	
Controls	**UW**	**PF**
Day1	1.81 ± 0.22	1.84 ± 0.22
24hr RT	1.81 ± 0.22	1.87 ± 0.22
24hr FR	1.78 ± 0.21	1.98 ± 0.18
Dried	1.03 ± 0.21	1.19 ± 0.39
Lipase PP (2×10^6 units / batch)		
Day1	3.45 ± 0.55	3.44 ± 0.56
Dried	3.33 ± 0.53	3.41 ± 0.52
Lipase WG (8.19×10^2 units / batch)		
Day1	2.05 ± 0.56	2.11 ± 0.64
Dried	1.94 ± 0.43	1.98 ± 0.57
Lipase Fungal (20×10^4 units / batch)		
Day 1	3.95 ± 0.63	4.01 ± 0.58
Dried	3.67 ± 0.59	3.79 ± 0.45

a Abbreviations are: WSN, white salted noodles; RT, storage at room temperature; FR, storage at 4°C, PP, porcine pancreas; WG, wheat germ

b Unit for lipase activity is given in µmoles of free fatty acids produced per gram of defatted flour per minute under the assay conditions

No significant change was seen in the levels of lipase activity during storage and more importantly following application of different processing conditions. The levels of lipase activity for control samples ranged from 1.03 ± 0.21 for dried to 1.81 ± 0.22 for fresh UW and 1.19 ± 0.39 for dried to 1.98 ± 0.18 for fresh PF., indicating that neither drying (hence, lower water level) or storage at 25°C and 4°C had significant effects upon the activity of this enzyme (Table 13.1). Although it is difficult to compare activities of enzymes from different sources, attempts have been made to ensure similar levels of addition from all three lipase sources so that their impact upon the quality of attributes of WSN can be fairly compared. Significantly higher activity was recorded when exogenous lipases have been added to the WSN. The activity ranged from 2.05 ± 0.56 and 2.11 ± 0.64 for WG lipase (UW and PF respectively) to 3.45 ± 0.55 and 3.44 ± 0.56 for PP lipase (UW and PF respectively) and 3.95 ± 0.63 and 4.01 ± 0.58 for fungal lipase preparation (UW and PF respectively) for fresh samples.

13.3 The effect of lipase on textural properties of WSN

In the preliminary experiments a series of levels of lipases were evaluated and textural properties were measured using only the flat cylinder probe (P/45). Results obtained for the two flours studied were similar, thus, only data for UW are presented here. The textural profile of raw and cooked samples for WSN treated with lipase from porcine pancreas is shown in Fig 13.1. It is observed in both graphs that lower levels of this lipase preparations result in softer noodles. A positive effect of the porcine pancreas preparation was seen when 1×10^6 units per batch of noodles were added to the formulation in the case of raw noodles and 2×10^6 units per batch in the case of cooked noodles. Softness, as one of the preferred characteristics for this type of noodles, was positively influenced when lower levels of the enzymes were used. Higher levels of addition did not appear to show further differences. Addition of 2×10^6 units per batch per batch was carried out for the remainder of the evaluations performed here.

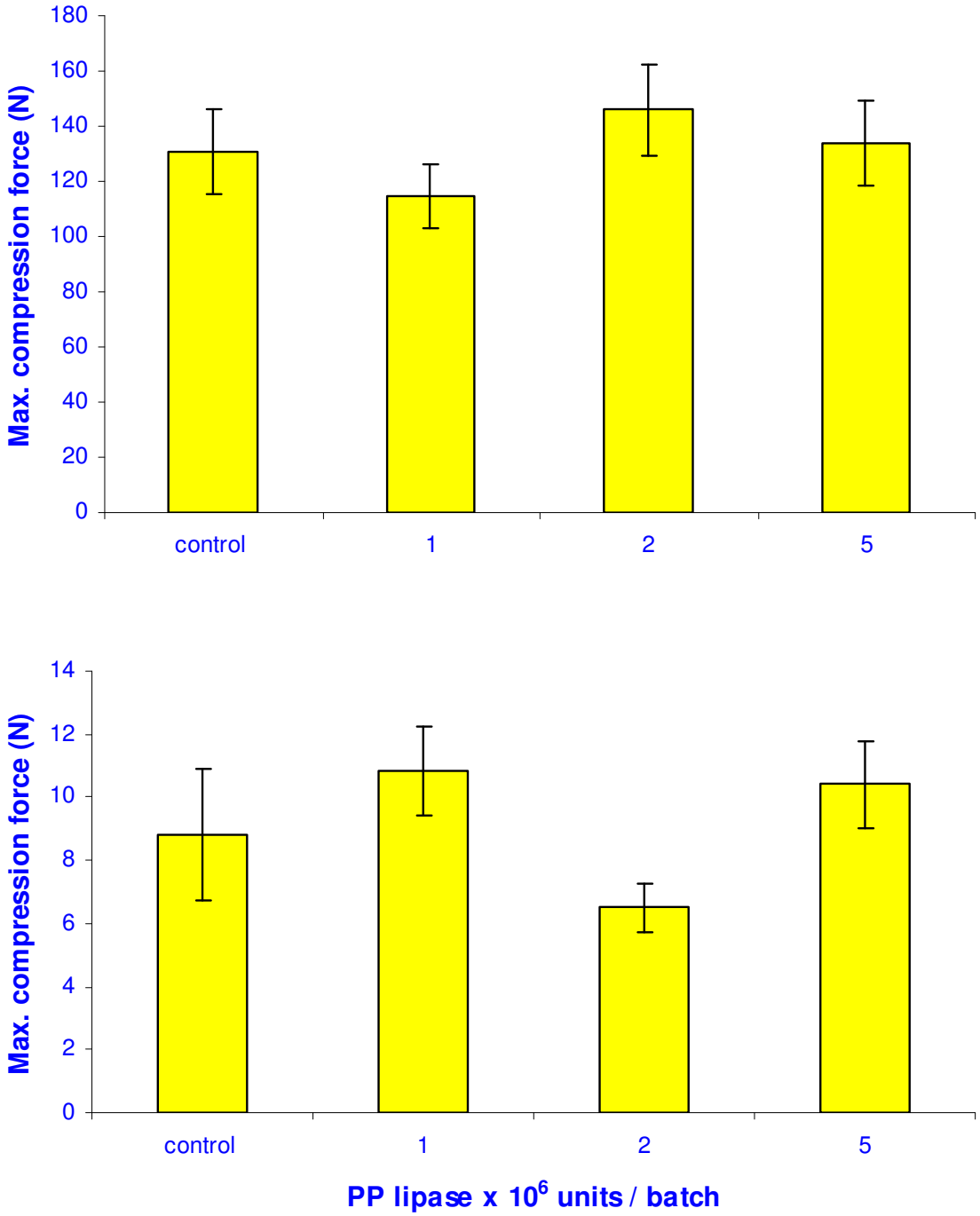


Fig 13.1 Hardness characteristics of fresh raw (top) and fresh cooked (bottom) WSN treated with lipase from porcine pancreas. Impact of various levels of the enzyme on the textural properties of WSN
Error bars represent standard deviation values. Measured using the flat cylinder probe (P/45)

Textural properties of WSN treated with lipase from wheat germ show that lower levels of this enzyme had a positive impact upon noodle softness (Fig 13.2). In both raw and

cooked noodles lower levels of addition resulted in softer noodles. Thus, addition of 20.5×10^2 units per batch was chosen to complete the study.

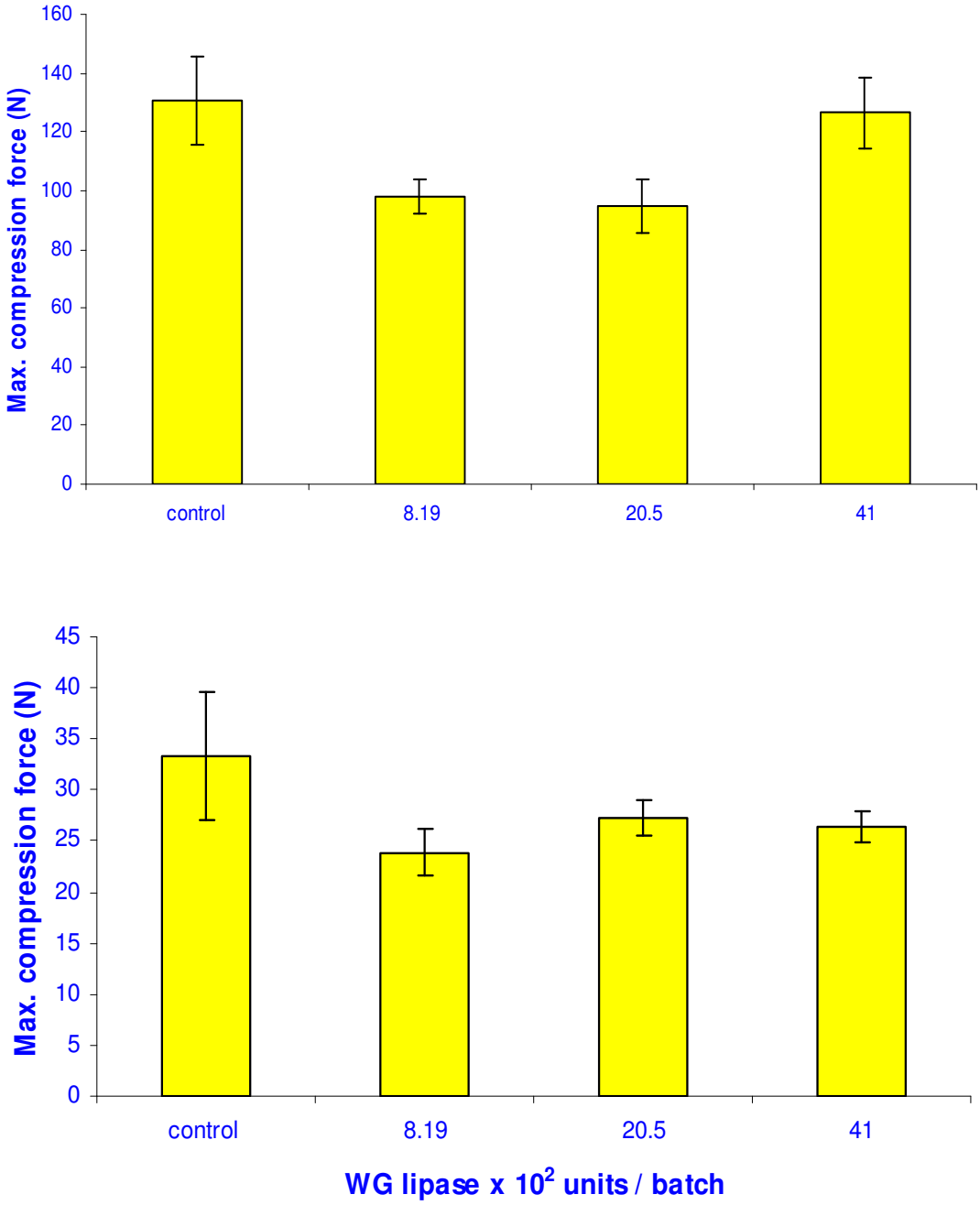


Fig 13.2 **Hardness characteristics of fresh raw (top) and fresh cooked (bottom) WSN treated with lipase from wheat germ. Impact of various levels of the enzyme on the textural properties of WSN**
Error bars represent standard deviation values. Measured using the flat cylinder probe (P/45)

Textural characteristics of WSN treated with fungal lipase are shown in Fig 13.3. Before cooking, no differences were recorded between control and the various treatments. Once noodles were cooked, the addition of 20×10^4 units per batch showed slight difference in terms of texture, resulting in softer noodles when compared to the control and other levels of addition (although the results were not statistically different). This level was subsequently chosen for all other studies.

For the fungal lipase, in addition to the preliminary test carried out using the flat cylinder probe (P/45), preliminary tests were done utilising the blade as well. Different levels of fungal lipase were evaluated for their influence on the textural properties of WSN. Amounts of addition ranged from 10×10^4 units per batch to 80×10^4 units per batch. There were no significant differences between controls and the enzyme treated noodles (Fig 13.4). In all cases a trend of slightly firmer noodles can be seen in enzyme treated noodles. In addition, higher levels of addition did not influence the textural properties of WSN differently from the control samples or those treated with lower levels of enzyme.

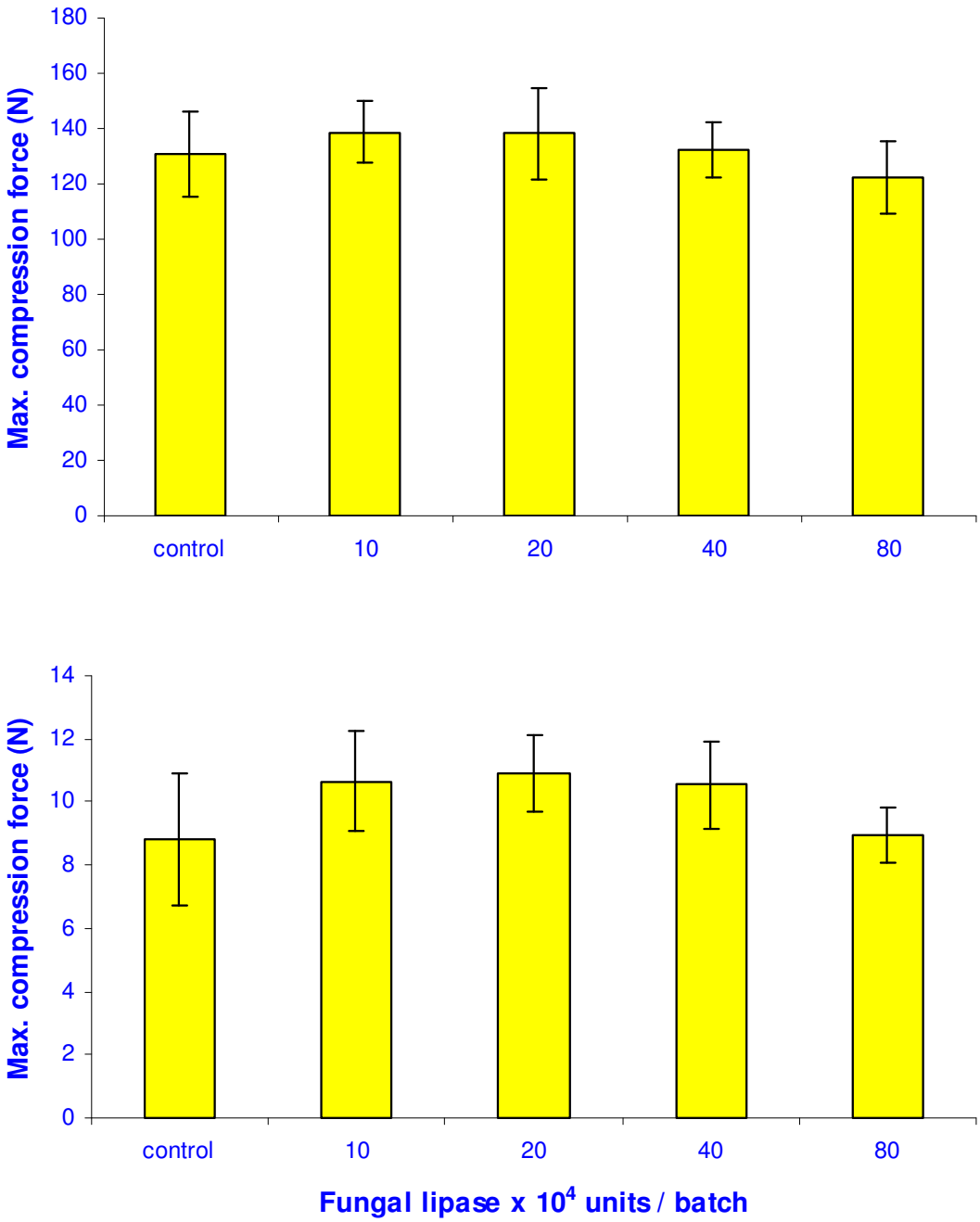


Fig 13.3 Hardness characteristics of fresh raw (top) and fresh cooked (bottom) WSN treated with fungal lipase. Impact of various levels of the enzyme on the textural properties of WSN

Error bars represent standard deviation values.
Measured using the flat cylinder probe (P/45).

Table 13.2 Effects of lipase from porcine pancreas on the textural properties measured by TA-XT2 for WSN made from UW

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.6[a]	130[a]	11.8[a]	2.42[a]
	LipasePP	46.0[a]	138[a]	12.9[a]	2.45[a]
	RT control	43.3[a]	134[a]	13.6[a]	2.55[a]
	LipasePP RT	36.1[a]	120[a]	14.0[a]	2.50[a]
	FR control	44.5[a]	136[a]	12.1[a]	2.44[a]
	LipasePP FR	37.9[a]	121[a]	12.1[a]	2.43[a]
Cooked	control	8.80[a]	33.3[a]	3.54[a]	0.70[a]
	LipasePP	10.2[a]	35.7[a]	4.81[a]	0.81[a]
	RT control	9.20[a]	34.8[a]	3.54[a]	0.70[a]
	LipasePP RT	9.42[a]	18.2[b]	4.06[a]	0.73[a]
	FR control	10.0[a]	37.0[a]	4.24[a]	0.78[a]
	LipasePP FR	8.31[a]	14.9[b]	4.60[a]	0.84[a]
Dried [b]	control	11.9[a]	39.5	6.01[a]	0.99[a]
	LipasePP	10.2[a]	28.8	5.58[a]	0.99[a]

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour; PP, porcine pancreas; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Level of addition was 2×10^6 units / batch

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Table 13.3 Effects of lipase from porcine pancreas on the textural properties measured by TA-XT2 for WSN made from PF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.2[a]	128[a]	11.9[a]	2.40[a]
	LipasePP	44.3[a]	133[a]	11.8[a]	2.51[a]
	RT control	38.1[a]	119[a]	13.0[a]	2.52[a]
	LipasePP RT	36.8[a]	122[a]	13.5[a]	2.45[a]
	FR control	39.4[a]	121[a]	12.9[a]	2.54[a]
	LipasePP FR	38.3[a]	123[a]	12.3[a]	2.46[a]
Cooked	control	9.10[a]	33.6[a]	3.30[a]	0.75[a]
	LipasePP	10.1[a]	34.5[a]	4.99[a]	0.85[a]
	RT control	9.50[a]	33.9[a]	3.21[a]	0.66[a]
	LipasePP RT	6.32[ab]	21.6[b]	3.95[a]	0.75[a]
	FR control	9.91[a]	34.1[a]	4.26[a]	0.80[a]
	LipasePP FR	5.66[b]	14.5[b]	4.87[a]	0.85[a]
Dried [b]	control	9.92[a]	34.1[a]	4.20[a]	0.83[a]
	LipasePP	9.32[a]	29.6[a]	5.64[a]	1.01[a]

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour; PP, porcine pancreas; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Level of addition was 2×10^6 units / batch

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Table 13.4 Effects of lipase from wheat germ on the textural properties measured by TA-XT2 for WSN made from UW

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.6[a]	130[a]	11.8[a]	2.42[a]
	LipaseWG	25.4	94.7	10.56[a]	2.39[a]
	RT control	43.3[a]	134[a]	13.6[a]	2.55[a]
	LipaseWG RT	46.1[a]	141[a]	12.56[a]	2.44[a]
	FR control	44.5[a]	136[a]	12.1[a]	2.44[a]
	LipaseWG FR	48.8[a]	144[a]	12.1[a]	2.37[a]
Cooked	control	8.84[a]	33.3[a]	3.54[a]	0.70[a]
	LipaseWG	7.80[a]	26.4[ab]	3.11[a]	0.67[a]
	RT control	9.23[a]	34.8[a]	3.54[a]	0.70[a]
	LipaseWG RT	6.80[a]	23.7[b]	2.45[a]	0.62[a]
	FR control	10.0[a]	37.0[a]	4.24[a]	0.78[a]
	LipaseWG FR	7.66[a]	24.9[b]	3.01[a]	0.75[a]
Dried [b]	control	11.9[a]	39.5	6.01[a]	0.99[a]
	LipaseWG	7.86[a]	25.5	6.58[a]	1.04[a]

a Abbreviations are: WSN, white salted noodles; UW, Ultra white; PP, porcine pancreas; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Level of addition was 8.19×10^2 units / batch

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Table 13.5 Effects of lipase from wheat germ on the textural properties measured by TA-XT2 for WSN made from PF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.2[a]	128[a]	11.9[a]	2.49[a]
	LipaseWG	32.5[a]	105[b]	11.03[a]	2.15[a]
	RT control	38.1[a]	119[ab]	13.0[a]	2.50[a]
	LipaseWG RT	40.2[a]	139[a]	12.35[a]	2.46[a]
	FR control	39.4[a]	121[a]	12.9[a]	2.51[a]
	LipaseWG FR	47.6[a]	137[a]	12.2[a]	2.32[a]
Cooked	control	9.12[a]	33.6[a]	3.34[a]	0.70[a]
	LipaseWG	7.43[a]	24.3[b]	3.03[a]	0.64[a]
	RT control	9.50[a]	33.9[a]	3.21[a]	0.66[a]
	LipaseWG RT	7.14[a]	22.5[b]	2.55[a]	0.76[a]
	FR control	9.94[a]	34.1[a]	4.28[a]	0.80[a]
	LipaseWG FR	8.17[a]	25.4[b]	2.98[a]	0.79[a]
Dried [b]	control	9.90[a]	34.1[a]	4.21[a]	0.81[a]
	LipaseWG	11.20[a]	38.7[a]	5.50[a]	1.00[a]

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour; PP, porcine pancreas; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Level of addition was 8.19×10^2 units / batch

Note: Means followed ny the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

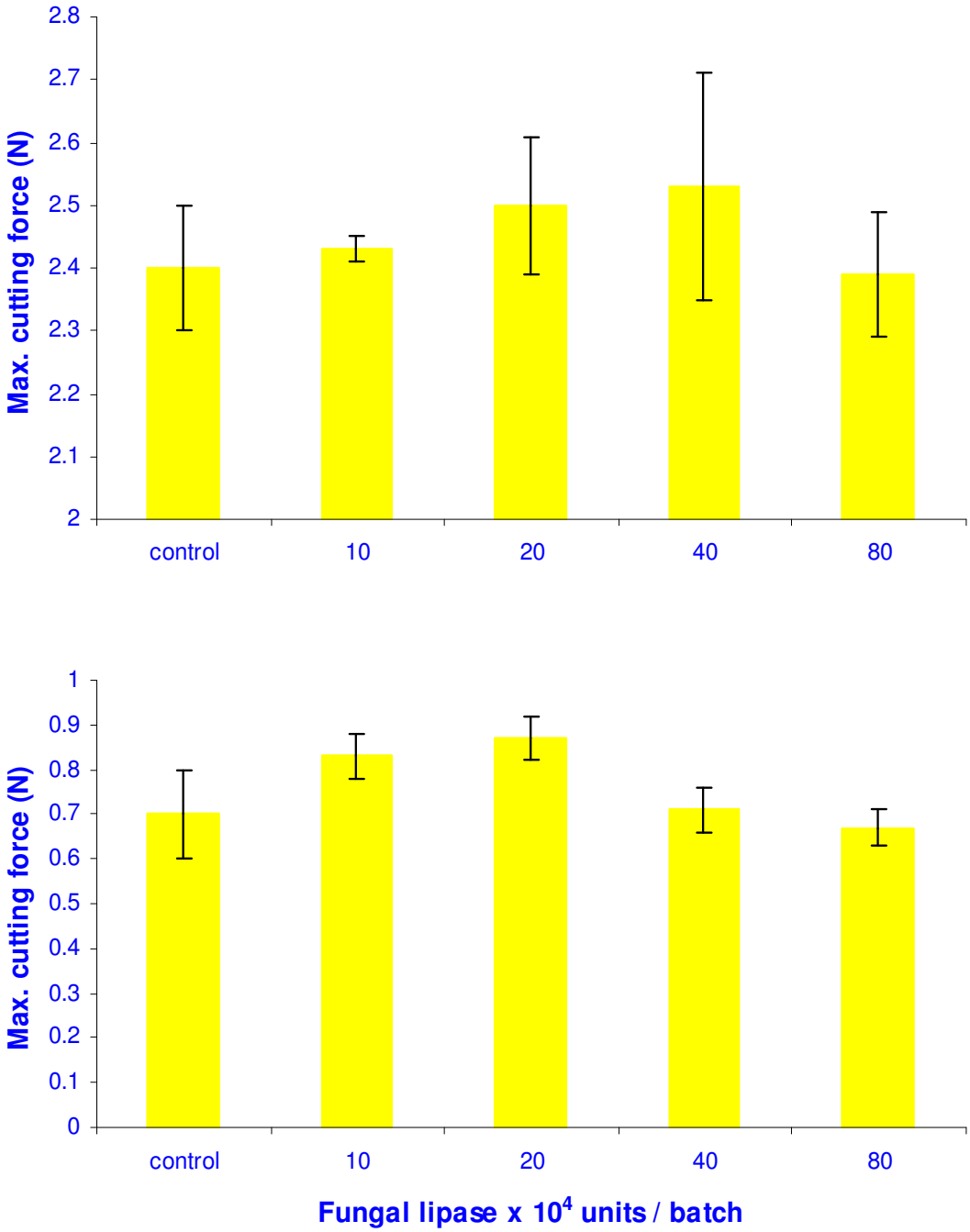


Fig 13.4 Firmness characteristics of fresh raw (top) and fresh cooked (bottom) WSN treated with various levels of fungal lipase. Impact of different levels of the enzyme on the textural properties of WSN

Error bars represent standard deviation values.
Measured using the blade attachment of the TA-XT2.

Table 13.6 Effects of lipase of fungal origin on the textural properties measured by TA-XT2 for WSN made from UW

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.6[a]	130[a]	11.8[a]	2.42[a]
	Lipase	45.3[a]	135[a]	15.4[a]	2.51[a]
	RT control	43.3[a]	134[a]	13.6[a]	2.55[a]
	Lipase RT	49.2[ab]	140[a]	16.8[b]	2.55[a]
	FR control	44.5[a]	136[a]	12.1[a]	2.44[a]
	Lipase FR	52.6[b]	122	16.8[b]	2.88[a]
Cooked	control	8.81[a]	33.3[a]	3.54[a]	0.70[a]
	Lipase	10.8[a]	35.6[a]	3.41[a]	0.75[a]
	RT control	9.20[a]	34.8[a]	3.54[a]	0.70[a]
	Lipase RT	10.9[a]	32.9[a]	2.46[a]	0.56[a]
	FR control	10.0[a]	37.0[a]	4.24[ab]	0.78[a]
	Lipase FR	11.4[a]	39.5[a]	5.56[b]	0.85[a]
Dried [b]	control	11.9	39.5[a]	6.01	0.99[a]
	Lipase	12.1	36.3[a]	8.22	1.16[a]

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Level of addition was 20×10^4 units / batch

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment

Table 13.7 Effects of lipase of fungal origin on the textural properties measured by TA-XT2 for WSN made from PF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.2^{a}	128^{a}	11.9^{a}	2.44^{a}
	Lipase	46.1^{ab}	138^{ab}	16.3^{b}	2.70^{a}
	RT control	38.1^{a}	119^{c}	13.0^{ab}	2.55^{a}
	Lipase RT	51.0^{b}	143^{b}	18.0^{b}	2.80^{a}
	FR control	39.4^{a}	121^{ac}	12.9^{ab}	2.54^{a}
	Lipase FR	53.1^{b}	146^{b}	17.3^{b}	2.85^{a}
Cooked	control	9.10^{a}	33.6^{a}	3.37^{a}	0.73^{a}
	Lipase	11.7^{a}	38.0^{ab}	4.95^{ab}	0.80^{a}
	RT control	9.58^{a}	33.9^{a}	3.21^{a}	0.66^{a}
	Lipase RT	10.8^{a}	31.4^{a}	4.47^{ab}	0.72^{a}
	FR control	9.99^{a}	34.1^{a}	4.20^{ab}	0.80^{a}
	Lipase FR	12.6^{a}	41.4^{b}	5.94^{b}	0.93^{a}
Dried [b]	control	9.90^{a}	34.1^{a}	4.27	0.80^{a}
	Lipase	12.2^{a}	35.2^{a}	8.11	1.13^{a}

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Level of addition was 20×10^4 units / batch

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

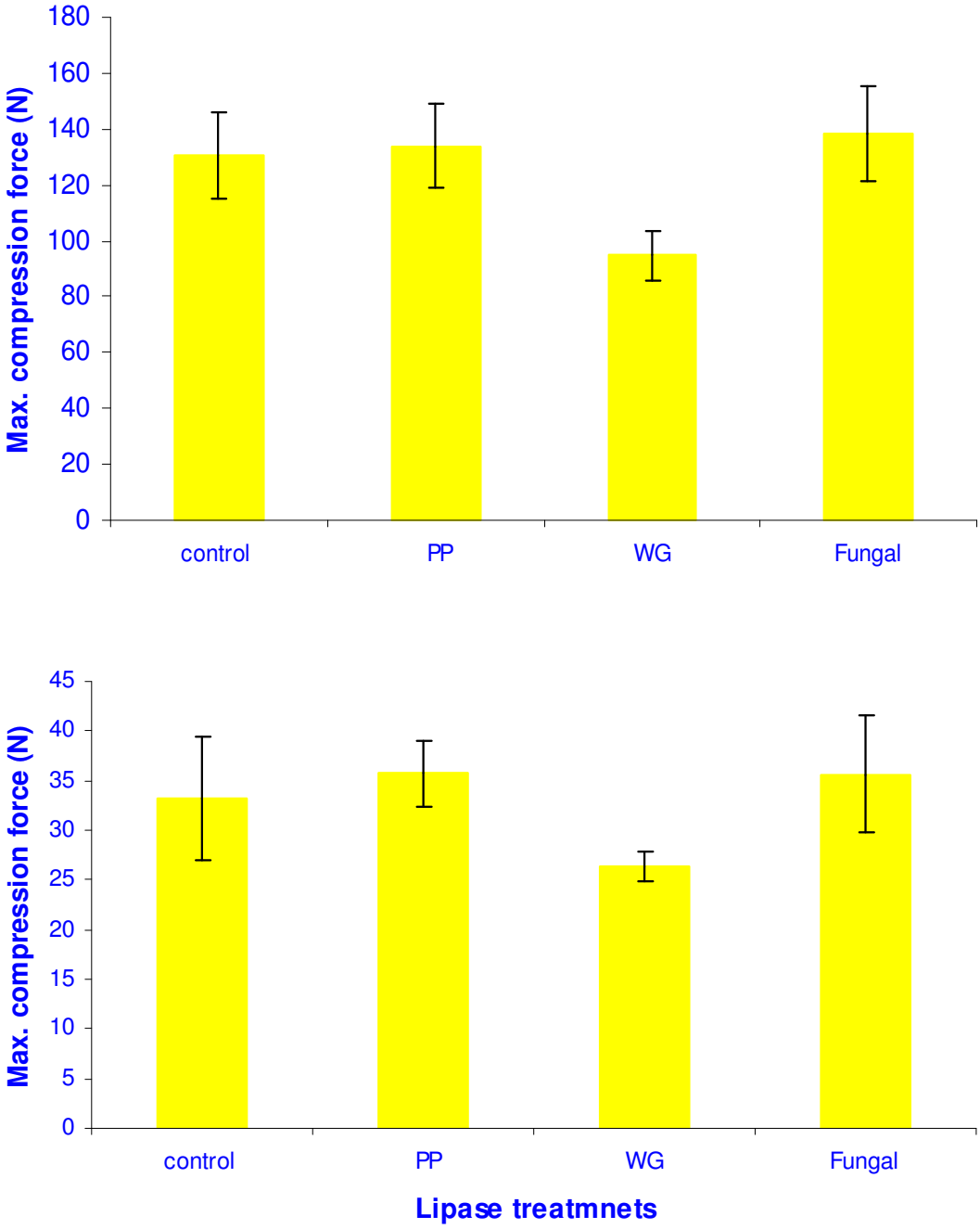


Fig 13.5 **Comparison of the different lipases used on the hardness characteristics of fresh raw (top) and fresh cooked (bottom) WSN. The levels of addition were: PP, 2×10^6, WG, 8.19×10^2 and fungal lipase 20×10^4 units per batch**

Error bars represent standard deviation values. Measured using the flat cylinder (P/45)

13.5 The effect of lipases on cooking yield

When samples were cooked, it was found that there were relatively little changes in cooking yields upon lipase addition (Tables 13.8 and 13.9)

13.6 Relationship between lipase and colour properties of WSN

All three preparations of lipase had an adverse impact on the colour properties of WSN (Figs. 13.6 – 13.9). This was more obvious in raw noodle sheets when L* and b* values were measured. Furthermore the difference between all different treatments was more obvious when noodles were stored at 25°C as compared to the storage at 4°C. The same pattern of discoloration was seen in both flours used (UW and PF) for making of WSN. The difference between treatments was less obvious when noodles were dried or cooked immediately after preparation (Figs 13.10 & 13.12).

Wheat germ (WG) lipase

Level of addition was 20.5×10^2 units per batch. The colour of WSN was most adversely effected by the addition of lipase from wheat germ. Noodles made with WG lipase preparation were dark yellow to light brown in colour at time zero and became darker brown during the storage. This lipase preparation resulted in darkest noodles compared to the control and other lipase treatments as manifested by lowest L* values (whiteness) (Fig 13.6) and highest b* values (yellowness) (Fig 13.7). These samples were darker than the control at time zero, (readings taken immediately after manufacture of noodle sheets) and discoloured over a period of time even further and stayed significantly darker than the control and other lipase treatments over a total period of 96hr. The rate of discolouration of noodle sheets was happening at a greater rate at 25°C as compared to the storage at 4°C, this was true for all treatments including the control. Drying of noodle sheets at 40°C for 30hr or boiling of noodles immediately after preparation (either fresh or dried and then boiled) prevented darkening to some extent. However, noodles with added WG lipase were darker than the controls for both flours, as indicated by lower L* and higher b* values (Figs 13.10 & 13.11).

Table 13.8 Cooked weight and cooked loss of WSN made from UW flour with and without lipase addition

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	2.3	58.7[a]	6.93[a]
fresh lipasePP	2.3	54.0[ab]	6.47[a]
fresh lipaseWG	2.3	53.0[b]	6.49[a]
Fresh lipase fungal	2.3	57.3[a]	6.86[a]
dried control	6.3	70.8[a]	8.84[a]
dried lipasePP	6.3	66.2[a]	9.90[a]
dried lipaseWG	6.3	68.9[a]	10.3[a]
dried lipase fungal	6.3	62.5[a]	8.26[a]

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour
Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

Table 13.9 Cooked weight and cooked loss of WSN made from PF flour with and without lipase addition

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	2.3	56.3[a]	6.38[a]
fresh lipasePP	2.3	55.7[a]	6.30[a]
fresh lipaseWG	2.3	55.8[a]	6.45[a]
fresh lipase fungal	2.3	59.1[a]	7.13[a]
dried control	6.3	70.25[a]	8.94[a]
dried lipasePP	6.3	66.8[ab]	9.90[a]
dried lipaseWG	6.3	68.0[ab]	9.77[a]
dried lipase fungal	6.3	62.6[b]	7.90[a]

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour
Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

Porcine pancreas (PP) lipase

Level of addition was 2×10^6 units per batch of noodles. Addition of this lipase preparation also resulted in darker (less white) noodle sheets, therefore the levels of addition used here having an adverse effect on the colour characteristics of WSN. At the

time of preparation and during the first couple of hours of storage of noodles the PP lipase preparation was similar in colour to the control samples (having similar L* values), however, greater darkening / discolouration was seen in these samples compared to the control, as the rate of discoloration was faster and to a greater extent indicated by higher b* values (Figs 13.6 – 13.9). Again the same pattern was seen for both flours used. The difference between these samples and control was less obvious when noodles were dried and dried and cooked immediately after preparations (Fig 13.10 & 13.11). Noodles supplemented with PP lipase were similar in colour to the control samples when cooked immediately after preparation, despite having slightly higher b* values indicating slightly more yellow products (Fig 13.11).

Fungal lipase

Level of addition in the case of fungal lipase was 20×10^4 units per batch. Addition of fungal lipase preparation to the noodle formulation resulted in colour that was closest to the control. Still, these treatments were darker at both storage temperatures, and discolured over the storage time (Figs. 13.6 – 13.9). This lipase preparation resulted in noodles being transparent yellow in colour. Both parameters measured (L* and b*) values were not adversely impacted by addition of this lipase preparation when noodles were dried or cooked immediately after preparation (Fig 13.10 & 13.11).

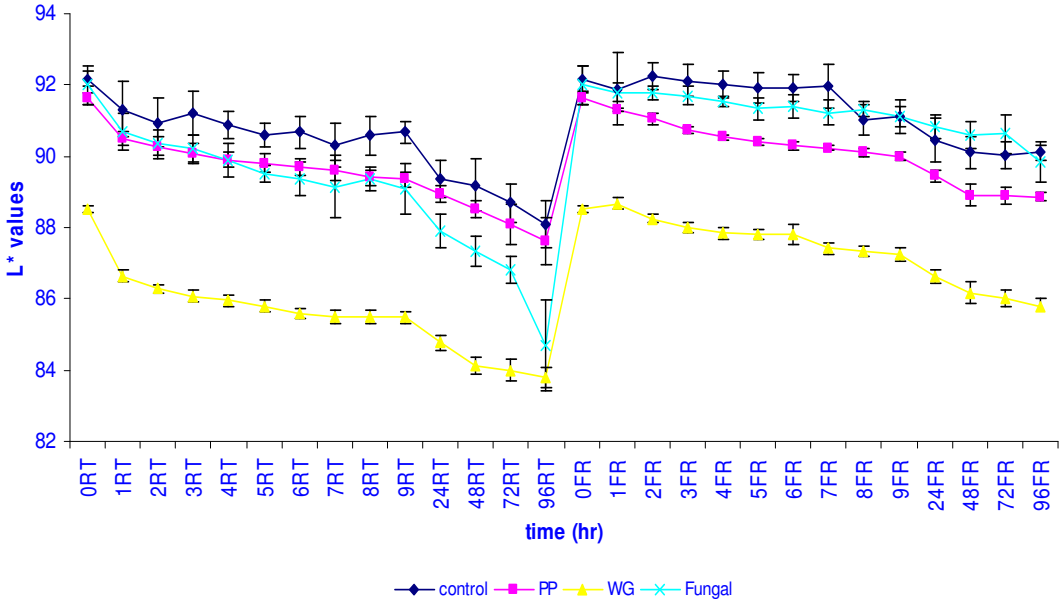


Fig 13.6 **Colour characteristics (L* values [whiteness]) of WSN made from UW stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

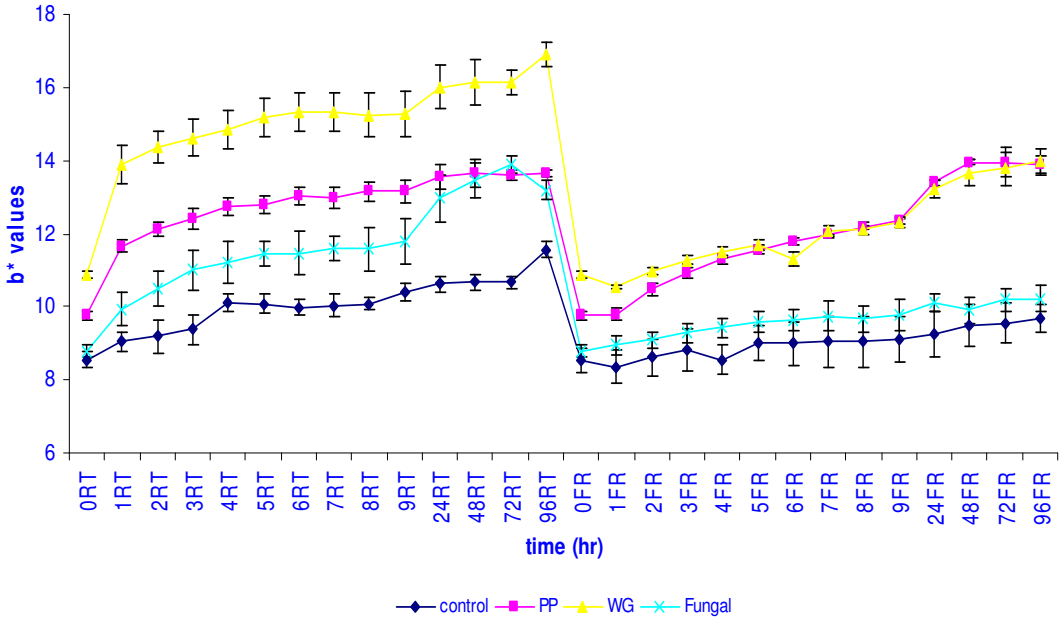


Fig 13.7 **Colour characteristics (b* values [yellowness]) of WSN made from UW stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

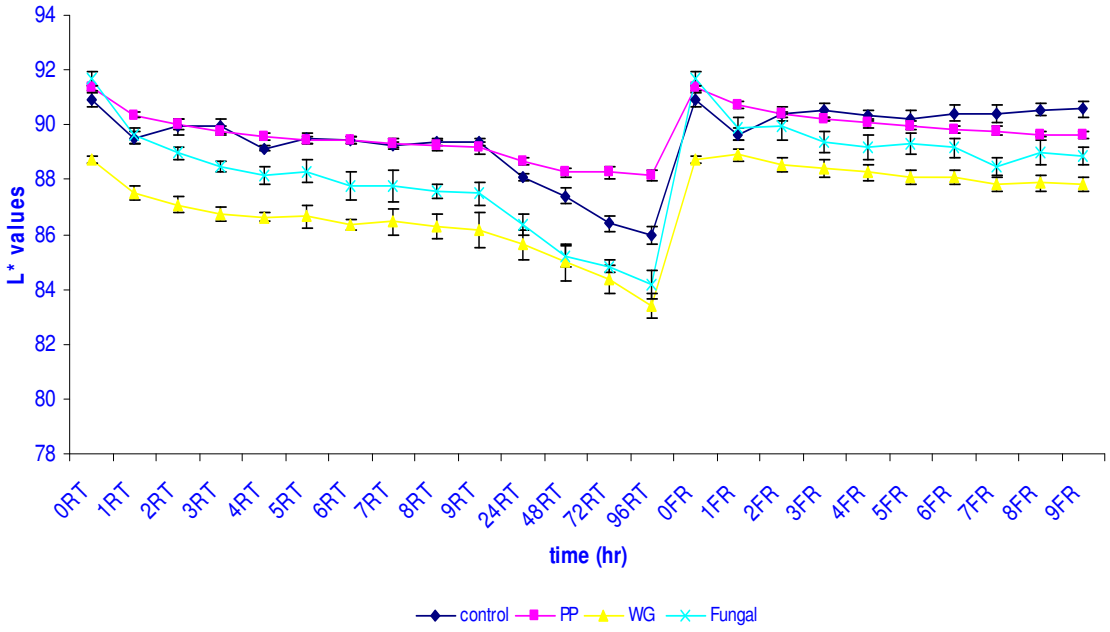


Fig 13.8 **Colour characteristics (L* values [whiteness]) of WSN made from PF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

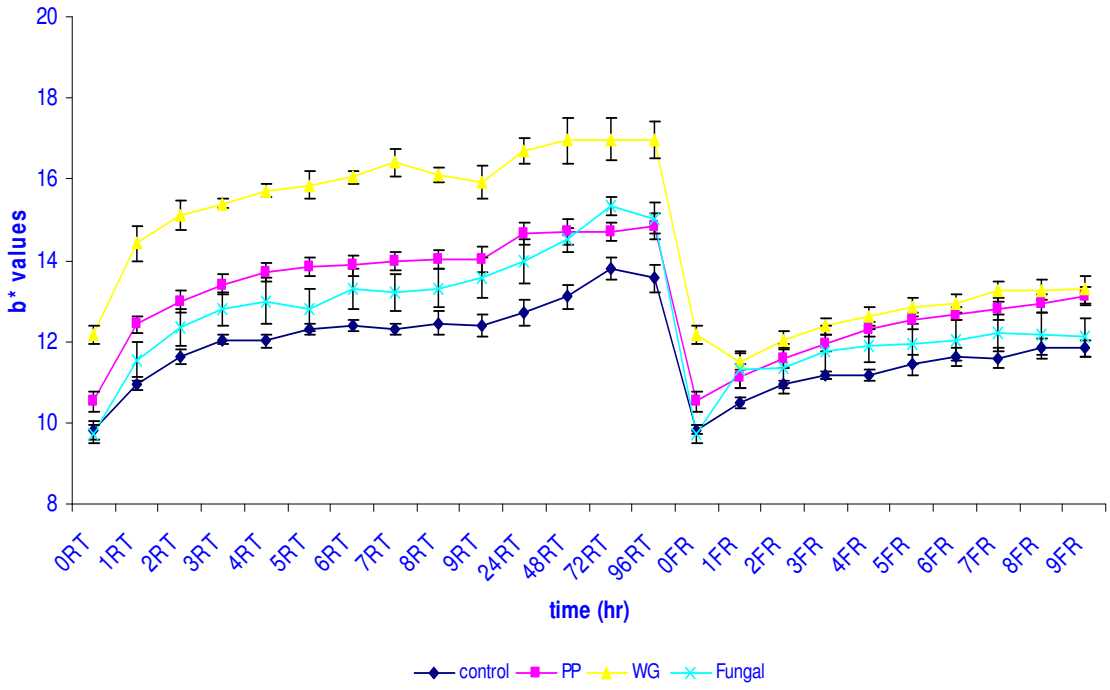


Fig 13.9 **Colour characteristics (b* values [yellowness]) of WSN made from PF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

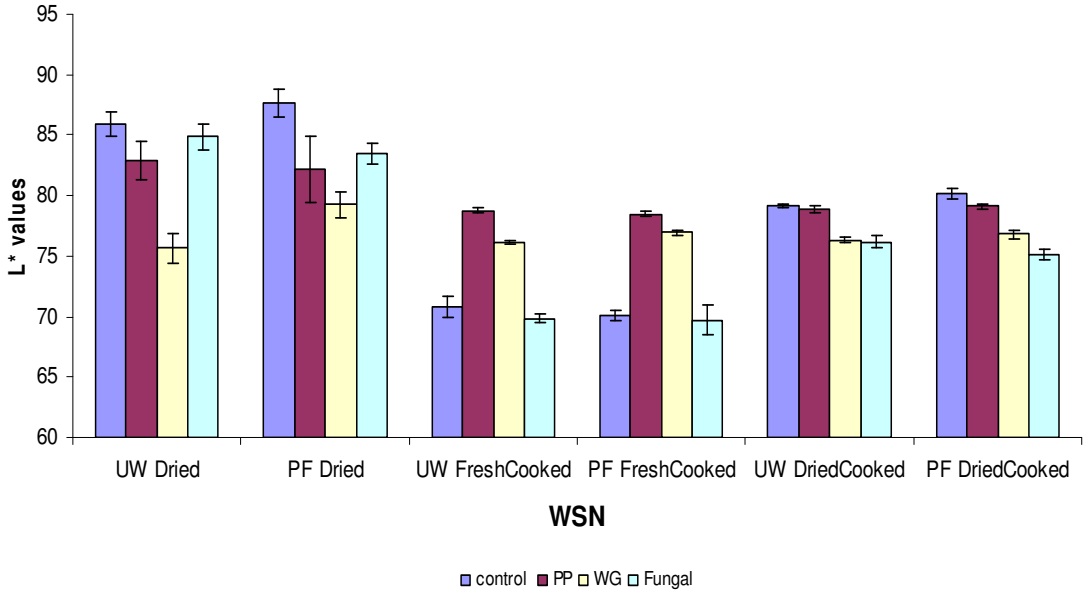


Fig 13.10 Colour characteristics (L* whiteness / brightness) of dried, fresh cooked & dried cooked WSN made from UW & PF
Error bars represent standard deviation values

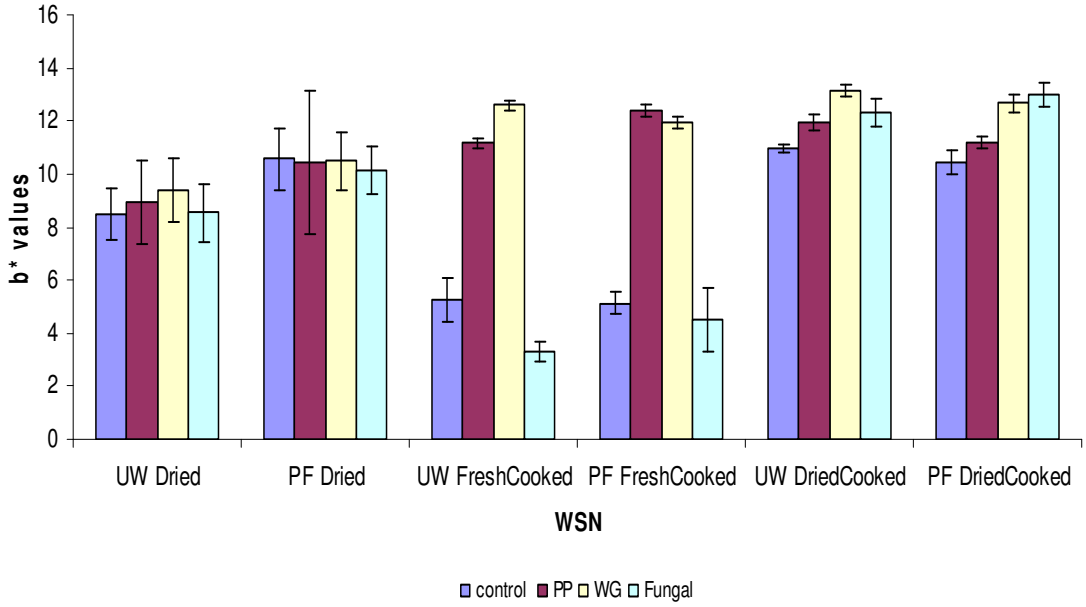


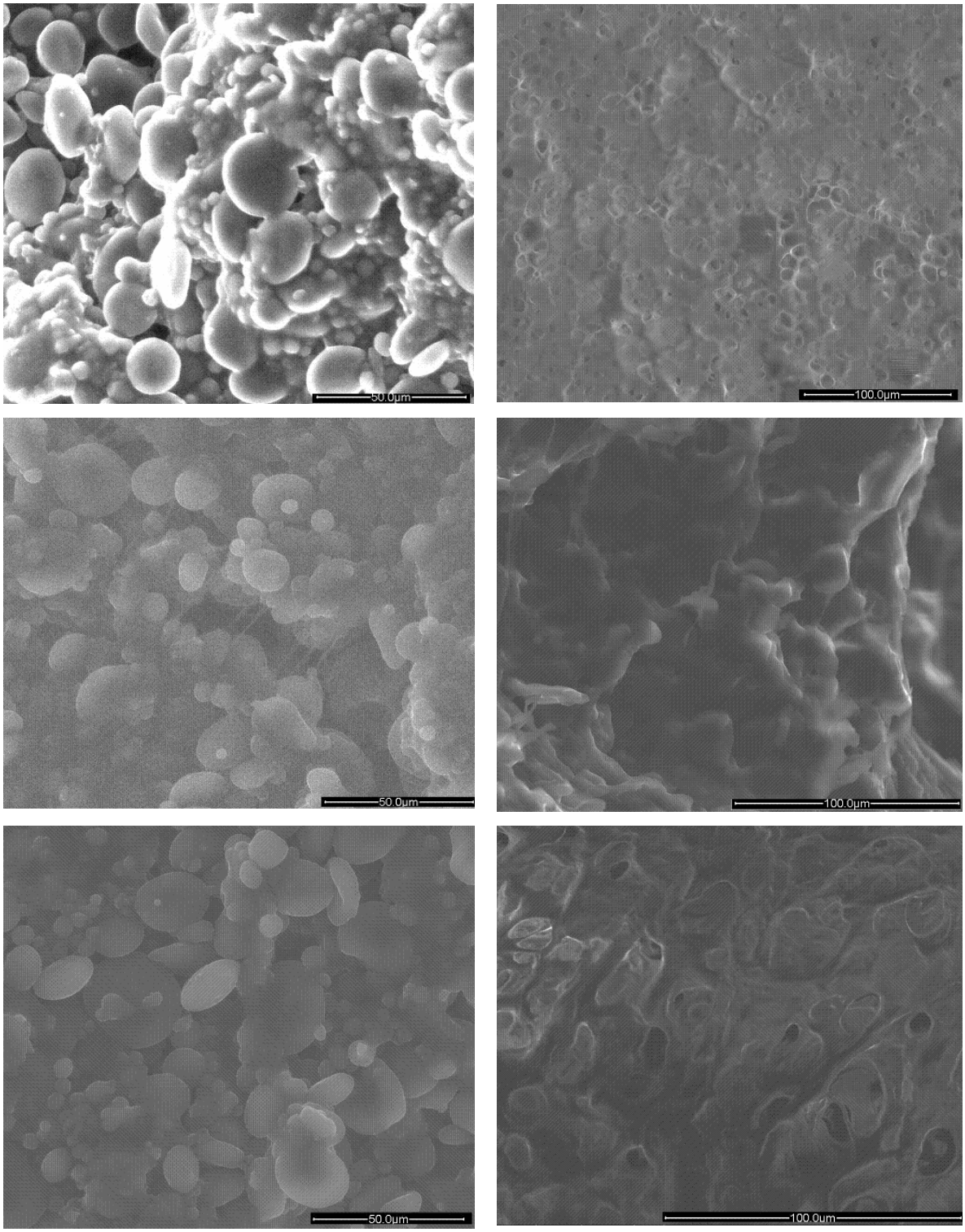
Fig 13.11 Colour characteristics (b yellowness) of dried, fresh cooked & dried and cooked WSN made from PF & UW
Error bars represent standard deviation values

13.7 Structural characteristics of WSN

The surface structure of raw noodles was studied using the ESEM mode of an electron microscope. In the case of all samples of raw noodles starch granules dominated the appearance of the surface although some gelatinization of starch granules was already visible in some lipase treatments especially in the samples incorporating fungal lipase and to some extent in the samples incorporating PP lipase (Fig 13.12). The images of control sample showed compact, lenticular to circular shaped starch granules. When looking at the samples treated with lipase from fungal origin it can be seen that slightly amorphous and not as compact images are obtained, with relatively irregular, depleted starch and gelatinisation of some starch on the surface. This was also seen to a similar extent in WSN incorporating PP lipase with many starch granules interspersed with gel-like regions. Noodles being treated with WG lipase were similar to the control samples, showing compact surface appearance with larger number of smaller appearing starch granules on the surface of noodles (Fig 13.12).

Upon cooking most of the starch gelatinizes and therefore the granular appearance is no longer clearly visible. Some evidence of granular structure remains in the control samples, (and to some extent in lipase treatments) where relatively even hollow regions can be seen within the protein network. Larger pockets are present in the network for samples treated with PP lipase (Fig 13.12). Drying of noodles did not have much effect of the surface appearance of control samples, showing overall again compact structure of granular starch. In samples treated with PP and especially in those incorporating fungal lipase preparation the overall structure is amorphous with many hole like regions within the structure, although some of the original granular formation of starch is still visible on the surfaces. Samples treated with WG lipase again showed surface similar in appearance to the control sample but having many smaller but compact starch granules in the surface. Cooking of dried noodles revealed that most of the starch in all samples but WG lipase treatments was gelatinised and its granular form is hardly noticeable within the gel-like surface and with the protein network (Fig 13.13). WG lipase treatments showed gelatinised starch within a protein network as well but to a less extent than the other samples and starch granules although too much a less extent than

before cooking are still visible on the surface, looking more like gel-like structures but showing the granular appearance with the protein network (Fig 13.13).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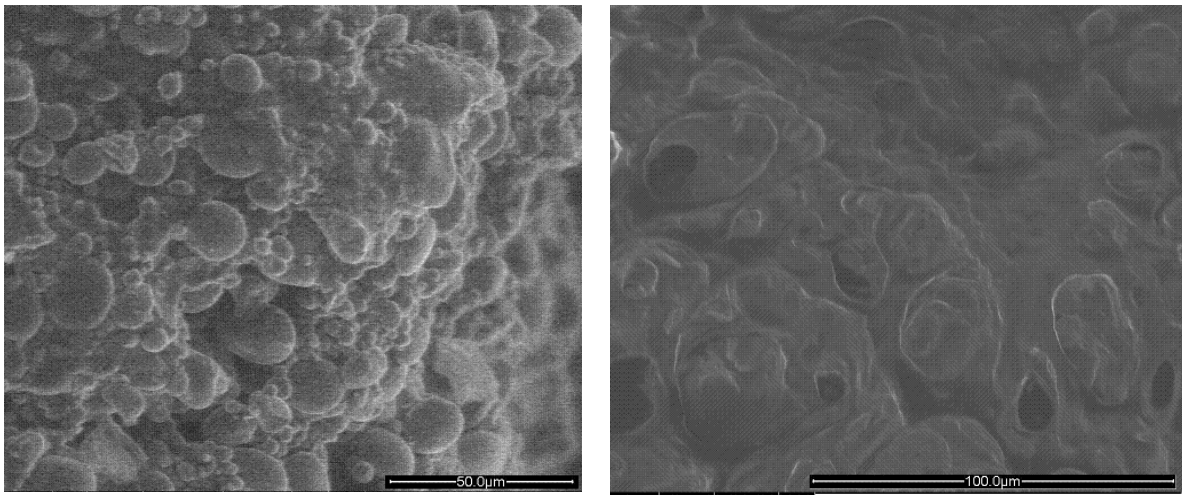
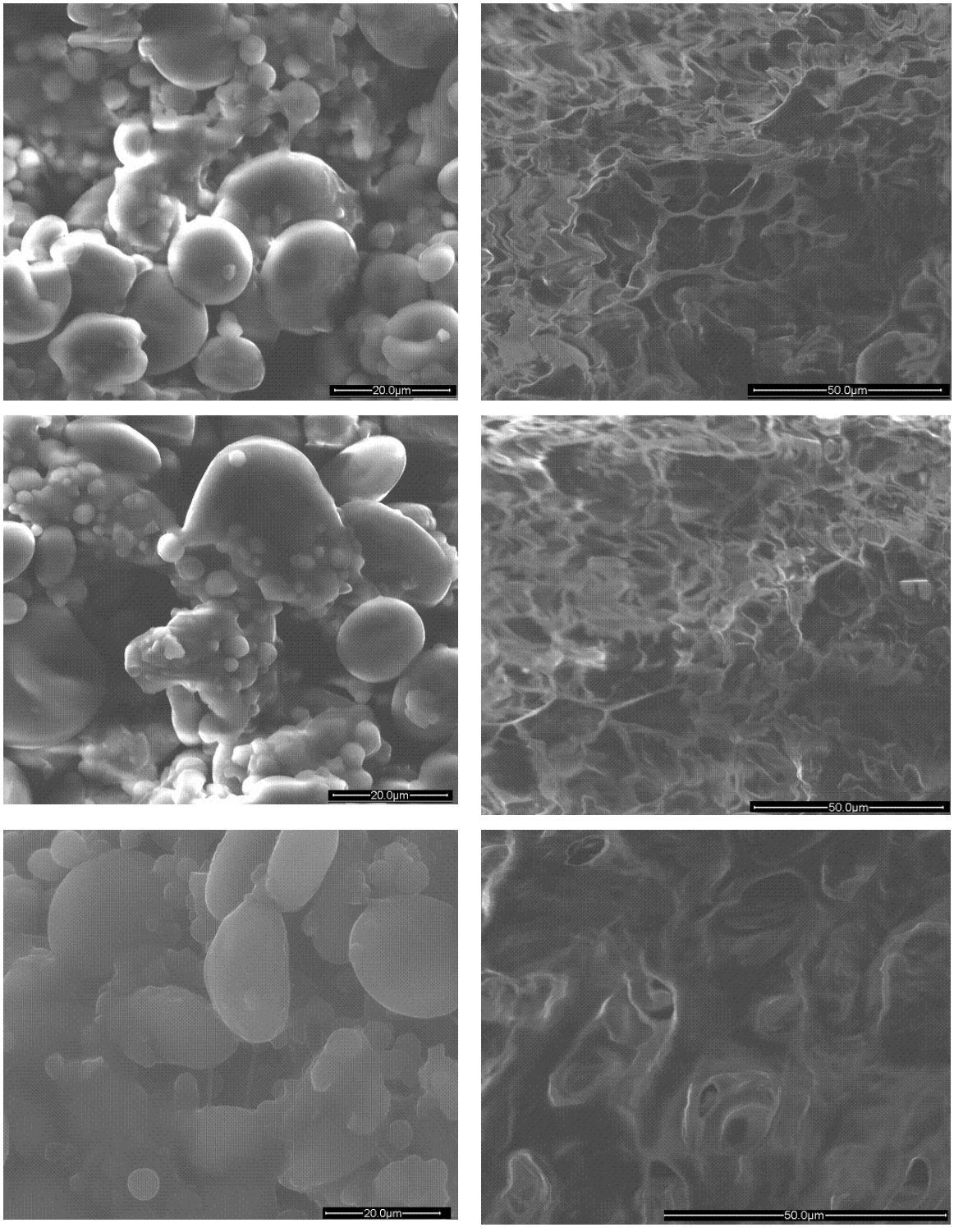


Fig 13.12 ESEM[a] images of fresh raw (left) and SEM[b] images of fresh cooked (right) WSN made form UW flour; Control, PP- lipase, Fungal lipase and WG – lipase (from top to bottom)

a ESEM, 6.0 Torr, 4°C, 800× Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 800× Mag., 5.0 spot size, 30kV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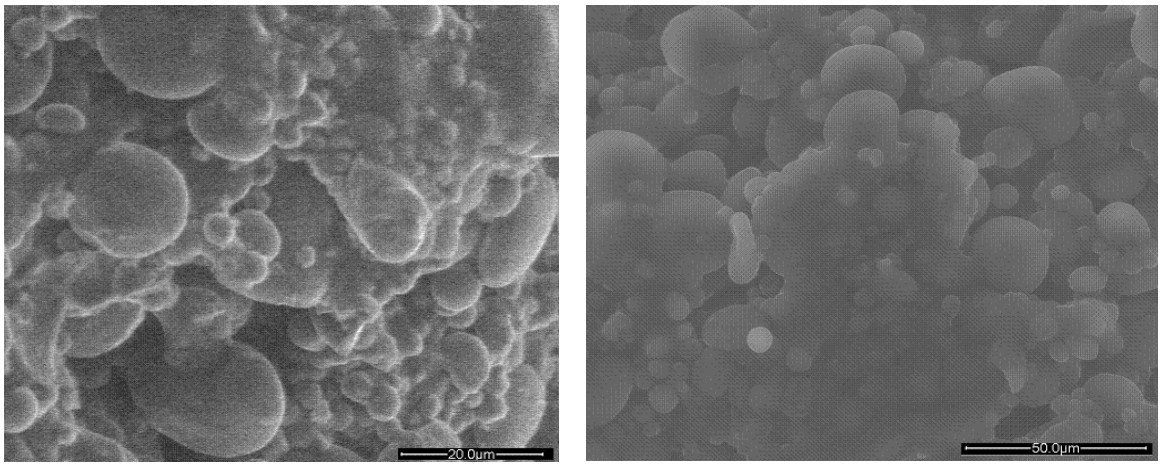


Fig 13.13 SEM images of dried[a] (left) and dried and cooked[b] (right), control, PP – lipase, Fungal lipase and WG – lipase from top to bottom

a SEM, 0.5 Torr, 23°C, 1600 × Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 1000 × Mag., 5.0 spot size, 30kV

13.8 Summary of results

Low levels of lipase present naturally in the flour indicate that at least as long as the wheat and flour are sound no adverse effect can be seen upon noodle processing. Although lipase acts primarily on lipids (its role in bread has been attributed to its actions on the small amounts of lipid that is routinely added to the bread formulation) in noodle formulation no fats are added, so the lipids present are very low levels naturally found in the flour. In addition it has to be said that the preparations used were partially rather than highly purified therefore, some side activities of other enzymes might have been present.

Textural properties of WSN were altered in one or the other way upon addition of each of the lipases. Three different addition of PP lipase were evaluated at first and these had different impact upon the properties of raw and cooked noodles. In terms of raw noodles the lowest level of addition (1×10^6 unites per batch) resulted in slightly softer noodles while the other levels of addition resulted in slightly harder noodles (2×10^6 and 5×10^6 units per batch). While for the raw noodles a medium level of addition resulted in slightly softer noodles while the other two levels resulted in slightly harder noodles (Fig 13.1). Lipase from WG was also evaluated at three different levels, here however, all levels appeared to result in slightly softer noodles for both raw and cooked noodles (Fig

13.2). For fungal lipase, four different levels of addition were evaluated and a similar pattern was observed for both raw and cooked noodles indicating slightly harder noodles upon the addition of this preparation (Fig 13.3). When some levels of this preparation were tested with the blade (measuring the cutting stress of noodles) a high correlation was observed (r^2 of 0.93). This indicates that both methods although measuring different textural properties are useful in the characterisation of texture of this type of product and can be applied in trying to understand noodle chewiness.

The addition of any of the lipase preparations had no adverse impact on cooking loss of WSN. Slightly higher losses in general were recorded for dried noodles (control included) as compared to the fresh samples.

Colour properties of WSN were adversely impacted by the addition of WG lipase, while PP and fungal lipase resulted in no significant changes to the colour properties of WSN. Control samples were whitest and brightest from all treatments at all times measured. Colour of WSN changed faster at 25°C compared to the storage of noodles at 4°C. Addition of WG lipase resulted in darkest and dullest noodle samples. Addition of PP resulted in no significant change in colour during storage at either 25°C or 4°C. WSN incorporating fungal lipase were transparent in appearance and had a surface appearance which could be described as glossy and oily. They were nevertheless bright yellow in colour.

The SEM showed relatively minor differences in starch appearance, with slightly different images seen in samples incorporating fungal and to some extent PP lipase. Overall, from a visual point of view, colour of control samples was regarded as best in terms of WSN quality attributes.

Chapter 14

Results and discussion: the effect of exogenous lipases on the quality parameters of Asian noodles prepared in the laboratory: Yellow alkaline noodles

The purpose of this chapter is to describe and discuss the results obtained when YAN were supplemented with varying levels of lipases from three different sources.

14.1 Introduction

The lipases used in the investigation of WSN are now incorporated into YAN and their impact upon the quality attributes studied. For this, the three lipase preparations were separately dissolved in distilled water and added to noodle formulations during the water addition stage. Since most plant lipases have neutral to alkaline pH optima, no major losses in enzyme activity were expected as the measured pH of this product was 10.05. The specific objectives have been to examine the effect of the selected exogenous lipases on quality particularly texture, surface structure and colour properties of YAN.

14.2 Levels of lipase in flour and stability during noodle processing

Relatively low levels of endogenous lipase were found in flour samples studied. Furthermore, no losses were recorded during noodle processing and storage under various conditions (Table 14.1). After additions of the exogenous lipases form all three sources, the enzyme remained active, and measured activity was approximately two folds higher, however, there was less activity when compared to the same levels of addition to the WSN (Chapter 13, Table 13.1), indicating that high pH conditions may have caused reduction in activity. Here the levels of activity ranged from 1.45 ± 0.18 for control samples; 1.42 ± 0.31 for WG lipase 2.15 ± 0.68 for PP lipase; and 2.22 ± 0.13 for fungal lipase (Table 14.1).

Table 14.1 Activity of lipase in control and supplemented YAN stored under different conditions

Treatment[a]	Lipase activity[b]
Controls	
Day 1	1.45 ± 0.18
24hr RT	1.61 ± 0.14
24hr FR	1.72 ± 0.27
Dried	1.18 ± 0.13
Lipase PP (2×10^6 units / batch)	
Day1	2.15 ± 0.68
Dried	2.09 ± 0.71
Lipase WG (8.19×10^2 units / batch)	
Day1	1.42 ± 0.31
Dried	1.35 ± 0.26
Lipase Fungal (20×10^4 units / batch)	
Day 1	2.22 ± 0.13
Dried	2.15 ± 0.19

a Abbreviations are: YAN, yellow alkaline noodles; RT, storage at room temperature; FR, storage at 4°C

b Unit for lipase activity is given in μmoles of fatty acids produced per gram of defatted flour per minute

14.3 Lipase from porcine pancreas: impact on YAN texture

Two different levels of porcine pancreas lipase preparation were evaluated first and the textural properties measured using the flat cylinder probe (P/45). Levels of initial addition were 2×10^6 and 5×10^6 units per batch of YAN. Immediately after preparation of YAN no marked differences were recorded between these treatments and control samples, all treatments being similar in noodle hardness. This applied for both raw and cooked samples (Fig 14.1). Storage at either 25°C or 4°C resulted in slightly softer noodles when lower levels of PP lipase preparation were used (2×10^6 per batch of YAN). This also was case for both raw and cooked noodles. Higher addition of PP lipase preparation (5×10^6 units per batch of YAN) appeared to have no impact before or after the storage (Fig 14.1).

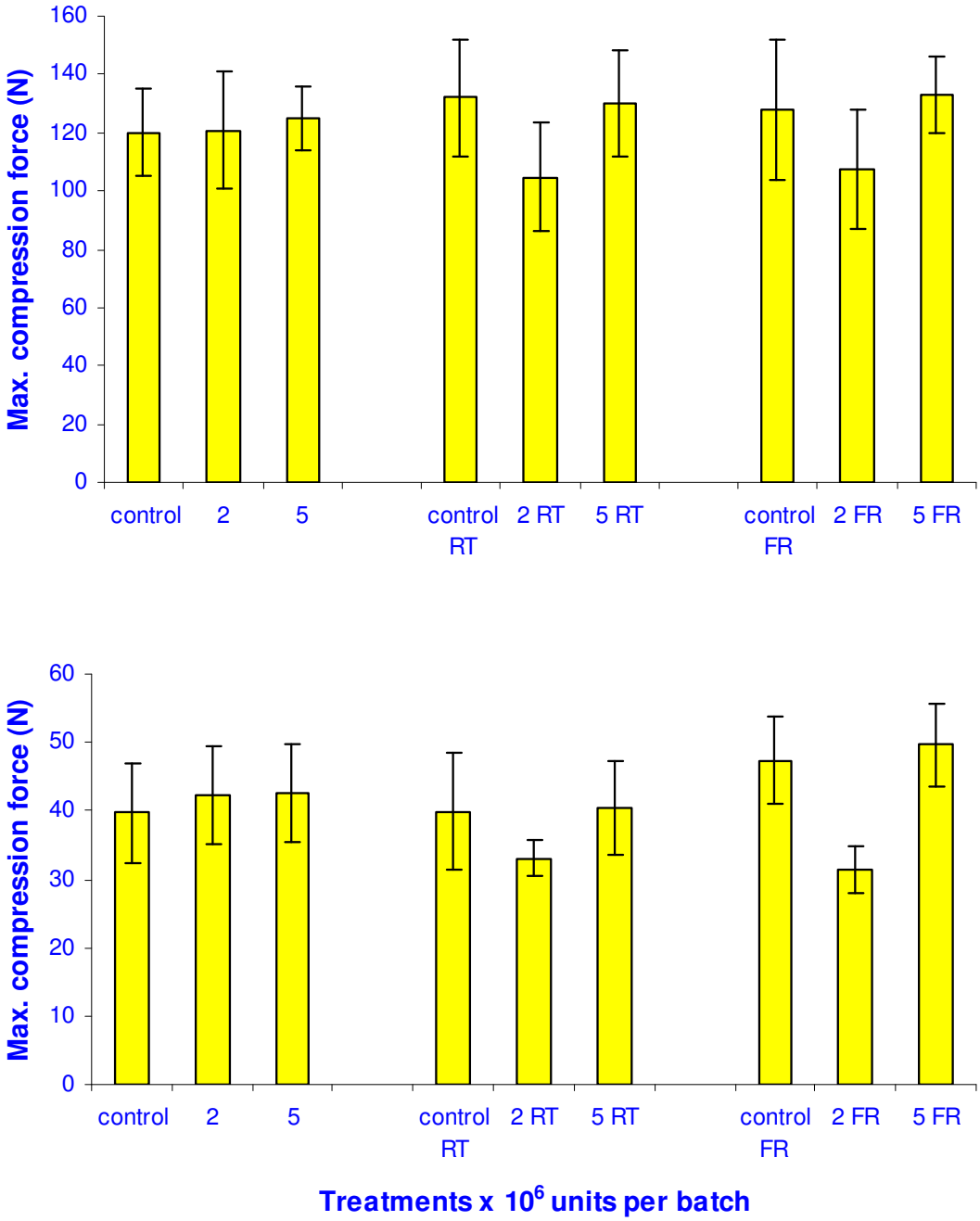


Fig 14.1 **Hardness characteristics of raw (top) and cooked (bottom) YAN treated with lipase from porcine pancreas. Impact of various levels of the enzyme on the textural properties of YAN**
Error bars represent standard deviation values. Measured using the flat cylinder probe (P/45)

The lower level of PP lipase addition was then used to complete the study and further textural properties of YAN were measured using the cutting (blade) attachment as well. The textural properties of YAN as measured using the blade attachment indicated slightly firmer noodles when compared to the control. This was true for all samples: raw, cooked before and after the storage (Table 14.2).

Table 14.2 Effects of lipase from porcine pancreas on the textural properties measured by TA-XT2 for YAN made from BGF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	36.1[a]	125[a]	11.0[a]	2.59[a]
	LipasePP	37.5[a]	125[a]	12.1[a]	2.66[a]
	RT control	40.1[a]	132[a]	12.4[a]	3.01[a]
	LipasePP RT	42.6[a]	130[a]	12.8[a]	3.11[a]
	FR control	39.5[a]	128[a]	11.1[a]	2.66[a]
	LipasePP FR	42.3[a]	133[a]	12.4[a]	2.79[a]
Cooked	control	10.6[a]	39.7[a]	3.50[a]	0.86[a]
	LipasePP	11.3[ab]	42.5[ab]	3.98[a]	0.91[a]
	RT control	10.7[a]	39.9[a]	4.58[a]	1.02[a]
	LipasePP RT	11.2[a]	40.5[a]	4.67[a]	0.97[a]
	FR control	13.4[bc]	47.4[b]	5.36[a]	1.11[a]
	LipasePP FR	12.7[ac]	49.6[b]	5.64[a]	1.21[a]
Dried [b]	control	12.4[a]	42.9[a]	5.84[a]	1.25[a]
	LipasePP	11.3[a]	40.7[a]	6.26[a]	1.42[a]

a Abbreviations are: YAN, yellow alkaline noodles; BGF, Baker's strong flour; PP, porcine pancreas; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Levels added 2×10^6 units / batch

Note: Means followed by the same letter are not statistically different at $P<0.05$ within the same column and treatment (raw-cooked-dried)

14.4 Lipase from wheat germ: The impact on YAN texture

Two levels of wheat germ lipase preparation were also evaluated in terms of textural properties of YAN. The texture of YAN was measured using the flat cylinder probe (P/45) in this initial stage only. Levels of WG lipase preparation were 8.19×10^2 and 20.5×10^2 units per batch of YAN. In the case of raw noodles none of the two additions evaluated showed different results in terms of noodle hardness (Fig 14.2). While again after cooking lower addition of WG lipase preparation resulted in slightly softer noodles (Fig 14.2). In addition noodles stored at lower temperatures resulted in firmer noodles when compared to the storage at 25°C.

For further experiments the lower level of addition was used and textural properties were measured using flat cylinder probe (P/45) and a cutting (blade) attachment. Again, textural properties measured with the blade attachment were opposite of that observed using the flat cylinder probe (P/45). Slightly firmer noodles were observed when textural characteristics wee measured using the blade attachment (Table 14.3).

14.5 Fungal lipase: The impact on YAN texture

Again two different levels of addition were evaluated (Figs 14.3 - 14.5). Fungal preparation was added to the YAN formulation at 20×10^4 and 40×10^4 units per batch of YAN. Both additions of fungal lipase preparation resulted in slightly harder noodles as measured using the flat cylinder probe (P/45) at time zero. After the storage at both temperatures only higher levels of addition 40×10^4 resulted in slightly harder noodles. When noodles were cooked immediately after preparation no great differences were seen between the treatments (Fig 14.3).

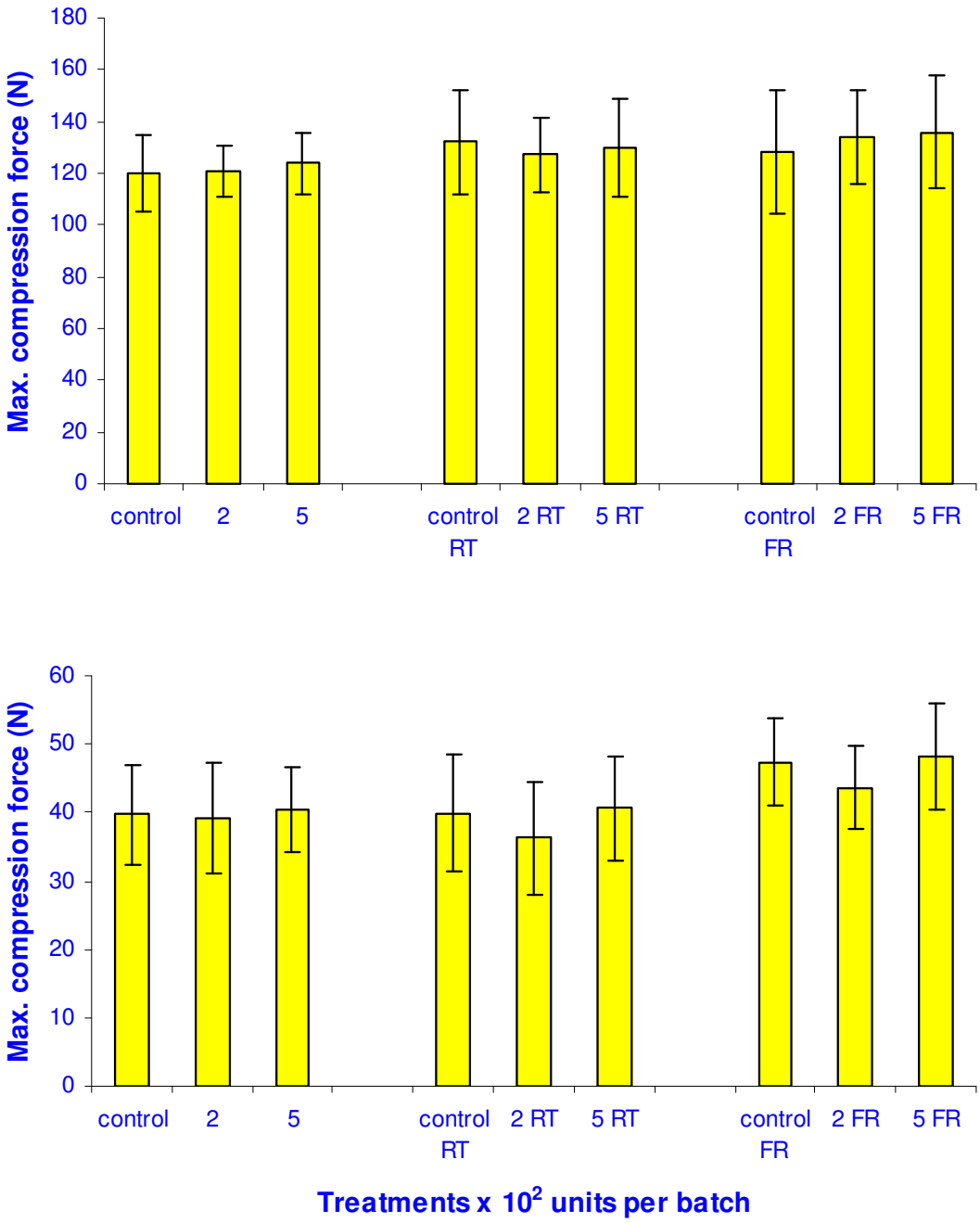


Fig 14.2 **Hardness characteristics of raw (top) and cooked (bottom) YAN treated with lipase from wheat germ. Impact of various levels of the enzyme on the textural properties of YAN**

Error bars represent standard deviation values. Measured using the flat cylinder probe (P/45)

Table 14.3 Effects of lipase from wheat germ on the textural properties measured by TA-XT2 for YAN made from BGF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	35.1[a]	120[a]	11.0[a]	2.59[a]
	LipaseWG	37.4[a]	124[a]	11.6[a]	2.66[a]
	RT control	40.1[a]	132[ab]	12.4[a]	3.01[a]
	LipaseWG RT	41.6[a]	129[ab]	12.6[a]	3.30[a]
	FR control	39.5[a]	128[ab]	11.1[a]	2.66[a]
	LipaseWG FR	42.9[a]	136[b]	11.4[a]	2.71[a]
Cooked	control	10.6[a]	39.7[a]	3.50[a]	0.86[a]
	LipaseWG	11.1[ab]	40.4[ab]	3.67[a]	0.93[a]
	RT control	10.7[a]	39.9[a]	4.58[a]	1.02[a]
	LipaseWG RT	11.3[ab]	40.7[ab]	4.66[a]	0.99[a]
	FR control	13.4[b]	47.4[b]	5.36[a]	1.11[a]
	LipaseWG FR	13.7[b]	48.2[b]	5.64[a]	1.16[a]
Dried [b]	control	12.4[a]	42.9[a]	5.84[a]	1.25[a]
	LipaseWG	12.0[a]	42.1[a]	6.66[a]	1.49[a]

a Abbreviations are: YAN, yellow alkaline noodles; BGF, Baker's strong flour; WG, wheat germ; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Levels added 8.19×10^2 units / batch

Note: Means followed by the same letter are not statistically different at $P<0.05$ within the same column and treatment (raw-cooked-dried)

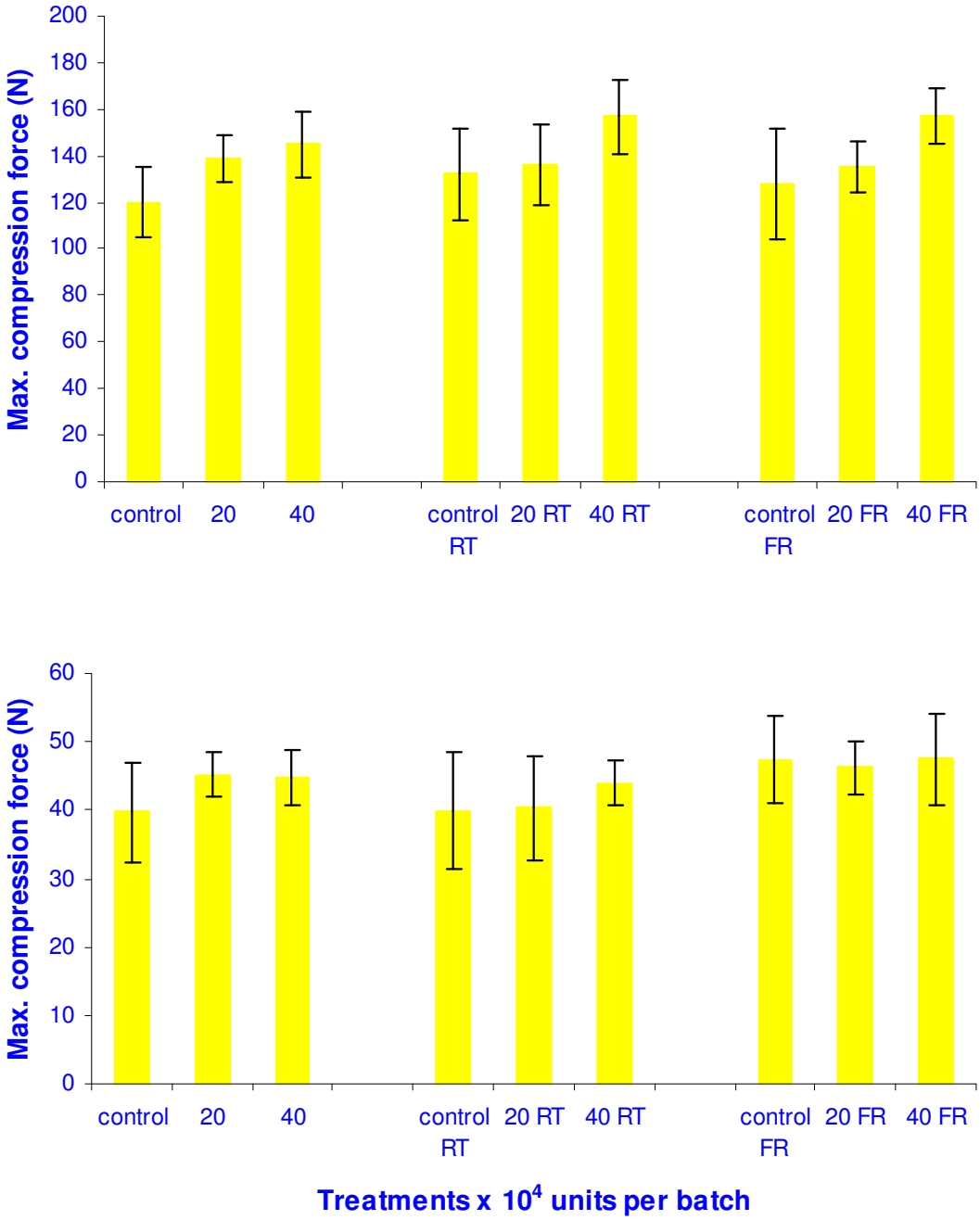


Fig 14.3 Hardness characteristics of raw (top) and cooked (bottom) YAN treated with lipase of fungal origin. Impact of various levels of the enzyme on the textural properties of YAN

Error bars represent standard deviation values. Measured using the flat cylinder probe (P/45)

The higher rate of fungal lipase addition was used to complete the study and evaluate the textural properties using the blade attachment as well. Addition of fungal lipase preparation resulted in noodles being slightly firmer when cutting stress of noodle strands was measured (Table 14.4).

Table 14.4 Effects of fungal lipase on the textural properties measured by TA-XT2 for YAN made from BGF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	35.1^{a}	120^{a}	11.0^{a}	2.59^{a}
	Lipase	47.4^{ab}	145^{b}	13.5^{a}	3.08^{a}
	RT control	40.1^{a}	132^{ab}	12.4^{a}	3.01^{a}
	Lipase RT	53.8^{b}	157^{b}	17.0^{b}	4.08^{a}
	FR control	39.5^{a}	128^{a}	11.1^{a}	2.66^{a}
	Lipase FR	51.3^{b}	151^{b}	17.1^{b}	3.94^{a}
Cooked	control	10.6^{a}	39.7^{a}	3.50^{a}	0.86^{a}
	Lipase	13.3^{ab}	45.2^{ab}	4.44^{ab}	1.00^{a}
	RT control	10.7^{a}	39.9^{a}	4.58^{a}	1.02^{a}
	Lipase RT	13.5^{ab}	43.9^{ab}	6.55^{c}	1.21^{a}
	FR control	13.4^{ab}	47.4^{b}	5.36^{abc}	1.11^{a}
	Lipase FR	14.7^{b}	47.5^{b}	7.23^{c}	1.33^{a}
Dried [b]	control	12.4^{a}	42.9	5.84^{a}	1.25^{a}
	Lipase	10.3^{a}	34.0	6.35^{a}	1.19^{a}

a Abbreviations are: YAN, yellow alkaline noodles; BGF, Baker's strong flour; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Levels added 20×10^4 units / batch

Note: Means followed by the same letter are not statistically different at $P<0.05$ within the same column and treatment (raw-cooked-dried)

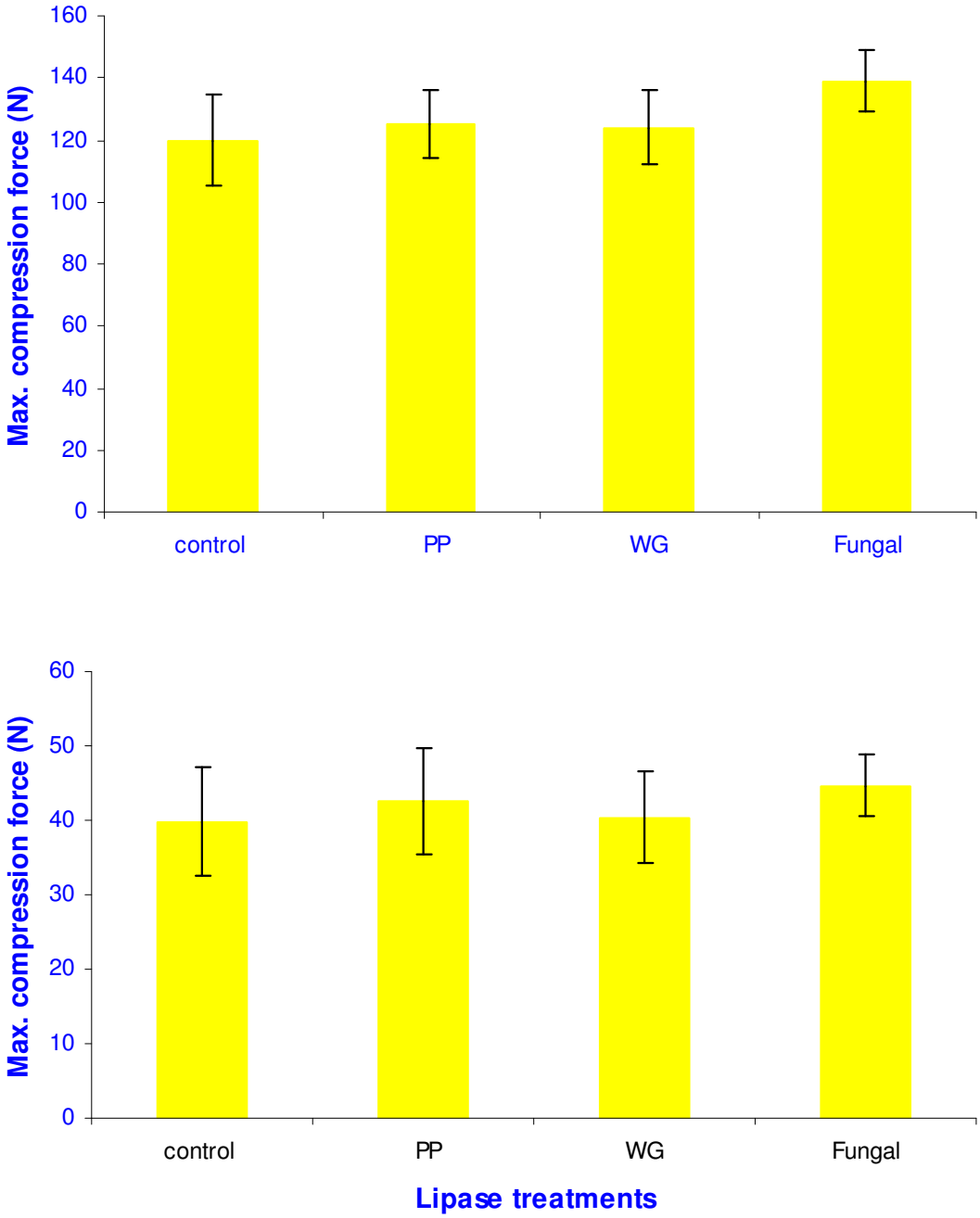


Fig 14.4 **Comparison of the impact of different lipases on the hardness characteristics of YAN (raw, top; cooked, bottom) used at the final levels of addition (PP, 2×10^6, WG, 8.19×10^2 and fungal lipase 20×10^4 unites per batch)**

Error bars represent standard deviation values. Measured using the flat cylinder (P/45)

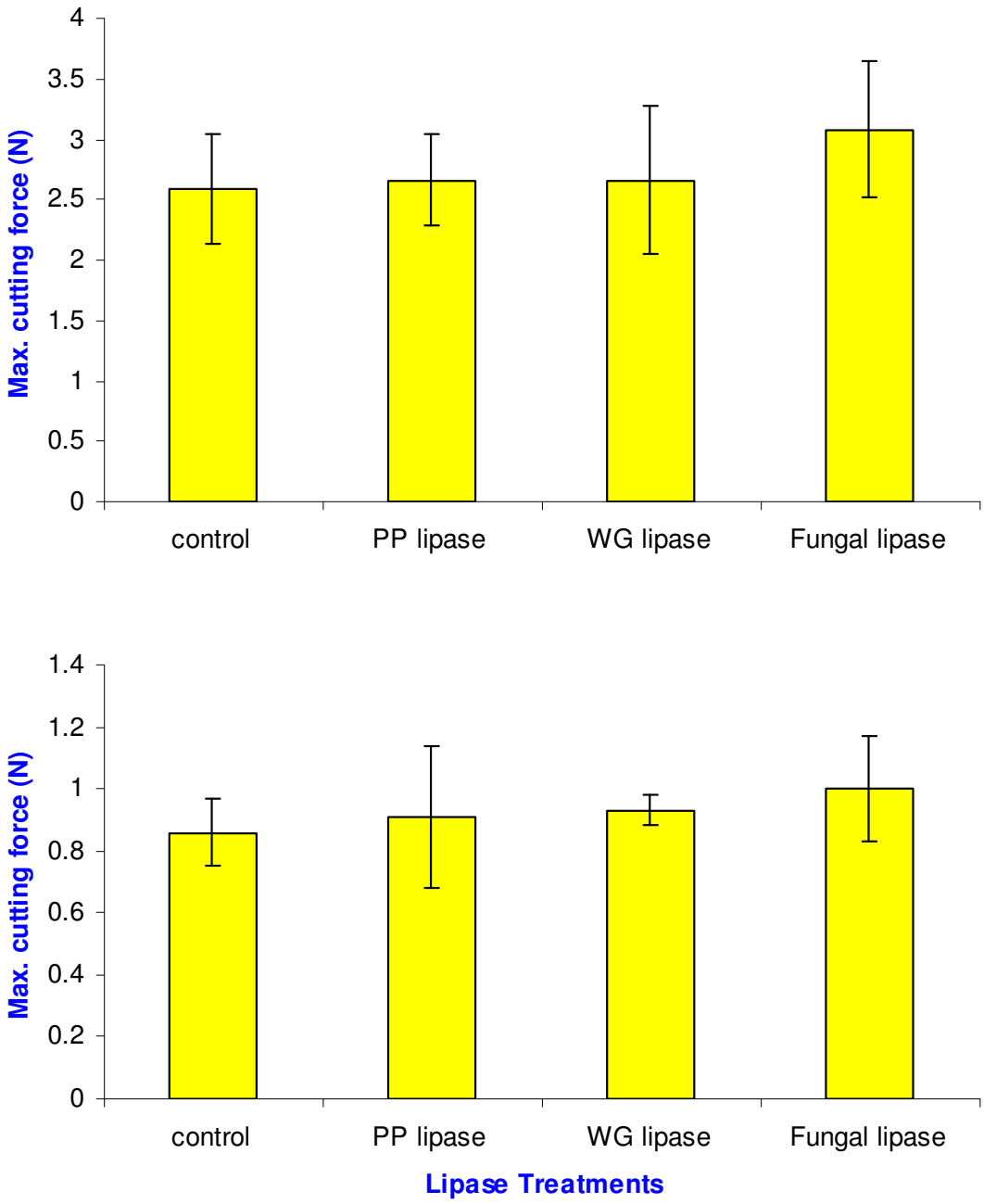


Fig 14.5 **Comparison of the impact of different lipases on the firmness characteristics of YAN (raw, top; cooked, bottom) used at the final levels of addition (PP, 2×10^6, WG, 8.19×10^2 and fungal lipase 20×10^4 unites per batch)**

Error bars represent standard deviation values. Measured using the blade attachment

14.6 The effect of lipase on cooking yield

When samples of noodles were cooked to the optimum point and analysed for weight increase and cooking loss (Table 14.5) the only differences observed were increases in losses for some of the treated dried noodles.

Table 14.5 Cooked weight and cooked loss of YAN made from BGF flour with and without lipase addition

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	4.30	24. 9[a]	9.67[a]
fresh lipasePP	4.30	23.7[a]	9.81[a]
fresh lipaseWG	4.30	24.2[a]	10.2[a]
fresh lipase fungal	4.30	22.5[a]	8.89[a]
dried control	9.00	72.07[a]	12.7[a]
dried lipasePP	9.00	67.2[a]	14.7[a]
dried lipaseWG	9.00	66.7[a]	15.4[ab]
dried lipase fungal	9.00	67.6[a]	15.9[b]

a Abbreviations are: YAN, yellow alkaline noodles; BGF, Baker's strong flour
Note: Means followed by the same letter are not statistically different at $P<0.05$ within the same column and treatment (raw-dried)

14.7 Influence of lipases on colour properties of YAN

Wheat germ, porcine pancreas and fungal lipase preparations were added to the YAN formulation at 20.5×10^2, 2×10^6 and 40×10^4 units pear batch respectively, and the colour characteristics were measured over a period of 96hr storage at different temperatures. At these levels of addition fungal lipase showed least adverse impact on colour properties of YAN. In all cases discoloration of noodle sheets was observed control included and it was more prominent and it occurred at a faster rate in noodles stored at 25°C as compared to the storage at 4°C (Figs. 14.6 & 14.7). Drying and cooking and drying and cooking immediately after the preparation of noodles prevented discoloration to some extent, however, noodles treated with PP and WG lipase were darker after cooking, drying and drying and cooking (as indicated by higher b*values) when compared to the control (Figs. 14.8 & 14.9). There were no specks in any of the samples.

Wheat germ (WG) lipase

Addition of WG lipase resulted in darkest noodles out of all treatments studied. Noodles treated with this lipase preparation were dark yellow to brown in colour at time zero (reading taken immediately after noodle sheets have been prepared), and noodle sheets discoloured further during the storage. This is indicated in Figs. 14.6 & 14.7 by lower L* and higher b* values for these treatments compared to other samples. Addition of WG lipase had an adverse impact on colour of dried, cooked and dried and cooked noodles as well, as indicated by high b* (yellowness) values (Figs. 14.8 & 14.9). This might indicate enzymes activity even at higher temperatures.

Porcine pancreas (PP) lipase

Whiteness (L* values) of noodle sheets were not adversely impacted by the addition of PP lipase to the YAN formulation. The values for this treatment were similar to the control, however, higher b* values were recorded during the storage at both temperatures for this treatment (Figs 14.6 & 14.7). Noodles treated with PP lipase were darkish yellow in colour. Only slightly more yellow than the control which is not necessarily an adverse effect. Similar was observed after drying and cooking, where noodles treated with PP lipase had similar L* values as the control but higher b* values, indicted more yellow products (Figs. 14.8 & 14.9).

Fungal lipase

Out of the three lipases at the levels studied here, fungal lipase resulted in colour characteristics that were most similar to control samples. YAN treated with fungal lipase were quite yellow in colour. YAN treated with fungal lipase had higher L* values than any other treatment control included, and in the case of storage at 25°C slightly higher b* values than control (Figs. 14.6 & 14.7). This shows that the addition of fungal lipase did not result in adverse colour characteristics of YAN. A similar pattern was seen after cooking or drying, where both L* and b* values were similar to the control samples, indicting similar colour characteristics to that seen in control samples (Figs. 14.8 & 14.9).

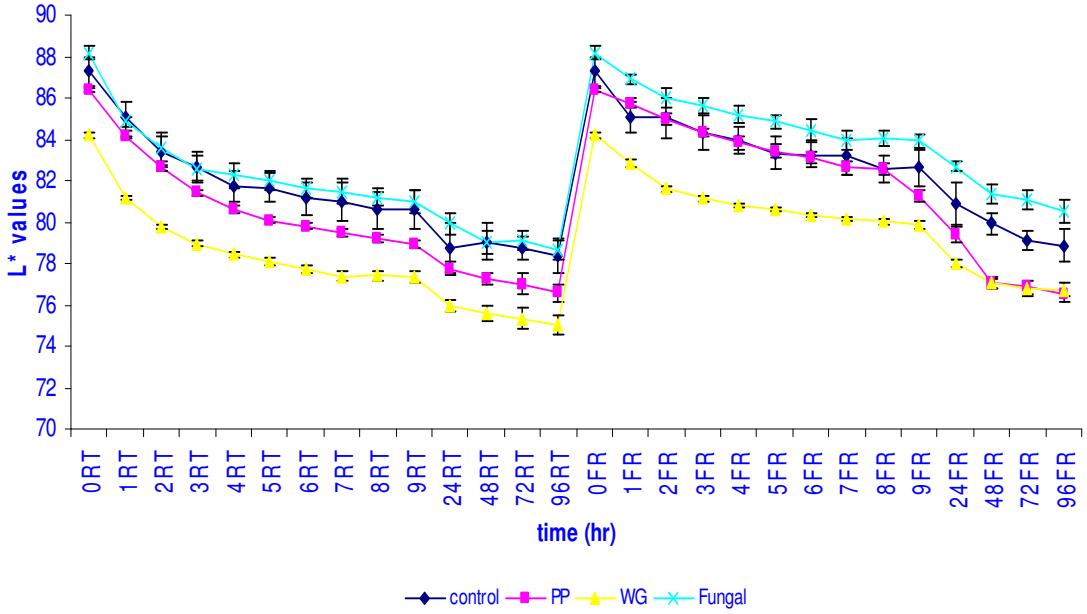


Fig 14.6 **Colour characteristics (L* values [whiteness]) of YAN made from BGF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

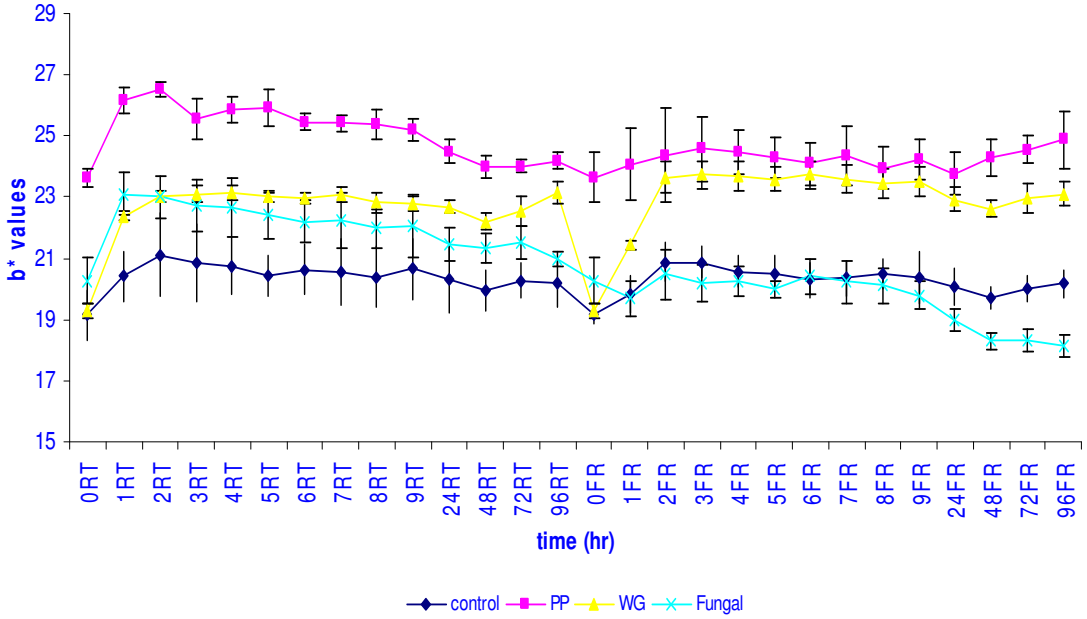


Fig 14.7 **Colour characteristics (b* values [yellowness]) of YAN made from BGF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

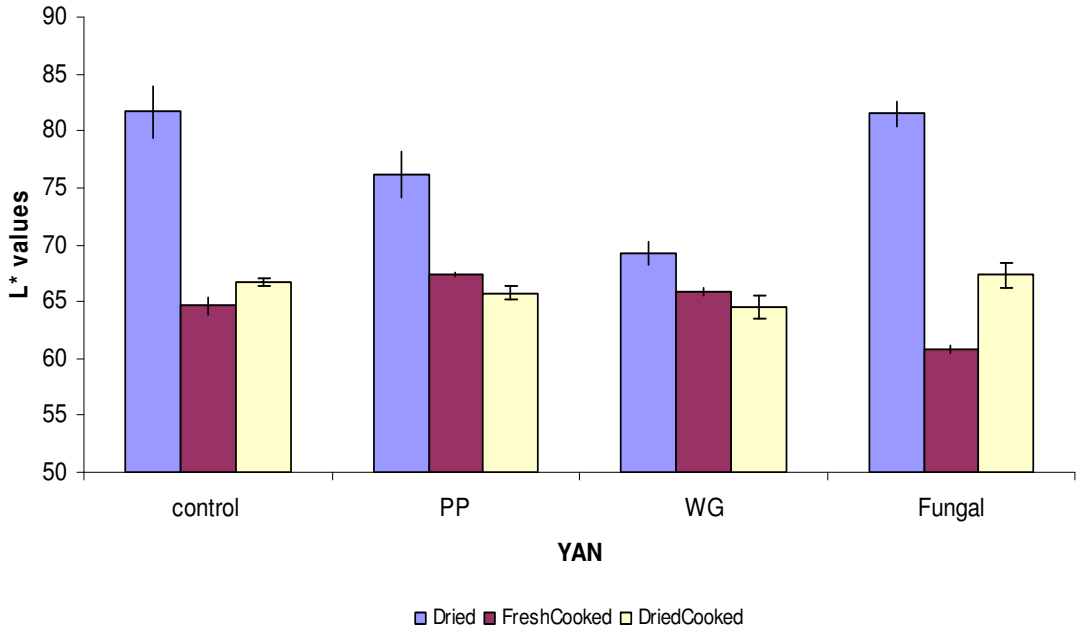


Fig 14.8 **Colour characteristics (L* whiteness /brightness) of dried, fresh cooked & dried cooked YAN made from BGF**
Error bars represent standard deviation values

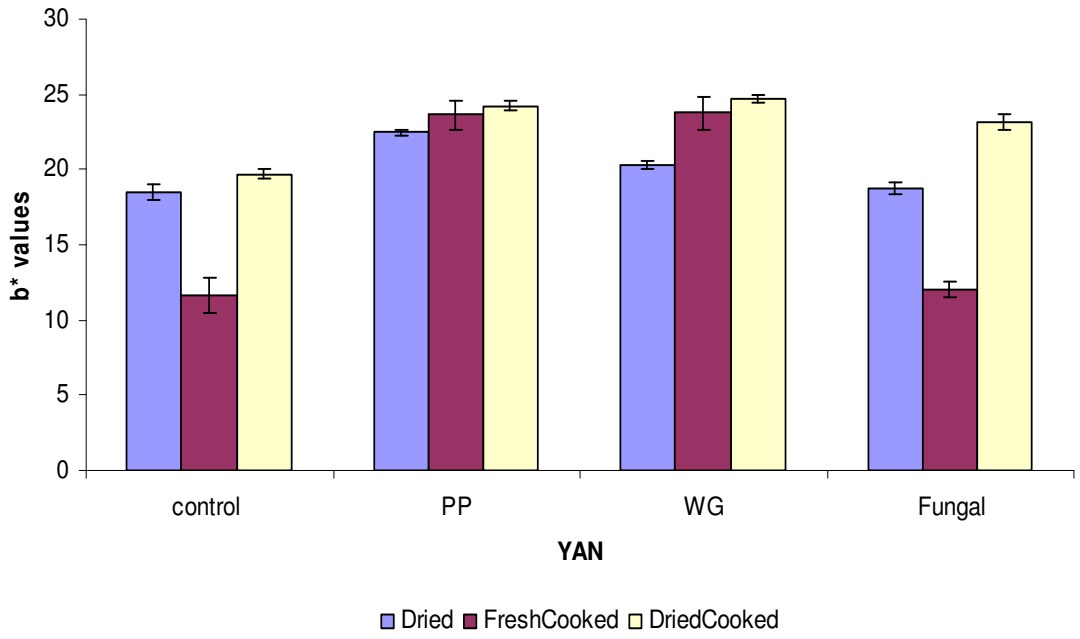
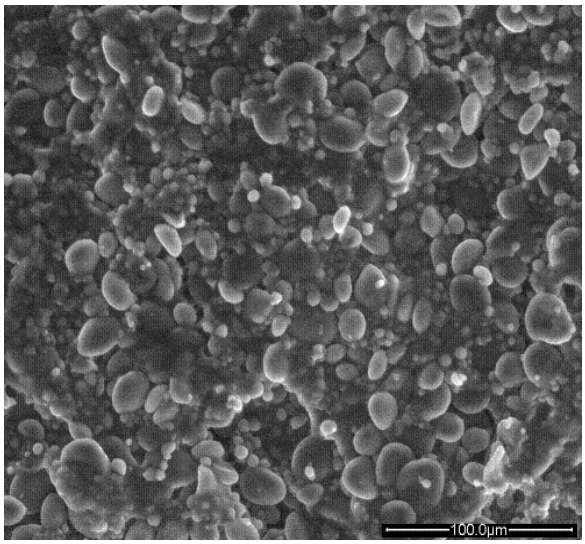
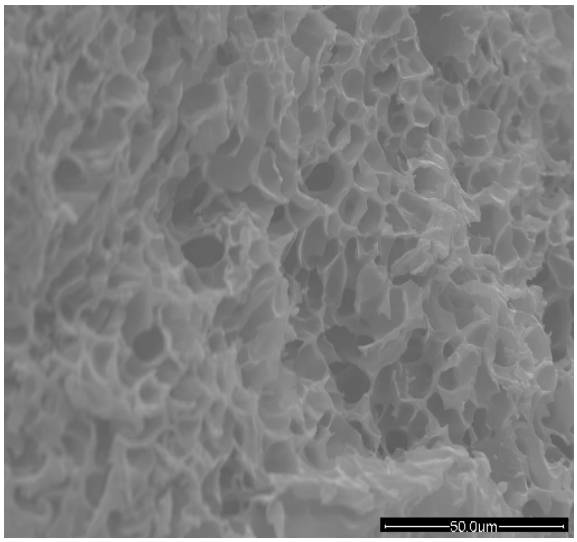


Fig 14.9 **Colour characteristics (b* yellowness) of dried, fresh cooked & dried cooked YAN made from BGF**
Error bars represent standard deviation values

14.8 Structural properties of YAN

Similar patterns in ESEM and SEM images were observed here as in the case of WSN and lipase treatments (Chapter 13). The surface structure of raw noodles was studied using the ESEM mode of an electron microscope while cooked samples were viewed using the SEM mode following sample preparation. Here as well in the case of raw YAN starch granules dominated the appearance of the surface although some gelatinization of starch granules was already visible in some lipase treatments especially in the samples incorporating fungal lipase and to some extent in the samples incorporating PP lipase (Fig 14.10). The images of control sample showed compact, lenticular to circular shaped starch granules. Looking at the samples treated with lipase from fungal origin it can be seen that slightly amorphous and not as compact images are obtained. Noodles being treated with WG lipase were similar to the control samples, showing compact surface appearance with larger number of smaller appearing starch granules on the surface of noodles (Fig 14.10).





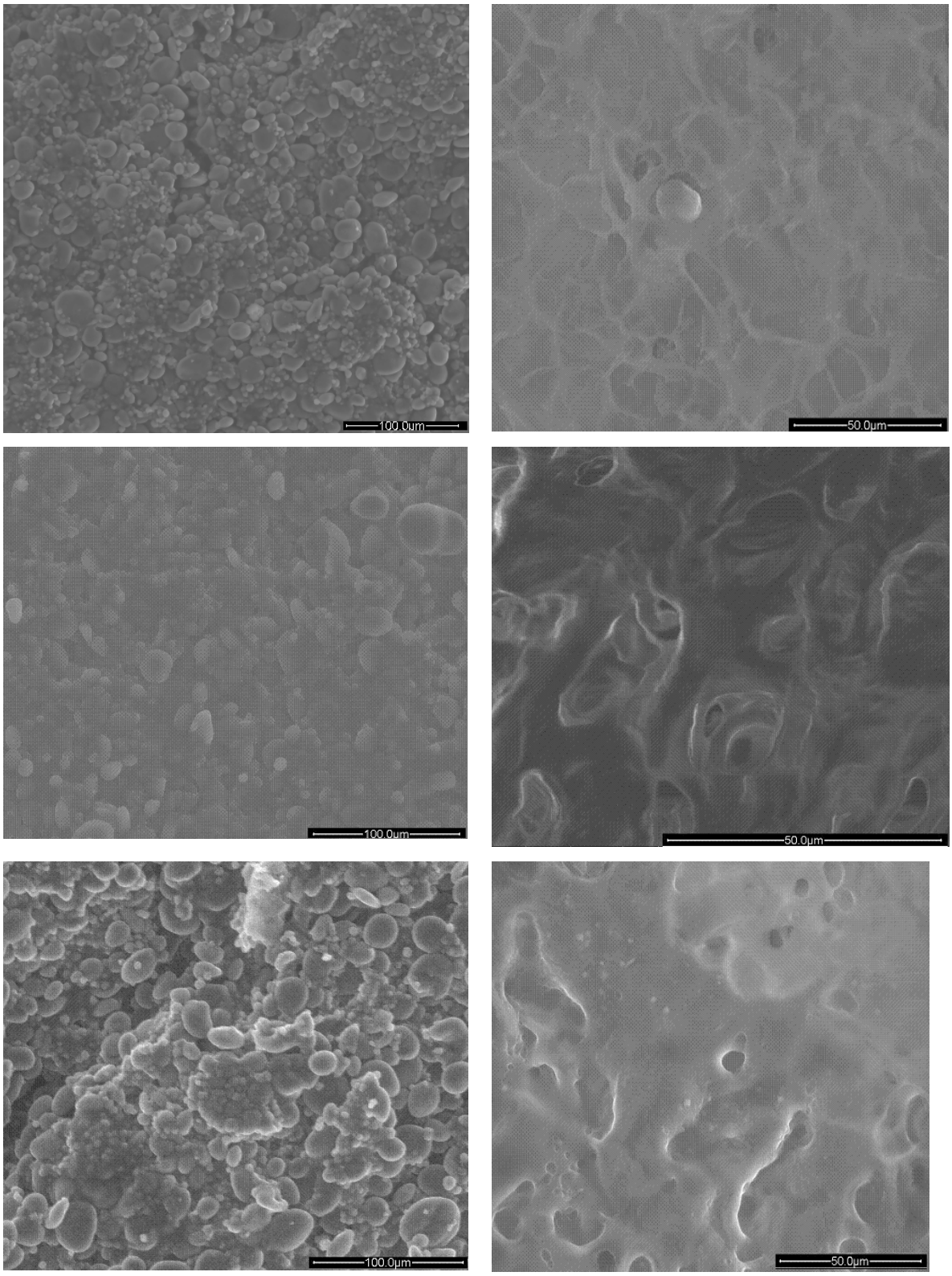
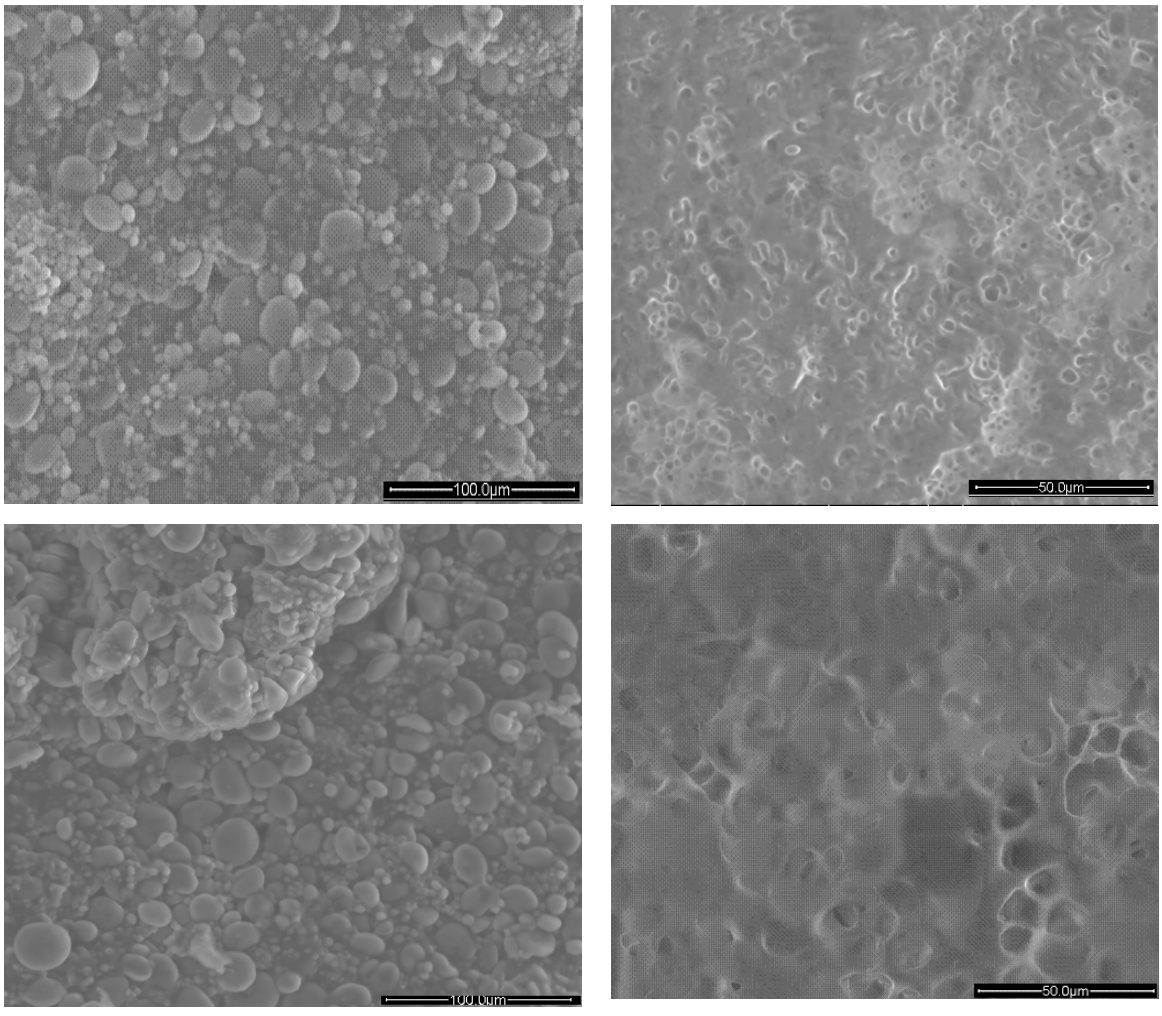


Fig 14.10 ESEM[a] images of fresh raw (left) and SEM[b] images of fresh cooked (right) YAN made form BGF flour; Control, PP- lipase, Fungal lipase and WG – lipase (from top to bottom)

a ESEM, 6.0 Torr, 4°C, 400× Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 1500× Mag., 5.0 spot size, 30kV

Cooking here resulted in complete starch gelatinisation with some form of granular starch being visible in control samples while the same cannot be said for samples incorporating lipase preparations (Fig 14.10). Drying of noodles did not have much effect of the surface appearance of control samples, showing overall again compact structure of granular starch for all samples (Fig 14.11). Cooking of dried noodles reviled that most of the starch in all samples. Some form of starch granularity can be seen in control and PP lipase samples, while samples incorporating WG and fungal lipase show no more evidence of granular starch within the gelatinised starch paste and the protein network (14.12).



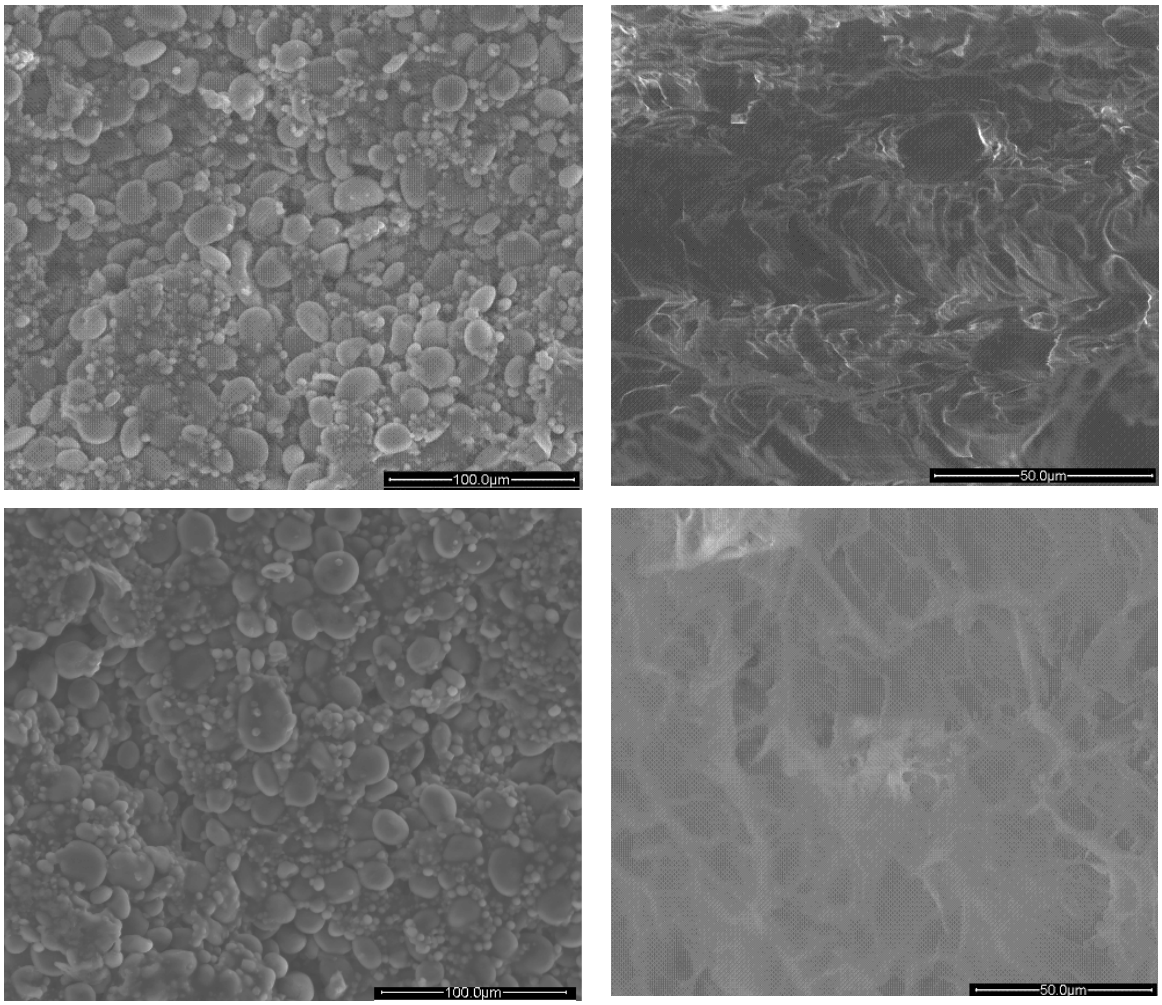


Fig 14.11 SEM images of dried[a] (left) and dried and cooked[b] (right), control, PP – lipase, Fungal lipase and WG – lipase from top to bottom

a SEM, 0.5 Torr, 23°C, 400 × Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 1500 × Mag., 5.0 spot size, 30kV

14.9 Summary of results

Slightly lower levels of lipase activity were found in YAN control and lipase treatments as compared to the same level of addition in the WSN, probably due to higher pH in these types of products than WSN. This is consistent with the enzyme preparations being more stable at lower (neutral pH) than at alkaline levels.

Addition of PP lipase (at a level of 2×10^6 units per batch) resulted in slightly softer YAN following the storage et either 25°C or 4°C, while no difference was recorded when textural properties were measured immediately after preparation. Incorporation of 5×10^6 unites per batch of the same preparation did not show any effects (Fig 14.1). This

was found to be true for both raw and cooked YAN. Both levels of WG lipase addition tested here showed no difference on textural characteristics of either raw or cooked YAN. Incorporation of fungal lipase indicated slightly harder noodles when textural properties were measured immediately after preparation (both raw and cooked YAN). However, after storage at 25°C theses treatments were the same in terms of hardness as control samples in terms of cooked noodles, while slightly harder noodles were obtained after storage at 25°C in the case of raw noodles being treated with fungal lipase. A similar effect was observed for the storage at 4°C and harder YAN (incorporating fungal lipase) were seen in the raw form, while upon cooking they were no longer different from the control samples (Fig 14.3).

Although lipases from different sources, can be difficult to compare due to their different activities and hence, different units for activity expression, it can be concluded that the end levels of lipases added to the YAN were at similar / comparable levels (Table 14.1). Therefore, from Fig 14.4 it can be concluded that none of the lipase preparations had any adverse effects on the textural properties of YAN. Both methods are meaningful and useful and can be used to further evaluate and understand rather difficult meaning of noodle chewiness.

Cooking loss of YAN was not adversely effected by the addition of nay three of the lipase preparations studied here. Higher cooking losses were seen in YAN as compared to the WSN (probably due to the addition of alkaline salts to the YAN formulation) and furthermore higher losses were recorded for dried when compared to the fresh noodles. However, no difference in cooking loss was due to any of the lipases.

In terms of colour properties of YAN same goes as for WSN, the worst case scenario was seen when WG lipase was added to the YAN formulation resulting in darkest noodles (L* and b* values) at all time, and discoloring even further after the elapse of 96hr (at a faster rate at YAN stored at 25°C compared to the 4°C storage). The other two preparations, PP and fungal lipase, were similar to control YAN. These control samples were whitest and brightest (higher L* and lower b* values) at all the time, although discoloration did occur in control samples as well. Noodles incorporating PP lipase were similar in appearance to the control samples, less bright perhaps, while

YAN incorporating fungal lipase were transparent and very yellow in colour but quite bright as well.

Only slight differences in starch appearance were seen in YAN incorporating fungal lipase, while the other two lipases appeared similar to the control samples when starch and noodle surface was studied using the ESEM / SEM. This was found to be true for all treatments, raw, cook, dried and dried and cooked.

Chapter 15

Results and discussion: Peroxidase and the quality parameters of Asian noodles prepared in the laboratory: White salted noodles

The purpose of this chapter is to describe and discuss the results obtained when peroxidase from horseradish was incorporated into WSN. The parameters studied included texture, colour and discolouration of noodle sheets during storage, structural as well as cooking characteristics.

15.1 Introduction

Oxidising agents have a beneficial effect on dough properties and attributes including volume, texture and crumb structure of baked products (Van Oort et al 1997). Replacement of chemical by enzymatic oxidisers (including POX) could provide the benefit of more specific and better controlled oxidation processes. It is proposed that although the making of bread and noodles have little in common the action of POX might be useful to improve the textural properties of Asian noodle products, furthermore, the application of such an enzyme might eliminate the use of other synthetic ingredients. In addition this enzyme seems to be crucial for colour quality as it effectively bleaches carotene. Hsieh and McDonald observed that a purified LOX from durum wheat endosperm also showed POX activity (Hsieh and McDonald 1984), thus indicating a direct link of POX to colour properties of wheat end products. It seems to negatively affect the yellow colour intensity of flour, probably by decreasing lutein content due to oxidation. This might be of significance for WSN noodles as clear snow-white (creamy white in some regions e.g. Japan) colour is preferred for this type of products. In the preliminary studies two preparations of POX were used from different suppliers (Sigma – Aldrich and MP Biomedicals, Inc.) both were sourced from horseradish (see Chapter 7). Initial trials included additions of different levels of the two preparations and the textural properties were measured using cylinder probe only. In the case of POX from Sigma –Aldrich two different levels were tried the initial 2.13×10^3U per batch of noodles (Figs 15.1 and 15.2) of POX preparation was estimated to be

double the amount already present in the flour. The starting point of addition for POX from MP Biomedicals was determined in the same way and was calculated to be approximately 36×10^3U per batch of noodles. In addition, higher levels of POX were tried out and subsequently the amount of 72×10^3U per batch of noodles was chosen to complete the study (Figs 15.3 and 15.4).

15.2 Levels of POX in flour and stability during noodle processing

Relatively high levels of POX have been measured in the flours studied (Chapter 11) and some losses in the original activity have been observed during noodle processing, but then relative stability of the enzyme has been obtained during the storage of noodles under various conditions (Table 15.1). The levels found in the two flours used for WSN ranged from 323 ± 15 for PF to 343 ± 19U of POX for UW. These levels naturally occurring in the flour dropped slightly during the WSN processing and ranged from 269 ±9 for fresh WSN made from PF to 305 ± 10U of POX for fresh WSN made from UW and even further drop was recorded for dried WSN (266 ± 33 for dried WSN made from PF and 292 ± 17U of POX for dried WSN made from UW) (Table 15.1). The levels of POX activity increased approximately double fold after the addition of exogenous POX, and were relatively stable during processing and the storage of WSN (Table 15.1).

15.3 Evaluation of two preparations of POX: The impact on texture of WSN

Although two POX preparations were from the same source (horseradish), both were tested. It was estimated that the addition of the two preparations would result in approximately same activity of POX so that the results can be directly compared.

15.4 POX preparations from Sigma - Aldrich

The effect of two different levels of addition of POX to the noodle formulation is shown in Figs 15.1 and 15.2. The liquid preparations of POX were added straight to the noodle formulation during the water addition stage of the processing. The textural differences were recorded using the cylinder probe (P/45) as noodle hardness Fig 15.1 and the flat blade as noodle firmness Fig 15.2. Similar findings were recorded in both cases noodle hardness as measured with the cylinder probe and noodle firmness as measured with the blade. In both cases no significant differences were seen among the treatments, although

the samples treated with POX appeared slightly firmer in some cases, and the dough was tougher to handle as compared to the control samples. It is speculated that the toughness of the dough after POX addition might be due to the action of cross – linking proteins and or attachment of arabinoxylans to gluten proteins.

Table 15.1 Activity of POX in control and supplemented WSN stored under different conditions

Treatment[a]	POX activity[b]	POX activity[b]
Controls	**UW**	**PF**
Day1	305 ± 10	269 ± 9
24hr RT	286 ± 7	314 ± 17
24hr FR	298 ± 10	273 ± 7
Dried	292 ± 17	266 ± 33
POX 72 × 10^3 U / batch added [c]		
Day1	763 ± 15	701 ± 13
24hr RT	759 ± 12	721 ± 11
24hr FR	736 ± 19	697 ± 14
Dried	767 ± 17	668 ± 23

a Abbreviations are: WSN, white salted noodles; POX, peroxidase; RT, storage at room temperature; FR, storage at 4°C

b Unit for POX is given as an increase in the absorbance measured @ 470nm per minute per gram of sample

c POX added was from MP Biomedicals, the amount was 72 × 10^3 U of POX per batch of noodles (200g of flour)

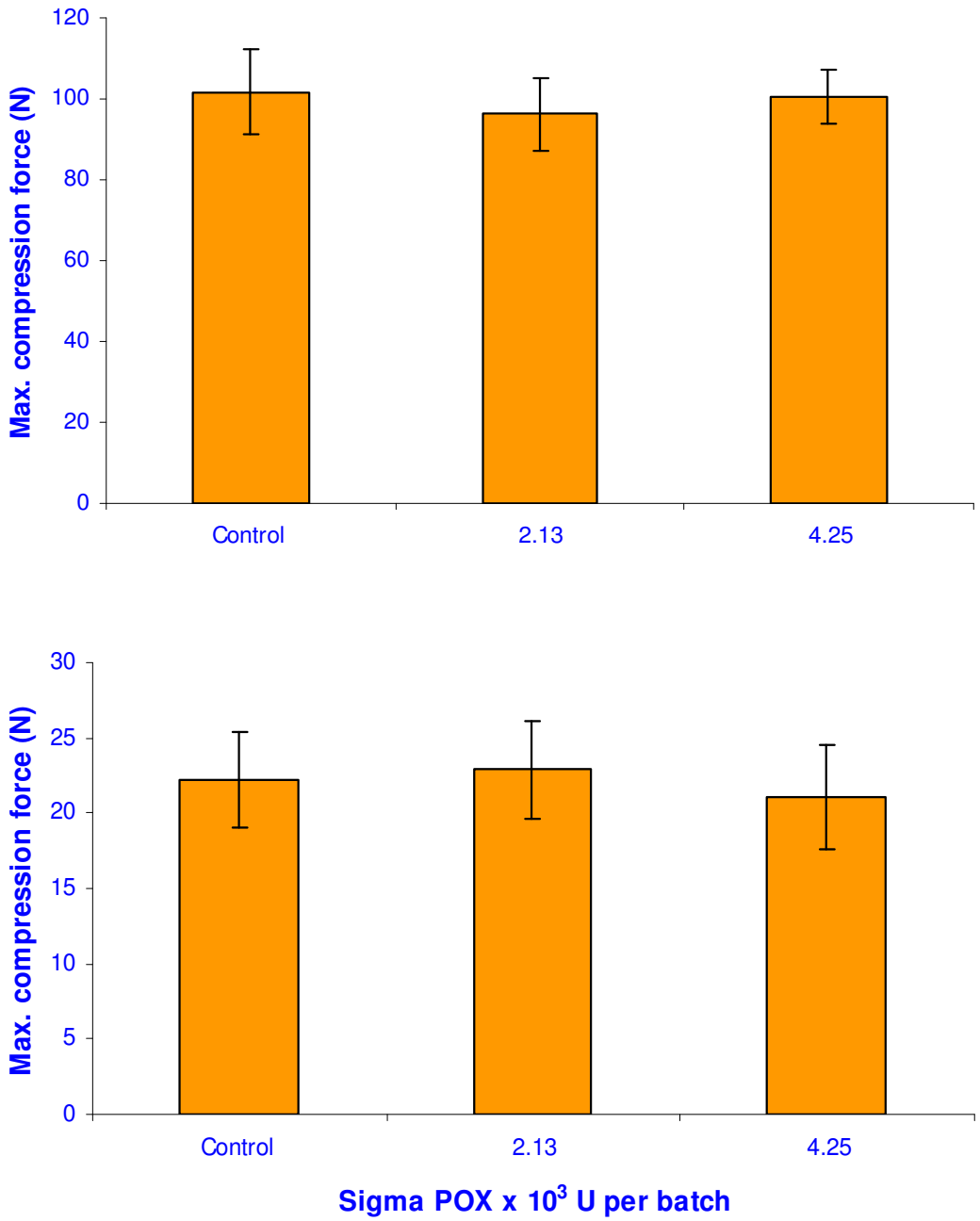


Fig 15.1 **Hardness properties of fresh raw (top) and fresh cooked (lower) WSN made from UW supplemented with POX (Sigma – Aldrich)**
Error bars represent standard deviation values. Measured with the flat cylinder probe (P/35)

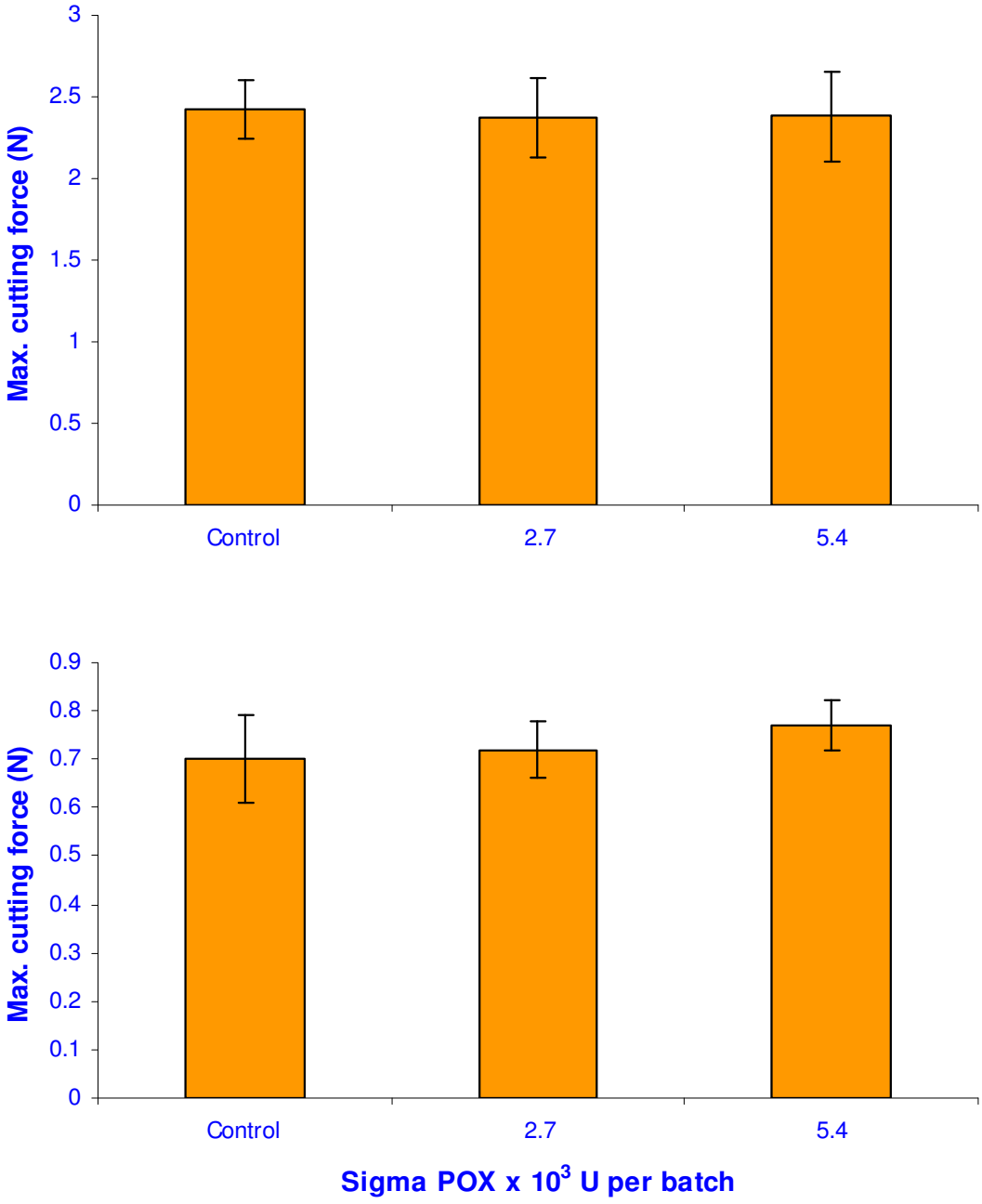


Fig 15.2 **Firmness properties of fresh raw (top) and fresh cooked (lower) WSN made from UW supplemented with POX (Sigma – Aldrich)**
Error bars represent standard deviation values. Measured with the flat blade attachment

15.5 POX preparation from MP Biomedicals

The freeze-dried powder preparations of POX from MP Biomedicals were dissolved in distilled water prior to addition to the noodle formulation. Hardness of noodles is shown in Fig 15.3 and these graphs indicate somehow opposite results as found previously when Sigma Aldrich preparations were used. Lover amounts of addition seem to result in softer noodles when hardness was measured and as the addition of POX increased so did the hardness values. The same was found for both raw and cooked noodle preparations.

On the other hand when the same preparations were used and noodle firmness was measured (blade method) Fig 15.4 opposite observations were made, i.e. lower amount of POX addition seemed to give slightly firmer noodles and the highest level of addition used resulted in less firmer noodles.

15.6 The effect of POX on textural properties of WSN

The remainder of this study was focused on the POX from Biomedicals. and the level used was 72×10^3U of POX for each batch of noodles (Tables 15.2 and 15.3).

The two methods used (blade –cutting and flat cylinder – compression) to evaluate textural properties of WSN and WSN supplemented with POX were highly correlated (data from Tables 15.2 & 15.3) (r^2 0.95) (Fig 15.5). Both measurements used were useful means in measuring firmness and hardness of these types of products.

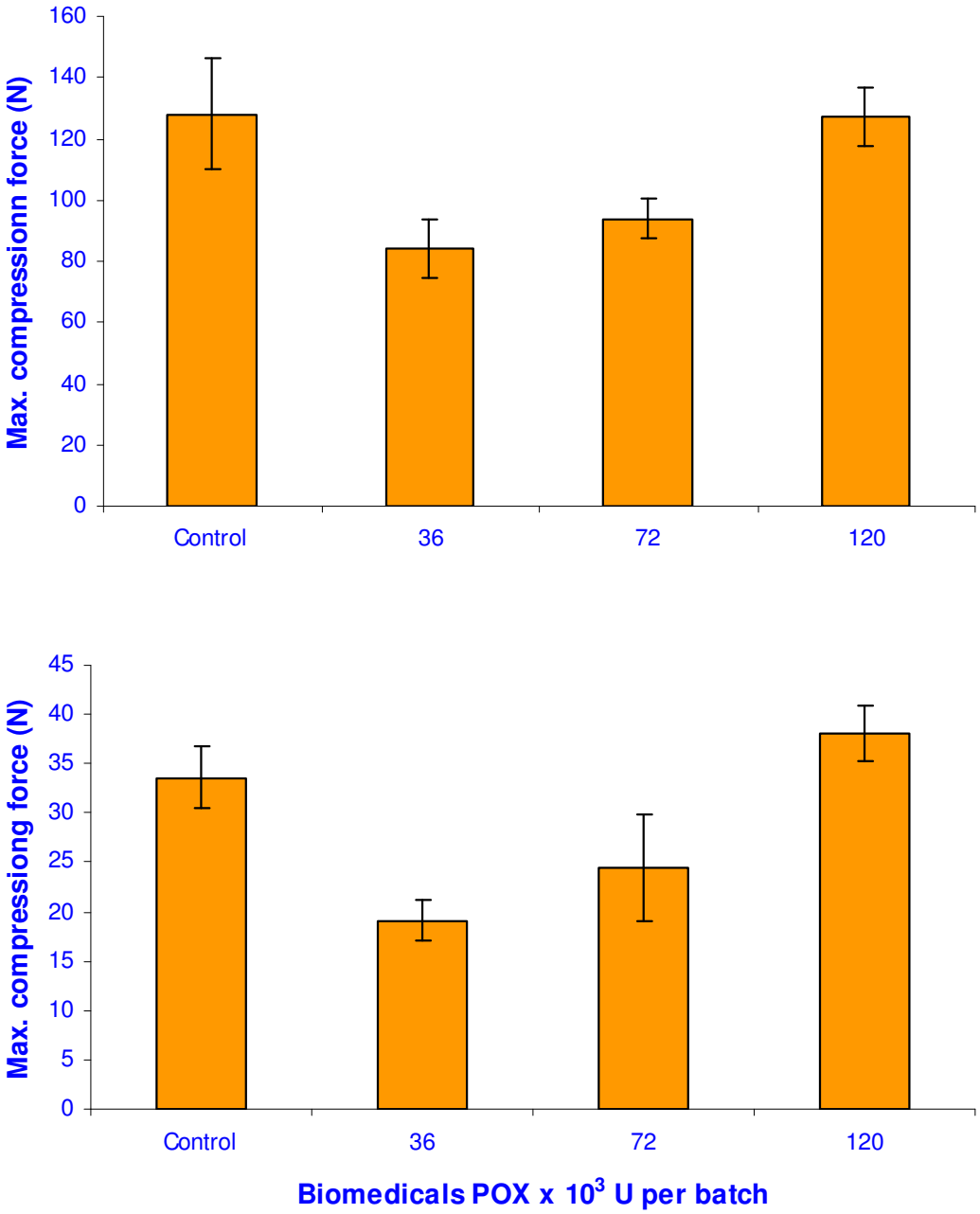


Fig 15.3 **Hardness properties of fresh raw (top) and fresh cooked (lower) WSN made from UW supplemented with POX (MP Biomedicals)**
Error bars represent standard deviation values. Measured with the flat cylinder probe (P/45)

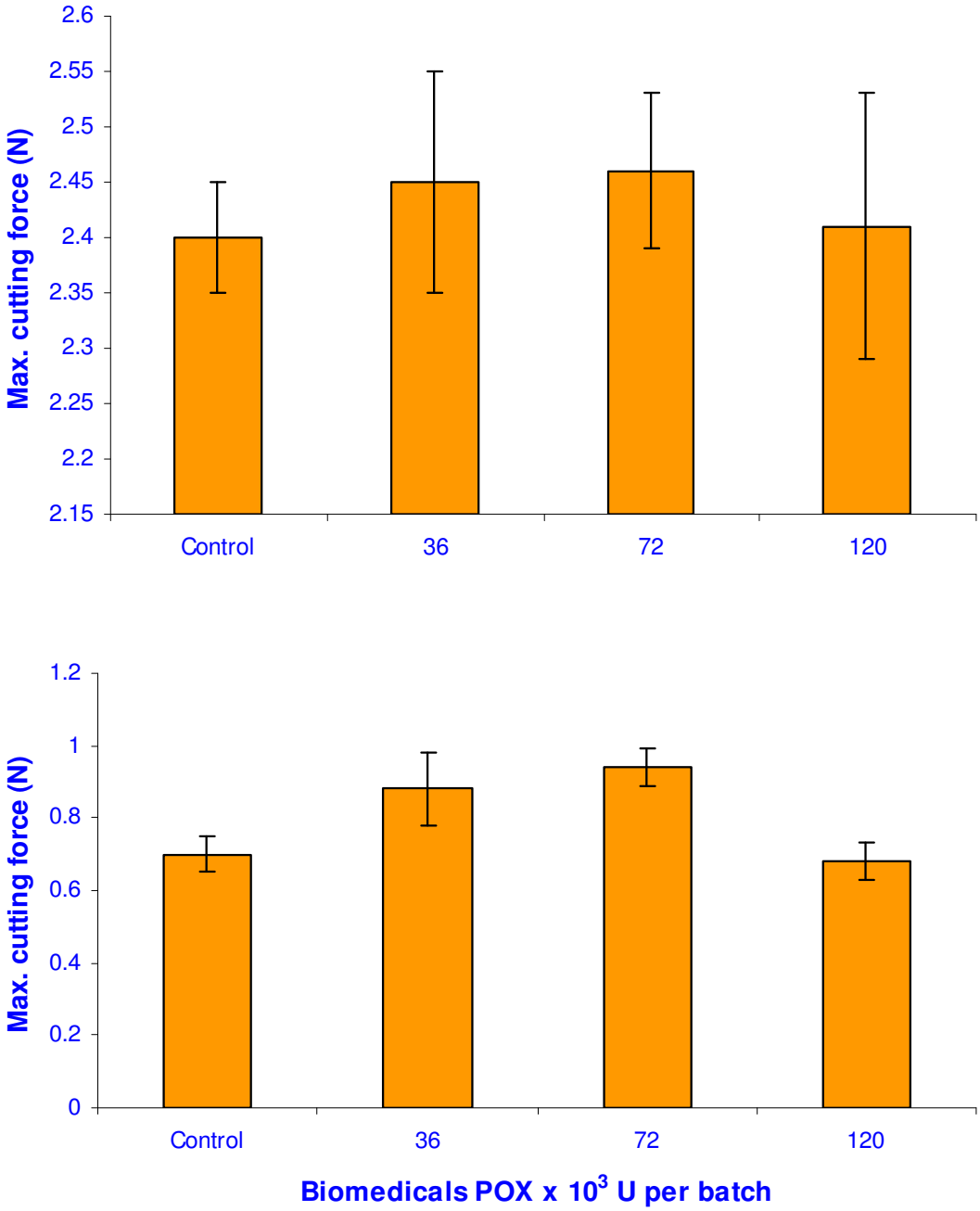


Fig 15.4 **Firmness properties of fresh raw (top) and fresh cooked (lower) WSN made from UW supplemented with POX (Biomedicals)**
Error bars represent standard deviation values. Measured with the blade

Table 15.2 Effects of POX on the textural properties measured by TA-XT2 for WSN made from UW

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.6[a]	130[a]	11.8[a]	2.42[a]
	POX	25.19[b]	93.8	13.3[a]	2.46[a]
	RT control	43.3[a]	134[a]	13.6[a]	2.55[a]
	POX RT	30.7[b]	125[a]	12.4[a]	2.45[a]
	FR control	44.5[a]	136[a]	12.1[a]	2.44[a]
	POX FR	43.8[a]	134[a]	12.6[a]	2.75[a]
Cooked	control	8.80[ab]	33.3[a]	3.54[a]	0.70[a]
	POX	6.25[a]	24.5	5.66[a]	0.94[a]
	RT control	9.20[b]	34.8[a]	3.54[a]	0.70[a]
	POX RT	8.44[ab]	33.5[a]	3.66[a]	0.77[a]
	FR control	10.0[ab]	37.0[a]	4.24[a]	0.78[a]
	POX FR	9.45[b]	35.9[a]	4.38[a]	0.85[a]
Dried [b]	control	11.9[a]	39.5[a]	6.01[a]	0.99[a]
	POX	10.5[a]	38.4[a]	4.76[a]	1.17[a]

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour; POX, peroxidase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Table 15.3 Effects of POX on the textural properties measured by TA-XT2 for WSN made from PF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.2[a]	128[a]	11.9[a]	2.40[a]
	POX	35.19[a]	101	13.0[a]	2.35[a]
	RT control	38.1[a]	119[a]	13.0[a]	2.55[a]
	POX RT	37.6[a]	121[a]	12.4[a]	2.67[a]
	FR control	39.4[a]	121[a]	12.9[a]	2.55[a]
	POX FR	42.5[a]	124[a]	11.6[a]	2.87[a]
Cooked	control	9.10[a]	33.6[a]	3.33[a]	0.70[a]
	POX	8.56[a]	31.9[a]	3.65[a]	0.86[a]
	RT control	9.55[a]	33.9[a]	3.21[a]	0.66[a]
	POX RT	9.44[a]	35.5[a]	3.48[a]	0.79[a]
	FR control	9.90[a]	34.1[a]	4.23[a]	0.81[a]
	POX FR	8.69[a]	31.9[a]	4.63[a]	0.89[a]
Dried [b]	control	9.90[a]	34.1[a]	4.22[a]	0.88[a]
	POX	10.3[a]	36.7[a]	5.68[a]	1.01[a]

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour; POX, peroxides; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum

Note: Means followed by the same letter re not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

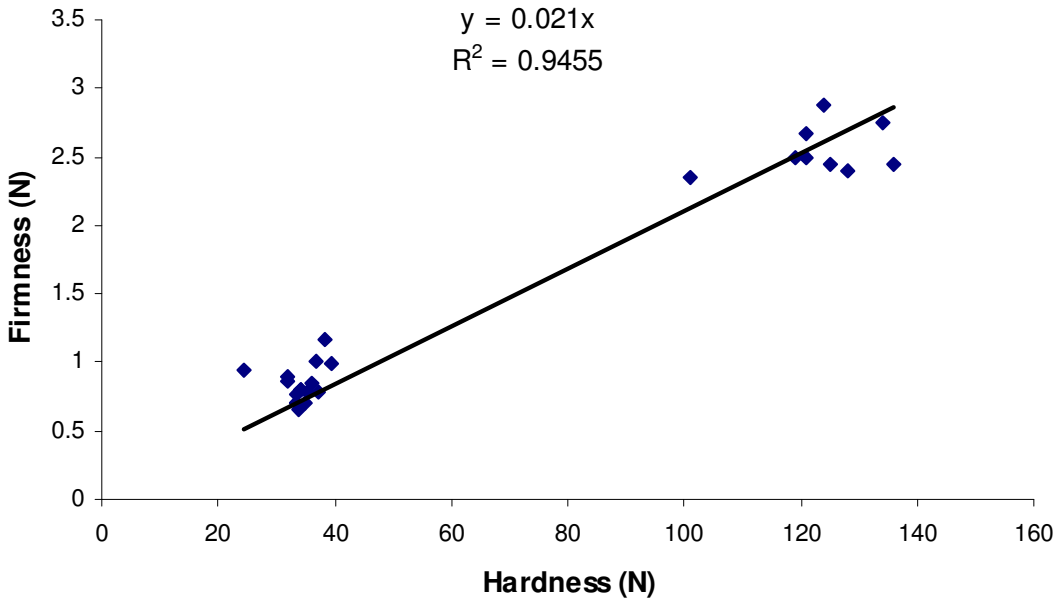


Fig 15.5 Correlation of firmness vs hardness of textural measurements
Data based on the mean values for firmness and hardness measurements of WSN (made from UW and PF, controls and POX treatments). Cooked and dried and cooked WSN

15.7 The effect of POX on cooking yield

Addition of POX preparation did not result in an increased cooking loss. The yield of noodles lost upon cooking for noodles incorporating the POX preparation was similar to the control samples. This was found to be true for both flours studied here and also for fresh as well as for dried WSN (Table 15.4 & 15.5).

Table 15.4 Cooked weight and cooking loss of WSN made from UW flour with and without addition of POX

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	2.3	58.7[a]	6.93[a]
fresh POX	2.3	51.1[a]	6.22[a]
dried control	6.3	70.8[a]	8.84[a]
dried POX	6.3	67.5[a]	8.66[a]

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour
Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)
Note: Level of addition was 72×10^3U / batch

Table 15.5 Cooked weight and cooked loss of WSN made from PF flour with and without addition of POX

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooked loss (%)
fresh control	2.3	56.7	6.93[a]
fresh POX	2.3	50.4	6.96[a]
dried control	6.3	70.25[a]	8.84[a]
dried POX	6.3	66.5[a]	9.21[a]

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour
Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)
Note: Level of addition was 72×10^3 U / batch

15.8 Relationship between POX and colour properties of WSN

POX more than any other enzyme preparation tested here had an adverse effect upon colour characteristics of WSN. At all times (from time zero until the end of storage) noodles incorporating POX preparation were visibly darker than control samples(this was true for both flours tested and also both storage temperatures) (Figs15.6-15.9). In all cases noodles incorporating POX were dark yellow (after 24hr they were orange / yellow) being highly unacceptable for these types of products. Especially at 25°C storage the L* values of POX treatments were very low (indicating darker and duller noodles) (Figs 15.6 & 15.8). Yellowness as indicated by b* values was in addition always significantly higher for POX treatments indicating darker noodles at both storage conditions (Figs 15.7 & 15.9).

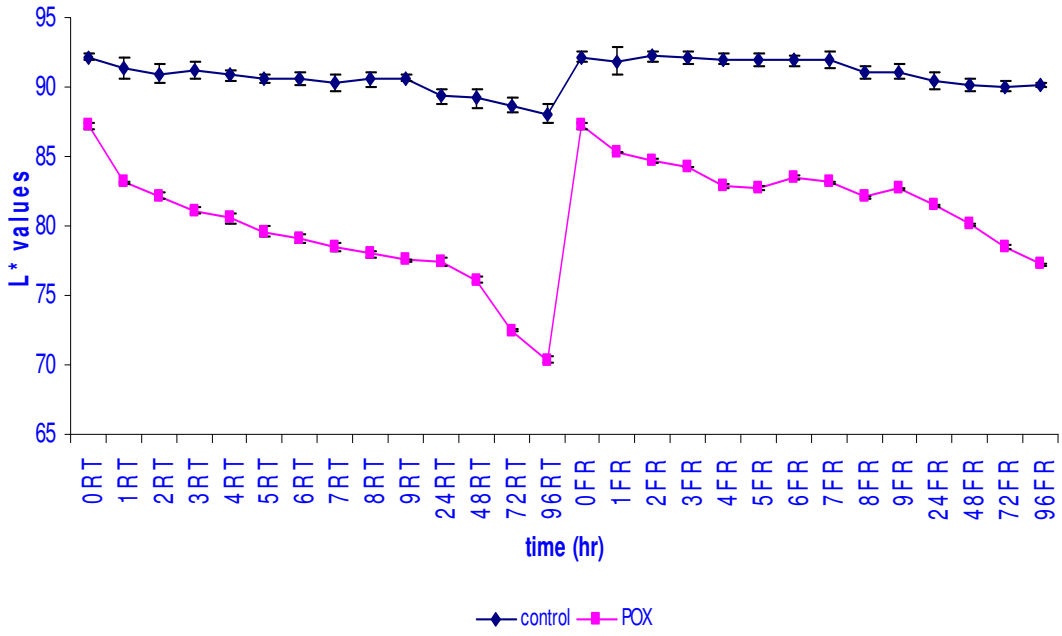


Fig 15.6 **Colour characteristics (L* values [whiteness / brightness]) of WSN made from UW stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

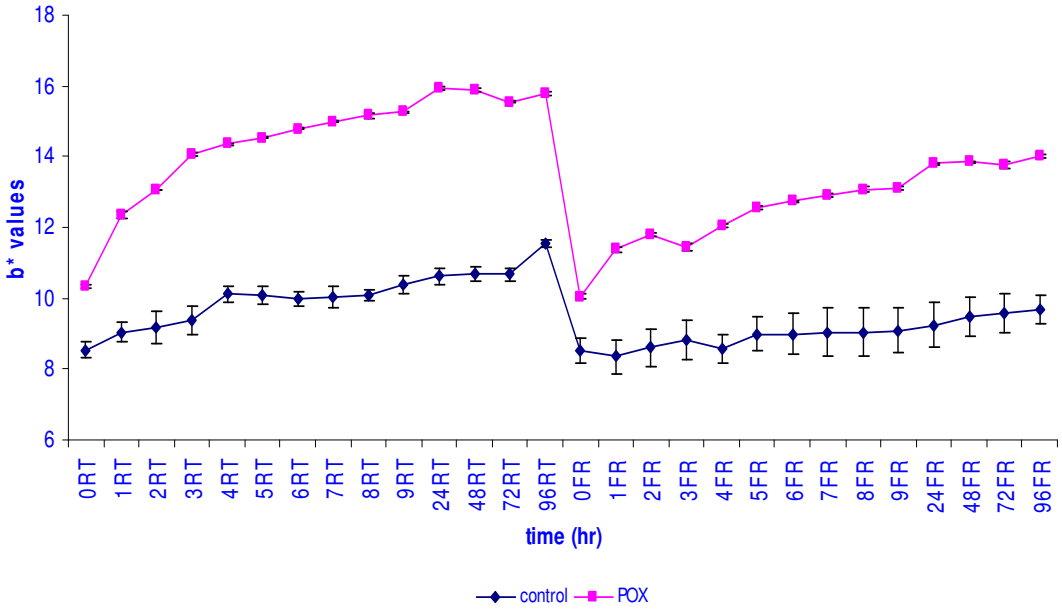


Fig 15.7 **Colour characteristics (b* values [yellowness]) of WSN made from UW stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

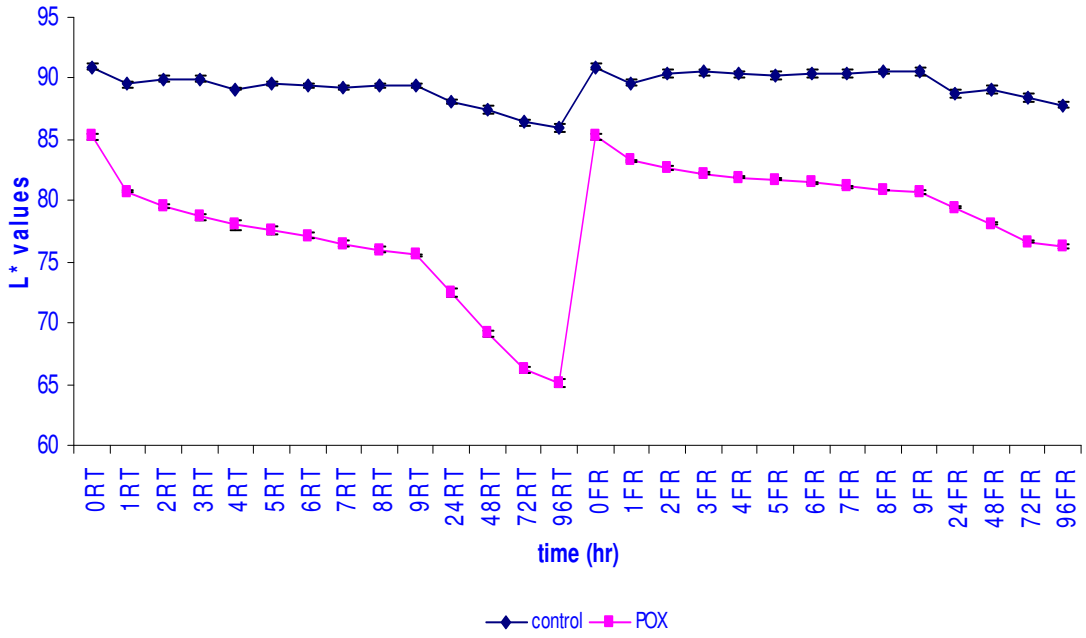


Fig 15.8 **Colour characteristics (L* values [whiteness]) of WSN made from PF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

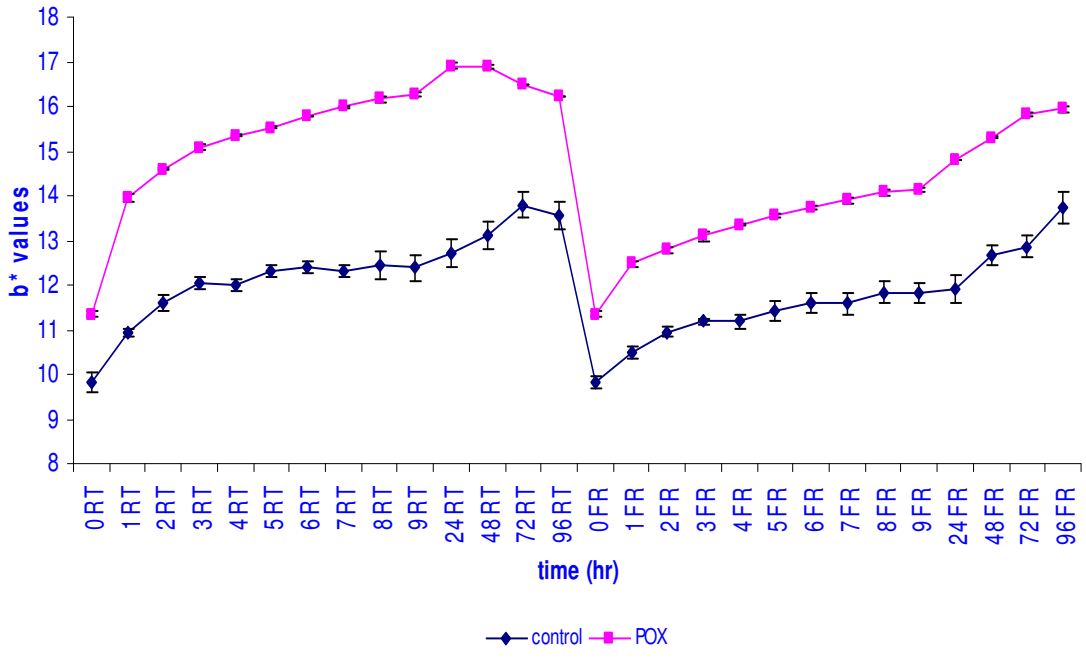


Fig 15.9 **Colour characteristics (b* values [yellowness]) of WSN made from PF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

Differences between control and POX treatments were not significantly different after cooking or drying immediately after preparation (Figs 15.10 & 15.11). Drying of noodle sheets immediately after preparation prevented yellow colour development in noodles incorporating POX therefore the differences in colour between dried controls and POX treated noodles are minimal (Figs 15.10 & 15.11).

Similar effects were seen after noodles were cooked immediately after preparation (Figs 15.10 & 15.11). Noodles incorporating POX had slightly higher b* values indicating only marginally more yellow products than control samples.

Colour of dried and cooked noodles was similar in all samples. Slightly lower L* values were recorded here for samples made from PF flour, however, the differences between controls and POX treatments again being not as obvious as in the fresh samples as colour development / darkening as caused by POX was probably suppressed by drying and or cooking (i.e. treatment at higher temperatures) (Figs 15.10 & 15.11).

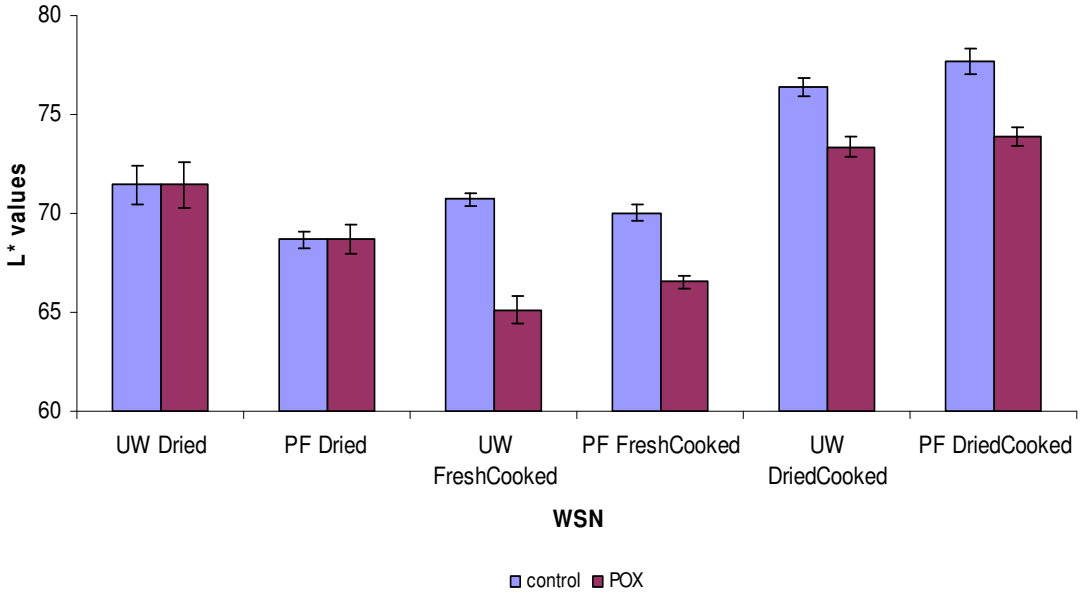


Fig 15.10 Colour characteristics (L* whiteness / brightness) of dried, fresh cooked & dried cooked WSN made from UW & PF
Error bars represent standard deviation values

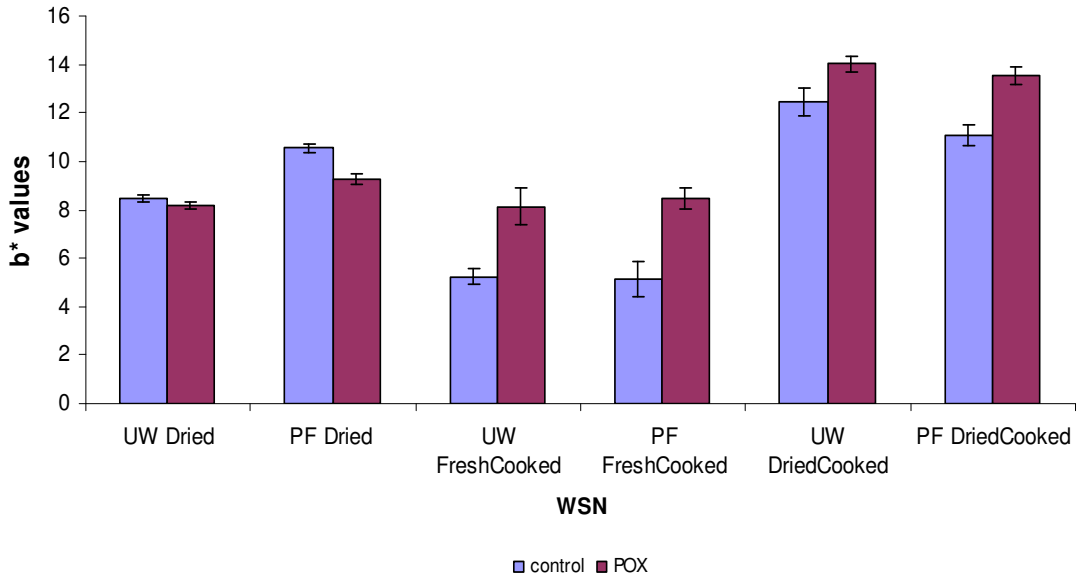


Fig 15.11 Colour characteristics (L* whiteness / brightness) of dried, fresh cooked & dried cooked WSN made from PF & UW
Error bars represent standard deviation values

15.9 Structural properties of WSN supplemented with POX

Structure of raw WSN was examined using the environmental scanning electron microscope (ESEM). In the case of raw noodles in both control and POX treatments fairly normal, lenticular and circular shaped, compact starch granules can bee seen. In the control the starch granules seem to be slightly smaller and closer to each other, while in the image of WSN incorporating POX preparation starch present appears partially gelatinised already and a paste-like surface can be noticed between the starch granules (Fig 15.12).

Images of cooked noodles were taken with low vacuum mode using the scanning electron microscope (SEM). The noodles were freeze-dried immediately after boiling (to the optimum cooking time) and stored at 4°C until analysis. Usage of low vacuum avoided gold coating, which might in addition to the ice formation on the surface further interfere with the actual surface structure of noodle samples. In the case of cooked noodles those incorporating POX preparation the starch appears completely gelatinised and the granular shape of the starch is no longer visible on the surface (Fig15.12).

The surface of WSN incorporating POX preparation appears more hollow and some starch granules appear gelatinised. After dried WSN have been cooked there was relatively little difference in the appearance between the control and POX treated noodles (Fig 15.13).

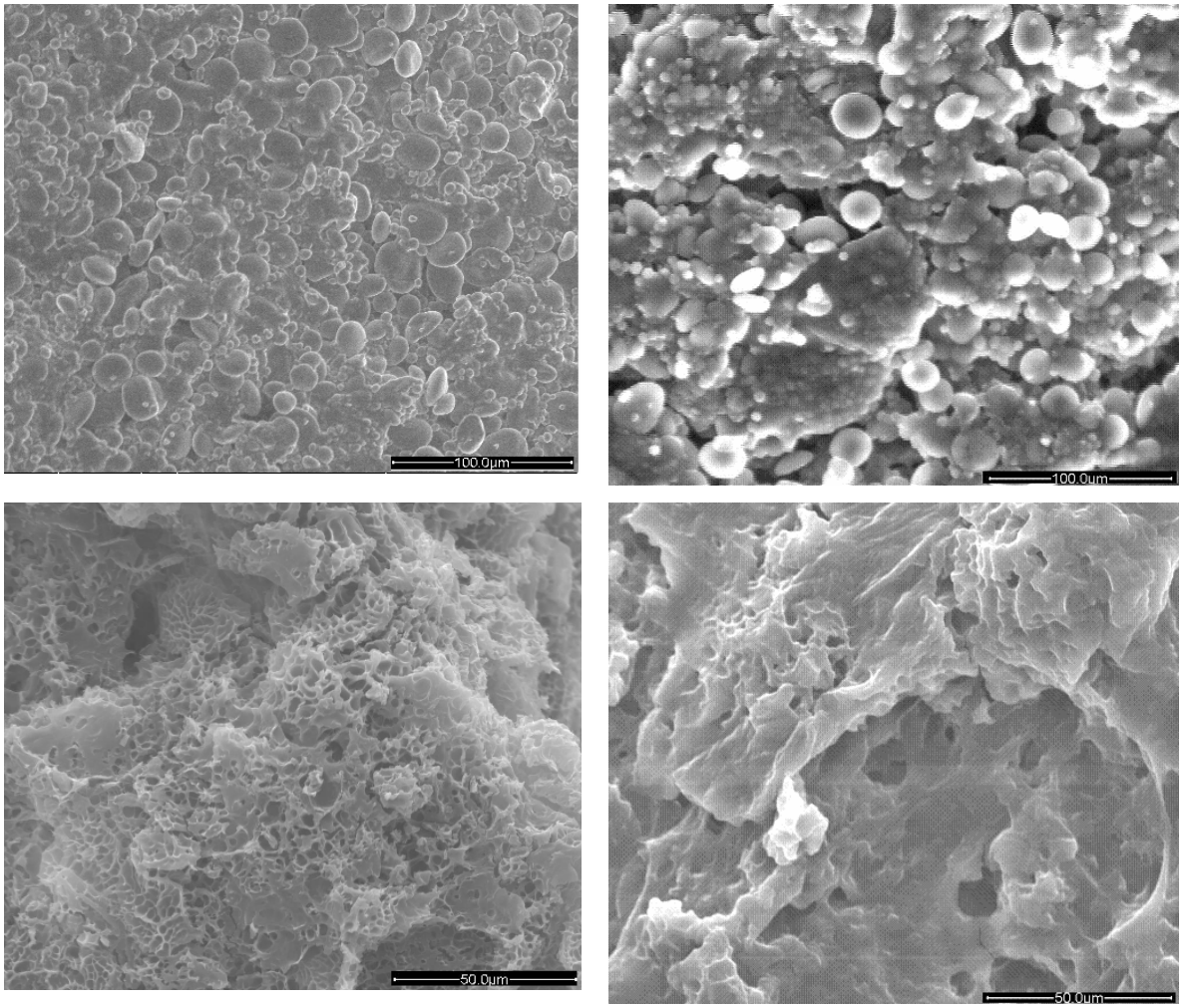


Fig 15.12 **ESEM[a] images of raw WSN [control and POX treatment, from left to right (top)] & SEM[b] images of cooked noodles [control and POX treatment, from left to right (lower)]**

a ESEM, 6.0 Torr, 4°C, 400× Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 1000× Mag., 5.0 spot size, 30kV

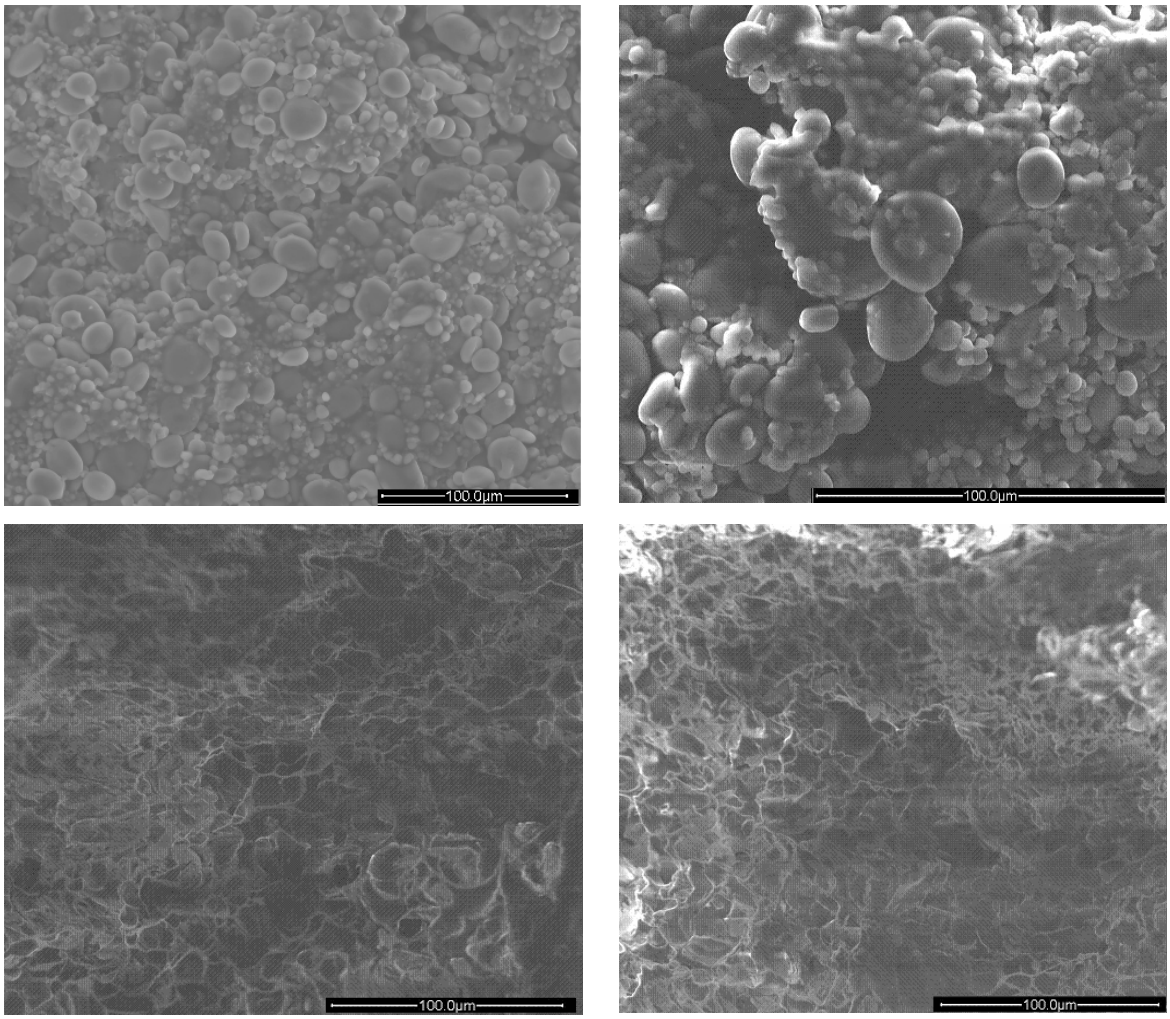


Fig 15.13 SEM[a] images of dried (top) and dried cooked (lower) WSN control and POX treatment (from left to right)

a SEM, 0.5 Torr, 23°C, 800× Mag. (top); 500× Mag. (lower), 5.0spot size, 30kV

15.9 Summary of results

As compared to other enzymes studied here, where levels of naturally present enzymes were relatively low and difficult to detect, in the case of POX the flours tested had relatively high levels of endogenous POX present. In addition, loss of activity was recorded after noodle processing indicating some instability of the enzyme. Although high levels were found originally in the flour, endogenous POX seemed not have the same effect as endogenous POX, since colour properties of control samples were not effected as adversely as POX treated noodles. Some level of discoloration was recorded in control samples as well, however, colour attributes were much more acceptable than in noodles incorporating POX. The primary conclusion from these experiments is that POX studied here is not acceptable for noodle processing as it adversely impacted upon

one of the most important quality attributes of Asian noodles. WSN incorporating POX were dark and dull in appearance and appeared very dark to orange yellow after 24hr of storage.

Of the two POX preparations tested in the preliminary experiments Sigma-Aldrich preparation resulted in similar textural characteristics for both methods used here (cylinder and blade). On the other hand, Biomedical POX resulted in quite different textural results for these two methods. Here lower amounts of addition resulted in slightly softer noodles (for both raw and cooked noodles) however, when using the blade attachment and when firmness was measured slightly firmer noodles were measured at the same level of POX addition (this was found to be true for both raw and cooked noodles).

Overall, for the textural evaluations, POX did not result in adverse effects, however, softness and hardness as measured by the texture analyser ideally should be linked to the sensory evaluation to be able to tell the preference in terms of softness and hardness. The addition of POX preparation did not have adverse effect upon cooking loss of WSN. Structural properties of noodles were slightly influenced by POX addition. The differences were more obvious in cooked and dried noodle samples.

Chapter 16

Results and discussion: Peroxidase and the quality parameters of Asian noodles prepared in the laboratory: Yellow alkaline noodles

The purpose of this chapter is to describe and discuss the results obtained during the preparation of noodles supplemented with peroxidase from horseradish. YAN were supplemented with peroxidase and the impact of the addition was assessed in terms of textural, structural and colour characteristics.

16.1 Introduction

Hsieh and McDonald reported on a POX activity in a purified LOX from durum wheat endosperm (Hsieh and McDonald 1984). Therefore indicating direct link of POX to colour properties of wheat end products. POX seems to negatively affect the yellow colour intensity of flour, probably by decreasing lutein content due to oxidation. In the preliminary studies on textural properties of YAN two different levels of POX (MP Biomedicals, Inc.) addition were used. The starting point of addition for POX estimated to be at least three times the level of POX initially assayed in the flour and noodle samples (Table 16.1). The textural properties were analysed using a flat cylinder probe (P/45) and a flat blade attachment.

16.2 Levels of POX in flour and stability during noodle processing

Relatively high levels of POX were recorded in the flours studied (this is in consistence with a study reported by Iori *et al* 1995. The activity of POX in the three flours studied ranged from 323 ±15 to 343 ± 19 and 425 ±16 for UW, PF and BGF respectively (Chapter 10). Slight loss in the activity from BGF flour to the YAN (371 ± 17) was recorded (Table 16.1). The activity increased approximately two fold after the incorporation of exogenous POX and no further losses were recorded during the storage under different processing conditions or during drying (Table 16.1).

Table 16.1 Activity of POX in control and supplemented YAN stored under different conditions

Treatment[a]	POX activity[b]
Controls	
Day1	371 ± 17
24hr RT	327 ± 13
24hr FR	322 ± 10
Dried	305 ± 22
POX 72 × 10^3 U / batch added[c]	
Day1	773 ± 6
24hr RT	751 ± 11
24hr FR	769 ± 15
Dried	738 ± 12

a Abbreviations are: YAN, yellow alkaline noodles; POX, peroxidase; RT, storage at room temperature; FR, storage at 4°C

b Unit for POX is given as an increase in the absorbance measured at 470nm per minute per gram of sample

c POX added was from MP Biomedicals, the amount was 72 × 10^3 U of POX per batch of noodles (200g of flour)

16.3 The effect of POX on the textural properties of YAN

In comparing treatment, some variations were seen in noodle texture using both cylinder and blade methods, with the later showing more obvious differences. Furthermore, with both attachments the same pattern was seen: YAN samples treated with POX were firmer (requiring higher force and showing higher resistance to compression) (Table 16.2). A similar trend was observed when noodles were cooked. Dried noodles analyzed with the blade attachment did not follow the same pattern (Figs 16.1 & 16.2). This method appeared to be more sensitive, possibly due to the relatively small sample area being tested (2mm in diameter) and the fact that five strands were evaluated each time as compared to two strands for the cylinder method. Slightly firmer noodles obtained upon treatment with POX might be a desirable effect since the evaluation of good noodle firmness / elasticity depends upon region and from country to country. Sensory evaluation might be a useful adjunct tool for the assessment of these treatments and their application in specific circumstances.

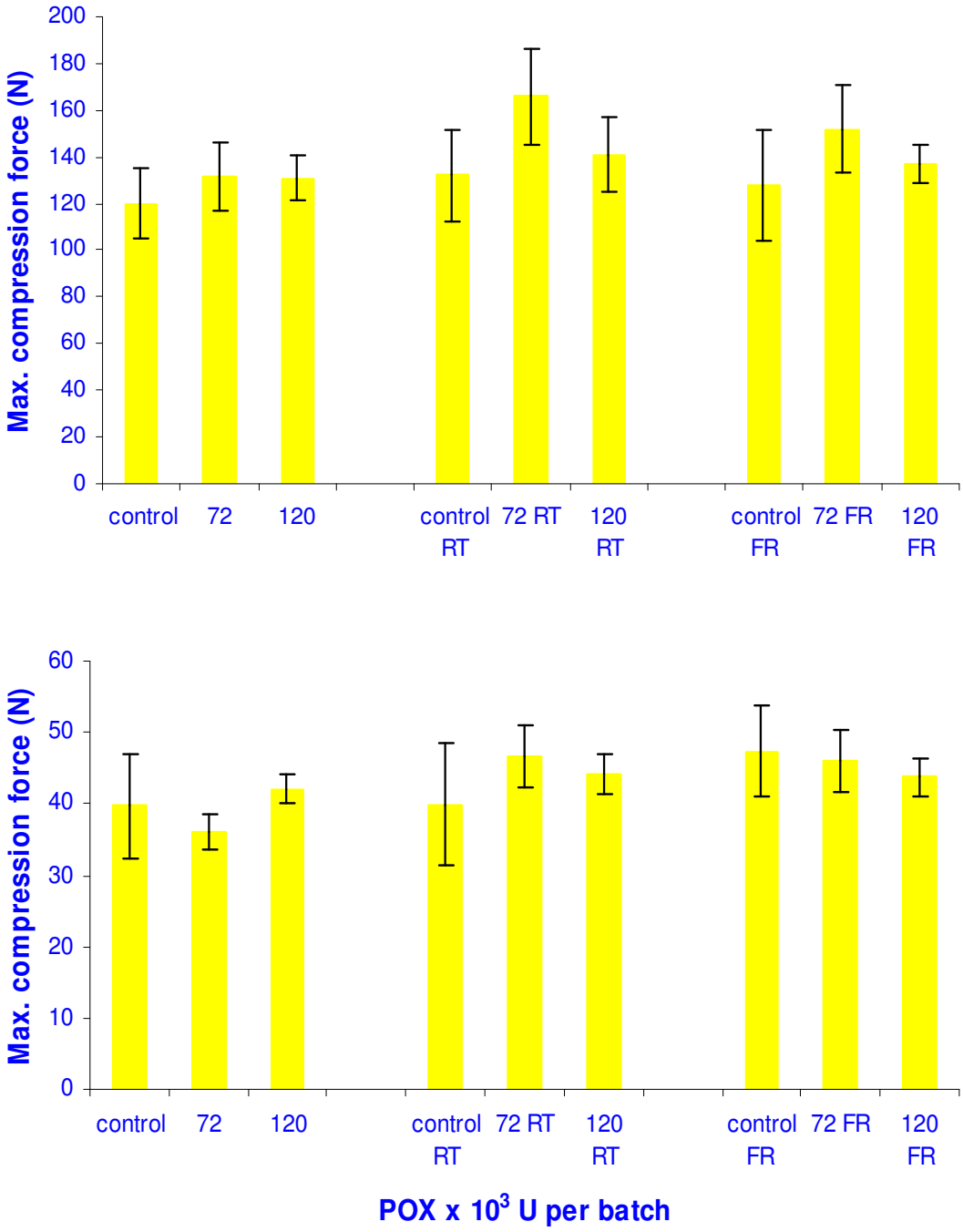


Fig 16.1 **Hardness properties of raw (top) and cooked (lower) YAN made from BGF supplemented with POX (MP Biomedicals, Inc.)**
Error bars represent standard deviation values.
Measurements taken with the flat cylinder probe (P/45)

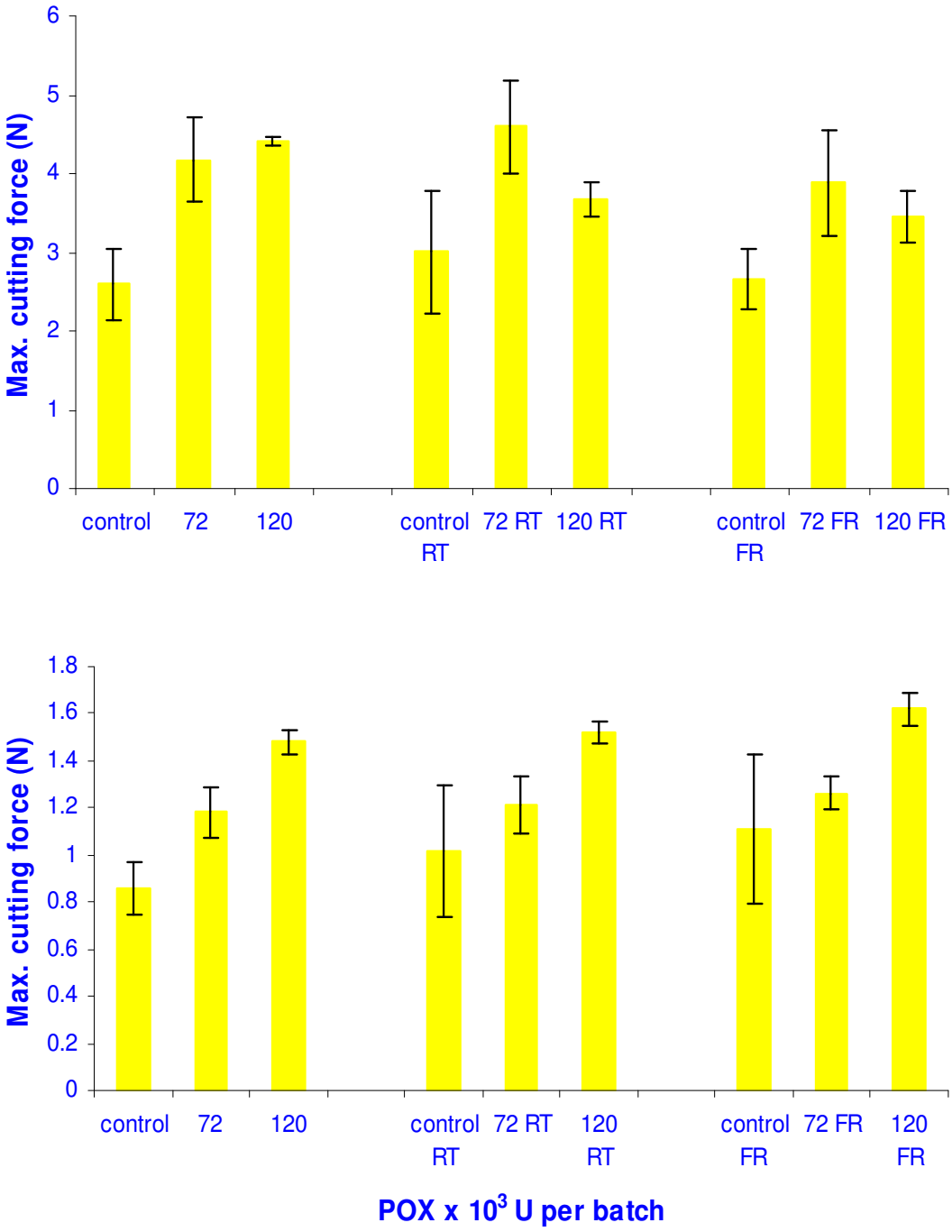


Fig 16.2 **Firmness properties of raw (top) and cooked (lower) YAN made from BGF supplemented with POX (MP Biomedicals, Inc.)**
Error bars represent standard deviation values.
Measurements taken with the flat blade attachment

Table 16.2 Effects of POX on the textural properties measured by TA-XT2 for YAN

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	36.1[a]	125[a]	11.0[a]	2.59[a]
	POX	41.4[a]	131[a]	18.8[b]	4.17[a]
	RT control	40.1[a]	132[a]	12.4[a]	3.01[a]
	POX RT	61.0	166[b]	22.8[b]	4.59[a]
	FR control	39.5[a]	128[a]	11.1[a]	2.66[a]
	POX FR	52.2	151[b]	18.3[b]	3.88[a]
Cooked	control	10.6[a]	39.7[ab]	3.50[a]	0.86[a]
	POX	10.6[a]	36.1[a]	6.17[b]	1.18[a]
	RT control	10.7[a]	39.9[ab]	4.58[ab]	1.02[a]
	POX RT	14.5[b]	46.7[b]	6.95[b]	1.21[a]
	FR control	13.4[ab]	47.4[b]	5.36[ab]	1.11[a]
	POX FR	14.2[b]	46.1[b]	7.48[b]	1.26[a]
Dried [b]	control	12.4[a]	42.9[a]	5.84[a]	1.25[a]
	POX	14.7[a]	47.4[a]	4.86[a]	0.94[a]

a Abbreviations are: YAN, yellow alkaline noodles; POX, peroxidase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Levels of addition were 72×10^3 U / batch

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

The findings for YAN supplemented with POX were similar to those for WSN (Chapter 15), with a strong correlation (r^2 0.92) between firmness (blade) and hardness (cylinder) performed using the TA-XT2 (Fig 16.3).

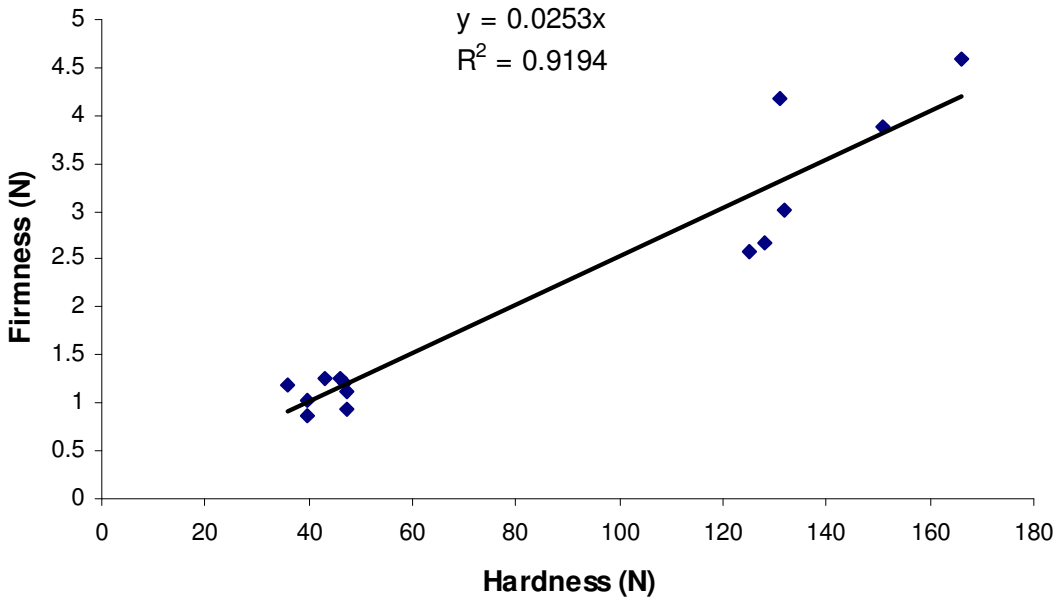


Fig 16.3 Correlation between firmness and hardness textural measurements
Data based on the mean values for firmness and hardness measurements of YAN (made from BGF, controls and POX treatments) for cooked and dried and cooked YAN

16.4 Relationship between POX incorporation and colour properties of YAN

Significant differences in noodle colour were seen. In Figs 16.4 & 16.5 L* and b* values are shown for raw noodles stored at 25°C and 4°C respectively. As might be expected, discoloration of noodle sheets occurred at a faster rate at 25°C as compared to storage at 4°C. At both storage temperatures noodles treated with POX appeared darker yellow to brown and this is not desirable for good quality noodles. In addition, it was noted that there were no specks present. Similar patterns were seen in cooked, dried and dried and cooked YAN (Figs 16.6 & 16.7) where there were substantial differences in L* values. In contrast, the b* values (yellowness) were quite similar for control and POX treatments. The enzyme POX had a negative impact on noodle colour, since dark yellow to brown colour was seen in POX treatments as compared to the clear, pale, bright yellow desired colour of good quality YAN. Negative effect of POX may have been caused by oxidation reactions decreasing the content of lutein.

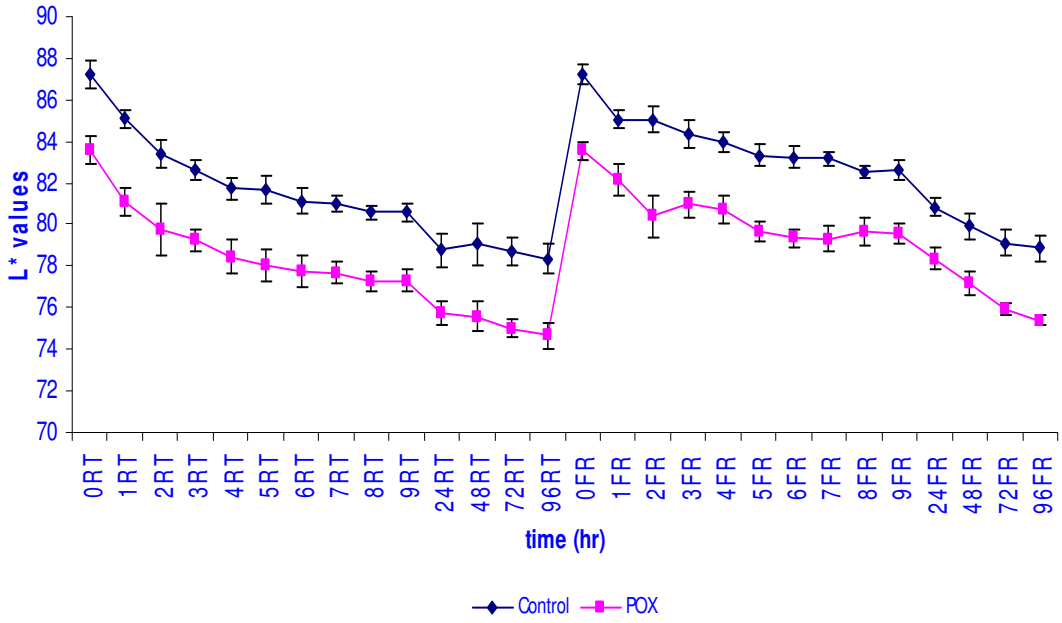


Fig 16.4 Discoloration of noodle sheets L* [whiteness] for noodles stored at 25°C and 4°C over a period of 96hr

Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

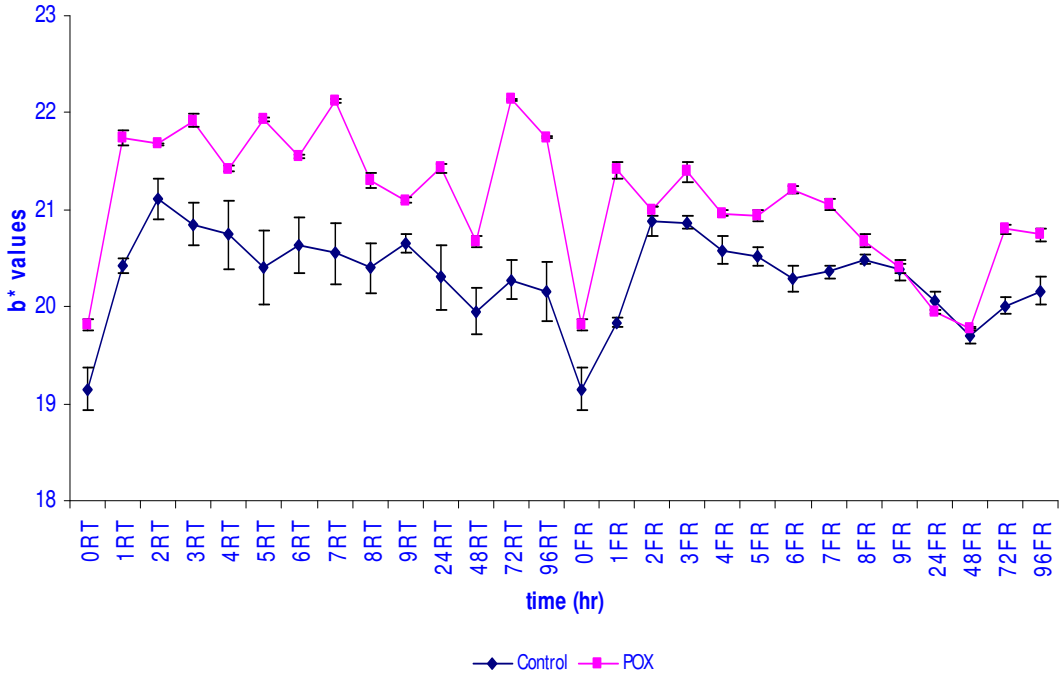


Fig 16.5 Discoloration of noodle sheets b* values (yellowness) for noodles stored at 25°C and 4°C over a period of 96hr

Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

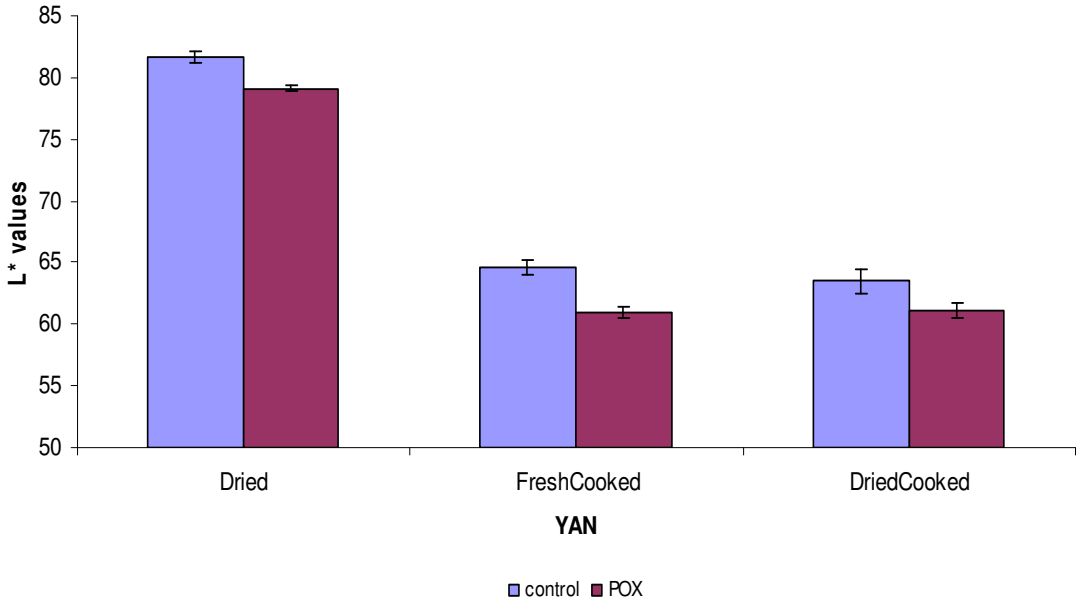


Fig 16.6 Colour characteristics (L* whiteness / brightness) of dried, fresh cooked & dried cooked YAN made from BGF
Error bars represent standard deviation values

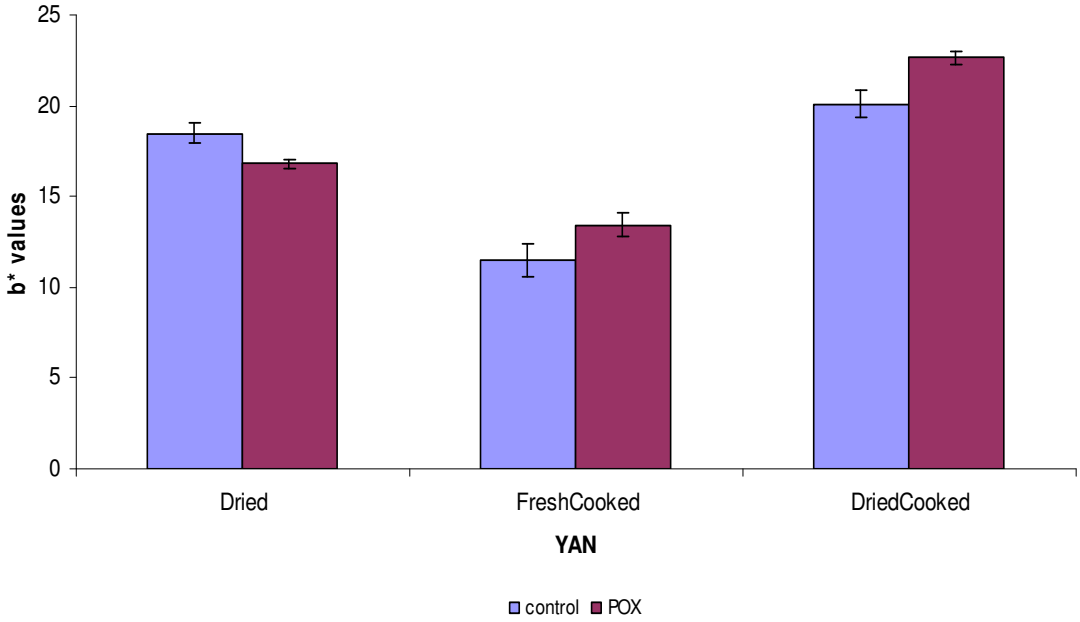


Fig 16.7 Colour characteristics (b* yellowness) of dried, fresh cooked & dried cooked YAN made from BGF
Error bars represent standard deviation values

16.5 The effect of POX on cooking yield

Higher cooking losses were recorded upon the addition of the enzyme to the noodle formulation for the dried noodle samples (Table 16.3). No differences were observed for water uptake.

Table 16.3 The impact of POX addition on cooking quality of YAN

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	4.30	58.7[a]	9.6[a]
fresh POX	4.30	54.2[a]	10.2[a]
dried control	9.0	72.1[a]	12.7
dried POX	9.0	69.5[a]	18.2

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

a Abbreviations are: POX, peroxidase; YAN, yellow alkaline noodles

16.6 Structural properties of YAN supplemented with POX

Structure of raw YAN was examined using ESEM. In both control and POX treatments starch granules appear lenticular and circular shaped, compact and typical of those found in wheat flour. In the control the starch granules seem to be slightly smaller and closer to each other. Relatively little protein network can be seen on the surface of both samples (Fig 16.8).

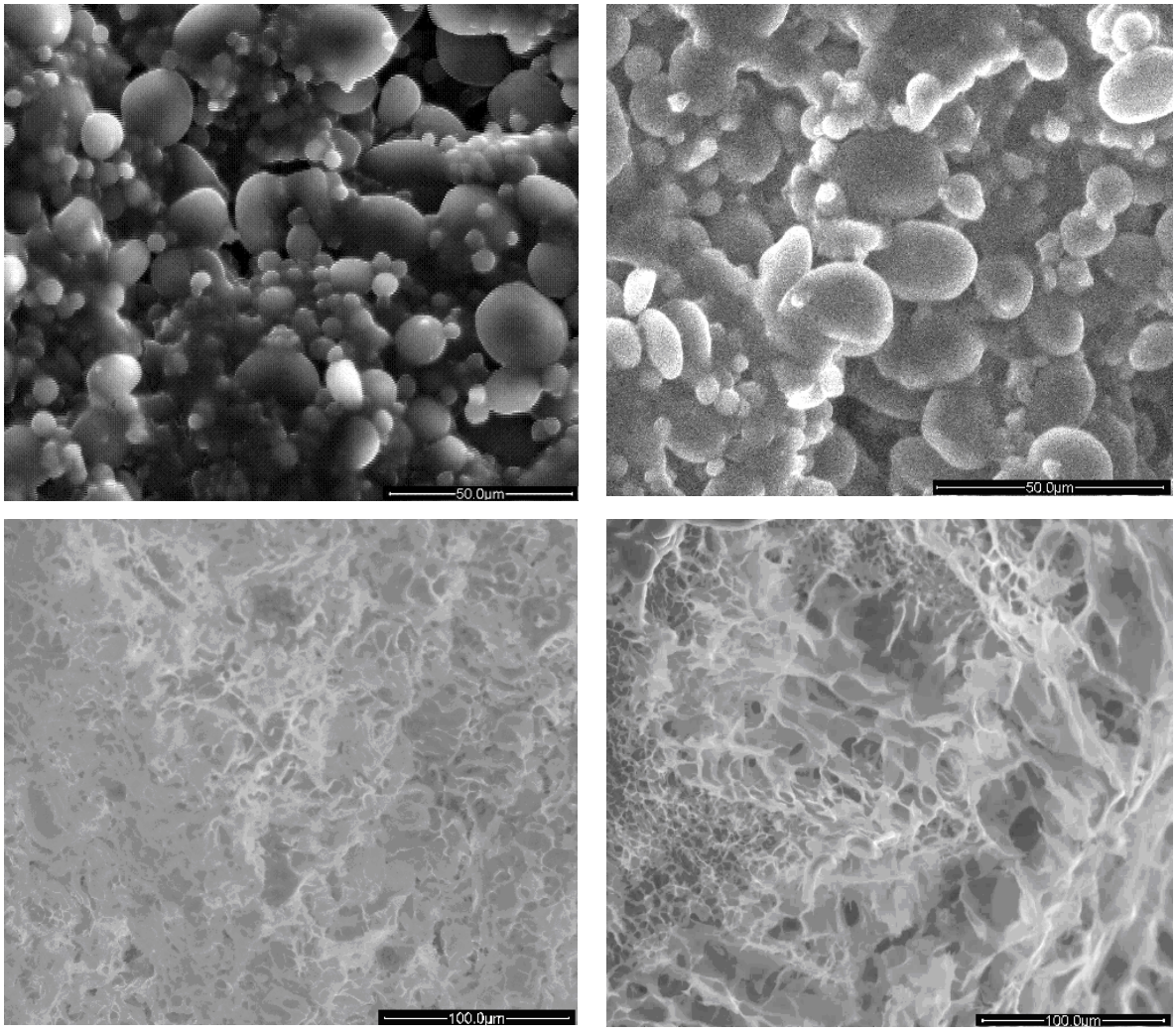


Fig 16.8 ESEM[a] images of fresh raw YAN control and POX treatment left to right (top) and SEM[b] images of fresh cooked YAN control and POX treatment left to right (lower)

a ESEM, 6.0 Torr, 4°C, 1000× Mag., 5.0 spot size, 30kV
b SEM, 0.5 Torr, 23°C, 400× Mag., 5.0 spot size, 30kV

Images of cooked noodles were taken with low vacuum mode using the scanning electron microscope (SEM). The noodles were freeze-dried immediately after boiling

(to the optimum cooking time) and stored at 4°C until analysis. Usage of low vacuum avoided the requirement for gold coating. The use of freeze drying minimized the likelihood of ice formation on the surface, which might also have interfered with the appearance of the surface structure of the samples. In the case of cooked noodles those treated with POX appear to have more hollow, amorphous structure in the protein network where most of the starch appears to be gelatinised in both cases (Fig 16.8). The more hollow structure in the case of POX treated YAN might be due to greater starch gelatinisation or removal of starch, in turn resulting in a less compact protein mass. Similar observations were made for dried and cooked and dried YAN (Fig 16.9).

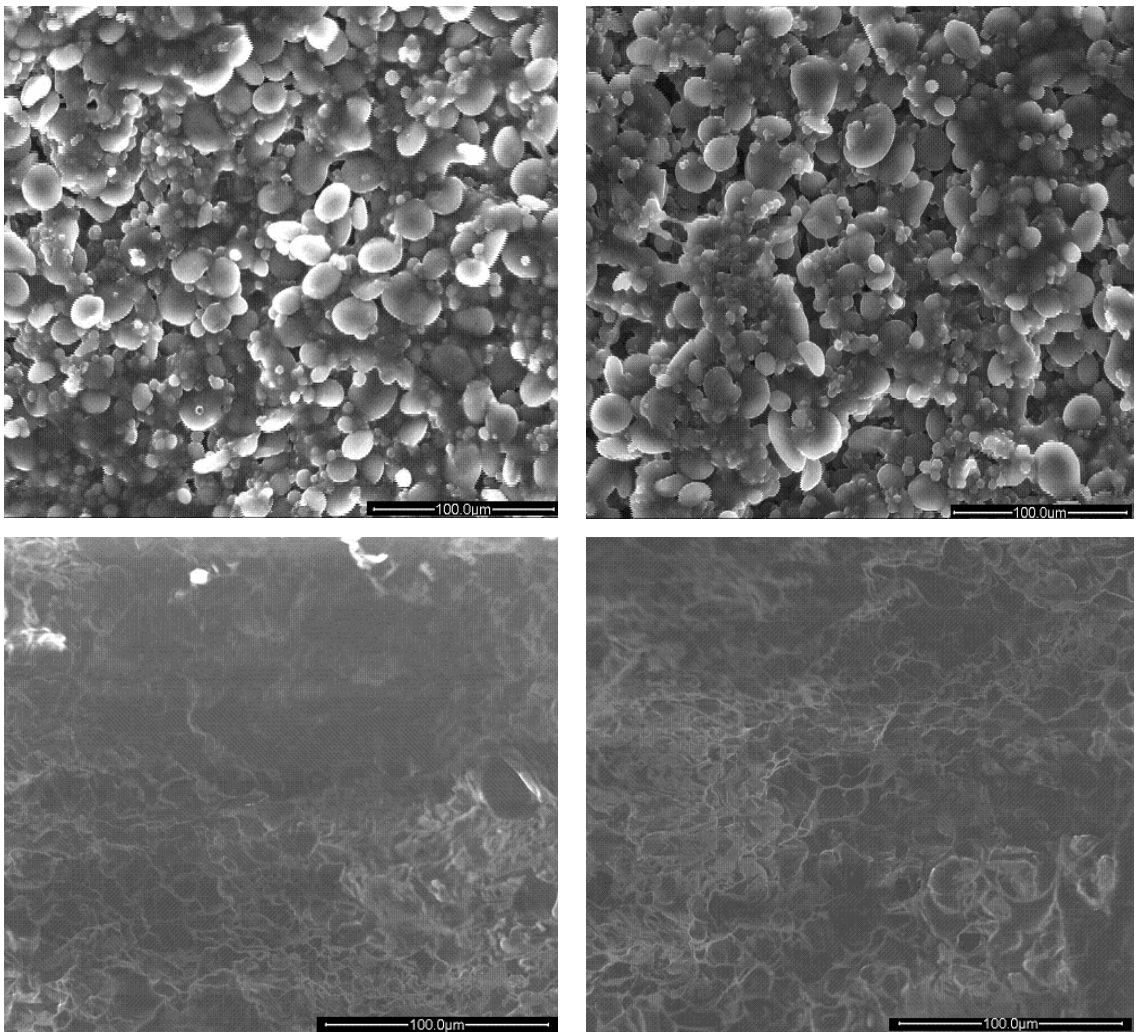


Fig 16.9 SEM images of dried[a] YAN (top) control and POX treatment (left to right) and dried and cooked[b] YAN (lower) control and POX treatment (left to right)

a SEM, 0.5 Torr, 23°C, 400 × Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 500 × Mag., 5.0 spot size, 30kV

16.7 Summary of results

High levels of naturally occurring POX in the flour did not seem to adversely impact upon any of YAN quality attributes, hence control samples appeared with good colour properties. This might be due to other natural enzymes present or to the fact that naturally POX might have different effects to the POX that was incorporated into the YAN formulation here. It is also possible that the commercial enzyme preparations contained other enzyme activities that had some impact. During processing, there was an initial loss in activity of the endogenous enzyme which decreased from 425 ± 16 (BGF) to 371 ± 17, although no further losses were recorded during storage under a variety of conditions.

The addition of exogenous POX preparation (at 72×10^3 units per batch) to the YAN formulation resulted in harder YAN after storage at 25°C for raw noodles while no differences were seen in cooked noodles or at storage at 4°C. Higher levels of addition 120×10^3 units per batch resulted in no differences as well when hardness of YAN of YAN was measured. Firmer noodles were found for both storage temperatures, and at both levels of addition when textural properties of noodles were examined using the blade method of the TA-XT2.

The addition of POX to the YAN formulation resulted in darker noodles (L* & b* values). Samples incorporating the POX preparation were darker than the control at all times and discoloured even further during the storage. YAN sheets were dark yellow to dark brown after the storage and had a very dull appearance. This negative impact of POX was seen in dried, cooked and dried and cooked YAN incorporating POX. Higher cooking loss was also seen in samples incorporating the POX preparation, for dried YAN. ESEM & SEM images showed relatively little difference between control and POX treated samples. Most of the starch appeared to be intact in its original granular form.

Chapter 17

Results and discussion: Ascorbic acid oxidase and the quality parameters of Asian noodles prepared in the laboratory: White salted noodles

The purpose of this chapter is to describe and discuss the results obtained during the preparation of WSN incorporating AAO from *Cucurbita* species. The impact of the addition of the enzyme on the quality of the noodles was assessed in relation to texture, structure, colour and cooking characteristics.

17.1 Introduction

The action of AAO in conjunction with the addition of ascorbic acid as an improver in bread making has been extensively studied. In contrast, its action and potential in noodle processing has not been reported. In preliminary experiments of this study varying levels of AAO have been added and textural properties of WSN evaluated. The effect of AAO has been studied without addition of ascorbic acid since ascorbic acid is not currently a common ingredient in the formulation of these types of products. The AAO studied was from *Cucurbita* species. The starting point of addition, 0.4×10^2 units per batch, was estimated to be approximately double the amount of AAO already present in the flour. Further levels of addition included 1.1×10^2 and 2.2×10^2 units of AAO per batch.

In terms of noodle hardness and firmness, there was relatively little variation found at any of the AAO levels used, although the addition of 1.1×10^2 units per batch gave slightly softer noodles. This was found for both raw and cooked samples when texture was measured with the cylinder probe (P/45) (Fig 17.1). In addition, visual examination and evaluation by touching of noodle strands indicated that the addition of 1.1×10^2 units per gram of AAO preparation was the most suitable. Therefore, this level of addition was used for further experimental evaluation of enzyme incorporation.

17.2 Levels of AAO in flour and stability during noodle processing

The two flours studied (PF and UW) were found to have similar levels of AAO activity (Chapter 10). Furthermore, the enzyme was found to be relatively stable after the flour has been processed into noodles, and also after the storage of these under various conditions(Table 17.1). Slightly lower activity was recorded following drying, while other storage conditions (room temperature ~ 25°C and 4°C storage) retained relatively high AAO activity.

Table 17.1 Activity of AAO in control and supplemented WSN stored under different conditions

Treatment[a]	AAO activity (U/g) activity[b]	
Controls	**UW**	**PF**
Day1	37.6 ± 3.7	38.5 ± 4.1
24hr RT	35.0 ± 2.4	33.7 ± 4.7
24hr FR	30.1 ± 3.3	34.3 ± 2.7
Dried	26.7 ± 6.4	25.6 ± 3.1
AAO (1.1×10^2 units / batch added[c)]		
Day1	43.7 ± 2.4	44.3 ± 2.8
24hr RT	41.3 ± 3.1	40.9 ± 3.4
24hr FR	41.6 ± 4.3	41.4 ± 4.7
Dried	39.3 ± 3.8	38.7 ± 3.5

a Abbreviations are: WSN, white salted noodles; AAO, ascorbic acid oxidase; RT, storage at room temperature (~25°C); FR, storage at 4°C
b Unit for AAO is (U/g), amount of activity that oxidises 1.0µg of ascorbic acid into dehydroascorbic acid under the assay defined conditions
c AAO added was from Sigma – Aldrich

17.3 The impact of varying levels of AAO on textural characteristics of WSN made from PF flour

The effect of various levels of AAO addition upon the textural properties of WSN is shown in Figs 17.1 and 17.2. The textural differences were recorded using the cylinder probe (P/45) as noodle hardness (Fig 17.1) and the flat blade as noodle firmness (Fig 17.2). More obvious differences were recorded when noodle hardness was measured

(Fig 17.1). Here, slightly softer products have been obtained upon the addition of 1.1 $\times 10^2$ units per batch of AAO preparations, while addition of lower or higher amounts of the same preparations showed no effect.

In terms of the firmness of WSN as measured with the blade attachment not much effect upon addition of any of the levels of AAO preparations was seen (Fig 17.2). In addition, visual examination and the dough behaviour during noodle processing as well as examination by touch indicated better / slightly softer noodles when addition of 1.1 × 10^2 units per batch of AAO was used. Since, softness is a primary issue for WSN, and softer and more elastic products are preferred it is believed that AAO might be used to enhance texture for these products even without the addition of ascorbic acid. Even greater improvement might be expected if ascorbic acid were to be added to the formulation. Further work would be required to investigate this possibility.

17.4 The effect of AAO on textural properties of WSN

When texture was measured during the storage of WSN, slightly firmer and harder noodles were observed at 4°C as compared to the storage at room temperature (~25°C) (Table 17.2). Furthermore, no significant differences were seen between the two flours (PF and UW) used in the preparation of WSN (Tables 17.2 and 17.3). Positively correlated results (r^2 0.96) were obtained from the two texture measurements (blade and cylinder) methods used here (Fig 17.3).

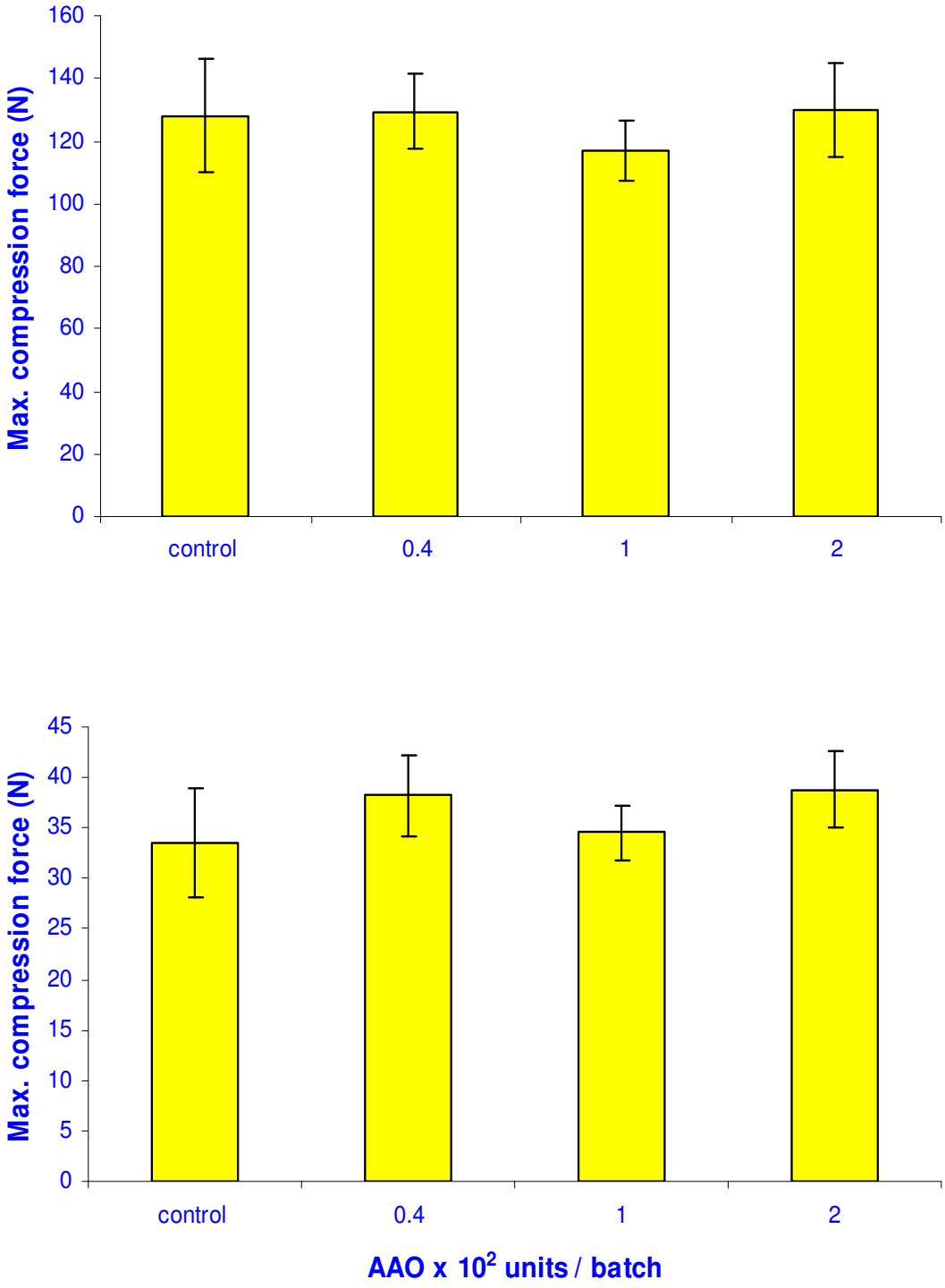


Fig 17.1 **The impact of various levels of AAO on the hardness properties of fresh raw (top) and fresh cooked (bottom) WSN made with PF flour**
Error bars represent standard deviation values.
Measurements taken using the cylinder probe (P/45)

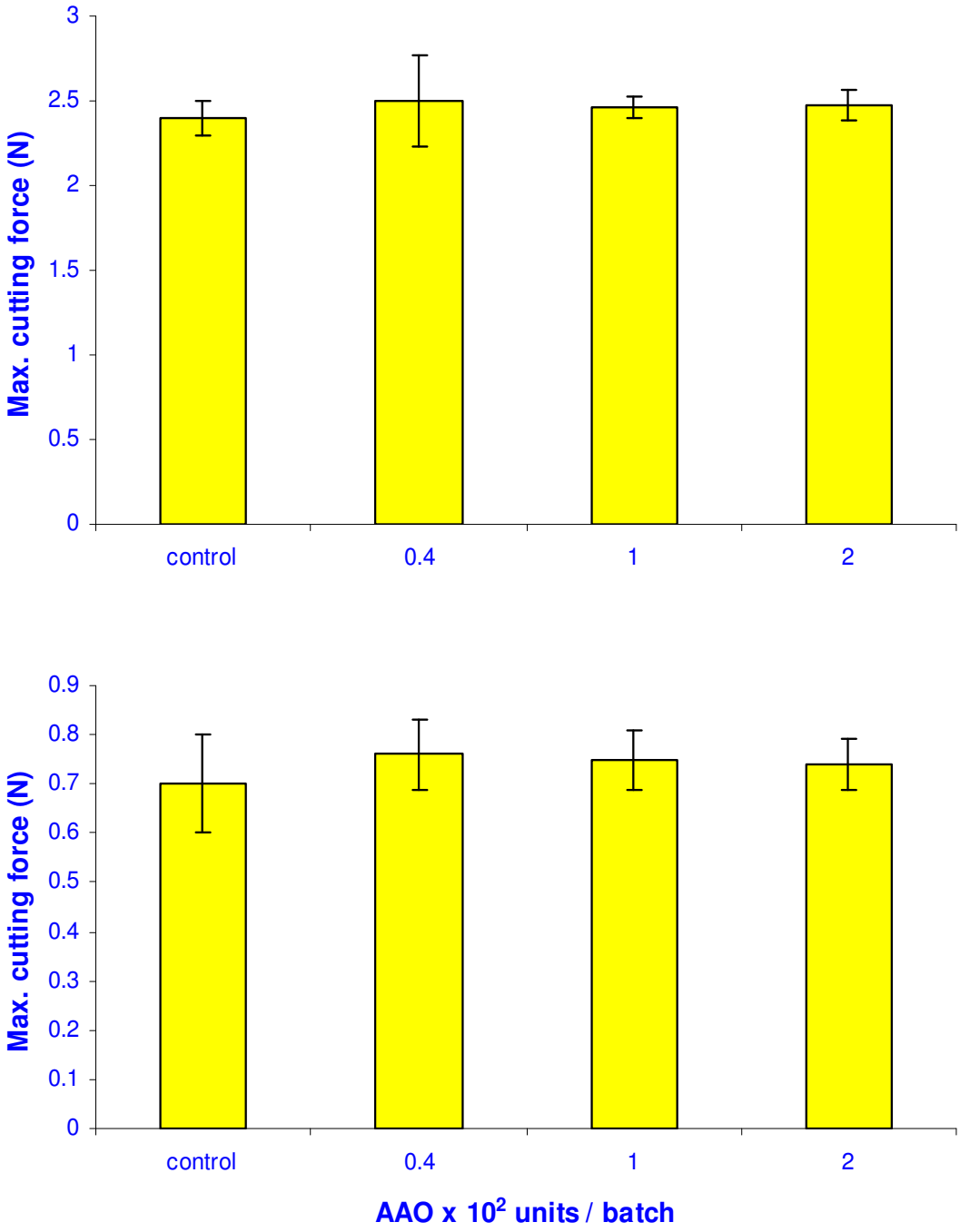


Fig 17.2 **The impact of various levels of AAO on the firmness properties of fresh raw (top) and fresh cooked (bottom) WSN made with PF flour**
Error bars represent standard deviation values.
Measurements taken using the blade

Table 17.2 Effects of AAO on the textural properties measured by TA-XT2 for WSN made from UW

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.6[a]	130[a]	11.8[a]	2.42[a]
	AAO	42.8[a]	127[a]	12.5[a]	2.68[a]
	RT control	43.3[a]	134[a]	13.6[a]	2.55[a]
	AAO RT	42.6[a]	130[a]	13.4[a]	2.62[a]
	FR control	44.5[a]	136[a]	12.1[a]	2.44[a]
	AAO FR	44.3[a]	135[a]	12.7[a]	2.65[a]
Cooked	control	8.80[a]	33.3[a]	3.54[a]	0.70[a]
	AAO	8.93[a]	32.4[a]	3.65[a]	0.77[a]
	RT control	9.20[a]	34.8[a]	3.54[a]	0.70[a]
	AAO RT	8.97[a]	34.6[a]	3.69[a]	0.72[a]
	FR control	10.0[a]	37.0[a]	4.24[a]	0.78[a]
	AAO FR	9.89[a]	36.42[a]	4.33[a]	0.85[a]
Dried [b]	control	11.9[a]	39.5[a]	6.01[a]	0.99[a]
	AAO	11.6[a]	38.6[a]	5.99[a]	0.86[a]

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour; AAO, ascorbic acid oxidase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Levels of addition were 1.1×10^2 units per batch

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Table 17.3 Effects of AAO on the textural properties measured by TA-XT2 for WSN made from PF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.2[a]	128[a]	11.9[a]	2.40[a]
	AAO	36.3[a]	117[b]	12.5[a]	2.59[a]
	RT control	38.1[a]	119[ab]	13.0[a]	2.53[a]
	AAO RT	37.9[a]	118[b]	13.6[a]	2.65[a]
	FR control	39.4[a]	121[ab]	12.9[a]	2.51[a]
	AAO FR	38.7[a]	120[ab]	13.1[a]	2.78[a]
Cooked	control	9.10[a]	33.6[a]	3.32[a]	0.70[a]
	AAO	9.88[a]	34.6[a]	3.33[a]	0.86[a]
	RT control	9.57[a]	33.9[a]	3.21[a]	0.66[a]
	AAO RT	9.73[a]	34.6[a]	3.64[a]	0.72[a]
	FR control	9.90[a]	34.1[a]	4.27[a]	0.80[a]
	AAO FR	9.22[a]	33.9[a]	4.33[a]	0.91[a]
Dried [b]	control	11.8[a]	39.3[a]	5.77[a]	1.02[a]
	AAO	11.4[a]	38.7[a]	6.34[a]	1.15[a]

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour; AAO, ascorbic acid oxidase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Levels of addition were 1.1×10^2 units per batch

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

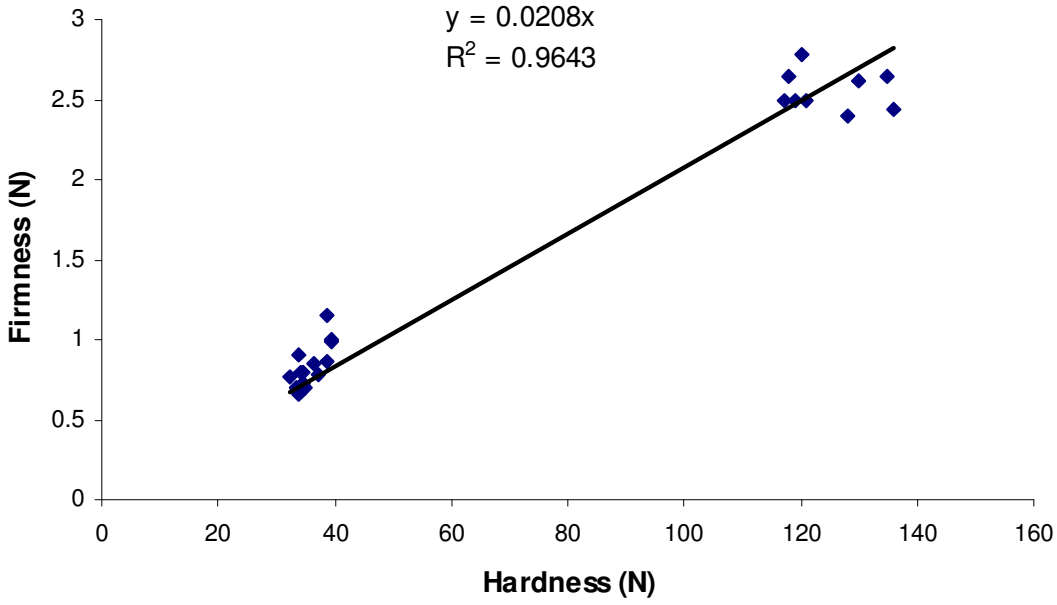


Fig 17.3 Correlation between firmness and hardness measurements of texture
Data based on the mean values for firmness and hardness measurements of WSN (made from UW and PF, controls and AAO treatments). Cooked and dried and cooked WSN

17.5 The effect of AAO on cooking yield

Upon the addition of the enzyme to the noodle formulation no differences were observed for either water uptake or cooking loss for the two flours used (Tables 17.4 and 17.5).

Table 17.4 Cooked weight and cooking loss of WSN made from UW flour with and without addition of AAO

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	2.3	58.7[a]	6.93[a]
fresh AAO	2.3	54.7[a]	5.35[a]
dried control	6.3	70.8[a]	8.84[a]
dried AAO	6.3	71.2[a]	8.35[a]

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour; AAO, ascorbic acid oxidase

Note: Level of addition was 1.1×10^2 units per batch
Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

Table 17.5 Cooked weight and cooking loss of WSN made from PF flour with and without addition of AAO

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	2.3	56.3[a]	6.93[a]
fresh AAO	2.3	54.3[a]	5.17[a]
dried control	6.3	70.25[a]	8.84[a]
dried AAO	6.3	69.4[a]	8.18[a]

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour; AAO, ascorbic acid oxidase

Note: Level of addition was 1.1×10^2 units per batch
Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

17.6 Relationship between AAO and colour properties of WSN

At both storage temperatures addition of AAO preparation resulted in slightly darker noodles compared to control (Fig 17.4). In addition, visually the enzyme treated noodles were slightly duller and lacked the brightness of the control samples consistent with the lower L* values (Fig 17.4). Discoloration was more obvious and it also occurred at a faster rate at 25°C as compared to the storage at 4°C. Loss of colour was obvious in control samples as well as in the noodle sheets incorporating the AAO preparation. Similar trends of colour loss can also be seen in the noodle yellowness (b* values), with slightly higher b* values from time zero throughout the whole storage seen in noodles incorporating enzyme AAO (Fig 17.5). The same trend was observed for both flours (Figs 17.4-17.7).

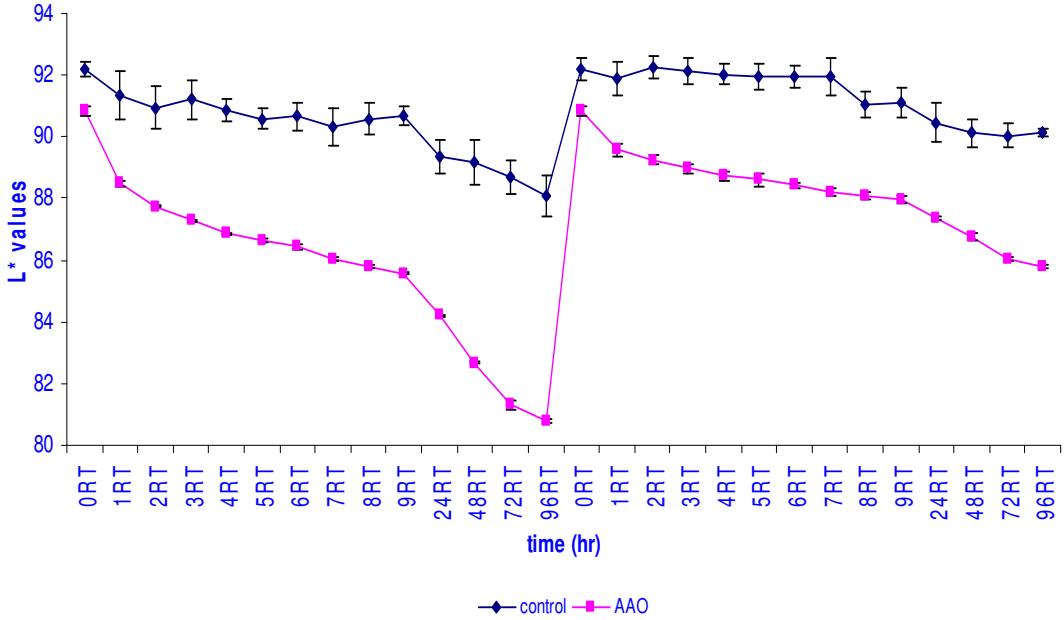


Fig 17.4 **Colour characteristics (L* values [whiteness]) of WSN made from UW stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

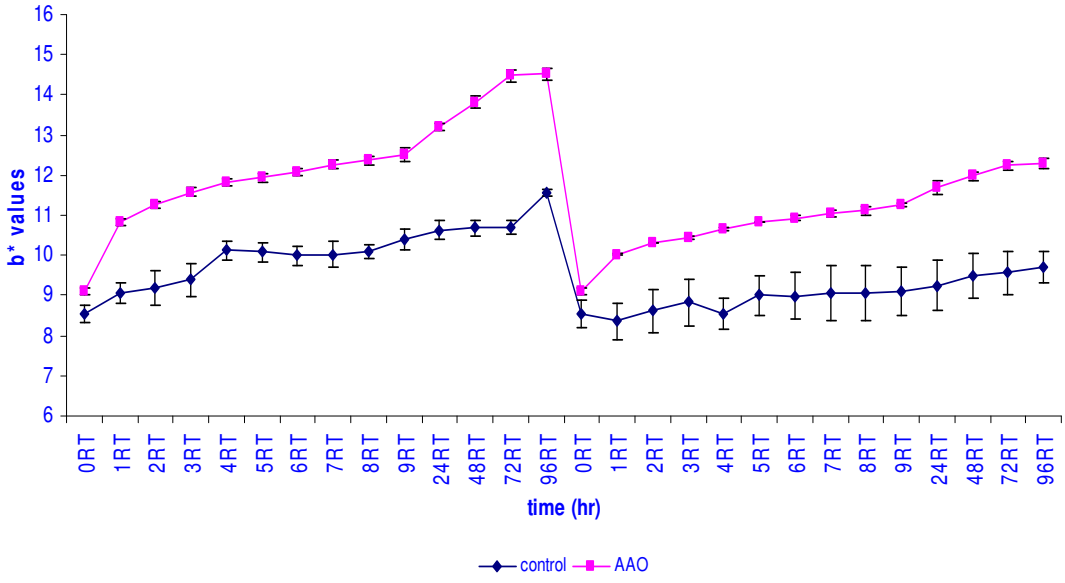


Fig 17.5 **Colour characteristics (b* values [yellowness]) of WSN made from UW stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

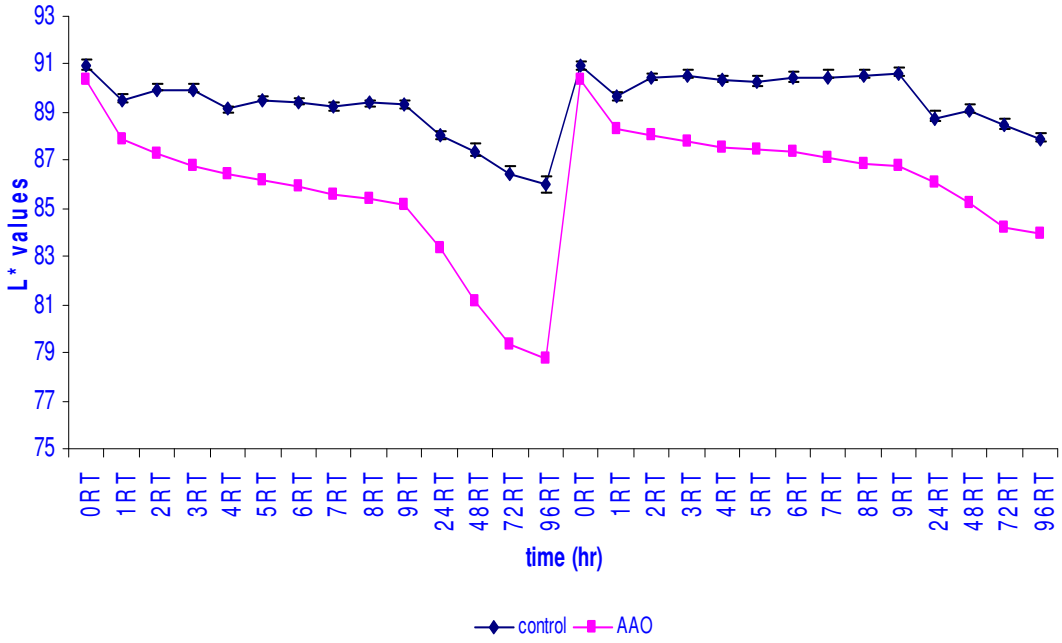


Fig 17.6 **Colour characteristics (L* values [whiteness]) of WSN made from PF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

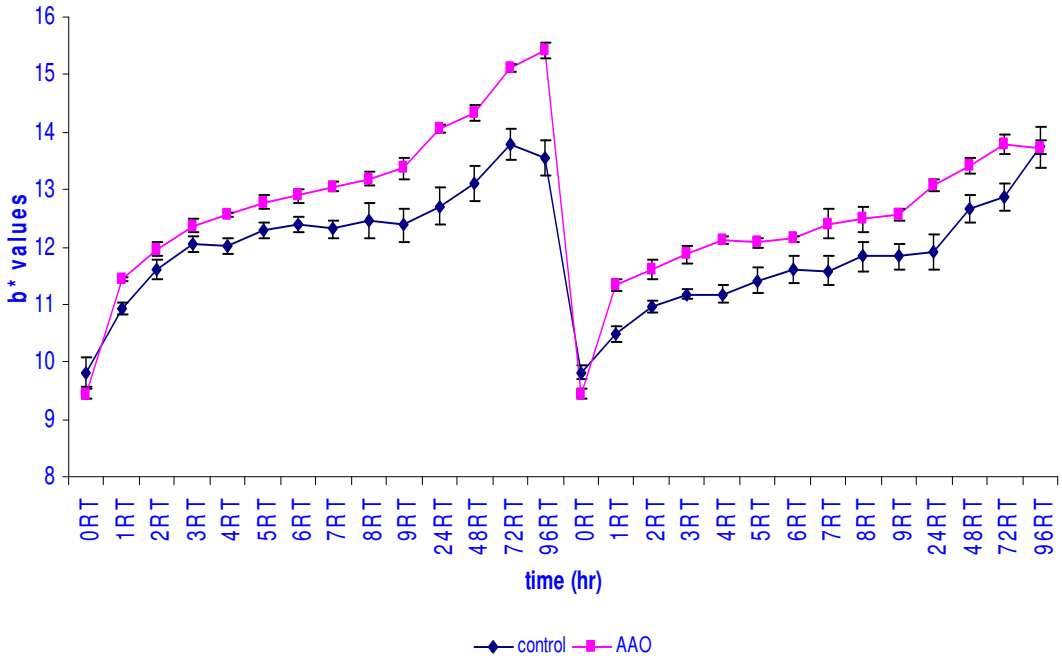


Fig 17.7 **Colour characteristics (b* values [yellowness]) of WSN made from PF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

Differences between the treatments were not as obvious however, when noodles were dried, cooked or dried and cooked immediately after preparation (Figs 17.8 & 17.9). Only in the case of dried noodles (using PF flour) were the differences between control and noodles incorporating AAO more obvious (Figs 17.8 & 17.9).

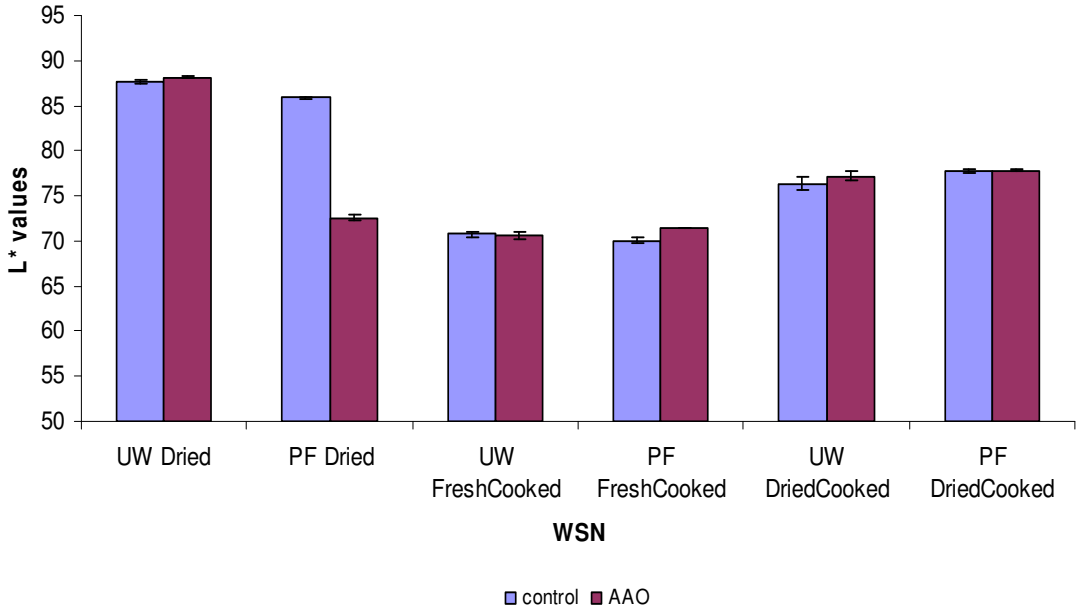


Fig 17.8 **Colour characteristics (L* whiteness / brightness) of dried, fresh cooked & dried cooked WSN made from UW & PF**
Error bars represent standard deviation values

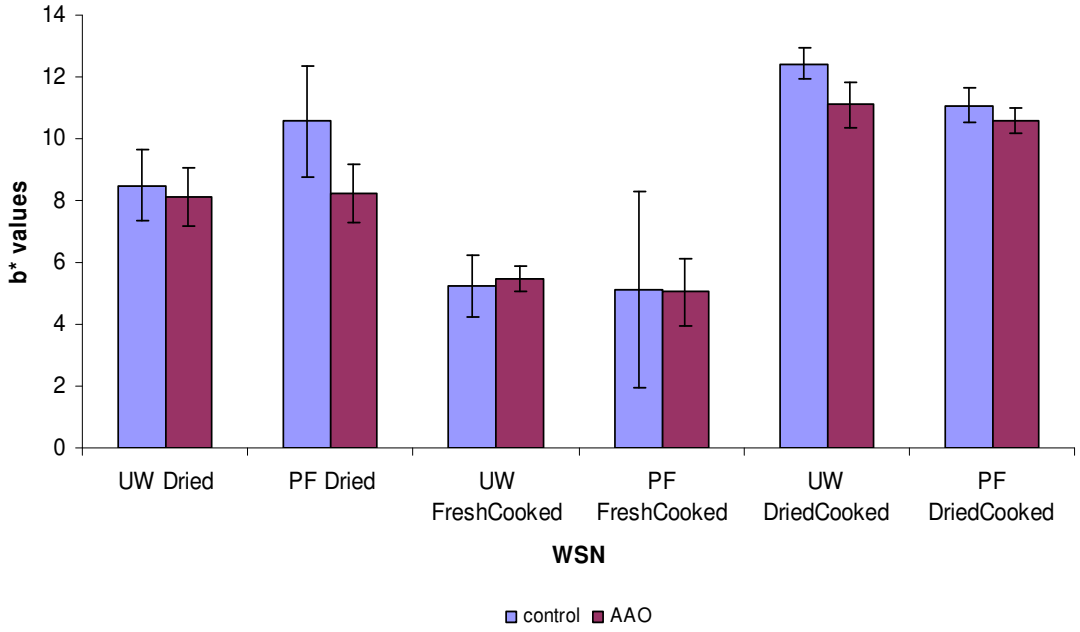


Fig 17.9 **Colour characteristics (b* yellowness) of dried, fresh cooked & dried cooked WSN made from PF & UW**
Error bars represent standard deviation values

17.7 Structural properties of WSN supplemented with AAO

Starch granules dominated the appearance of the surface of raw noodles. The images of control sample showed compact, lenticular to circular shaped starch granules. Noodles incorporating AAO showed similar surface appearance and did not differ greatly from control samples (Fig 17.10).

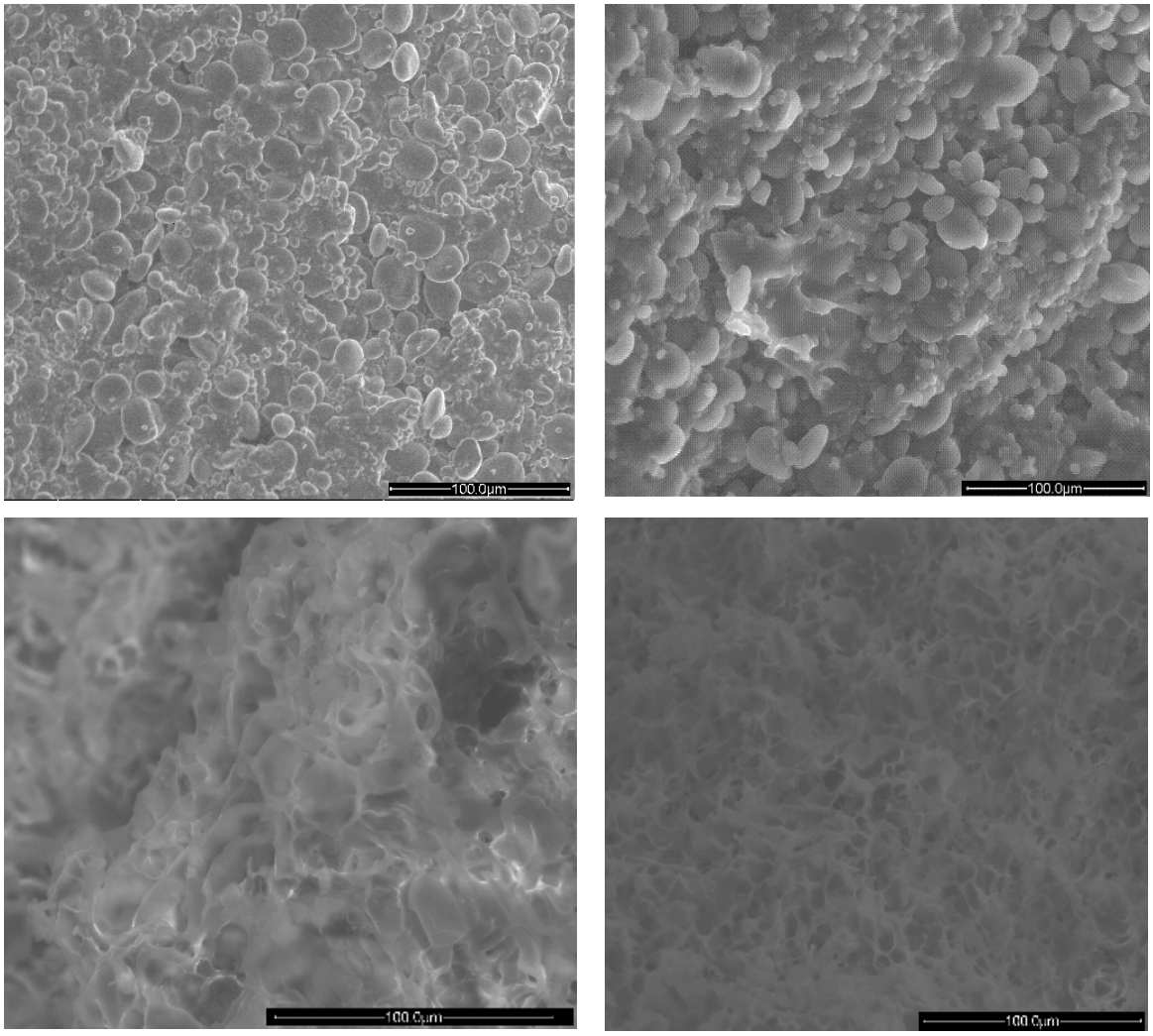


Fig 17.10 ESEM[a] images of raw WSN [control and AAO treatment, from left to right (top)] & SEM[b] images of cooked WSN [control and AAO treatment, from left to right (lower)]

a ESEM, 6.0 Torr, 4°C, 400× Mag., 5.0 spot size, 30kV
b SEM, 0.5 Torr, 23°C, 600× Mag., 5.0 spot size, 30kV

Upon cooking most of the starch gelatinised and the surface appearance is no longer dominated by the granular starch. However, some evidence of granular structure remains in both samples with no differences between control samples and noodles

incorporating AAO (Fig 17.10). Drying of noodles did not impact on the surface appearance of control samples, again showing compact structures of granular starch (Fig 17.11). Following cooking, dried noodles had gelatinised starch on the surface within the protein network with no visible difference in structural appearance between control and noodles incorporating AAO (Fig 17.11).

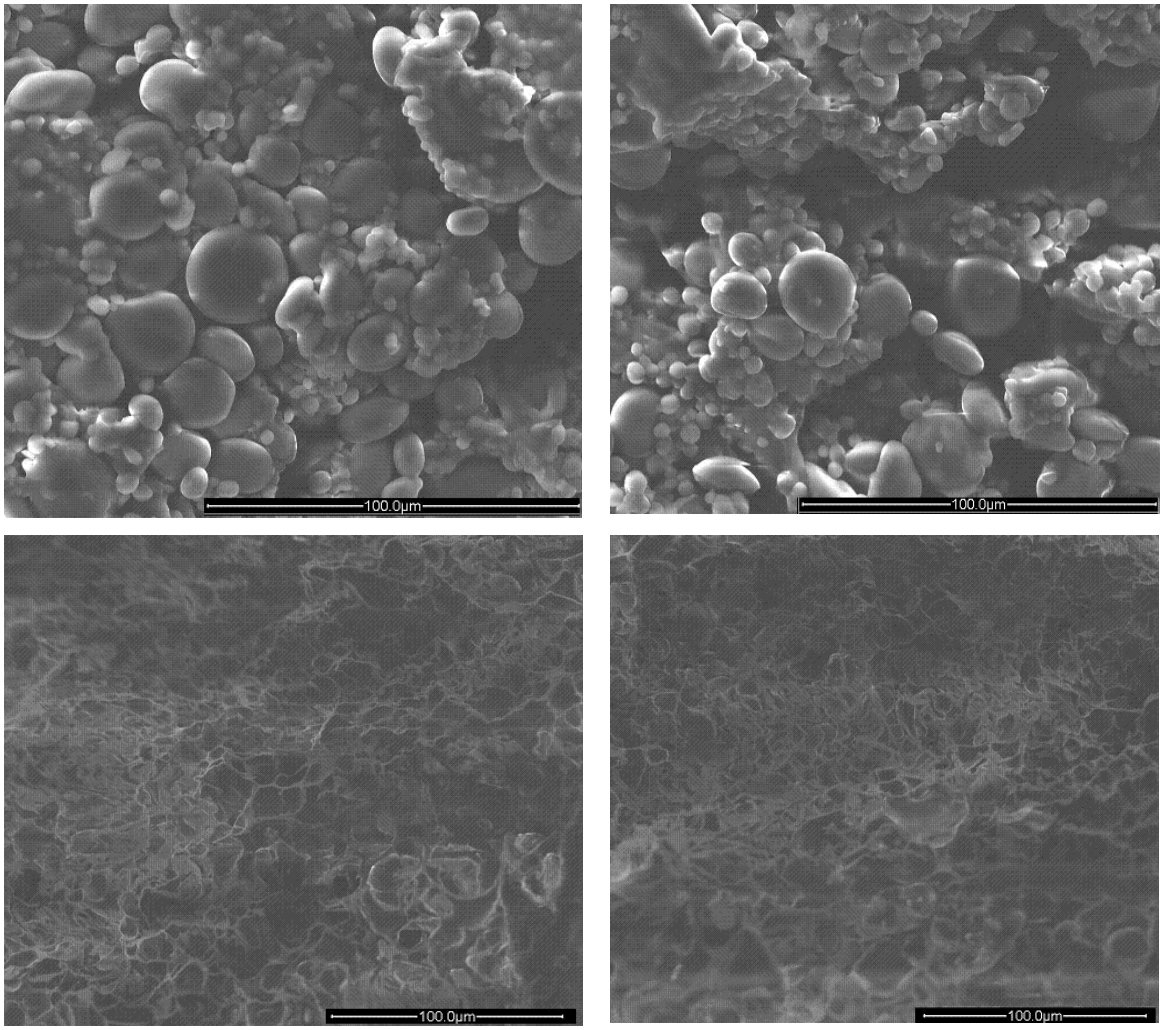


Fig 17.11 SEM[a] images of dried WSN control and AAO treatment (top) left to right and dried cooked WSN control and AAO treatment from left to right (bottom)

a SEM, 0.5 Torr, 23°C, 800× Mag. (top); 500× Mag.(lower), 5.0spot size, 30kV

17.8 Summary of results

The levels of endogenous AAO found in the two flours studied were relatively low and no losses in enzyme activity was recorded during processing or storage of WSN under a variety of conditions. Different levels of AAO addition tested in the preliminary experiments showed relatively minor differences between controls and WSN incorporating the AAO preparation. Furthermore, no significant differences were found in textural properties of noodles as a result of different storage temperatures.

WSN incorporating AAO were darker at all times than the controls, and differences in colour were more obvious in terms of L* than b* values. Discoloration of WSN sheets was faster and more obvious at 25°C compared to the storage of noodles at 4°C. Although as indicated by the Minolta readings differences in L* values were significant, WSN samples treated with AAO were visually acceptable and were as bright in appearance as control samples. Drying and cooking of WSN immediately after preparation resulted in no significant differences in terms of both L* and b* values. The addition of AAO preparation to the WSN formulation did not result in any adverse effect upon the cooking qualities of WSN. Furthermore the structural characteristics of WSN appeared to be unaltered by the enzyme preparation.

Chapter 18

Results and discussion: Ascorbic acid oxidase and the quality of Asian noodles prepared in the laboratory: Yellow alkaline noodles

The purpose of this chapter is to describe and discuss the results obtained for YAN incorporating a preparation of AAO from *Cucurbita* species.

18.1 Introduction

It might be expected that the impact of AAO on YAN would be different to that seen in WSN largely due to the different pH conditions within these products. While the pH of WSN is relatively close to neutral (~ 6.1) that of YAN is very high compared to almost all other food products with the measured value for YAN prepared in the laboratory found to be 10.1. The higher pH is due to the alkaline salts added to the formulation and this might partially inactivate the enzyme or decrease its effect. The optimum pH range of AAO is reported to be around 6.0 (Every et al 1995), therefore, a shift in pH to 10 might result in the enzyme having limited action. On the other hand, it might be suggested that slightly firmer noodles are be preferred for YAN as compared to WSN, and AAO might influence the texture and appearance in such a way to enhance the firmness and elasticity. In preliminary experiments two different levels of enzyme addition were evaluated (1.1×10^2 and 2.2×10^2 units per batch). Addition of the lower of these two was chosen for further experimentation as it resulted in softer noodles than addition of 2.2×10^2 of AAO per batch, handling was easier and these noodles scored more highly from a visual point of view.

18.2 Levels of AAO in flour and stability during noodle processing

The measured levels of AAO for BGF flour were similar to that of PF and UW flours (Chapter 10). AAO activity in YAN (33.4 ± 3.9) was slightly lower as compared to the WSN (37.6 ± 3.7 for UW and 38.5 ± 4.1 for PF). Furthermore, here the addition of 1.1×10^2 units of AAO preparation per batch of YAN did not had great impact on the overall activity of AAO (35.2 ± 4.5), probably due to the relatively alkaline conditions in these

noodles. The levels of AAO in dried noodles were much lower when compared to fresh noodles or those stored at either 4°C or 25°C (Table 18.1).

Table 18.1 Activity of AAO in control and supplemented YAN stored under different conditions

Treatment[a]	AAO activity[b]
Controls	
Day1	33.4 ± 3.9
24hr RT	32.5 ± 3.1
24hr FR	32.6 ± 4.1
Dried	27.7 ± 5.8
AAO 1.1 × 10^2 units / batch added[c]	
Day1	35.2 ± 4.5
24hr RT	34.6 ± 5.1
24hr FR	34.3 ± 3.7
Dried	30.9 ± 3.6

a Abbreviations are: YAN, yellow alkaline noodles; AAO, ascorbic acid oxidase; RT, storage at room temperature; FR, storage at 4°C

b Unit for AAO is (U / g), amount of activity that oxidises 1.0μg of LAA to DHAA acid under the assay defined conditions

c AAO added was from Sigma – Aldrich

18.3 The impact of varying levels of AAO on textural characteristics of YAN

The effect of the two different levels of AAO addition upon the textural properties of YAN is shown in Figs 18.1 and 18.2. The textural differences were recorded using the cylinder probe (P/45) as noodle hardness Fig 18.1 and the flat blade as noodle firmness Fig 18.2. A tendency of slightly harder noodles was seen upon the addition of AAO preparation to the YAN formulation (Fig 18.1). This was observed for both raw and cooked YAN. Similar observations were made when noodle firmness was measured, again slightly firmer noodles were seen upon the addition of the enzymes, here the addition of 2.2 × 10^2 units per batch having slightly greater effect (Fig 18.2).

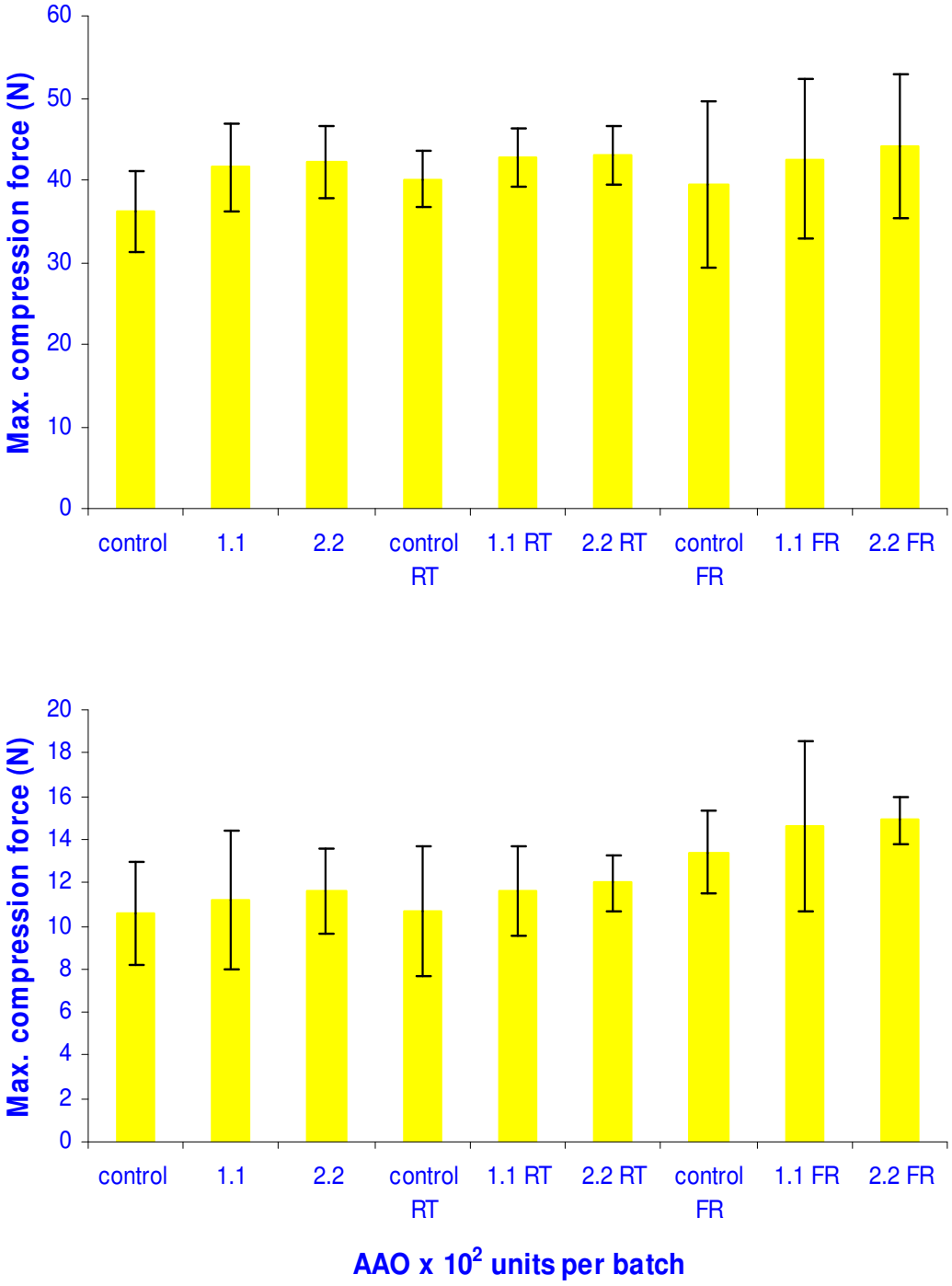


Fig 18.1 **The impact of various levels of AAO on the hardness properties of raw (top) and cooked (bottom) YAN**
Error bars represent standard deviation values.
Measured using the cylinder probe (P/45).

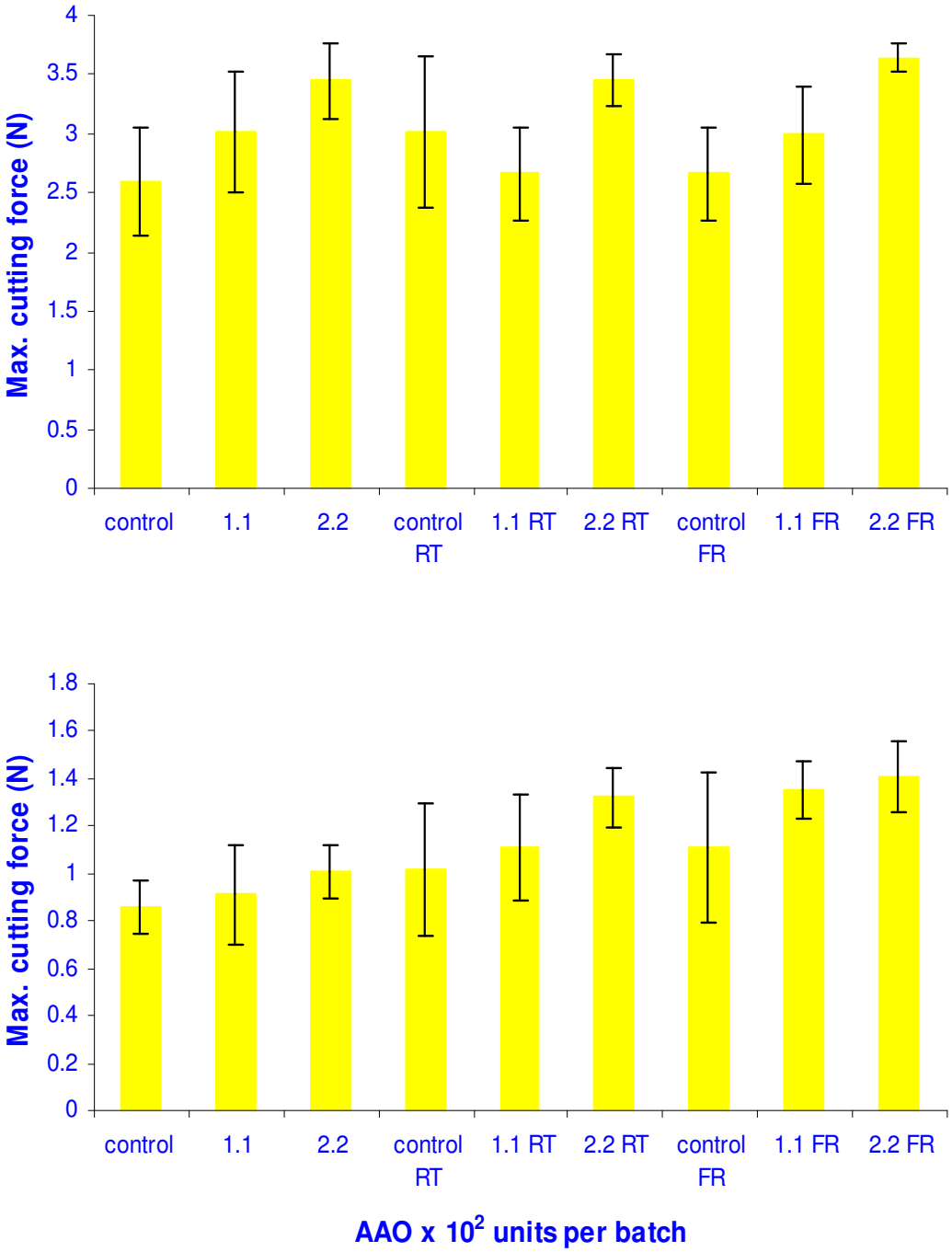


Fig 18.2 **The impact of various levels of AAO on the firmness properties of raw (top) and cooked (bottom) YAN**
Error bars represent standard deviation values.
Measured with the bladeattachment.

18.4 The effect of AAO on textural properties of YAN

The storage of YAN under various conditions resulted in similar finding like for the fresh noodles (Table 18.2). Addition of the enzyme preparation resulted in slightly firmer noodles, furthermore, the storage at 4°C resulted in slightly firmer noodles as well.

Table 18.2 Effects of AAO on the textural properties measured by TA-XT2 for YAN

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	36.1[a]	125[a]	11.0[a]	2.59[a]
	AAO	41.6[a]	131[a]	11.9[a]	3.01[a]
	RT control	40.1[a]	132[a]	12.4[a]	3.01[a]
	AAO RT	42.7[a]	135[a]	13.3[a]	3.60[a]
	FR control	39.5[a]	128[a]	11.1[a]	2.66[a]
	AAO FR	42.6[a]	136[a]	11.9[a]	2.99[a]
Cooked	control	10.6[a]	39.7[a]	3.50[a]	0.86[a]
	AAO	11.2[a]	40.6[a]	3.88[a]	0.91[a]
	RT control	10.7[a]	39.9[a]	4.58[a]	1.02[a]
	AAO RT	11.6[a]	42.6[ab]	5.01[a]	1.11[a]
	FR control	13.4[a]	47.4[ab]	5.36[a]	1.11[a]
	AAO FR	14.6[a]	49.8[b]	5.78[a]	1.35[a]
Dried [b]	control	12.4[a]	42.9[a]	5.84[a]	1.25[a]
	AAO	13.6[a]	44.6[a]	5.98[a]	1.31[a]

a Abbreviations are: YAN, yellow alkaline noodles; AAO, ascorbic acid oxidase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum. Level of addition was 1.1×10^2 units per batch

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Correlation between the firmness and hardness textural measurements was positively correlated (r^2 0.95) indicating that both textural measurements were useful in determining textural properties of YAN (Fig 18.3).

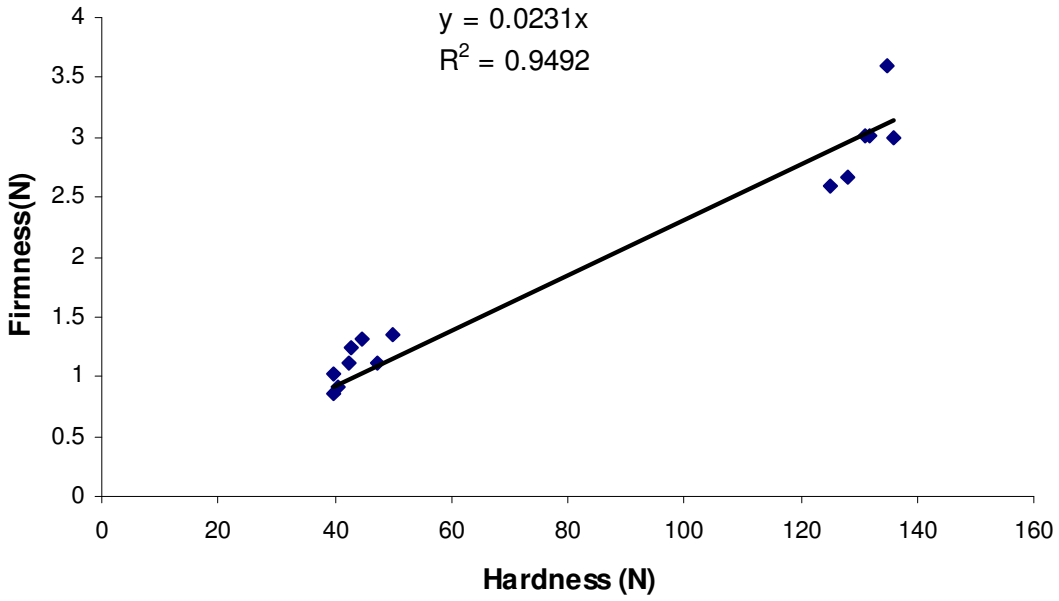


Fig 18.3 Correlation of firmness and hardness textural measurements
Data based on the mean values for firmness and hardness measurements of YAN (made from BGF, controls and AAO treatments). Cooked and dried and cooked YAN

18.5 The effect of AAO on cooking yield

Upon the addition of the enzyme to the noodle formulation no differences were observed for either water uptake or cooking loss (Table 18.3).

Table 18.3 Cooked weight and cooking loss of YAN made from BGF flour with and without addition of AAO

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	4.30	58.7[a]	9.63[a]
fresh AAO	4.30	56.3[a]	9.60[a]
dried control	9.00	72.1[a]	12.7[a]
dried AAO	4.30	71.9[a]	12.1[a]

a Abbreviations are: YAN, yellow alkaline noodles; BGF, Baker's strong flour; AAO, ascorbic acid oxidase
Note: Level of addition was 1.1×10^2 units per batch
Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

18.6 Relationship between AAO and colour properties of YAN

Visually the difference between control and noodles incorporating AAO preparation were not readily detectable, however, statistically lower L* values were recorded for noodles treated with AAO and stored at room temperature (Fig 18.4). Differences in L* values for noodles stored at refrigeration temperatures and differences in b* values were not significant (Figs 18.4 & 18.5). The trend of slightly darker noodles when AAO was added to the formulation was seen at both temperatures as indicated by lower L* (whiteness) and slightly higher b* values (yellowness) (Figs 18.4 & 18.5).

No obvious differences between the treatments were seen when noodles were dried immediately after proportion (Fig 18.6). Whiteness of noodles was not different (L* values) while yellowness was more prominent in control samples as indicated by slightly higher b* values (Fig 18.6).

Both the L* and b* values were almost identical for control and AAO treatments when noodles were cooked immediately after preparation (Figs 18.6 & 18.7). Similar results were obtained for dried and cooked noodles, where no differences between the treatments were seen (Figs 18.6 & 18.7).

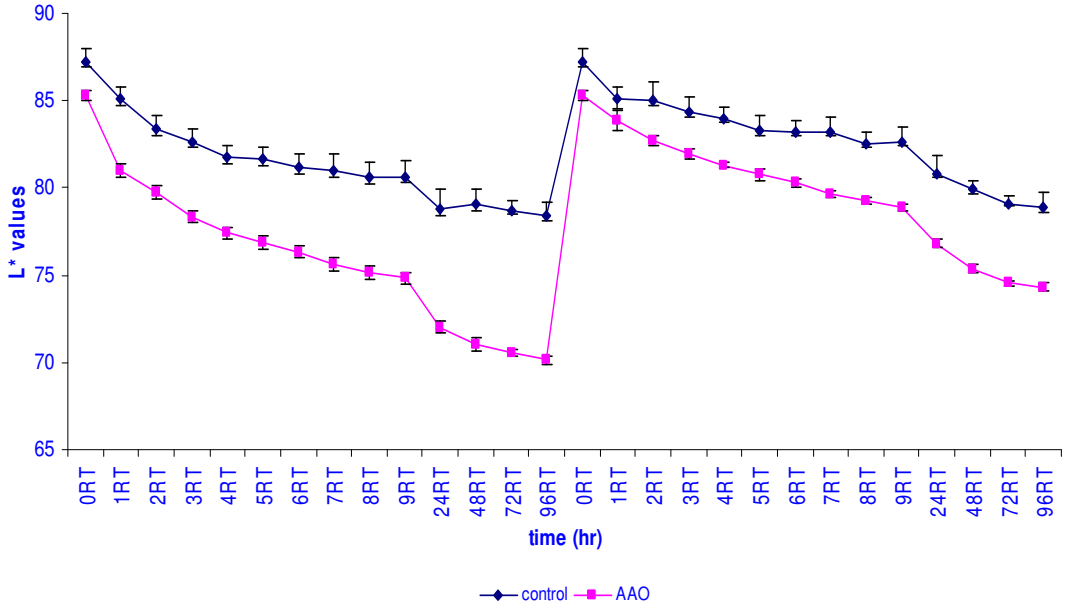


Fig 18.4 **Colour characteristics (L* values [whiteness]) of YAN made from BGF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

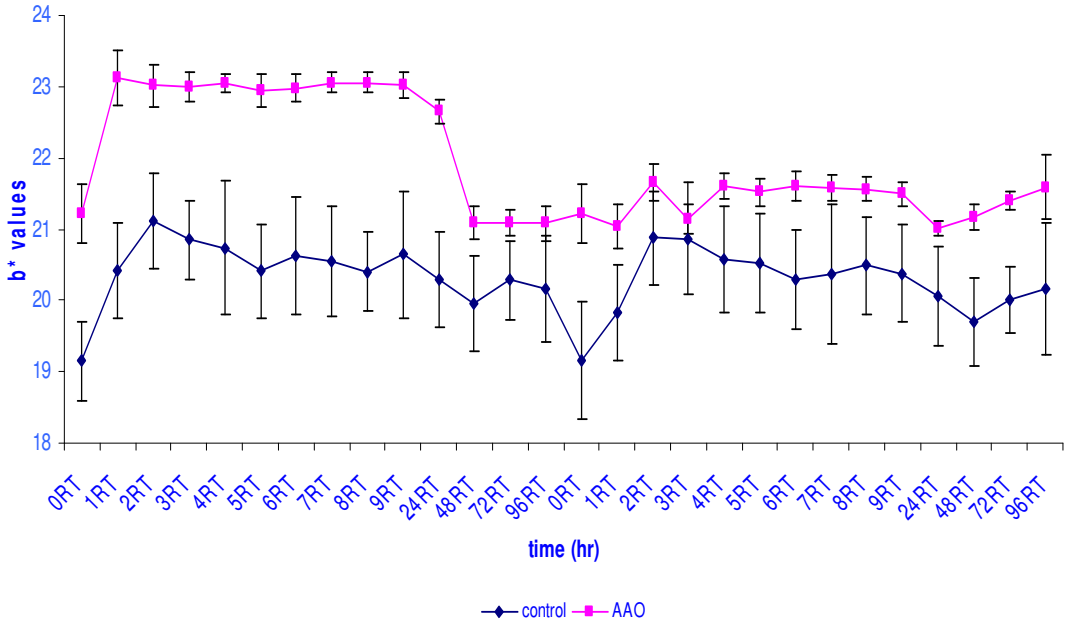


Fig 18.5 **Colour characteristics (b* values [yellowness]) of YAN made from BGF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

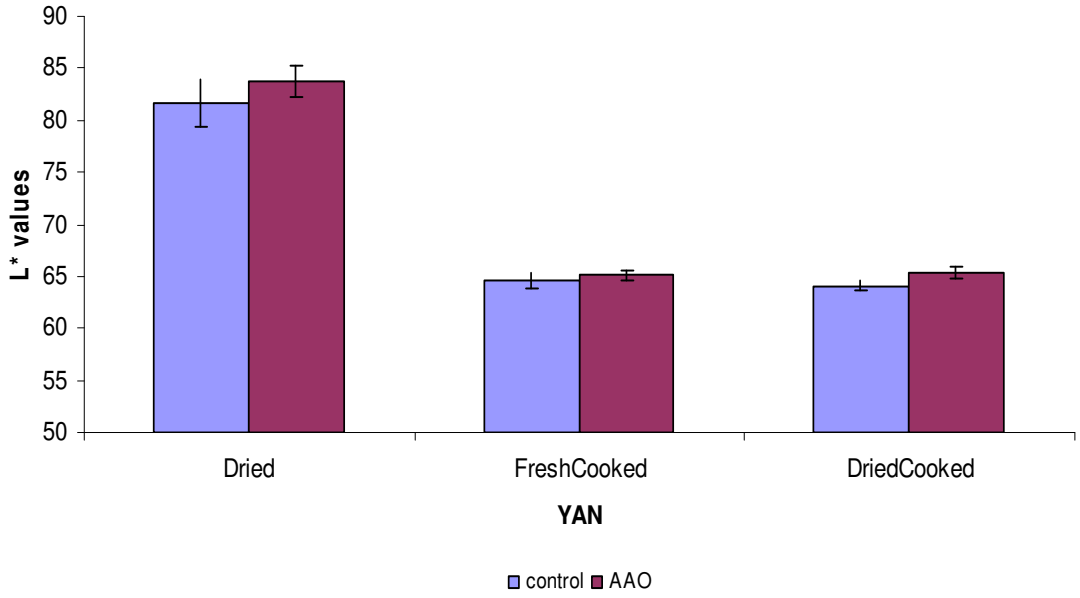


Fig 18.6 **Colour characteristics (L* whiteness / brightness) of dried, fresh cooked & dried cooked YAN made from BGF**
Error bars represent standard deviation values

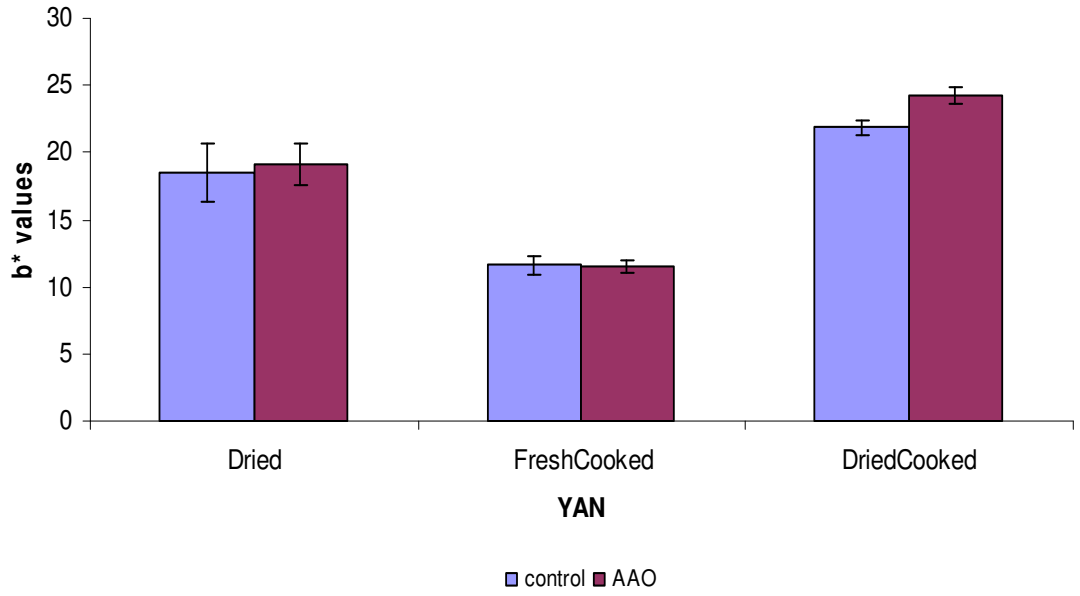


Fig 18.7 **Colour characteristics (b* yellowness) of dried, fresh cooked & dried cooked YAN made from BGF**
Error bars represent standard deviation values

18.7 Structural properties of YAN supplemented with AAO

The structural properties of YAN incorporating AAO were not effected in any way when compared to the control. The surface appearance of raw YAN was dominated by starch granules. The images of the control sample showed compact, lenticular to circular shaped starch granules. Noodles incorporating AAO showed similar surface appearance and did not differ greatly from control samples (Fig 18.8). Some evidence of granular starch remains visible on YAN surface after cooking, however, the appearance of the image is dominated by the starch paste within the protein network. This can be said for both control and YAN incorporating AAO preparation (Fig 18.8). The process of drying itself did not have much effect of the surface appearance of control samples, showing overall again compact structure of granular starch (Fig 18.9). Furthermore no differences in between the controls and AAO treated YAN can be seen. Cooking of dried noodles again showed gelatinised starch on the surface within the protein network wit no visible difference in structural appearance between control and YAN incorporating AAO (18.9).

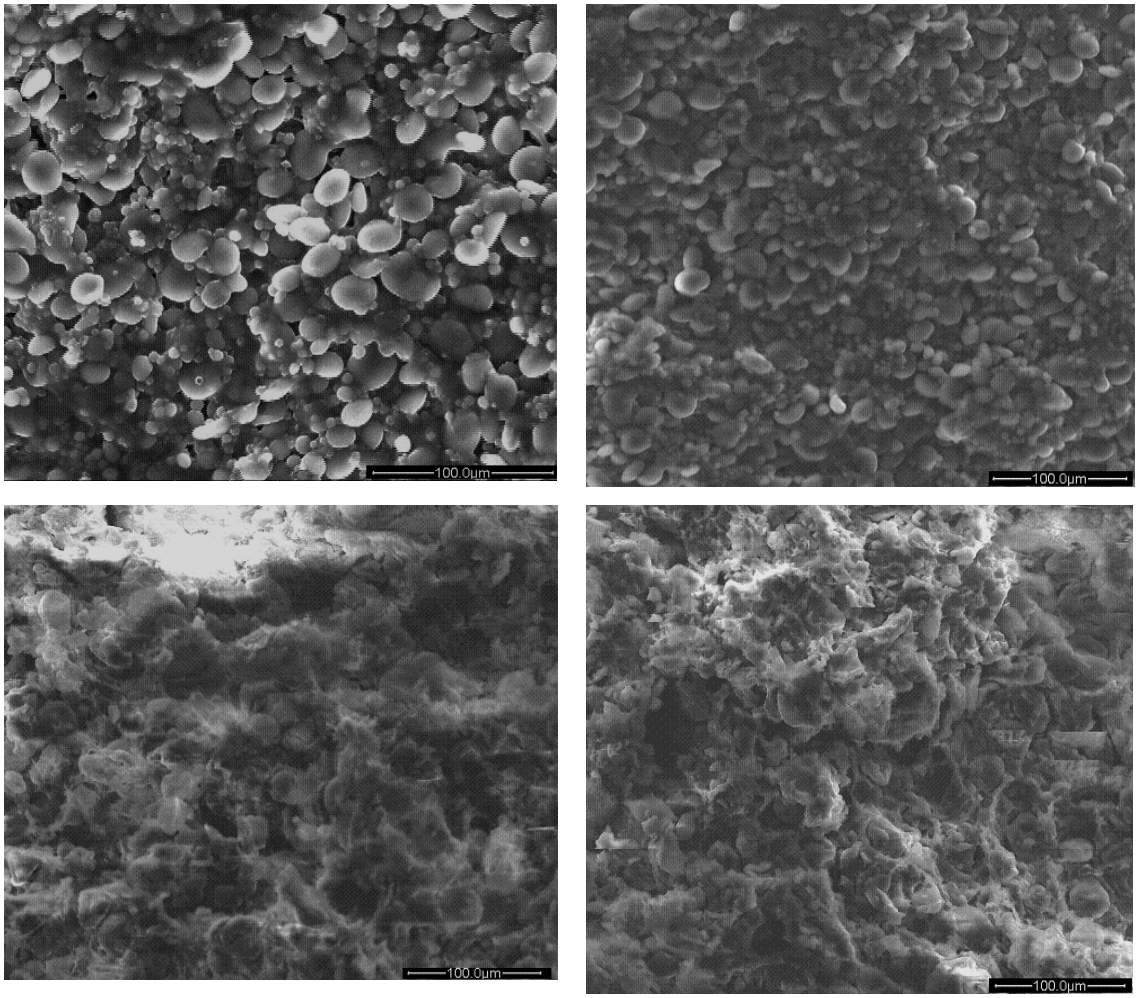


Fig 18.8 ESEM[a] images of fresh raw YAN (top) and SEM[b] images of fresh cooked YAN (bottom) made form BGF flour; control, and AAO treatment (from left to right)

a ESEM, 6.0 Torr, 4°C, 300× Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 500× Mag., 5.0 spot size, 30kV

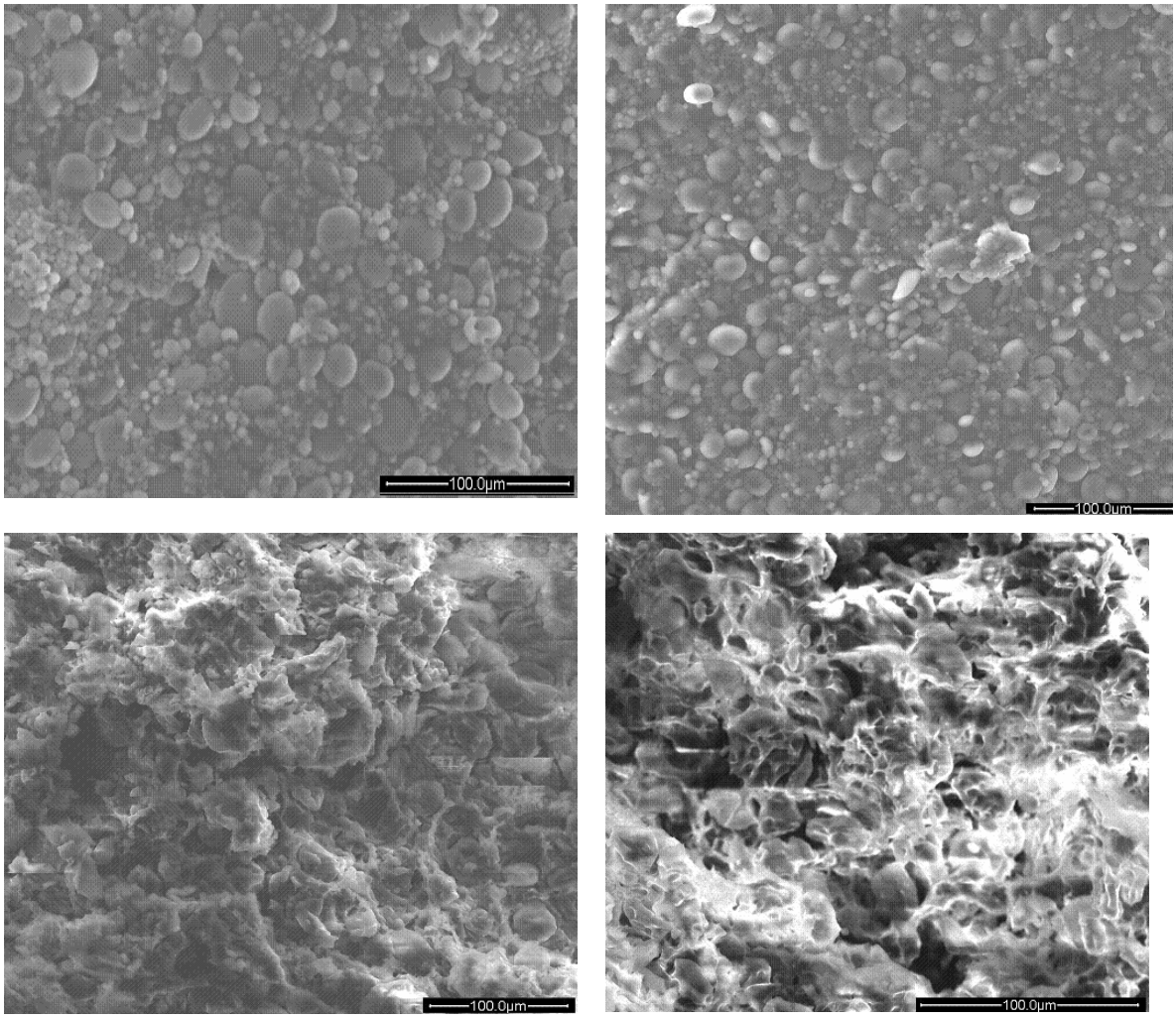


Fig 18.9 SEM images of dried[a] YAN (top) and dried and cooked[b] YAN (bottom) control and AAO treatment (from left to right)

a SEM, 0.5 Torr, 23°C, 300 × Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 500 × Mag., 5.0 spot size, 30kV

18.8 Summary of results

Processing of YAN, where pH is higher than that for WSN, did not result in any loss of AAO activity. In preliminary experiments where different levels of addition were tested, a tendency for harder and firmer YAN was observed, although the differences were not great. Overall no adverse effect upon textural characteristics of YAN was seen.

The addition of AAO preparation to the YAN formulation did not adversely impact upon cooking properties of YAN. Discolouration was seen in all samples, including

controls. YAN incorporating AAO tended to have slightly darker appearance, these differences corresponding with more obvious impacts on L* compared to b* values. The surface appearance of YAN observed by electron microscopy was similar for controls and AAO treatments.

Chapter 19

Results and discussion: Lipoxygenase and the quality of Asian noodles prepared in the laboratory: White salted noodles

The purpose of this chapter is to present results for WSN to which a lipoxygenase preparation from soy was added.

19.1 Introduction

It has been reported that the enzyme lipoxygenase (LOX, EC 1.13.11.12) has a variety of effects in wheat flour dough. One of these is an increase in mixing tolerance in combination with a general enhancement in dough rheological properties (Faubion and Hoseney 1981). Never-the-less the enzyme is best known as a bleaching agent. This action is believed to involve the oxidation of pigments and unsaturated fatty acids by oxygen. Whilst the significance of the enzyme for bread and pasta quality has been researched in considerable detail (Faubion and Hoseney 1981; Miller and Kummerow 1948; McDonald 1979; Matsuo 1987), less is known of its role, influence and potential in noodle products (Edwards et al 1989; Matsuo 1987). Accordingly the objective of this phase of the study has been to examine the effect of an exogenous LOX preparation from soybean on textural and colour properties of WSN.

19.2 Levels of LOX in flour and stability during noodle processing

LOX activity in flours and noodles was assayed by a modified spectrophotometric procedure (Eriksson 1967; Surrey 1964; Rani et al 2001; Hsieh et al 1984 and Zimmerman and Vick 1970, see Chapter 7 for details). In the two flours studied here (Chapter 10) and subsequently in WSN the levels of LOX recorded were relatively low, 29.3 ± 6.2 for PF and slightly higher 34.9 ± 8.6μmol of hydroperoxidase formed per minute per gram of flour for UW (Table 19.1). No significant losses were seen in the enzyme activity upon the storage of noodles under various conditions. Higher LOX activity was recorded when the soybean preparation was added to the formulation (Table 19.1).

Table 19.1 Activity of LOX in control and supplemented WSN stored under different conditions

Treatment[a]	LOX activity[b]	
Controls	**UW**	**PF**
Day1	34.9 ± 8.6	29.3 ± 6.2
24hr RT	33.4 ± 3.9	30.6 ± 3.4
24hr FR	32.6 ± 4.1	31.6 ± 3.3
Dried	30.3 ± 2.3	28.6 ± 3.4
LOX (1.85×10^3 units per batch added[c])		
Day1	38.3 ± 3.5	39.4 ± 2.1
24hr RT	38.6 ± 3.3	40.1 ± 1.2
24hr FR	40.9 ± 3.1	42.6 ± 2.3
Dried	39.8 ± 1.6	40.4 ± 2.4
LOX (3.70 × 103 units per batch added[c])		
Day1	45.5 ± 3.6	46.8 ± 2.9
24hr RT	44.8 ± 4.3	47.2 ± 2.8
24hr FR	44.3 ± 3.6	47.6 ± 2.5
Dried	43.6 ± 3.6	46.6 ± 1.9

a Abbreviations are: WSN, white salted noodles; LOX, lipoxygenase; RT, storage at room temperature; FR, storage at 4°C

b Unit for LOX is μmol of hydroperoxidase formed per minute per gram of flour under the defined assay conditions

c LOX added was from Sigma – Aldrich

19.3 The impact of varying levels of LOX on textural characteristics of WSN made from PF flour

In the preliminary experiments two different levels of LOX addition were assessed. The soy bean preparation was dissolved with 5.0mL of the extraction buffer (50mM sodium phosphate pH 7.5) and then 1.85×10^3 and 3.70×10^3 units per batch added. Visually and in handling terms little difference was seen between controls and enzyme treatments. Hardness of WSN was measured with the flat cylinder P/45 probe. The treatments were similar to the controls in both the raw and cooked product, showing a tendency for less hard noodles in the raw form (Fig 19.1). Firmness of WSN was assessed using the cutting attachment blade of the TA- XT2. Here a tendency of firmer WSN was seen upon the addition of LOX. Addition of 1.85×10^3 units per batch

seemed to result in slightly firmer noodles than addition of 3.70 × 10^3 units per batch, but both being firmer when compared to the control samples. This was observed for both raw and cooked WSN (Fig 19.2).

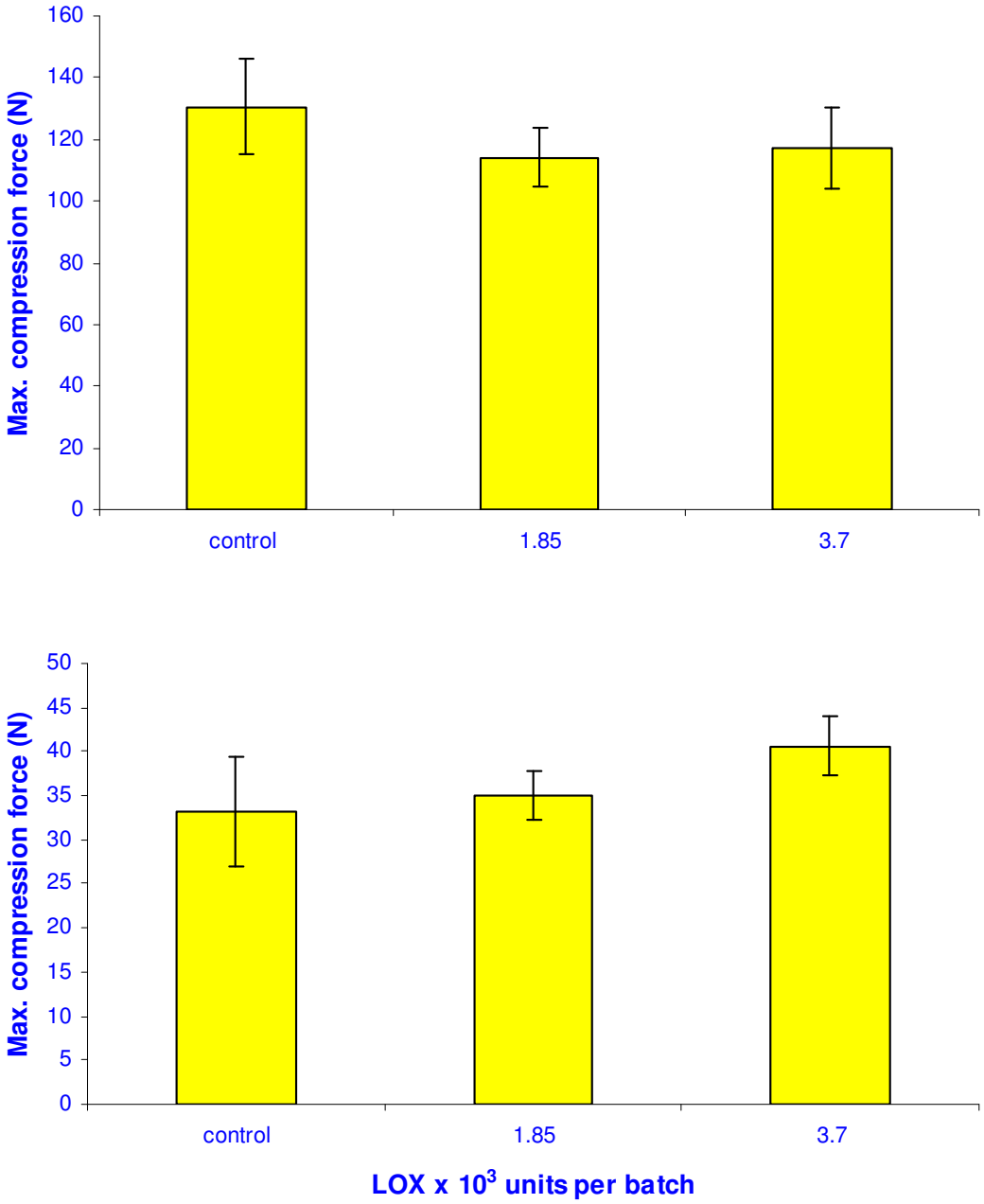


Fig 19.1 **Evaluation of different levels of LOX addition on hardness properties of fresh raw (top) and fresh cooked (lower) WSN made from UW**
Error bars represent standard deviation values.
Measured using the P/45 flat cylinder probe

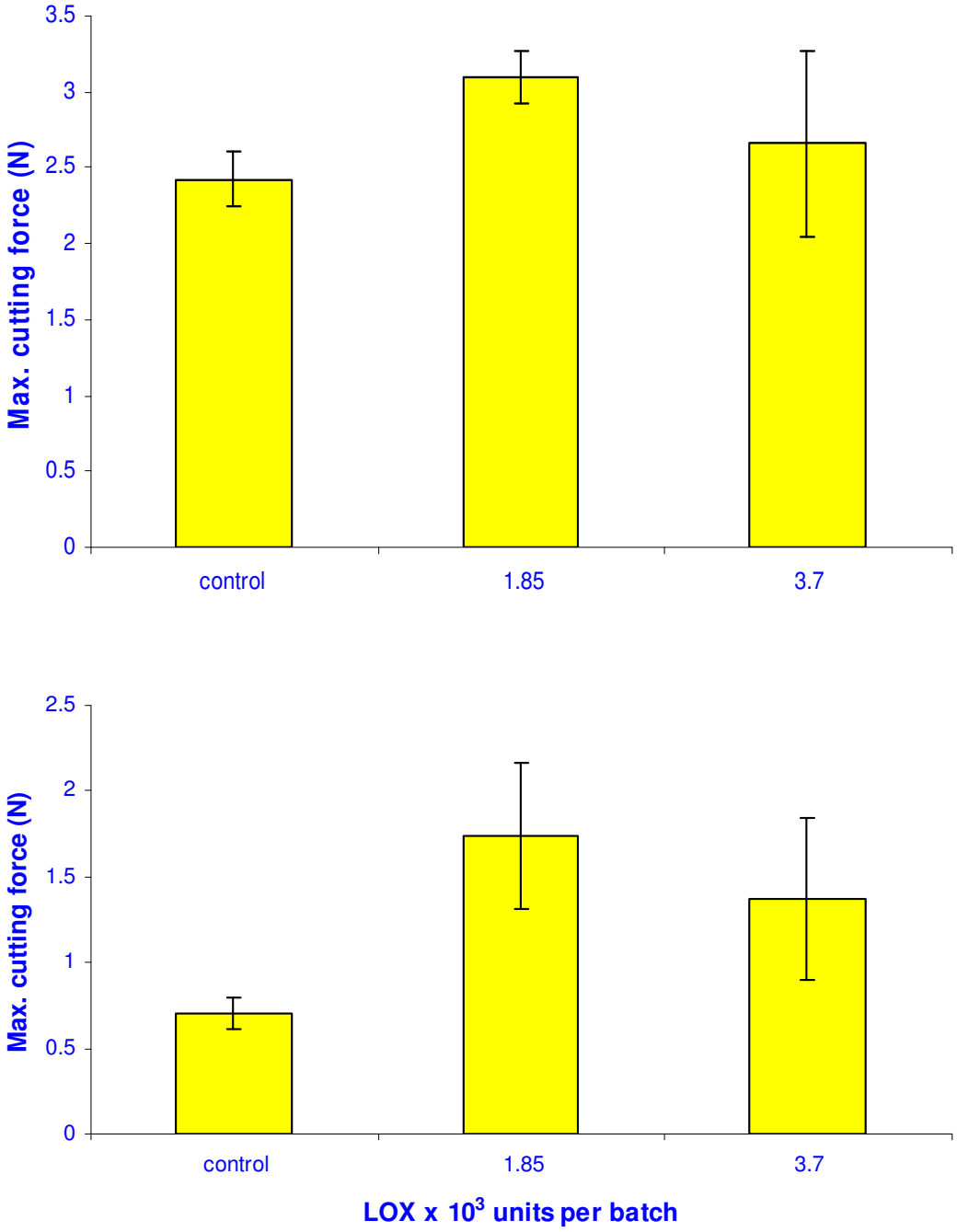


Fig 19.2 **Evaluation of different levels of LOX addition on firmness properties of fresh raw (top) and fresh cooked (lower) WSN made from UW**
Error bars represent standard deviation values.
Measured using the flat blade attachment

Both levels of enzyme addition were evaluated after noodles have been stored under various conditions and results of these measurements are presented in Tables 19.2 – 19.5. Again through storage at either room temperature or at 4°C or in the case of dried

noodles no obvious differences were recorded between controls and WSN treated with LOX. Furthermore, there was no difference between the two flours (UW and PF) used in the making of WSN. A tendency of slightly firmer and harder noodles was seen upon the storage of noodles at 4°C compared to the storage at room temperature (~25°C) and also dried noodles showed the same tendency (Tables 19.2 – 19.5).

Table 19.2 Effects of LOX (1.85×10^3 units per batch) on the textural properties measured by TA-XT2 for WSN made from UW

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.6^{a}	130^{ab}	11.8^{ab}	2.42^{a}
	LOX	34.7^{a}	114^{ac}	8.51^{ac}	2.74^{a}
	RT control	43.3^{a}	134^{b}	13.6^{b}	2.55^{a}
	LOX RT	46.6^{a}	141^{b}	12.5^{bc}	2.48^{a}
	FR control	44.5^{a}	136^{b}	12.1^{bc}	2.44^{a}
	LOX FR	39.8^{a}	128^{bc}	11.8^{bc}	2.14^{a}
Cooked	control	8.80^{a}	33.3^{a}	3.54^{a}	0.70^{a}
	LOX	10.2^{ab}	35.1^{a}	2.56^{a}	1.20^{a}
	RT control	9.20^{ab}	34.8^{a}	3.54^{a}	0.70^{a}
	LOX RT	13.2^{b}	43.3^{a}	3.13^{a}	0.68^{a}
	FR control	10.0^{ab}	37.0^{a}	4.24^{a}	0.78^{a}
	LOX FR	13.5^{b}	45.9^{a}	4.12^{a}	0.65^{a}
Dried [b]	control	11.9^{a}	39.5^{a}	6.01	0.99^{a}
	LOX	10.3^{a}	35.3^{a}	3.87	1.31^{a}

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour; LOX, lipoxygenase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Table 19.3 Effects of LOX (3.70×10^3 units per batch) on the textural properties measured by TA-XT2 for WSN made from UW

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	41.6^{a}	130^{a}	11.8^{ab}	2.42^{a}
	LOX	39.5^{a}	118^{b}	8.72^{ab}	2.66^{a}
	RT control	43.3^{a}	134^{a}	13.6^{c}	2.55^{a}
	LOX RT	47.6^{a}	138^{a}	10.2^{abc}	2.92^{a}
	FR control	44.5^{a}	136^{a}	12.1^{c}	2.44^{a}
	LOX FR	40.7^{a}	123^{ab}	7.71^{b}	2.52^{a}
Cooked	control	8.80^{a}	33.3^{a}	3.54^{a}	0.70^{a}
	LOX	9.56^{a}	40.6^{bc}	3.79^{a}	1.37^{a}
	RT control	9.22^{a}	34.8^{a}	3.54^{a}	0.70^{a}
	LOX RT	9.62^{a}	32.6^{a}	3.18^{a}	1.10^{a}
	FR control	10.0^{a}	37.0^{ac}	4.24^{a}	0.78^{a}
	LOX FR	12.6^{a}	41.3^{bc}	3.35^{a}	1.20^{a}
Dried [b]	control	11.9^{a}	39.5^{a}	6.01^{a}	0.99^{a}
	LOX	12.2^{a}	36.5^{a}	4.48^{a}	1.00^{a}

a Abbreviations are: WSN, white salted noodles; UW, Ultra white flour; LOX, lipoxygenase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Table 19.4 Effects of LOX (1.85×10^3 units per batch) on the textural properties measured by TA-XT2 for WSN made from PF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	**control**	41.2[a]	128[a]	11.9[a]	2.46[a]
	LOX	39.6[a]	120[a]	11.2[a]	2.56[a]
	RT control	38.1[a]	119[a]	13.0[a]	2.59[a]
	LOX RT	39.4[a]	122[a]	12.6[a]	2.65[a]
	FR control	39.4[a]	121[a]	12.9[a]	2.58[a]
	LOX FR	40.3[a]	125[a]	13.1[a]	2.78[a]
Cooked	**control**	9.12[a]	33.6[a]	3.30[a]	0.79[a]
	LOX	10.2[a]	34.2[a]	3.22[a]	0.87[a]
	RT control	9.54[a]	33.9[a]	3.21[a]	0.66[a]
	LOX RT	10.3[a]	34.6[a]	3.33[a]	0.69[a]
	FR control	9.91[a]	34.1[a]	4.29[a]	0.84[a]
	LOX FR	10.5[a]	35.2[a]	3.89[a]	0.79[a]
Dried [b]	**control**	11.8[a]	39.3[a]	5.74[a]	1.06[a]
	LOX	12.2[a]	39.8[a]	5.93[a]	1.12[a]

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour; LOX, lipoxygenase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Table 19.5 Effects of LOX (3.70×10^3 units per batch) on the textural properties measured by TA-XT2 for WSN made from PF

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	**control**	41.2[a]	128[ab]	11.9[a]	2.46[a]
	LOX	40.5[a]	123[ab]	9.01[ab]	2.71[a]
	RT control	38.1[a]	119[b]	13.0[a]	2.59[a]
	LOX RT	44.3[a]	136[a]	10.1[ab]	2.88[a]
	FR control	39.4[a]	121[ab]	12.9[a]	2.57[a]
	LOX FR	42.1[a]	125[ab]	7.55[b]	2.44[a]
Cooked	**control**	9.10[a]	33.6[a]	3.39[a]	0.74[a]
	LOX	9.66[a]	41.3[a]	3.69[a]	1.47[a]
	RT control	9.58[a]	33.9[a]	3.21[a]	0.66[a]
	LOX RT	9.89[a]	34.1[a]	3.45[a]	1.13[a]
	FR control	9.97[a]	34.1[a]	4.29[a]	0.87[a]
	LOX FR	11.6[a]	42.3[a]	3.44[a]	1.34[a]
Dried [b]	**control**	11.8[a]	39.3[a]	5.70[a]	1.05[a]
	LOX	12.6[a]	38.5[a]	4.56[a]	1.23[a]

a Abbreviations are: WSN, white salted noodles; PF, P-Farina flour; LOX, lipoxygenase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to the optimum

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Positive correlations (r^2 0.90) was found for textural measurements of WSN between two methods evaluated (Fig 19.3).

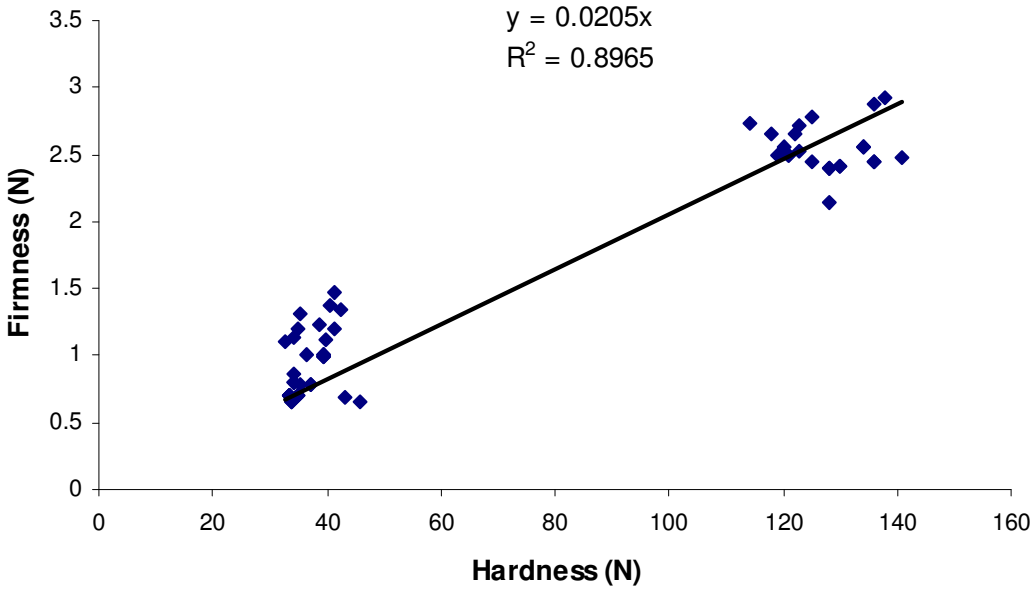


Fig 19.3 Correlation of firmness vs hardness of textural measurements
Data based on the mean values for firmness and hardness measurements of WSN (made from UW and PF, controls and LOX treatments)

19.4 The effect of LOX on cooking yield

Cooking characteristics of WSN were assessed by methods modified from Grant *et al* 2004 and Chakaraborty *et al* 2003. Noodles were cooked to their optimum cooking time and cooking properties presented as percentage yield lost and percentage of water uptake (Tables 19.6 and 19.7). In general higher losses in the yield of WSN were seen in dried noodles as compared to the fresh samples, in addition slightly higher losses were seen when 3.70×10^3 units of LOX preparation were added to a batch of WSN. No differences in yield losses were seen in relation to the two flours used (Tables 19.6 and 19.7).

Table 19.6 The impact of LOX addition on cooking quality of WSN made from UW flour

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	2.3	58.7	6.93[ab]
fresh LOX 1.85 × 10^3U	2.3	52.4[a]	5.88[a]
fresh LOX 3.70 × 10^3U	2.3	51.9[a]	7.25[b]
dried control	6.30	70.8[a]	8.84[a]
dried LOX 1.85 × 10^3U	6.30	67.8[a]	8.85[a]
dried LOX 3.70 × 10^3U	6.30	69.6[a]	8.72[a]

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

a Abbreviations are: LOX, lipoxygenase; WSN, white salted noodles; UW, Ultra white flour

Table 19.7 The impact of LOX addition on cooking quality of WSN made from PF flour

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	2.3	56.3[a]	6.38[a]
fresh LOX 1.85 × 10^3U	2.3	53.4[ab]	6.08[a]
fresh LOX 3.70 × 10^3U	2.3	52.5[b]	6.95[a]
dried control	6.30	70.3[a]	8.94[a]
dried LOX 1.85 × 10^3U	6.30	69.6[a]	9.15[a]
dried LOX 3.70 × 10^3U	6.30	69.9[a]	9.12[a]

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

a Abbreviations are: LOX, lipoxygenase; WSN, white salted noodles; PF, P-Farina flour

19.5 Relationship between LOX and colour properties of WSN

Discolouration of noodles sheets was observed for all samples including controls (Figs 19.4 - 19.7) although there was no development of speckiness in any case. Formulations incorporating LOX darkened less, and at a slower rate when the level of addition was 3.70 × 10^3U. Darkening occurred at a faster rate and was observed earlier in noodles stored at 25°C as compared to those stored at 4°C. Control samples were white and off-white respectively at time zero and, after 96 hours, the colour was an unattractive beige. On the other hand LOX treatments were initially clear and bright white but gradually

lost brightness during storage. Surprisingly high b* values were recorded for noodles treated with lipoxygenase (Fig 19.5) indicating more yellow products and this was observed for both flours used (UW and PF). The two flours showed different results: PF noodles treated with LOX were slightly darker than the control samples (Figs 19.6 & 19.7) while in the case of UW the opposite was observed. Noodles made from UW showed better colour properties, with a clearer and brighter white colour.

The colour of dried noodles was also measured and the results are shown in Figs 19.8 & 19.9. There were relatively minor difference in terms of L* values and again the same pattern was seen for the two flours used, noodles made from PF and treated with LOX were slightly darker in colour than control samples. The differences seen in b* values were not as great once noodles had been dried.

The colour attributes of boiled noodles were measured when fresh products were cooked immediately following preparation. Here, for noodles made from both flours, enzyme treated noodles were whiter and brighter in colour than controls as indicated by higher L* values (Figs 19.8 & 19.9). However, again unexpectedly high b* values were recorded for LOX treated noodles for both flours used. The primary impact of the enzyme was on the cooked samples which were lighter in colour but had higher yellowness readings. Similar effects were observed for dried and cooked WSN incorporating the LOX preparation (Figs 19.8 & 19.9).

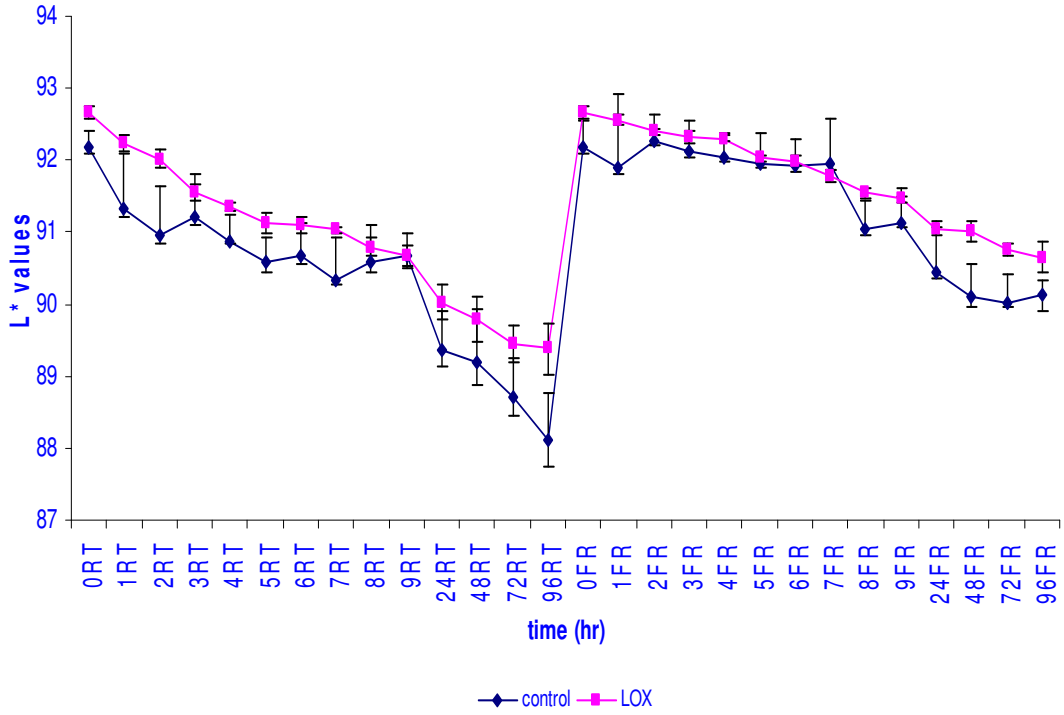


Fig 19.4 **Colour characteristics (L* values [whiteness]) of WSN made from UW stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

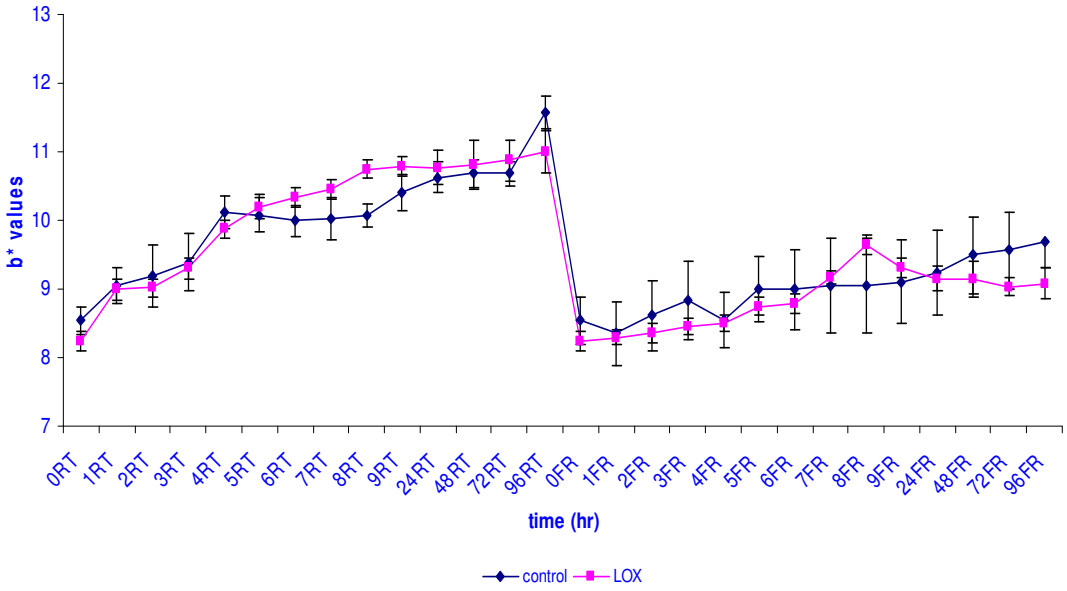


Fig 19.5 **Colour characteristics (b* values [yellowness]) of WSN made from UW stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

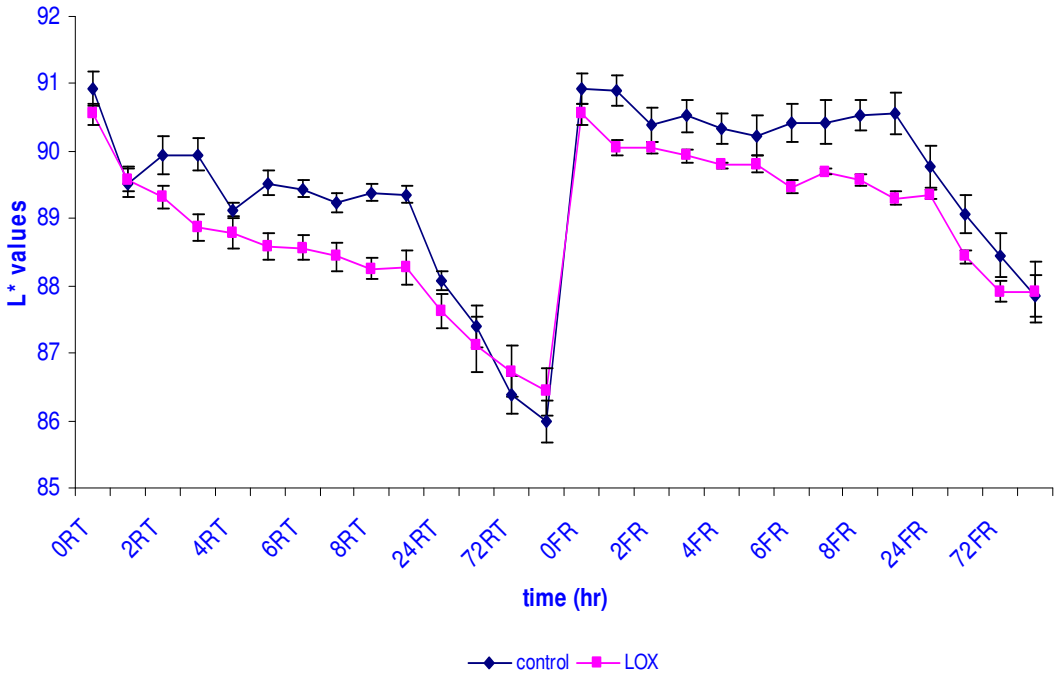


Fig 19.6 **Colour characteristics (L* values [whiteness]) of WSN made from PF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

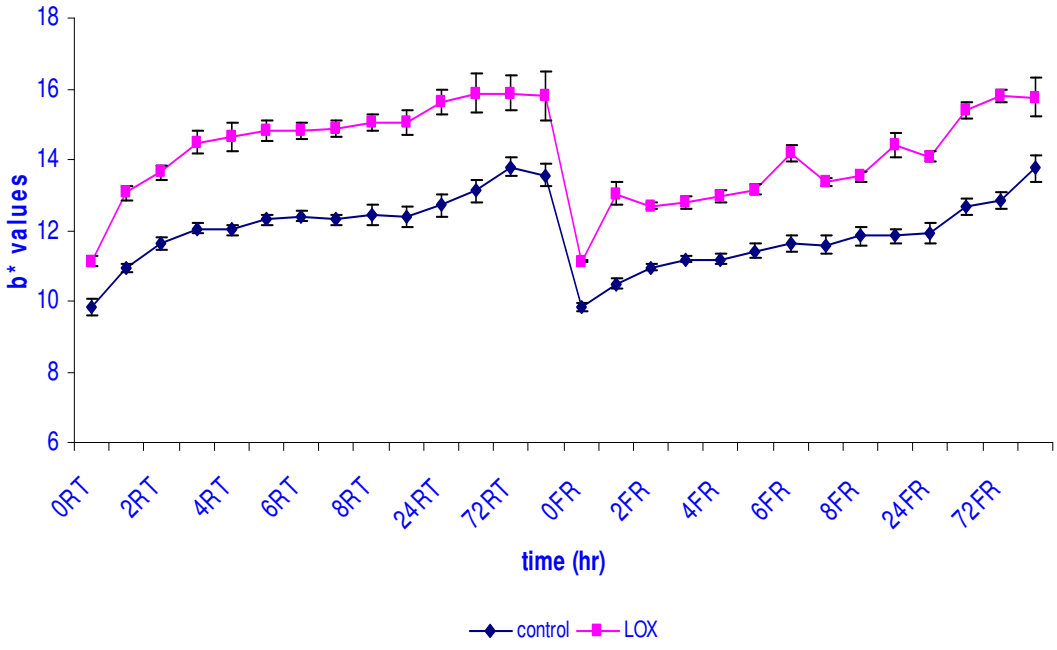


Fig 19.7 **Colour characteristics (b* values [yellowness]) of WSN made from PF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

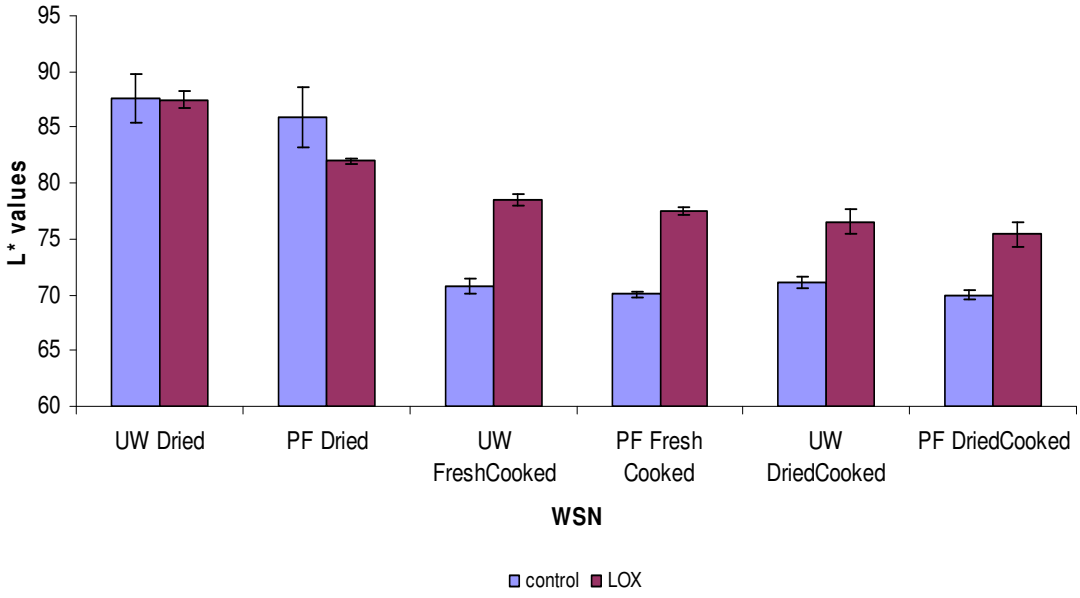


Fig 19.8 Colour characteristics (L* whiteness/ brightness) of dried, fresh cooked & died cooked WSN made from UW & PF
Error bars represent standard deviation values

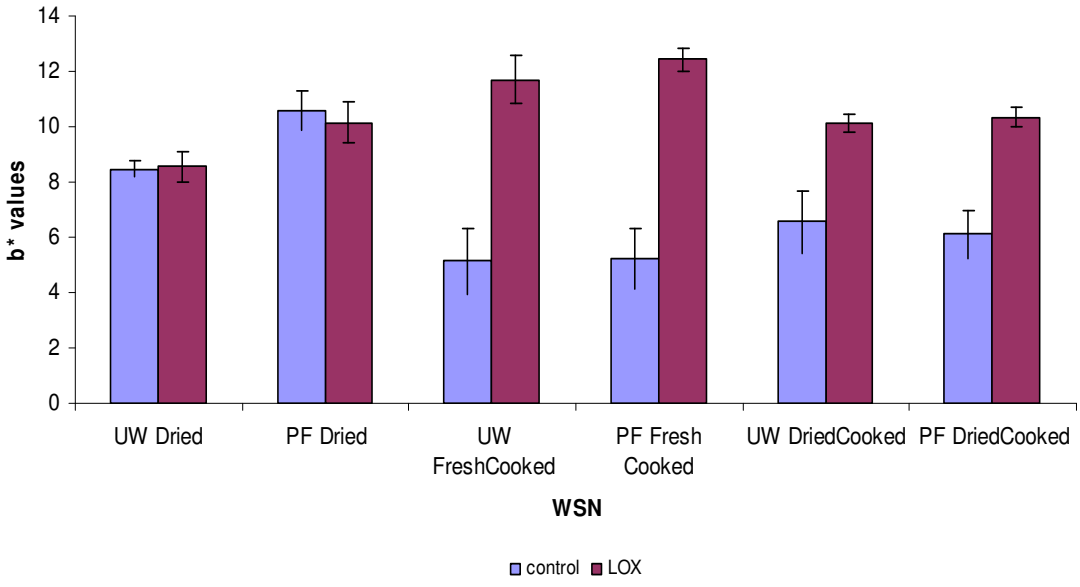


Fig 19.9 Colour characteristics (yellowness) of dried fresh cooked & dried cooked WSN made from PF & UW
Error bars represent standard deviation values

19.6 Structural properties of WSN supplemented with LOX

For all samples of raw noodles, starch granules dominated the appearance of the surface. The control showed compact, lenticular to circular shaped starch granules. When looking at the samples treated with LOX smaller and not as compact starch granules are seen on the surface, clearly showing a more extensive protein network between the starch granules than seen in control samples (Fig 19.10). Upon cooking most of the starch gelatinises and therefore the granular appearance is no longer clearly visible. Some evidence of granular structure remains in the control samples, where relatively even, hollow regions can be seen. The pockets appear to be larger but fewer in number for samples treated with LOX (Fig 19.10).

Similar observations were made for dried (uncooked) WSN. Granular starch dominated the appearance of the surface, the control, however, showing more compact, lenticular shaped granules. The samples incorporating LOX had starch granules which were not as compact on the surface. These also showed more hollow structure between the starch granules (19.11). Cooked dried noodles incorporating LOX preparation also had more open, hollow structure, where all the starch has gelatinised and no granular starch is visible within the protein network (19.11).

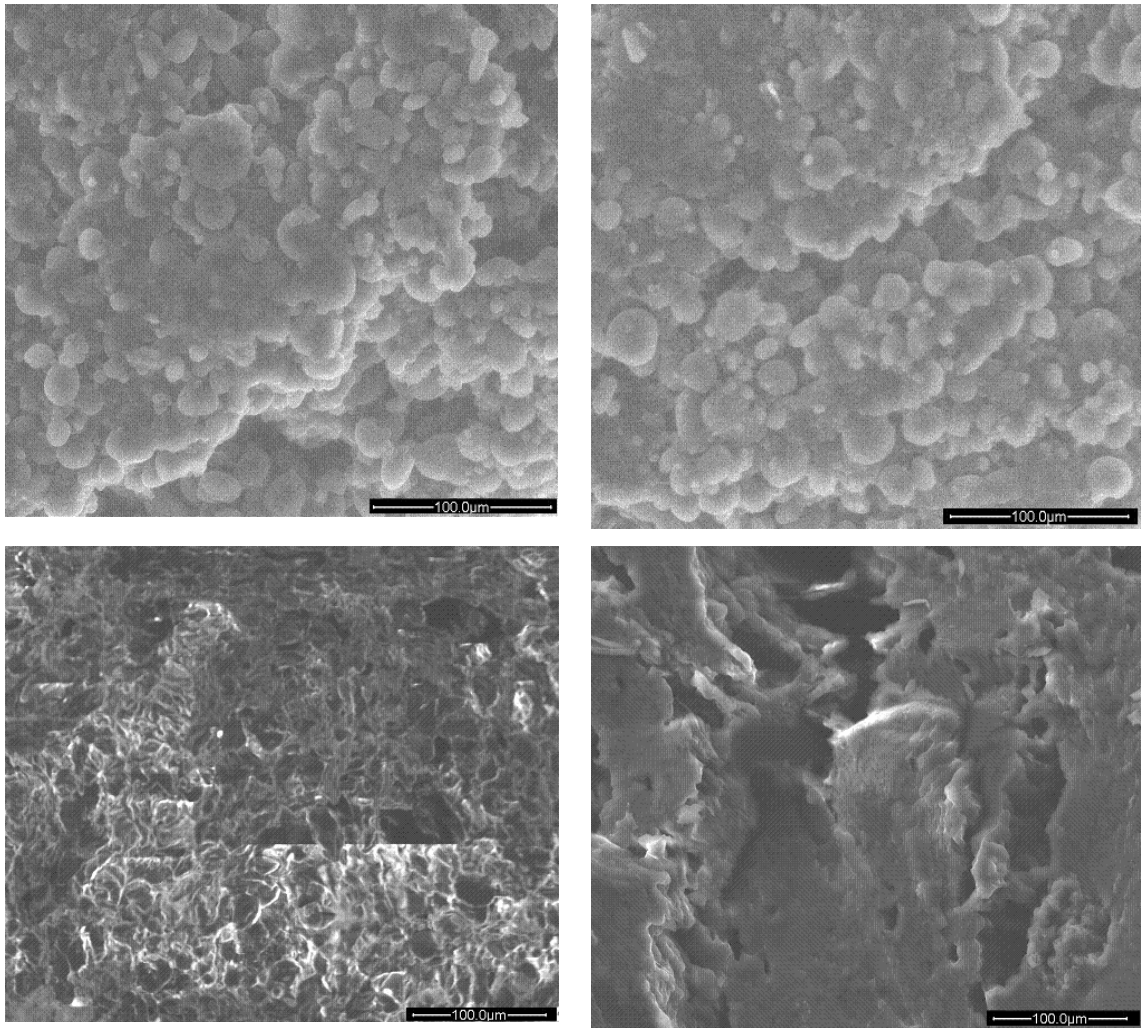


Fig 19.10 **ESEM[a] images of raw WSN [control and LOX treatment, from left to right (top)] & SEM[b] images of cooked WSN [control and LOX treatment, from left to right (lower)]**

a ESEM, 6.0 Torr, 4°C, 400× Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 300× Mag., 5.0 spot size, 30kV

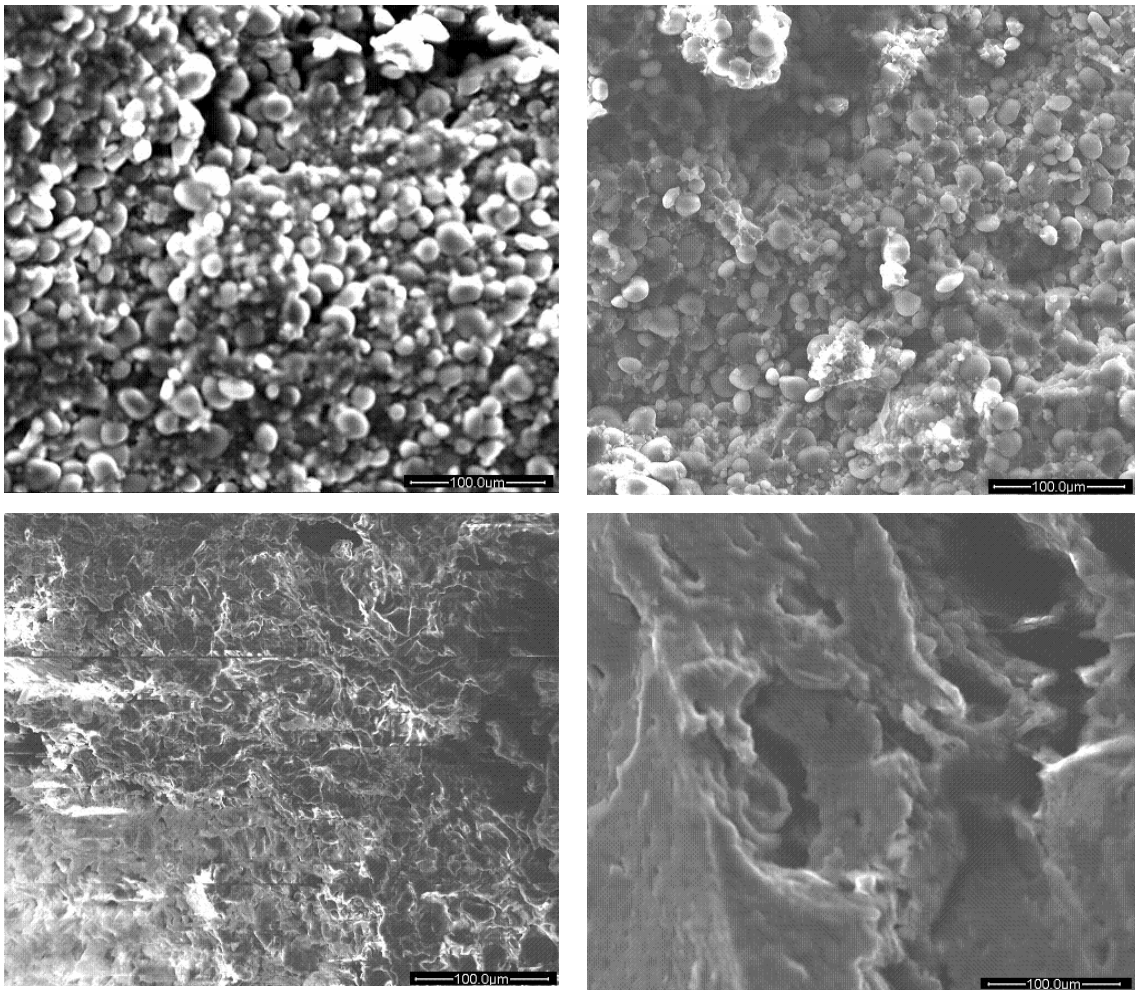


Fig 19.11 SEM[a] images of dried WSN control and LOX treatment (top) left to right and dried cooked WSN control and LOX treatment from left to right (bottom)

a SEM, 0.5 Torr, 23°C, 300× Mag.; 5.0spot size, 30kV

19.7 Summary of results

When a LOX preparation from soybean was incorporated into a WSN formulation, the product firmness and hardness characteristics were only slightly altered, at both levels of addition. The compression or softness of noodles as measured using the flat cylinder (P/45) probe indicated that the LOX addition resulted in slightly harder noodles meaning the noodles are chewier. The measurements on cutting force (relating to the bite characteristics of noodles) assessed using the blade, indicated firmer noodles upon LOX addition. This firmness (or higher force to cut) is also directly related to the chewiness properties of these types of products with the greater firmness corresponding

to chewier noodles. Both of these measurements although measuring different properties are useful to an understanding the complicated meaning of noodle chewiness.

Upon storage of noodles L* values of raw noodle sheets deteriorated for controls and treated samples, particularly at 25°C versus 4°C. It is possible that the changes observed during storage may reflect the presence of enzyme activities other than LOX. Whilst the hypothesis of achieving whiter (brighter) noodle sheets was achieved with LOX addition, means of preventing concurrent discolouration would have to be found. WSN treated with LOX were whiter when compared to the control samples, however, over a period of time became darker at a similar rate to control samples.

The addition of LOX to the WSN formulation did not have any adverse effects upon the cooking quality attributes of WSN. ESEM & SEM images revealed that LOX might have some impact upon starch as more of the granules appeared to be in some way damaged or hydrolysed in both raw and cooked samples treated with LOX. Changes in the structural appearance were seen in samples incorporating LOX, however, these structural changes did not seem to be reflected in the textural data for the noodles.

Chapter 20

Results and discussion: Lipoxygenase and the quality of Asian noodles prepared in the laboratory: Yellow alkaline noodles

The purpose of this chapter is to present results for the quality of YAN to which a lipoxygenase preparation from soy bean was added.

20.1 Introduction

Due to the action of alkaline salts upon the xanthophylls and possibly caratenoids from flour a yellow colour is developed. Over time upon storage of noodles darkening increases and noodles become dark yellow to brown. In the context of the current study, it was hypothesised that the bleaching effect of lipoxygense might have a positive effect upon noodle discoloration and thereby minimise this problem of discolouration. Although the pH of these noodles is relatively high (approx 10.1) lipoxygenase is expected to have an impact since its pH optimum is reported to be relatively high at around 9.0.

In preliminary experiments two different levels of enzyme addition were evaluated (1.85×10^3 and 3.70×10^3 units per batch). The resultant textural properties of the noodles were assessed with the flat cylinder (P/45) probe and the blade attachment of the texture analyser. When the cylinder probe was used for both raw and cooked YAN the higher addition of LOX preparation resulted in noodles which were less hard compared to the controls. However, the opposite effect was found when textural properties were measured using the blade with firmer noodles at higher levels of the enzyme usage (Figs 20.1 & 20.2).

20.2 Levels of LOX in flour and stability during noodle processing

In BGF flour as well as in the PF and UW (compare with Chapter 10) relatively low levels of LOX were recorded. Similar levels were found in the YAN with 26.13 ± 4.12µmol of hydroperoxide formed per minute per gram of sample made from BGF

(Table 20.1). Slightly lower activity of LOX was seen in dried noodles, but no significant losses were seen during processing or upon storage under any of the defined conditions studied. Addition of soy bean enzyme preparation seemed had little impact on LOX activity, since measured activity upon addition of 1.85×10^3 and 3.70×10^3 units per batch was measured to be only slightly higher than in control samples 28.6 ± 3.5 and 29.4 ± 3.3 respectively (Table 20.1).

Table 20.1 Activity of LOX in control and supplemented YAN stored under different conditions

Treatments[a]	**LOX activity**[b]
Controls	
Day1	26.1 ± 4.1
24hr RT	23.5 ± 3.6
24hr FR	23.7 ± 4.6
Dried	21.7 ± 4.2
LOX 1.85×10^3 U / batch added[c]	
Day1	28.6 ± 3.5
24hr RT	28.3 ± 3.7
24hr FR	27.7 ± 4.7
Dried	27.6 ± 5.1
LOX 3.70×10^3 / batch added[c]	
Day1	29.4 ± 3.3
24hr RT	28.8 ± 4.6
24hr FR	28.6 ± 4.5
Dried	27.9 ± 4.3

a Abbreviations are: YAN, yellow alkaline noodles; LOX, lipoxygenase; RT, storage at room temperature; FR, storage at 4°C
b Unit for LOX is μmol of hydroperoxide formed per minute per gram of sample
c LOX added was from Sigma – Aldrich

20.3 The impact of varying levels of LOX on textural characteristics of YAN

In the preliminary assessment of YAN the two levels of LOX addition were evaluated in terms of textural properties of YAN, using the blade and the flat cylinder (P/45) probe. Hardness of YAN (as measured with the P/45 probe) was not significantly different

between controls and the treatments (Fig 20.1). Noodle firmness as measured with the blade showed also no obvious differences between controls and treatments, however, in this case in both raw and cooked samples addition of 3.70×10^3U per batch resulted in slightly firmer noodles when compared to the control and treatment with LOX addition at 1.85×10^3U per batch (Fig 20.2).

20.4 The effect of LOX on textural properties of YAN

Similar findings were seen throughout the storage of noodles under various storage conditions with relatively little differences between the different temperatures of storage conditions (Tables 20.2 & 20.3).

The correlation obtained between the two textural methods (blade vs cylinder), was positive (r^2 0.94), (Fig 20.3).

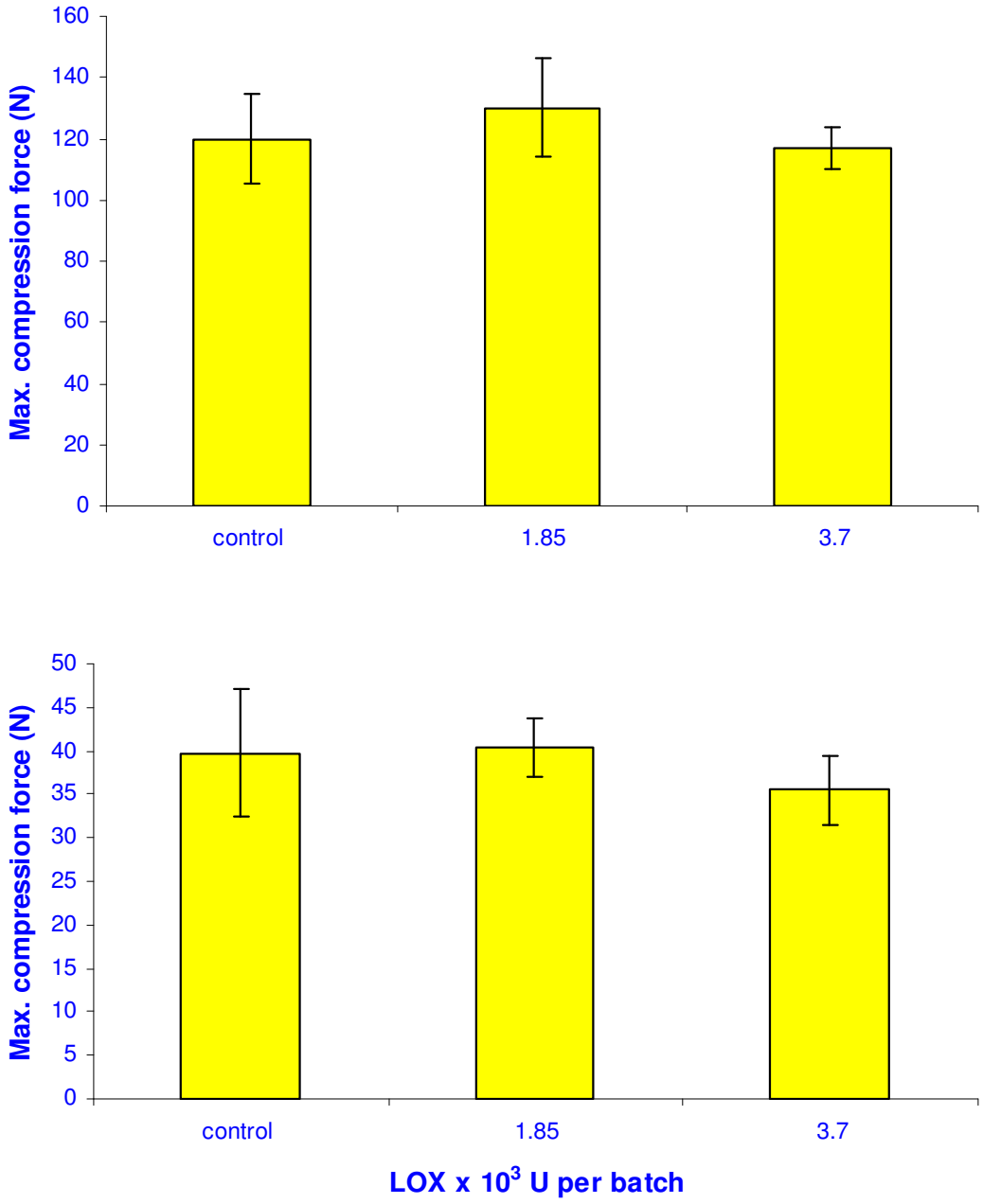


Fig 20.1 **Evaluation of different levels of LOX addition on hardness properties of fresh raw (top) and fresh cooked (lower) YAN**
Error bars represent standard deviation values. Measured using the P/45 flat cylinder probe

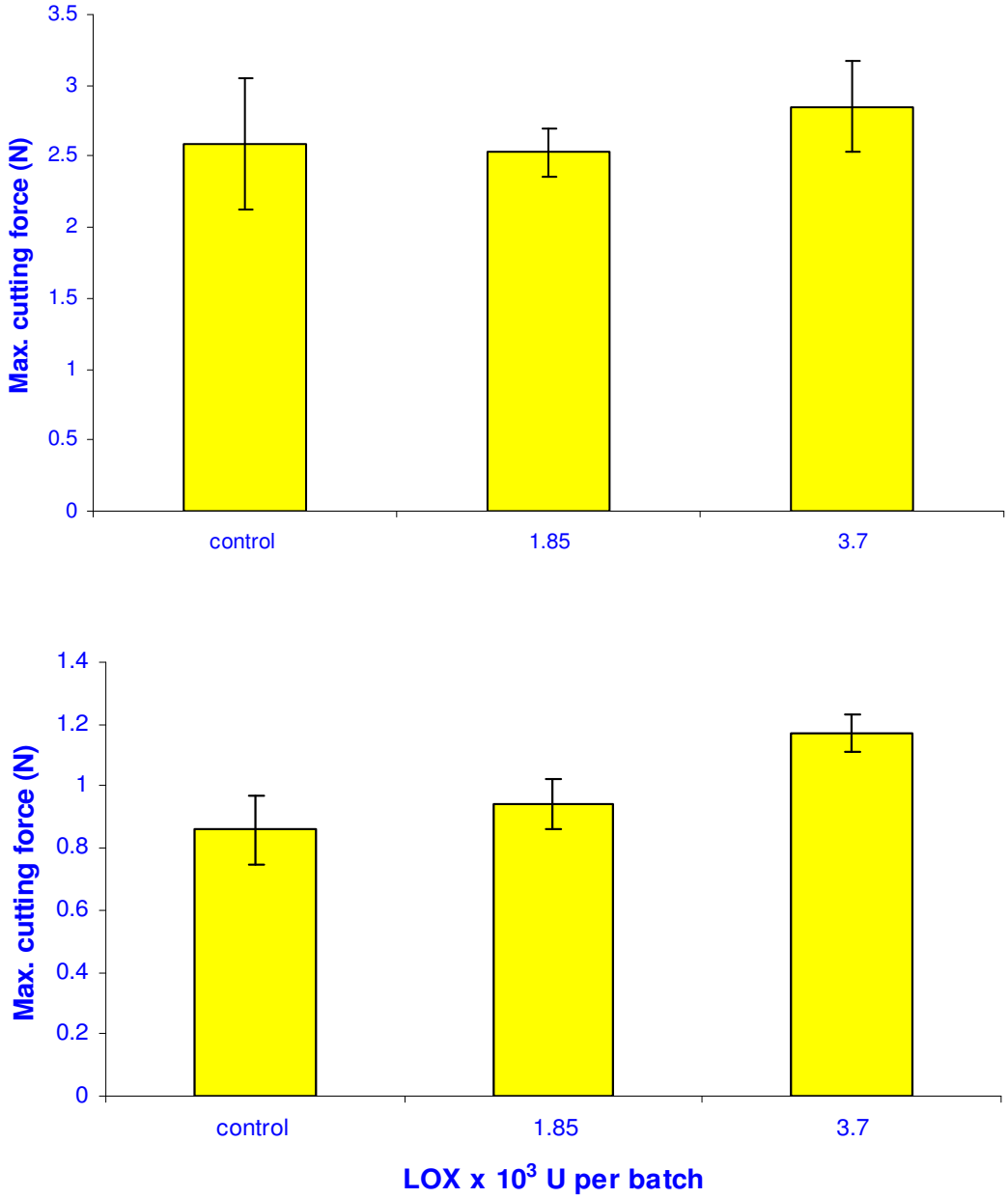


Fig 20.2 **Evaluation of different levels of LOX addition on firmness properties of fresh raw (top) and fresh cooked (lower) YAN**
Error bars represent standard deviation values. Measured using the flat blade attachment

Table 20.2 Effects of LOX (1.85×10^3 U / batch) on the textural properties measured by TA-XT2 for YAN

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	36.1[a]	125[a]	11.0[a]	2.59[a]
	LOX	39.5[a]	130[a]	6.05[b]	2.53[a]
	RT control	40.1[a]	132[a]	12.4[a]	3.01[a]
	LOX RT	40.2[a]	130[a]	6.89[b]	2.79[a]
	FR control	39.5[a]	128[a]	11.1[a]	2.66[a]
	LOX FR	37.9[a]	125[a]	5.85[b]	2.69[a]
Cooked	control	10.6[a]	39.7[a]	3.50[ab]	0.86[a]
	LOX	11.6[ab]	40.4[a]	2.18[ac]	0.94[a]
	RT control	10.7[a]	39.9[a]	4.58[ab]	1.02[a]
	LOX RT	11.9[ab]	40.7[a]	2.45[abc]	1.12[a]
	FR control	13.4[ab]	47.4[a]	5.36[b]	1.11[a]
	LOX FR	14.0[b]	47.0[a]	2.14[ac]	1.05[a]
Dried [b]	control	12.4[a]	42.9[a]	5.84[a]	1.25[a]
	LOX	13.2[a]	43.6[a]	4.65[a]	1.15[a]

a Abbreviations are: YAN, yellow alkaline noodles; LOX, lipoxygenase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

Table 20.3 Effects of LOX (3.70×10^3 U/ batch) on the textural properties measured by TA-XT2 for YAN

Treatment[a]		Hardness (P/45)		Firmness (blade)	
		Total E to compress (N.s)	Maximum compression force (N)	Total E to work (N.s)	Maximum cutting force (N)
Raw	control	35.1[a]	120[a]	11.0[a]	2.59[a]
	LOX	33.7[a]	117[a]	6.79[b]	2.85[a]
	RT control	40.1[a]	132[b]	12.4[a]	3.01[a]
	LOX RT	35.0[a]	121[a]	6.31[b]	2.56[a]
	FR control	39.5[a]	128[ab]	11.1[a]	2.66[a]
	LOX FR	39.7[a]	129[ab]	6.47[b]	2.52[a]
Cooked	control	10.6[a]	39.7[a]	3.50[ab]	0.86[a]
	LOX	15.7[b]	55.5	2.42[ab]	1.17[ab]
	RT control	10.7[a]	39.9[a]	4.58[ab]	1.02[a]
	LOX RT	12.5[ab]	41.4[a]	2.41[b]	1.11[ab]
	FR control	13.4[ab]	47.4[a]	5.36[a]	1.11[ab]
	LOX FR	13.0[ab]	43.3[a]	3.17[ab]	1.36[b]
Dried [b]	control	12.4[a]	42.9[a]	5.84[a]	1.25[a]
	LOX	12.2[a]	41.5[a]	5.65[a]	1.22[a]

a Abbreviations are: YAN, yellow alkaline noodles; LOX, lipoxygenase; (N.s), Newton second; (N), Newton; E, energy; RT, storage at room temperature; FR, storage at 4°C

b Measurements taken after cooking to optimum

Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-cooked-dried)

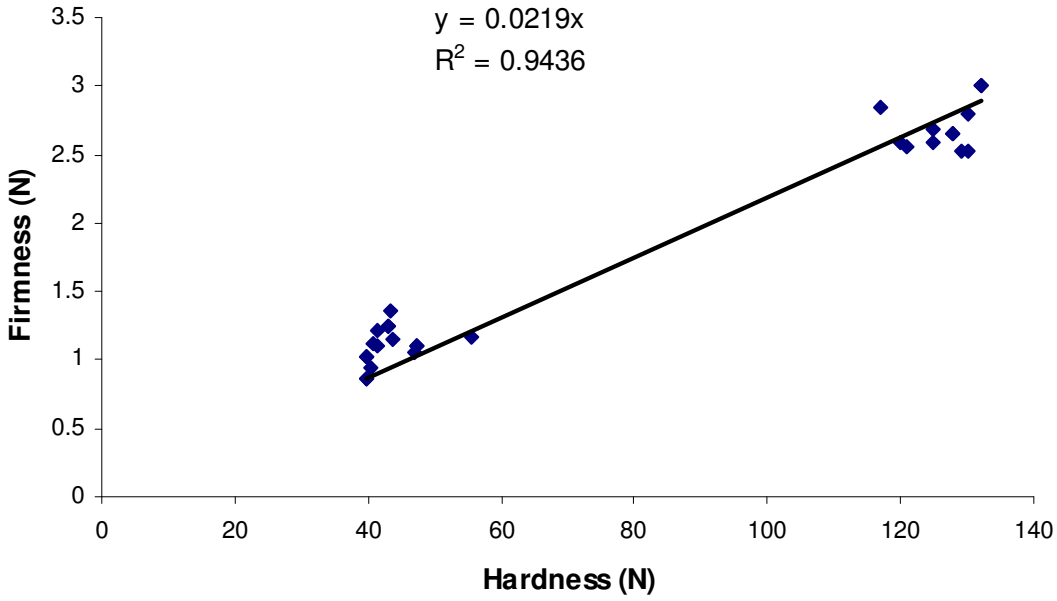


Fig 20.3 Correlation between firmness and hardness measurements of texture
Data based on the mean values for firmness and hardness measurements of YAN (made from BGF, controls and LOX treatments)

20.5 The effect of LOX on cooking yield

Cooking characteristics were determined by comparing the yield lost upon cooking and subsequently by comparing the water uptake during cooking (Table 20.4). For fresh noodles, addition of 3.70×10^3U of LOX resulted in higher loss (12.9 ± 1.2) compared to controls (9.63 ± 0.02). For dried noodles, slightly higher loss occurred in noodles supplemented with 1.85×10^3U (13.1 ± 0.2) of LOX per batch compared to 12.7 ± 0.6 for control.

20.6 Relationship between LOX and colour properties of YAN

In all samples discolouration of noodles sheets was observed and this including controls where no enzyme had been incorporated (Figs 20.4 & 20.5). It was also observed that there was no development of speckiness in any case. Formulations incorporating LOX darkened less, and at a slower rate when the level of addition was 3.70×10^3U. Furthermore, noodles incorporating LOX were whiter all times when compared to the control samples (Fig 20.4). Unexpectedly higher b* values were observed for LOX treatments (Fig 20.5). It is noted that the same effect was observed for WSN (Chapter 19). Darkening occurred at a faster rate and was observed earlier in noodles stored at

25°C as compared to those stored at 4°C. Control samples were light yellow and darker yellow respectively at time zero and, after 96 hours. On the other hand LOX treatments were initially a clear, bright yellow but gradually lost clarity and yellowness during storage.

Table 20.4 Cooked weight and cooked loss of YAN made from BGF flour with and without LOX addition

Sample[a]	Cooking time (min)	Cooked weight (% increase)	Cooking loss (%)
fresh control	4.30	58.7[a]	9.63[a]
fresh LOX 1.85×10^3U	4.30	59.1[a]	8.77[a]
fresh LOX 3.70×10^3U	4.30	59.6[a]	12.9
dried control	9.00	72.1	12.7[a]
dried LOX 1.85×10^3U	9.00	66.2[a]	13.1[a]
dried LOX 3.70×10^3U	9.00	65.2[a]	12.2[a]

a Abbreviations are: YAN, yellow alkaline noodles; BGF, Baker's strong flour
Note: Means followed by the same letter are not statistically different at $p<0.05$ within the same column and treatment (raw-dried)

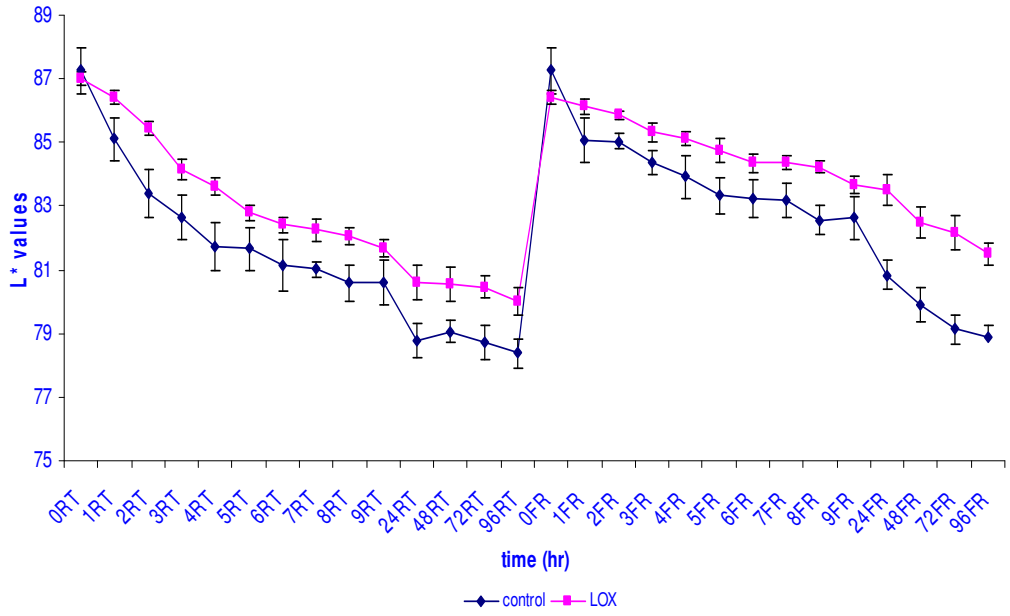


Fig 20.4 **Colour characteristics (L* values [whiteness]) of YAN made from BGF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

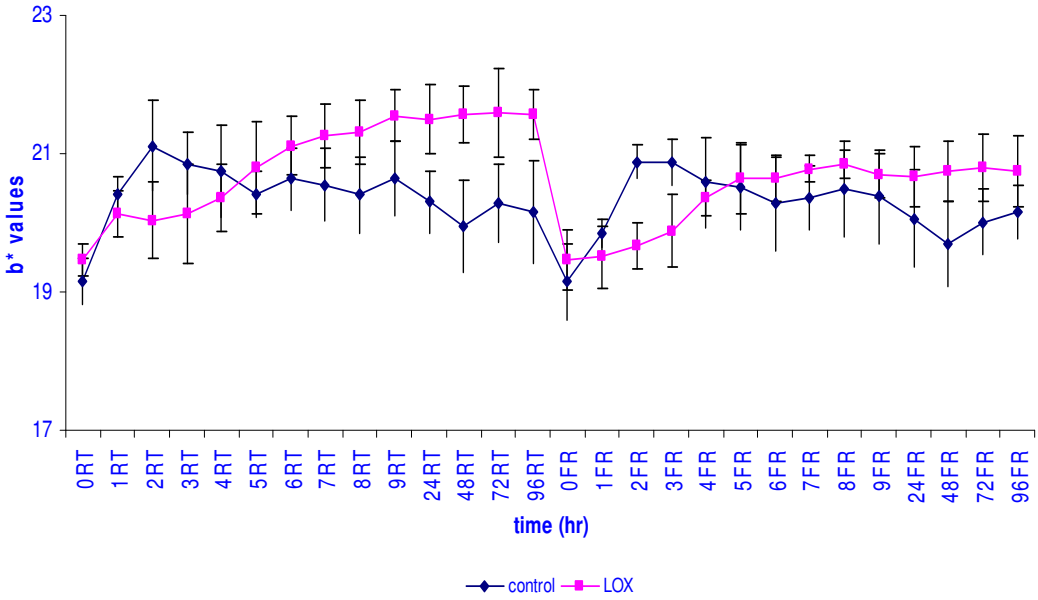


Fig 20.5 **Colour characteristics (b* values [yellowness]) of YAN made from BGF stored at 25°C and 4°C**
Error bars represent standard deviation values
RT, Room Temperature; FR, 4°C storage

When noodles were dried at 40°C for 30hr and this procedure was commenced immediately after preparation of the fresh noodle sheets, differences in colour were minimal. Slightly higher L* values were recorded in noodles supplemented with LOX, while there were no differences in b* values between LOX treatments and control samples (Figs 20.6 20.7).

For noodle samples that were cooked immediately after preparation, only minor differences in colour were seen (Figs 20.6 & 20.7). Slightly higher L* values were recorded for noodles incorporating LOX, while again b* of LOX treatments were significantly higher than in control samples.

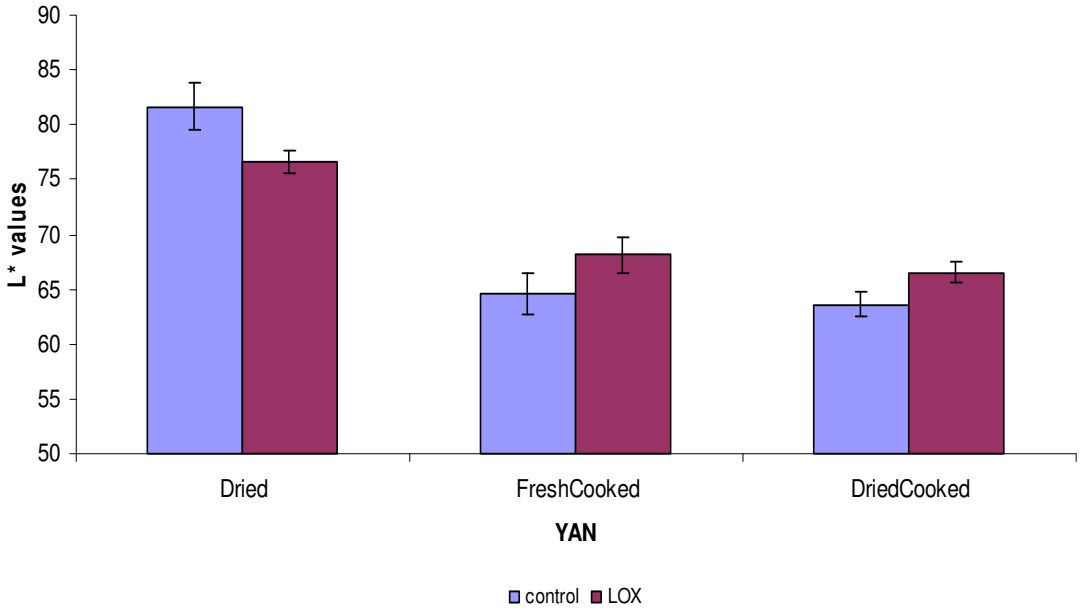


Fig 20.6 **Colour characteristics (L* whiteness / brightness) of dried, fresh cooked & dried cooked YAN made from BGF**
Error bars represent standard deviation values

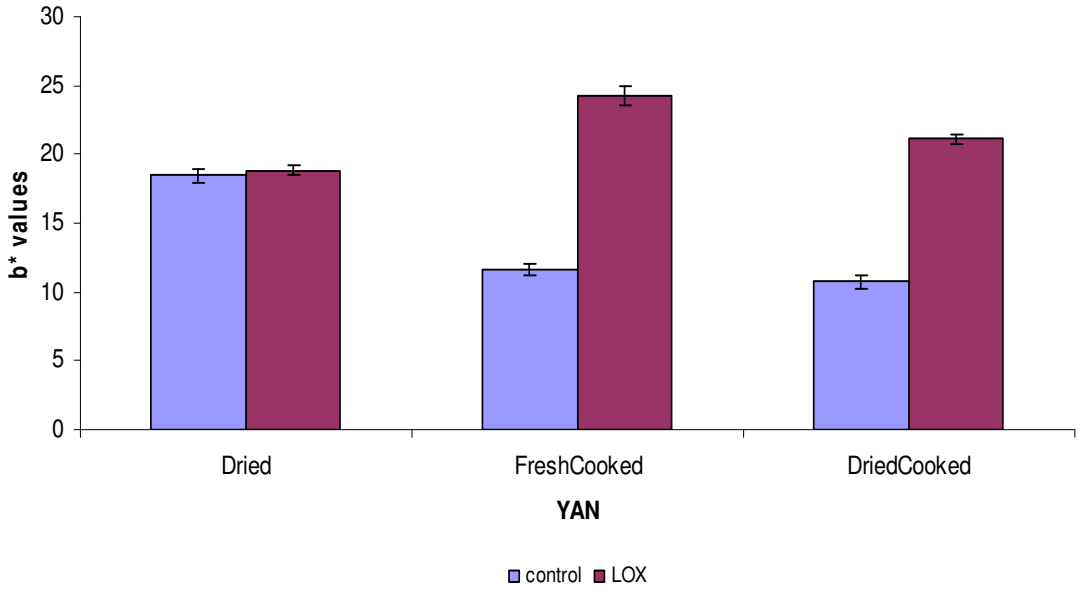


Fig 20.7 **Colour characteristics (b* yellowness) of dried, fresh cooked & dried cooked YAN made from BGF**
Error bars represent standard deviation values

20.7 Structural properties of YAN supplemented with LOX

The surface structure of raw noodles was studied using the ESEM mode of an electron microscope. In the case of raw noodles the appearance of control the typical patterns seen throughout this study. Similar observations were made for YAN incorporating the LOX preparation where slightly more open structure was seen and there was a gel network between the clearly visible circular starch granules. Relatively little protein network is visible on the surface of raw YAN (Fig 20.8). Upon cooking most of the starch gelatinises and therefore no granular structure remains visible. Hollow pockets of gluten can be seen in the control, while some larger empty pockets between the protein network are seen in noodles incorporating LOX preparation (Fig 20.8).

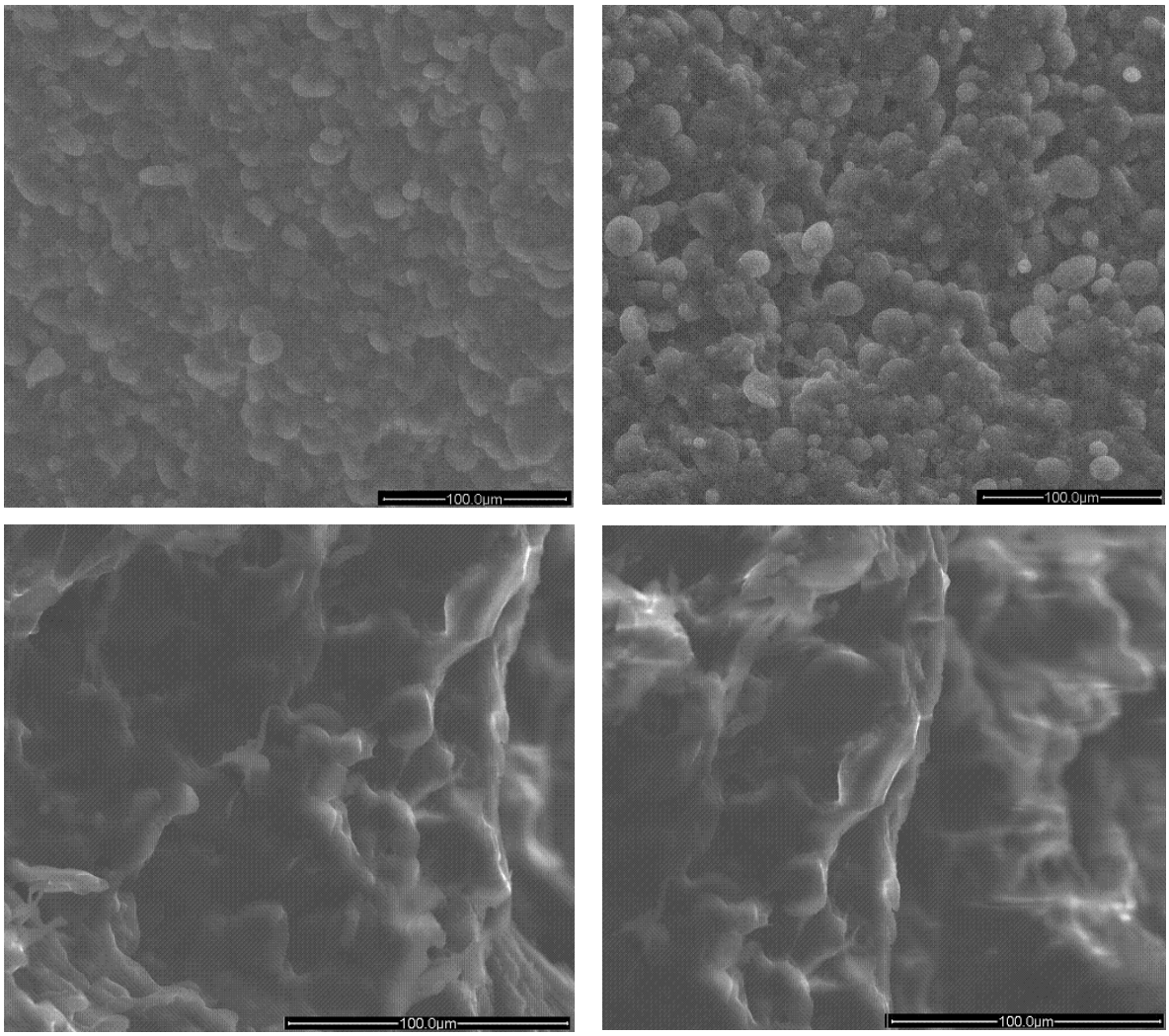


Fig 20.8 ESEM[a] images of fresh raw YAN (top) and SEM[b] images of fresh cooked YAN (lower) made form BGF flour; control and LOX treatment (from left to right)

a ESEM, 6.0 Torr, 4°C, 400× Mag., 5.0 spot size, 30kV
b SEM, 0.5 Torr, 23°C, 600× Mag., 5.0 spot size, 30kV

For dried YAN in both control and when LOX preparation was incorporated, compact granular starch dominates the surface appearance (Fig 20.9). Starch granules in both samples appear circular in shape and not damaged or gelatinised. The images of cooked dried YAN on the other hand show a greater difference between the two. The control YAN appears to show gelatinised starch embedded within the protein network and some granular form of starch remains visible and there are even pockets on the surface. In contrast, the noodles incorporating the LOX preparation appear more hollow, all of the starch appears gelatinised with much more open structure in between the gelatinised starch and the protein network (Fig 20.9).

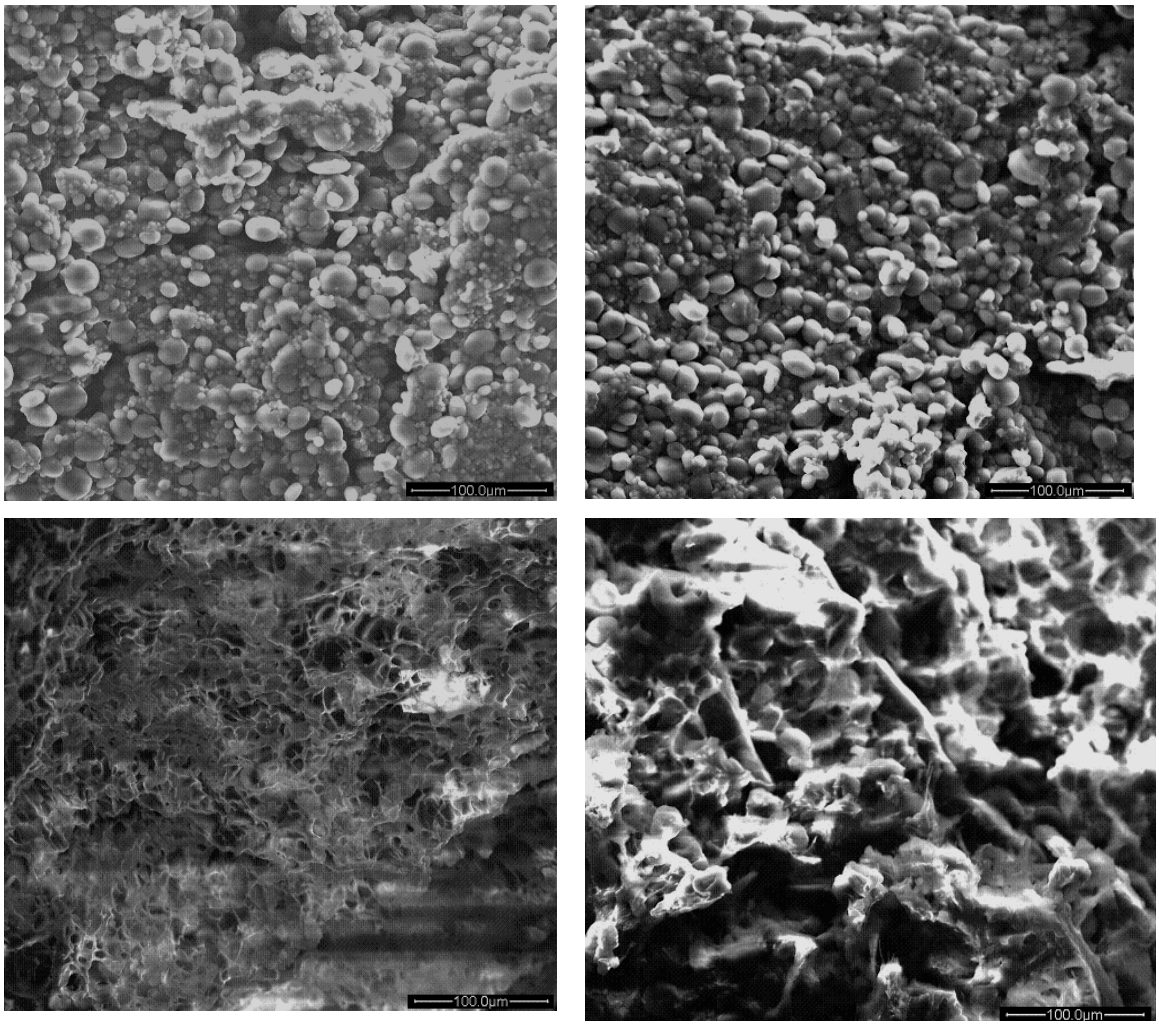


Fig 20.9 SEM images of dried[a] YAN (top) and dried and cooked[b] YAN (lower) control and LOX treatment (from left to right)

a SEM, 0.5 Torr, 23°C, 300 × Mag., 5.0 spot size, 30kV

b SEM, 0.5 Torr, 23°C, 300 × Mag., 5.0 spot size, 30kV

20.8 Summary of results

The exogenous LOX used in this study was reasonably stable during YAN processing and storage, although the activity levels recorded in the noodles were slightly lower than those found for WSN (Chapter 19). This effect was observed for both the naturally occurring LOX as well as the LOX preparation from soy bean added to the formulation. This is probably due to the higher pH conditions of YAN as compared to the WSN.

The two methods used to evaluate textural properties of noodles showed no differences between control and YAN incorporating LOX preparation. The data indicate that both of these methods are useful tools to evaluate textural properties of cooked and dried and cooked YAN.

On visual assessment, slightly whiter YAN were observed when LOX was added into the formulation. However, the differences measured instrumentally were not significantly different. Samples also discolored over a period of time as did control samples. The effect was more obvious and happened at a faster rate in noodles stored at 25°C as compared to the 4°C storage. Surprisingly and not easy to explain was the observation that all YAN incorporating LOX preparation clearly showed higher b* values indicating greater yellowness. These observations confirm the trend seen in WSN (Chapter 19).

Control noodles and YAN incorporating the LOX preparation were very bright in appearance and there were no specks present. Once the noodles were cooked, dried or dried and cooked there were no differences observed using either visual inspection or instrumental assessment of colour. The preparation of LOX added to the YAN formulation did not adversely impact upon the cooking characteristics of YAN.

Some minor differences were seen in structural appearances of YAN using electron microscopy. These were more obvious in cooked and dried and cooked noodles, where samples incorporating LOX preparation where the structure had a more open and hollow appearance.

Chapter 21

General discussion and conclusions

The purpose of this final chapter is to summarise the results obtained during the current study, draw final conclusions and make recommendations for further research into enzymes and Asian noodles.

21.1 Introduction

A survey of the scientific literature undertaken during the early stages of this study indicated that the enzymes selected for investigation here are currently either added to bakery formulations for loaf breads or are typically involved during the baking processes for loaf breads. In the study of enzymes during the manufacture of noodles, it might prove difficult to compare enzymes to each other even if they coming from the same source due to the different assays available and different units used in the presentation of activity data. Available information indicates that low levels of some of these enzymes might cause undesirable effects upon the quality characteristics of pasta and noodles. There is research available indicating that the most obvious undesirable impacts probably relate to the action of amylases and polyphenol oxidases. The investigation here has focused on Asian noodle products which are a major staple food globally. The issue of the enzymes naturally present in flour is explored along with the practicality of addition of exogenous enzymes to the two types of Asian noodles studied here, WSN and YAN.

The results described in this thesis fall into five broad areas. These are:

1. Selection and validation of enzyme analysis procedures
2. Analysis of wheat flour samples
3. Studies of the stability of the enzymes in Asian noodles prepared in the laboratory under controlled conditions
4. Studies of the effect of endogenous and exogenous enzymes on the quality attributes of Asian noodles

5. Evaluation and application of textural and scanning electron microscopy methods used for these types of products.

The results for each of these is now reviewed as a basis for presenting the primary conclusions of this project and a discussion of areas recommended for further research.

21.2 Selection and validation of enzyme assays

Whilst method development was not the primary emphasis of this project, considerable effort was made to select and validate suitable methods for enzyme assays. Firstly, various published methods which had potential for the assay of enzymes in wheat and wheat products were evaluated.

For the measurement of each of the individual enzymes (α-amylase, lipase, LOX, AAO, and POX) one procedure was selected and investigated. Each was thoroughly evaluated in terms of repeatability and comparison to the data previously published (where possible). In each case this necessitated the comparison of extraction procedures and the results clearly show that sample extraction and treatment protocols are very important to the reliable determination of each of the enzymes studied.

One of the conclusions from the evaluation of the enzyme assays was that the published procedures required adaptation and optimisation in order to obtain the most useful results for the particular samples being studied here. Another is the desirability of the adoption of standard international units so that enzyme activities can be compared more readily. Whilst there is an ongoing requirement to review and refine analytical methods, the procedures adopted for the current study gave reasonably reliable results and facilitated the thorough investigation of the enzymes and their stability reported here.

21.3 Analysis of wheat flour samples

A series of wheat flour samples were assayed for various enzyme activities during the course of this study. The purpose of these analyses was three-fold: firstly to ensure the validity of the extraction and analytical methods for flour and flour-based foods; secondly to allow comparison with published data for flour, and thirdly as a basis of

comparison for the various noodle products made from the flours during the subsequent phases of the study.

In considering the significance of the results for flours and their application in noodlemaking, it is likely that some of the variability in the results for the different flours reflects differences in milling extraction rates. It is known that each of the enzymes tends to be concentrated in the outer layers of the grain, primarily the bran. In addition, lower flour extraction rates are typically preferred for noodle making.

21.4 Studies of the stability of the enzymes in Asian noodles prepared in the laboratory under controlled conditions

Initially, procedures for preparation of each of the two styles of Asian noodles in the laboratory were set up and used under controlled conditions. These were selected to reflect formulations and processes widely used in commercial manufacture. It must, however, be recognised that considerable variation can be found in the practices adopted between different countries, between specific regions within countries and even individual manufacturers.

In this study, noodles were made from commercial flours and in many of the experiments the formulation included exogenous enzymes. It is noted that the levels of addition were selected following the assay of the endogenous activities in the flours. In selecting the level of addition, the starting point was to add sufficient of the particular endogenous preparation of enzyme so that the total activity in the product was double that occurring in the original flour sample.

It is noted that during the course of this research various flours were used and that some variations were found from batch to batch. In all cases appropriate precautions were taken and blank treatments used to ensure the validity of the results. The primary purposes of this phase of the study have been to assess the effects of the enzymes, along with the impact of storage.

The relative losses of enzyme activities during processing are summarised in Table 21.1. Of those studied, the enzyme demonstrating the least stability was POX for which the losses occurred in both types of noodles. Higher α-amylase losses were expected for

YAN due to the higher pH values and hence lower enzyme stability. However, no losses were found for either style of noodles. Some decline was recorded for LOX but only for WSN. The optimum pH reported for cereal LOX is around 9 thus this might explain greater LOX stability for YAN compared to that observed during processing of WSN.

Table 21.1 A comparison of relative losses of endogenous enzyme activities during processing of the two styles of Asian noodles

	α-amylase	LOX	POX	AAO	Lipase
WSN	No loss	15.5	21.6	No loss	No loss
YAN	No loss	No loss	12.7	No loss	No loss

Notes
1 Based on data from Tables: 10.1, 10.2, 10.4, 10.5, 10.6, 10.7, 10.9, 10.10, 10.12 & 10.13
2 All relative loss values are expressed as percentages compared to the levels of the endogenous enzymes in the flours analysed

White salted and yellow alkaline noodles are often dried after preparation and for this step enzymes were particularly stable, except POX, where again high losses have been recorded (Table 21.2). In assessing the factors which might be used to explain the results for each enzyme, there is a considerable body of literature concerning the stability including pH and temperature as the two main factors. The results reported from the current study can be explained in terms of the pH characteristics of the enzymes and the pH conditions existing in the two styles of noodles.

21.5 Studies on the storage of Asian noodles prepared in the laboratory

It was decided to further investigate the loss of selected enzymes during short term storage. Noodles were stored for up to 24hr at either 25°C or 4°C. Noodles were also dried at 40°C for 30hr. Different patterns of loss were observed for the various combinations of enzymes, noodle style and storage conditions (Table 21.2):

- The decline in AAO activity was quite marked in some cases, reaching thirty percent for noodles dried at 40°C.
- Moderate losses were found for all cases of the enzyme POX for both WSN and YAN.
- In contrast, relatively low losses were found for other enzymes studied.
- In most instances losses were higher for YAN than WSN.

- Drying at 40°C for 30hr caused higher enzyme losses compared to storage at 25°C and 4°C.

Table 21.2 A comparison of relative losses of the enzymes in WSN and YAN during short term storage

Storage conditions	α-amylase	Lipase	LOX	POX	AAO
YAN					
4°C	No loss	No loss	No loss	11.9	23.6
25°C	No loss	No loss	No loss	13.2	19.0
40°C	26.4	18.6	0.46	17.9	34.9
WSN					
4°C	No loss	1.7	No loss	6.2	0.69
25°C	No loss	No loss	0.89	2.3	6.7
40°C	18.5	43.1	4.7	4.3	29.0

Notes **1** Based upon data presented in Tables 10.2, 10.5, 10.7, 10.10 & 10.13
2 All relative loss values are expressed as percentages compared to the levels of the enzymes in the laboratory made noodles at the time of preparation

21.6 Studies on the textural properties of Asian noodles prepared in the laboratory

Many studies over the years have reported on the textural properties of cooked noodles measured either using the Instron Universal Testing Machine or various other texture analysers which operate in a similar way by compressing or cutting a sample with some form of probe or blade attachment (Rice and Caldwell 1998; Sasaki et al 2004; Park and Baik 2004; Acone et al 1999; Janto et al 1989; Yu and Ngadi 2004; Ross et al 1997; Miskelly 1984; Miskelly and Moss 1985; Oh et al 1983; Oh et al 1985a; Rho et al 1988; Martin et al 1998; Kovacs et al 2004; Edwards et al 1993). In the literature review no indications or discussions were found for the textural characteristics of raw, uncooked noodles. Although cooked noodles are the form in which consumers are going to consume the product, if means can be found to measure textural properties of uncooked noodles these can be used as an indication or predictive measure of the textural quality in the final, cooked product.

This study included a comparative evaluation of two textural methods. For this the textural properties of raw, cooked and dried as well as cooked noodles were measured. When cylinder and blade data were compared for the cooked and dried and cooked noodles the results were positively correlated. However, evaluation of textural properties of raw noodles requires further research, and possibly evaluation of different attachments. Based upon the observations made during the current study, consideration might be given to a device that would hold raw noodles so that they do not move during the measurement to prevent bending of noodles and therefore giving false or imprecise readings.

In addition further investigations relating the results of instrumental measurements and sensory evaluation are proposed, as often it is not known what level of softness or hardness is preferred by particular groups of consumers including those within specific regions. The addition of enzymes generally resulted in softer or harder noodles compared to the controls, and data from sensory panel testing would be needed to evaluate the desirability and acceptability of the products.

21.7 Studies on the structural properties of Asian noodles prepared in the laboratory

Although there have been relatively few studies of structural properties of noodles or pasta products, most previously published research has utilised SEM in conjunction with some form of coating to examine the surface appearance of these products (Wu 2002; Moss et al 1987; Hensen and Brismar 2003; Shelke et al 1990; Dexter et al 1979). In the current study, SEM was used for dried noodles (without sample preparation), cooked as well as dried and cooked noodles (noodles were freeze dried prior to evaluation) and ESEM was used for fresh noodles (without any requirement for sample preparation). In preliminary experiments various SEM modes were evaluated at high and low pressure, and the structure of noodle samples was evaluated with and without the application of coating treatments. In addition, the Cryo-mode of an SEM was evaluated and images compared (Chapter 9).

Due to the clearer images obtained and also the relative ease of sample preparation, SEM and ESEM without the application of any coating was selected as the most effective approach. Therefore for the investigation of all experimental enzyme

treatments these were used for all samples. It is noted that for freeze dried samples there still remains the issue that it is not always clear if any changes in the structural appearance are due to the enzyme treatment or to the freeze drying procedure. In the latter case removal of water from the sample causes some shrinkage and may change the appearance. None-the-less, in this study it was found that SEM and ESEM provided useful tools for comparison of treatments. The resultant images were generally very clear and provided interesting observations complementing the other instrumental analyses and measurements obtained.

21.8 Studies on the colour characteristics of Asian noodles prepared in the laboratory

The majority of studies undertaken on Asian noodles have focussed on the colour properties of these products. Recent research has concentrated on improving colour and colour stability of Asian noodles as well as identifying which flour components, processing steps or ingredients have adverse effects upon colour and colour stability (Mares and Campbell 2001; Moss et al 1986; Kruger et al 1992; Morris et al 2000; Kruger et al 1994a; Bhattacharya et al 1999b; Baik et al 1995b; Miskelly 1984; Kruger et al 1994b; Corke et al 1997; Borrelli et al 1999; Zhang et al 2005).

In the current study, colour properties of WSN and YAN were studied under different storage conditions in order to thoroughly evaluate the impact of the various enzymes. As expected, the results obtained in this study confirm that temperature is an important factor in discolouration of noodle sheets. Products made from the three flours evaluated here (PF, UW and BGF), had good initial colour and brightness, however, darkened during storage. This occurred at a faster rate and was more prominent at 25°C compared to 4°C. Refrigeration suppressed the process of discolouration. None of the enzyme preparations evaluated here appeared to be able to have a substantial impact of enhancing colour or colour stability of Asian noodles. Generally darker noodle sheets were obtained when enzymes were added to the noodle formulation, and furthermore there were no strong positive impacts on colour of any treatment.

It is recognised that the level of purity of the enzyme preparations may explain some of the observations. The may have been other activities present impacting upon the attributes of noodles prepared during the study. The enzymes were not specifically

selected as having been highly purified. The reason for this was that the practical application of preparations in commercial production of Asian noodles would probably include at least some other activities and highly purified enzymes would be very expensive unless new production approaches became available.

21.9 Major conclusions

The final conclusions of this study are summarised here:

1. In the selection and validation of suitable methods for analysis of Asian noodles, it is important to ensure that suitable sample preparation and extraction procedures are applied for each of the individual enzymes.

2. The activities of the enzymes in the three different wheat flours were found to be quite similar, and the levels of endogenous enzymes were relatively low except in the case of peroxidase.

3. Based upon studies of Asian noodles made under laboratory conditions it was found that generally the enzymes other than peroxidase showed either no loss or relatively minor losses during processing while slightly higher losses of enzyme occurred upon drying compared to the other two storage temperatures.

4. All of the enzyme preparations resulted in discoloration of noodle sheets, with the most adverse effect on noodle colour attributes seen when peroxidase as well as lipases from wheat germ and fungal sources were added to noodle formulations.

5. The cooking quality of both WSN and YAN was most adversely affected when bacterial amylase was incorporated into the noodle formulation. Significant loss in noodle yield was also recorded when POX was added to the formulation of dried noodles.

6. The action of α-amylase is not the primary factor influencing texture of WSN and YAN prepared from flour milled from sprouted wheat.

7. Textural properties of WSN and YAN as measured here using the cylinder and blade attachments of a TA-XT2, showed positive correlations when firmness was correlated against hardness for cooked as well as dried and cooked noodles. However, the correlations were less strong for raw noodles, indicating a need for further investigations into the application of compressive methods and different attachment options for the evaluation of Asian noodle characteristics.

8. The structural properties of WSN and YAN were adversely affected upon addition of bacterial amylase to the formulation while other enzymes had minor effects upon structural properties of cooked and dried and cooked noodles.

21.8 Possible areas for future research

This study has concentrated on two particular styles of Asian noodles and on selected enzymes. There are a number of issues which remain to be resolved and warrant further study. These are briefly described here:

It would be of value to extend the approach used here to other flour and cereal grain foods. One specific example is of Asian steamed products e.g. steamed breads which represent a staple food in various parts of Asia. As with noodles, these products are popular and also vary widely in formulation, process and have substantial regional variations in consumer preferences. Again the clarity, uniformity and colour of the surface are of significance to the appeal of the product.

Of greatest immediate interest would be instant noodles which have some similarities with the products studied here in terms of both formulation and processing approaches. The term instant noodles refers to a group of related products which are becoming increasingly popular at least partly due to the convenience for the consumer. Less research into these foods has been reported in the scientific literature and there is certainly potential for enzyme preparations to provide enhanced quality attributes.

In the investigation of enzyme effects in food products, there are a series of considerations regarding the level of purity and the sources of enzymes. One of the primary decisions made in the studies reported here was to use commercial enzyme

preparations. Whilst the preparations had been partially purified, it was not the purpose in the current study to purify the enzymes of particular interest. The results at least partially reflect the activity of interest, although it is acknowledged that other enzymes may have been present and influenced the quality attributes that were evaluated. Further studies into the impact of highly purified enzymes would be of interest as this may clarify the effects of the individual enzymes.

A further aspect of the impact of enzymes in foods is the influence of enzymes having the same activity but which have been obtained from distinct biological sources. In the case of some of the enzyme preparations studied here, only one preparation was used or preparations from the one source. In the cases of lipase and α-amylase, a variety of sources, including both plant and microbial, were utilized. The results confirmed the general observation that there are some differences in the impact of the same enzyme obtained from a differing species. Some of the results obtained in the current study are explained in terms of the pH and thermal stability properties of the enzymes from the various sources. In this context there is a strong basis for further studies that would consider other sources of enzymes. On specific example is the lipase group of enzymes where there are a range of recently released commercial products which appear to vary in terms of either substrate specificity, or in the nature of the products of hydrolysis.

The other broad area which might be considered is that of looking at enzymes that were not included amongst those reported upon here. There are a range of enzymes present in wheat flour which may have some significance for product quality, along with others which have potential for use as processing aids during processing of wheat based foods.

Another significant issue which relates to the involvement of enzymes in product quality is that of sprouting. Although not central to the current research, the results reported here have been interpreted as demonstrating that the enzyme α-amylase may not be the primary cause of the problems encountered in the utilization of flour from sprouted wheats for noodle manufacture. The tests routinely used in the classification and segregation of wheat for the identification of grain that has been subjected to pre-harvest sprouting are based upon the action of amylolytic enzymes. In order to enhance our understanding of the significance of sprouting and the most appropriate uses of sprouted grain, it is recommended that further studies be pursued. These should focus

on those enzymes which might impact upon both colour and textural attributes of the various styles of Asian noodles. It may also be useful to investigate the reasons behind the observations that durum wheat processing and products are less sensitive to the presence of sprouted grains than are Asian noodles.

In addition another enzyme which was not directly study here is PPO. This has been studied in considerable detail in relation to the colour and colour stability of noodles. Its role in discolouration of noodle products is more or less well understood. However, the issue of colour and colour stability still remains. Thus further research is warranted to achieve further reduction of PPO in wheat grain, to understand genetic and environmental factors responsible for non-PPO darkening and the relationship between ash and colour. In addition further research might usefully consider any interactions between PPO and other enzymes including peroxidase and lipoxygenases. The distribution within the grain and the involvement of other chemical components including enzyme substrates may assist us to understand the complex factors influencing the visual appearance of noodles, and possibly other products as well.

In conclusion, there have been rapid developments in our knowledge over recent years in the areas of noodle processing and packaging as well as the factors that influence their appearance and consumer appeal. The research reported here is the first systematic investigation of a series of enzymes and their effects on these foods. It is hoped that this work might form the basis of further studies of Asian noodles and related foods, ultimately leading to the enhancement of their quality attributes and at the same time might contribute to the ongoing enjoyment of these products.

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Note: The referencing style adopted in this thesis is that recommended for the journal Cereal Chemistry, by the publishers, the American Association of Cereal Chemists (AACC). The format is that provided in the instructions to authors at www.aaccnet.org. and accessed 12 June 2005.

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