Detection of rancidity in peanuts

Sophe Williamson

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DETECTION OF RANCIDITY
IN PEANUTS

By
Sophe Williamson
BSc(Hons)

HONOURS RESEARCH PROJECT

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EDITH COWAN UNIVERSITY
Faculty of Communication, Health & Science
FOOD SCIENCE & NUTRITION PROGRAM
Mount Lawley Campus
USE OF THESIS

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ABSTRACT

As peanuts age, the lipid component deteriorates, and the peanuts become rancid. The Health Department of Western Australia has received numerous complaints from consumers who are unsatisfied with the quality of peanuts they have purchased from certain grocers. Presently there is no standard methodology for detecting rancidity in peanuts, thus enabling grocers to continue selling peanuts of poor quality. In an attempt to overcome this problem, a standardised methodology needs to be developed, which will enable offending grocers to be prosecuted.

The three methodologies trialed were the Peroxide Value (PV), Acid Value (AV), and Fatty Acid Profile (FAP) methods. In order to test these methodologies fresh raw peanuts were aged under various environmental conditions, primarily heat and light. The aging process occurred over an 8 week period, with samples being tested for PV, AV, and FAP periodically. The 40°C, and 60°C peanut samples were found to have little on the quality of the peanuts. It was found that the peanuts exposed to sunlight aged more rapidly than any of the other peanut samples. Overall the peanuts were found to be more susceptible to rancidity when exposed to heat, oxygen, and light at the same time, rather than exposure to only dry heat.

The PV, AV, and FAP methodologies were all effective in detecting changes in the peanut oil. On the basis of the results from this study, none of the methodologies trialed could be singled out as being the best method for detecting rancidity in peanuts. However, the PV, and AV methods were found to be faster, and less costly than the FAP method. It is suggested that using the PV, and AV methods for determining rancidity in peanuts would provide enough evidence for prosecution. If further evidence is required, then perform an FAP test, otherwise it is best suited for other purposes, since the PV, and AV adequate evaluate the quality of peanuts.
I certify that this thesis does not, to the best of my knowledge and belief:

(I) incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education;

(ii) contain any material previously published or written by another person except where due reference is made in the text; or

(iii) contain any defamatory material.

Sophe Lee Williamson

Date: 13th November 1998
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1. BACKGROUND STATEMENT

As the year 2000 approaches food quality has become a growing concern for consumers. The Health Department of Western Australia in association with the Food Chemistry Centre are concerned with the ‘freshness’ of food products purchased as bulkfoods, particularly peanuts. Rancidity develops as peanuts age, as a result of a series of hydrolytic and/or oxidative reactions. In recent years it has been brought to the attention of the Health Department that consumers are unsatisfied with the quality of peanuts purchased from certain vendors. Sensory evaluation alone does not provide enough evidence to enable prosecution of manufacturers and retailers who sell rancid peanuts. It is therefore necessary to identify an analytical method that would stand up in a court of law, effective in the detection of rancidity in peanuts.

Peanuts referred to as ‘groundnuts’ or ‘goobernuts’ are seeds that are encompassed in a straw-coloured pod, grown by the *Arachis hypogaea* plant of the Leguminosae family. The peanut is an annual legume, that is grown in warm soil in tropical or sub-tropical climatic locations. Kingaroy is the peanut capital of Australia located in Queensland, about 200 kilometres north west of Brisbane. The townsite of Kingaroy is dominated by the presence of large silos, storing the yearly yield of 40,000 to 60,000 tonnes of peanuts produced in this region (Reader's Digest, 1995). Internationally around 20 million tonnes of peanuts are produced annually, with the largest producer being India, a country in which the majority of the peanut crops are consumed locally (Rogers, 1990). The United States, China and Argentina are also major peanut producers, however a large portion of the peanut crop from these three countries is exported (Sanders *et al.*, 1993).

A wide range of degradation products are formed as the lipid component of the peanut becomes rancid. To prolong the onset of rancidity, peanuts should be stored away from moisture, oxygen, metal catalysts, light and heat. In the marketplace peanuts are stored in various materials including plastics, metal foils, paper and fabrics. These materials are used individually or in combination to for the desired peanut package. Vacuum packs, foil lined paper packets, plastic wraps, and resealable plastic containers are all current methods
manufacturers use to package peanuts. Consumers are often unaware of the important the role that packaging plays in protecting peanuts from quality degradation. As consumers often purchase peanuts based on their visual appearance, manufacturers do not always view quality preservation as the most important factor when designing peanut packaging.

Bulkfood storage bins in supermarkets have been subject to recent criticisms concerning hygiene and preservation of food quality. The current policy of the Australian New Zealand Food Authority (ANZFA) stipulates that the country of origin for peanuts to be sold as bulkfoods must be displayed on the bulkfood bin (ANZFA, 1997). It is concerning however, that this authority makes no mention of stipulating use-by dates for bulkfoods. As peanuts age they become increasingly susceptible to rancidity, resulting in the development of a distinct off-flavour and odour. Since the shelf-life of bulkfood peanuts is not stipulated on the bulkfood bins often consumers unwittingly purchase rancid peanuts.

There is a need to identify an efficient, accurate and reliable method for detecting rancidity in peanuts. As yet there is no single test of determining rancidity that has been universally accepted as a standardised method (Rayner et al., 1998). It is the purpose of this study to identify and test analytical methodologies for detecting rancidity in peanuts.
2. LITERATURE REVIEW

2.1 PEANUTS

The peanut or groundnut as it is commonly called in various parts of the world and comes from the *Arachis hypogaea* plant. In fact the peanut is not a real nut, instead seeds are encompassed in a straw-coloured soft pod. Each elongated pod is about 5cm in length and contains between 2-4 seeds which are coated with a reddish-brown skin (Rogers, 1990, p. 319). Peanuts are high in protein (22-33%) and fat (44-56%) (Sanders *et al.*, 1993), and provide a good source of vitamin E.

There are several varieties of peanuts classified into four categories; Spanish types, Runner types, Virginia types, and Valencia types (Woodroof, 1973). The Virginia variety are larger in size than the other peanut varieties, and thus the most marketable peanuts (Rossell and Pritchard, 1991). The smaller peanut varieties such as the Spanish peanuts are used for oil extraction, and are particularly good for making peanut butter.

Peanuts are grown underground, hence the name groundnut where they grow along a long tendril. To harvest peanuts the entire plant is removed from the soil and the peanuts are left to dry for a few days on the surface of the ground. Following this initial drying process, the pods are collected and placed in the windrow for 1-3 days for further drying using mechanical driers (Weiss, 1983). Peanuts can be purchased with or without the shell/pod, skinned or deskinned, raw or roasted and as a variety of by-products, including peanut butter and peanut oil.

2.2 Peanut Oil

Peanut oil is composed of about 95% triacylglycerol, which is mainly made up of palmitic, oleic and linoleic fatty acids. The remaining 5% of the oil is made up of phospholipid and
sterols (Sanders et al., 1993). It is pale yellow in colour with a distinct nutty aroma and flavour (Rogers, 1990). Peanut oil is 100% fat, unlike peanuts it contains no protein, but it is a high quality oil with a melting point of 0.5-2.2°C and a smoke point of 226°C. This high smoke point means that degummed peanut oil is an excellent deep-frying fat that can be clarified to be re-used numerous times. Peanut oil is largely an unsaturated fat with 32-33% polyunsaturated, 46-49% monounsaturated and 16-18% saturated fat contents (McWilliams, 1993, p.287.).

2.2.1 The Commercial Extraction of Oil from the Peanut

Peanut oil is extracted from the peanut through a series of processes which involves the crushing of the soft outer shell of the peanuts, cleaning, grading and removing peanuts that do not meet quality specifications based primarily on visual characteristics. Often the skin is removed from the peanuts to avoid the bitter flavour resulting from the presence of tannins in the peanut skin. The peanut germ or heart of the peanut which is located at one end of the peanut kernel is also removed as it too imparts a bitter flavour (Anon, 1983).

Commercially peanut oil is extracted through hydraulic (mechanical) press, expeller and solvent extraction. It is further refined to remove undesirable colour and aroma from the oil by undergoing alkali refining, bleaching and deodorising processes. In order to use peanut oil for deep-frying purposes it has to be degummed so that it does not become frothy when heated to such high temperatures. This frothiness makes the oil unsuitable for continuous deep-frying operations (Anon, 1983).

2.2.2 The Laboratory Extraction of Oil from the Peanut

In the laboratory it is impractical to attempt to pilot the same process of oil extraction as is done on a commercial level. The most commonly used extraction method used in the laboratory is solvent extraction. In the past solvents such as acetone, azeotropic mixture (acetone, hexane and water), liquid carbon dioxide, or ethanol and isopropanol have been used for lipid extraction. Today hexane is the most commonly used solvent.
The advantages of hexane as a solvent include; (i) it does not impart an odour into the oil, (ii) it is easily evaporated out of solution as the boiling point of hexane is 68.9°C, which is less than that of most oils, (iii) it is relatively inexpensive compared to other solvents such as liquid carbon dioxide, and (iv) the methodology necessary for the extraction is simple to perform (Allen and Hamilton, 1994). The only real limitation with hexane as a solvent is that it is highly flammable, so care must be taken when heating the hexane to form a vapour.

Prior to adding the solvent to the peanuts, they have to be ground into small particles, the smaller the particles the greater their surface area. Solvent penetration of the peanut mash is increased with increased surface area, thus enabling a higher concentration of oil to be extracted from the mash. In the study by Rittner, 1984, examining the solvent extraction of soybean oil by extrusion. It was found that flaking rather than grinding of oil-bearing materials enhances the rate of oil extraction, and the amount of oil extracted. However, Rittner, 1984, had access to a peanut press-cake, which is a machine that is used commercially to flake peanuts prior to extrusion. In the present study such a device is not available, but this is unlikely to have a significant effect on the amount of oil extracted from the peanuts as grinding is adequately effective.

Suggested methods for grinding peanuts include the use of a food processor, coffee grinder, or mortar and pestle. The solvent can be added directly into the mortar following grinding, which is advantageous to minimise oil loss. The use of a food processor or coffee grinder is more likely to result in oil loss, as there are more components in which the peanut is exposed during grinding, and it is impractical to attempt to rinse each of these components with solvent. Also addition of solvent to these device may cause reactions with the plastics and/or metals that make up the equipment, thus interfering with the oil extraction. The mortar and pestle grinding method would minimise the amount of oil lost, however it would be more difficult to grind the peanuts into a fine mash.

After grinding the solvent would be added to the mash, and this mixture would be transferred into a gravity filter. The filtrate contains the oil and solvent, and the residue contains the carbohydrate and protein portions of the peanut. To remove the solvent from the oil, the mixture is heated above the boiling point of the solvent, evaporating it out of the
solution leaving behind the peanut oil (Pomeranz and Meloan, 1994). To recycle the hexane, a rotatory extractor can be used which enables the evaporated solvent to be condensed back into a liquid and caught in a separate flask. The condensed solvent can then be re-used, thus reducing wastage and the cost involved in the solvent extraction process.

2.3 RANCIDITY

Rancidity is the chemical deterioration of fats and oils, as a result of hydrolytic and/or oxidative changes in the fat (Pomeranz and Meloan, 1994). Rancid fats and oils have a distinctive off-flavour and odour. In the food industry, rancidity is becoming an increasingly important issue in the manufacture of quality foods.

2.3.1 Oxidative Rancidity

Oxidative rancidity results from the changes that occur from reactions with atmospheric oxygen. This is a three step process, where by: (i) the fat absorbs oxygen from the air, (ii) peroxides form in the fat, and (iii) the peroxides breakdown to form secondary products. The off-flavour and odour associated with rancidity are characteristic of the secondary degradation products produced in the fat, such as aldehydes and ketones (Rossell and Pritchard, 1991).

2.3.1.1 Factors Affecting Hydroperoxide Formation

The formation of hydroperoxides in an oil is initiated by the formation of free radicals which combine with oxygen and an unsaturated group. This reaction produces hydroperoxides and another free radical, which begins a chain of events (propagation). The reaction is terminated when all the free radicals are destroyed, and come together to form a non-reactive molecule (Gunstone and Padley, 1997).
Hydroperoxide formation in an oil is dependant on a variety of environmental, physical and chemical factors. Oxidative rancidity affects the more unsaturated fatty acids first. Polyunsaturated fats polymerise or breakdown to form smaller molecules with fewer double bonds (Allen and Hamilton, 1994). The degree of saturation of an oil determines its susceptibility to the formation of hydroperoxides, so when highly unsaturated oils are exposed to oxygen they readily oxidise.

The rate of oxidation in oils is accelerated with an increase in temperature. The exposure of an oil to light promotes the formation of free radicals which are responsible for initiating autoxidation (Gunstone and Padley, 1997). These two factors, temperature and light can be regulated during the storage of oils. In order to minimise the effect heat and light on oils they should be stored under ambient conditions, in a dark coloured glass bottle. The storage conditions suitable for peanuts, and peanut oil is discussed in section 2.5.

A high moisture content of a fat is associated with free fatty acid formation and hydrolytic rancidity. However, a low moisture content accelerates oxidation due to the absence in water activity sending the fat into 'hydration protection' mode (Gunstone and Padley, 1997, p. 187). A water activity level of 50% saturation indicates that the solubility is best, as at this level of water activity the destruction of free radicals is optimal.

Another group of pro-oxidants are metal catalysts, as they accelerate the rate of free radical formation and the breakdown of hydroperoxides. The most active metal catalysts include; copper (Cu), iron (Fe), nickel (Ni), manganese (Mn), Chromium (Cr), Zinc (Zn), and Aluminium (Al) (Gunstone and Padley, 1997). To preserve the quality of an oil, care must be taken during processing to ensure that these metals do not come in contact with the oil.

Antioxidants unlike pro-oxidants (light, metal catalysts, oxygen concentration, heat, and moisture), suppress the formation of free radicals and accelerate the formation of a non-reactive molecule. There is a natural antioxidant component of most oils and fat laden foods. Sometimes the natural antioxidant level of an oil is not protective enough against environmental conditions, so a synthetic antioxidant is added. The most common antioxidants used to extend the shelf-life of oils are BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene). It should be noted that antioxidants can not prevent
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hydrolytic or ketonic rancidity, they purely act as a barrier to oxygen to prevent the formation of hydroperoxides in the fat or oil (Allen and Hamilton, 1994).

### 2.3.1.2 Induction Period

Autoxidation occurs in two phases, the first phase is known as the induction period. During this period the oxidation rate is slow and steady. Once oxidation has proceeded to a specific point, the rate of oxidation increases markedly, this is the beginning of the second phase. Foul flavour and odour are not associated with the induction period where oxidation is minimal (Patterson, 1989). These adverse characteristics can be detected at the beginning of the second phase, and develop in strength during this period (Allen and Hamilton, 1994). The addition of an antioxidant can extend the induction period, thus increasing the shelf-life of the product.

The induction period for an oil can be determined by measuring the peroxide value of the oil at various stages of deterioration, and then plotting this data against time on an oxidation curve. The rancimat which is discussed in section 2.3.6, is an automated method useful in constructing oxidation curves to determine the induction period of a specific oil or fat.

### 2.3.2 Hydrolytic Rancidity

Hydrolytic rancidity is most often caused by a combination of moisture and enzymes (Allen and Hamilton, 1994) interacting with the fat or oil. The glycerides in the oil hydrolyse when they react with water to form free fatty acids (FFA), diglycerides, monoglycerides and glycerol (Gunstone and Padley, 1997). All of these degradation products cause undesirable change in the quality of the oil, so that the flavour and odour of the oil become unpleasant. The rate of hydrolysis is determined by the fatty acid content of the oil, the type of oil, the amount of dissolved water in the oil, and the storage conditions to which the oil is exposed (Gunstone and Padley, 1997).
An enzyme called lipase is present in both animal and plant tissues. The presence of this enzyme enables the lipolysis of lipids to from FFA and glycerol, triggering hydrolytic rancidity (McWilliams, 1993). Crude oils are particularly sensitive to lipase, so manufacturers briefly heat treat oils to desensitise these enzymes and prevent extensive splitting of triglycerides (Patterson, 1989). Polyunsaturated FFA are susceptible to further degradation by oxidative enzymes. The interaction of products formed from the breakdown of FFA and the products from the breakdown of hydroperoxides, results in the formation of keto acids, methyl ketones and lactones, all having an individual, yet distinct pungent flavours (Patterson, 1989).

The primary environmental conditions that affect hydrolytic rancidity in stored fats are temperature and moisture (Allen and Hamilton, 1994). Hydrolysis is accelerated by increasing temperature, with every 10°C rise in temperature, the rate of hydrolysis is expected to double. This is an important factor to consider when storing fats and oils, as even a small rise in storage temperature increases the liberation of FFA from the oil or fat, thus having a catalytic effect on hydrolytic rancidity (Gunstone and Padley, 1997).

The rate of hydrolysis is dependant on the water content of the oil, the higher the concentration of water the greater the rate of hydrolysis. Moisture is more readily absorbed from the environment in oils or fats where the FFA content is high. The amount of water saturating the oil depends on the molecular weight of the triglyceride that forms the basis for the oil, in peanuts this is oleic acid (Gunstone and Padley, 1997).

It is expected that as the molecular weight of a lipid increases the rate of hydrolysis decreases, as lower molecular weight lipids have an increased saturation solubility for water. According to Gunstone and Padley, 1997, the rate of hydrolysis in peanut oil is less than that of palm oil, coconut oil, palm acid, coconut acid and butter acid, respectively. It is thought that hydrolytic rancidity is a problem for oils based on lauric acid, like coconut and palm kernels (Allen and Hamilton, 1994). Peanut oil is based on oleic acid, which has a much lower molecular weight (282) than lauric acid (200), and has a lower level of water solubility (Gunstone and Padley, 1997). In the literature by Patterson (1989), and Pomeranz and Meloan (1994), it is suggested that high molecular weight lipids, such as peanut oil, do
not produce the same type of off-flavour and odour as low molecular weight lipids, such as coconut acid.

The increased amount of free fatty acids liberated from an oil can be detected by using the acid value methodology, which is explained in section 2.3.3 of this report. To detect the rancidity in oils and fats it is more common to use methods of determining oxidative rancidity, than methods of determining hydrolytic rancidity (Pomeranz and Meloan, 1994). In the literature by Patterson (1989), it is explained that an increase in FFA content of an oil indicates that (i) the oil was stored inappropriately, and (ii) oxidation has occurred. Based on the literature reviewed it would appear that oxidative rancidity commonly accompanies hydrolytic rancidity.

2.4 METHODS OF DETECTING RANCIDITY

Rancid fats are of poor quality and of little market value, so to prevent financial loss it is in the manufacturers best interest to be able to detect rancidity. Peroxide value, acid value, and fatty acid profiles are useful methods of detecting oxidised and/or hydrolysed fats in foods. Although there is the possibility that all three of these methods are suitable for detecting rancidity in peanuts, based on the literature reviewed, a decision must be made as to which of these methods is likely to produce the most accurate and reliable results.

2.4.1 Peroxide Value (PV)

As oxidation is the most common cause of rancidity, it is reasonable to assume that as an oil ages, the first undesirable product to form is a hydroperoxide. The determination of peroxide values (PV) involves chemical analysis of hydroperoxide content of the oil. It is represented numerically as the number of milli-equivalents of oxygen per kilogram in which potassium iodide (KI) is oxidised to iodine (Lezerovich, 1985; Rossell and Pritchard, 1991). It is expected that a freshly refined fat would have a PV of less than 1 unit. A fat that has a PV greater than 10 units is considered rancid. (Allen and Hamilton, 1994). The most common method of determining peroxide values is by iodometric titrations. The
colorimetric method, electrochemical technique and high performance liquid chromatography (HPLC) are alternative methods that can be practised in determining peroxide values. Lezerovich, 1985, which compared spectrophotometric methods of PV analysis with the standardised iodometric method, and found the iodometric method to be more precise.

The iodometric titration is thought to be the most appropriate PV method for detecting rancidity in peanuts, is documented in AOAC Methods Cd 8-53 (1990). The reagents used for iodometric titrations are acetic acid-chloroform solution (HOAc-CHCl₃), saturated potassium iodide solution (KI) and sodium thiosulfate standard solution (Na₂S₂O₃). Specific volumes of the sample oil are mixed with a set volume of HOAc-CHCl₃, and a set volume of KI and water are added. This solution is titrated with 0.1N Na₂S₂O₃ until it becomes colourless, and then a set volume of 1% starch is added turning it blue in colour. The titration continues until this blue colour vanishes, this is the end-point (AOAC, 1990).

It should be noted that the AOAC iodometric method is not the only recognised iodometric method, other standardised methods include the British Standard Method BS 684:2.14 and ISO 3960 (Allen and Hamilton, 1994). In Australia, the American Chemists' methods of oil analysis are the most recognised, so for the purpose of this study the AOAC methods of PV determination are applicable.

The major limitation of the iodometric method of PV determination is that very low peroxide values are not detected, making it difficult to determine the end point. This problem can be resolved by conducting the electrochemical technique on the samples in question. The titration step is replaced with a platinum electrode which maintains a constant potential in the sample solution, so as to reduce the liberated iodine. This process requires purified nitrogen to be passed through the solution to eliminate air (Lezerovich, 1985). The anisidine value (AnV) test, measures the aldehyde content in oils. This test is a useful accompaniment to PV tests, as it enables early detection of the substances primarily responsible for producing the off-flavours and unpleasant odours associated with rancidity (Gunstone and Padley, 1997). However, the AnV is not a recognised standardised methodology, and is a source of controversy among the scientific community (Allen and Hamilton, 1994; Gunstone and Padley, 1997; Rossell and Pritchard, 1991).
Peroxide values are good guides to assessing the quality of oil, however there are several disadvantages associated with PV determination. Results obtained can be misleading such that a low PV may have resulted from fast breakdown of hydroperoxides, rather than slow hydroperoxide formation. A false low PV may also result from unsaturated bonds in the fatty acids absorbing iodine (Rossell and Pritchard, 1991). The flavour and stability of an oil with a high PV may remain unaffected by the increased presence of hydroperoxides, as these hydroperoxides can breakdown into secondary products which do not alter the taste (Gunstone and Padley, 1997). Disadvantages also exist in the experimental design, where care must be taken when sampling and handling samples, ensuring that contact with air is minimised to avoid ‘oxygen error’ (Gunstone and Padley, 1997). The presence of oxygen in the titrated solution can result in iodine separating out of the KI solution, resulting in the PV of the oil sample being falsely high (Rossell and Pritchard, 1991).

The use of peroxide values in assessing the quality of highly unsaturated oils is not recommended. Although peanut oil is generally referred to as an unsaturated fat, it is not regarded as being highly unsaturated in the same context as unhydrogenated fish oils (Allen and Hamilton, 1994). Highly unsaturated fats are unstable, so reactions occur instantaneously meaning that the hydroperoxides quickly breakdown into secondary products. This means that even after the fat has undergone significant oxidation, the PV remains low, although the fat is likely have become rancid. This problem is may not be experienced when determining the PV of peanut oil.

Peroxide values are an effective method of detecting the degree of oxidation of fats and oils. The PV methodology is highly empirical, so the AOAC method must be followed exactly in order to minimise error, and produce reliable results. Based on the literature reviewed PV determination is an efficient and cost effective methodology, which when performed correctly could become a useful tool for detecting rancidity in peanuts.
2.4.2 Acid Value (AV)

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralise the free fatty acids (FFA) in 1g of the sample fats or oils (Rossell and Pritchard, 1991). It is the purpose of acid value methodology to measure the extent to which hydrolysis has liberated the FFA from the parent glyceride molecule, thus enabling the detection of hydrolytic rancidity. According to the Food Standards Codes, the acid value for edible oils and fats must be less than 2 (ANZFA, 1997).

The acid value methodology described by the AOAC methods (1990), specifically identifies butterfat as the medium in which the methodology is applicable. It is expected that the methodology used in the acid value determination of saturated fats, would be effective when applied to unsaturated fats, such as peanut oil. The reagents required are; 1:1 alcohol-ether mixture, 0.1N or 0.5N potassium hydroxide solution, and 1% methanoic phenolphthalein solution. The fat or oil sample is dissolved in the alcoholic-ether solvent, and approximately 1 ml of the methanoic phenolphthalein solution is added. This is titrated with potassium hydroxide solution until the sample solution maintains a faint pink colour for at least 10 seconds. The acidity of the fat or oil sample is determined from the amount of used titrant (Rossell and Pritchard, 1991).

The efficiency and accuracy of the determination of the acid value of oilseeds such as peanuts, is dependant on the rate and completeness of free fatty acid extraction (Berezin et al, 1996). Berezin et al., (1996), developed a reagent set consisting of two reagents, and assessed the completeness of FFA extraction from oilseeds, for the purpose of acid value determination. Their comparison of the reagent set with a standard extraction solvent, indicated that the reagent set did not improve the amount of FFA extracted, but was effective in shortening the extraction time. The extraction of the oil and FFA from peanuts can be achieved using hexane as the solvent. The ground peanut and hexane mixture are heated above hexanes boiling point, thus resulting in it evaporating out of solution leaving behind the oil and FFA.
The literature reviewed suggests that the acid value methodology is an effective method in determining oil quality. In peanuts it is expected that oxidative rancidity would play a greater role in oil degradation than hydrolytic rancidity, especially in supermarket bulkfood storage where the peanuts are regularly exposed to air, and other contaminants.

### 2.4.3 Fatty Acid Profiles (FAP)

Fatty acid profiles determine the percentage concentration of each specific fatty acid present in fats/oils. The FAP method is useful for identifying specific fats and oils, and determining the degree of deterioration the fat/oil has undergone. This method is not a conventional method used to detect rancidity in foods, however scientists have found that there are specific fatty acids that change in concentration as the food deteriorates. Literature suggests that as peanuts age the ratio of oleic/linoleic acid increases (Sanders et al., 1993). The FAP methodology enables these changes in fatty acid concentration to be detected, and thus may be useful in determining rancidity in peanuts.

Fats and oils are natural esters, formed from glycerol, and fatty acids. The esters in fats and oils are called triglycerides (Garnett, 1985). The FAP procedure requires a small volume of fat/oil to be diluted with water, which is boiled to evaporate the natural ester out of the solution. Alcohol is then added to the oil, to trigger an acid plus alcohol yielding an ester plus water reaction. The ester is evaporated, only to be condensed back into the oil solution. Boron trifluoride is added, and allowed to reflux for a few minutes, prior to adding hexane, which is also allowed to reflux. Saturated sodium chloride is added to the oil solution once it has cooled, to cause the solvent and fatty acid to float to the top of the solution, so it can be pipetted off into a vial containing anhydrous sodium sulphate (Youngberg, 1998). On completion of this procedure the fatty acid profiles can be determined using gas chromatography (GC).

In rancidity determination the GC method is commonly used to measure volatile hydrocarbons such as pentane, which have been formed as a result of the breakdown of peroxides. It has also been found to be particularly useful in detecting trace amounts of low molecular weight aldehydes, which are characteristic of advanced rancidity (Allen and
Detection of Rancidity in Peanuts

Hamilton, 1994). In has only been in recent times that GC has been considered an appropriate and effective method for constructing fatty acid profiles for fats/oils, in order to identify trends between specific fatty acid concentrations, and the age of the fat/oil.

2.4.4 Rancimat

The rancimat is a rapid automated method used to determine the oxidative stability of fats and oils. It has the ability to measure an oil's resistance to oxidative rancidity by determining the induction period (IP). The IP is the time prior to the rapid deterioration of an oil, therefore an oil that reacts quickly has a shorter induction period than one that reacts slowly, the latter being the most stable (Allen and Hamilton, 1994). Measurements by the rancimat are based on the detection of volatile secondary products which have formed as a result of the decomposition of hydroperoxides.

The rancimat enables the induction phase, in which negligible amounts of secondary products are formed, and an oxidation phase, where hydroperoxides breakdown into volatile products of the fat to be determined. The operation of the rancimat involves six samples placed in the machine, and each sample analysed simultaneously. A stream of air at a predetermined temperature interacts with the fat sample in an attempt to encourage oxidation. The machine determines the peroxide values of the fat sample at varying time intervals, or records the time for the fat to reach a predetermined peroxide value, depending on the researchers methodology.

The results obtained from the rancimat when testing oils that incorporate the antioxidants BHA or BHT are of little value, since both antioxidants are highly volatile. During the rancimat process BHA or BHT is swept up into the air steam that passes through the system, thus removing it from the oil. The results then reflect the speed in which the antioxidants can be swept out of the oil, rather than the oxidative resistance of the oil-antioxidant combination (Allen and Hamilton, 1994).

The OSI (oxidative stability index) apparatus is the American equivalent to the European rancimat. The OSI apparatus is thought to have made several improvements on the
rancimat. The OSI, unlike the rancimat can analyse 24 samples simultaneously at two different temperatures between 40°-200°C. It also has more sturdy electrical sensors which last an extended period of time and provide higher mechanical stability. The expensive glassware used to hold the samples in the rancimat have been replaced in the OSI apparatus with inexpensive disposable glass tubes, to reduce both cost and problems associated with cleaning away residual oil (Allen and Hamilton, 1994).

The rancimat and OSI apparatus are used to determine the induction period of fats and oils. Accelerated aging of sample oils or fats takes place in the rancimat or OSI apparatus using heat. At various stages the PV is determined, and plotted against time on a graph called an oxidation curve. There should be a clear distinction shown on the oxidation curve for the induction period and rapid oxidation stage of the oil aging process. Analysing peanuts using the rancimat or OSI apparatus would be beneficial. These machines provide accurate, precise and reliable methods for detecting rancidity and the expectant shelf-life of foods. It is unfortunate that in Western Australia access to the rancimat or OSI apparatus is limited, in fact it is doubtful that there is either of these machines in this State. Although this technology would be a beneficial investment in areas involved in large scale rancidity testing, but for the purposes of periodic rancidity research the capital investment would be too high, as these are very costly machines.

2.4.5 Electronic Nose

The electronic nose or aromascan analyses complex vapours (aroma) using an array of sensors (Hodgins, 1997). It is useful as an alternative methodology to gas chromatography and sensory analysis. The electronic nose is made up of three primary components; sensory array, conversion of sensor signals, and software analysis (Hodgins, 1997).

The electronic nose requires between six and twelve non-selective sensors which are able to monitor a large number of vapours. In detecting rancidity, a few of the sensor types would be specifically designed to detect rancid odours, while the remaining sensor types would be suitable for the sample being tested, in this case peanuts.
The analysis of data by the electronic nose includes fingerprinting the aroma from the sensory array, by taking the individual sensor outputs at any specific time during the test period. The data provided by the aromascan can be as simplistic or comprehensive as desired, and can be subject to a variety of statistical tests and graphical representations (Hodgins, 1997).

A major disadvantage of the electronic nose is that it can not distinguish the nature of the complex vapours, or the degree to which the vapours are sensory acceptable. The electronic nose does not come cheap, at around $70,000 dollars each, it is likely that only large food companies would make such a costly investment. In Western Australia access to electronic nose technology in the food industry is negligible, the only existing aromascan in this State belongs to the mining industry, and thus is unsuitable for food aroma analysis. This is somewhat unfortunate as many food manufacturers and researchers would find the aromascan technology useful for detecting poor quality food products.

Applications of the aromascan technology in the food industry are vast. It is useful in assessing the quality of raw ingredients prior to processing, so as to avoid “off-odours” tainting the final product. This also enables the food manufacturers to identify suppliers who are providing inferior quality raw ingredients. Product development and product simulation would be made easier using the electronic nose, by providing fingerprints for all ingredients or products, and matching these prints with those that consumers have found sensory acceptable (Hodgins, 1997).

In Hodgins, 1997 review of the electronic nose system, she suggests that detecting rancidity in nuts prior to their use in the food industry could significantly reduce the rate of product spoilage. Hodgins performed an electronic nose analyses of fresh walnuts and rancid walnuts, using a 10-sensor conducting polymer array. It was found that significant differences existed between all 10-sensors, indicating that all walnut samples were of varying degrees of rancidity. The author concluded that this system offered a rapid routine assessment of the degree of walnut rancidity. It is expected that the electronic nose would be equally as effective a method for detecting rancidity in peanuts.
2.4.6 Sensory Evaluation

This method provides a descriptive flavour analysis of the food being studied. Sensory evaluation is seen by some researchers (Bett and Boylston, 1992) as being the most accurate method of determining rancidity in foods. Although many researchers (Hodgins, 1997; Pomeranz and Meloan, 1994) disagree arguing that sensory evaluation is a subjective method, as flavour analysis is always biased by the sampler, no matter how experienced the person is in sensory evaluation.

Sensory evaluation relies on descriptive terms such as nutty, beany, rancid, painty and so on, to describe the sensory sensations experienced when tasting a specific food (Allen and Hamilton, 1994). A panel of eight to twelve people, who are trained in sensory evaluation is often the preferred manner in which sensory evaluation is used. Panellists complete a questionnaire which asks the panellist to indicate on a measurable scale, such as a fifty point differential scale, where the sample being tested would be placed, for each descriptive term. A wider study can be conducted using the same questionnaire format, but instead of trained panellists, a random sample is taken from the general population (Lawson, 1985).

There is no doubt that if done correctly sensory evaluation is an effective method of determining rancidity in foods. As sensory evaluation is subjective, such that what one person finds rancid, another person finds edible, it would not stand up in a court of law. Analytical methods enable the establishment of a benchmark, which indicates the point at which a food is considered rancid. It is for this reason that an analytical method for detecting rancidity in peanuts has been chosen, in preference to sensory evaluation in this study.

2.5 FACTORS AFFECTING THE SHELF-LIFE OF PEANUTS

As peanuts are between 46-56% oil, autoxidation of the lipid portion of the nut is the major contributor to negative flavour changes. Literature suggests that as peanuts mature the oil content, oleic/linoleic (O/L) ratio and triacylglycerol content increase, while other
components such as free fatty acids, polar lipids, monoacylglycerols and diacylglycerols decrease (Sanders et al., 1993, p. 290). According to Bett and Boylston (1992), the larger the peanut size, the less susceptible it is to rancidity. They found that smaller peanuts, and immature peanuts had a greater potential for off-flavour development, due to the presence of increased concentrations of aldehydes, alcohols and ketones.

Light, and heat are both factors that influence the shelf-life of peanuts. Light promotes oxidation in peanuts, especially shorter wavelength light, which penetrates the peanuts with much greater intensity (Allen and Hamilton, 1994). Elevated storage temperatures shorten the shelf-life of peanuts, a 10°C increase in storage temperature can double the rate of oxidation. Freezing peanuts in an airtight container extends their shelf-life, as the peanuts retain their freshness and eating quality. Ideally peanuts are best stored at 18.5°C in an airtight container in a dry cool place.

In commercial storage operations peanuts may undergo a variety of biochemical reactions, affecting their nutritive value, sensory acceptability and appearance. After harvest, care must be taken to ensure that the peanuts are kept well ventilated, and the moisture level in the storage house or silo is controlled. This minimises the possibility of aflatoxin contamination, and preserves the quality of the peanuts by maintaining low levels of FFA and carbonyl compounds (Sanders et al., 1993). Shelled peanuts are very susceptible to changes in moisture in the storage environment, as the moisture content of the peanut increases, off-flavours and a darkish pigment develops.

Supermarket bulkfood bins create prime conditions for peanut degradation. The constant opening of the bulkfood container exposes the peanuts to oxygen, moisture, microbes, vermin and foreign materials. As oxygen and moisture interact with the lipid portion of the peanut causing it to degrade and become rancid, it is concerning that bulkfood operations are not more rigidly regulated. Presently proprietors only have to state the country of origin of the peanuts in the bulkfood containers (ANZFA, 1997). There are no regulations in place to insist that the date of harvest or expected shelf-life of the peanuts is stated on the bulkfood bins. The decrease in peanut shelf-life during bulkfood storage conditions, as well as lack of regulation in this area, enables consumers to unwittingly purchase rancid peanuts.
2.5.1 Packaging of Peanuts and Peanut Oil

Packaging plays an important role in the preservation of peanuts. Plastics, foil wraps, paper and fabric all form the basis for packaging materials used in the peanut industry. The most effective method for packaging peanuts prevents oxygen, light, metal catalysts, and moisture from interacting with the peanuts. Vacuum packaging using a non-catalytic foil lined wrap or a dark coloured plastic would provide ideal packaging mediums. The high cost of this method limits its use in the peanut industry, it is more common for peanuts to be packaged in plastics or foil lined paper.

Consumers often purchase peanut in clear plastic packages in preference to bulk stored peanuts, with the belief that the plastic wrap preserves the quality of the nuts. Unfortunately this is not always the case as clear plastics do not eliminate light from the package, which can interact with the peanuts causing them to degrade (Allen and Hamilton, 1994). It is expected that dark coloured plastics wraps, and non-catalytic foil lined paper which has been exteriorly coated with a plastic film, are the most effective and cost efficient packaging methods to preserve peanut quality.

Oils are highly empirical and unstable so it is important to store them correctly to prevent rancidity. Peanut oil is best packaged in a dark glass bottle and stored in a dark place. Clear glass and plastics are not recommended as the oil is susceptible to light deterioration. Metal tins are also unsuitable to store oils, as not only do some metals catalyse oil deterioration, after a period of time the oil develops a distinct, undesirable metallic flavour.

2.5.2 Accelerating the Aging Process of Peanuts

Autoxidation can be accelerated by heat, irradiation, metal catalysts, oxygenation and/or enzyme action. Heating is commonly used where samples are heated under controlled conditions at high temperatures. A method to accelerate the deterioration of peanut oil, is to heat the oil at 100°C for twenty hours, taking samples every two hours to test the degree of degradation. It is expected that the early stages of deterioration occur between 0-10 hours
Detection of Rancidity in Peanuts

(Allen and Hamilton, 1994). If the same heat treatment was attempted on the peanut kernel, rather than the peanut oil, it is expected that the time required for the lipid component of the peanuts to deteriorate would be increased.

The use of high temperatures to accelerate oxidation can produce misleading results, since most oils age under ambient storage conditions at 20-25°C. Aging of peanuts at room temperature is slow, such that it can take several or more months from harvest to rancidity. Research time constraints dictate the method by which peanuts are aged. It is suggested that by heating oil to 60°C for 72 hours, rather than 100°C for 20 hours, would still accelerate the aging of the oil, but reduce the concentration of degradation products produced from the heating process (Allen and Hamilton, 1994). The same principle can be applied to the aging of the peanut kernel, but rather than oxidation occurring after 72 hours exposure to 60°C, it is expected that the early stages of oxidation would be detected during the second week of exposure to 60°C.

In examining storage conditions the aging process of the peanuts would be best accelerated at a temperature of 40°C, since in the summer months, it is not uncommon for this temperature to be reached. It is expected that for every 10°C rise in temperature, the rate of rancidity development is doubled (Gunstone and Padley, 1997). If this is true, then it would be expected that at 40°C the peanuts would age at a rate which is 4 times lower than peanuts heat at 60°C. Since peanuts are best stored around 20-25°C, it is probable that the peanuts stored at both 40°C, and 60°C would age at a considerably faster rate, particularly at the latter temperature.

The rate of oil deterioration depends on the surface area of the oil, such that the greater the surface area exposed to the atmosphere, the faster the rate of oil degradation (Gordon and Mursi, 1994). The aging process of peanut oil could be accelerated by pouring small volumes of the oil into open petri dishes at ambient temperature. This would increase the surface area of the oil exposed to air, so that the rancidity process would be accelerated by oxygen rather than heat. Leaving an oil containing petri dish in the sunlight, would further accelerate the aging process, due to oxygen, heat, and light interacting with the oil (Gordon and Mursi, 1994). Exposing peanut kernels to sunlight is likely to decrease the shelf-life of the peanuts, and thus achieve a greater rate of oil deterioration in a shorter period of time.
Moisture is important in the development of hydrolytic rancidity, and when combined with heat this process is rapidly accelerated. Moist, warm environments cause deterioration in the oil quality of peanuts, and initiate the growth of undesirable aflatoxins and moulds (Gunstone and Padley, 1997). It would be useful to determine the rate at which peanuts age under humid conditions, yet in a laboratory situation humidity is often difficult to simulate.

2.6 JUSTIFICATORY STATEMENT

Peanut growers, suppliers and vendors need to be accountable for the quality of the peanuts they sell. Consumers purchase peanuts with the expectation that the peanuts are of good eating quality. If the purchased peanuts are rancid, then the consumers expectations have not been met, and she/he has the right to seek compensation. Legally it is difficult to prosecute suppliers or vendors for selling poor quality peanuts, due to the absence of a standardised methodology for determining rancidity. In order to prevent suppliers and vendors from continuing to sell rancid peanuts, a standardised methodology for detecting rancidity in peanuts needs to be established.
3. RESEARCH DESIGN

3.1 AIMS OF THE STUDY

3.1.1 General Aim

⇒ To detect rancidity in peanuts using Peroxide Value (PV), Acid Value (AV) and Fatty Acid Profile (FAP) methodologies.

3.1.2 Specific Aims

♦ To determine the best storage conditions for preserving the shelf-life of peanuts.
♦ To extract the oil from the peanuts without altering the chemical composition of the oil.
♦ To accelerate the aging process of the extracted peanut oil.
♦ To test the extracted peanut oil for rancidity using Peroxide Value method.
♦ To test the extracted peanut oil for rancidity using Acid Value method.
♦ To determine the Fatty Acid Profile for the extracted peanut oil.
♦ To identify the methodology (PV, AV or FAP) that is most effective in detecting rancidity in peanuts.

3.2 METHODS AND PROCEDURES

3.2.1 Sampling of Peanuts

Fresh Peanuts
The raw peanuts were supplied by The Peanut Company of Australia, (Kingaroy, Queensland). These peanuts were presumed to be fresh, since the peanut company receives peanuts directly from growers. The variety of peanuts chosen was 'Streeton 1 Raw Trade-Stirr, as it is the most common variety available for purchase in Western Australia.
The peanuts were tested for rancidity on arrival to the laboratories to ensure that they were fresh. The aging process of the peanuts was accelerated by exposing fresh peanuts to heat and light for a period of eight weeks.

**Old Peanuts**
A 3 year old sample of peanuts, 'Peters 95' was obtained, and tested for rancidity, using PV and FAP tests.

**Freezing**
A sample of 50-60g of the fresh peanuts were frozen at -20°C for eight weeks. In week 8, the peanuts were removed from the freezer and left to thaw at room temperature. The oil was then extracted for PV, AV and FAP tests.

**Recommended Storage Conditions**
The remaining fresh peanuts were placed in an airtight container and stored in a cool dark place. During week 8, the oil from 50-60g sample of peanuts was extracted and tests conducted for PV, AV and FAP.

**Light Exposure**
Approximately 100g of fresh peanuts were spread in a single layer on an aluminium tray and placed outside in the sunlight for eight weeks. During the eighth week the peanut oil was extracted and PV, AV and FAP tests were conducted.

A second tray containing a single layer of about 100g of the fresh peanuts was placed in a well lit area of the food laboratory, where the fluorescent lighting could be left on for 24 hours a day for the entire eight week period. Similarly to the sunlight exposed peanuts, the peanuts exposed to fluorescent lighting were tested for PV, AV and FAP during the eighth week.
**Heat Treatments**

Twenty small disposable aluminium trays were each filled with 50-60g of the fresh peanuts. 10 trays containing peanuts were placed in an oven which was preheated and set at 40°C. The remaining 10 trays were placed in another oven which was preheated and set at 60°C.

The peanut oil was extracted on the day the peanuts were removed from the ovens. Peroxide Value (PV) and Acid Value (AV) tests were performed upon completion of the oil extraction procedure, refer to section 3.2.2. Fatty Acid Profiles (FAP) were performed in batches, so the oil remaining after the completion of PV and AV tests were placed in a labelled test tube and frozen at -20°C until required. A tray of peanuts from the 40°C oven and 60°C oven were tested for PV and AV daily during the first week of the incubation period. In the following 2-8 weeks, the peanuts were tested for PV and AV on a weekly to fortnightly basis.

It should be noted that all heated peanut samples when removed from the ovens were left to cool to room temperature prior to grinding for oil extraction.

**3.2.2 Oil Extraction Procedures**

Two oil extraction methods were trialed.

**3.2.2.1 Method 1: Chloroform/Methanol Oil Extraction Methodology**

**Reagent Preparation**

I. Chloroform/Methanol (CH₃Cl/MeOH) - A 2:1 vol/vol solution was prepared by combining 1L of CH₃Cl with 500ml of MeOH.

II. Sodium Chloride Solution (NaCl) - A saturated solution of NaCl was prepared by heating a beaker of distilled water and adding solid NaCl until no more would dissolve.
Detection of Rancidity in Peanuts

Procedure

1. 50-60g of peanuts (with skins) were ground into a mash using a coffee grinder. Care was taken to avoid over grinding the peanuts in order to prevent too much of the oil being lost and the peanut mash becoming clumpy.

2. The peanut mash was placed in a blender, and 300ml of the prepared CH₃Cl/MeOH was added. The mixture was blended for 1-2 minutes.

3. The blended mixture was slowly poured into a filter paper lined Buchner funnel which was connected to an aspirator. The mealy components of the peanut (carbohydrate and protein) formed the residue, while the CH₃Cl/MeOH and oil were collected and poured into a separatory filter.

4. Saturated NaCl was added to the separatory filter containing the CH₃Cl/MeOH and oil. This was shaken vigorously for approximately 1 minute, and then left to stand until phase separation appeared to be complete (1.5-2 hours).

5. The water layer being more dense than the oil and CH₃Cl layer sank to the bottom of the separatory filter, where it was tapped off and discarded.

6. The remaining solution in the separatory filter was washed with more saturated NaCl, shaken and left to stand until further phase separation occurred. The water layer was again tapped off and discarded. This step was repeated to ensure phase separation was complete. This process took less than 1 hour.

7. The oil was separated from the CH₃Cl by a rotary extractor. CH₃Cl (62°C) having a significantly lower boiling point (BP) than peanut oil (>226°C) was evaporated out of the solution.

8. The remaining oil was placed in a 70°C oven for 5 minutes to ensure all of the chloroform was removed.

9. The oil was left to stand until it cooled to room temperature.

Summary of CH₃Cl/MeOH Methodology

A good quantity of oil was extracted from the 50-60g peanut samples, however this method was very time consuming, as the phase separation stages (steps 4-6) took between 2-3 hours to complete. This was the major limitation of the CH₃Cl/MeOH method.
3.2.2.2 Method 2: Hexane Oil Extraction Methodology

Procedure
1. Similarly to the chloroform/methanol oil extraction method, 50-60g of peanuts (with skins) were ground in a coffee blender until a powdery mash was formed.

2. The peanut mash was poured into a blender, and approximately 100ml of hexane was added. This mixture was blended for 1-2 minutes.

3. The blended peanut and hexane mixture was slowly poured into a filter paper lined Buchner funnel which was connected to an aspirator. The mealy components of the peanut (carbohydrate and protein) formed the residue, while the hexane and oil were collected and poured into a bulb flask.

4. The hexane (BP=68.9°C) was evaporated out of the peanut oil (BP>226°C) using a rotary extractor.

5. The remaining oil was placed in a 70°C for 5 minutes to ensure that all the hexane had been removed.

6. The oil was left to stand until it cooled to room temperature.

Summary of Hexane Methodology
The hexane method was not only effective in extracting a good quantity of oil from each 50-60g peanut sample, but it was also rapid, since each extraction was completed within 20 minutes. This was the chosen oil extraction methodology for all of the heat treated and light exposed peanut samples.

3.2.3 Peroxide Value Determination

The peroxide value methodology used for the detection of rancidity in the extracted peanut oil, is a modified method based on the AOAC method Cd 8-53 (1990). The procedure used are as follows:
Reagents

I. Acetic acid-chloroform solution (HOAc-CHCl₃) - 3 vols HOAc mixed with 2 vols CHCl₃

II. Potassium iodide solution (KI), saturated - A saturated solution of KI was formed by heating distilled water and adding KI until no more would dissolve. This solution was prepared in small volumes (<5ml) as it was needed.

III. Sodium thiosulphate (Na₂S₂O₃) - 0.1N prepared standard solution.

Procedure

1. Approximately 5.00g peanut oil was weighed into 200ml conical flask.
2. To this 30 ml of HOAc-CHCl₃ was added, and the mixture was swirled until the oil dissolved.
3. Then 0.5 ml of saturated KI solution was added and the solution was left to stand for 1 minute, shaking occasionally.
4. Finally, 30 ml of distilled water (H₂O) and about ⅛ of a teaspoon of vitex indicator was added, and the solution turned purple in colour.
5. The 0.1N Na₂S₂O₃ was slowly titrated into the oil solution, until the purple colour disappeared. This marked the end point of the reaction.

Calculation

The Peroxide Value (milliequiv. peroxide/kg sample) was calculated using the following formula:

\[ PV = S \times N \times \frac{1000}{m} \]

where \( S \) = volume (ml) of Na₂S₂O₃ (blank corrected)
\( N \) = normality Na₂S₂O₃
\( m \) = mass of oil (g)
3.2.4 Acid Value Determination

Reagents

I. Ethanol, neutralised - The ethanol was neutralised by adding 2 drops of phenolphthalein indicator, and then gradually adding drops of the 0.1N NaOH until the ethanol turns (very) faint pink in colour.

II. Sodium Hydroxide (NaOH) - 1.0N prepared standard solution.

Procedure

1. Approximately 3.00g of peanut oil was weighed into a 200ml conical flask.
2. 50ml of neutralised ethanol was warmed in a hot water bath, and poured into the peanut oil. To this 2ml of phenolphthalein indicator was added, and the solution became milky white in colour.
3. 0.1N NaOH was titrated into the oil and ethanol solution, which was shaken vigorously. When the solution retained a pinkish colour for at least 30 seconds the end point reading was recorded.

Calculation - based on oleic acid.

The Acid Value (milliequiv. KOH/1g sample) was calculated using the following equation:

\[
\text{Acid Value} = \frac{\%\text{FFA} \times \text{Oleic}}{\text{m}}
\]

\[
\%\text{FFA (free fatty acids) Oleic} = \frac{S \times N \times 28.2}{m}
\]

where  
\(S\) = volume (ml) of NaOH  
\(N\) = normality of NaOH  
\(m\) = mass of oil (g)  
28.2 = standard value for oleic acid  
1.99 = standard value used to convert FFA Oleic to Acid Value
3.2.5 Fatty Acid Profiles (FAP)

This methodology was written and conducted by Mr Bruce Youngberg from the Chemistry Centre of Western Australia. The FAP methodology he used was as follows:

Reagents
I. Caustic methanol - 1g of NaOH was dissolved in 50 ml of methanol.
II. Boron Trifluoride
III. Hexane
IV. Saturated NaCl - excess NaCl solid was dissolved in heated distilled water.
V. Anhydrous Sodium Sulphate

Procedure for FAP
1. 0.1 ml of peanut oil was diluted with 20 ml of distilled water.
2. 1 ml of the oil solution was pipetted into a 50 ml round bottom flask containing some boiling chips. The ester was boiled off and immediately esterified.
3. 4 ml of caustic methanol was added.
4. The vapour was then liquefied and boiled for 10 minutes.
5. 5 ml boron trifluoride was through a condenser.
6. Again the vapour was liquefied and boiled for another 3 minutes.
7. This mixture was then cooled.
8. The flask was disconnected and enough NaCl was added so that the solvent floated on the top.
9. The solvent was transferred into a 25 ml vial which contained anhydrous sodium sulphate.
10. This was shaken and stored in the freezer until used.
11. The analysis of fatty acids was conducted using gas chromatography.

Conditions for Gas Chromatography (GC)
BPX 70 column
- Initial temperature 100°C, held for 5 minutes. Ramp 2.5 degrees/minute.
Detection of Rancidity in Peanuts

- Final temperature 150°C, held for 2 minutes. Ramp 1.5 degrees/minute.
- Final temperature 220°C, held for 1 minute. Ramp 6 degrees/minute.
- Final temperature 260°C, held 5 minutes.

- Injector temperature 260°C
- Detector temperature 275°C
- Chart speed 0.7cm/minute
- Attenuation 0
- Area Reject 2000
- Head Pressure 200 kPa

**Standards**

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4. RESULTS

The titration results, and calculations for the PV, and AV tests are shown in Appendix A, and B, respectively. Appendix C shows the fatty acid profile results, such that the concentration of each free fatty acid in the peanut oil is known. The concentration of oleic and linoleic acid in the peanuts oil, are displayed in both Table 1, and Table 2. Changes in the concentration of these fatty acids is more strongly associated with the rancidity process, than the other fatty acids detected using the FAP methodology.

The following table is relevant to sections 4.1 to 4.4 inclusive. Table 1 shows the peroxide value, acid value, oleic acid and linoleic acid contents of six samples which have been stored under varying conditions as discussed in the methods section 3.2.1.

<table>
<thead>
<tr>
<th>Peanut Samples</th>
<th>Peroxide Value (PV)</th>
<th>Acid Value (AV)</th>
<th>Fatty Acid Profiles (FAP)</th>
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</thead>
<tbody>
<tr>
<td>(1)</td>
<td>1.51</td>
<td>0.44</td>
<td>47.6 30.6</td>
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<td>110.00</td>
<td>-</td>
<td>36.1 35.4</td>
</tr>
<tr>
<td>(3)</td>
<td>1.54</td>
<td>0.51</td>
<td>47.6 30.4</td>
</tr>
<tr>
<td>(4)</td>
<td>1.56</td>
<td>0.46</td>
<td>47.6 30.5</td>
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<td>1.70</td>
<td>0.54</td>
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</tr>
<tr>
<td>(6)</td>
<td>10.20</td>
<td>0.70</td>
<td>48.1 29.5</td>
</tr>
</tbody>
</table>

* PV is measured in ml equivalents O₂/kg fat
* AV is measured in ml equivalents of KOH required to neutralise 1g of fat

(1) Fresh raw peanuts  (4) Peanuts stored under recommended conditions
(2) Old peanuts - 'Peters 95'  (5) Peanuts exposed to laboratory light
(3) Peanuts frozen and stored at -20°C  (6) Peanuts exposed to sunlight
4.1 Fresh Peanuts

The fresh raw peanuts had a low PV (1.51) and AV (0.44). The oleic acid content was 47.6%, and the linoleic acid content was 30.6%. These results were as expected, thus verifying that the raw peanuts were fresh.

4.2 Old Peanuts

The old peanut sample was found to have an extremely high PV of 110. Peanuts are classified as rancid with a PV of 20, so the 'Peters 95' sample was definitely not of eating quality. The fatty acid profile of the 'Peters 95' sample showed an increase in linoleic acid (35.4%), and a decrease in oleic acid (36.1%) contents, compared to the linoleic acid (30.6%), and oleic acid (47.6%) contents of the fresh peanut sample. This would suggest that as peanuts age the concentration of oleic acid decreases, whilst the concentration of linoleic acid increases.

4.3 Peanuts Stored at -20°C

The peanuts that were frozen at -20°C for 8 weeks were found to have a low PV (1.54), and low AV (0.51). The oleic acid content (47.6%), and the linoleic acid content (30.4%) were similar to that of the fresh peanuts. However, these results were as expected, since freezing inhibits the onset of rancidity, by prolonging the aging process of the peanuts. The eating quality of the peanuts was not affected by freezing, such that the peanuts were of similar eating texture and flavour to the fresh peanuts. It is expected that raw peanuts would remain of good eating quality for at least 5 years if kept frozen at -20°C (Shewfelt and Young, 1977). Once the peanuts have been removed from the freezer it is expected that they would begin to age similarly to the peanuts stored at room temperature.

4.4 Peanuts Stored Under Recommended Conditions

The peanuts were stored in an airtight container (glass bottle) and placed in a dark, cool place. The PV (1.56) and AV (0.46) were both low, as expected. The oleic acid (47.6%), and linoleic acid content (30.5%) of the peanut sample stored under recommended conditions were very similar to that of the fresh peanut sample, and frozen peanut sample.
4.5 Light Exposed Peanuts

The intensity of sunlight, particularly ultra violet rays is greater than that of laboratory light. It was therefore expected that the peanuts exposed to sunlight would age faster than those exposed to laboratory light. The results obtained from both the sunlight exposed peanuts, and laboratory light exposed peanuts supported this theory.

Laboratory Light Exposed Peanuts

The peanuts exposed to laboratory light had a similar AV (0.54) to the frozen peanut sample (0.51), which was slightly higher than the AV of the fresh peanuts (0.44). The oleic acid (47.3%) content was a little lower than that of the fresh peanuts (47.6%), and linoleic acid (30.8%) content was slightly higher than that of the fresh peanuts (30.6%). These small differences in oleic and linoleic acid content are likely to be insignificant.

The PV (1.70) was higher than the PV of the fresh peanuts (1.51), frozen peanuts (1.54), and the peanuts stored under recommended conditions (1.56). This could indicate that the peanuts could be in the early stages of rancidity, where hydroperoxides are being formed, or due to experimental error. The latter is the most likely scenario because the PV test is not reliable at low PV.

Sunlight Exposed Peanuts

The PV of the peanut sample exposed to sunlight was 10.2, which was significantly higher than the PV of the fresh peanut sample (1.51). This indicates that lipid oxidation has taken place, producing an increased amount of hydroperoxides. The AV (0.70) of the sunlight exposed peanuts was also higher than that of the fresh peanut sample (0.44). The oleic acid content (48.1%) was higher, and linoleic acid content (29.5%) was lower than the oleic (47.6%), and linoleic (30.6%) acid content of the fresh peanuts. Although it would appear that hydrolytic rancidity may have been initiated, in actual fact this is unlikely to be the case. It is a source of concern that the oleic/linoleic acid ratio of the sunlight exposed peanuts was the reverse to that of the old peanuts. The old peanuts showed an increased linoleic acid content (35.4%), and a decreased oleic acid content (36.1%), on comparison to the fresh peanuts. The sunlight exposed peanuts showed the opposite relationship, where the oleic acid content had increased, and the linoleic acid content had decreased.
It is reasonable to assume from the PV of the sunlight exposed peanuts that they have undergone some oxidative changes. The act of leaving the peanuts in the sun for 8 weeks has exposed the peanuts to more than one environmental variable capable of triggering autoxidation. It is likely that a combination of light, heat and air was responsible for initiating hydroperoxide formation. It is possible that the oxidative changes that have occurred in the peanuts, have not affected the composition of free fatty acids. Changes in fatty acid profiles are more likely to result from hydrolytic changes in the peanuts. The AV indicated that the sunlight exposed peanuts were relatively fresh, and this was in turn supported by the oleic and linoleic acid contents. Based on the raised PV, and the low AV, it is reasonable to suggest that the sunlight exposed peanuts have been more susceptible to oxidative rancidity, than hydrolytic rancidity.

It should be noted that the PV (10.2) did not indicate that the peanuts were rancid, but did suggest that they were deteriorating in eating quality. The sunlight had initiated the formation of peroxides, and thus commenced the oxidative rancidity process. The PV level of the peanuts need to increase to 15 before being considered rancid.

4.6 Heat Treated Peanuts

The peanuts tested were found to be relatively stable throughout the 8 week period from the “heating treatments”. Table 2 shows the duration of time that the peanuts were exposed to heat at either 40°C or 60°C, having little or no effect on accelerating the aging process of the peanuts. In fact it is reasonable to assume that any small variations in the results were due to experimental error, rather than the onset of lipid oxidation or hydrolysis.
Table 2: PV, AV and FAP Results for Peanuts Heated at 40°C, and 60°C

<table>
<thead>
<tr>
<th>Date Removed from Ovens</th>
<th>Peroxide Value (PV)</th>
<th>Acid Value (AV)</th>
<th>Fatty Acid Profiles (FAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40°C</td>
<td>60°C</td>
<td>40°C</td>
</tr>
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<td>08/09/98</td>
<td>1.87</td>
<td>1.90</td>
<td>0.40</td>
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<td>1.47</td>
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<tr>
<td>04/11/98</td>
<td>1.16</td>
<td>2.38</td>
<td>0.58</td>
</tr>
</tbody>
</table>

* PV is measured in ml equivalents O₂ /kg fat
* AV is measured in ml equivalents of KOH required to neutralise 1g of fat

The peanuts from both, the 40°C and 60°C, produced similar results. The duration time at each temperature did not have any significant effect on rancidity. The temperature difference of 20°C between 40°C and 60°C failed to age the peanuts at different rates. It was expected that for every 10°C rise in temperature the rate of rancidity would be doubled (Gunstone and Padley, 1997). This was not shown to be the case as indicated in Table 2.
5. DISCUSSION AND CONCLUSION

Consumer complaints concerning the purchase of rancid peanuts, has been of concern to the Health Department of Western Australia. In the past it has been difficult to prosecute proprietors for selling poor quality peanuts, due to the absence of a standardised methodology for detecting rancidity in peanuts. Unfortunately the results from this study (refer to Table 1, and 2) were unable to identify a particular methodology from Peroxide Value (PV), Acid Value (AV), or Fatty Acid Profiles (FAP), as to which was the most effective in determining rancidity in peanuts.

Attempts to accelerate the aging process of the peanuts over an 8 week period were made, using heat and light as the environmental controls. It is evident from the results shown in Table 1, and Table 2 that this was an insufficient period of time to age fresh raw peanuts to the degree where rancidity could be detected. The only peanut sample to show any signs of becoming rancid was the sunlight exposed peanuts, which increased in PV value. Surprisingly the peanuts stored at 40°C, and 60°C, remained relatively fresh throughout the entire 8 weeks of being exposed to these temperatures. It was expected, that the rate of rancidity in the peanuts would double for every 10°C rise in temperature (Gunstone and Padley, 1997). Since the optimum storage temperature for peanuts is between 20-25°C, then at 40°C, and 60°C the rate of rancidity was expected to be rapid, especially at the latter temperature.

The are numerous explanations which may explain the failure of the peanuts to age at 40°C, and 60°C. Gunstone and Padley (1997) based their conclusions regarding the affect of heat on the rate of rancidity development in lipids, on oils. If peanut oil was aged using heat, then it would be expected to age at a significantly faster rate than if the peanut kernel was aged. This was taken into account by the researcher when attempting to age the peanut kernels using heat, however the aging process occurred at a much slower rate than expected, thus failing to have a significant effect on peanut rancidity during the 8 week period.
Detection of Rancidity in Peanuts

Since the attempts to accelerate the aging process of peanuts were rather unsuccessful, it was impossible to determine the most effective methodology (PV, AV, or FAP), for detecting rancidity in peanuts. The peroxide value method proved successful in detecting oxidative changes in the peanuts. This was seen in the sunlight exposed sample where the PV had significantly increased, while the AV had remained relatively constant. A major limitation of the iodometric PV method was that it was difficult to determine the end point of the reaction at low PV values. This affected the precision of PV results at low levels of rancidity.

The results from the AV methodology appeared to be more precise than the results from the PV methodology for each sample and its duplicates, especially at low levels of rancidity. It is not known how effective this method is at high AV, as the highly rancid 'Peters 95' sample was not AV tested, due to an insufficient quantity of the extracted oil. It is possible for the AV of an oil to be low, even after considerable oxidation has taken place (Lezerovich, 1985). The AV, and PV results for the sunlight exposed peanuts, support this theory, as the increased PV indicates that oxidation has occurred, while the AV has remained relatively constant. It is for this reason that it would be beneficial to test peanuts for rancidity using AV and PV methodologies. This would ensure that the peanuts were tested for both hydrolytic and oxidative rancidity.

The FAP methodology is more complex, and time consuming than the PV, and AV methods. A certain degree of skill is required to perform FAP tests, and while there is no standardised methodology for FAP testing, the procedure used has proved useful for determining the concentration of each fatty acid in the peanut oil samples. The FAP, like the AV detects the concentration of free fatty acids in the oil. However, the FAP is a more specific test than the AV, as it identifies the concentration of each fatty acid present in the oil. The AV is based on the most predominant fatty acid present in the oil, for peanut oil this is oleic acid. The FAP methodology is useful for detecting rancidity in peanuts, however PV and AV tests appear to be equally as effective, and yet are less complex, take a shorter period of time to perform, and are lower in cost. It is suggested that when the concentration of each specific fatty acid in the peanut oil does not need to be known, then simply using the AV test to determine the oleic acid concentration of the oil is sufficient to assess its rancidity status.
Overall the results from this study found that raw peanuts were relatively stable, and could withstand long term exposure to increased temperatures. It is therefore fair to assume that rancid raw peanuts, have either been stored incorrectly for a long period of time, or are old. If stored under optimum environmental conditions peanuts may remain of sound eating quality for up until 5 years (Shewfelt and Young, 1977). So the sale of rancid peanuts could suggest that suppliers, and/or grocers have stored the peanuts beyond their use-by date. To prevent rancid peanuts being sold, then standardised methods to detect rancidity, and thus act as a deterrent to those dodgy peanut suppliers, and grocers, who persist in selling poor quality peanuts.

The PV, AV, and FAP methodologies were all effective in determining the degree of lipid deterioration in the peanuts. It is difficult to identify a singular methodology as being the most effective, as all three method analysed different changes occurring in the peanut oil. The PV test measured the quantity of peroxides formed, the AV test measured the concentration of free fatty acids, based on oleic acid, and the FAP test measured the concentration of each specific fatty acid in the peanut oil. Overall the PV, and AV methods were less time consuming, and easier to perform than the FAP method. It is expected that knowledge of the PV, and AV of the peanut oil would provide enough evidence to identify the rancidity status of the oil. Therefore, it is suggested that prosecutors require both the PV, and AV values to be determined for all peanut samples, in order to detect rancidity in the peanuts.
6. RECOMMENDATIONS

Based on the results of this study, the following recommendations can be made:

- Moisture and heat are critical environmental conditions which can affect the stability and shelf-life of peanuts. It would be useful to create a humid environment in which to store raw peanuts, and periodically test the peanuts for PV, AV and FAP. The results of such a study would be useful to peanut growers, as peanuts are often cultivated in regions where humidity is high, such as Kingaroy in Queensland.

- Since it was found that the end point of the iodometric PV methodology was often difficult to determine at low PV, it is suggested that the electrochemical PV technique be trialed. The electrochemical PV method as explained in the literature review, section 2.4.1, enables very low PV to be detected more accurately (Lezerovich, 1985).

- Roasted peanuts are more readily available in supermarket bulkfood bins, than raw peanuts. Therefore, it would be useful to test roasted peanuts for rancidity.

- Construction of standard curves for peanut rancidity based on PV and AV would be useful. Two curves would exist, one for PV, and the other for AV. As the age of the peanuts increases, so too does the degree of rancidity. The use of standard curves would enable the researcher to plot the determined PV or AV results on their corresponding curves, and from this determine the age of the peanuts. To prosecute offending proprietors for selling rancid peanuts, the use of standard curves would enable the prosecutor to make a fairly accurate estimation of the peanuts age.

- It would be interesting to conduct a random study where peanut samples are purchased from a variety of supermarkets, health food stores, and other grocers, to be analysed using PV, AV and FAP methods.
7. CRITICISMS OF THE STUDY

Based on the results of this study, the following criticisms can be made:

- Difficulty was experienced in locating peanuts that were of known age, or known to be rancid. It was found that suppliers discarded peanuts that were not of good quality. The PV, AV and FAP analysis of peanuts of known age would have enabled standards to be determined. This would have made it easier to reach conclusions from the research, particularly those involving the effectiveness of the PV, AV and FAP methodologies for detecting rancidity in peanuts.

- The 8 week time period available for completing this research was a major limiting factor. It became evident as time progressed that the peanuts were relatively stable, and would therefore require a longer period of time to be affected by the various aging techniques employed. Unfortunately, the period of time required to accelerate the aging process of the peanuts was poorly judged by the researcher. In reflection a 20 week time period would have been more appropriate.

- The peanut variety of the rancid sample ('Peters 95') was not known, and therefore maybe of a different variety to that of the fresh raw peanuts. Different peanut varieties have varied contents of FFA, so it is probable that the oleic and linoleic acid ratio would be different for each variety. This is not thought to be a significant limitation for this study, however in future studies using the same peanut variety, or subjecting differing varieties to the same test conditions may be beneficial.

- The results obtained from this study are typical of raw peanuts only, roasted peanuts are expected to age differently, as they have already been exposed to high temperatures during the roasting process.
Experimental error is another limitation of this study. At low PV the end point of the reaction was difficult to determine, thus resulting in error. The results from the AV, and FAP tests were just as equally affected by experimental error, which may have occurred throughout any of the stages of rancidity determination. A degree of experimental error is expected for all analytical procedures, however minimisation of error is an important consideration in obtaining accurate and precise results.
REFERENCES


Rogers, J. (1990). What food is that?: and how healthy is it? Sydney: Lansdowne Publishing Pty Ltd.


Detection of Rancidity in Peanuts

BIBLIOGRAPHY


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APPENDICES

Appendix A: Peroxide Value Results

Appendix B: Acid Value Results

Appendix C: Fatty Acid Profile Results
## Appendix A: Peroxide Value Results

### Peroxide Value Results for the Fresh Peanuts, Old Peanuts, Frozen Peanuts, Peanuts Stored Under Recommended Conditions, Peanuts Exposed to Laboratory Light, and Sunlight Exposed Peanuts

<table>
<thead>
<tr>
<th>Date Samples Tested</th>
<th>Mass Oil (g)</th>
<th>Final Titre</th>
<th>Initial Titre</th>
<th>Vol (ml) Na₂S₂O₅</th>
<th>Peroxide Value</th>
</tr>
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The Peroxide Value (milliequiv. peroxide/kg sample) was calculated using the following formula:

\[ PV = \frac{S \times N \times 1000}{m} \]

where  
- \( S \) = volume (ml) of Na₂S₂O₅ (blank corrected)  
- \( N \) = normality Na₂S₂O₅  
- \( m \) = mass of oil (g)
### Peroxide Value Results for Peanuts Heated at 40°C

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The Peroxide Value (milliequiv. peroxide/kg sample) was calculated using the following formula:

\[
PV = S \times N \times \frac{1000}{m}
\]

where  
- \( S \) = volume (ml) of Na₂S₂O₃ (blank corrected)  
- \( N \) = normality Na₂S₂O₃  
- \( m \) = mass of oil (g)
### Peroxide Value Results for Peanuts Heated at 60°C

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<th>Mass Oil (g)</th>
<th>Final Titre</th>
<th>Initial Titre</th>
<th>Vol (ml) Na₂S₂O₃</th>
<th>Peroxide Value</th>
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The Peroxide Value (milliequiv. peroxide/kg sample) was calculated using the following formula:

\[
PV = \frac{S \times N \times 1000}{m}
\]

where  
- \( S \) = volume (ml) of Na₂S₂O₃ (blank corrected)  
- \( N \) = normality Na₂S₂O₃  
- \( m \) = mass of oil (g)
## Appendix B: Acid Value Results

### Acid Value Results for the Fresh Peanuts, Frozen Peanuts, Peanuts Stored Under Recommended Conditions, Peanuts Exposed to Laboratory Light, and Sunlight

#### Exposed Peanuts

<table>
<thead>
<tr>
<th>Date Samples Tested</th>
<th>Mass Oil (g)</th>
<th>Final Titre</th>
<th>Initial Titre</th>
<th>Vol (ml) NaOH</th>
<th>Acid Value</th>
</tr>
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<tr>
<td>Peanuts Frozen at -20°C</td>
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<td>Recommended Storage Conditions</td>
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<td>Peanuts Exposed to Lab Light</td>
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</table>

The Acid Value (milliequiv. KOH/1g sample) was calculated using the following equation:

\[
\text{%FFA (free fatty acids) Oleic} = \frac{S \times N \times 28.2}{m} \\
\text{Acid Value} = \frac{\text{%FFA} \times 1.99}{m}
\]

where \(S\) = volume (ml) of NaOH; \(N\) = normality of NaOH; \(m\) = mass of oil (g); 28.2 = standard value for oleic acid; and 1.99 = standard value used to convert FFA Oleic to Acid Value.
Detection of Rancidity in Peanuts

Acid Value Results for Peanuts Heated at 40°C

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<th>Date Samples Tested</th>
<th>Mass Oil (g)</th>
<th>Final Titre</th>
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<th>Vol (ml) NaOH</th>
<th>Acid Value</th>
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<td>2.20</td>
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</table>

The Acid Value (milliequiv. KOH/1g sample) was calculated using the following equation:

\[
\text{%FFA (free fatty acids) Oleic} = \frac{S \times N \times 28.2}{m}
\]

\[
\text{Acid Value} = \%\text{FFA} \times 1.99
\]

where \( S \) = volume (ml) of NaOH; \( N \) = normality of NaOH; \( m \) = mass of oil (g); 28.2 = standard value for oleic acid; and 1.99 = standard value used to convert FFA Oleic to Acid Value.
Detection of Rancidity in Peanuts

Acid Value Results for Peanuts Heated at 60°C

<table>
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<tr>
<th>Date Samples Tested</th>
<th>Mass Oil (g)</th>
<th>Final Titre</th>
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<th>Vol (ml) NaOH</th>
<th>Acid Value</th>
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<td>2.00</td>
<td>0.20</td>
<td>0.36</td>
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<td>09/09/98 1</td>
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<td>Average AV</td>
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<td>0.46</td>
</tr>
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<tr>
<td>04/11/98 1</td>
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<td>Average AV</td>
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<td></td>
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</tr>
</tbody>
</table>

The Acid Value (milliequiv. KOH/1g sample) was calculated using the following equation:

\[
\%\text{FFA (free fatty acids)} \times \text{Oleic} = \frac{S \times N \times 28.2}{m}\]

\[
\text{Acid Value} = \%\text{FFA} \times 1.99
\]

where \(S\) = volume (ml) of NaOH; \(N\) = normality of NaOH; \(m\) = mass of oil (g); 28.2 = standard value for oleic acid; and 1.99 = standard value used to convert FFA Oleic to Acid Value.
Appendix C: Fatty Acid Profile Results

Fatty Acid Profiles for the Sunlight Exposed Peanuts, and the Heat Treated Peanuts
Dated 04/11/98, and 13/10/98

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<th>4/11/98</th>
<th>13/10/98</th>
<th>13/10/98</th>
</tr>
</thead>
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Fatty Acid Profiles for the Fresh Peanuts, Old Peanuts, Frozen Peanuts, Peanuts Exposed to Laboratory Light, and Heat Treated Peanuts


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