The Cause of Bitter Flavour Development in Toasted Rolled Oats (*Avena sativa* L.)

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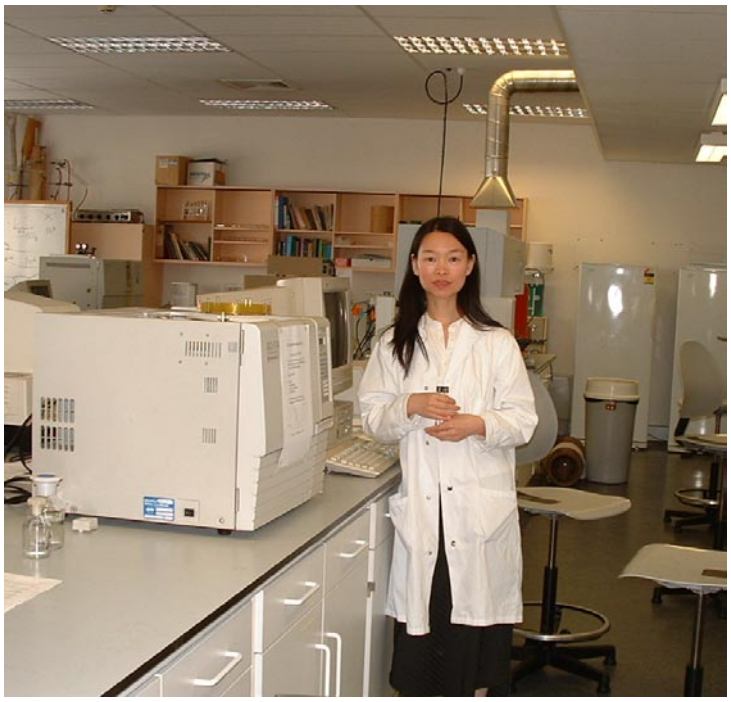

Abstract

Hubbard Foods Limited of Auckland makes a variety of oats-based value-added products. In the preparation of a range of products at Hubbard Foods, technical staff have become aware of a bitterness problem that sporadically appears in toasted oats. Toasting involves dry heating to about 150°C resulting in the golden colour and flavour development necessary for range of products. Bitterness development has been described in the literature, but Hubbard staff are necessarily focussed on production issues, rather than on a sporadic problem seemingly outside the scope of production variables. The author of this thesis set out to identify the cause and suggest a remedy.

Prior research with oats has shown that bitterness and associated off-flavours are linked to the accumulation of free fatty acids, their volatile oxidation products, and possibly amino acids and certain phenols. Oats are distinguished from related grains by their high relative fat content, about seven percent, and an associated very active lipase. The free fatty acids stem from the lipase activity that should be, but may not be, inactivated at source in Australia. This is achieved in the milling process by physical disruption and moist heating to a temperature at which the enzyme is denatured. However, residual lipase activity may adversely affect oats quality during time in storage and transit.

A number of analytical methods for cereals were adapted to match the constraints of time and resources. These methods were for colour, moisture, peroxidase activity, *p*-anisidine, fat and free fatty acids content, composition of fatty acids, total phenols, volatiles, and bitterness as perceived by an analytical sensory panel of four people. Determination of lipase activity is very expensive, so peroxidase activity is commercially used as an indicator. If the latter is inactive, the former will necessarily be also inactive.

The designed methods were first applied to 17 oats lots passing through the Hubbard environment, where 14 were paired raw and toasted. The values of moisture, fat content, free fatty acids content and total phenols were within the normal limits expected for commercial lots of oats compared with the previous studies. Not much variation was observed among the 17 oats lots, with the exception of lot DWHE25. Lot DWHE25 was a faulty product, which had high moisture content, high free fatty acids content, and tasted very bitter. The results suggested that moisture content, free fatty acids and bitterness were usually correlated. In spite of the differences encountered and the clues provided by extremes, the data generated

from Hubbard oats lots did not provide enough variation in quality to lead to a definitive chemical model of bitter flavour development. But perhaps crucially, it was found that most samples as received from Hubbard Foods were peroxidase-active which conflicted with the results reported on specification sheets prepared by the oats supplier. These specifications accompanied each lot delivered to Hubbard Foods. Therefore, the supplier's method was examined and was found to be deficient in one critically important respect. Their method omitted the key reactant hydrogen peroxide. Therefore, it is possible that the lipase was active in many of the samples.

Therefore, some experiments were conducted where raw oats, from Hubbard Foods and a supermarket, were treated with water additions and stored for a period to examine the effect of moisture content on the quality and flavour deterioration on subsequent storage. Water-treated oats were toasted to simulate a typical Hubbard process, yielding a total of 58 samples with carrying moisture contents.

The data set was statistically analysed to identify the cause of bitterness and the means of its control. The free fatty acids content, volatile compounds particularly hexanal, and total phenols increased with moisture content and storage time. The correlations between chemical analysis and sensory test indicated that free fatty acids positively correlated with bitterness ($r = 0.71$), and hexanal was also positively correlated. Total phenols did not appear to correlate with bitterness. Oats lots with high peroxidase activity tended to have the poorest quality, strongly implicating residual lipase activity as the critical factor. There were no important interactions between water addition and toasting for most of the experiments. Therefore, it seems likely that the toasting procedures at Hubbard Foods are not responsible for bitterness formation. The cause(s) of bitterness is certainly at source, with a faulty peroxidase test strongly implicated.

Table of Contents

List of Figures

List of Tables

Statement of originality

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institution of higher learning, except where due acknowledgement is made in the acknowledgements.

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Date……

Chapter 1

Introduction

1.1 Foreword

Oat (*Avena sativa* L.) is a cereal grain adapted to cool, dry growing conditions. Before the early 20th century, oats[1] were used as a feed source rather than as a human foodstuff. In recent years, oats as a food item has gained increased popularity in Western diets due to perceived or real health benefits, principally related to the bran content of oats. Thus oats are regarded as a nutritious foodstuff supplying high quality protein and significant quantities of vitamins and minerals (Welch, 1994). Additionally, Welch stated that besides their nutritional attributes, oats products can improve gastrointestinal function, modulate glucose metabolism, and decrease blood cholesterol. According to the U.S. Food and Drug Administration (1997), "*Soluble fibre from foods such as oat bran, rolled oats or oatmeal and whole oat flour, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease*".

However, oats quality, particularly flavour, deteriorates in some circumstances, and this is most obvious after toasting, a treatment where oats are toasted to about 150°C, resulting in browning and related limited caramelisation. The initially raw oats with no significant flavour defects become bitter. The cause of bitterness in toasted oats used by Hubbard Foods Limited of Auckland is the topic of this thesis.

[1] Oat is the correct term for this grain, but the plural form is in common use.

1.2 History of oats

The oat (*Avena sativa* L.) is a species of cold-tolerant cereal grain, and the expression of oats refers to the dry, mature seeds of this plant. Oats have been cultivated for about 2,000 years in various regions throughout the world (Ensminger, Ensminger, Konlande, and Robson, 1994). In Europe, oats were grown as an important commercial crop in many countries including Britain, Germany and the Scandinavia. In the early 17th century, the Scottish settlers introduced oats to North America (WHFoods, n.d.). In New Zealand, oats were similarly introduced by Scottish settlers and were mainly grown in Otago and Canterbury in the South Island (McLintock, 1966).

Oats rank fifth as an economically important cereal crop in the world production after wheat, rice, corn and barley (Kiple and Ornelas, 2000). Oats production stands at about 25 million tonnes in recent years (Home Grown Cereals Authority, 2002). The Russian Federation, United States, Canada, Germany, and Finland are the largest commercial producers of oats.

Historically, oats were used as a feed source for animals, especially for poultry and horses. Currently, only five percent of cultivated oats is used for human consumption (Williams, 2003), with the remainder destined for stock feed. Oats are popular as a breakfast food in Western countries in generally two forms, porridge and as an ingredient in muesli, granola, and similar presentation styles. In all these forms the oats are crushed or rolled and otherwise processed to increase palatability. In the West oats are contemporarily considered a ‘health’ food.

1.3 Genuine and perceived health benefits of oats

In discussing the health benefits of particular foods, it is important to distinguish between foods and diets. It appears self-evident that there are neither good foods nor bad foods, just good and bad diets. However, the argument can be advanced that a significant inclusion of a particular food in a diet will promote better long-term health. Health claims for particular foods – and there are very many – are tacitly based on this argument, but the notion that a particular food is intrinsically healthy is what the public at large is bombarded with. The sources of these claims are usually the commercial organisations seeking to increase sales of their product, such as American Oats, Inc. However, in many if not all cases, other related foods may fulfil the same role. For example, many or most of the ‘health benefits’ of oats

are shared by other grains perhaps represented by commercial interests with smaller promotional budgets. The statements presented below must be seen in light of these concepts.

Oats are valued as a nutritious grain which offers considerable health benefits. First of all, oats contain a high concentration of dietary fibre. Studies have shown that oat bran can help lower blood low density lipoproteins ('bad' cholesterol), and reduce the risk of heart disease. The soluble fibre (called beta-glucan) from oats also helps stabilise blood glucose levels, which can benefit people with non-insulin-dependent diabetes. Research result from Saltzman et al. (2001) at Tufts University in Boston showed that blood pressure and total cholesterol level may be lowered in six weeks by consuming oats-rich diets.

A high percentage of desirable complex carbohydrates in oats may reduce the risk of colon, breast, and prostate cancer, and lower bowel problems such as constipation (American Oats, Inc., 2001).

Oats also contain high concentrations of protein. Oat protein is nearly equivalent in quality to soy protein which has been shown to be equal to that of meat, milk, and egg protein by World Health Organization (Lasztity, 1999).

Additionally, the high lipids (fats) content in oats provides a high level of energy value. The fatty acid composition of oat lipids is of considerable interest because of the nutritional significance of unsaturated fatty acids (normally esterified as triacylglycerols), which are essential fatty acids in human diets (Peterson, 1992), and have been connected with long life and general good health (American Oats, Inc., 2001).

1.4 Processing of oats

Generally, oats are harvested once a year in late summer or autumn. At seed maturity the field-dry grain is harvested. Then the oats are stored in the whole form before processing (Ensminger et al., 1994). Oat products are usually sold as milled grains. Oats milling for human consumption typically involves several steps which are presented in the flow diagram (Figure 1).

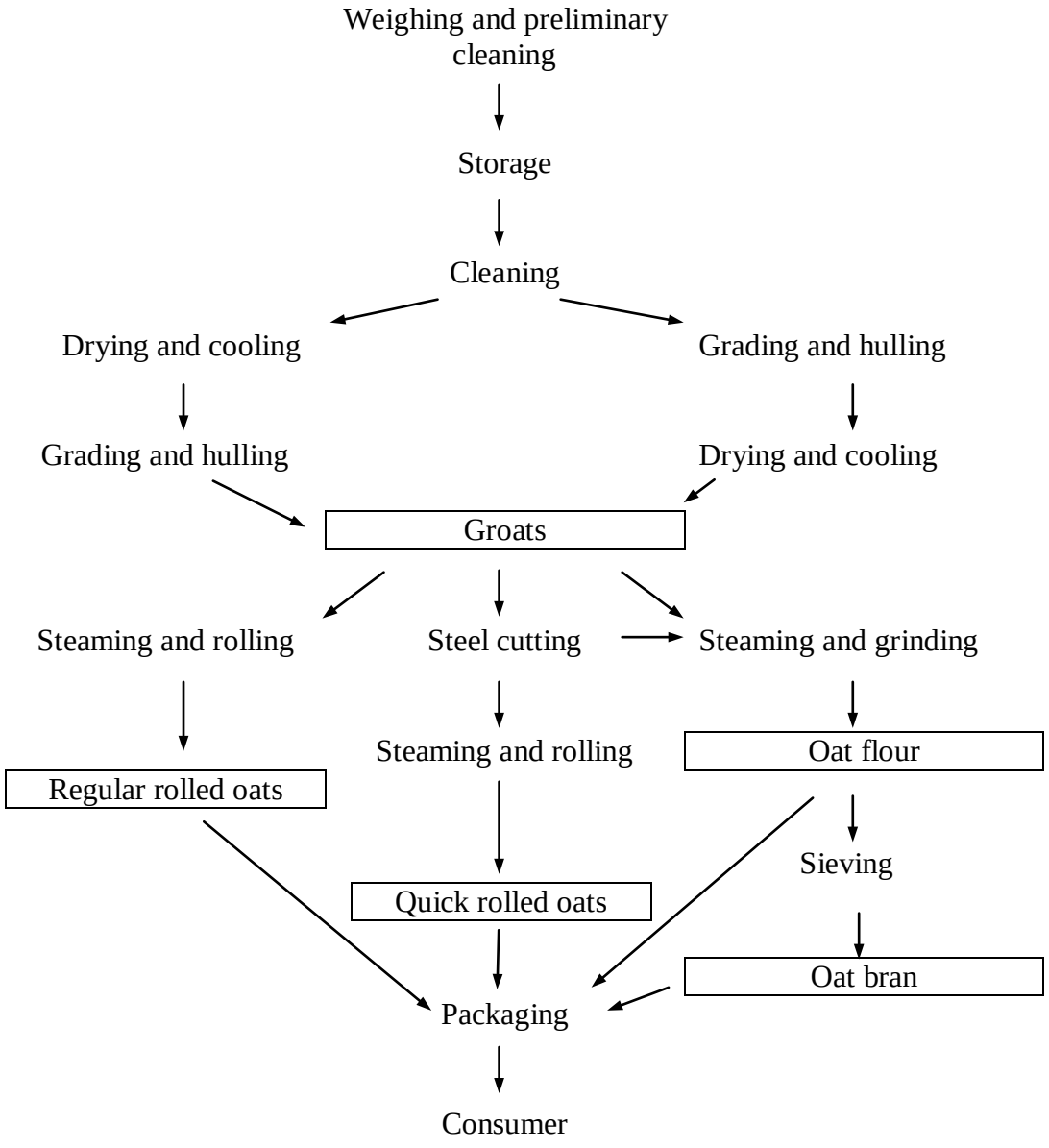


Figure 1. Flow diagram of typical oats milling. Intermediates and final products are in boxes (modified from Deane and Commers, 1986)

Foreign materials such as dust, stems, weed seeds, and oats that are unsuitable for milling are first removed in cleaning process. After cleaning, the grain is called clean milling oats (Environmental Protection Agency, 1995). The drying process reduces the moisture content of oats to between 7 and 8.5 % by weight. This is achieved by heating at 75°C for 60 to 90 minutes (Belitz, Grosch, and Schieberle, 2004). After drying, the oats are cooled by air circulation. The hulling machine separates the so-called kernel, the main source of carbohydrate, from the surrounding hull. Subsequently, the kernels are subjected to a range of cutting, rolling and heat processes to generate a number of product types. Rolling is achieved by passing the oats through rollers to crush the kernels into a flat or flaked form (Ensminger et al., 1994). Oats are heat treated with steam at various stages during processing to inactivate an endogenous lipase and to soften the kernel prior to rolling (Zmidzinska, Cenkowski, and Arntfield, 2006).

The production procedure for final oat products varies with the product range of the food manufacturer. At Hubbard Foods, for example, raw oats are toasted according to defined temperature-time profiles. There are four ovens (zones) which are set at different temperatures. Raw oats are conveyed to batch ovens starting in a 150°C-zone, passing through a 160°C-zone, another 150°C-zone, and then arriving at 125°C-zone. After final heating at 125°C-zone, toasted oats are collected as the main source for breakfast cereals and muesli bars. The procedure takes about 30 minutes in total and requires mixing of oats during heating to minimize toasting variation.

1.5 Composition of oats

Like other cereals, oats contain carbohydrate, protein, water, fat, amino acids, and other trace compound classes (McKechnie, 1983). Carbohydrate is the primary constituent of oats comprising from 45 to 62 % of the whole grain by weight (Paton, 1977). The protein content of oats is higher than that of many other cereals (15 to 20 %) (Robbins et al., 1971).

Importantly, the fat (lipid) content of oat groats is also higher than that of many other cereals. According to Morrison (1978) and Åman and Hesselman (1984), typical percentage free lipid contents of cereals are: oats groats, 5.0 to 9.0; wheat, 2.1 to 3.8; rice, 0.8 to 3.1; corn, 3.9 to 5.8; barley, 3.3 to 4.6; and millet, 4.0 to 5.5. The fatty acid composition of acyl lipids of oats is shown in Table 1. The major fatty acids in oats are the unsaturated fatty acids oleic (18:1) and linoleic (18:2) acids. Each comprises about 38 % of total fatty acids, which are normally presented in esterified as triacylglycerols.

Table 1. Average fatty acid composition of acyl lipids of oats (Adapted from McKechnie, 1983)

Acyl lipid	Weight (%)
Myristic 14:0	0.6
Palmitic 16:0	18.1
Stearic 18:0	2.0
Oleic 18:1	37.6
Linoleic 18:2	39.5
Linolenic 18:3	1.0
Gadoleic 20:1	1.4

1.5.1 Oat lipids

In animals and plants, storage lipids are composed of glycerol esterified to fatty acids. The latter can be saturated or unsaturated. An unsaturated fatty acid is a fatty acid in which there is one or more double bond (–HC=CH–) in the fatty acid alkyl chain (Belitz et al., 2004). For example, oleic acid has one double bond and linoleic acid has two, each of which is in a defined position in the chain. Fatty acids can also occur as ‘free’ fatty acids where they are no longer esterified. Free fatty acids derive from the breakdown of a triglycerol into its components, in the limiting case glycerol and three free fatty acids (Belitz et al., 2004).

While contemporary nutritional interest in oats has concentrated on dietary fibre, oat lipids will clearly have a major nutritional impact where oats constitute a significant part of a diet, due to quantity ingested and the quality of the fatty acids (Youngs and Püskülcü, 1976; Karunajeewa, Tham, Brouwer, and Barr, 1989; Molteberg, Vogt, Nilsson, and Frolich, 1995).

In contrast to these positive nutritional attributes, oats are also adversely affected by lipid-related problems, and these hamper the development of new food applications of oats (Zhou, Robards, Glennie-Holmes, and Helliwell, 1999). The most significant result of lipid reactions is their effect on sensory properties (Jacobsen, 1999). Lipid hydrolysis to generate free fatty acids, and oxidation of these and remaining esterified fatty acids, generate undesirable flavour products. The content of free fatty acids, for instance, constitutes a measurable portion of the lipid fraction (Molteberg et al., 1995) and it has been shown that an excessive amount of free fatty acids or a greater proportion of unsaturation adversely affect the storage and flavour quality of oats (Hutchinson and Martin, 1955).

1.5.2 Lipid stability

There are a number of potential factors which can affect the stability of lipids. The three key factors are moisture content, enzyme activity, and temperature at every step from harvest to consumption.

Water (moisture) is a major constituent in many foods. Water supports a huge array of chemical reactions which occur in food products. Moisture content is a measure of how much water is in a portion of the substance relative to the substance itself. By contrast, water activity is the relative availability of water in a substance. It is defined as the vapour pressure of water in the headspace above the substance divided by that of pure water at the same temperature (Wikipedia, 2006). Although the two measures are different, moisture content is positively correlated with water activity (Bell, 2002).

Water affects both the enzyme activation and the thermodynamic equilibrium of the enzymatically-catalysed reaction. Different lipases have different water activity values at which activation occurs. The literature on microbial lipases suggested that these values are well below a water activity of 0.3 (Wehtje and Adlercreutz, 1997). When water activity exceeds 0.3, enzymatic activity and other reactions can readily occur (Labuza, 1970) (Figure 2).

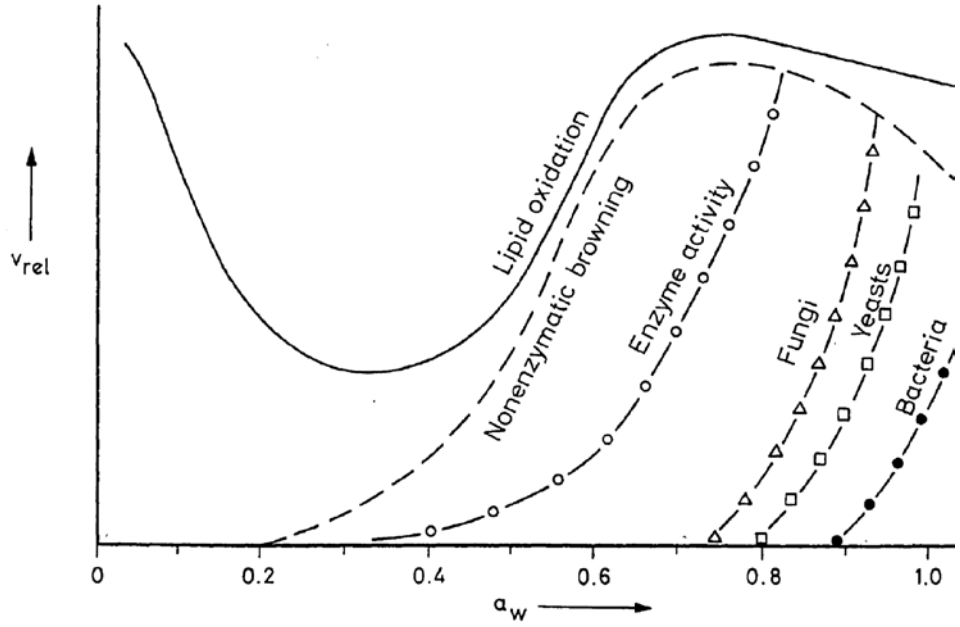


Figure 2. Stability map of foods as a function of water activity (modified from Labuza, 1970), where v_{rel} means relative reaction velocity, a_w is water activity

Not only do oats have a high unsaturated lipid content, but also have an extremely active lipid enzyme system. The lipolytic enzymes in oats are 10 to 15 times more active than those in wheat (Matlashewski, Urquhart, Sahasrabudhe, and Altosaar, 1982). Before milling, oat lipids are stored in encapsulated globules which protect them from enzyme activity. Once the oats are milled or ground without deactivating the enzymes, the lipids start hydrolysis to produce free fatty acids. Generally speaking, accumulation of free fatty acids constitutes hydrolytic rancidity by way of flavour. The free fatty acids have flavour which can render the food unacceptable. The hydrolysis reaction is accelerated by higher temperatures, but at all times an active lipase has to be present.

The majority of fatty acids in oats contain one to three double bonds. The double bonds regions of fatty acid represent areas of chemical reactivity (Belitz and Grosch, 1999). These groups readily undergo oxidation reactions that are often assisted by lipid system enzymes (Figure 3). A lipoxygenase (linoleic acid oxygen oxidoreductase) occurs in many plants. It catalyses the oxidation of some unsaturated fatty acids (Belitz et al., 2004, p. 205). The lipoxygenase reaction rate in different cereals varies greatly. Compared with that in barley and wheat, the reaction in oats is slow. Barley and wheat have high lipoxygenase activity (Fretzdroff and Seiler, 1987; Lehtinen, Kaukovirta-Norja and Laakso, 2000).

In oats, a particular peroxidase activity is responsible for the conversion of hydroperoxides to relevant hydroxyacids (Figure 3) (Biermann and Grosch, 1979). These hydroxyacids have been implicated in the bitter taste associated with the enzymatically active oat containing active lipid enzymes (Biermann, Wittmann and Grosch, 1980).

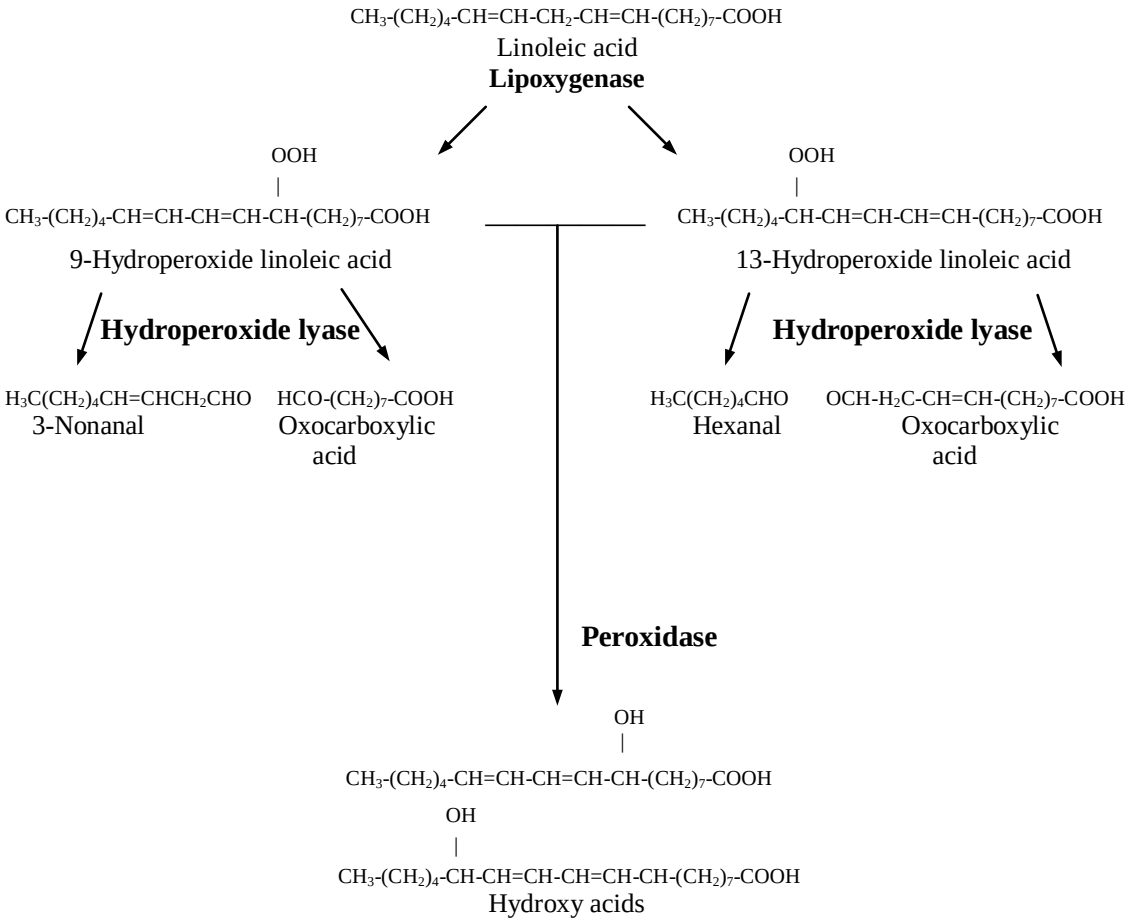


Figure 3. Simplified scheme of the reactions in the enzymatic oxidation of linoleic acid in oats (Lehtinen, 2003 modified from Noordermeer, Veldink and Vliegenthart, 2001 and Biermann et al., 1980)

Reaction rates increase with increasing temperature because the activation energy barrier is exceeded more at higher temperature. However, the effect of temperature will be more complex if there is more than one enzyme present because the temperature dependency varies with each enzyme. There is, however, a limit to the increase because higher temperatures ultimately lead to a sharp decrease in reaction rates. This is due to the denaturation of protein structure resulting from the breakdown of the bonds that stabilise the three dimentional structure of the proteins (Belitz et al., 2004). Thus each enzyme activity has its optimum temperature.

In terms of thermal denaturation of lipid system enzymes, peroxidase is more stable than lipoxygenase and lipase, as shown in Figure 4 for potato tuber as a model (Belitz et al., 2004). There are no known equivalent data for oats. If oats enzymes follow the same pattern as potato enzymes – and this seems likely – it follows that if the oats peroxidase has been destroyed by heat, the other lipid system enzymes will also have been inactivated (Belitz et al., 2004).

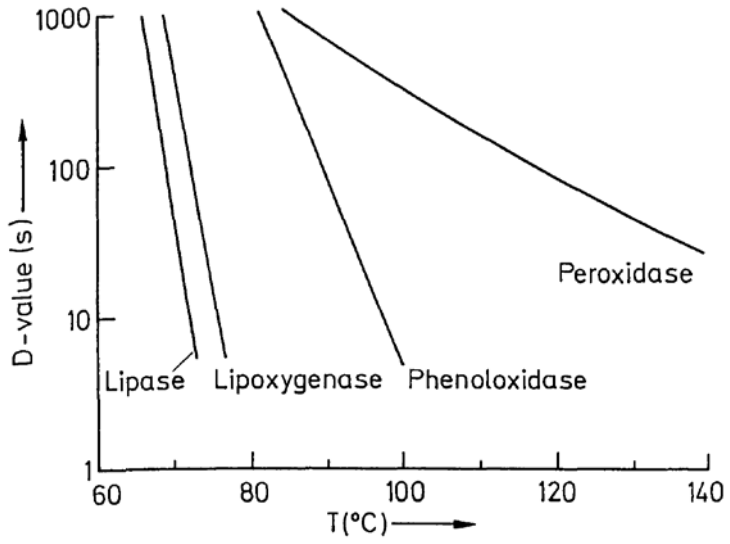


Figure 4. Thermal inactivation (90%) of enzymes present in potato tuber (Belitz et al., 2004, p. 135)

1.6 Flavour of oats and sources of bitterness

Flavour is the sensory impression of a food, which is determined mainly by taste, odour, and textural feeling (Belitz et al., 2004). Flavour compounds are categorised into two classes: those responsible for taste and those responsible for odours. Compounds responsible for taste are generally non-volatile compounds at room temperature and those responsible for odours are volatiles. The four important basic taste perceptions are provided by: sour, sweet, bitter, and salty compounds (Belitz et al., 2004).

Chemical reactions occurring in food system significantly affect food flavour (Heiniö, 2003). Off-flavours, also known as flavour defects, can be problematic for food products. In general, many of the off-flavours arise during food storage and preparation, caused by either foreign substances or reactions of food constituents (Belitz et al., 2004). For example, hydrolytic rancidity is associated with the presence of water, while oxidative rancidity requires the presence of oxygen. Of the three major compenents of food: fats (lipids), carbohydrates and proteins, the lipid compenent is the one most associated with rancidity (Hamilton, 2005).

Very little literature is available on the perceived flavour of raw oats (Heiniö, 2003), whereas numerous studies on the flavour of processed oats have been documented. Oat groats as harvested from the field lack flavour (Zhou, Robards, Glennie-Holmes and Helliwell, 1999). However, high protein and particularly high fat content are features of oats which influence flavour perception indirectly (Heiniö, 2003). During milling, the whole grain is rolled by grinding or flaking. This occurs in the presence of air at elevated temperatures, which makes the grain susceptible to the development of oxidative and hydrolytic rancidity. According to Biermann et al. (1980), this causes the development of a bitter taste.

Bitter tastes derive from many compounds including amino acids, peptides, alkaloids and fatty acids. In the case of oats, a considerable literature points to free fatty acids as the cause of bitterness (Heiniö, Lehtinen, Oksman-Caldentey and Poutanen, 2002), although contributions from other compound classes cannot be excluded.

1.6.1 Fatty acids as a potential source of bitterness

Lipids in general and free fatty acids in particular are potential precursors for flavours, particularly for unfavourable ones. Free fatty acids in oats enhance formation of bitterness (Pomeranz, 1983) and unsaturated free fatty acids in oats are susceptible to oxidation to form undesirable aromatic components (Galliard, 1989). The degradation of fatty acid hydroperoxides contributes to bitter flavour. As mentioned previously, oats contain lipoxygenases with lipoperoxidase activity. This activity can degrade hydroperoxy acids to hydroxyl derivatives which taste bitter (Belitz et al., 2004).

An example of the formation of bitter compounds from fats is shown in Figure 5. The taste threshold values are tabulated in Table 2.

Table 2. Taste of unsaturated fatty acids and oxidized fatty acids (Adapted from Belitz et al., 2004, p. 162, 210)

Compound	Threshold value for bitter* taste (mmol l^{-1})
Oleic acid	9 – 12
Linoleic acid	4 – 6
Linolelaidic acid	11 – 15
γ-Linolenic acid	3 – 6
α-Linolenic acid	0.6 – 1.2
Arachidonic acid	6 – 8
13-Hydroxy-cis-9, trans-11-octadecadienoic acid	7.6 – 8.5
9-Hydroxy-trans-10, cis-12-octadecadienoic acid	6.5 – 8.0
9,12,13-Trihydroxy-trans-10-octadecenoic acid	0.6 – 0.9
9,10,13-Trihydroxy-trans-11-octadecenoic acid	0.6 – 0.9

*The taste is described as bitter, burning, pungent

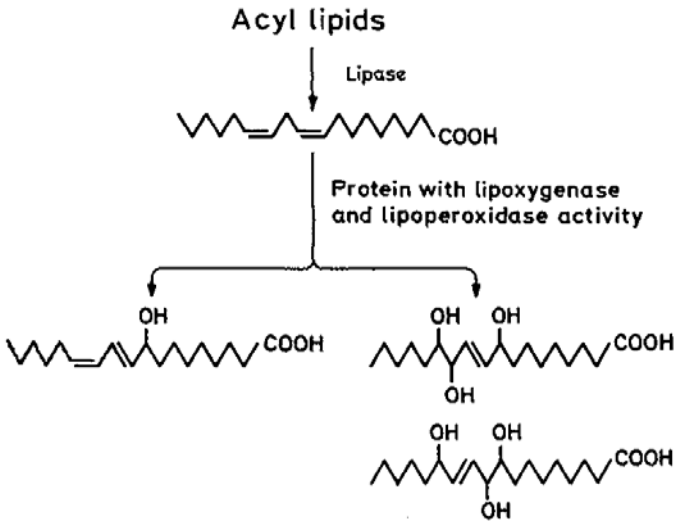


Figure 5. Formation of bitter tasting compounds in oats (Belitz et al., 2004, p. 699)

1.6.2 Volatile compounds as indicators of bitterness

Volatile compounds are responsible for odours and therefore cannot be directly responsible for bitterness, a sensation of taste. However, their presence may be correlated with bitter tastes. According to Belitz et al. (2004), volatile compounds are formed by the autoxidation of unsaturated fatty acids (e.g. oleic acid, linoleic acid, and linolenic acid). The volatile compounds in oats as reported by Maga (1978) are mainly aldehydes, ketones and alcohols, resulting from heat assisted oxidation of fatty acids. Some well known oxidation products are aliphatic aldehydes, such as pentanal, hexanal, and nonanal, formed by oxidation of fatty acids.

The most abundant volatiles in oats include hexanal, pentanal, 2, 4-decadienal, octa-3, 5-dien-2-one and 1-pentanol (Heiniö, 2003). From the study of Sjövall, Lapveteläinen, Johansson and Kallio (1997), the major volatiles in stored oats flour extrudate were hexanal, decane, 2-pentylfuran and nonanal. These chemicals can contribute to flavours or off-flavours (Heydanek and McGorrin, 1981) depending on their concentration. Fritsch and Gale (1977) showed that rancid odours occurred in ready-to-eat oat cereals when the hexanal concentration reached 5-10 ppm. The formation of volatile lipid oxidation products was shown, by headspace analyses of hexanal, to be dependent on the manufacturing process, but hexanal concentration was not related to the concentration of free fatty acids.

1.6.3 Phenol compounds as a potential source of bitterness

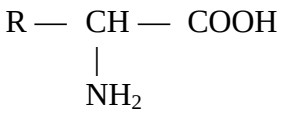
Phenols (phenolics) is a class of chemical compounds consisting of a hydroxyl functional group (-OH) attached to an aromatic hydrocarbon group (Wikipedia, 2006). The simplest one is phenol (C_6H_5OH).

Phenols are non-volatile compounds which are widely distributed in plants. Phenol compounds in plants are diverse in structure but are characterised by hydroxylated aromatic rings (e.g. flavan-3-ols). Many plant phenols are polymerised into large molecules such as the proanthocyanidins (condensed tannins) and lignins (Sahelian, n.d.).

Phenolic acids and lignin are degraded thermally or decomposed by microorganisms into phenols, which are then detected in food (Belitz et al., 2004, p. 376). The release of phenols depends strongly on the moisture content, temperature and duration of processing (Dimberg, Molteberg, Solheim and Frölich, 1996; Molteberg, Magnus, Bjørge and Nilsson, 1996a). Although free phenolic acids are minor compounds in cereals, they may influence the flavour perception of oats even in small amounts (10 - 90 mg kg^{-1}) (Dimberg et al., 1996). Phenolic acids can contribute objectionable flavours, especially astringency, at taste threshold levels of 40 - 90 ppm (Maga and Lorenz, 1973). Several phenolic acids such as *p*-coumaric acid, vanillin, *p*-hydroxybenzaldehyde and coniferyl alcohol may contribute to the rancid, intense flavour of oats (Molteberg et al., 1996a).

1.6.4 Amino acids as a potential source of bitterness

Amino acids are nitrogen-containing compounds that are the building blocks of proteins. There are about 20 amino acids in a protein hydrolysate (Belitz et al., 2004). With a few exceptions, their general structure is:



Hydrolytic processes that occur in protein-rich food yield free amino acids which contribute to flavour. Bitter tastes have been related to a high content of hydrophobic amino acids with hydrophobic side chains (Matoba and Hata, 1972; Cliffe and Law, 1990; Belitz et al., 2004). The bitter taste amino acids are generally within the L-isomers (Table 3) that dominate in

nature. L-tryptophan and L-tyrosine are the most bitter amino acids (threshold value: 4-6 mmol l^{-1}).

Table 3. Taste of amino acids (L-isomers) in aqueous solution at pH 6-7 (Belitz et al., 2004)

Amino acids	Taste	
	Quality	Intensity*
Alynine	sw	12 – 18
Arginine	bi	
Asparagine	neu	
Aspartic	neu	
Cystine	neu	
Glutamine	neu	
Glutamic acid		
Glycine	sw	25 – 35
Histidine	bi	45 – 50
Isoleucine	bi	10 – 12
Leucine	bi	11 – 13
Lysine	bi	80 – 90
Methionine	sulphurous	
Phenylalanine	bi	5 – 7
Proline	sw	25 – 40
Serine	sw	25 – 35
Threonine	sw	35 – 45
Tryptophan	bi	4 – 6
Tyrosine	bi	4 – 6

sw-sweet, bi-bitter, neu-neutral
*Recognition threshold value in mmol l^{-1}.

1.6.5 Peptides as a potential source of bitterness

Peptides are major agents responsible for bitterness (Aubes-Dutau, Capdevielle, Seris and Combes, 1995). The formation of bitter peptides can create undesirable tastes in foods. As might be expected from Table 2, the bitterness of peptides is caused by the hydrophobic amino acid residues (Ney and Gisela, 1986). The sensory properties of peptides are presented in Table 4.

Table 4. Taste of peptides in aqueous solution at pH 6-7 (Belitz et al., 2004)

Peptide**	Taste	
	Quality	Intensity*
Gly-Leu	bi	19 – 23
Gly-D-Leu	bi	20 – 23
Gly-Phe	bi	15 – 17
Gly-D-Phe	bi	15 – 17
Leu-Leu	bi	4 – 5
Leu-D-leu	bi	5 – 6
D-Leu-D-Leu	bi	5 – 6
Ala-Leu	bi	18 – 22
Leu-Ala	bi	18 – 21
Gly-Leu	bi	19 – 23
Leu-Gly	bi	18 – 21
Ala-Val	bi	60 – 80
Val-Ala	bi	65 – 75
Phe-Gly	bi	16 – 18
Gly-Phe	bi	15 – 17
Phe-Gly-Phe-Gly	bi	1.0 – 1.5
Phe-Gly-Gly-Phe	bi	1.0 – 1.5

bi-bitter
*Recognition threshold value in mmol l^{-1}.
**L- configuration if not otherwise designated.

There are many reports on protein hydrolysis giving bitter tastes from hydrophobic peptides. For example, enzymatic hydrolysis of proteins in general results in bitter tastes (Saha and Hayashi, 2001), because during hydrolysis low molecular weight peptides composed of hydrophobic residues are generated. Hydrophobic amino acids residues located in the interior of protein molecules are exposed by fragmentation with proteases. Ishibashi et al. (1987a, 1987b) concluded that the marked bitter taste in peptides is caused by the hydrophobicity of Phe, or Tyr, or Leu molecules. Bitterness is more intense when Phe or Leu residues are located at the C-terminal of di- and tripeptides.

1.7 Effects of industrial processing on oats quality and flavour degradation

In the last three decades there have been a number of research studies simulating industrial process conditions to monitor the effects of processing on oats quality, particularly flavour degradation. These investigations focused on the contribution of lipids and their role as flavour generators in oats (Forss, 1972; Min and Smouse, 1985; Supran, 1987).

Molteberg et al. (1995) investigated the effects of storage time, moisture content, and heat treatment procedure on the content and composition of free fatty acids in oats. One group of oat samples were stored at three levels of relative humidity, 30, 55, and 80 %, respectively. The content of free fatty acids in oats was found to be positively correlated with relative humidity and storage time. In contrast, the lipid content was lower at 80 % relative humidity than that at 30 % after 16 months of storage. Another group of oat samples were dry toasted at 100°C for 10 minutes, showing that the free fatty acid concentration and titratable acidity was reduced by an average of 50 % during heat processing. The reduction was explained as a reduction in linolenic acid resulted to increasing content of volatiles, such as hexanal.

Heiniö et al. (2002) inspected certain raw and processed oats during a 12-month period. Changes in sensory attributes, lipid composition, and the amount of volatile and phenols were observed. The flavour changes in the stored, raw oats were obvious and perceived as a more bitter flavour, whereas that of processed oats slowly became less cereal, more musty and less sweet, and slowly more bitter during storage. The chemical changes causing rancidity and bitterness developed faster in the raw oats. Lipid hydrolysis occurred rapidly during the first six months of storage, but less than half of the original triacylglycerol fraction was hydrolysed into free fatty acids. Volatile lipid oxidation products such as pentanal, hexanal, and 2-pentylfuran were present in the headspace and increased 10-fold during storage both in the raw and processed oats.

Sensory variation in oats due to variety, storage and heat treatment were studied by Molteberg et al. (1995, 1996b). Heat-treated dehulled oats had the highest intensities of oat odour, oat flavour, fresh odour and fresh flavour. Oats heat-treated with hulls in place, however, had the highest rancid odour and flavour and bitterness. Statistically, the sensory variation was described partly by variation in phenolic compounds, such as p-coumaric acid, vanillin, and p-hydroxybenzaldehyde, free fatty acids and moisture content.

Molteberg et al. (1996a) studied sensory and chemical effects of lipid oxidation in raw and heat-treated oat flours. Heat-treated oat flours were more stable against lipid oxidation than raw oat flours. Compared with raw flours, heat-treated flours had lower levels of total fatty acids, free fatty acids, and most flavours, particularly bitterness and astringency.

Further oxidative changes can lead to the formation of volatile oxidation products, notably carbonyl compounds which are claimed to be largely responsible for the odour of rancid oats. However, the kinetics of the development of rancid flavour is different in different kinds of oat products (Ekstrand and Gangby, 1993). It is affected by many factors because the reaction can take a number of paths. Perhaps predictably, temperature, moisture and light, as well as the presence or absence of prooxidants and antioxidants, influence the reaction.

Sides et al. (2001) monitored the volatile profile of oats during processing, using solid phase microextraction (SPME) of headspace followed by gas chromatography-mass spectrometry (GC-MS). The study did not yield any insights into the causes of rancid flavour for example, but did serve to identify the sorts of compounds present in stored and processed oats, a range of alcohols, aldehydes, alkyl benzenes and ketones.

More recently, Larsen, Lea and Rødbotten (2005) studied the sensory changes in extruded oat stored under different packaging, light and temperature conditions. The results indicated that the sensory quality of the oats was highly dependent on the oxygen transmission rate of the plastic films, and on light exposure. There were no rancid odours or flavours developed in oats during three months of dark storage in a nitrogen atmosphere at 23°C (Larsen, Magnus, and Wicklund, 2003). Oats packaged in medium- and high- oxygen transmission films, and exposed to light at 38°C, developed the highest degree of rancidity and bitter flavour.

Significant variations have been reported in the lipase activity of different oat cultivars (Frey and Hammond, 1975; Miller, Fulcher and Altosaar, 1989; Lee and Hammond, 1990; Ekstrand, Gangby and Akesson, 1992). Oats has remarkable lipase activity compared with other cereals, even before germination (Matlashewski, Urquhart, Sahasrabudhe and Altosaar, 1982; Urquhart, Brumell, Altosaar, Matlashewski and Sahasrabudhe, 1984; Youngs, 1986) and significant lipolysis can occur even at low moisture levels (Frey and Hammond, 1975) in broken or crushed oats.

Theoretically, lipases catalyse the hydrolytic deterioration where triglycerides or phospholipids are converted into free fatty acids which are much more susceptible to lipid oxidation than the original lipids (Molteberg et al., 1995). However, the study of Lehtinen et al. (2003) presented a different effect. They undertook a research on the effects of heat treatment on lipid stability in processed oats. They found that lipid oxidation and evolution of volatile oxidation products were not correlated with the residual lipase activity in whole kernels or kernel fractions. The results indicated that the overall mechanism of lipid deterioration in oat products has not been completely understood nor are the lipid species susceptible to these reactions identified (Lehtinen et al., 2003).

1.8 Aim of the study

Hubbard Foods Limited of Mangere, New Zealand, makes a variety of cereal-based, value-added products with oats. However, sporadic bitterness in toasted oats is responsible for costly product rejection. Prior research with oats has shown that the undesired off-flavours are positively related to the accumulation of free fatty acids, their volatile oxidation products, and possibly certain phenols.

Amino acids and peptides may contribute to bitterness, but prior research on these two classes is very limited (Heiniö, 2003). With the limited time available, it was decided to concentrate on the previous implicated compounds.

The aim of this research was to investigate flavour degradation in oats as received by Hubbard Foods from its Australian supplier, and to the effect of the value on oats quality particularly, and then develop reliable methods which can be used by Hubbard Foods to monitor quality of oats routinely.

The main objectives of this study were to:

- Monitor the physical and chemical quality of raw and toasted oats routinely received and processed at Hubbard Foods
- Determine peroxidase activity in raw and toasted oats
- Determine the variation of phenols in raw and toasted oats
- Study the effect of moisture content on the fat content
- Study the effect of moisture content on the free fatty acid content
- Determine variation of volatile fat oxidation products in raw and toasted oats

- Analyse the composition of free fatty acids in bitter oat samples

The research consisted of eight analyses which were variously applied to oats as received from Hubbard Foods and oats bought in a supermarket, while moisture addition, storage and toasting generated another level of treatments. The analyses were: colour variation of raw and toasted oats in Hunter colour space (CIE, 1986) that uses the L*, a*, and b*; moisture variation of raw and toasted oats; peroxidase activity in oat samples; variation of fat content, composition of fatty acids and free fatty acids; variation of phenols in oat samples; *p*-anisidine value in oats; volatile compound variation of raw and toasted oats; and bitter flavour variation of raw and toasted oats.

However, before these analyses could be executed, methods had to be developed that could be used in a university research environment with time and safety constraints that would not necessarily apply in an industrial laboratory with more resources. The development of these methods is the subject of the next chapter.

Chapter 2

Materials and Methods Development

2.1 Oats and treatments

The oats are grown in southern Australia, and are accepted by the Blue Lake Milling Company, Bordertown, South Australia according to its receiving standards (Appendix I). Subsequently, the company supplies processed raw oats to Hubbard Foods and other commercial buyers. The oats are delivered to Hubbard Foods in 25 kg plastic-wrapped paper sacks on pallets, which are stored in the warehouse until toasting according to defined temperature-time profiles to suit different products.

For the present study, the oats were sampled by Hubbard Foods personnel and couriered to the Wellesley site of the AUT where most work was done. The samples, typically 0.5 kg, were sometimes paired, that is, the raw oats had a matching sample of toasted oats.

On occasions, oats were also sourced from supermarkets (Pak'n Save, Auckland) in the form of retail branded rolled oats. The sources and code numbers of all oats samples are listed in Appendix II.

As the project progressed, it was realised that the samples obtained from Hubbard Foods did not provide enough variation in quality to develop a plausible chemical model of bitter flavour development. Therefore, some experiments were conducted where raw oats were treated with minor water additions to generate possible flavour deterioration on subsequent storage. A laboratory toasting procedure was also developed to simulate a typical Hubbard Foods process.

2.2 Water addition and toasting

Small volumes of water were added to oats to raise the moisture content from about 9 to 14 percent by weight. The required volumes of water were finely and evenly spread onto oats that were further mixed before sealing in polyethylene plastic bags and storage in the dark at room temperature. Polyethylene is impermeable to water but not to oxygen. After storage for several weeks, the samples were variously analysed raw and toasted.

Toasting was done in a convection oven, model MOV – 112F (Sanyo, Japan). The oven temperature was set at 150°C. The total toasting time was one hour by which time the colour of the toasted oats matched those typically obtained from Hubbard Foods.

2.3 Physical and chemical analyses

Most of the methods used for chemical analysis were adapted from the American Association of Cereal Chemists (AACC, 2000) or the American Oil Chemists' Society Official Methods (AOCS, 1998). Several of them were modified in order to suit oats as a grain, to suit the equipment available at AUT, and to suit time and safety constraints. For example, the AACC official method for Soxhlet extraction of fat calls for a 16 hour extraction with (flammable) petroleum spirit. If fat extraction were the sole analysis, the official method could be followed exactly, but with many analyses to do and with constrained time access to the laboratory, the extraction period had to be shortened, as will be described later.

2.3.1 Colour measurement

Colour is an important attribute to the food industry. Often colour and flavour is directly related. For example, if the product looks dark it will probably taste burnt; if it looks light it will probably taste underdone. In some cases, colour variation and moisture content is also related (Good, n.d.).

Colour measurement was achieved by Hunter colour system. The Hunter colour system is based on the concept of a colour space with the colour defined by three coordinates (Coultate, 2002), L*, a*, and b* values. The vertical coordinate L* is lightness from 0 (total light absorbance and therefore completely black) through grey (50) to 100 (complete light reflectance); the horizontal coordinate a* is greenness/redness, from – 60 (green) through grey to + 60 (red); an orthogonal horizontal coordinate b* is blueness/yellowness from – 60 (blue) to + 60 (yellow) (Figure 6).

Hue angle refers to the gradation of colour within the visible spectrum of light. Hue angle is arctangent (b*/a*) determined by rotation about the a* and b* axes.

Chroma is the intensity of a specific hue: a highly saturated hue has a vivid, intense colour, while a less saturated hue appears more muted to the point of the vertical continuum of black, grey, white. Chroma is calculated as $\sqrt{(a^{*2} + b^{*2})}$. Thus, L* (luminosity or reflectivity), hue

angle and chroma are values that theoretically describe all perceived light. Thus in Figure 6, in L* a* b* colour space, the tip of the thick arrow is defined by its lightness (70 on a scale of 0 to 100), its redness (+26 on a scale of –60 to +60) and yellowness (+15). The hue is arctangent 15/26 (= 30°) and the chroma, or intensity, is the length of the thick line, √(152 + 262) (= 30).

A Hunter colorimeter, ColorFlex (Hunter Associates, Virginia, U.S.), was used to measure the colour. Oats were held in a filled cylindrical glass dish (Schott, Germany) measuring 2.5-inch (64-mm) and placed in the illuminant path (sample port), and then covered with an opaque metallic black shroud. D65/10° was selected the illuminant/observer combination to measure daylight colour expressed as L*, a*, b*.

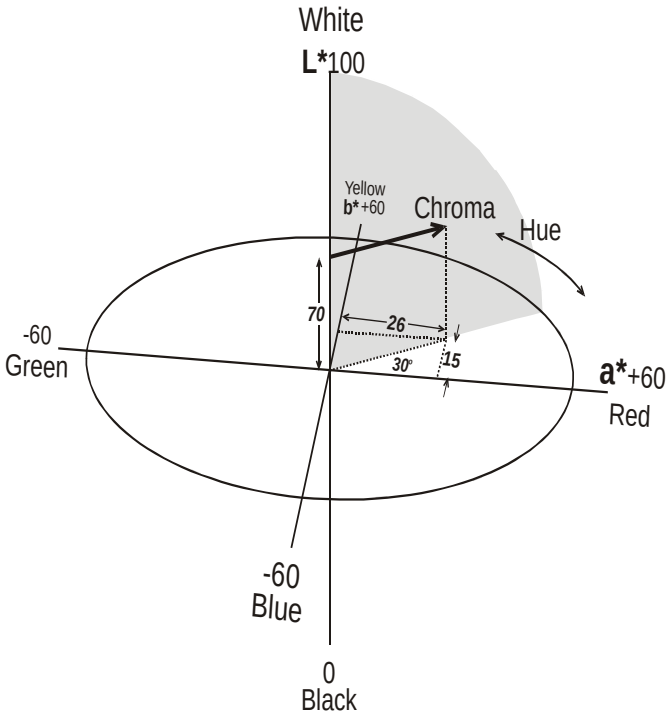


Figure 6. Hunter colour space (modified from Young and West, 2001, p. 52)

2.3.2 Moisture content

The moisture content of dried oats is normally about 10 % (w/w) (McKechnie, 1983). Higher moisture content could promote lipase activity potentially leading to flavour problems. Hence, a routine moisture content test was essential.

The AOCS official method Aa 3-38 was adapted to determine moisture content. The official method calls for heating to 130ºC for three hours, but this was changed to 110ºC for five hours. A preliminary time trial showed that dried weights were stable at five hours (Appendix III). An aluminium moisture dish containing about 10 g oats sample was weighed accurately and dried in a laboratory oven (Wilton, New Zealand). The dishes were cooled in a desiccator and reweighed. The percent moisture content was calculated as follows.

$$\text{Moisture content (\%)} = \frac{100 \times \text{Loss in mass (g)}}{\text{Original sample mass (g)}}$$

2.3.3 Peroxidase activity

When processing oat products, the activity of the endogenous lipase(s) is an important indicator for maintenance of adequate flavour in stored products. In theory, lipase should not be active in processed raw oats because the thermal denaturation step in the milling process should denature the enzyme. Peroxidase activity also can be used as an indicator of lipase activity because peroxidise has greater thermal stability than lipase (Belitz and Grosch, 2004, p. 134). Thus if peroxidase activity cannot be detected, lipase will also be inactive. For this reason, the Blue Lake Milling, which supplies Hubbard Foods, routinely tests for peroxidase activity, which is much easier to test for than lipase activity.

To test for peroxidase activity, AACC official method 22-80 (AACC, 1999) was applied. Ascorbic acid (reagent grade, AC0515, Scharlau), the blue dye 2,6-dichloroindophenol (36180, Fluka), and hydrogen peroxide (30 %, v/v) were used in this test.

Oat samples were dry-blended in a Waring-style domestic blender to pass through a 20-mesh (0.85 mm) sieve. Two 1 g samples served as test sample and blank. These were placed in 125 ml Erlenmeyer flasks, to which was added 50 ml of deionised water. Each was swirled to mix. Two millilitres of ascorbic acid solution (1 mg ml^{-1}) and 3 ml of 2,6-dichloroindophenol solution (0.2 mg ml^{-1}) were then added. Hydrogen peroxide (0.1 ml, 30

% v/v) was added to the test flask but not to the blank. After heating in a water bath at 38 ºC for 10 min, the colour was observed against a white background without shaking. The method defines three semi-quantitative levels of peroxidase activity: –, no apparent change in colour of solution, indicating adequate heat processing; 0, a change in appearance of solution to grey or light blue; +, a definite blue colour formation, indicating peroxidase activity.

In routine testing, however, it was found more useful to define five levels of activity due to the different colour intensity in peroxidase positive samples. The following system was adopted: 0 = no activity, with increasing activity between 1 and 4 according to colour intensities. This scale was used throughout this project. The numerical scale was also useful for statistical analysis.

2.3.4 Fat content, free fatty acids and composition of fatty acids

The fat content in milled oats is about seven percent. The dominant form is triacylglycerols, which are mixed esters of fatty acids and glycerol. They are neutral. Hydrolysis of acylglycerols generates free fatty acids, which increases acidity that can be measured by a titrimetric procedure with alkali.

Fat content and free fatty acids determinations were respectively based on AACC method 02-01A (1999) and AOCS Ca 5a-40 (1997). The fat extraction time was reduced from 16 to five hours for logistical reasons, but trials revealed that the time reduction had minimal effect on fat content determinations (data not shown). Another issue was effect of oats particle size on fat extraction. A sample of oats was dry-blended with a Waring-style blender to approximately ⅛ the size of the original oats. This was achieved in two minutes. Duplicates of unblended and blended oats were extracted in parallel for five hours. The quantity of fat extracted from the unblended and blended oats (smaller particles) were 7.41 ± 0.28 and 7.54 ± 0.35 % by weight, respectively (Appendix IV). However, the difference was not significant and unimportant. Oats were therefore not blended before extraction.

Fat was extracted using the Soxhlet system (Figure 7). About 10 g of an accurately weighed unblended oats sample was placed in a paper thimble (25×80 mm, Advantec, Japan), extracted with 90 ml petroleum spirit (40-60°C boiling range, Scharlau) at rate of one siphon about every three minutes. After five hours extraction, the spirit was evaporated over a

boiling water bath. The dry flask was reweighed to determine the percent fat content calculated as

$$\text{Fat content (\%)} = \frac{100 \times \text{Extract mass (g)}}{\text{Oats sample mass (g)}}$$

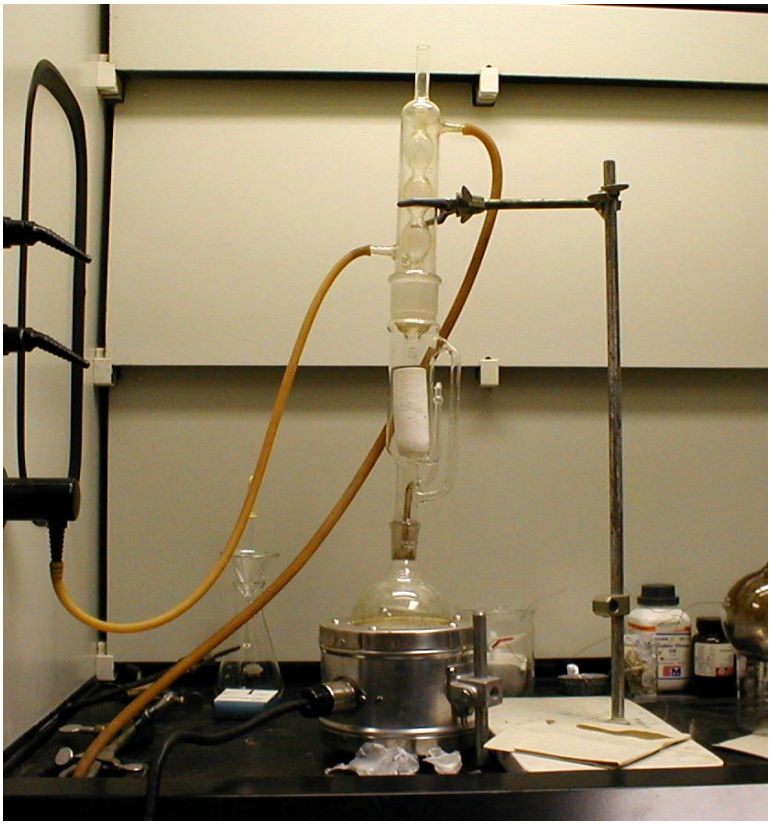


Figure 7. Soxhlet extraction equipment, comprising a vertical (reflux) condenser, and a paper thimble in the extraction chamber. The chamber has a siphon sidearm to return fat-enriched solvent to the boiling flask

The free fatty acids determinations (AOCS Ca 5a-40) were modified to suit the acidity range and sample size of oats.

In pilot tests, the free fatty acids level for oats was found to be in the range of 2 to 12 % of total fat. Referring to Table 5, the concentration of NaOH best suited to titration was 0.25 M. However, because the weight of fat extracted from a 10 g oat sample was less than 1 g, the concentration was reduced to 0.05 M. The NaOH solution was accurately standardised using the AOCS H 12-52 method.

Table 5. Free fatty acids range, solvent volume and strength of alkali (AOCS Ca 5a-40, 1998)

Free fatty acid range (%)	Sample (g)	Solvent (ml)	Strength of alkali (M)
0.00 to 0.2	56.4 ± 0.2	50	0.1
0.2 to 1.0	28.2 ± 0.2	50	0.1
1.0 to 30.0	7.05 ± 0.05	75	0.25
30.0 to 50.0	7.05 ± 0.05	100	0.25 or 1.0
50.0 to 100	3.53 ± 0.001	100	1.0

For free fatty acid determinations, the fat extracted from 10 g of oats was dissolved in 50 ml of an equal volume mixture of toluene and 95 % (v/v) ethanol. One millilitre of phenolphthalein solution (1 % in 95 % ethanol) was added as the endpoint colour indicator. The solution was titrated with 0.05 M standard sodium hydroxide until a faint pink colour was obtained that persisted for at least 1 min. Free fatty acids were calculated as percent oleic acid as the equation shows below:

$$\text{Free fatty acids as oleic (\%, w/w)} = \frac{\text{Titre} \times 0.05 \times 282 \times 100}{\text{g} \times 1000}$$

where titre is the volume (ml) of NaOH solution required, g is the fat weight, 0.05 is molarity of the NaOH solution, and 282 g $mole^{-1}$ is the molecular mass of oleic acid.

The fatty acid composition was determined following a modified method of Bligh and Dyer (1959, cited by Watanabe, n.d.). Fat extraction was performed using the Soxhlet extraction technique as described above. Subsequently, 20 mg of fat was placed in a screw top test tube and resuspended in 1 ml of chloroform. Methanolic NaOH solution (3 ml, 6 % w/v) was added and the sealed tube vigorously shaken. The tube was subsequently heated in a water bath at 100°C for 20 min. After cooling, 5 ml deionised water and 2 ml diethyl ether (HPLC grade, BDH) were added. The tube was shaken for 2 min. Once settled, the total upper aqueous phase was discarded. One millilitre of concentrated HCl was added for sample acidification. Then 2 ml of diethyl ether was added and the mixture shaken. The upper diethyl ether phase containing the total fatty acids was recovered for methylation.

About 1.5 ml of 14 % w/v boron trifluoride (S4427563, Merck) in methanol was added to the fatty acids in diethyl ether, mixed with 50 µl of the internal standard tricosanoic acid (T6543,

Sigma, 100 mg ml^{-1} in diethyl ether) sealed, then heated at 100°C for 20 min. After cooling, 5 ml water and 2 ml diethyl ether were added and mixed. Once settled, the top diethyl ether phase containing fatty acid methyl esters (FAMEs) was recovered for gas chromatography-mass spectrometry (GC-MS). The procedures are summarised in Appendix V.

FAMEs analysis was performed on a gas chromatograph GC-17A (Shimadzu, Japan) equipped with QP-5000 quadrupole mass spectrometer (Shimadzu, Japan). Separation was achieved on a CP-Wax column (25 m, 0.25 mm i.d., 0.20 μm film thickness) (Varian, U.S.), using helium (zero grade, BOC, Auckland) as the carrier gas at 1 ml min^{-1}. A suitable temperature program to resolve the main fatty acids in oats was as follows: start at 100°C, increase to 150°C at 15 °C min^{-1}, then to 200°C at 1.5 °C min^{-1}, hold for 20 min. The column flow rate was 50 ml min^{-1}. The split ratio was 28 with an injection volume of 1 μl. The interface temperature between column and detector was 260°C. The detector voltage was 1.5 V, the mass range was 35-400 m z^{-1} using the total ion count mode, and the scan rate was 0.8 sec $scan^{-1}$.

Standard F.A.M.E. Mix C4-C24 (18919-1AMP, Supelco, U.S.) was used as an external standard. One microlitre of 10 mg ml^{-1} F.A.M.E. Mix was injected into the injection port. The retention time of each component was evaluated.

Fatty acid peaks were identified by comparing their GC retention times to external standard, with confirmation by analysis mass spectrometric fragmentation patterns using the probability-based matching software NIST147 (National Institute of Standards and Technology, U.S.).

2.3.5 *p*-Anisidine value

The AOCS official method Cd 18-90 can be used to determine the amount of aldehydes (principally 2-alkenals and 2, 4-dienals) in animal and vegetable fats and oils, by reaction of *p*-anisidine in acetic acid with aldehydes in an oil. The absorbance of the resulting complex is measured at 350 nm.

Fat that had been Soxhlet-extracted from 10 g of oats was dissolved in 25 ml iso-octane. Five millilitres of this solution was treated with 1 ml of the *p*-anisidine reagent (A1006, Sigma) and mixed. After 10 min, the absorbance of the solution was measured at 350 nm in a quartz cuvette, against an iso-octane reference also containing the *p*-anisidine reagent. An

equivalent weight of domestic soyabean oil was used as reference material to evaluate this method. The results of applying this method to extracted fat (unsuccessful in the event) are reported in the next chapter.

2.3.6 Total phenols

The Folin-Ciocalteu method (Slinkard and Singleton, 1977, reported by Waterhouse, n.d.) was used to measure total phenols. In outline, an alcoholic extract of the sample was treated with Folin-Ciocalteu reagent (F9252, Sigma) and saturated sodium carbonate solution in that order. The blue colour that develops was measured by absorbance at 765 nm. Gallic acid was used to construct a calibration curve (Figure 8).

The standard curve shows a linear relationship between absorbance and concentration within at least the range 0 to 500 mg of gallic acid l^{-1}. The phenols concentration in methanolic solution was determined using the standard curve, and the results calculated as milligrams of phenols per gram of oats.

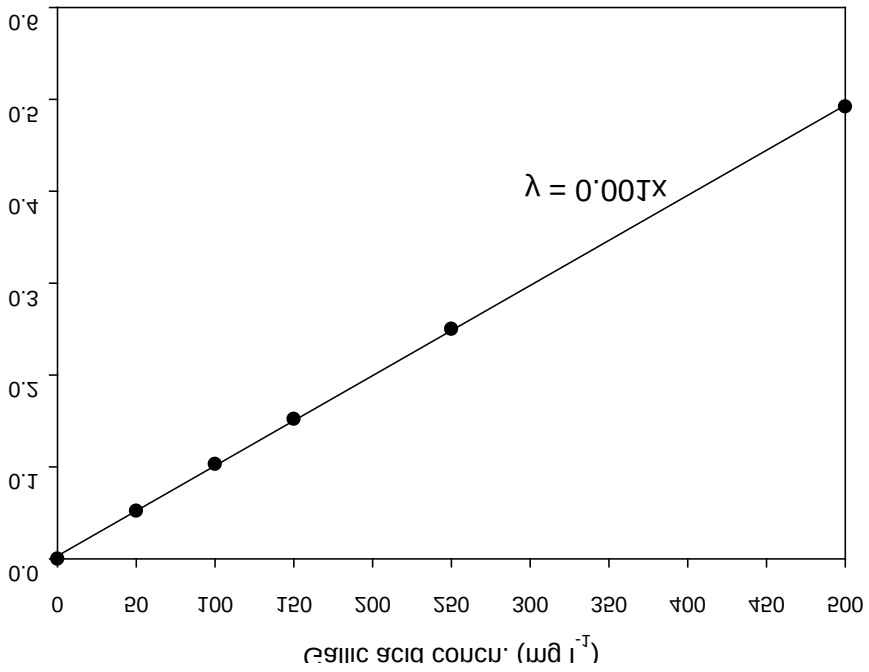


Figure 8. Folin-Ciocalteu gallic acid standard curve for total phenols determination at 765 nm

Phenols are soluble in methanol and in ethanol. A preliminary trial was done with three oats lots, each extracted with methanol and ethanol for 48 hours respectively to find which was

the more efficient solvent. Methanol was the more effective (Appendix VI). Two oats samples were then extracted with methanol for up to 96 hours to determine a suitable extraction time. Forty eight hours was chosen (Appendix VII).

In routine execution of this method, 20 µl of a 48-hour methanolic extract of oats (10 g extracted with 50 ml of methanol) was pipetted into a plastic cuvette, while 20 µl of water served as a blank. To each was added 1.58 ml water, and 100 µl Folin-Ciocalteu reagent (F9252, Sigma). After four minutes, 300 µl of saturated sodium carbonate solution was added. After two hours, the absorbance was measured at 765 nm against the blank.

2.3.7 Volatile compounds

The primary oxidation products of unsaturated fatty acids are the hydroperoxides – highly reactive compounds that decompose rapidly, yielding a complex mixture of potentially volatile compounds such as hydrocarbons (ethane, pentane etc.), aldehydes (pentanal, hexanal, 2-octenal, 2-nonenal, etc.) and ketones, which affect the overall quality of a food containing unsaturated fats. Aldehydes are particularly important in relation to flavour alteration and from a toxicological perspective (Frankel, 1980).

A solid phase microextraction – gas chromatograph (SPME-GC) technique was developed to determine headspace pentanal, hexanal and 2-pentylfuran, and nonanal. The way SPME fibres work is shown in Figure 9.

The effects of SPME fibre exposure and desorption times on the resulting GC profile was examined before routine analyses were performed. It was also found that minor increases in the moisture content of oats samples did not affect the performance of the fibres (data not shown). The fully developed method was as follows.

A carboxen^{TM}/ polydimethylsiloxane (CAR/PDMS, 50/30 µm) fibre [57328-U] (Supelco, U.S.) was used for SPME headspace analysis. The fibre was used with a manual fibre assembly holder [57330-U] (Supelco, U.S.). The fibre was conditioned at 250°C for one hour before each use. In the method developed here, a 10 g oats sample was weighed into a wide, round-bottom glass tube (10 cm height, 32mm i.d.) and sealed using aluminium foil held in place with a rubber band. The resulting headspace occupied about 50 % of the tube's volume. The reason is that the sensitivity of headspace sampling is best when the headspace volume is small. The fibre extracts more sample, faster, and with greater efficiency

(Supelco, n.d.). The sealed tube was placed in an aluminium DK20 block heater (VELP Scientifica, Italy), which was maintained at 60ºC. The conditioned SPME fibre assembly was exposed in the headspace to a depth of 2 cm 15 minutes to trap volatile compounds. The fibre was then was retracted, removed from the glass tube and desorbed for six minutes in the Shimadzu GC-17A injection port set at 250°C.

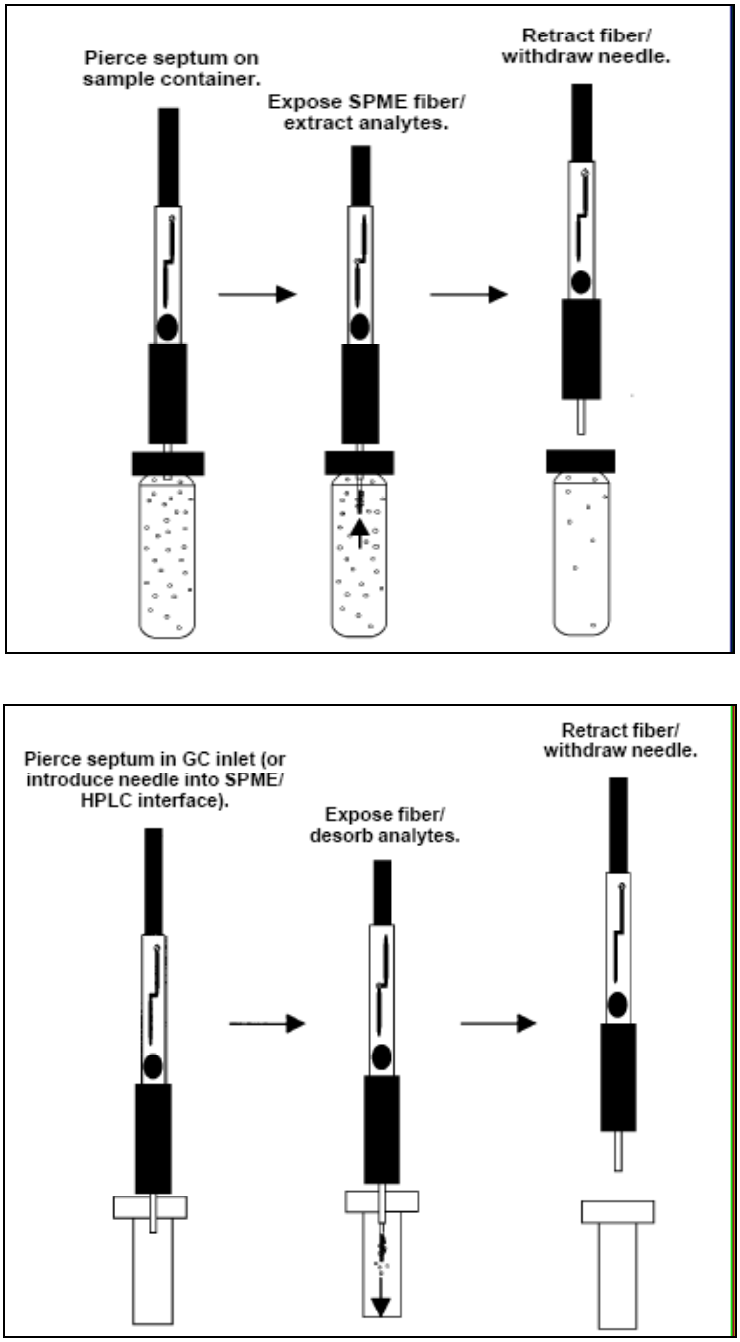


Figure 9. The way SPME fibres work (Supelco, n.d.). The upper diagram shows the absorption procedures and the lower one desorption procedures

Separation was achieved on a CP-Wax column (30 m, 0.32 mm i.d., 0.15 μm film thickness) (Varian, U.S.) that led to a flame ionisation detector. The oven temperature program was: start at 30°C, hold for 2 min, ramp to 120°C at 5°C min^{-1}, and then to 200°C at 15 °C min^{-1}, and hold for 10 min. The splitless model was applied for headspace analysis. The column pressure was 50 kPa and the helium flow rate was 1.8 ml min^{-1}. The resulting linear gas velocity was 28 cm sec^{-1}.

To compensate for the variation in the performance of the SPME fibre and flame ionization detector, all responses of duplicated samples were standardised to the response of an external standard, pentanal present in an equivolume mixture of four references, pentanal, hexanal, 2-pentylfuran and nonanal. Duplicate 0.2 μl samples of the standards mixture were accurately transferred into the round-bottom glass tubes containing no oats, using an analytical microsyringe. The tubes were then treated as if they were oats replicates. The subsequently desorbed volatiles were used to monitor the retention time of the four volatiles. The peak area of pentanal was selected as the standard to calculate the relative ratios of the four volatiles.

2.4 Sensory studies

2.4.1 Sensory evaluation

Sensory evaluation of food is a scientific method using the senses of taste, smell, sight, touch and hearing to examine the flavour properties of food (Anonymous, 1975, cited by Stone and Sidel, 2004). Based on the definition, sensory evaluation involves the measurement and evaluation of the flavour, and also involves the analysis and interpretation of the responses.

Flavour is the human response to odour and taste as respectively perceived by the olfactory receptors in the nasal cavity and the taste receptors in the mouth. The responses have a time component: the food can often be smelt before it enters the mouth; taste is perceived during chewing or transient retention in the buccal cavity; odour is perceived retronasally during chewing or retention, and on swallowing. Moreover, the sensations of flavour can linger for seconds, tens of seconds and even minutes are after swallowing.

2.4.2 Methods for bitterness testing

Bitterness is regarded as a sensory defect for many if not most products (Rouseff, 1990). It has been observed that the ability to detect bitterness varies widely within the general population. Some individuals are extremely sensitive to bitterness, whereas others can detect bitterness only at very high concentration (Prescott and Tepper, 2004).

Measuring of bitterness in solid food is difficult. As one of the four common taste sensations, bitterness is mainly detected on the back of the tongue, the back of the hard palate and in the pharynx (Figure 10). Because this sensation is perceived towards the rear of the oral cavity, many foods do not taste bitter until swallowed; moreover, the intensity is frequently strongest as an aftertaste. In general, the slower the reaction time, the longer the persistence. The oral response time to bitter compounds is slower than for the other common tastes, being 1.082 sec as compared with 0.307 sec (salt), 0.446 sec (sweet) and 0.536 sec (sour) (Fenwick, Curl, Griffiths, Heaney and Price, 1999).

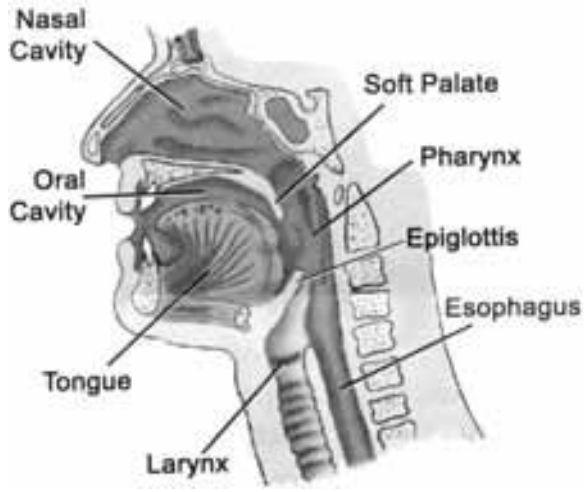


Figure 10. Anatomy of oral cavity (Korow, n.d.). After the teeth chew the food, the tongue pushes the bolus to the pharynx

Bitterness testing was performed both at AUT and at the Hubbard Foods site in Mangere using scaling methods. The evaluation of bitterness for dry toasted and untoasted oats was a challenge because the oats has to be chewed dry, and the perception of bitterness, if any, developed with increasing time of chewing. This was an unpleasant and unpopular task.

The method used at AUT was category scaling method (Meilgaard et al., 1991) with a bitterness scale from 0 to 2 (Table 6). Four panellists including two staff and two postgraduate students, all with a food science background and able to regularly attend the tests, were selected to conduct bitterness testing as required. Oat samples, variously as received from Hubbard or treated at AUT, were randomised for presentation order and served to panellists. About one spoonful of sample was tasted and score recorded. Water was served to rinse the mouth between samples.

Table 6. The flavour profile descriptive analysis method used at AUT (numerical type category scale anchored with words)

Numerical value	Word anchor
0	Not bitter at all
0.5	Slight bitter
1	Bitter
1.5	Moderate-strong bitter
2	Very bitter

Measures of bitterness were also undertaken at Hubbard Foods by seven food technologists. Their method was also category scaling which defined as: 0, meets Hubbard standard (target/reference quality); 1, minor improvement needed; 2, major improvement needed; 2.5, not meeting specification, major sensory defect; 3, not to Hubbard quality. (It must be pointed out that the descriptive terms in this scale are not entirely suitable for assessing bitterness in oats because there is no known way to achieve 'major improvement needed', short of discarding the bitter oats in favour of non-bitter oats.) In the event, Hubbard data were not used because reports were too sporadic, and there was no guarantee of control procedures in their evaluations. However, they may have been conducted adequately.

2.5 Data analysis

Microsoft Excel was used for all data handling and preliminary analysis. For industrial oats samples, the data are presented as bar graphs where means of duplicate determinations are presented for each oats lot. These individual means were used to calculate means and standard deviations (or percent coefficient of variation) for raw and toasted oats. Correlation coefficients were calculated as required, and scatter plots are discussed after inspection.

For laboratory generated samples, a t-test was used to compare the colour of industrial toasted oats with those generated in the laboratory. A two-way analysis of variance was routinely using Minitab Release 14 (Minitab Inc., Pennsylvania), where oats lot was a block factor in analysing the effects of water addition. As with industrial oats samples, correlation coefficients between variables were calculated and scatter plots made. These are discussed with respect to the results of analysis of variance to develop models of bitterness development.

Chapter 3

Results and Discussion for Industrial Oats Samples as Received

3.1 Introduction

The starting point to identify the cause(s) of bitterness in oats was to monitor samples of oats lots as received by Hubbard Foods with the methods described in Chapter 2. It was hoped that this approach would reveals chemical clues as the cause(s), which could lead to experimental verification. In the event, some clues were found and these led to work described in Chapter 4. What follows here is a systematic study of typical oats lots used in oats products by Hubbard Foods.

3.2 Results of physicochemical and sensory tests

3.2.1 Colour

The colour of 17 raw and toasted oats samples received from Hubbard Foods were measured using the ColorFlex. All were paired samples except where two had no toasted equivalents, and another had no untoasted equivalent. The daylight colour parameters L*, a*, b* were recorded, and a* and b* values were transformed into hue angle and chroma. The mean values with coefficients of variation percent are in Table 7.

Table 7. Mean colour values and coefficient of variation percent for pooled raw and toasted oats

	Raw oats[1] (n = 9)		Toasted oats[1] (n = 8)	
Lightness, L*	72.09	(2.8)	62.38	(5.0)
Hue angle, arctangent (b*/a*)	1.36	(0.7)	1.30	(2.3)
Chroma ,$\sqrt{(a^{*2} + b^{*2})}$	26.17	(13.0)	30.45	(10.0)

[1]Values are means and (coefficient of variation percent)

The basic colour (hue angle) of the oats was unchanged by toasting, but toasting caused an increase in variability as revealed by the higher coefficient of variation percent. Lightness decreased on toasting and was more variable. The colour intensity (chroma) increased on

toasting, but was similarly variable. One lot of toasted oats, DWHE25, was exceptionally dark and was particularly bitter. This lot is further discussed below.

3.2.2 Moisture content

The moisture contents of 17 industrial lots were measured (Figure 11). Lots OQ3225 and OR3725 were only raw samples, while lot DWHE25 was only toasted.

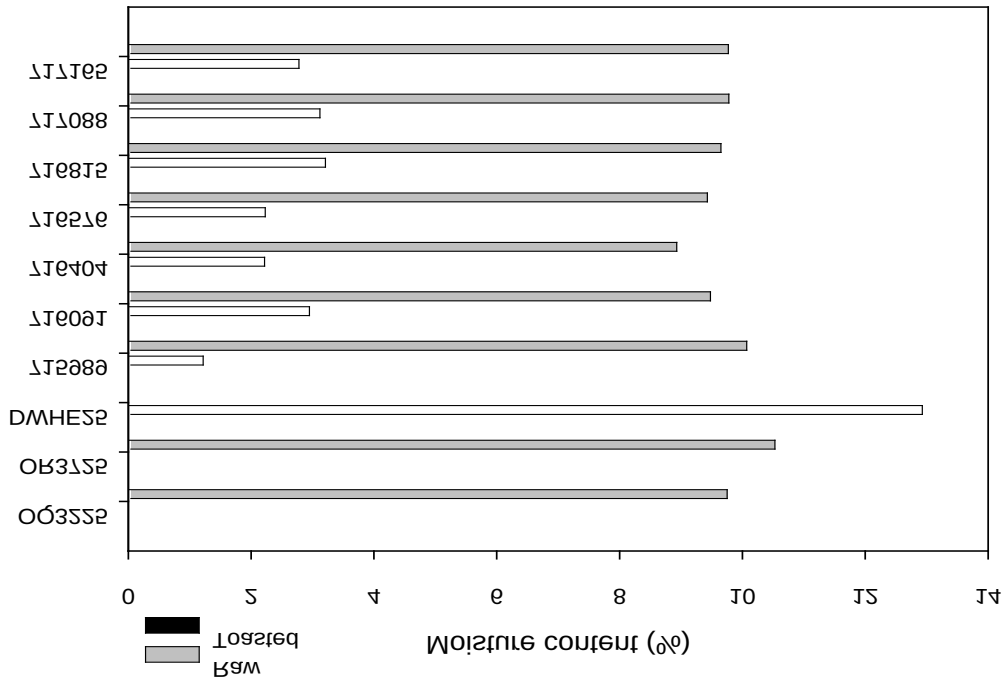


Figure 11. Moisture content of industrial oats samples. Values are means of duplicates

The moisture contents of raw oats were about 10 % (w/w) which approached the maximum recommended storage moisture condition (Hellevang, 1994). Hellevang (1994) recommended that for dehulled oats, a low moisture content (< 10 %) could allow long-term storage without significant deterioration. Toasting reduced the moisture content markedly, such that the content was normally less than 3.5 % (Figure 11), but values were proportionately more variable than for untoasted oats.

The moisture content of toasted lot DWHE25 was a very high level 13 %. This sample was declared bitter by Hubbard staff, and the sensory panel at AUT agreed. It was also very dark as noted above. A clue to bitterness in this lot was that the high moisture content after toasting suggested a very high, but unknown, content before toasting.

3.2.3 Fat content and free fatty acids content

Fat content and free fatty acids content of 17 industrial lots were shown in Figure 12. The mean fat content of raw oats was 7.24 ± 0.21 % by weight, while that of most toasted oats was 7.78 ± 0.12 %. The increase is almost certainly due to the lower moisture content of toasted oats, so the proportion of weight due to fat increases.

Two lots, 717165 and 717088, had an unusually high fat content in the toasted form, about 12 % (w/w). It was found that sesame seeds were mixed with oats in those two samples, but only in toasted oats, not in raw oats. The fat content of lot DWHE25 was low, about 1.5 % by weight.

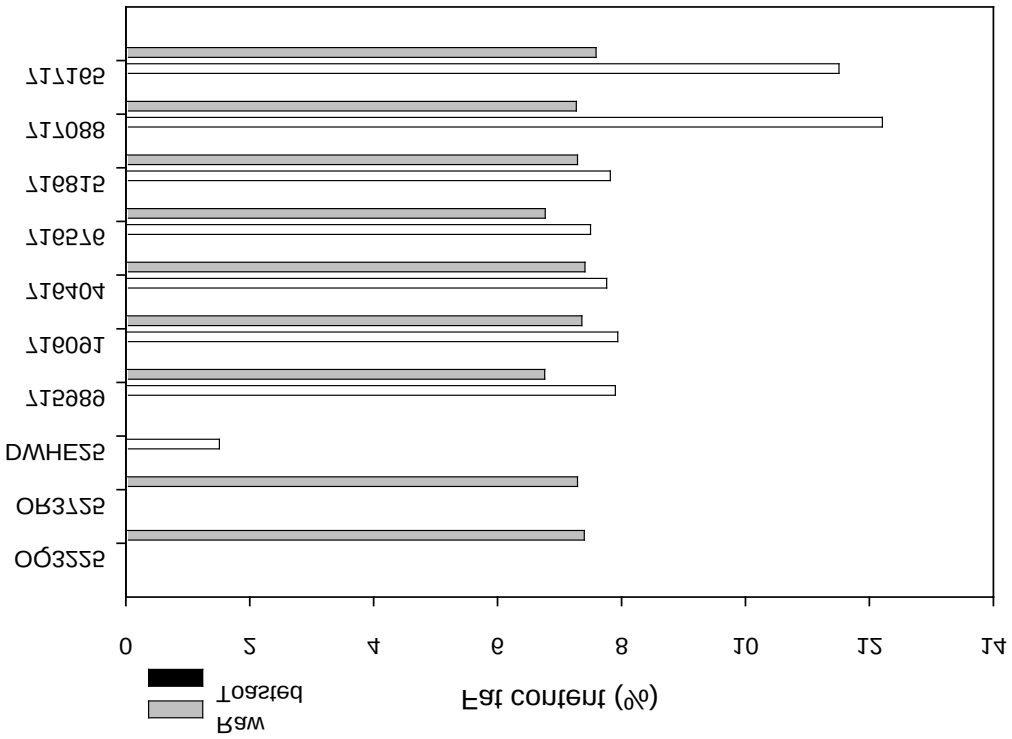


Figure 12. Fat content of industrial oats samples. Values are means of duplicates

Not much variation in free fatty acid content was observed among the 17 oats lots, with the exception of lot DWHE25 (Figure 13). The mean free fatty acid content of raw oats was 3.40 ± 0.59 %, expressed as grams of oleic acid equivalents per 100 g of oats, while that of toasted oats was 3.23 ± 0.29 %. The content of free fatty acids was about 5 % of total fats in the study of Heiniö et al. (2001).

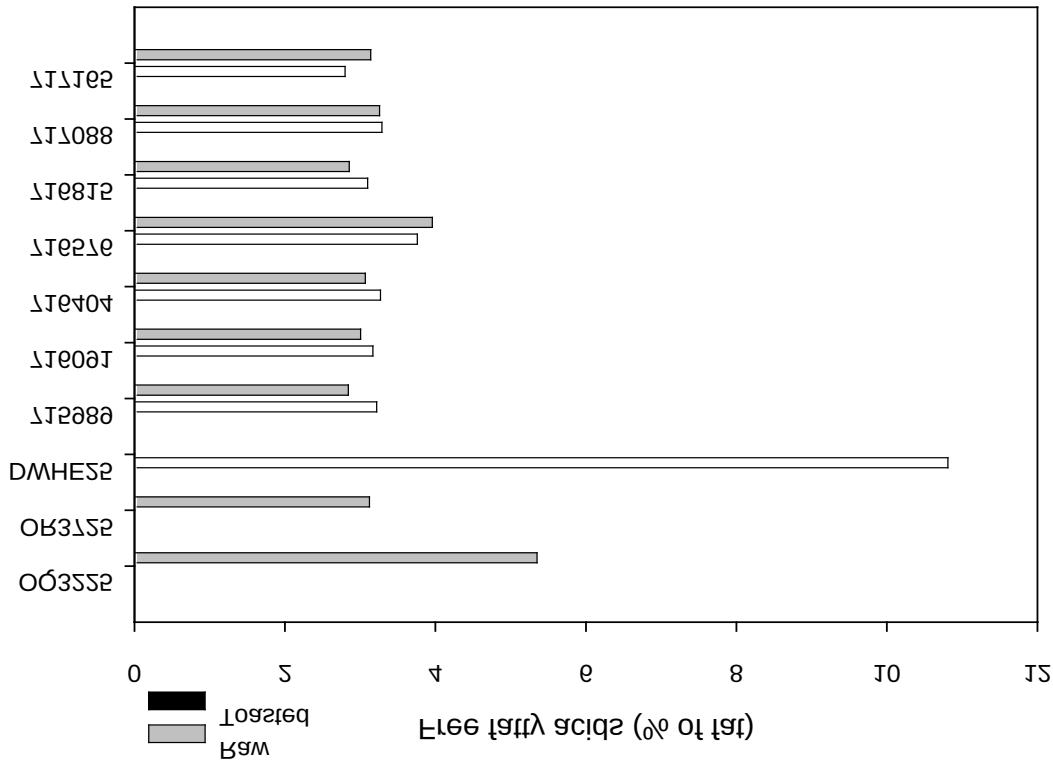


Figure 13. Free fatty acids content of industrial oats samples. Values are means of duplicates

The toasted lot DWHE25 – which also had a very high moisture content (see Figure 11) – had low fat content (1.50 %, w/w) but very high level of free fatty acids, 10.80 % of oats fat. It was clearly a faulty product, and as noted earlier, this lot was very bitter.

3.2.4 *p*-Anisidine value

The preliminary trial showed that the *p*-anisidine value test was applicable for the soyabean oil but not for the fat extract from oats. Under conditions where the *p*-anisidine value of soybean oil was around 1 (a dimensionless value), oats fat returned a negative value. The oats test was plagued with brown precipitates.

The fat in solution, even though passed through a 0.45 µm micro-filter was still slightly cloudy. The absorbance of the fat solution was 0.934. The fat solution with anisidine treatment resulted in a clear solution but with a dense brown precipitate (Figure 13). The absorbance of this solution was 0.389. The calculation to determine *p*-anisidine value of oats fat returned a negative answer.

That suggested the fat extract was contained with materials of unknown status that confounded with the result, so the test was not applied to oats fat.

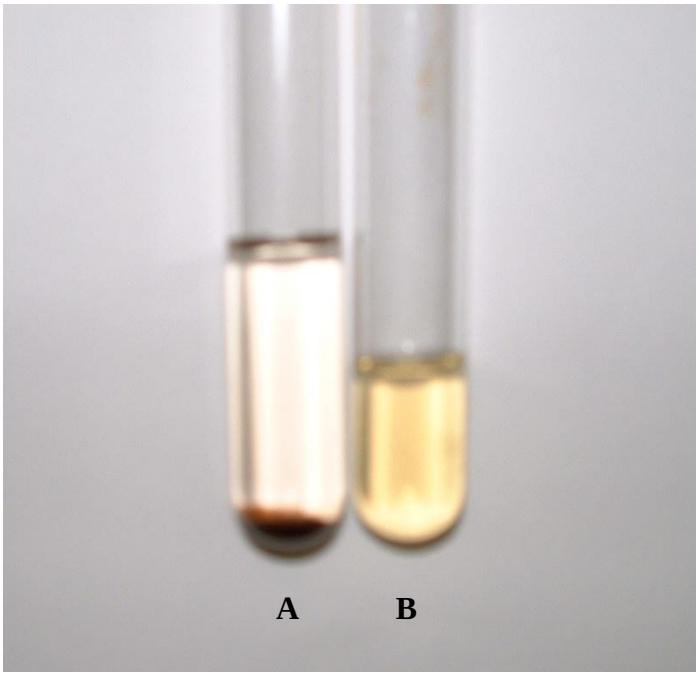


Figure 14. Reaction of oats fat and soybean oil with *p*-anisidine reagent treatment. A, brown precipitate in oats fat solution treated with *p*-anisidine; B, soybean oil with *p*-anisidine treatment

3.2.5 Total phenols

The phenols concentration in methanolic solution was determined using the standard curve (Figure 8), and the results calculated as milligrams of phenols per gram of oats.

The results showed that the variation of total phenols in 17 lots was high (Figure 15). The average content was 0.21 ± 0.09 mg g^{-1} and 0.32 ± 0.03 mg g^{-1} for raw and toasted oats, respectively. The values were lower than those reported by Heiniö et al. (2001). They found that the concentration ranges of phenols in two cultivars were 0.30 to 0.50 mg g^{-1} and 0.40 to 1.40 mg g^{-1}.

In the present study, toasted oats had a higher phenols content than untoasted oats, again probably due to the lower moisture content after toasting. In contrast to the results with fats and free fatty acids, lot DWHE25 did not present an unusual result, immediately suggesting that phenols were not the cause of bitterness.

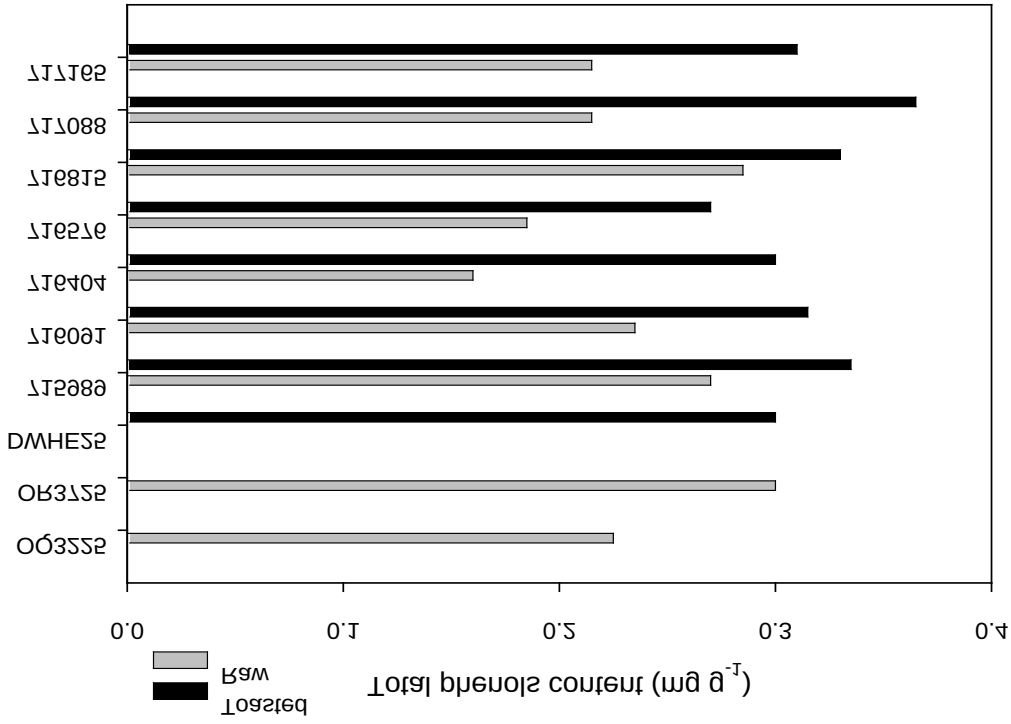


Figure 15. Total phenols content of industrial oats samples. Values are means of duplicates

3.2.6 Peroxidase activity

The peroxidase test based on the AACC 22-80 method was positive for most of the raw oats. The colour change (blue colour formation) was observed in seven of nine lots, and of these three were intensively blue to the point of scoring 3 or 4 on the numerical scale (Table 8). The blue colour variation was shown in Figure 16.

Any blue colour indicates the presence of peroxidase activity. These results are in direct conflict with the results reported on specification sheets prepared by the oats supplier that accompanied each lot delivered to Hubbard Foods.

Therefore, the method used by the supplier was examined (Appendix VIII) and was found to be deficient in one crucially important respect. The method omitted the key reactant hydrogen peroxide.

Figure 16. The colour observation of peroxidase testing. From left to right, increasing colour intensity indicates increasing peroxidase activity

A positive peroxidase response in any oats lot means that it is entirely possible for the oats lipase to be still active in those lots (Table 8). As noted in Chapter 2, if peroxidase is truly inactive, then lipase will also be inactive because the former is more heat stable. Active lipase is a potential cause of oat lipid oxidation.

Table 8. Peroxidase activity scores in raw oats as determined by the AACC 22-80 method, but with a numerical scoring scale

Lot	Activity[1]
717165	2
717088	2
716815	4
716576	2
716404	3
716091	0
715989	1
DWHE25[2]	
OR3725	2
OQ3225	3

[1]Activity increases in the sequence 0 to 4 where 0 means no trace of activity. [2]This lot had no untoasted sample

3.2.7 Bitterness

Data from Hubbard Foods were very fragmentary, which is a common outcome of in-house sensory testing in a demanding production-driven environment. Panellists are often scattered throughout the factory and focused on other tasks when tasting is required.

Bitterness data obtained at AUT are summarised in Figure 17. Most of the raw oats lots were not bitter or only slightly bitter, whereas toasted oats were consistently more bitter where paired lots could be compared. Bitterness perception was highly variable between panellists. This was probably due to the slow response for bitterness to develop in the mouth, and differing individual sensitivity.

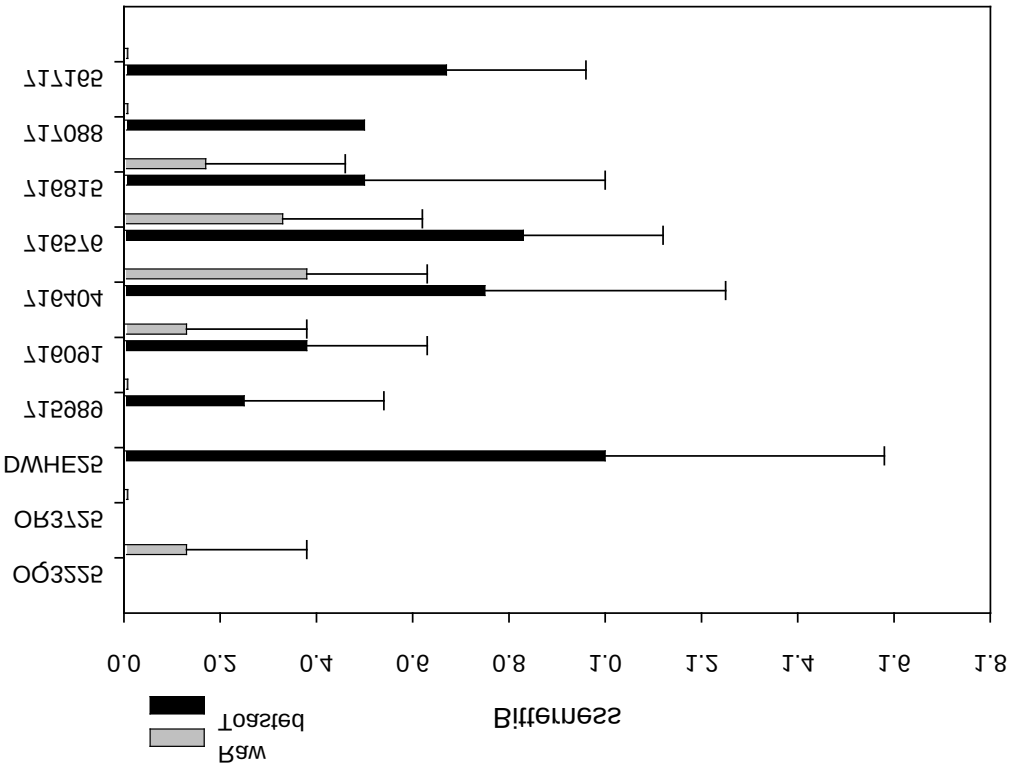


Figure 17. Bitterness and standard deviations for industrial oats samples (AUT data), where the standard deviation bars represent variation due to panellist. The bitterness scale was set from 0 to 2. In four cases, the very small values represent 0

3.3 Discussion of physicochemical and sensory tests

Although data were limited there was an opportunity to correlate the bitterness data with the physicochemical results (Table 9), at the same time realising that any outlying data points would strongly affect correlations. Many of the apparent correlations between raw and toasted oats were conflicting, but these differences may be real and important at a molecular level. Equally though, the bitterness levels were very low in the raw oats, so the correlations for raw oats may be just statistical noise.

Bitterness was higher in toasted oats, and that is where the technical problem occurs. The important correlations – if any – are the higher positive and negative values. Thus, lighter oats (more reflective) were more bitter, but there is no obvious causal link. A less intense toasting colour (chroma) was positively associated with bitterness, as were higher moisture contents. Chroma and moisture content may be linked, because wetter oats may be slower to brown. Thus, cause might be traced to moisture content through some biochemical

mechanism. If a lipase were continually active, and were supported by a higher moisture content, then the free fatty acids might be expected to be positively correlated with bitterness, as is plausible from Table 9. There is no obvious model linking a high fat content in toasted oats with low bitterness, unless the fat had become oxidised.

Total phenols do not appear to be related to bitterness (Table 9) (r = -0.22), which conflicts with Heiniö et al. (2001). They concluded that total phenols was the only sensory attribute correlating significantly with the bitter flavour, whereas in their work free fatty acids did not correlate significantly with any of the sensory attributes evaluated.

Table 9. Correlation coefficients between physicochemical results and bitterness in industrial oats lots

Physicochemical measure	Bitterness	
	Raw oats	Toasted oats
Lightness	-0.33	0.69
Hue angle	-0.01	0.02
Chroma	0.17	-0.71
Moisture	-0.73	0.66
Fat content	-0.18	-0.52
Free fatty acids	0.22	0.67
Total phenols	-0.63	-0.22

As noted above, the weak links between free fatty acids and bitterness may have their origins in peroxidase activity, because if peroxidase remains active then lipase may also be active. This link is examined in Table 10.

Table 10. Correlation coefficients between peroxidase activity and free fatty acids, bitterness in industrial oats lots

	Peroxidase activity	
	Raw oats	Toasted oats
Free fatty acids	0.25	-0.04
Bitterness	0.42	0.33

The peroxidase activity was weakly but positively correlated to the concentration of free fatty acids and bitterness in raw oats (r = 0.25) (Table 10). For toasted oats, free fatty acids

concentration did not correlate with peroxidase activity (r = -0.04). It remains possible that free fatty acids indirectly linked to peroxidase activity, may undergo peroxidation to secondary oxidation products that may themselves be bitter (r = 0.33).

The correlations are also expressed in scatter plots, which will show the effects of any outlying data. Figures 17, 18 and 19 present the detail of the correlations between the physicochemical results and bitterness.

Figures 17 and 18 plot the physicochemical results of raw oats against bitterness in raw and toasted oats. For raw oats (Figure 18), only free fatty acids and peroxidise activity appeared positively related to bitterness, but only very weakly. Total phenols content was not positively related. For toasted oats (Figure 19), these three relationships were confirmed. Indeed, total phenols appeared negativity related.

Figure 20 plots physicochemical results in toasted oats against bitterness in those toasted oats. At first sight some relationships appear useful, but many were critically dependent on a single data point (lot DWHE25). Thus a single value of 10.8 % effectively defined the correlation between free fatty acid and bitterness. Similarly, a single moisture content value of 12.93 % and a single fat content value of 1.51 % defined the correlations. Figure 20 also plots bitterness in raw and toasted oats. The graph hints that raw oats with a slight bitter flavour will have continuous, even stronger bitterness after toasting.

In summary, the limited data suggest that phenols are not responsible for bitterness, but that moisture content, peroxidise activity, and free fatty acids content are involved. With these possibilities in mind, attention was then directed to generating bitterness in industrial and retail oats samples by water addition to simulate higher moisture contents (Chapter 4).

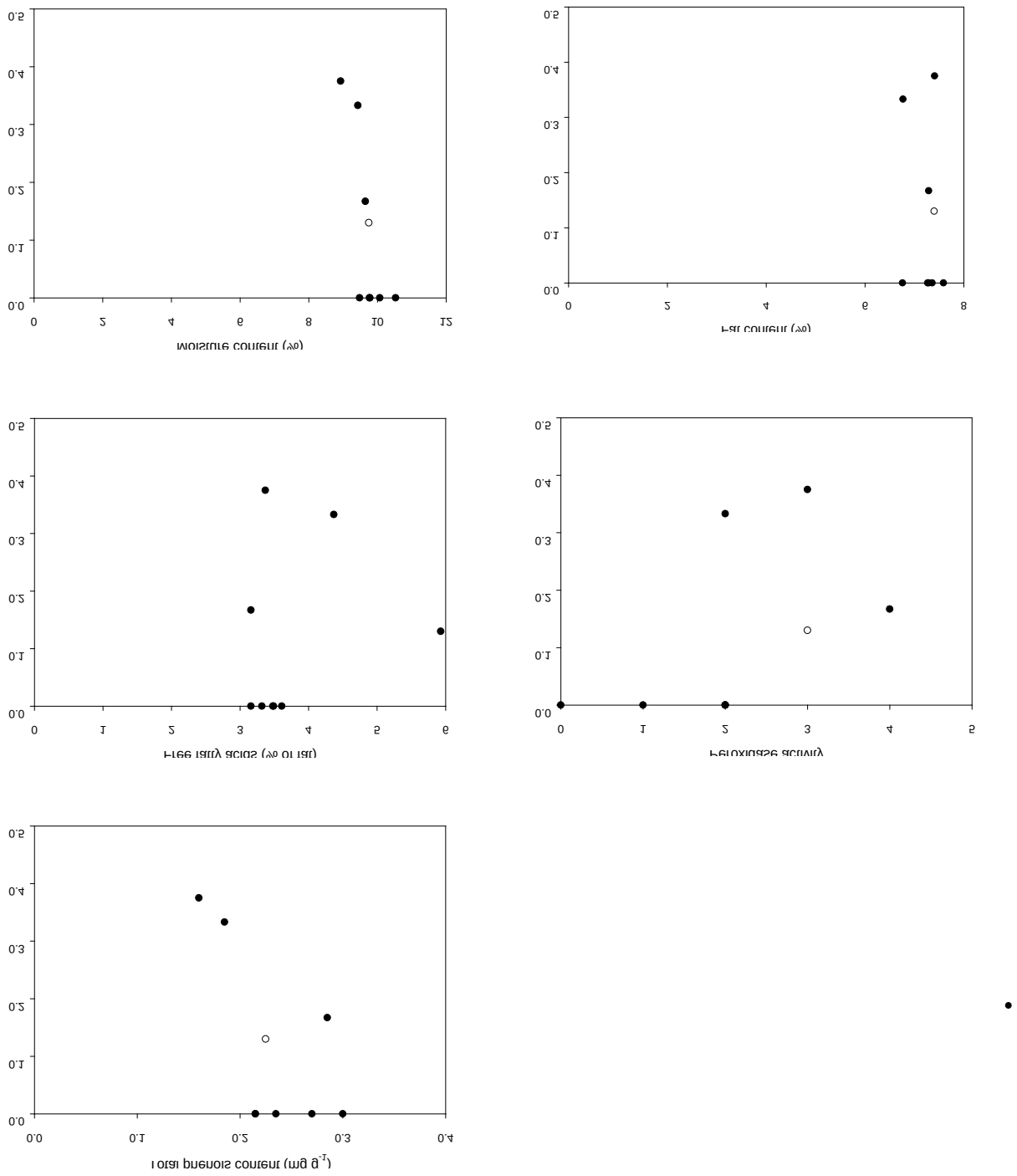


Figure 18. Scatter plots between physicochemical results and bitterness in raw industrial oats samples

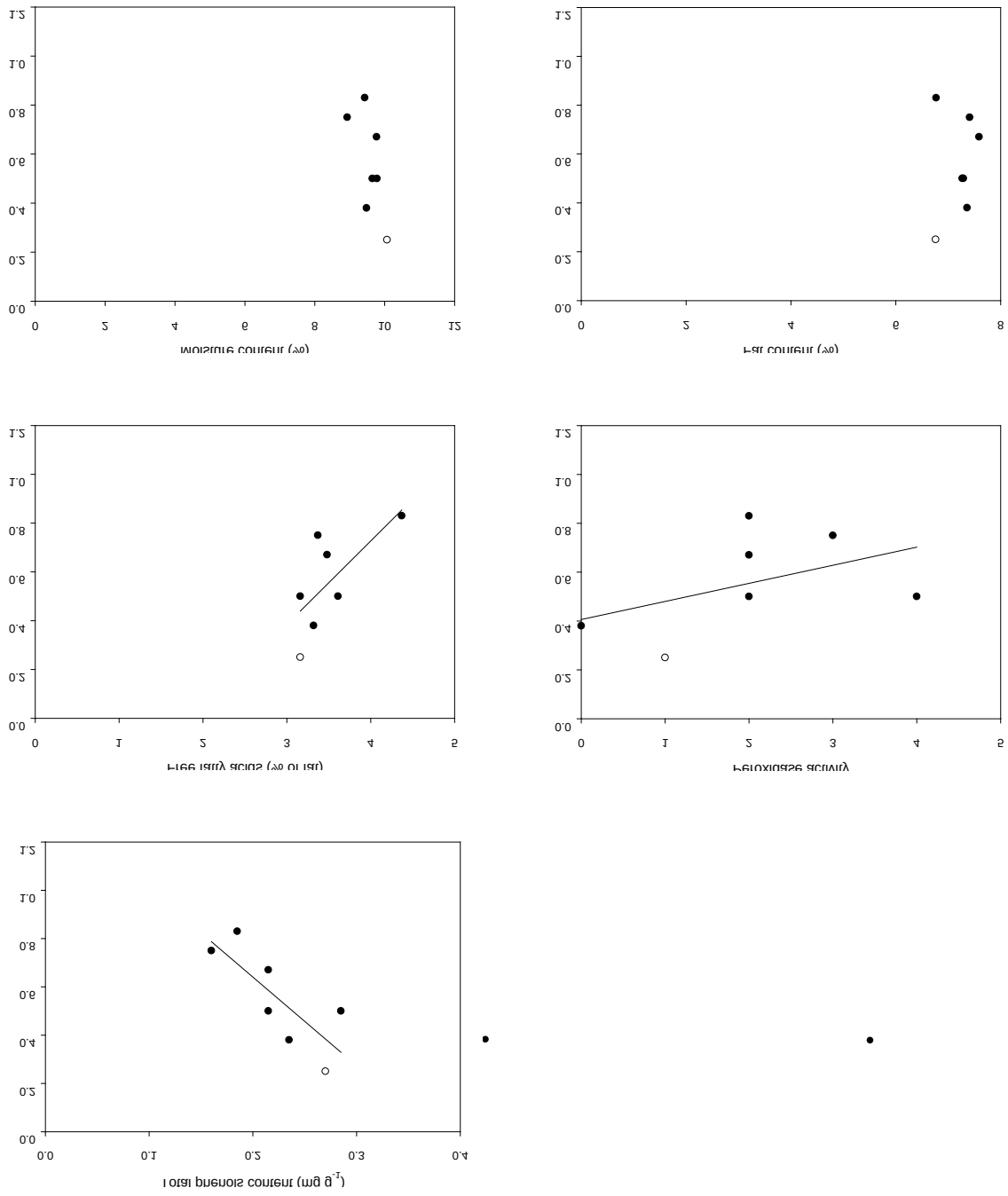


Figure 19. Scatter plots between physicochemical results of raw oats and bitterness of toasted paired oats samples. Correlation lines are shown where there is a suggestion of a significant relationship

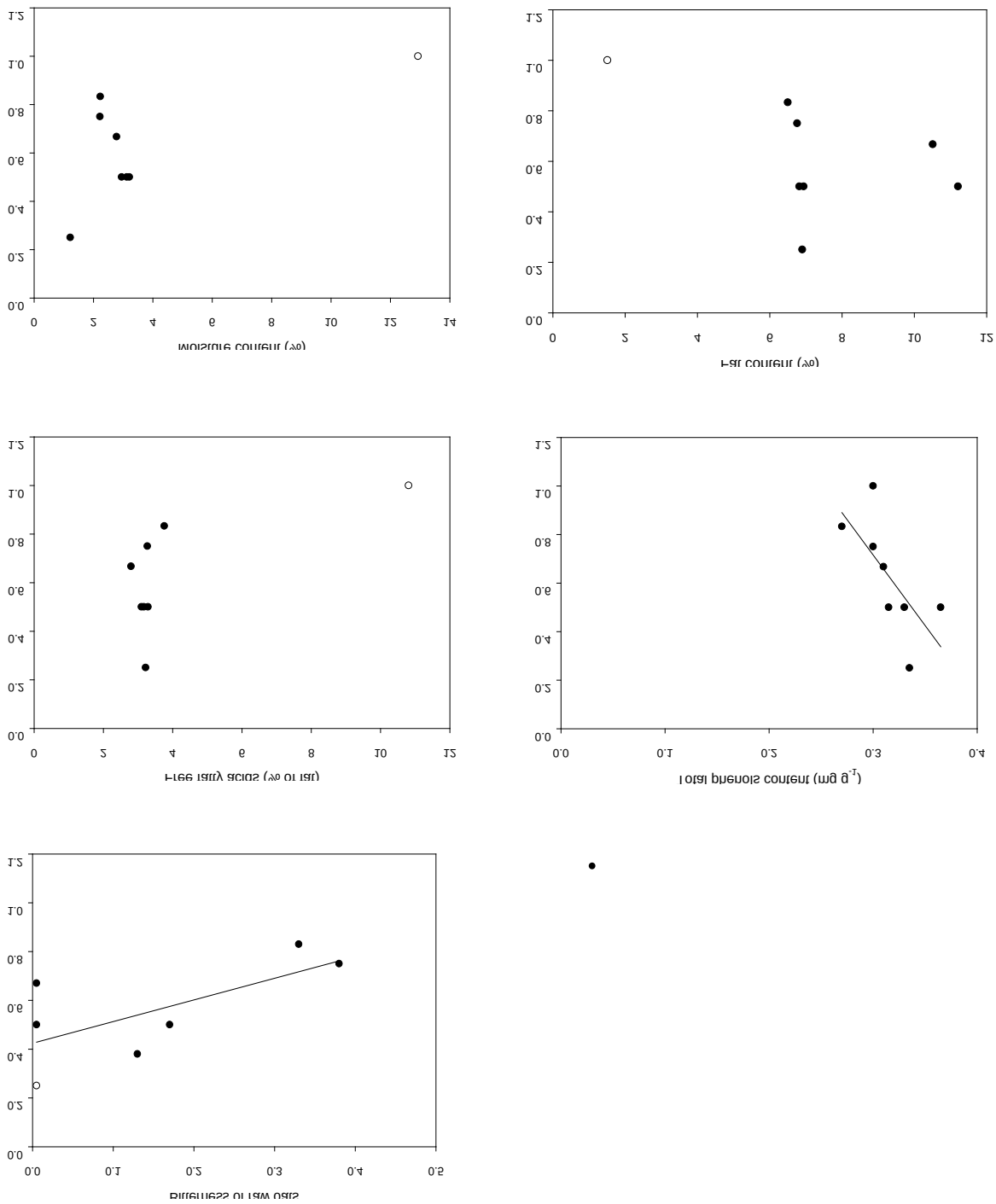


Figure 20. Scatter plots between physicochemical results and bitterness in toasted industrial oats samples. Correlation lines are shown where there is a suggestion of a significant relationship

Chapter 4

Results and Discussion for Laboratory Generated Samples

4.1 Introduction

In Chapter 3, analysis of the limited data set found there were no clear causal relationships between physiochemical measures and oats bitterness, but some measures were nonetheless implicated in bitterness, in particular, free fatty acids and residual peroxidase activity. A possible causal model is that residual peroxidase activity might indicate residual lipase activity, which in higher moisture oats samples would lead to fat hydrolysis and subsequently peroxidation of free fatty acids. These latter reactions would be linked to bitterness. In this chapter, experimental models were developed to test this model.

Higher moisture oats lots were prepared from Hubbard Foods oats and also oats sourced from a supermarket (four lots, PK7942A, B, C, and D, from Pak'n Save, Auckland). The method for controlled water addition and toasting was described in Chapter 2, yielding about 50 treatments of paired raw and toasted oats.

4.2 Results of physicochemical and sensory tests

4.2.1 Peroxidase activity

The peroxidase activities of the Hubbard oats were previously shown in Table 8 (Chapter 3). The peroxidase activity of the four retail oats lots were similarly determined by the AACC 22-80 method. All retail oats lots were peroxidase positive. Lots PK7942A, B, and D had similar scores '2', respectively, while lot PK7942C had a higher score '3'.

The peroxidase activities of the four retail lots and the five selected Hubbard oats which were used for higher moisture treatment are shown in Table 11, which shows that the selected lots had a wide range of peroxidase activity.

Table 11. Peroxidase activity scores in selected raw oats as determined by the AACC 22-80 method, but with a numerical scoring scale

Lot	Activity[1]
PK7942D	2
PK7942C	3
PK7942B	2
PK7942A	2
716815	4
716576	2
716404	3
716091	0
OQ3225	3

[1]Activity increases in the sequence, 0 (no trace of activity) to 4 (high activity)

4.2.2 Colour

The colours of the 50 laboratory-generated toasted lots were compared with those of 17 as-received industrially toasted oats lots, by a non-paired t-test with Minitab 14 (Appendix IX). There were no significant differences for lightness, hue angle or chroma ($P > 0.05$ for each attribute). Therefore, the colour values of toasted laboratory-generated oats samples matched those of industrially toasted oats from Hubbard Foods, indicating that the laboratory-generated oats would represent an adequate model.

4.2.3 Fat content and free fatty acids content

Molteberg et al. (1995 and 1996) indicated that the fat content was reduced at high relative humidity and the free fatty acids content in oats was positively correlated with relative humidity and storage time.

In the present study, six oats lots, OQ3225, 716091, 716404, 716576, PK7942A, and PK7942B (Hubbard and retail) were selected to examine the effect of added water. In these trials, the added water contents were 0, 3 and 6 % by weight. The statistical analyses compared six oats lots at three water addition levels, 0, 3 and 6 % added water, stored for two weeks then toasted. Therefore, there were a total of 18 raw treatments and 18 toasted treatments.

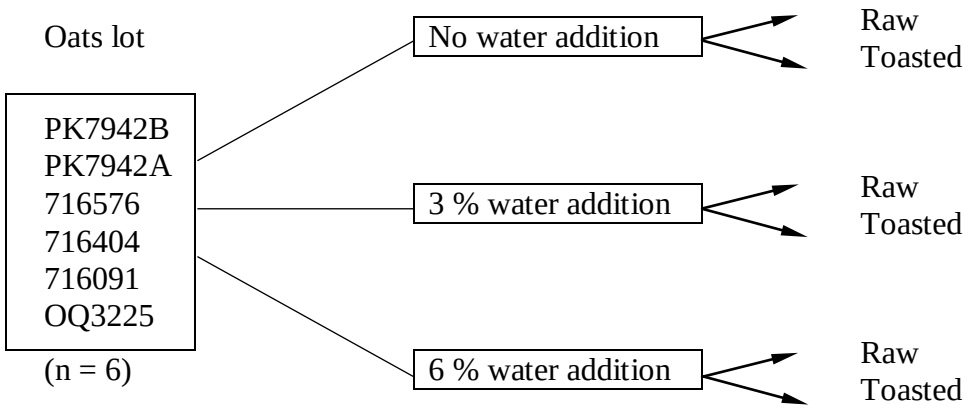


Figure 21. Experimental design for laboratory generated samples

The values presented in Table 12 are mean fat contents of six lots within each water addition treatment. A two-way analysis of variation (Appendix X) showed that the mean fat contents were significantly different both between oats lots for both raw and toasted oats ($P < 0.001$ and 0.01 respectively). After the effect of the variance caused by oats lots was removed by the analysis, the fat content of raw and toasted oats was found to decrease with increasing water addition ($P < 0.001$ and < 0.05, respectively) (Table 12). Tukey's test applied to raw and toasted also confirmed the significant differences caused by water addition (Appendix XI). (No individual standard deviations are presented for the means because those values would only express the large variance due to oats lots. But the relevant pooled standard deviations were 1.07 and 1.52 % for raw and toasted, respectively.)

The decrease in fat content could be simply due to the increase in weight due to water addition, but may also include true fat loss.

Toasting caused an average 0.65 (t-test) ($P = 0.006$) percentage point decrease in fat content, but this paired difference is similar for all oats lots and all water contents.

There was no significant interaction between water addition and toasting. Because toasting to 150°C for one hour would drive off all free water, the reduction in fat content in toasted oats where water had been added was real.

Table 12. Mean fat content (percent of oats weight) for laboratory generated oats samples

Water addition (%)	Raw oats[1] (n = 6)	Toasted oats[1] (n = 6)
0	6.89	6.73
3	6.59	5.86
6	6.40	5.35
Statistical effect[2] of:		
Water addition	***	*
Toasting	**	
Interaction (water/toasting)	NS	

[1]Values are means, for which the pooled standard deviations are 1.07 for raw and 1.52 for toasted. [2]NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

The same two-way analysis of variance was applied to free fatty acids content, expressed as percent of fat. Oats lots significantly affected the content in both raw and toasted samples ([2]data not shown). With this variation removed it was found that the free fatty acids content increased with increasing water addition in raw and toasted oats (Table 13). The overall effects were significant at $P < 0.05$ and $P < 0.01$, respectively. Toasting caused an increase in free fatty acids content ($P < 0.001$), but there was no significant interaction between water addition and toasting. Since water addition had significant effects on both raw and toasted oats, and toasting also had a significant effect, the lack of interaction implies that whatever changes in free fatty acid were caused by water addition, the effect of toasting was strictly proportional to those changes.

Table 13 treats the water addition as a category factor in the two-way analysis of variance, but that water addition was ultimately manifest as continuous data in the form of moisture content in raw oats. This presents another way of looking at the results of water addition (Figure 22), where the moisture content of raw oats, rather than that of toasted oats, is of more interest. Once toasted the final moisture contents of oats will almost certainly be independent of the initial moisture contents. Recall that the bitterness hypothesis is that hydrolysis of fats would proceed during storage of raw oats and during the early stages of toasting, in both cases under the influence of moisture content at that time.

[2] The analysis details for Tables 12, 13, 15, 16 and 17 are exemplified by the results in Appendices X and XI.

Table 13. Mean free fatty acids as oleic acid (percent of fat) for laboratory generated oats samples

Water addition (%)	Raw oats[1] (n = 6)	Toasted oats[1] (n = 6)
0	4.71	5.46
3	4.98	6.33
6	5.24	6.82
Statistical effect[2] of:		
Water addition	*	**
Toasting	***	
Interaction (water/toasting)	NS	

[1]Values are means, for which the pooled standard deviations are 1.48 for raw and 1.14 for toasted. [2]NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Correlations between free fatty acids content and moisture content of the raw oats are shown in Figure 22. There was a positive linear relationship between free fatty acids content and moisture content for both raw and toasted oats samples, which was clearer for toasted oats, as was expected from the statistical analysis in Table 13.

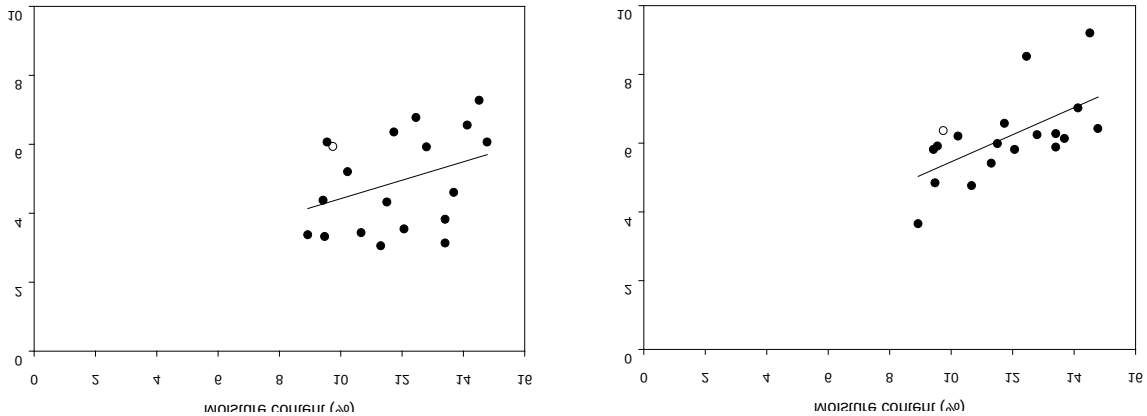


Figure 22. Correlation between free fatty acids content and untoasted moisture content of laboratory generated oats samples. The correlation for toasted oats was significant ($P < 0.01$)

To investigate the effect of storage time on the fate of fat in oats, lots PK7942D and 716815, both with peroxidase activity '2' and '4' respectively, were treated with water to 5 % addition, and stored at room temperature for 0, 2, 4, and 6 weeks. They were then toasted

and analysed to determine the effect of storage time. Data were not analysed statistically because there were only two replicates, each with a different peroxidase activity.

For both lots, numerical values for fat content decreased and free fatty acids increased with storage time (Figure 23). Figures 23 a and c show the details for lot PK7942D paired raw and toasted oats. The fat content decreased and free fatty acids increased slightly with the increasing of water addition. Figures 23 b and d are for lot 716815 paired raw and toasted samples. Generally, the changes in toasted samples were more obvious, particularly in lot 716815 (Figures 23 b and d). It is possible, but not proven, that the higher peroxidase activity in lot 716815 would reflect a higher residual lipase activity that would in turn promote fat hydrolysis.

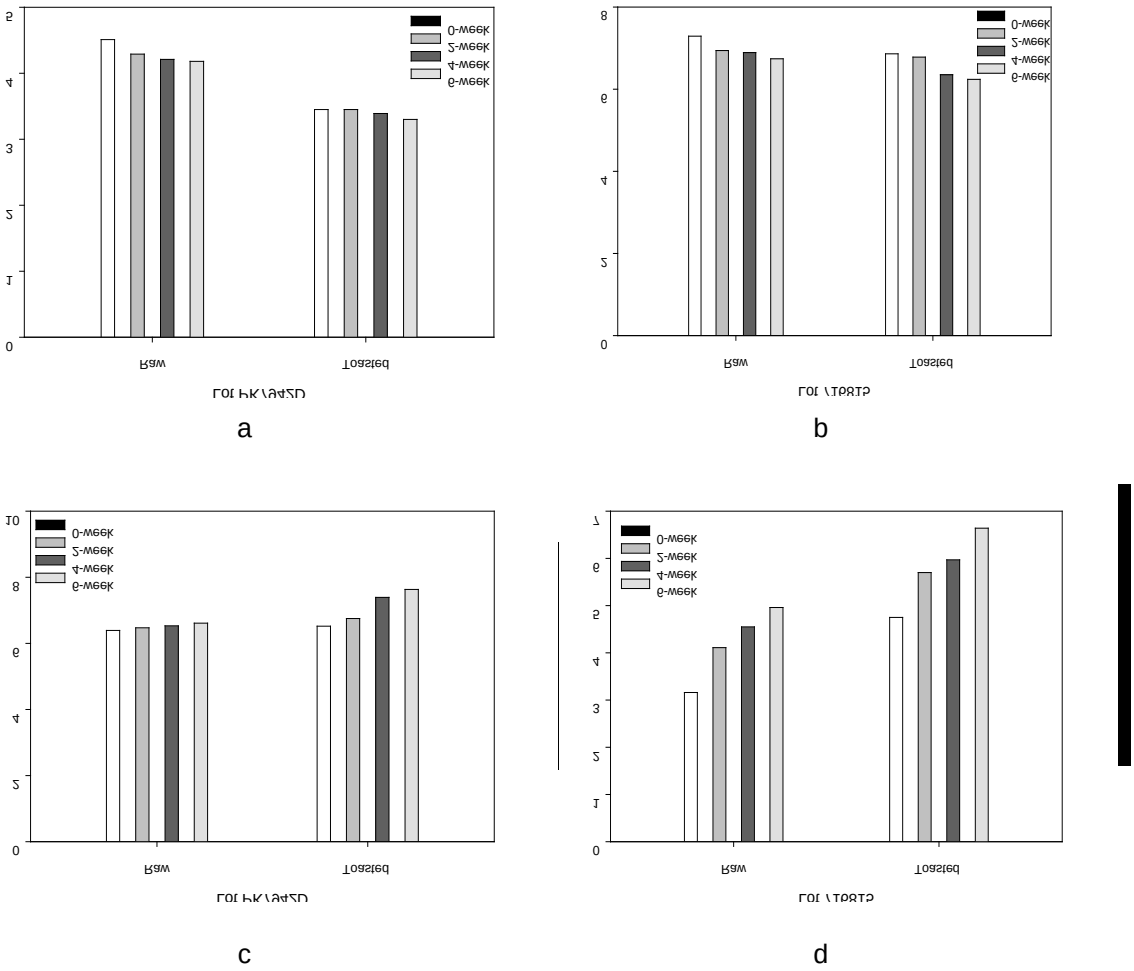


Figure 23. Effect of storage time on fat content and free fatty acids content

4.2.4 Fatty acids composition

Using the same two oats lots (PK7942D and 716815), analysis of fatty acid methyl esters (FAMEs) identified four principal fatty acids in oats. These were palmitic (16:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acid (Figure 24). The mass spectra were displayed in Appendix XIII. Oleic and linoleic acid were the major fatty acids, consistent with the findings of McKechnie (1983).

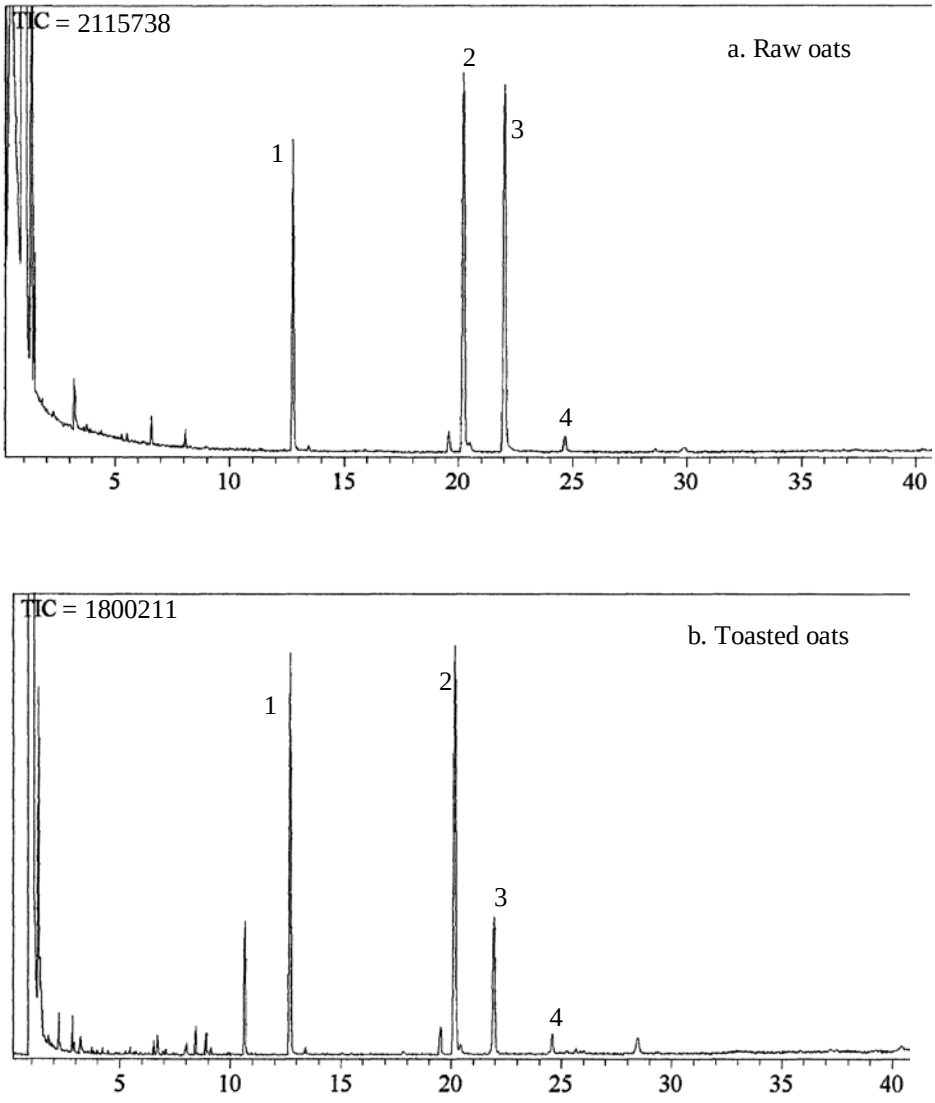


Figure 24. Typical chromatograms of FAMEs analysis by GC-MS, for raw (a) and toasted (b) oats, in this case lot 716815. 1, palmitic acid (16:0); 2, oleic (18:1); 3, linoleic (18:2); 4, linolenic (18:3)

Heiniö et al. (2001) pointed that an increase in the amount of free fatty acids indicates the hydrolysis of lipids, while a decrease in the degree of unsaturated indicates the oxidation of lipids. After toasting, the contents of oleic and linoleic acid decreased relative to palmitic (Figure 24), shown here for lot 716815 but also seen in lot PK7942D. In particular, the content of linoleic acid fell severely. The results indicate that toasting has a considerable effect on the stability of unsaturated fatty acids.

These data were quantified with respect to the internal standard, tricosanoic acid (Table 14). The amount of the four principal fatty acids was calculated as relative ratios to the tricosanoic internal standard. The percentage decrease after toasting was 19 for palmitic acid, 48 for oleic, 71 for linoleic, and 67 for linolenic.

Table 14. Ratios of fatty acids in raw and toasted oats for lot 716815 relative to the tricosanoic internal standard

	Relative ratio		Percentage decrease after toasting
	Raw oats	Toasted oats	
Tricosanoic acid	100	100	
Palmitic acid	32	26	19
Oleic acid	56	29	48
Linoleic acid	63	18	71
Linolenic acid	1.8	0.6	67

4.2.5 Total phenols

A two-way analysis of variance was similarly applied to total phenols. The effect of oats lots was significant for raw oats ($P < 0.05$), but not for toasted oats. With the statistical effect of oats lots removed, phenols increased with increasing added water in raw oats ($P < 0.01$), which contrasts with the results for fat (Table 12), where means decreased. The effect of added water was not significant in toasted oats (Table 15). Over the six lots, the overall mean content was 0.24 and 0.36 mg g^{-1} for raw and toasted oats, respectively (Table 15). The increase was significant ($P < 0.01$). The higher value for toasted oats may simply reflect their lower moisture content.

Table 15. Mean total phenols content (milligram per gram of oats) for laboratory generated oats samples

Water addition (%)	Raw oats[1] (n = 6)	Toasted oats[1] (n = 6)
0	0.18	0.35
3	0.25	0.34
6	0.29	0.38
Statistical effect[2] of:		
Water addition	**	NS
Toasting	**	
Interaction (water/toasting)	NS	

[1]Values are means, for which the pooled standard deviations are 0.05 for raw and 0.03 for toasted. [2]NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Figure 25 plots the correlation between phenols in raw and toasted oats against the moisture content of the raw oats. The respective raw and toasted correlation coefficients were 0.81 and 0.36 (Figure 25).

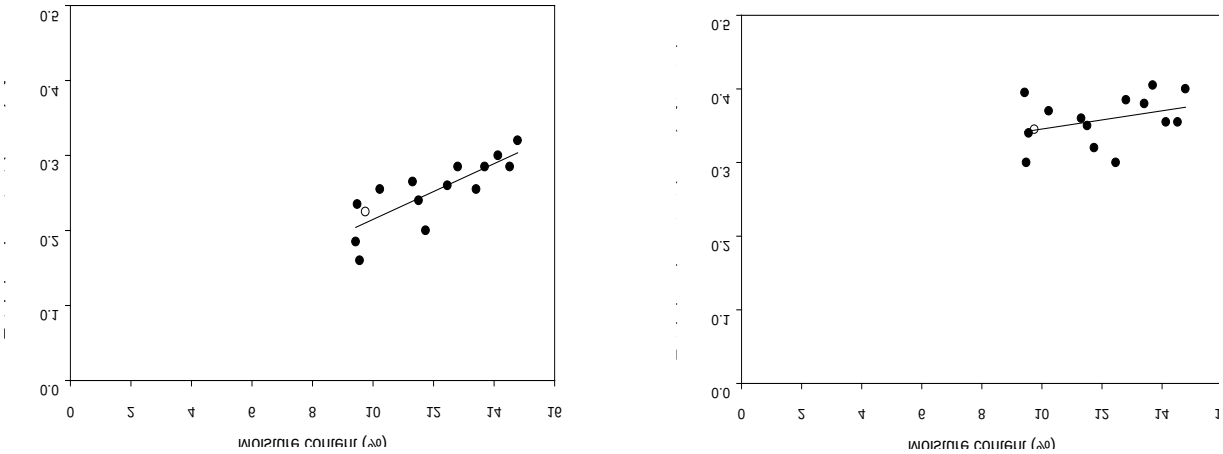


Figure 25. Correlations between phenols in raw and toasted oats and the untoasted moisture content of laboratory generated oats samples. The correlation for raw oats was significant (P < 0.01).

4.2.6 Volatile compounds

The standard heating time prior to exposure of oats samples to SPME fibres was determined in a time course experiment. Five equal weight samples of lot DWHE25 were heated for 1, 2, 3, 4 hours or overnight and each exposed for 15 minutes. Heating beyond one hour was of

no advantage (Appendix XIII). In a related series of trials, a 15 minute fibre exposure was found to be adequate, as was a six minute desorption time. There were no significant differences in the efficiency of the SPME fibre with minor increases of the moisture content of oats samples.

The analysis of volatile lipid oxidation products released from oats heated to 60 °C for one hour was performed using the SPME-GC technique, with a 15 minutes fibre exposure. A typical chromatogram of the test samples is presented in Figure 26. The aldehydes, pentanal, hexanal, and nonanal, as well as 2-pentylfuran were well resolved and identified by retention times of authentic standards and confirmed by mass spectrometry.

A two-way analysis of variance revealed that the variation of relative headspace volatiles between different oats lots was not significant for pentanal, hexanal, and nonanal in raw oats, but differences were observed for 2-pentylfuran ($P < 0.05$, data not shown). In toasted oats, pentanal and 2-pentylfuran were affected by oats lot (both $P < 0.05$, data not shown).

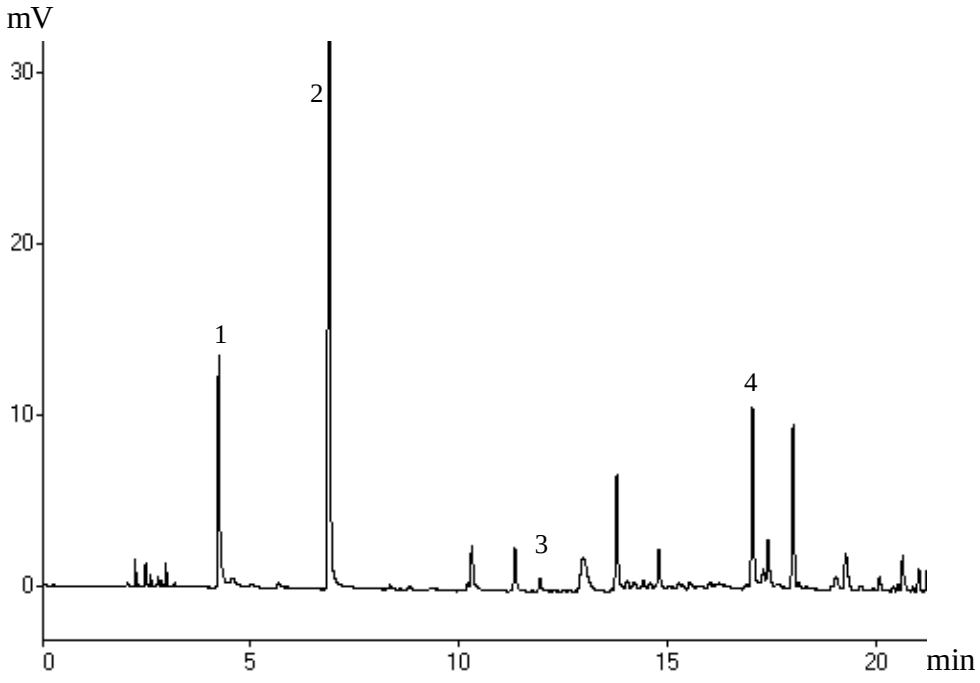


Figure 26. Chromatogram of oats (lot 716815) headspace obtained by SPME. 1, pentanal; 2, hexanal; 3, 2-pentylfuran; 4, nonanal

For both raw and toasted oats, only hexanal showed significant changes ($P < 0.05$) with water addition (Table 16), where increases occurred. Hexanal was also the most dominant volatile as is clear from Figure 26, and was almost certainly derived from the loss of linoleic acid (Belitz et al., 2004). Toasting caused large increases in volatiles ($P < 0.001$), except for 2-pentylfuran (NS).

There was no interaction between water addition and toasting. As with free fatty acids in Table 13, this implies that the changes in hexanal was caused by water addition to raw oats, and that the effect of toasting was strictly proportional to those changes. In other words toasting directly mirrored what already occurred in raw oats.

Table 16. Effect of added water on the headspace concentration of volatiles of laboratory generated oats lots relative to a pentanal external standard. Values are relative ratios (area of volatile compound/area of standard pentanal).

Water addition (%)	Pentanal[1]		Hexanal[1]		2-Pentylfuran[1]		Nonanal[1]	
	Raw (n = 6)	Toasted (n = 6)	Raw (n = 6)	Toasted (n = 6)	Raw (n = 6)	Toasted (n = 6)	Raw (n = 6)	Toasted (n = 6)
0	0.02	0.30	0.18	1.15	0.03	0.07	0.03	0.50
3	0.03	0.30	0.40	1.56	0.06	0.08	0.11	0.50
6	0.04	0.32	0.37	1.76	0.08	0.09	0.09	0.57
Pooled SD[1]	0.02	0.17	0.13	0.35	0.07	0.04	0.07	0.02
Statistical effect[2] of:								
Water addition	NS	NS	*	*	NS	NS	NS	NS
Toasting	***		***		NS		***	
Interaction (water/toasting)	NS		NS		NS		NS	

[1]Values are means, with pooled standard deviations (SD).
[2]NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

4.2.7 Bitterness

The perception of bitterness was highly variable between panellists, especially for toasted oats samples. This is reflected in the high standard deviations, as illustrated in Figure 27.

Table 17. Mean panellist bitterness values and standard deviations for laboratory generated oats samples

Water addition (%)	Raw oats[1] (n = 6)	Toasted oats[1] (n = 6)
0	0.20	1.08
3	0.28	1.50
6	0.36	1.70
Statistical effect[2] of:		
Water addition	NS	**
Toasting	***	
Interaction (water/toasting)	*	

[1]Values are means, for which pooled standard deviations are 0.15 for raw and 0.20 for toasted. [2]NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Panellist means of bitterness data of laboratory generated oats lots were statistically analysed (Table 17). The factor of oats lots was not statistically significant, and this is also clear by inspection of Figure 27. Water addition to 6 % had no significant effect on bitterness of raw oats (Table 17). However, addition caused a significant increase ($P < 0.01$) in bitterness in toasted oats, increasing from a mean of 1.08 to 1.70 bitterness units (Table 17). Most of the raw oats lots were only slight bitter, whereas bitterness was about 5-fold higher after toasting ($P < 0.001$).

Bitterness in toasted oats was significantly correlated with moisture content of raw oats (Figure 28) for which r = 0.85 ($P < 0.001$). Some samples were very bitter. For example, the bitterness score of toasted lot OQ3225 with 6 % added water was judged to have exceeded the defined bitterness scale by all panellists, but scores were nonetheless recorded as 2.0. There was an interaction between water addition and toasting treatment ($P < 0.05$, Table 17), which is particularly clear in Figure 28, which plots the correlation between bitterness and moisture content. The response in raw oats was almost flat while that in toasted oats was steep. This confirms that any bitterness in raw oats was unimportant. Only toasting

mattered. It was concluded that toasting revealed any latent bitterness, particularly in high moisture content oats samples.

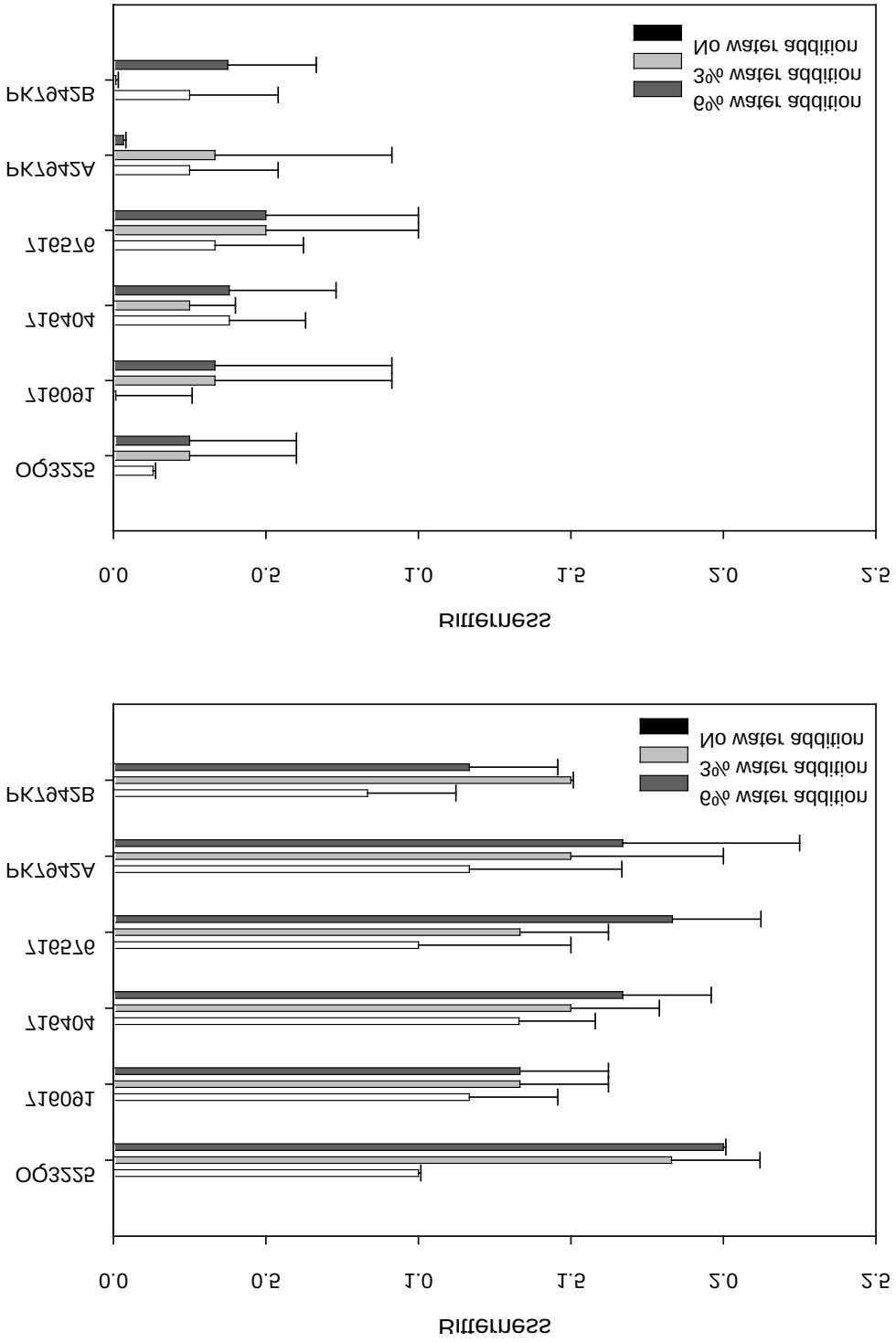


Figure 27. Bitterness and standard deviations for laboratory generated samples. The bitterness scale was 0 = not at all bitter, to 2 = very bitter

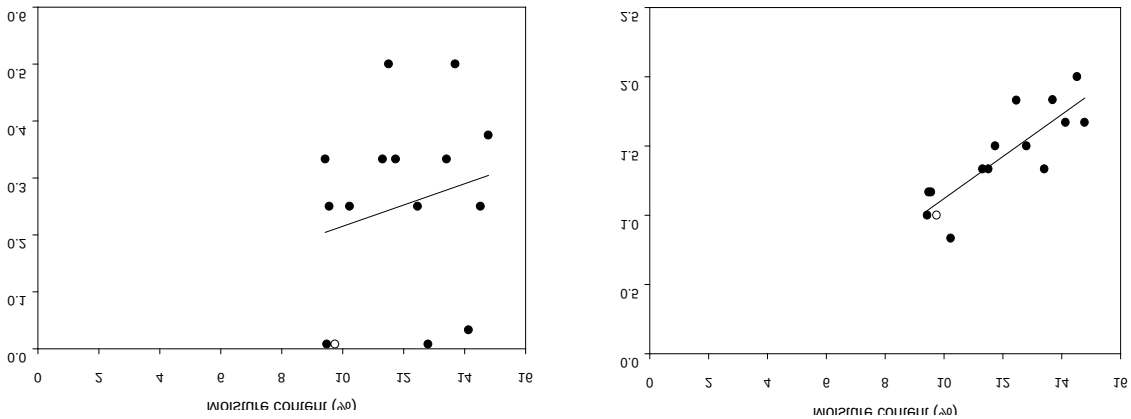


Figure 28. Correlation between bitterness and untoasted moisture content of laboratory generated oats samples. The correlation for toasted oats was significant ($P < 0.001$).

4.3 Correlation and discussion of physicochemical and sensory data

Based on the findings of Chapter 3, the work on laboratory generated oats lots focused on fat content, free fatty acids content, volatile compounds, total phenols content, and the correlations between these attributes with bitterness.

The study of 58 paired, raw- and toasted-oats samples returned complete sets of data. For each of the raw and toasted treatments, water addition and storage data were pooled to represent a range of conditions that might be encountered in industrial processing. The correlations between chemical measures and bitterness are shown in Table 18. The main focus was on bitterness in toasted oats, because bitterness values were much higher here (Table 17) and that is the commercially important product.

The most striking positive correlation was between free fatty acids content and bitterness in toasted oats, r = 0.71 The fatty acid oxidation products hexanal and nonanal were also positively correlated well with bitterness. The correlation between fat content in toasted oats and bitterness was a negligible 0.08, but for raw oats was -0.36. The reason for the high negative correlation may be as follows. Water addition and storage caused a decrease in fat content (Table 12), presumably because free fatty acid formation would be accelerated. The hydrolysis and subsequent oxidation products would likely be positively correlated with

bitterness. However, this model depends on the extent to which free fatty acids would be included in the measure of fat and the extent of oxidation. Total phenols were poorly correlated with bitterness in toasted oats (Table 18). Peroxidase activity was weakly related to bitterness in untoasted oats (r = 0.02), but positively correlated with bitterness in toasted oats (r = 0.43).

Table 18. Correlation coefficients between chemical measures and bitterness in laboratory generated oats lots

Chemical measure within raw and toasted categories	Bitterness	
	Raw oats (n = 29)	Toasted oats (n = 29)
Fat content	-0.36	0.08
Free fatty acids	0.09	0.71
Pentanal	0.12	-0.10
Hexanal	0.08	0.30
2-Pentylfuran	0.12	0.13
Nonanal	0.15	0.26
Total phenols	0.27	-0.02
Peroxidase activity (raw oats only)	0.02	0.43

The relationships between the various chemical measures and bitterness are further inspected using scatter plots in Figure 29 and Figure 30. The scatter plots are unimpressive for raw oats. The poor relationship between bitterness and free fatty acids and peroxidase activity were consistent with results in Chapter 3 where industrial oats samples had no special treatment. In Chapter 3 the total phenols content was negatively correlated with bitterness in raw oats (r = -0.63), but this result was not confirmed here (Table 18, Figure 29).

The correlation coefficient (0.71) between bitterness and free fatty acids in toasted oats was certainly high, but inspection of the plot in Figure 30 shows that the relationship is strongly dependent on two data points. Nonetheless, the result was consistent with the results in Chapter 3. In contrast, total phenols of toasted oats had no relationship with bitterness, contrasting with results in Chapter 3, where there was a negative relationship between total phenols and bitterness (r = -0.22).

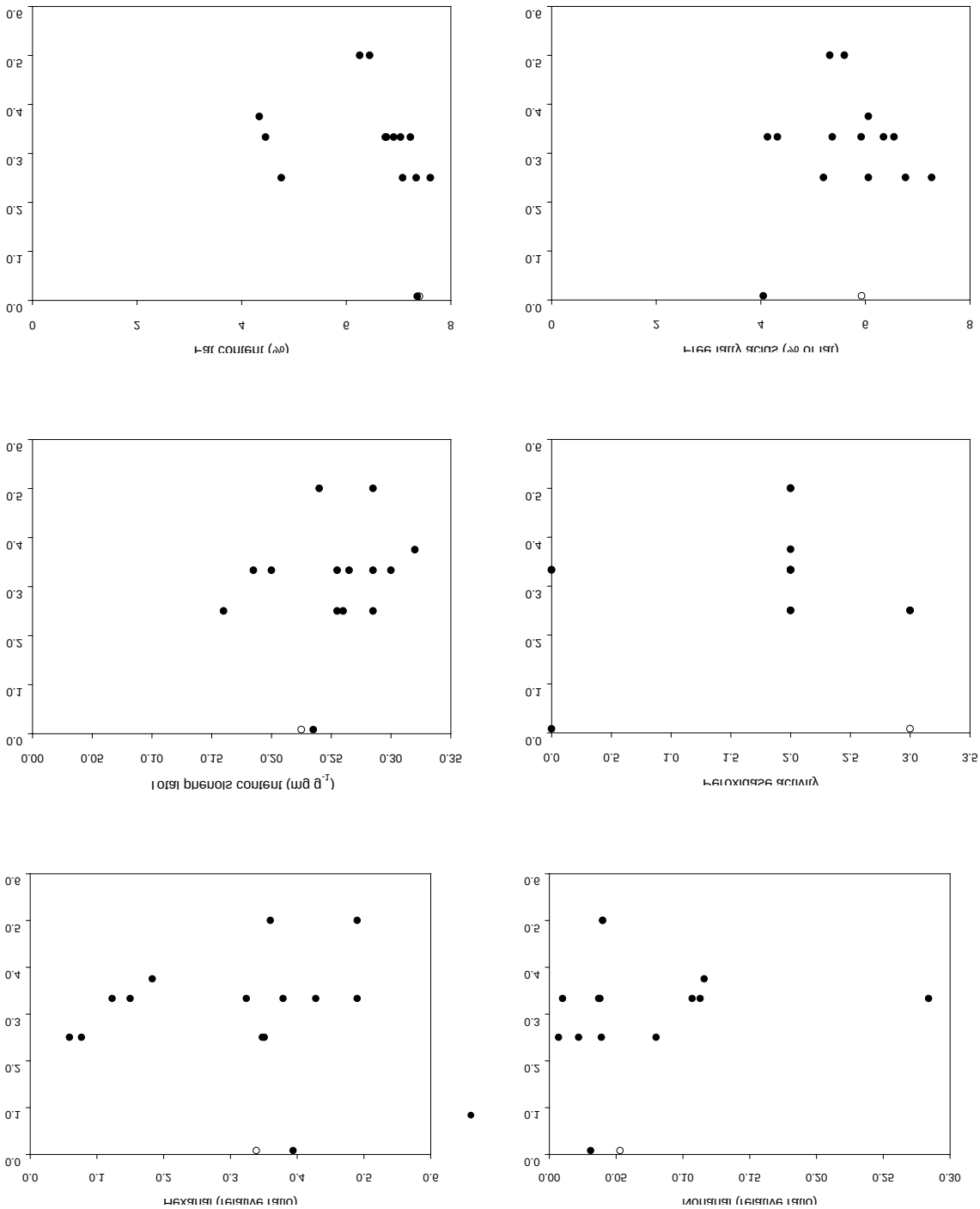


Figure 29. Scatter plots between physicochemical results and bitterness in raw laboratory generated oats samples

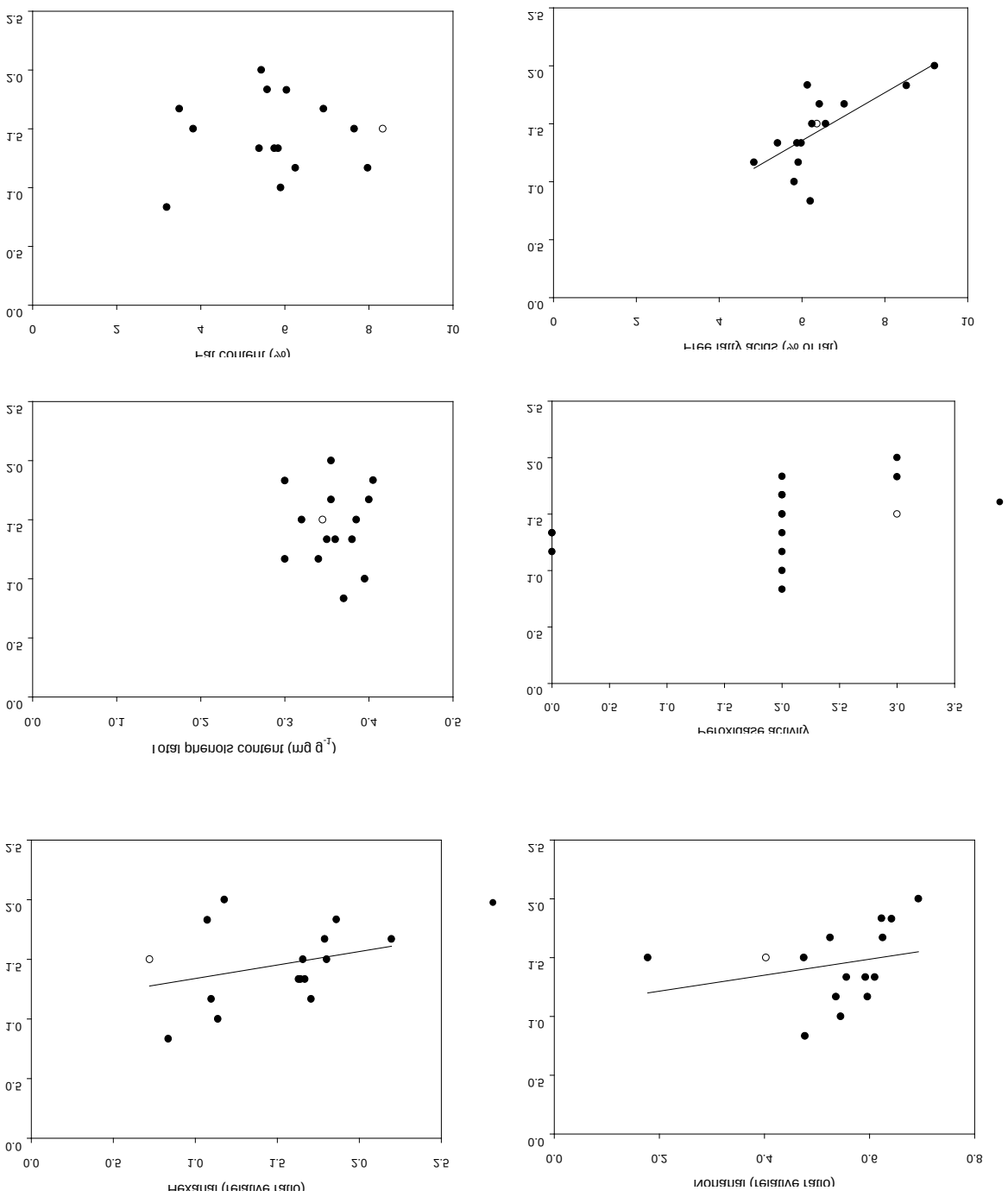


Figure 30. Scatter plots between physicochemical results and bitterness in toasted laboratory generated oats samples. Correlation lines are shown where there is a suggestion of a significant relationship

Chapter 5

Overall Discussion and Recommendations

5.1 Introduction

In the present research project, the cause(s) of bitter flavour in oats quality was studied by examining eight objective factors. These were colour, moisture content, fat content, free fatty acids, peroxidase activity, total phenols content, volatile oxidative products, and bitterness. The results for industrial oats samples as received were described in Chapter 3, while the results for laboratory generated model samples were described in Chapter 4.

The collective outcome of this work indicated that moisture content, free fatty acids, and peroxidase activity were positively related with bitterness. Total phenols content showed conflicting results.

In this chapter, the results obtained in the present study are compared with the results of other studies with oats. Within the limitations of the study the most likely cause of the bitterness problem is identified and a solution is recommended.

5.2 Total phenols

The function of phenols in oats is unclear, but given the antioxidative activity of phenols (Welch, 1995; Kähkönen et al., 1999; Decker, Beecher, Slavin, Miller and Marquart, 2002), protection of fats seems to be a likely role. For example, vanillin is an antioxidant used in complex foods containing polyunsaturated fatty acids to mask undesirable off-flavour development (Burri, Graf, Lambelet, and Löliger, 1989).

Oats contain non-volatile, low molecular weight phenolic compounds, which contribute to sensory properties (Durkee and Thivierge, 1977; Dimberg, Molteberg, Solheim and Frølich, 1996). The free phenolic acids with low molecular weight, contribute significantly to objectionable flavour, even at low level (10-90 mg kg^{-1}) (Maga and Lorentz, 1973; Huang, and Zayas, 1991).

Liberation of phenols may be highly dependent on moisture content, time and temperature during processing. Dimberg et al. (1996) detected 11 phenols in oats and identified seven of

them, including caffeic acid, p-coumaric acid, ferulic acid, vanillic acid, p-hydroxybenzaldehyde, vanillin, and coniferyl alcohol. They found that storage at a high relative humidity (80 %) resulted in significantly higher levels of vanillic acid, p-coumaric acid, and especially caffeic acid. The present data were not inconsistent with those results.

In the present study, release of phenols was affected by moisture content and heat treatment. The content of phenols increased with the increasing of moisture in raw oats, and also increased after toasting. However, there was no interaction between high moisture content and toasting. Total phenols content did not appear to be related to bitterness in the present study. The correlation between phenols and bitterness was negative, so phenols are unlikely to be the cause of bitterness.

5.3 Fat and total free fatty acids

Fats in food are regarded as flavour precursors. Based on its unique physical and chemical properties, fats can undergo complex chemical changes via hydrolysis and further oxidation to generate numerous compounds both desirable and undesirable, during processing, storage, and handling (Fennema, 1996).

Fats hydrolysis (lipolysis) may occur by enzyme action (Woolley and Petersen, 1994) or by heat and moisture, in both cases resulting in the liberation of free fatty acids (Fennema, 1996). During lipolysis, triglycerides are hydrolysed first to diglycerides, then to monoglycerides and finally to fatty acids (Belitz et al., 2004). The release of short-chain fatty acids by hydrolysis is responsible for the development of an undesirable rancid flavour, such as in raw milk and dairy products (Fennema, 1996).

With a 5-fold higher fat content (Percheron and Löliger, 1990) and 10 to 15-fold higher lipolytic activity compared to other cereals like wheat (Matlashewski et al., 1982), oats are much more prone to develop fat degradation products. As reported by Outinen (1999), no lipid hydrolysis was observed in intact whole oats during subsequent storage. Even dehulled oats showed only minor lipid hydrolysis (Peltonen-Sainio, Kontturi, Rajala, Kirkkari and Lehtinen, 2003). But, after more extensive processing, the native organisation of oats is changed. These changes render fats more susceptible to deteriorative reactions. Fat hydrolysis starts rapidly and free fatty acid accumulation occurs within one day of processing if moisture content is high (Liukkonen, Montfoort and Laakso, 1992; Lehtinen, 2003). Since

the lipase catalysed hydrolysis was initiated by milling, it is possible that different milling methods would impart intermediates and final oats products with different tendencies to form free fatty acids. However, no data are available in the scientific literature on this subject, even though it could enable an additional means to control lipid hydrolysis (Lehtinen, 2003).

In the present study, the fat content of both raw and toasted oats expressed as percentage of weight decreased with increasing moisture content and storage time. The decrease may be due to two reasons: increasing moisture content which contributes to weight and/or deteriorative chemical reactions.

Free fatty acids present another level of complexity. Several research papers (Frey and Hammond, 1975; Welch, 1977; Sahasrabudhe, 1979; Liukkonen, Montfoort, and Laakso, 1992; Molteberg et al., 1995) have reported that during storage of oats processed to a variety of endpoints, the free fatty acids content increased significantly in improperly stored or handled samples. For example, Welch (1977) reported that high moisture grain yielded free fatty acids levels of up to 16 % of fat after seven months storage in comparison with 4 % in dry grain. Using oat flours prepared from heated-treated and non-heat-treated grain, Liukkonen et al. (1992) reported that fat hydrolysis was slow during storage at moisture contents around 7 to 11 %. However, increasing the water content up to 50 % markedly increased the rate of fat hydrolysis, and therefore free fatty acid accumulation, within a day. With model systems of oats products, Lehtinen (2003) showed that the extent of fat hydrolysis occurring during dry storage can be effectively predicted by following the accelerated hydrolysis of fat at high water activities. However, Molteberg et al. (1996a) found that although free fatty acid content generally increased with storage time of raw oat flours, long term storage to 42 weeks resulted in the eventual decrease in free fatty acids content as a fraction of dry matter. The most extensive reduction occurred in linoleic and linolenic acids, but even the content of oleic acid was significant reduced. These results strongly suggest that fatty acid oxidation was also occurring.

In the present study with industrial oats samples as received and with laboratory generated samples, a strong correlation was observed between raw oats moisture content and free fatty acids content expressed a percentage of fat or dry weight. The free fatty acids content increased with increasing of moisture content and storage time, especially in peroxidase positive oats lots. However, the content of unsaturated fatty acids suffered relative reduction

to saturated fatty acids. Overall, the present results are consistent with the findings of the above studies on moisture content and storage effects.

The literature reports on the effect of heating oats during production of rolled oats and similar products is limited to moist heating as is encountered during normal processing. There is no literature on the effects of toasting to elevate temperatures. The present study has shown that increases the free fatty acid content as fraction of fat occurred on toasting. Whether this is caused by enzymic or non-enzymic hydrolysis cannot be determined from the present data, but the latter seems much more likely for two reasons. First is the inherent instability of lipase at high temperatures. The second reason can be inferred from Table 13 where there was no statistical interaction between water addition and toasting. This means that although water addition caused an increase in free fatty acids in raw oats this did not cause additional problems on toasting. The problems were merely proportional to the damage done in raw oats due to moisture.

5.4 Fatty acid profile and volatile fat oxidation products

In oats, a large portion of fatty acids are unsaturated. According to Molteberg et al. (1995; 1996a), the free fatty acids profile comprises 40 % oleic and linoleic acid each, 15 % of palmitic acid, and 1.5 % of stearic and linolenic acid each. The proportions of these free fatty acids did not change much in a variety of processing treatments that did not include toasting. In the present study, where toasting was included, four principle fatty acids were identified – palmitic, oleic, linoleic, and linolenic acid (Table 14). Oleic and linoleic dominated, and the relative reduction of unsaturated free fatty acids after toasting indicates an increasing content of volatile oxidation products at the expense of those fatty acids. The relative content of the unsaturated oleic and linoleic acids decreased markedly, while the content of linoleic acid fell severely where there was a 71 % decrease after toasting.

In the study of Molteberg et al. (1995), free fatty acids content increased with moisture and storage, but the proportions of the dominant fatty acids did not change much. Evidently, toasting is required to markedly affect the proportions. Nonetheless, Molteberg et al. (1995) demonstrated an increase in the major fat oxidation product, hexanal, with moisture, processing and storage time.

In the present study, water addition had no significant effects on the release of volatiles, but toasting had significant effects (Table 16). Toasting generally enhanced the release of volatiles. In particular, the headspace concentration of hexanal was increased by 5-fold after toasting, probably as a direct results of linoleic acid oxidation (Belitz et al., 2004).

Extending these results to the problem of bitterness was explored by correlation analyses. Hexanal, 2-pentylfuran, and nonanal were positively correlated with bitterness. Hexanal was the one with the highest correlation to bitterness of toasted oats (Table 18). Compared with the findings of Molteberg et al. (1996a), the results were comparable. They reported that in heat-treated oats samples, the coefficient of correlations between volatiles and bitterness were 0.47 for hexanal, 0.46 for 2-pentylfuran, and 0.53 for nonanal.

Table 16 also shows that there was no significant statistical interaction between water addition and toasting for volatiles generation. This parallels the result for total free fatty acids (Table 13), further implying that the cause of deterioration of fats stems from moisture, and probably residual enzyme activities in raw oats. However, there was water addition/toasting interaction for bitterness (Table 17), which does not comply with the notion that toasting only mirrors the damage done by added moisture. This matter is unresolved, but given the variability in bitterness of raw oats samples (Figure 28) the matter may not be important.

5.5 Enzyme activities

Whereas the secondary products of lipid peroxidation, mainly volatile compounds like aldehydes and ketones are strongly flavour active (Belitz et al., 2004), the primary products of autoxidation, the monohydroperoxides, are odourless and tasteless.

As was shown in Figure 3, lipoxygenase activity, which generates monohydroperoxides, is an early step in the development of favour active oxidation products. Lipoxygenase activity in raw oats is low compared to that in other cereals (Lehtinen, 2003), being less than 50 μmol min^{-1} 100 mg of $flour^{-1}$, compared with values of 630 and 1200 respectively for wheat and barley. However, Lehtinen (2003) found that this activity was still sufficient to trigger oxidation of unsaturated fatty acids in non-heat-treated oats when moisture contents were high. According to Galliard (1994) and Molteberg et al. (1996a), fat oxidation can be

triggered by the presence of lipoxygenase, and further enhanced by high moisture content particularly when oats are exposed to heat, air, and light.

Lipoxygenase was not measured in the present study. However, it was found that peroxidase activities were unacceptably high in many oats lots analysed, probably due to inadequate heating during processing. This could mean that lipoxygenase activity, which although more heat labile (Belitz et al., 2004), might still remain unacceptably high, so leading to fat oxidation. This will be especially true when lipase is also active.

When processing oat products, the activity of lipase is an important indicator for the maintenance of flavour quality in stored products. Whereas lipase will clearly be active as temperature increases during thermal processing (not toasting), there should be no residual lipase activity in processed raw oats, because the heat treatment should finally denature the enzyme. Peroxidase is an indicator of residual lipase activity because peroxidase has greater thermal stability than lipase, and lipoxygenase (Figure 4) (Belitz et al., 2004). Thus, if peroxidase is inactive, then lipase will necessarily be inactive, thus minimising fat hydrolysis and subsequent oxidation. The data in Figure 4 are for potato tuber but the pattern will probably be similar to that in oats. Certainly the use of peroxidase as an indicator of oats quality (AAAC 22-80 method) implies that its activity after heat treatment will reflect the activity of lipase.

In the present study, most raw oats samples received from Hubbard Foods had active peroxidase, meaning that there was no guarantee the oat lipase or lipoxygenase were inactive. Moreover, a peroxidase activity in oats is responsible for the conversion of hydroperoxides to equivalent hydroxyacids (Biermann and Grosch, 1979). Biermann et al. (1980) proposed that these hydroxyacids are partially responsible for the bitter taste associated with the enzymically active oats.

5.6 Bitterness in Hubbard oats products

The results from the present study showed that peroxidase activity, free fatty acids, and volatile oxidation products, were all positively correlated with bitterness. Residual lipoxygenase and lipase activity, which are precursors to free fatty acids formation and volatile oxidation products, were most likely cause(s) of resulting bitterness. Exactly what

fat product (free fatty acids, hydroxyacids, volatiles) or combination is direct responsible for the flavour changes cannot be determined from the present data.

The standard way to avoid lipolysis and subsequent oxidation is to heat treat the oats during processing to destroy the very active lipase (Ekstrand, Gangby, Akesson, Stollmaa, Lingnert and Dahl, 1993). This is done in the milling stage, but if the process is unsuccessful, many months may elapse before bitterness is noted in downstream processed products. In any event, trace amounts of lipase may remain even after heat treatment, and during the heating step, the enzyme's activity will be enhanced at least until it is completely destroyed at toasting temperatures.

Proof of lipase denaturation is provided by the peroxidase test, and the Australian supplier sent data showing that the peroxidase was inactive in all samples received. However, in the present study, the peroxidase test based on the validated AACC 22-80 method was positive for many samples, which is in direct conflict with the results obtained by Blue Lake Milling. Therefore, it is possible that the lipase is active in many of the samples. The results indicate free fatty acids stem from the residual lipase activity that should be but may not be controlled at source in Australia. Or, residual lipase activity may adversely affect oats during time in storage and transit. Certainly it seems likely that the toasting procedures at Hubbard Foods are not responsible as was revealed in the water addition experiments. The problem develops upstream and toasting merely serves to expose the problem.

5.7 Recommendations

According to the scientific literature and the present study, residual enzyme activity in the mature oat kernel triggers lipid deterioration, which causes the decrease in the sensory quality of processed oats during storage. Several enzymes, lipase, lipoxygenase and peroxidase are together involved in hydrolytic and oxidative rancidity. Therefore, testing for enzymic activity is important for both oats suppliers and food manufacturers.

Appendix VIII presents the test procedures used at Blue Lake Milling. There are two mistakes in this method, one of nomenclature and one of design. First, this method is described as a test procedure for lipase, but it is rather a peroxidase test method that can only infer lipase activity. The other mistake – which is crucial – is that the method omits the key reactant hydrogen peroxide. The incorrect method will lead to meaningless results.

It is recommended that Hubbard Foods should point out this severe deficiency to Blue Lake Milling. Hubbard Foods and Blue Lake should work together to establish a peroxidase test that will reliably monitor this critical control point in the oats supply chain.

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Appendix I. Oats supplier, Blue Lake Milling Pty Ltd, Australia

Blue Lake Milling Pty Ltd
OATMILLS OF AUSTRALIA

A.C.N. 007 968 318 A.B.N. 30 007 968 318
RAMSAY TERRACE, (P.O. BOX 443) BORDERTOWN S.A. 5268
PHONE (08) 87520 111, FAX (08) 87522 967
INTERNATIONAL FAX (61 8) 87522 967

PRODUCT SPECIFICATION
32 THOU QUICK OATS (0.74-0.90mm)

COMPANY DETAILS

Blue Lake Milling Pty Ltd is a privately owned company located in the rural township of Bordertown, South Australia that complies with Manufacturing Best Practices in all processes.

Blue Lake Milling received HACCP Certification in 2000, ISO Certification in 2004 and is also certified with the National Association of Sustainable Agriculture (NASAA) under certification number: 5178P.

SOURCING OF RAW MATERIAL

Blue Lake Milling oats are sourced from Australian Growers who have declared their crop to be free of genetically modified origins. The locality of the grain can vary from year to year, with the climate influencing procurement localities.

The oats sourced must meet Blue Lake Milling's strict receiving standards, which seeks to attain the largest available kernel from the raw oat to enhance its established quality criteria.

PRODUCT DESCRIPTION

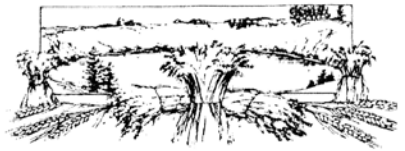
The Quick Oats are derived from sound milling oats that have undergone a hulling process to remove the outer husk. The kernel is cut into desired lengths and best described to as oat chips. The oats chip is subjected to steam injection for stabilisation prior to passing through large steel rolls to form a flake or rolled product. The "Quick" in the product description is related to flake size, thickness and cooking time.

The manufacturing process encompasses metal extraction and detection systems through strategic earth magnet installations and screening techniques which will detect 2.5mm ferrous and 3.5mm non ferrous spherical in a 10, 15 or 25kg bag.

FLAVOUR / ALLERGENS

This product is of typical oaten flavour, free from rancid, bitter and other foreign taints.
It contains no egg, peanut, tree nut or milk products.
Gluten is present in this product.

PACKAGING OPTIONS Multiwalled paper sacks with polyethylene lining, machine sewn and packaged in 10kg, 15kg, 25kg, and 1000kg bulk bags or tailored to meet customer requirements.



PRODUCT SPECIFICATION
32 THOU ROLLED OATS 0.74 - 0.90mm

MOISTURE	12.5% Maximum	CALIBRATED OVEN METHOD
SIEVE	3.35mm screen >80%	RETSCH VIBRATOR
	2.00mm screen <15%	(5 minute duration)
	1.70mm screen <5%	
	1.18mm screen < 5%	
	Tray <5%	
THICKNESS	0.74 - 0.90mm	MICROMETER STANDARD TEST
BULK DENSITY	33.1-36.9kg/hl	CHRONDROMETER STANDARD TEST
ENZYME ACTIVITY	Negative	CATECHOL METHOD
HUSK	0.05g Max 100g	PICKING
FOREIGN MATERIAL (Other cereal grains)	0.06g Max 100g	PICKING
NATURAL GRASSES	0.02g Max 100g	PICKING
GELATINISED GRAINS (over processed)	0.02 Max 100g	PICKING
MICROBIOLOGICAL RESULTS	COLIFORM <10/gram TPC<500/gram Y&M <100/gram	IN HOUSE TESTING USING PETRIFILM PROCEDURE

Certificate of Assurance and/or Conformance is available upon request
NUTRITIONAL DATA~TYPICAL ANALYSIS ON 100 grams

ENERGY	1600 KJ	SODIUM	3mg
PROTEIN	10.5%	SUGAR	0.0
TOTAL FAT	8.5%	CARBOHYDRATES	61.0g
SATURATED FAT	1.5%	DIETARY FIBRE	10.0g

This analysis may alter slightly depending on growing and climatic conditions

HALAL STATUS- Compliant Company Statement available

KOSHER STATUS- Compliant

SAFETY AND STORAGE DATA

There are no known risks associated with the handling or processing of this product. Product should be stored under constant, cool, dry conditions to maintain optimum freshness of product. Our recommended shelf life for this product is twelve (12) months after the production date but we will accept no responsibility for infestation fourteen days after the product has been accepted into the customer's storage facilities.

Approved by BLM **Date** / / **Accepted by** **Date** / /
Signature _____ **Signature** _____

PRODUCT SPECIFICATION

32 THOU QUICK OATS (0.74-0.90mm)

MOISTURE	12.5% Maximum	CALIBRATED OVEN METHOD
SIEVE	3.35mm screen 10-40%	RETSCH VIBRATOR
	2.00mm screen 45-80%	(5 minute duration)
	1.70mm screen- <15%	
	1.18mm screen- < 10%	
	Tray >5%	
THICKNESS	0.74-0.90mm	MICROMETER STANDARD TEST
BULK DENSITY	37.5-42.5kg/hl	CHRONDROMETER STANDARD TEST
ENZYME ACTIVITY	Negative	CATECHOL METHOD
HUSK	0.05gMax 100gram	PICKING
FOREIGN GRAINS	0.06g Max 100gram	PICKING
FOREIGN MATERIAL (Other cereal grains)	0.06g Max 100 gram	PICKING TEST
NATURAL GRASSES	0.02g Max 100 gram	PICKING TEST
GELATINISED GRAINS(over processed)	0.02g Max 100 gram	PICKING TEST
ENZYME ACTIVITY	Negative	CATECHOL METHOD
MICROBIOLOGICAL RESULTS	COLIFORM <10/gram TPC <500/gram Y & M <100/gram	INHOUSE TESTING USING PETRIFILM PROCEDURE

Certificate of Assurance and/or Conformance is available upon request

NUTRITIONAL DATA~TYPICAL ANALYSIS ON 100 grams

ENERGY	1600 KJ	SODIUM	3mg
PROTEIN	10.5%	SUGAR	0.0
TOTAL FAT	8.5%	CARBOHYDRATES	61.0g
SATURATED FAT	1.5%	DIETARY FIBRE	10.0g

This analysis may alter slightly depending on growing and climatic conditions

HALAL STATUS-Compliant Company Statement available

KOSHER STATUS- Compliant

SAFETY AND STORAGE DATA

There are no known risks associated with the handling or processing of this product. Product should be stored under constant, cool, dry conditions to maintain optimum freshness of product. Our recommended shelf life for this product is twelve (12) months after the production date but we will accept no responsibility for infestation fourteen days after the product has been accepted into the customer's storage facilities.

Approved by BLM **Date / /** **Accepted by** **Date / /**

Signature _____ **Signature** _____

Appendix II. The sources and code numbers of all oats samples

Oat code[1]	Source	Percent moisture added	AUT storage time[2] (weeks)	Toasted at AUT	Process date	Receive date	Test date
OQ3225	Hubbard Foods Ltd.	0		No	1/11/2005	14/12/2005	15/12/2005
OQ3225*		0		Yes	13/04/2006	13/04/2006	1/05/2006
OQ3225[3%]		3	2	Yes	13/04/2006	13/04/2006	1/05/2006
OQ3225[6%]		6	2	Yes	13/04/2006	13/04/2006	1/05/2006
OR3725	Hubbard Foods Ltd.	0		No	21/11/2005	14/12/2005	10/01/2006
DWHE25	Hubbard Foods Ltd.	0		No	29/06/2005	28/11/2005	
715989	Hubbard Foods Ltd.	0		No	6/03/2006	8/03/2006	9/03/2006
716091	Hubbard Foods Ltd.	0		No	13/03/2006	16/03/2006	16/03/2006
716091*		0		Yes	9/05/2006	9/05/2006	25/05/2006
716091[3%]		3	2	Yes	9/05/2006	9/05/2006	25/05/2006
716091[6%]		6	2	Yes	9/05/2006	9/05/2006	25/05/2006
716404	Hubbard Foods Ltd.	0		No	4/04/2006	7/04/2006	7/04/2006
716404*		0		Yes	12/04/2006	12/04/2006	1/05/2006
716404[2%]		2	2	Yes	12/04/2006	12/04/2006	1/05/2006
716404[4%]		4	2	Yes	12/04/2006	12/04/2006	1/05/2006
716404[6%]		6	2	Yes	12/04/2006	12/04/2006	1/05/2006
716576	Hubbard Foods Ltd.	0		No	18/04/2006	21/04/2006	24/04/2006
716576*		0		Yes	9/05/2006	9/05/2006	25/05/2006
716576[3%]		3	2	Yes	9/05/2006	9/05/2006	25/05/2006
716576[6%]		6	2	Yes	9/05/2006	9/05/2006	25/05/2006
716815	Hubbard Foods Ltd.	0		No	15/05/2006	18/05/2006	18/05/2006
716815*		5	0	Yes	22/05/2006	22/05/2006	25/05/2006
716815[5%2w]		5	2	Yes	22/05/2006	22/05/2006	9/06/2006
716815[5%4w]		5	4	Yes	22/05/2006	22/05/2006	27/06/2006
716815[5%6w]		5	6	Yes	22/05/2006	22/05/2006	13/07/2006

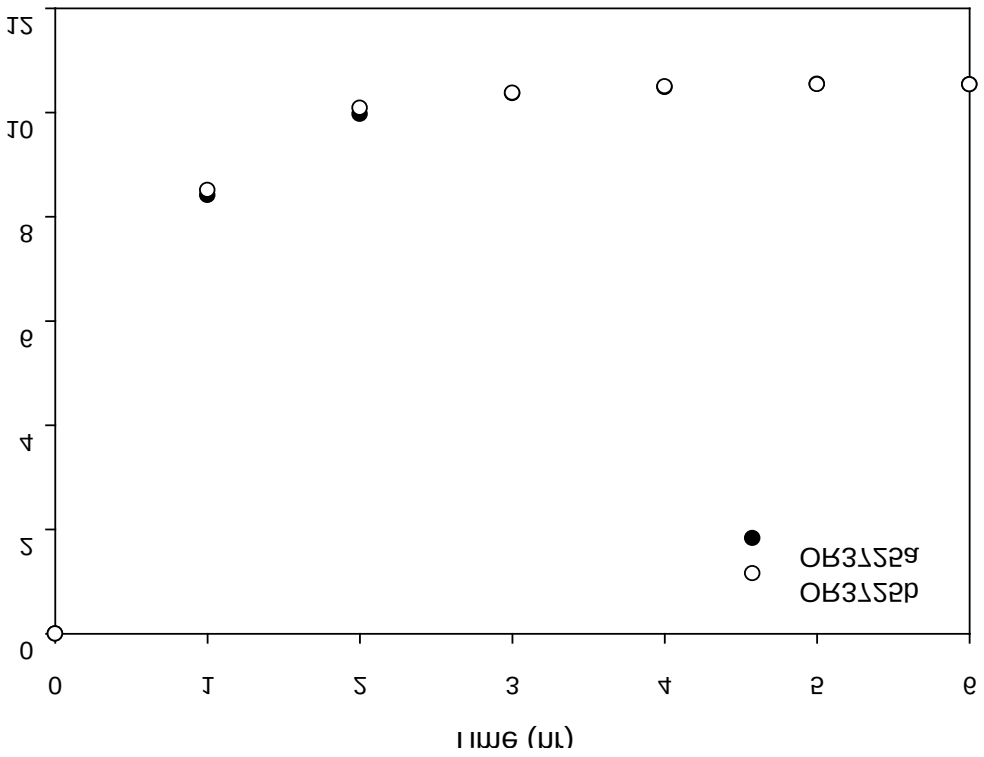
Oat code[1]	Source	Percent moisture added	AUT storage time[2] (weeks)	Toasted at AUT	Process date	Receive date	Test date
717088	Hubbard Foods Ltd.	0		No	30/05/2006	1/06/2006	19/06/2006
717165	Hubbard Foods Ltd.	0		No	6/06/2006	8/06/2006	20/06/2006
717500	Hubbard Foods Ltd.	0		No	6/07/2006	11/06/2006	3/07/2006
PK7942A	Pak'n Save, Auckland	0		No		22/04/2006	24/04/2006
PK7942A$^{3\%}$		3	2	Yes	9/05/2006	9/05/2006	25/05/2006
PK7942A$^{6\%}$		6	2	Yes	9/05/2006	9/05/2006	25/05/2006
PK7942B	Pak'n Save, Auckland	0		No		22/04/2006	24/04/2006
PK7942B$^{3\%}$		3		Yes	9/05/2006	9/05/2006	26/05/2006
PK7942B$^{6\%}$		6		Yes	9/05/2006	9/05/2006	26/05/2006
PK7942C	Pak'n Save, Auckland	0		No		13/05/2006	25/05/2006
PK7942D	Pak'n Save, Auckland	0	0	No		13/05/2006	25/05/2006
PK7942D$^{5\%2w}$		5	2	Yes	22/05/2006	22/05/2006	9/06/2006
PK7942D$^{5\%4w}$		5	4	Yes	22/05/2006	22/05/2006	27/06/2006
PK7942D$^{5\%6w}$		5	6	Yes	22/05/2006	22/05/2006	13/07/2006

[1]Oats samples from same oat code, but different treatments are defined by different superscripts; all were paired with raw and toasted samples, except where lot DWHE25 had no untoasted

[2]Time period between water addition treatment and toasting or testing for raw samples

[*]Non-water addition oats toasted at AUT

Appendix III. Time course of moisture content testing

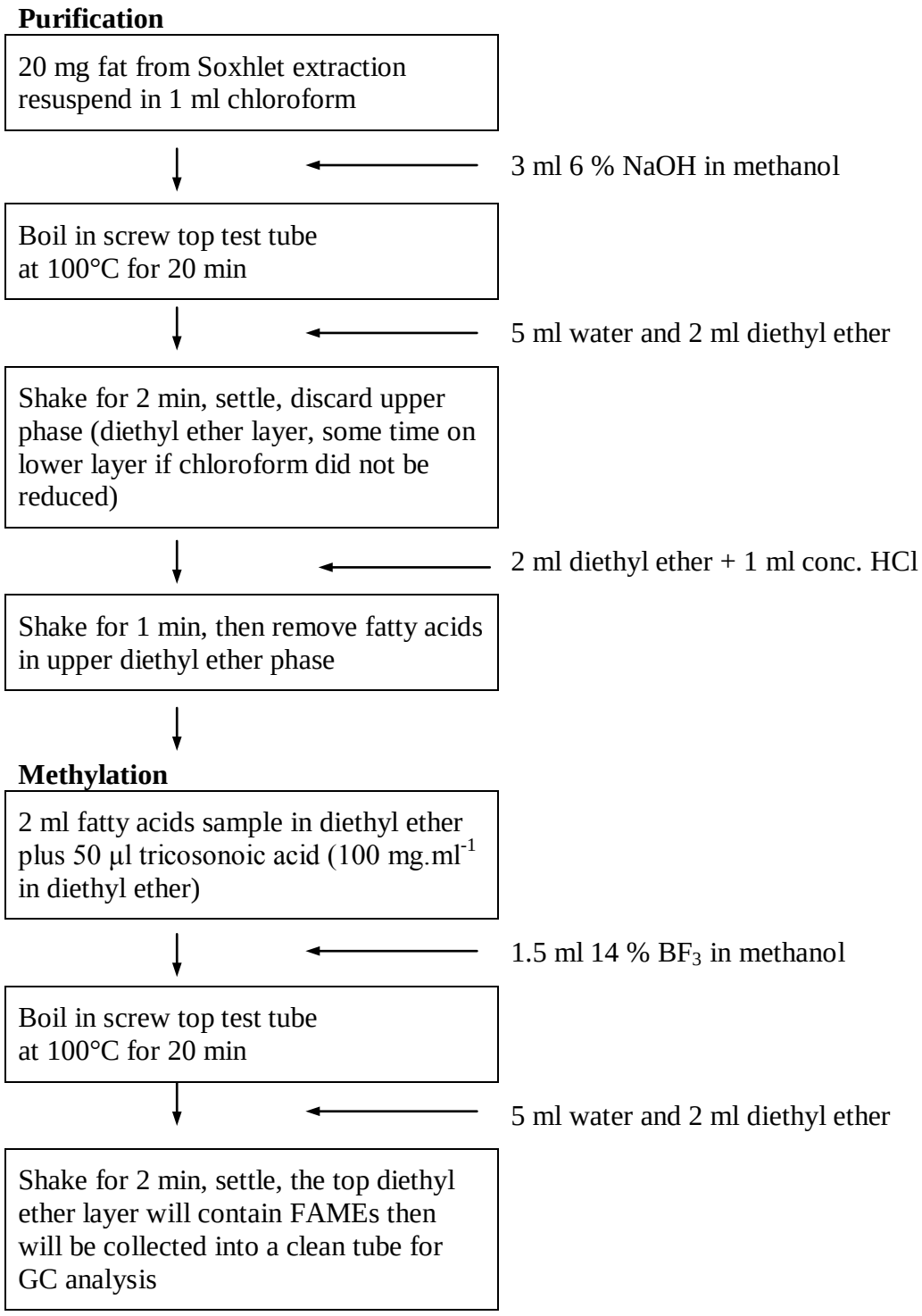


Appendix IV. Sample size and fat extraction efficiency

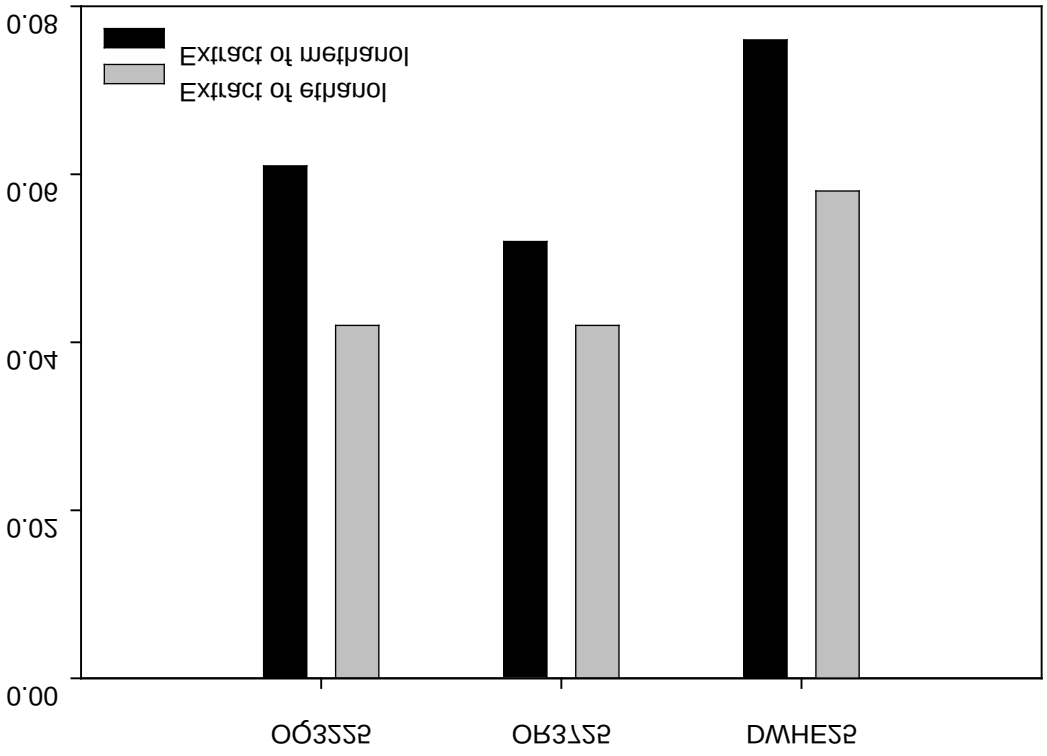
In the preliminary trial, two oat samples were dry-blended using a Waring-style blender, then extracted duplicate to see whether the sample size would affect the efficiency of extraction. The results showed that the percent of fat extract from blended oats with smaller size was constant higher than that from original oats. However, the difference was not significant. The average fat contents of the two samples were 7.41 ± 0.28 % and 7.54 ± 0.35 % by weight, respectively.

Appendix V.

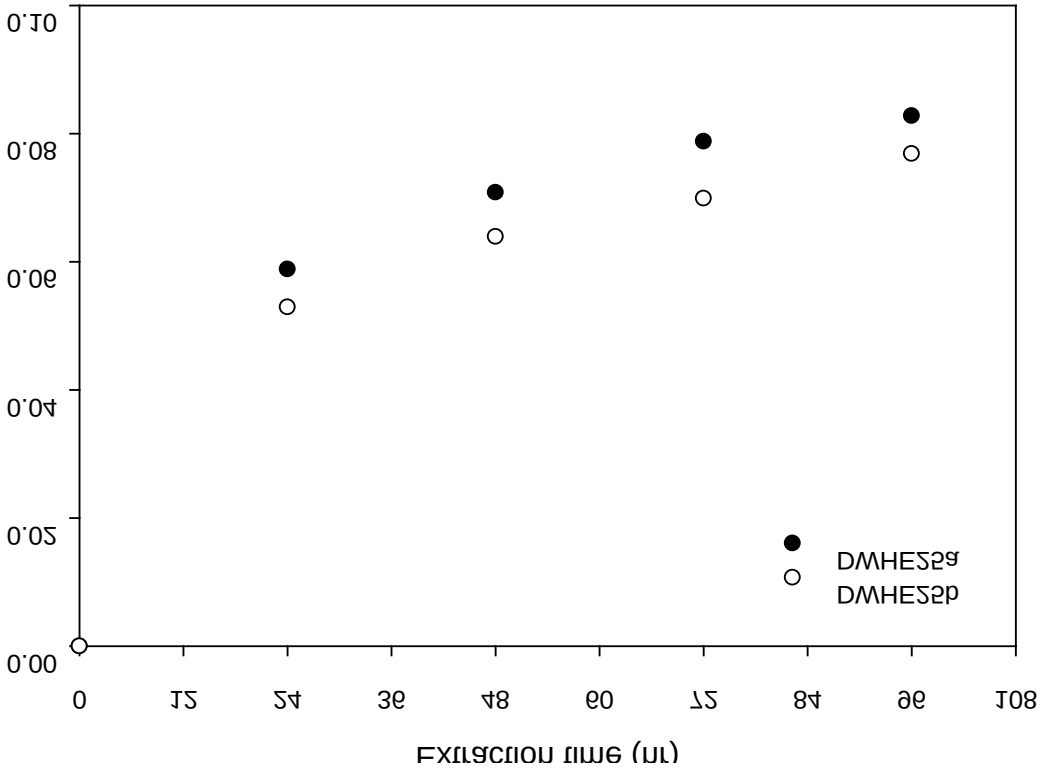
Purification and methylation procedures of crude fat (modified from Bligh and Dyer, 1959)



Appendix VI. Phenolics extraction solvent testing



Appendix VII. Time course of phenols extraction with methanol



Appendix VIII.

BLM
blue lake milling pty ltd

TEST PROCEDURES OATMILL

LIPASE: Lipase testing is to be done for every sample.

CATACHOL METHOD:

Place 1g of oats in a test tube and add 15ml of distilled water and 1 ml of catachol solution and shake vigorously. Leave in a dark cupboard for 30 minutes. The pipette tip is to be changed at the start of each shift and a new batch of Catachol to be made up.

CATACHOL SOLUTION:

Mix 1g of Catachol crystals with 40ml of distilled water, in an amber bottle, shake vigorously. Put in a dark cupboard. Double quantity can be made by doubling the mix.

REACTION

Clear to White indicates there is no activity (negative).

Yellow-Pink-Brown indicates there is activity (positive).

Lipase is critical therefore if lipase is positive then action must be taken immediately the product must be rejected, labeled and put in the designated area, a Non Conformance Report must be completed.

Appendix IX. Statistical analysis of colour results

One-Sample T: L* Raw oats

```
Test of mu = 71.9 vs not = 71.9


Variable      N     Mean   StDev  SE Mean        95% CI            T      P
L* Raw oats  35  71.8960  2.4919   0.4212  (71.0400, 72.7520)  -0.01  0.992
```

One-Sample T: L* Toasted oats

```
Test of mu = 66.46 vs not = 66.46


Variable          N     Mean   StDev  SE Mean        95% CI            T
L* Toasted oats  35  66.4566  3.5054   0.5925  (65.2524, 67.6607)  -0.01
    P
0.995
```

One-Sample T: H* Raw oats

```
Test of mu = 1.3633 vs not = 1.3633


Variable      N     Mean    StDev  SE Mean        95% CI            T      P
H* Raw oats  38  1.36329  0.01296  0.00210  (1.35903, 1.36756)  -0.00  0.998
```

One-Sample T: H* Toasted oats

```
Test of mu = 4.0683 vs not = 4.0683


Variable          N     Mean    StDev  SE Mean        95% CI            T
H* Toasted oats  37  4.06827  0.41079  0.06753  (3.93131, 4.20524)  -0.00

Variable            P
H* Toasted oats  1.000
```

One-Sample T: C* Raw oats

```
Test of mu = 25.91 vs not = 25.91


Variable      N     Mean   StDev  SE Mean        95% CI            T      P
C* Raw oats  38  25.9126  2.7912   0.4528  (24.9952, 26.8301)  0.01  0.995
```

One-Sample T: C* Toasted oats

```
Test of mu = 30.06 vs not = 30.06


Variable          N     Mean   StDev SE Mean        95% CI            T
C* Toasted oats  37  30.0589  2.4388  0.4009  (29.2458, 30.8721)  -0.00
    P
0.998
```

Appendix X.

Two-way ANOVA: Raw fat versus Oats, Water addition

```
Source          DF       SS       MS       F      P
Oats             5  17.2633  3.45266  662.42  0.000
Water addition   2   0.7160  0.35801   68.69  0.000
Error           10   0.0521  0.00521
Total           17  18.0314

S = 0.07220   R-Sq = 99.71%   R-Sq(adj) = 99.51%


                  Individual 95% CIs For Mean Based on
                  Pooled StDev
Oats        Mean  -----+---------+---------+---------+----
716091   7.00667                                (-*)
716404   7.16667                                  (-*)
716576   6.49333                          (*)
OQ3225   7.27333                                    (*)
PK7942A  7.29333                                    (*)
PK7942B  4.52000  (-*)
                  -----+---------+---------+---------+----
                     4.80      5.60      6.40      7.20


                   Individual 95% CIs For Mean Based on
Water              Pooled StDev
addition     Mean  ----+---------+---------+---------+-----
0         6.88500                                (---*---)
3         6.59167             (---*---)
6         6.40000  (---*---)
                   ----+---------+---------+---------+-----
                     6.40      6.56      6.72      6.88
```

Two-way ANOVA: Toasted fat versus Oats, Water addition

```
Source          DF       SS       MS     F      P
Oats             5  28.3368  5.66735  8.63  0.002
Water addition   2   5.8176  2.90882  4.43  0.042
Error           10   6.5648  0.65648
Total           17  40.7192

S = 0.8102   R-Sq = 83.88%   R-Sq(adj) = 72.59%


                  Individual 95% CIs For Mean Based on
                  Pooled StDev
Oats        Mean  -----+---------+---------+---------+----
716091   5.82667               (-----*------)
716404   6.67000                    (------*-----)
716576   5.74333              (------*-----)
OQ3225   6.60333                    (-----*------)
PK7942A  7.51333                         (------*-----)
PK7942B  3.50000  (------*-----)
                  -----+---------+---------+---------+----
                     3.2       4.8       6.4       8.0


                   Individual 95% CIs For Mean Based on
Water              Pooled StDev
addition     Mean  --+---------+---------+---------+-------
0         6.72500                  (--------*--------)
3         5.85500        (--------*--------)
6         5.34833  (--------*--------)
                   --+---------+---------+---------+-------
```

```
                  4.80      5.60      6.40      7.20
```

One-Sample T: Difference

```
Test of mu = 0 vs not = 0


Variable     N      Mean     StDev   SE Mean          95% CI            T      P
Difference  18  0.649444  0.882120  0.207918  (0.210777, 1.088112)  3.12  0.006
```

Appendix XI.

Tukey Simultaneous Tests
Response Variable Raw fat
All Pairwise Comparisons among Levels of Water addition
Water addition = 0 subtracted from:

Water addition	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	-0.2933	0.04168	-7.04	0.0001
6	-0.4850	0.04168	-11.64	0.0000

Water addition = 3 subtracted from:

Water addition	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
6	-0.1917	0.04168	-4.598	0.0026

Tukey Simultaneous Tests
Response Variable Toasted fat
All Pairwise Comparisons among Levels of Water addition
Water addition = 0 subtracted from:

Water addition	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	-0.5817	0.2442	-2.382	0.0615
6	-0.9308	0.2442	-3.813	0.0020

Water addition = 3 subtracted from:

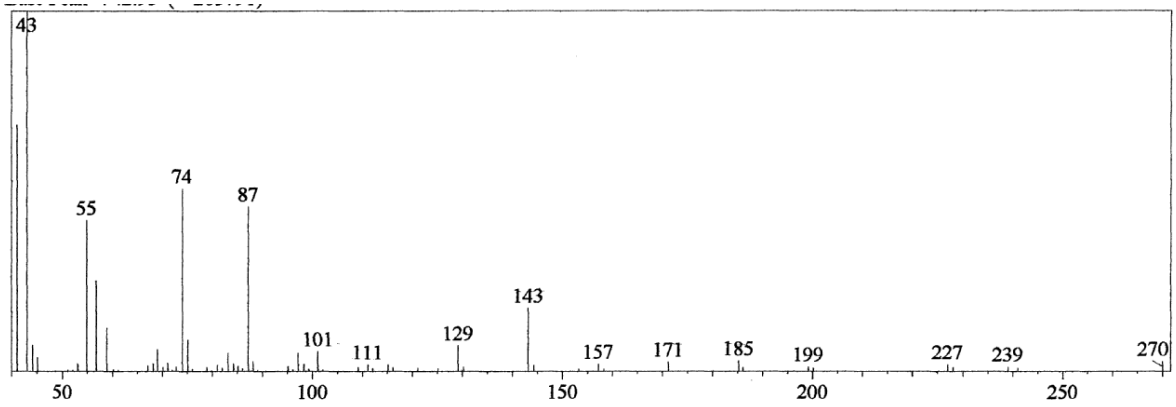
Water addition	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
6	-0.3492	0.2442	-1.430	0.3401

Appendix XII.

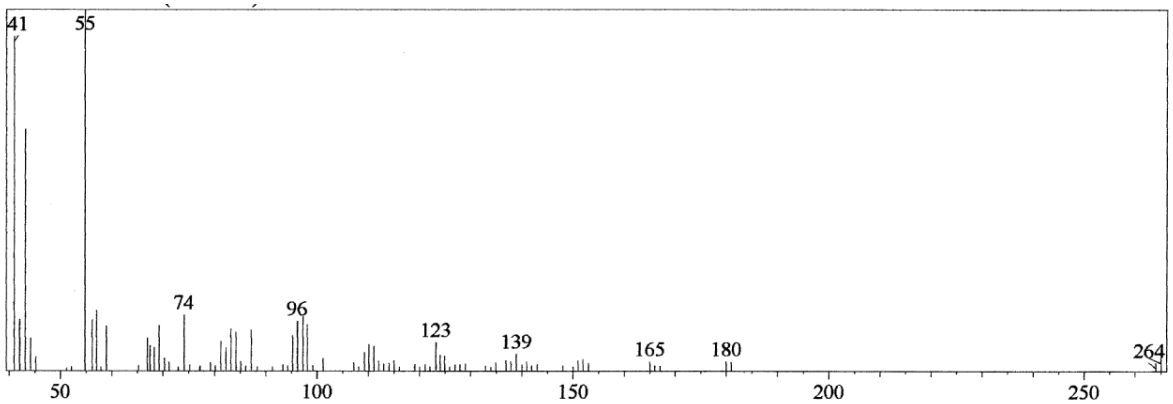
The mass spectra of identified objective fatty acids

The mass spectra of those fatty acid compounds that were identified with comparison to an authentic compound. Peaks are numbered as in Figure 24. RT = gas chromatograph retention time (minutes).

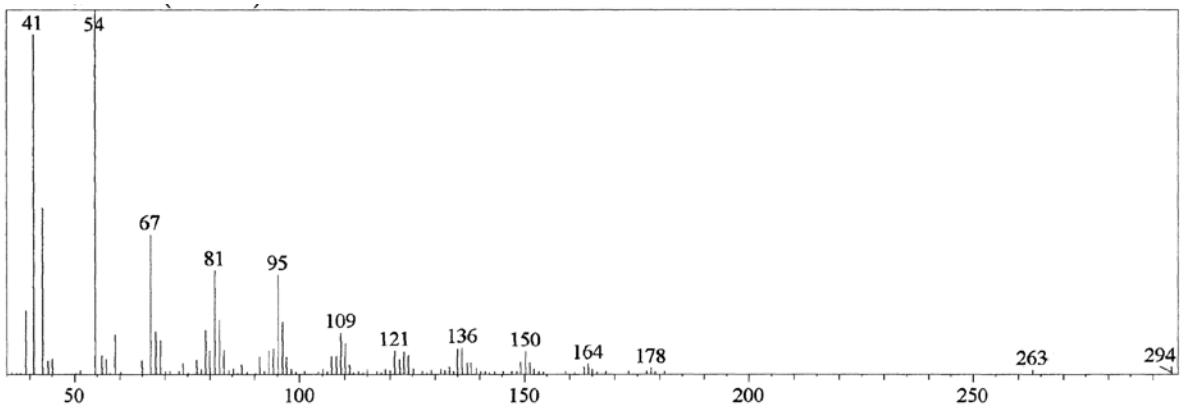
Peak 1. RT 12.700 C 16:0
Scan #: 1501
Base Peak: 42.95 (265791)



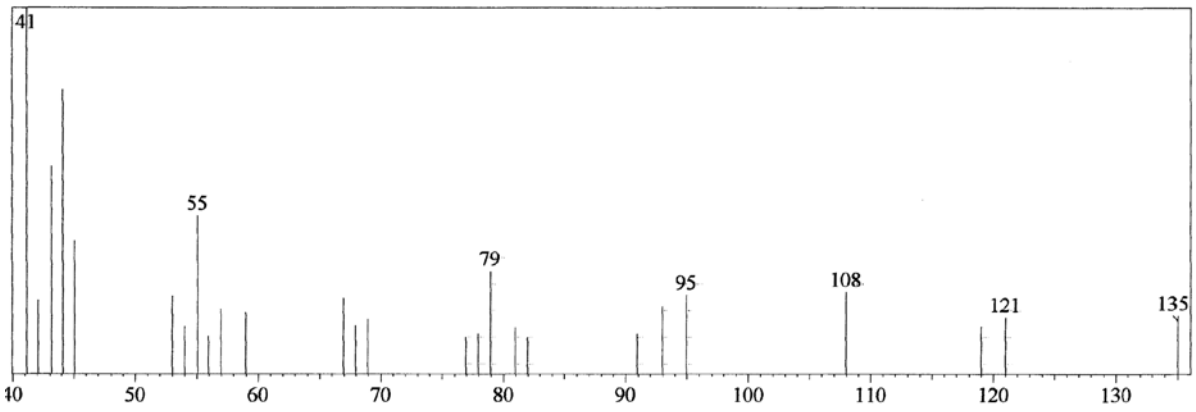
Peak 2. RT 20.133 C 18:1
Scan #: 2393
Base Peak: 54.75 (140528)



Peak 3. RT 22.158 C 18:2
Scan #: 3135
Base Peak: 54.45 (306889)



Peak 4. RT 24.633 C 18:3
Scan #: 3233
Base Peak: 41.10 (11570)



Relative reaction rate

Appendix XIII.

Chromatographic multiple comparison of time course of oats heating preparation. From the front to the back, heating time is 1, 2, 3 and 4 hrs, and 24 hrs, respectively. The relative response at 5.5 min was similar for 1,2, 3 and 4 hrs (bottom graph), and although higher at 24 hrs, 1 hr heating time was chosen in the interests of speed.